## 2-10 year follow-up of the cemented Lubinus SP II hip prosthesis

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**Introduction**
Total hip arthroplasty with cemented Lubinus SP II prosthesis is well analyzed in the Sweden National Hip Arthroplasty Register. There are only few reports from the other countries concerning survival analysis of this implant in short and mid-term follow-up.

**Materials and Methods**
During the years 1996-2003 1446 primary total hip arthroplasties in 1300 patients were performed in our hospital using Lubinus SP II prosthesis. The age of patients varied between 28-95 years (mean 64.9 years). Mean time of follow-up at the evaluation was 5.3 years (range 2-10 years). Clinical and roentgenological examinations of patients were performed regularly. Radiolucent lines in Gruen zones of stem were estimated.

**Results**
During the follow-up 25 hips underwent revision (revision burden 1.69%). The reasons for revision were: deep infection - 11 cases (0.8%), aseptic loosening - 9 cases (0.6%) among them 5 for both component and 4 for acetabular component loosening, periprosthetic fracture - 1 case, fracture of the stem - 1 case and hip dislocation - 5 cases (0.3%). Aseptic loosening of the stem was characterized by evaluation of Gruen zones. Radiolucent lines in zones 1 to 7 (all zones) were occurred in 1 case, in zones 1 and 7 - 1 case and in zones 2-6 - 3 cases.

**Conclusions**
The follow-up data demonstrated excellent behaviour of Lubinus SP II stem in follow-up 2-10 years period. Only in 5 cases out of 1446 primary operations the stem loosening was detected. Aseptic loosening as a reason for stem revision occurred in 0.6%, deep infection in 0.8% and dislocation in 0.3% of cases. We recommend this prosthesis for more wide use in orthopaedic clinics.

## 10 years experience with cemented LINK Lubinus SPII Hip System – no aseptic loosening

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Very good clinical long-term results of the Lubinus SPII hip prosthesis stem were reported in the literature. Unfortunately the end-point for failure in this investgations is revision. A prosthesis still in place, in our opinion, does not mean success. Our investigation included a questionare (WOMAC)

and clinical and radiological examination to create the Harris hip score. Within a period between April 1994 and December 2003 we implanted 309 Lubinus SPII hip prostheses in 301 patients. The preoperative diagnosis was osteoarthritis in 84,9%, rheumatoid arthritis in 6,3%, degenerative changes secondary to developmental dysplasia in 4,8% and trauma sequel in 4%. The average age was 64,3 years (32 – 88 years) at the time of operation. All patients had a primary THR performed. 293 patients (301 hips) received a cemented Lubinus acetabular component, only in 8 hips (2,6%) uncemented acetabular components was used. Second and later third generation cementing technique was employed. Eigth patients died in the meantime and 3 could not be reached. Remaining 290 patients (298 hips) were followed up. The folow-up was 3-11 years, with an awerage of 92 months. Of those 290 patients, revision surgery was performed only in one case due to septic complication 1 month after surgery. All patients were doing well, with good pain relief and high satisfaction. Mean Harris hip score was 87,2 points. At last visit our patients had a mean WOMAC score of 78 (pain -17, stiffness – 9, and physical function – 52). 2 stems had an area of radiolucency in Gruen zones 1 and 7, but were asymptomatic. The clinical results showed significant differences between patients with one affected hip and those with the musculosceletal co-morbidity.

Conclusion. The SP II femoral component has exellent mid- to long-term follow-up clinical and radiographic results. Excluding 1 revision for infection, the survival rate in our study was 100%.

## 12 years experience with Lubinus THR in Latvia

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**Purpose**
The aim of the study was to establish the incidence of revisions after primary cemented THR in Lubinus implant group and to compare the results in Latvia with data in hip registries in other countries.

**Material**
In Latvia from 1994 till 2006 in total 1737 cases with Lubinus implants primary cemented and THR were done. The Lubinus Classic Plus and Lubinus SP-2 implants were used. All patients in this group were older then 55 years. In case of narrow femoral canal the Lubinus Classic Plus implants were used and in case of wide femoral canal the Lubinus SP-2 implants were used.

The revision cases from both implant groups were registered. After clinical and radiological evaluation it was divided into two groups: septic and aseptic loosening.

**Results**
There were 38 revisions performed, 97,82% of THR with Lubinus implants had not revised in 12 years. The cause for revision in 1,32% cases were aseptic loosening, but in 0,86 % of cases deep infection.

**Conclusion**
The founded results showed that the Lubinus implants could be safely used in primary THR. The results in Latvia in total correlates with the results published in Swedish hip registry and the hip registries from other countries. Most differences were found in cumulative frequency of revisions by deep infection. In Latvia it was higher.

# Cementless Zweymüller hip endoprosthesis – mid-term results and radiological evaluation

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**Introduction**
Cementless Zweymüller prosthesis has been introduced in 1979 and has gained considerable popularity. This study evaluates the mid-term results of cementless Zweymüller prosthesis in total hip arthroplasty with 1-9 year follow-up.

**Materials and Methods**
During the years 1998-2005 293 primary cementless total hip arthroplasties in 267 patients were performed in the Tartu University Hospital using Zweymüller prosthesis. The age of patients varied between 15-67 years (mean 44.8 years). Mean time of follow-up at the evaluation was 4.0 years (range 1-9 years). Radiographic evaluation included determining radiolucent lines, sclerosis, osteolysis in the Gruen zones, stem subsidence and position of stem. Survival analysis was done according Kaplan-Meier at 9 years using prosthetic revision as the endpoint.

**Results**
During the follow-up 11 prosthesis underwent revision (revision burden 3.6%). The reasons for revision were: deep infection - 1 case (0.3%), aseptic loosening - 3 cases (1.0%), hip dislocation - 2 cases (0.6%), perioperative bone fractures – 4 cases (1,3%) and intraoperative technical errors – 1 cases (0.3%).

**Conclusions**
The follow-up data demonstrated good behaviour of cementless Zweymüller prosthesis in follow-up 1-9 years period. Aseptic loosening as a reason for revision occurred in 1.0%, deep infection in 0.3%, dislocation in 0.6% and periopeartive bone fractures in 1.3% of cases. We conclude that survival rate for the uncemented Zweymüller prosthesis is 96,2%.

# Infectious Complications, Treatment Outcome, Risk Factors and Microbiology Following THR

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**Introduction**
Successful treatment of prosthetic joint infections often requires multiple surgical intervention and prolonged antimicrobal therapy.

**Objectives:** To determine the incidence, treatment outcome, causative agents and risk factors of the infectious complications.

**Materials and Methods**
1) Between January 2002 and December 2006, 3284 primary THR were performed. 2)We assessed 94 patients (50 women and 44 men) aged 26-85 (mean age 66 years) treated for deep periprosthetic infections in this period. All of the examined patients had undergone prosthetic replacement during 1987-2006. 3)63 (67%) of these patients were first operated in our clinic, while 31 (33%) came from other hospitals. 4)We have reviewed the clinical and laboratory records of these patients in order to analyse the complications, to consider risk factors and bacteriology. 5)Bacteriological samples were

gained in all 94 casies. Antimicrobial suspectibility was tested by the disc diffusion test.

**Results**

Follow-up 1 mth to 17 years:
Early infection (up to 1 mth) - 17 cases
Late infection - 63 cases
Positive i/op culture - 8 cases
Acute hematogenous infection - 6 cases
Surgical treatment:
Debridgement - 8 cases (50% success rate)
Removal of prosthesis – 17 (89% success rate)
Two-stage revision – 50 cases (94% success rate)
One-stage revision – 13 cases (85% success rate)

Staphylococcal monoinfection was obtained in 53 cases (56%) and in 13 cases - methicillinresistant coagulase-negative staphylococci were found that estimated 14 percent of thise cases. Methicillinresistant staphylococcus aureus was found in 2 cases only. The other isolated causative agents were Streptococcus spp. (8), Enterococcus spp. (6), E. coli (2), Pseudomonas aeruginosa (2), Acinetobacter baumani (2), Salmonella (1), Pasteurella multocida (1), Enterobacter spp. (3), Klebsiella spp. (2), Proteus spp. (1) and others. No microorganisms were isolated from 6 samples. 3 patients were treated with long-term antibiotic therapy.

**Conclusions**

1)The incidence of infectious complications following THR (2002-2006), at Hospital of Traumatology and Orthopaedics are 1,3%. 2)The recommendent treatment method for deep periprosthetic infection is two-stage reimplantation. 3)Staphylococci were a predominant group. The leading infectious agents were methicillinsensitive staphylococcus aureus, methicillinresistant coagulase-negative staphylococci and streptococci.

## Thursday, 6 Sept — BONE METABOLISM

# Mikkeli Osteoporosis Index (MOI) identifies osteoporosis and fracture risk in young pmp woman

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**Objectives**

To identify osteoporosis and fracture risk factors in young postmenopausal women we adjusted Fracture Index (FI) with BMD by increasing the weight thresholds nonlinearly from 57 kg to 80 and decreased the age thresholds by 10 years.

**Material and methods**

After stepwise multiple regression in the population based Kuopio FPS cohort, 434 women aged 65-72, and in a cohort of 300 low-energy fracture patients, women aged 45-79, MOI includes 7 risk factors: age over 54 y. (1 point/5 y., max 6 p), weight below 80/71/64/58 kg (1/2/3/4 p), earlier adult fracture, maternal hip or spine fracture and smoking (2 p each), shortening by 3/5 cm (1/2p) and need of arms when rising from chair (2 p), max 20p. We compared the AUROC for osteoporosis with FI and ORAI in the FPS cohort and in the fracture cohort, extended to 430 patients. We validated MOI further in the population based Kuopio OSTPRE-study cohort, 1125 women aged 48-

59 with 10-year follow-up. The fractures in the OSTPRE population/10 years were recorded by postal questionnaires and validated from X-rays / medical records.

**Results**

The AUROC for osteoporosis of the femoral neck in FPS cohort, in fracture patients, and in OSTPRE cohort at baseline and 10 years later was with MOI 67/0,73/ 0.78/0,79, with FI 0,56/0,68/0.72 /0,77 and with ORAI 0,62/0,72/0.83/0,76. In the Cox survival analysis the fracture-free time was 2.5 times shorter within category MOI 7-11 p than with MOI 0-6 (HR 0,4, p< 0.01; Fig.1) In osteoporotic-osteopenic (T-score <-1) and in non-osteopenic women the risk was identical (HR 0,4). ORAI and FI did not predict fractures in this cohort.

**Conclusions**

MOI contains all independent risk factors of FI and identifies both osteoporosis and fracture risk in young PMP women.

Fig.1 Prediction of fractures with MOI. Cox regression model(n=1125).

## Consecutive assessment of bone metabolism improves decision-making for identifying women with risk of developing osteoporosis

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One of the important challenges in the management of osteoporosis is to identify women who are at high risk of developing osteoporosis and fragility fractures. We evaluated if assessment of bone metabolism at multiple occasions can identify such women in a population-based sample of 1044, 75-year-old women. Seven bone turnover markers were assessed at baseline, 1, 3 and 5 years (n=573). Bone mineral density (BMD) was measured at baseline and five years. Change in BMD was evaluated in women with constantly high (highest tertile), intermediate or low turnover over the five year period.

Decrease in total body BMD was significantly greater in women who had constantly high turnover when compared to women with intermediate or constantly low turnover (p for trend <0.01). In the high turnover group, BMD change was -2.3% - -2.7% and in the low turnover group -0.2% - -1.0%, depending on marker. The differences were most pronounced when classification was based on resorption markers. Women with constantly high turnover (S-CTX) also had greater decrease in hip BMD (-8.3%) compared to women with intermediate (-6.0%) or low turnover (-5.1%, p=0.010). Differences were similar in the subgroup of women with osteopenia (n=285). During the five-year follow-up, almost half (49.1%) of osteopenic women with constantly high S-CTX progressed to osteoporotic level of femoral neck BMD (T-score ≤-2.5). For osteopenic women with constantly low S-CTX, the incident rate of progression was 28% (p=0.03).

In conclusion, women who had constantly high turnover lost significantly more bone than women with constantly low turnover, also in the subgroup of osteopenic women. Our results suggest that high bone metabolism at consecutive assessments may improve identification of women at high risk for bone loss and subsequent risk of osteoporosis, and assist in targeting preventive measures. This should be of special clinical interest in women with osteopenia.

# Bisphosphonates as stimulators of longitudinal bone growth in the organ culture model of fetal rat metatarsal bones

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**Background**
Bisphosphonates are widely used anti-resorptive drugs in adult population. In children, their use is mainly limited to patients with osteogenesis imperfecta. However, there has been some concern about their long-term effects on growing skeleton.

**Aim**
We aimed to study the effects of two commonly used bisphosphonates, alendronate (Aln) and pamidronate (Pam) on bone growth using in vitro fetal rat metatarsal organ culture model. Furthermore, we studied the possible potential of these drugs to prevent glucocorticoid-induced growth retardation.

**Methods**
Long-term cultures of E20 fetal rat metatarsal bones were performed in the presence of Aln or Pam and bone growth was analyzed. The effects on chondrocytes were studied in more detail, including proliferation by BrdU incorporation, differentiation by collagen type X immunohistochemistry, and apoptosis by TUNEL. In some experiments, bones were cultured in the presence of bisphosphonates in combination with dexamethasone (Dexa).

**Results**
Bisphosphonates were observed to have dose-dependent effects on longitudinal bone growth in 47 day-cultures of fetal metatarsal bones. High concentrations of bisphosphonates (0.1 – 1 mM) blocked longitudinal bone growth (by 99% and 65% with Aln and Pam, respectively), while low concentrations (10 – 100 nM) had no effect. Interestingly, at a clinically relevant concentration (1 μM) both bisphosphonates significantly stimulated longitudinal bone growth ($p<0.001$ for Aln vs. control, $p<0.05$ for Pam vs. control). This effect was due to increased chondrocyte proliferation, while differentiation or apoptosis were not affected. However, bisphosphonates did not prevent dexamethasone-induced growth retardation in fetal rat metatarsal bones.

**Conclusion**
Based on our in vitro data, both alendronate and pamidronate appear safe to use in growing children, at least regarding to effects on bone growth. However, they may not be the treatment of choice for the prevention of glucocorticoid-induced growth retardation.

# Total Skeletal Uptake of 99mTc-Methylene Diphosphonate is Correlated to Biochemical Markers of Bone Turnover

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**Background and Objectives**
The skeletal uptake of 99mTc labelled methylene diphosphonate (99mTc-MDP) is used for diagnosis of pathological bone uptake. This study was done

to study the association between nine biochemical markers of bone turnover with total skeletal uptake (TSU) of 99mTc-MDP.

**Methods**

22 healthy postmenopausal women (52-80 years) volunteered to participate. Fractures within 0.5-2 years prior to the study were recorded. Total body bone mineral density (BMD) was measured. After injecting 99mTc-MDP, scintigraphic images were obtained at 3 minutes and 5 hours to measure whole body radioactivity. The TSU of 99mTc-MDP after 5 hours was calculated and expressed as a percentage of the radioactivity of the 3 minutes image.

Serum bone specific alkaline phosphatase (S-Bone ALP) and three different assays for serum osteocalcin (OC) as bone formation markers; serum tartrate resistant acid phosphatase 5b (S-TRACP5b) and serum C-terminal cross-linked telopeptides of type I collagen (S-CTX-I) as bone resorption markers and also three different assays for urinary OC (U-OC) were analysed.

**Results**

The median TSU of 99mTc-MDP was 23 % (range 5 to 48 %). Women with a history of fracture (n=8) had lower total body BMD (p=0.041), higher TSU of 99mTc-MDP (p=0.048), and higher bone formation markers (p=0.017-0.034) than women without fractures (n=14).

All bone turnover markers were significantly correlated, with the TSU of 99mTc-MDP with r-values from 0.52 (p=0.013) to 0.90 (p<0.001). The two bone resorption markers had numerically higher correlations (S-TRACP5b r=0.90 and S-CTX, r=0.80) with TSU of 99mTc-MDP, than the bone formation markers (S-Total OC, r= 0.72, and S-Bone ALP, r= 0.66). The TSU of 99mTc-MDP did not correlate with age, weight, body mass index or BMD.

**Conclusions**

Biochemical markers of bone turnover are strongly correlated with the skeletal metabolism as measured by TSU of 99mTc-MDP.

## Influences of Type 1 Diabetes on Bone Mineral Density and Bone Metabolism

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In epidemiologic trials, type 1 diabetes is associated with an increased fracture risk. The potential mechanisms linking glucose metabolism and bone quality are still uncompletely investigated. We investigated the influence of current glycemic control on bone mineral density (BMD) and bone metabolism in type 1 diabetes in a cross-sectional study. We studied 128 unselected patients with type 1 diabetes from our outpatient clinic: 65 premenopausal women (41 +/- 8 years) and 63 men (45 +/- 10 years), the duration of diabetes was 21.8 +/- 10.6 years. All patients received a standard questionnaire on diabetes history and risk factors for osteoporosis. Parameter of bone metabolism were analysed (ionised calcium, osteocalcin, c-terminal telopeptide of type I collagen (CTX), Osteoprotegerin (OPG), soluble receptor of nuclear factor-kappaB ligand (sRANKL)). BMD was measured using DXA (Lunar Prodigy Advance) at lumbar spine (LS), right femoral neck (FN) and total hip (TH).

Osteoporosis (T-score <-2.5 SD) at the LS (FN;

TH) was found in 6 (5; 2) %. Osteopenia (T-score between -1 and -2.5 SD) at the LS (FN; TH) was found in 38 (38; 28) %. BMD was not associated with cigarette smoking or a familial history of osteoporotic fractures. For comparison of two different groups of glycemic control, a median split was performed (1st, 5.5 to <7.6 %; 2nd 7.6 to 11.3 %). No difference was seen for bone mineral density at all measuring points. Bone resorption- and formation markers were equally in both groups. Interestingly, sRANKL was significantly higher in the group with poorer metabolic control ($p<0.05$). In conclusion, deteriorated BMD is a clinical significant and commonly underestimated problem in type 1 diabetes. Increased sRANKL is associated with poor glycemic control whereas OPG remains unchanged. This results in a lower OPG-to-RANKL ratio which may contribute to increased bone resorption. However, current metabolic control was not associated with BMD.

## Bone mineral density in Estonian population

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### Background

Osteoporosis is one of the leading health problems in the developed world in terms of the number of people affected, largely due to the ageing population. There is no data on osteoporosis prevalence in Estonia.

### Objectives

The aim of our study was to report the prevalence of low bone mineral density (BMD) in Estonian population in an age group where peak bone mass has been presumably acquired.

### Methods

The study was population based. 560 subjects aged 25 to 70 years were randomly selected from the list of 2 family physicians in northern Estonia (latitude N). Total of 306 subjects (176 women and 130 men) agreed to participate (response rate 55%). The mean age was 48.9 ±12.2 years. BMD was measured using computer assisted dual energy X-ray absorptiometry (Lunar DPX-IQ, GE Lunar) in the lumbar spine and the femoral neck. For diagnosis WHO criteria for osteopenia (T-score from -1.1 to -2.4) and osteoporosis (T-score ≤ -2.5 SD) were used.

### Results

In the lumbar spine osteopenia was present in 25% (n=76) and osteoporosis was present in 8% (n=24) of the subjects. In the femoral neck osteopenia was present in 11% (n=34) and osteoporosis in 1% (n=3) of the subjects. There was no statistically significant gender difference in osteopenia and osteoporosis prevalence for either anatomical region.

### Conclusion

Around a third of adult Estonian population has impaired BMD measured by DEXA bone densitometry. Measures should be implemented to achieve a higher bone mineral density in people living at northern latitudes (59°N) to prevent possible osteoporotic fractures. Our study also indicates that DEXA measurement of the lumbar spine is more sensitive region when screening for changes in bone mineral density.

# Imaging of bone from the macro to the micro-molecular scale

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Innovations in imaging approaches continue to enlarge the spectrum of morphologic and functional aspects that can be assessed in vivo. The clinical diagnostic examination of patients and the research-oriented investigation of the pathophysiology of skeletal disorders require imaging techniques that allow visualization of the skeleton at different scales: from the organ level to tissue, cellular, and subcellular levels, depicting morphology and function. Progress in the field of imaging technologies resulted in methods suited for clinical investigation of patients in vivo, non-invasive methods for preclinical animal studies and sophisticated functional and molecular imaging methods for both in vivo and ex vivo characterization of bone status have been introduced.

At the *macroscopic scale* the mechanical function of individual bones can now be assessed by 3-D volumetric spiral CT approaches. The image-data collected can be analyzed using Finite Element Models to calculate breaking strength under simulated impacting forces. This allows more accurate identification of subjects at risk for fracture and the monitoring of progress in fracture healing.

At the *microscopic scale* micro-CT has seen impressive advances with ever increasing image resolution – some devices are now suited for nano-CT imaging. This technology allows studies on the effects of bone turnover in normal and diseased tissue, including metabolic bone disorders such as osteoporosis but also of arthritis and skeletal tumours and metastases. Examinations of living animals enable the non-invasive longitudinal monitoring of skeletal effects of therapeutic interventions.

Finally, *molecular imaging*, i.e. the visualization of molecular, biochemical or cellular processes with radiological methods: to date this method is mostly restricted to animal studies. However, the achievements seen here are impressive: localized visualization of molecular and physiological information, e.g. imaging of labelled osteoblasts and their precursors, monitoring of the effects of hormones or gene therapy, or an earlier identification of skeletal metastases. Substantial research is still required to bring these advances to the clinic but the prospects for better individualized patient care based on combined molecular imaging and therapy are most exciting.

In this presentation we will provide an overview of state-of-the-art skeletal imaging approaches and outline perspectives for their future development.

## Rankl-dependent local and systemic bone resorption. Role of joint overloading

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Optimal, moderate and pulsating loading (MPL) induce anabolic response in contrast to immobility, which causes decline in chondrocytes (ChC) and osteoblasts (OB) activity. However, excessive and permanent overloading (EPOL, as in obesity, varus or valgus knee) trigger in them catabolic reaction very similar to inflammatory conditions. Osteoclasts (OCs) react differently: immobility stimulates generalized bone resorption, but localized EPOL stimulated OCs to resorb of subchondral bone, and enabled this way bone marrow invasion.

Changes in the cartilage and subchondral bone are the effect of activation of the Nuclear Factor kappaB (NFkappaB) in ChC and OB, which causes a secretory reaction strictly dependent on the kind of stimulus.

In ChC MPL, as do growth factors TGF-alfa and IGF-1, induce secretion of collagen type II and Glycosaminglycanes. EPOL, as do inflammatory cytokines IL-1, PGE2 and TNF-beta in Rheumatoid Arthritis (RA), stimulate secretion of chondrolytic enzymes and leads to ChC apoptosis. This „bad" activation of ChC may be inhibited by small doses of steroids, some non-steroid anti-inflammatory drugs, flavonoids, vitamins E and C, glucosamine, diacerrheine, sulfasalazine, leflunomide (and probably calcitonin, strontium, and OPG?).

In OB MPL, as do TGF-alfa, IGF-1 and estrogen, stimulate collagen type I and osteoprotegerin (OPG, decoy RANKL receptor) secretion. However, EPOL, as do IL-1beta, PGE-2 and others, force RANK-Ligant secretion, which having bound with the RANK receptor, stimulates the maturation and activity of OC. OPG bonds with RANKL preventing OCs growth and activity. Interestingly, nociceptive pain itself via substance P stimulates bone resorption by RANKL-dependent mechanism. RANK is also present on the surface of ChC.

Hence, physiological bone remodeling and skeletal homeostasis demand RANKL – OPG balance, in both generalized and localized conditions. Excessive RANKL leads to bone loss and needs anti-RANKL, anti-resorptive treatment.

## TRAPER Mouse - a Model to Study Estradiol Effects Effects on Osteoclasts

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Osteoporosis is a common ailment among postmenopausal women negatively affecting quality of life and causing substantial costs due to bone fractures and their consequences. In cell culture systems estradiol has been shown to enhance osteoprotegerin production on osteoblasts thus inhibiting osteoclast activation and maturation. However, the potential direct estradiol effects on osteoclasts are poorly known. Neither it is known what are the putative differences of estrogen

signaling via the estrogen receptor alpha or –beta in this cell population.

To evaluate these subjects in vivo we have generated the TRAPER-mouse, where the ER-alphagene can be inactivated in myeloid cells such as osteoclasts by utilizing the cre - lox recombination system. The transcription of cre-recombinase is controlled by the promoter of the tartrate resistant acidic phosphatase (TRACP) gene. Within the TRAPER strain both cre+ and cre- individuals are formed allowing comparison of cre+ mice to genetically similar cre- control mice.

In TRAPER mice only slight differences could be detected between cre+ and cre- mice until 1 year age, when cre+ female mice display spontaneous bone loss. At earlier ages the large variation in bone assay results between individuals may mask some real phenotypic differences. E g. some, but not all female cre+ mice respond strongly to ovariectomy. The findings in TRAPER mice may point to estrogen effects on myeloid cell maturation. Estradiol is known to inhibit the final maturation of other types of myeloid cells e.g. dendritic cells. It can be postulated that the regulatory condition of the myeloid cell population in cre positive TRAPER mice is unstable thus the manifestation of cre+ phenotype can be somewhat sporadic at earlier ages and become more apparent upon aging.

## Analysis of the inhibitor sensitivity of the lysosomal proton pump from human osteoclasts and macrophages

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Osteoclasts dissolve bone mineral by acidifying the extracellular resorption lacuna. The acidification is carried out by the vacuolar type of proton pump (V-ATPase), a multimeric protein consisting of at least twelve different subunits that mediates acidification of many vesicular compartments. The major question is whether the osteoclast V-ATPase can be selectively inhibited. Interestingly, mutations in the a3 isoform of the 116 kDa subunit seem to specifically affect bone resorption, although a3 mRNA is expressed in all tested cells.

Human in vitro differentiated osteoclasts were cultured and the localization of a3 was studied by immunofluorescence staining. To further study the a3-containing compartment, we isolated it as latex bead-containing phagolysosomes from osteoclasts, and for comparison from THP.1 macrophages. The acidification, and its sensitivity to V-ATPase inhibitors, of these phagolysosomes were studied with Acridine orange. The sensitivity of bone resorption and degradation of horseraddish peroxidase to V-ATPase inhibitors were estimated.

We found that a3, which is in resorbing osteoclasts polarised towards the ruffled border, localises to the intracellular lysosomal compartment in nonresorbing osteoclasts. Moreover, a3 colocalises partly with cathepsin K but not with MMP-9 nor with early endosomes. Phagocytosed latex beads entered the a3-positive compartments and when isolated, the acidification of this compartment was highly sensitive to inhibition by SB242784, a tissue selective inhibitor that prevents bone resorption in animal models of osteoporosis. However, also macrophage phagolysosomes were a3-positive and their acidification was roughly equally sensitive to SB242784. We speculate that a3 may be a general lysosomal protein and that SB242784 is selective for lysosomal V-ATPase rather than osteoclast-selective. Nevertheless, bone resorption is significantly more sensitive to inhibition than degradation of horseraddish peroxidase in macrophages, suggesting that other, yet speculative factors, such as the buffering capacity of the mineral contribute to the increased sensitivity of osteoclast V-ATPase.

# Gene inactivated OSTER mice develop osteoporosis ERalpha

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Sex steroids are known to affect bone mineralization/demineralization. The effect of estrogen on bone is fairly well known on tissue level but the exact cell population(s) mediating the estrogen effect is poorly understood. Estrogen receptors, ERalpha and -beta, are present in all bone cell populations. Estrogen inhibits formation of osteoclasts in mixed cell cultures but not in cultures of pure osteoclast precursor cells. Testosterone, on the other hand, has a direct inhibitory effect on osteoclast differentiation. We have generated novel genetically manipulated mouse strain, where ERalpha gene can be inactivated in specific cell populations by utilizing the cre-lox recombination system. The transcription of cre-recombinase is controlled by osteocalcin (OC) gene promoter. This leads to activation of the cre-recombinase in osteoblasts.

In this work we have measured the long bones of the OSTER (OC-cre ERalpha flox) mice by pQCT and microCT to determine in long bones the trabecular and cortical bone mineral content. Comparison of results is made between cre positive mice and genetically similar cre negative control mice.

The results indicate that cre positive adult female OSTER mice spontaneously develop striking osteoporosis. Male mice show only slight differences between cre positive and negative individuals.

The results support the current knowledge how osteoblasts regulate the function of bone resorbing osteoclasts. RANK-L signaling is crucial for osteoclast formation and activity. Osteoblasts competitively inhibit RANK-L signaling by secreting a decoy molecule osteoprotegerin (OPG) which is upregulated by estradiol. Thus, diminished estradiol signaling on osteoblasts may lead to decreased OPG production and increased osteoclasts activity. Further analyses on the phenotypes of these mouse strains are currently ongoing and OSTER-mouse derived bone cell lines are being developed.

# A rapid method to visualise mesenchymal stem cells and bone marrow mononuclear cells with iron oxide label and MRI

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**Background**

Porcine mesenchymal stem cells and bone marrow mononuclear cells are used to improve bone healing, remodellation and to regenerate infarcted cardiac muscle in several studies. In this study we wanted to visualise the transplanted cells in vivo using iron oxide label. We also optimised the conditions for cultured mesenchymal stem cells and mononuclear cells in two different labelling methods. Finally, we inserted labelled mononuclear cells into tissue samples retrieved from a pig, to see how they show in contrast to the surrounding tissue.

**Materials and methods**

Porcine bone marrow cells were generated from the tibia of a pig under anaesthesia. Mononuclear fraction was isolated with ficoll-paque method and labelled with (resovist) iron oxide nanoparticles. Different labelling methods were tested, namely differing electroporation conditions and incubating the cells for 1 hour and using a rotator to improve diffusion of label into cells. The cells were then washed twice, and filtered to remove precipitate metal. The 10 million labelled cells were then embedded into agar gel for MRI imaging. The best method was selected, and cells were labelled and inserted into fresh tissue samples retrieved from a recently deceased pig. The tissues were then analysed by MRI.

**Results and conclusions**

Our study suggests the selected method is fast and safe, and there is no need for electroporation to get good labelling. The mesenchymal stem cells of the mononuclear fraction also retain their osteoblast differentiation ability, which suggests the cells are largely unharmed. Thus, our data suggests that iron oxide-mri is a good method for labelling mesenchymal stem cells and marrow mononuclear cells in clinical studies as the label does not affect the long term survival or bone formation of the cells in vitro.

## Rab13 regulates trafficking of a unique vesicle population in osteoclasts

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Osteoclasts are bone resorbing multinucleated cells that undergo strong changes in cell polarization and heavy vesicular trafficking during resorption cycle. These events require precise orchestration in order to maintain the special characteristics of different membrane domains in osteoclasts. Rab proteins are small GTPases involved in regulation of all steps of vesicle trafficking. The presence and function of some of the members of the Rab family has already been described in osteoclasts.

Rab13 gene expression is highly upregulated during human osteoclast differentiation from peripheral blood monocytic cells. This suggests an important and possibly resorption specific function for Rab13 protein in osteoclasts. To further reveal its function in osteoclasts, we performed immunolocalization studies for Rab13 and various osteoclast and vesicular trafficking markers.

Rab13 localization appeared to be completely distinct from Cathepsin K and TRACP positive vesicles. Also vesicles containing degraded bone particles travelling from ruffled border for release at the functional secretory domain were devoid of Rab13. This indicates that Rab13 may not be involved in the trafficking of bone degradation products through the cell. In addition, Rab13 did not associate with early endosomes or recycling endosomes labelled by EEA1 or TRITC-labeled transferring, respectively. This is congruent with previous reports of Rab13 in other cell types.

As conclusion, Rab13 appears to regulate the trafficking of a unique vesicle population rather small in size located at the periphery of upper parts of osteoclasts. The vesicle type and the cargo remain yet to be identified.

## Direct Rab11-Rac1 interaction regulates the exit of recycling vesicles from the perinuclear compartment

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Rab GTPases play many important roles in the regulation of vesicular transport. Rab11 has been shown to regulate the slow endocytic recycling pathway. Here, we report that another small GTPase Rac1, directly interacts with Rab11a. This was demonstrated in the bacterial two-hybrid system, pull-down experiments and co-immunoprecipitation assays. We further showed that Rab11 and Rac1 colocalized at perinuclear recycling compartment and punctuate recycling vesicles through the cytoplasm. Importantly, when we followed the transferrin uptake and recycling, we saw a clearly increased Rab11a-Rac1 colocalization at the basolateral plasma membrane in Hela cells which expressed GFP-Rab11 and Dsred-Rac1, and also in primary resorbing osteoclasts. Colocalization could also be visualized at the endocytic recycling vesicles and perinuclear recycling compartment. Furthermore, an intensive triple colocalization of Rab11, Rac1 and transferrin at the leading edge of the migrating osteoclasts suggests that the direct Rab11-Rac1 interaction may be involved in cellular migration. In addition, we were able to show that only the GTP-bound and wild type forms of Rab11a but not GDP-bound form colocalized with Rac1 at recycling vesicles and plasma membrane in Hela cells after transferrin internalization. Moreover, after 30 min of transferrin internalization the perinuclear recycling compartment segregated into recycling vesicles revealing an increased triple colocalization of Rab11, Rac1 and transferrin receptor. Importantly, in the GDP Rab11 and Rac1 expressed Hela cells, the perinuclear structures were even more condensed than in non-treated cells. GDP Rab11 and Rac1 were partially overlapping and retained in the big spot-like membrane clusters, also preventing the exit of transferrin receptor. Our data suggests that direct GTP Rab11a and Rac1 interaction is required not only for Rab11 controlled targeting and fusion to the plasma membrane, but it is also involved in the exit of recycling vesicles from perinuclear recycling compartment.

## FGF-8b induces the NGFI-B subfamily of nuclear receptors in preosteoblastic cells

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The NGFI-B subfamily of orphan nuclear receptors consists of three members, NGFI-B, Nurr1 and Nor1 (NR4A1, NR4A2, and NR4A3, respectively). Nurr1 has previously been shown to have an important role in the central nervous system but its role in peripheral tissues has remained unclear. Nurr1, NGFI-B and Nor1 are expressed in osteoblasts and their expression can be induced as immediate early genes by parathyroidhormone (PHT). Osteopontin and osteocalcin have been reported as osteoblastic target genes for the NBFI-B subfamily. In addition, Nurr1 has been shown to stimulate the differentiation of osteoblasts.

FGF-8b is a growth factor known to stimulate the proliferation and differentiation of preosteoblasts. As both Nurr1 and FGF-8b has been linked to

osteoblast differentiation, and as Nurr1 has target genes marking different stages of osteoblast maturation, we asked whether FGF-8b acts through the NGFI-B subfamily of nuclear receptors.

We found that in MC3T3-E1 preosteoblastic cells FGF-8b induces the mRNA expression of Nurr1, NGFI-B and Nor1 as immediate early genes in a dose-dependent manner. FGF-8b activates multiple signaling pathways through different FGF receptors in osteoblasts, and we have analysed their involvement in Nurr1 induction.

Treatment of cells with 25 ng/ml FGF-8b stimulated cell proliferation. Transfection of wild-type Nurr1 alone increased cell proliferation, as transfection of dominant-negative form of Nurr1, which represses the activity of Nurr1, NGFI-B and Nor1 target promoters, inhibited the stimulation by FGF-8b.

In conclusion, we suggest that the expression of the members of the NGFI-B subfamily of nuclear receptors is induced by FGF-8b and that these receptors are involved in preosteoblast proliferation.

## Intercellular calcium signals induced by mechanical stimulus in primary osteocytes

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Osteocytes are the most abundant bone cells and are ideally located in the bone tissue to form a cellular network in the mineralised bone matrix. One hypothesis for the function of osteocytes is to sense and transmit mechanical signals to other bone cells and thus regulate bone modelling and remodelling. We had previously shown that isolated primary osteocytes could form cellular networks in culture and express gap junction proteins, like connexin 43. They also form functional gap junctions. Intercellular calcium waves are calcium transients that spread from one cell to another in response to different stimuli.

Primary osteocytes were isolated from rat bones and were cultured on glass bottom dishes or glass coverslips for 2 days. Cells were loaded with Fura 2. Imaging was performed with the Olympus MT 20. Calcium waves were induced by stimulating a single cell with a glass microinjector tip, which was fixed to a micromanipulator system. In order to test the role of gap junctions in the regulation of intercellular calcium waves we used gap junction specific inhibitor (AGA), which was added 2 hours before the stimulus and cells were kept in 3;M AGA throughout the experiment. Our data demonstrate that the calcium wave from a single osteocyte can be transmitted to the several adjacent cells in the cellular network of primary osteocyte culture. Whenever gap junctions were are blocked by AGA, calcium signalling was also inhibited. These results suggest that intercellular calcium waves can be one of the signals that are responsible for signal transductions of loading also in vivo.

# β-hydroxy-β-methylbutyrate (HMB) improves volumetric bone mineral density, morphometric and mechanical properties of tibia in male turkeys

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Current genetic selection for higher body weight gain and massive muscle development in poultry results in insufficient adaptation of skeletal system to heavy body weight. As a consequence of an impaired adaptation of bones, the skeleton is not able to fulfill protective, supportive and locomotory functions in fast growing poultry. This study was performed to investigate effects of β-hydroxy-β-methylbutyrate (HMB) administration on skeletal system properties in turkeys. Thirty two male turkeys were randomly divided into two weight-matched groups at the age of 6 weeks. The first group of birds consisted of control turkeys (N=16), while calcium salt of HMB (0.05 g/kg BW/day) was administered orally to the experimental group (N=16). The turkeys were kept under standard rearing conditions and slaughtered at the age of 20 weeks to analyse tibia in terms of bone geometry, volumetric bone mineral density (vBMD) of the trabecular and cortical bone, as well as maximum elastic strength and ultimate strength. Moreover, assessment of free amino acids concentrations in plasma was performed. Obtained results showed 6.3% increase of vBMD of the trabecular and cortical bone compartments of tibia in response to HMB treatment ($p \leq 0.01$). Cross-sectional area and second moment of inertia significantly increased in HMB-treated turkeys by 21.3% and 49.0%, respectively ($P<0.01$). HMB improved maximum elastic strength and ultimate strength of the tibia by 27.2% and 28.3%, respectively (both $P=0.01$). Amino acid analyses showed that HMB increased plasma levels of amino acids such as proline, glutamate, leucine, isoleucine, valine, alanine, aspartate, phenylalanine and cysteic acid (all $P<0.05$). All these results indicate that long-term administration with HMB improves vBMD, geometrical and mechanical properties of skeletal system in fast growing turkeys. This study supports our previous hypotheses postulating enhanced amino acid synthesis, especially proline and leucine, as an important factors responsible for improved quality of skeletal system.

## Risk Factors for Hip Fractures in 48-57 -year old Women- a 14 Year Follow-up Study

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Hip fractures typically occur among elderly women. The aim of this study was to describe risk and protective factors for early postmenopausal hip fractures.

The baseline questionnaire was sent to 14220 women of the OSTPRE-cohort in 1989 and a total of 13100 questionnaires were returned. Bone mineral density (BMD) was measured from a cohort of 3222 women. The follow up data at 5, 10 and 15 years was obtained from 12376, 11504 and 10269 subjects, respectively. The questionnaires contained questions about medication, health disorders, health behavior, falls and fractures. The subjects were followed to their latest reply of questionnaire or to their hip fracture. All self-reported fractures were validated by perusal of medical records. The mean age (SD) of the cohort was 52.4 (2.9) years at baseline. The mean follow up time (SD) was 14.0 (3.0) years. A total of 76 hip fractures were reported by 74 women (0.6 %).

Statistically significant differences at baseline between fractured (n=74) and unfractured groups (n=12302) were found in age, body mass index (BMI), smoking status, number of prescribed medications and chronic illnesses, femoral neck BMD, menopausal status, amount of regular exercise, history of hip fracture in mother, sister and brother. In the Cox model, statistically significant risk factors for hip fracture were age (HR = 1.17 ), current smoking (HR = 2.78 ), lifelong years of smoking (HR = 1.04), daily amount of cigarettes (HR = 1.05) and number of prescribed medications (HR = 1.26). Protective factors for hip fracture were BMI (HR = 0.89), femoral neck BMD (HR = 0,43) and regular exercise (HR = 0.57).

Although hip fracture is quite rare among early menopausal women, there are several risk factors associated with it. Especially smoking and use of medications predispose middle-aged women to a hip fracture.

## Operative treatment of the proximal humeral fractures

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**Background**
Proximal humeral fractures with displacement, comminution or dislocation of the humeral head represent a difficult clinical challenge. Controversy persists concerning the preferred operative treatment method. The present study was undertaken to evaluate: 1) shoulder function after open reduction and internal fixation with T plate,

2) most common disadvantages of this treatment method.

**Methods**

Shoulder pain and function was retrospectively assessed for 76 patients after operation with minimum 12 months follow-up. We evaluated radiographs of the shoulder joint and shoulder function using Constant score.

**Results**

After an average of 18 months of follow-up,sixty five (86%) had good or excellent results and 11 patients (14%) had a poor results according to Constant score.

**Conclusion**

Open reduction and internal fixation with T-plate in most cases is good operative treatment method for displaced proximal humeral fractures. The most common disadvantage of this technique is subacromial impingement due to plate and proximal screws. This can affect shoulder rehabilitation and cause restriction of range of motion.

## Analyses of the proximal femoral fracture treatment from 1955 to november 2005

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**Aims**

The purpose of this study is to analyse of the epidemiological and treatment aspects of proximal femoral fractures during the 10 – year period in North - Estonia Regional Hospital in Tallinn. How do the time factors of treatment and treatment options influence morbidity and mortality?

**Methods**

The case reports from 1995 to November 2005 with diagnosis S 72.0, S 72.1 and S 72.2 (IDC-10) were analyzed. The data concerning epidemiology, treatment methods, mechanism of the injuries, associated chronic diseases, admission time to the hospital and to the operating theatre, date of death were collected.

**Results**

1628 cases of primary proximal femoral fracture were found. The number of the patients who have been operated on during the first 6 hours after admission to the hospital has increased up to 49 %. At the same time the number of postoperative complications has decreased up to 6 %.

94 % of cases were treated operatively and related to this, from 2001 to 2003 the treatment cost has doubled.

Mortality rate after hospitalisation in this period was 40%, 63% among of them were operated. In different kinds of operative treatment mortality rate was approximately 13%.

From 1996 the DHS, DCS and SEP devices, from 2001 extraordinary TEP procedure have become widely used in our hospital and the treatment options have been changed to be more operative.

First 2 hours of preoperative time in hospital have arised from 25% to 85%. This fact has also remarkable influence on the shortening of the hospital stay and decreasing of the mortality on the time of hospital stay. There is also important part on the after-treatment department with professional personal.

Mortality rate have two spices in all treatment options, except TEP procedure: first before 6 moths after treatment and second between first and fifth year after treatment.

## The functional outcome of surgically treated unstable pelvic ring fractures

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The orthopaedic literature contains few studies evaluating the long-term outcomes of surgically treated unstable pelvic fractures. The purpose of our study was to determine the factors that may influence the clinical and functional outcomes of such fractures.

364 patients with unstable pelvic fractures, treated from 1992 to 2005 were retrospectively reviewed. The mean patients age was 38 years (range 18 to 82). 220 patients were men and 144 women. The commonest cause was a road traffic accident (N=174). There were 116 type-C and 248 type-B fractures according to Tile's classification. 21 of the fractures was open. 55% of patients sustained additional injuries. External fixation as a definite management was applied to 31 fracture; 291 patients were treated operatively with internal fixation and 42 with a combination of both. Surgical reduction was scored from the post-operative radiographs and Majeed's score was used to assess the clinical outcome. The mean follow-up was 7,5 years (range 15 to 2 years). 21 patient developed superficial infection. The mean hospital stay was 25,7 days (range 5 to 98 days). All the patients had either good or excellent radiological reduction, however 25 of them had a fair functional outcome according to Majeed's criteria. Five patients died 2 years after surgery from causes unrelated to pelvic injury and we were unable to trace 12 patients. Four patients with type-C3 fracture underwent hip replacement, 2-7 years following surgery. 32 patients complained of pelvic pain and 12 had sexual dysfunction. 70% of patients returned to their original job, however the level of pain affected their performance accordingly. The management of unstable pelvic fractures is challenging; initial haemodynamic stabilisation takes preference and significantly reduces mortality. Associated injuries are common and they might further compromise the functional outcome. Open reduction and internal fixation yields a satisfactory outcome, however rehabilitation period is prolonged

## Minially Invasive Management of Distal Metaphyseal Tibial Fractures and Locking Compression Plate

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In a prospective study the LCP 4,5/5,0 (Synthes) system was used to treat 27 patients. In 22 cases the MIPPO technique was used. After 6, 12 and 24 months, clinical, radiological and biomechanical follow up assessment was carried out on 27 patients with distal tibial metaphyseal fractures. After 12 month period the Olerude-Molander score was 88 and AOFAS 95 out of possible 100. No functional aftetreatment was used. Full weightbearing was started after 12 (16 – 6) week. The loss of the correction of talocrural angle postoperatively was important in 3 cases, loss of correction more than 5 degrees. Malalignment of the tibial shaft was definied as >5 degrees of varus/valgus, or >10 degrees anterior/posterior angulation. In one case pseutoarthrotic formation

was registed. No complication from wound side was found. Patients satisfaction rate with treatment was 93%. If to compare the circumference of thigh and calf after 6 and 12 months there was no significant difference. After 24 months we noticed a significant growth in thigh circumference and there was a substantial growth of muscle strenghth in both injured and normal limb. If to compare the respective results of muscle stenghth and of the circumference of the limb after 6 and 12 months, the decrease in both cases, then it clearly indicates the need for the prevention of muscle atrophy, and because of that, the need for coordinated rehabilitative treatment already in the earliest phases of postoperative treatment. The low rate of loss of correction of tibial axis in postoperative period and patients satisfaction high rate shows that LCP in combination with MIPPO is a helpful tool to treat distal metadiaphyseal comminution fractures of tibia. The high primary stability in combination with newly developed minimal-invasive techniques are bases for a functional aftertreatment and rapid bony consolidation with a low complication rate.

## Surgical options for tibial shaft non-union management

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Fractures of tibial shaft, in spite of surgical technique improvements, remain the risk for serious complications. Late complications, such as delayed union, non-union and pseudarthrosis need long term treatment and use of complicated surgical techniques. Only

high-qualified orthopaedic surgeon can successfully treat patients with these bone defects. Information for 3 years (2004-2006) study is aggregated in the work. Examined was the clinical material of Hospital of Traumatology and Orthopaedics, patients (34) with non-united fractures and pseudarthrosis of tibial shaft.

The average posttraumatic time before treatment varies from 2 to 48 months (average time 10 months). Examined patients were 16 to 72 years old (middle age- 39 years), male - 26, female - 8. 19 examined cases included open fractures. Before hospitalization in our hospital patients were treated using external fixators- 13 cases, UTN- 7, osteosynthesis with plate fixation- 8, conservative treatment- 6. As a result of trauma or treatment process, bone defects evolved in 4 patients.

The most common methods of treatment in cases of fracture and pseudarthrosis of tibial shaft: UTN used in 23 cases, osteosynthesis with plate fixation in 7 cases and external fixation in 4 cases. Out of 34 cases with tibial non-unions and pseudarthrosis in 8 cases bone grafting operation, in 4 cases bone transport was performed. Evaluating the results of treatment also in consideration were taken x-rays and functional results, complication analysis was performed and recommendations for complication reduction were developed.

**Conclusions**

1. In treatment of non-united fractures and pseudarthrosis of tibial shaft most common method of treatment- osteosynthesis with UTN.
2. In cases of tibial bone defect as the most suitable treatment must be considered osteosynthesis with ring system external fixator.
3. In cases of closed fractures of tibial shaft, plate osteosynthesis more commonly leads to non-union of the fracture than other treatment methods.

# Vitamin D supplementation inhibits seasonal variation of calcitropic hormones and maintains coupled bone turnover

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Vitamin D is suggested as a coupling factor for bone remodelling. When compared to women, men are believed to have more stable bone remodelling, thus they are considered less susceptible to the seasonal variation of calcitropic hormones.

The aim of this study was to determine whether there exists seasonal variation in the bone remodelling markers of healthy adult men and to estimate which vitamin D intake is required to prevent it.

Subjects (N=54) were healthy Finnish men, aged 21-49 years, from the Helsinki area. This was a vitamin D RCT for 6-mo, in which subjects recieved either 20 µg, 10 µg, or placebo. Trial lasted from November to April. Serum 25-OHD, iPTH, BALP and TRACP were analysed together with the radial BMD by pQCT with repeated measures ANCOVA.

A seasonal variation was noted in S-25-OHD and S-iPTH ($p<0.001$ and 0.012, respectively) but not in the bone remodelling markers. Vitamin D supplementation increased S-25-OHD concentration dose-dependently, the mean dose-response based on compliance was 1.5 (1.2) nmol/l/µg. The supplementation inhibited the winter elevation of PTH ($p=0.035$), decreased S-BALP concentration ($p=0.044$), whereas the S-TRACP was not affected. At the baseline the bone remodelling markers correlated with each other ($r = 0.309$, $p = 0.031$), but at 6 mo, a similar correlation was noted only in the groups of 10 µg and 20 µg.

Healthy adult men are exposed to seasonal variation of calcitropic hormones, but not bone remodelling. Supplementation improved vitamin D status and inhibited the elevation of PTH, but also decreased BALP concentration. The bone turnover remained coupled in supplemented groups, but not with placebo. Both the lowered and accelerated bone turnover may impair skeletal health, but the results concerning peripheral radial BMD needs to be analysed before confirming that. A total intake of 17 µg/d is required to prevent the seasonality of calcitropic hormones in men.

# Body Fat Distribution, Bone Mineral Measurements, and Hormone Therapy in Postmenopausal Women

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Women with android distribution of body fat are associated with higher bone mineral density (BMD). However, only limited information is available on the association between body fat distribution and central BMD in postmenopausal women as well as the modifying effect of hormone therapy (HT).

In this cross-sectional population-based study regional body composition as well as BMD of 198 postmenopausal women (mean age 67.5 (1.9 SD), mean BMI 27.1 (3.9 SD)) were assessed by dual X-ray absorptiometry (DXA, Prodigy). Ninety-six of women had never used HT, whereas the rest were either current or past users of HT (n=102).

Of the two body composition parameters (fat and lean mass), fat mass was the main determinant of postmenopausal bone mass. Only spinal BMD, not femoral neck, values correlated positively with trunk-leg fat-ratio. Among non-users of HT higher spinal BMD values were shown with increasing trunk-leg fat-ratio after adjustment with total body fat and lean mass. This effect was not evident with ever-users of HT. In conclusion, higher trunk-leg fat-ratio was associated with spinal BMD, but not with hip BMD values in postmenopausal women. In subgroup analysis this association was confirmed to never-users of HT.

# Urinary osteocalcin and other markers of bone metabolism: the effect of risedronate therapy

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## Background

Serum osteocalcin (S-OC) is used as an index of bone formation. Nevertheless, some urinary fragments of OC seem to reflect bone resorption (Srivastava et al., 2002, Ivaska et al., 2005).

## Objective

To investigate the short-term changes in urinary midfragments of osteocalcin (U-MidOC) and other bone turnover markers in response to risedronate therapy.

## Methods

The study group consisted of 19 patients with postmenopausal osteoporosis, aged 49-66 years. 54 premenopausal women served as controls. Lumbal bone mineral density (BMD) was measured before and after 12-months risedronate therapy. Urinary osteocalcin was measured by the U-MidOC ELISA assay for midfragments, other bone markers (S-P1NP, S-OC, S-CTx-I) by electrochemiluminescence assay.

**Results**

At baseline, U-MidOC values were correlated only with S-OC. After 1st month of therapy the decrease was similar in the levels of U-MidOC and S-CTx-I. A decrease in formation markers was observed after 1-3 months. During therapy the strongest correlation was found between the changes in U-MidOC and S-CTx-I.

Individuals with positive response for BMD (> 3%) after 12-months therapy had significantly lower baseline values of U-MidOC than non-responders (p=0.005). A significant correlation was observed between baseline values of U-MidOC and lumbal BMD response (rho= -0.571, p=0.026, n=15). No such correlation was found with S-CTx-I. Strong correlation was found between the 1-month decrease in U-MidOC and 12-months change in BMD (rho=0.742, p=0.004, n=13).

**Conclusions**

During the risedronate therapy, U-MidOC behaved similarly to S-CTx-I as a resporption marker. Still, in individual patients they had a different pattern. Therefore we suppose that these two markers reflect different aspects of bone resorption.

U-MidOC might be useful in monitoring antiresorptive therapy.

Baseline and 1-month U-MidOC values might also have a predictive value for the response in lumbal BMD after 12-months risedronate therapy.

## Low bone mass in girls with Rett's syndrome

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**Introduction**

Rett's syndrome (RS) is progressive disease of central nervous system, which appears in girls, who do not suffer from any disorders during first 6 monthes of life. In the course of the illness severe mental retardation, microcephaly, hyperventilation, apnea, hand steroeotypies, decreasing mobility, losing of expressive and understanding language are observed.Morover, patients with RS are at risk of bone metabolic diseases. The aim of the study is to evaluate case report of 5 girls with RS who present clinical symptoms of low bone mass, like bone fractures and pain, arrthralgias, gait disturbances.

**Material and methods**

Girls with RS were examined with dual x-ray energy absorptiometry method in total body (BMD TB) and spine (BMD S) programmes. Osteoporosis was diagnosed for Z-score values below –2,0 and osteopenia when Z-score ranged from –1,0 to –2,0.

**Results**

In all children low bone mass was diagnosed - in 3 osteoporosis and in 2 osteopenia. The firs girl, 11-years old, with dicreased mobility had Z-score BMD TB: -5,31. The second 18-years old, who underwent two bone fractures had BMD TB: -3,9; BMD S: -4,2. The third one 14-years old with bone pains BMD TB: -1,5. Two next (7 and 9 years of age)BMD S -1,3 and -2,3, respectevely.

**Conclusions**

Low bone mass can be the symptom in the course of Rett's syndrome, therefore it seems to be usefull to diagnose patients for osteopenia or osteoporosis.

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# Assessment of bone mass in children in the moment of diagnosis of lymphoproliferative disease

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**Introduction**

The diagnosis of lymphoproliterative disease in children is connected with the necessity of glucocorticoid treatment, which can impair the bone mineral density and growth. The aim of the study is to assess bone mass in children in the time of diagnosis of lymphoproliferative disease.

**Patients and methods**

The stdudy comprised 17 children, aged 6-18 years; 8 girls and 9 boys. In 11 acute lymphoblastic leukaemia, in 1-acute myeloblastic leukaemia, in 3- Hodgin's lymphoma and in 2 non-Hodgin's lymphoma were diagnosed.Bone mineral density (BMD)was examined by dual X-ray energy absorpiometry method (DXA)in total body and spine programmmes.Osteoporosis was diagnosed for Z-score values below -2,0 and osteopenia when Z-score ranged from -1,0 to -2,0. Heel ultrasound examination was done in 12 children with Achilles plus Solo apparatus;absolute and relative values of speed of sound (SOS), broadband ultrasound attenuation (BUA) and automatically calculated Stiffness index were analysed. Statistical analysis was done by Statistica v.6.0. Correlations were evaluated by Spearmann's method.

**Results**

The low bone mineral density by DXA was assessed in 9/17 children - in 3 it was osteoporosis and in 6 - osteopenia. 6/12 patients had lowered at least one of the ultrasound parameters. Trere were ststistically significant correlations between BMD spine and absolute values of all ultrasound parameters, as well as between bone mineral content in total body and spine and BUA.

**Conclusions**

1. It seems to be important to examine bone mass already at the beginning of the lymphoproliferative disease in children and adolescents. 2. Patients with low bone mass should be treated parallely with the therapy of the basis disease and the rest of them should be given calcium and vitamin D as prophylaxis of bone mineral disturbances.

The study was partly financed by Medical University of Lodz grant no 50211317

# Prediction of fracture susceptibility in children basing on Idiopathic Juvenile Osteoporosis

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The results of investigations performed in our study are based on clinical observation of 61 IJO children, including 34 girls (13.6±3.1; 7-18 yrs) and 27 boys (14.3±3.3; 5-18 yrs) (1). The analysis of skeleton was based on DXA measures of total body (TB) and lumbar spine (S) bone mineral content (BMC) and density (BMD). Additionally pQCT Total and Trabacular volumetric densities have been measured. During the phase of fractures and bone pain (acute phase of IJO) in the group of IJO children mean Z-scores for TBBMD and SBMD were significantly lower than Z-scores observed during recovery from IJO. Furthermore, both genders during Acute IJO had significantly lower volumetric density assessed using pQCT. In contrast, during recovery from disease, marked increase in Total vBMD and Trabecular vBMD was noted irrespective of gender.

During acute stage of IJO TBBMC/LBM Z-scores of -2.95±1.15 and -2.56±1.49 were noted in girls and boys respectively. The corresponding SBMC/LBM Z-scores were -2.66±1.07 and -2.22±1.62, in girls and boys. During the recovery from IJO TBBMC/LBM and SBMC/LBM Z-scores of -1.07±0.99 and -0.91±1.16 and of -1.15±1.40 and -0.68±1.45 were noted in girls and boys, all significantly higher than during the acute phase respectively ($p<0.0001$). Moreover, pQCT assessed Strength Strain Index (SSI-P) values were also significantly decreased during the phase of bone pain and fractures. In contrast during the recovery from the disease a muscle-bone relationship tended to normalize, giving the Z-scores for SBMC/LBM and TBBMC/LBM ratios as well as SSI-P values markedly closer to these of healthy controls, what coincided with lack of bone pain and new fractures.

It should be stressed up that low values of relative bone strength indices highly likely reflect markedly increased risk for fracture.

## Risk factors of hip dislocation after primary total hip replacement (THR) – clinical and radiological analysis

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**Purpose/Introduction**

According to literature data hip dislocation occurs in 2-9% patients after primary THR.

Most commonly it is caused by component malposition, soft tissue disbalance et.c.

The purpose of our study was clinical and radiological analysis of the risk factors determining hip dislocation after THR.

**Materials and Methods**

We retrospectively analysed 2626 THR cases treated at the Hospital of Traumatology and Orthopaedics (HTO) in the period of 2000-2003. We found 59 hip dislocations (20 in male and 39 in female patients).

In the clinical analysis we included surgical diagnosis, patient condition, leg length, surgery data, cause of dislocation.

Radiological analysis included: plain x-ray of pelvis; hip AP, LL (before surgery, after surgery, dislocation, reposition, revision surgery); lumbar spine AP, LL; CT scan according to evaluation protocol.

For data evaluation SPSS were used.

**Results**

In our hospital (2000-2003) hip dislocation had occurred in 2,2% of primary THR cases.

Clinical results.

Early postoperative dislocations (up to 6 weeks) – 28,8%;

Late and recurrent dislocations – 86,4%;

Untoward risk factors: alcohol – 35,6%; mental disorders – 8,5%; previous surgery – 16,9%

of dislocation cases.

Radiological results.

Acetabular component malposition was found in 35,6% of dislocation cases.

**Discussion/Conclusion**

1. Dislocation rate. In our clinical and radiological analysis we found it 2,2%. This result corresponds with the data of literature.
2. The main risk factors were found to be - previous surgery of hip, alcohol abuse, mental diseases and implant malposition.

## Management of femoral bone deficits in stem revision after THR

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**Purpose**

To analyze the midterm results after THR stem revisions width several types of bone deficits.

**Material and methods**

The clinical results of 147 THR revision cases threaded between 1998 and 2006 had analyzed.

The femoral bone deficits had assessed according to AAOS classification.

In 79 cases with proximal femur, defects (I, II, IIIA types) Zweimuller revision or normal stems had used.

In 65 cases with more severe or diaphiseal femur defects (IIIB, IV type), ribbed modular MP Reconstruction stems and bone grafting were used. The follow up protocol includes clinical and X-ray examination.

**Results**

1. In-group with proximal femur defects (I, II, IIIA types) there no postoperative complications was founded in 94, 9% of cases. Rerevision had needed in 4 cases (infections – 3, fracture – 1). Other complications: 1 fracture was healed conservative, 1 patient dead in the early postoperative period.
2. In group with more severe or diaphiseal femur defects (IIIB, IV type) there no postoperative complications was founded in 83, 0% of cases. Rerevision was needed .in 7 cases ( infections 1, stem subsidence 2, recurrent dislocations 4). Other complications:1 fracture and, 3 dislocations had treated conservative.
3. The clinical assessment according MerleD`Aubigne score shows 74% of very great improvement, 18% great improvement and 8% fair improvement.
4. The X-ray evaluation shows the no radiolucent zones in 76% of cases, radiolucent line in1 or2 Gruen zones in 15% of cases and radiolucent line in more then 2 zones in 9% of cases.

**Conclusions**

The used tactic of bone deficits management in midterm period leads to good and satisfactory results in majority of cases. The clinical assessment shows god functional outcome in 92% of cases. Radiological signs of loosening had found in 9% of cases. In other cases, the bone on grown, restoration and in some cases new bone formation of proximal femur had found. The most common complications leading to rerevision were infection.

## Short outcome after minimally invasive midvastus total knee replacement comparison with conventional technique

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**Background and objectives**

Minimally invasive surgery is getting more interest in literature recently. However the reports regarding the effectiveness of this particular technique are controversial. There are reports in the literature that minimally invasive technique is associated with increased number of technical mistakes. Advantages of this technique are less postoperative pain and faster rehabilitation. The aim of our randomized trial was to evaluate the effectiveness of this particular technique compared with conventional total knee replacement.

**Methods**

In randomized controlled trial we analyzed 70 knee osteoarthritis patients admitted for elective total knee replacement. All patients were randomized to do surgery using minimally invasive or conventional technique. Depuy P.F.C Sigma PS endoprosthesis was used in all cases. We analyzed operation time, blood loss, the stability of prosthetic knee, range of motions 1-6 days after surgery. After 6 and 12 week we evaluated total knee replacement patiens according KSS score. For radiographic examination we performed anterior and lateral x-rays 2-ed day after the

surgery. After 12 week anterior, lateral and axial x-rays we performed.

**Results**

Preoperative data such as severity of osteoarthirtis, range of motions, KSS score did not differ within the groups. Minimally midvastus group was associated with longer operation time, faster recovery of range of motions, higher KSS score 6 weeks after surgery. 12 week after total knee replacement we did not find any differences between the groups. We did not observed any complications or critical errors in all studied patients. Component position and leg axis deviation did not differ between the groups. We conclude that minimally invasive midvastus approach is safe method and is associated with faster rehabilitation than conventional total knee replacement.

## Is 4-hour clamping drainage useful comparing with unclamped drainage after total knee arthroplasty?

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**Background and objectives**

The use of closed suction drainage after total knee arthroplasty (TKA) is controversial. Many authors reported disadvantages such as greater need for blood transfusion of closed suction drainage as compared with knees without any drainage. Another alternative of drainage after TKA analyzed in literature is clamping closed suction drainage system for a certain period of time. However there are leak of data about the severity of knee osteoarthritis (OA) and it's correlation with blood loss during and after the surgery. The aim of our study was to compare the differences in 4-hour clamped and unclamped groups after TKA in severe OA cases, which require more aggressive surgical technique and associated with greater blood loss during and after TKA.

**Methods**

In prospective, randomized trial we analyzed 60 patients undergoing total knee replacement. Ramdomly selected patients were divided in 2 groups. In group 1 (30 patients), drainage was clamped for the first 4 postoperative hours. In group 2 (30 patients), drainage was not clamped. Radiological OA grade was assessed according Burnett's radiographic atlas. The evaluation was based on a number of radiographical changes and graded in stages from 1 to 21 (the higher the grade, the more severe radiographical OA). Only the patients with 16 OA severity score were included in the study. In all cases the same type of incision and implant was used.

**Results**

The decrease of hematocrit after the first postoperative day was significantly less in group 1, as compared with non clamped group 2. The average postoperative hemorrhage fluid loss trough drains was significantly less after 6 and 24 postoperative hours in group 1. The need for blood transfusions was also less (p=0,0521) in group 1 - 6(20 %), than in group 2 - 13(43 %). We conclude that clamping the drainage in the first 4 postoperative hours is recommended in TKA patients in severe OA cases.

# Relationship between analgesia and neuroendocrine and inflommatory responses after knee arthroplasty

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**Objective**
To evaluate clinical efficacy and immune responce of different epidural analgesic medications in patients undergoing knee replacement surgery.

**Background data**
Studies have prevously shown that epidural analgesia significantly reduces the pain, with a lower catecholamine and cytokine responce associated with major joint surgery. This study compares the effect of epidural analgesia with local anaesthetics, opiates and glicocorticoids on pain relief and immune response in patients with knee replacement.

**Materials and methods**
We studied 50 ASA 2 patients undergoingknee replacement surgery in a randomized and prospective manner. Combined spinal epidural analgesia was performed. Before the end of surgery Group 1 patients received Solu-Medrol 40 mg and Fentanyl 0,05mg. All solutions were diluted with NaCl 0,9% till 10 cc. In the group 4 patients didn"t receive any epidural analgesia and were treated with repeated boluses of opiates.

In the ward patients received mixture of Solu-Medrol 20 mg with Morphine 0,5 mg (1), or Marcaine 25 mg with Morphine 0,5 mg (2) or Solu-Medrol 20 mg with 0,05 mg of Fentanyl (3) into epidural space in the fixed hours. If analgesia were unsatisfactory, rescue analgesic was Marcaine 0,5 5 ml epidurally.

Sensory level, motor blocade, pain, MAP, HR, SatO2, respiratory rate, sedation was assessed after introduction for 24 hours. Changes of Na, K, Cl, blood sugar, CRP level, cortisol, IL-6, IL-10were measured before incision and 24 hours aftertransportation to surgical ward. Also side effects like PONV, hipotension and other problems were registered.

**Results**
Pain score was significantly lower in Group 1 without top-up injections. Side effects mostly were linked to Morphine"s action. Glucose level was statisticaly comparable in all groups during first 24 hours, but CRP and IL-6

# Isometric force production capacity of knee extensor muscles before and six months after unilateral total knee replacement

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The purpose of the present study was to evaluate voluntary isometric force production, relaxation and activation capacity of the knee extensor muscles before and six months after unilateral total knee arthroplasty (TKA). TKA was performed in ten women with primary knee osteoarthritis (OA) using the condular endoprostheses. Patients were compared with ten age- and gender-matched asymptomatic subjects as controls. Isometric maximal voluntary contraction (MVC) force, rate of force development at 50% of MVC (RFD50) and their ratio to body mass, half-relaxation time (HRT)

and voluntary activation (VA) of the knee extensor muscles were recorded in patients for operated and non-operated leg before and six months after TKA. In controls, only dominant leg was assessed. The clinical examination in patients was performed using the Knee Society System (KSS) scores and pain intensity was assessed by visual analogue scale. Isometric MVC force in patients for operated leg was lower ($p<0.05$) before and 6 months after TKA as compared with the non-operated leg (31 and 32%, respectively) and controls (48 and 44%, respectively). Patients had lower ($p<0.05$) VA of the knee extensor muscles in operated leg 6 months after TKA as compared to controls. Significant increase ($p<0.05$) of KSS clinical scores and the tendency for increase of explosive force production of the knee extensor muscles in operated leg were observed 6 months after TKA (RFD50 was 60% lower before TKA and 40% lower 6 months after surgery as compared to controls). When compared with preoperative value, HRT prolongation ($p<0.05$) was noted 6 months after TKA in knee extensor muscles of both legs in patients. In conclusion, the present study suggested that patients with knee OA had reduced force generation ability of the knee extensor muscles before TKA and the improvement of explosive force was noted 6 months after surgery.

## Saturday, 8 Sept FRACTURES

## The idea of A. I Seppo is alive

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It is time to evaluate not only great meaning of endoprosthesis replacement, both surgical aid in destructive diseases of hip joint and dissatisfaction with prolonged results of this treatment method. Aseptic instability of endoprosthesis (from 5 to 60%) attracts particular attention. This process is causes of revision operations. Endoprosthesis replacement, made in the age of 20-40, will be not a single operation for patients.

With the purpose of removal of endoprosthesis replacement in young patients, we returned to idea of A.I. Seppo - to prolong anatomic and functional life of hip joint by dipping

distruction device. It can be realized by use of saved reparative activity and by bone remodeling in young patients. It is necessary to make conditions for regular unloading of joint in saved function, but it is excluded, because of one of the main pathogenetic moment of distraction progression in joint – its overload. In accordance with idea of A. Seppo, we made distraction device, allowing shunting of axial load from caxal bone to trochlear area.

Experimental researches in test machine, which were made on plastic models, allowed making sure, that in different phase of step, joint is unloaded on 63 % without distraction, saving stability. Exclusion of interpressure on artricular ends and creation of cartilage space with adjusted size, provide opportunity of regeneration of cartilaginous surface, it was confirmed by researches of A. Seppo (1962), G. Lavrischeva (1964). Modern results of morphological investigations show, that conditions of distraction with width of chink - 1, 5 mm, induce to regeneration of stem cells in subchondral part of artricular end.

We have an experience of preservation of functional and anatomic life of hip joint for more than 300 patients and removal of endoprosthesis replacement on 5-10-15-20 and more years.

## Application of vertebroplasty for strengthening of transpedicular fixation

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**Introduction**
Transpedicular fixation (TPF) in patients with poor bone quality is unsafe because of high risk of skrew displacement. We combined TPF with vertebroplasty (VP) for safer skrew anchorage.

**Material/Method**
Simultaneous VP and TPF was performed in 7 patients. 4 patients suffered from osteoporosis determined vertebral fractures with severe spinal canal stenosis, 2 patients were operated on due tu multiple myeloma and 1 patient – due to multiple brest cancer metastases. In osteoporotic patients posterior decompression and TPF with TSRH 3D implants was combined with VP of fractured vertebra. In myeloma and metastasis patients after decompression VP was done in the vertebras above and below and pedicular screws were inserted in the vertebras prior to complete cement polymerization. The grade of pain perception was evaluated by using of visual analogue scale (VAS).

**Results**
In all operated patients good pain reliefe (VAS from 8,7 to 3,3) was achieved. There were no signs of kyphosis progression or screw displacement 6 month after surgery.

**Conclusions**
1.VP performed simultaneously with TPF in patients with poor bone quality provides good pain relief and stable fixation.
2. VP combined with TPF in patients with poor bone quality increases safety of surgical treatment and facilitates early rehabilitation and mobilization of patients.

## Surgical treatment results of spinal tumors and metastases

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Oncological care today with different treatment options has given tumor patient longer expectancy and also quality of life. Last 10 years have changed spinal tumor treatment, spinal surgeons have reached their hand to help oncologists. No oncology center today can exist without spinal surgeon, because spinal metastases are on the first place of skeletal metastases. New implants and operative techniques give our patients better quality of life.

Up today spinal tumor patients were treated by painkillers, chemo- and radiotherapy. The main operative procedure in past for treatment of the spinal metastases was laminectomy and results didn`t differ from those not operated. It is undrestandable why most of spinal metastases patients were treated by radiotherapiest.

Tumor in spine destroys normal bone, which results in pathological fracture. 80 % tumors locate in vertebral body, 20 % in posterior structures. In pathological fracture the anterior support dissapears and result is pain, worsening in standing and weakening in laying down. These mechanical problems can be solved by stabilisation of spine.

Bone fragments or tumor can invade the spinal canal. These patients get radiculopathies up to paresis and can be helped by decompression and followed by stabilisation.

Most of spinal tumor patients are offered palliative care, because of far developed disease. Absolutely new challenge to spinal surgeons is en-block resection of solitary tumors and metastases.

Still the effectiveness of treatment depends on correct oncological diagnosis. Tumor treatment depends on patients general condition, neurological status, amount and localisation of metastases. With scoring systems and team of doctors we try to help patients with life expectancy more than three month.

Operative treatment of spinal metastases by nowadays standards started in Estonia in 2004 with Oulu spinal surgeon Pirkka Mäkela. Now we are performing all the surgeries by ourselves and first results are presented to you.

## The technique and results of periacetabular osteotomy

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Patients with symptomatic hip dysplasia are commonly young women. Until the present time their treatment has consisted in symptomatic cure with NSAID`s and hip arthroplasty after onset of secondary osteoarthrosis. Altough hip endoprotheses last about 20 years these young patients are subjected to revision arthroplasty as they will outlive their implant. The new trend is bioregenerative surgery where the natural joint is preserved and biomechanics is corrected by pelvic osteotomy. The latter does not exclude the possible need for arthroplasty in the future but certainly postpones it for many years.

Periacetabular osteotomy is a jointpreserving procedure which corrects deficient femoral head coverage by a dysplastic acetabulum. Polygonally shaped juxtaarticular osteotomy ensures vascular blood supply to the acetabular fragment. It facilitates extensive acetabular reorientation leaving the posterior column of the acetabulum intact, thus enabling minimal internal fixation and maximally early patient mobilization.

The best candidate for periacetabular osteotomy is a young patient with symptomatic hip dysplasia with no secondary osteoarthrosis.

All patients operated by us claimed that under similar circumstances they would undertake the procedure again. Periacetabular osteotomy is a modern method of treating symptomatic hip dysplasia. On the basis of our experience and short-term follow-up we conclude that periacetabular osteotomy yields universally good results.

## Bone modeling by sol-gel coated functional Nitinol intramedullary nails

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We investigated bone response to functional sol-gel coated intramedullary Nitinol nails applying continuous bending force to femoral bone. Effect of nailing direction was also under study.

Nickel-titanium shape memory metal alloy, Nitinol, is a functional material whose shape and stiffness can be controlled with temperature. The metal undergoes a complex crystalline-to-solid phase

change called martensite-austenite transformation. As the metal in high-temperature (austenite) phase is cooled, the crystalline structure enters the low-temperature (martensite) phase, where it can be easily bent and shaped. As the metal is reheated, its original shape and stiffness are restored.

Pre-shaped intramedullary Nitinol nails with two different curvature radius, and also straight nails, were implanted in the cooled martensite form in the medullary cavity of the right femur in 40 Sprague-Dawley rats. Intramedullary nailing was done from a proximal to a distal direction; the nail entered the bone from the trochanteric groove. Body temperature restored the austenite form, causing a bending force to the bone.

Anteroposterior radiographs did not reveal any bowing or shortening of the bone in the different experimental groups after 24 weeks. This was confirmed by bone length measurements.

Results from histomorphometry clearly demonstrated that bending force together with sol-gel coating resulted in highest bone contact formation around the implant stem and, respectively, showed significantly less fibrous tissue around the implant. On the two forces tested, the higher bending force gave better results on implant attachment to the surrounding bone. Straight intramedullary nails were poorly attached to bone, even with the sol-gel coating.

In conclusion, intramedullary nailing from trochanteric groove together with constant bending force applied to surrounding bone by sol-gel coated Nitinol implant seems to be an interesting way to enhance bone attachment to metal implant, without chances in macroscopic structure of the implanted bone.

## Rod-through-plate fixation of sheeps diaphyseal fractures

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Nowadays, in orthopaedic surgery for the treatment of fractures of tubular bones of small animals both conservative and operative methods are used. The main factors that ensure speedier healing of a bone fracture are precise reposition, stable fixation of the fracture and as early mobilisation of the limb as possible. The principles of combined fixator were first introduced by A. Seppo in 1979 in humans. In animals, it is extremely important that the construction used for fixing the broken fragments has maximum strength and is compact and stable, because we cannot expect animals to stay immobile during the process of recovery. The rod-through-plate fixator is made of stainless steel and consists of a support plate, two curved lamellae and two cortical bone screws. The method increases the fixator's shoulder on account of the intra- and extramedullar osteosynthesis of the bone, which in turn reduces pressure in the area of the fracture and guarantees the required strength. Thus, the main aim of the method is to maximally reduce the traumatization of soft tissues during the operation and ensure strong fixation of bone fragments.

Experimental bone fractures were induced in the central third of the diaphysis of the sheep's femur and the regeneration of the bone tissue was studied using radiographical and morphological methods at week 10.

The working hypothesis is that attaching the rod-through-plate fixator on the fracture of a long tubular bone will contribute to the development of a positive proportion of connective tissue/ cartilage tissue/ osteoid tissue in the process of reparative regeneration. Preliminary results with active periostal bone tissue formation and decreased amount of cartilage tissue (8-10%) in callus tend to support the hypothesis.

# Associations between urinary C-telopeptide fragments of type II collagen (U-CTX-II) and knee osteophytes, joint space narrowing and osteoarthritis (OA) progression in subjects with early knee OA

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## Introduction

OA is characterized by increased degradation of articular cartilage. There is evidence that U-CTX-II is associated with the prevalence and progression of radiographic knee and hip OA.

## Objective

To investigate the behaviour of U-CTX-II in population-based cohort with early knee OA.

## Methods

A symptomatic population cohort aged 32-55 years (97 women, 51 men) and 25 non-symptomatic individuals were studied. Radiographs from tibio-femoral (TF) and patello-femoral (PF) joints were graded for OA: 53% had grade I, 34% grade 0. U-CTX-II was measured in duplicates by ELISA.

## Results

In postmenopausal women, the U-CTX-II medians were higher than in pre-menopausal subjects, 224 and 133 ng/mmolCrea, respectively (p=0.0001).

In premenopausal women, correlations were found between U-CTX-II and BMI (rho=0.457, p=0.001). In women, a correlation was observed between the level of U-CTX-II and TF osteophytes in the right knee, remaining significant after adjustment for menopausal status (ß=0.250, p=0.012) and BMI (ß=0.263, p=0.016). In men, significant positive correlations between U-CTX-II and TF osteophytes as well as PF osteophytes were observed. In premenopausal women, an association was found between U-CTX-II and PF joint space narrowing (JSN) in the lateral compartement (rho=0.353, n=44, p=0.019), being significant after adjustment for BMI (ß=0.301, p=0.033).

The subjects with progression of PFOA during 3 years (n=18) had higher levels of U-CTX-II compared to the subjects without radiographic OA (n=19, p=0.01). The measurements of U-CTX-II were conducted only in follow-up. No significant association was found between OA grade and the level of U-CTX-II.

## Conclusions

1. Cartilage destruction assessed by U-CTX-II correlated with:
   - TF and PF osteophytes in men and women
   - Narrowing of PF joint in premenopausal women
2. 3-years follow-up demonstrated that PFOA "progressors" had higher U-CTX-II values compared to subjects without radiographic OA.
3. For interpretation of the U-CTX-II results one has to consider the important contribution of gender, postmenopausal status and BMI.

## Characterization of novel factors expressed during cartilage differentiation in developing mouse limb

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The aim of this project is to characterize novel factors expressed during cartilage differentiation in the developing mouse limb and to evaluate their role in chondrogenesis and in extracellular matrix assembly. We have used microarray approach to evaluate gene expression profiles of 34 000 annotated genes and 6000 EST clones in developing mouse epiphyseal cartilage during embryonic limb development to search for novel cartilage genes. From this data we have indentified a novel EST clone (Clone 1) that was highly upregulated during late mouse limb chondrogenesis. This EST clone exhibited similar expression pattern with cartilage oligomeric matrix protein (COMP) and chondroadherin (CHAD). Predicted by in silico analysis, Clone 1 is a transmembrane glycoprotein and it has several putative extracellular O-N-acetylgalactosamine (O-GalNAc) glycosylation sites and a putative intracellular O-N-acetylglucosamine (O-GlcNAc) glycosylation site. O-GlcNAc glycosylation implies that the protein may have a role in cell signaling. Clone 1 is conserved in all vertebrate classes from mammals to fishes and sequence homology is high especially in C-terminal part of protein. According to Northern and in situ hybridization studies, Clone 1 is solely expressed in cartilage tissue in mice at stages E16.5 and E18. In mouse epiphyseal cartilage Clone 1 is expressed from stage E16.5 to at least 10 months of age. Currently we are preparing antibodies and establishing functional in vitro cell culture studies to learn more about the role of this gene in chondrogenesis and cartilage homeostasis.

## Assessment of the knee function in subjects with early knee osteoarthritis: population based longitudinal study

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### Background

Development of knee osteoarthritis (KOA) is slow insidious, possibly with nonlinear or phasic progression (Sharif at al 2004). Our follow-up of the middle-aged cohort during three years by advanced WOMAC index - KOOS, revealed significant decrease in some self-reported functional abilities (Tamm et al., 2006).

### Aim

To evaluate functional abilities of the knee joints in the same cohort using objective methods.

### Methods

160 subjects from symptomatic Elva population with mean age 45 (35-54) years were studied. Functional performance of the legs was assessed by stair climbing test (SCT, steps in cm) and by range of motion (ROM) of knee and hip joints (in degrees). Radiographs of the tibiofemoral,TF joint and axial radiographs of the patellofemoral, PF joint were taken at baseline and three years later.

### Results

At baseline, the SCT results of the signal knee

(complaints dominantly in left or right) were significantly worse ($p<0.015$) in comparison with contralateral knee. After 3 years the functional abilities were better, but an association appeared between SCT and radiographic TFOA in the right knee.

Some decrease in knee extension of the signal knee appeared only on follow-up study. Flexion of knee joints correlated with TFOA already at baseline. Active Laseque manouver with the right leg demonstrated significantly lower ROM both at baseline and three years later. Surprisingly, decrease in hip flexion correlated with TFOA at baseline (right side) and three years later (both sides).

**Conclusions**

1.Some of the subjects had lower ability for stair climbing of the signal knee.The correlation between the SCT results and radiographic TFOA was weak but significant.

2.Some decrease in knee extension and flexion might be a sign for manifest radiographic OA in future.

3.Correlation between TFOA and decreased hip mobility might refer to involvement of both joints by early OA.

## Association between early knee osteoarthritis and adam12 gene polymorphisms in estonian population

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**Background**

One of the recognized candidate genes of osteoarthritis (OA) is the metallopeptidase domain 12 (ADAM12) gene. We investigated the potentiale role of two single nucleotide polymorphisms (SNP) of the ADAM 12 gene in susceptibility to knee OA and its progression.

**Methods**

adam 12_48(C/G ) and adam12_int(C/T) polymorphisms were determined by restriction fragment length polymorphism in a population based cohort consisting of 118 symptomatic subjects with radiographic features of OA in the tibiofemoral and/or the patellofematal joint and of 71 non-symptomatic subjects. For estimation of OA progression, X-ray investigation was repeated three years later. In statistical calculations the data were adjusted for age and body mass index.

**Results**

We found strong association between adam 12_48(C/G) polymorphism and patellofemoral OA in male patients (OR=3.55, 95%CI 1.29-9.76, p=0.014), genetic risk was related to CC homozygosity (OR=11.68, p=0.043). The adam12_48(C/G ) SNP also affected presence of advanced stages osteophytes in the whole group. The risk was more related to occurrence of osteophytes in the patellofemoral joint, especially on patellar margins (OR =2.77, 95%CI 1.02 -7.53, p= 0.046). In OA progression the most significant association was found between joint space narrowing of the tibiofemoral joint and adam12_48(C/G) SNP in women (OR=2.66, p=0.018). No significant association was observed between adam12_48(C/G) SNP and the progression traits in men. The adam12_int(C/T) polymorphism was not related to OA susceptibility or progression traits. Haplotype GC(adam12_48/int) was associated with reduced risk of development of osteophytes in patellofemoral joint (OR=0.24, 95% CI 0.064 - 0.94, p=0.041). The protective effect was more prevalent concerning osteophytes in women group (OR=0.20, p=0.06), but with radiological OA in men (OR=0.10, p=0.046) also.

**Conclusion**

Adam12_48(C/G) polymorphism may affect occurrence of knee OA (mainly due to development of patellar osteophytes) and OA progression. Activation of various pathogenetic mechanisms in OA seems to be gender-dependent.

# Spatial variation of ultrasound backscattering is significantly related with the mechanical properties of trabecular bone

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In general, ultrasound parameters on osteoporosis diagnostics are measured as a mean value within a region of interest (ROI) or as a value at single measurement point.

In the present study, we investigated diagnostic potential of spatial variation of ultrasound parameters for prediction of bone mechanical properties. Further, the effect of time window length (measure for the depth of ultrasound information in bone) on the ultrasound backscattering was characterized.

Human trabecular bone samples (n=20) were measured with a scanning ultrasound (2.25 MHz) instrument. Apparent Integrated Backscattering (AIB) and Integrated Reflection Coefficient (IRC) were determined from a single point at the ROI centre and as a mean value within ROI (88 mm2). Spatial variation of ultrasound parameters within the ROI was quantified as standard deviation (SD). Additionally, to investigate the effect of signal windowing, AIB was analysed using five different time windows (tw) from 1 to 5 microsecond at one microsecond steps.

Ultimate strength of the samples was determined with destructive testing.

Mean value of AIB within ROI predicts ultimate strength strongly only with a short time window ($r = 0.62$, tw = 1 microsecond) whereas SD of AIB within ROI predicts ultimate strength strongly also with long time window ($r = -0.82$, tw = 4 microsecond). Interestingly, the linear combination of mean IRC and SD of AIB within the ROI improved the prediction of bone ultimate strength significantly ($r = 0.92$).

All ultrasound parameters showed significant variation within the ROI. In addition, variation of AIB was a strong predictor of ultimate strength. However, signal windowing affected significantly spatial variation and diagnostic potential of AIB.

In conclusion, our findings suggest that the measurement of spatial two-dimensional parametric maps could yield information on bone quality not extractable from single point measurements. This highlights the diagnostic potential of parametric imaging.

# Different techniques of induction of bone growth by using beta-tricalcium phosphate matrix and multipotent femoral canal cells

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This study was carried out to investigate directing and accelerating of bone tissue growth, which are important aspects in modern mending of complicated fracture healing and treating of bone tissue disintegrating diseases. Three components are considered to be necessary for bone tissue engineering: matrix/scaffold, progenitor (stem) cells and inductive agents. We compared the bone growth in six types of implants (beta-tricalcium phosphate matrixes) implanted in subcutaneous pouches or close to the femoral head of male Wistar rats: 1. implants immersed in 0.9% sodium chloride solution (control implants), 2. implants with the progenitor cells from femoral canal, 3. implants immersed in inductive BMP-2 solution, 4. implants with the progenitor cells from femoral canal + BMP-2 solution, 5. implants immersed in inductive BMP-2 solution and implanted closed to the femoral head, 6. implants immersed in inductive BMP-2 solution and implanted closed to the femoral head while leaving the femoral canal opened for better access of the femoral canal cells. All the necessary progenitor cells were taken from the femoral canal during the implantation procedure. Implants were removed 21 days after operation and dissected following principles of stereology. Presence of bone or cartilage or connective tissue was evaluated by hematoxylin eosin histochemistry.

## Results

Bone formation was only found in the implants where BMP-2 was introduced (the other implants consisted mostly of connective tissue). However, no distinctive differences were found between the implants where cells and BMP-2 were introduced and between the implants where just BMP-2 was used. Percentages of the bone tissue out of all the implant were as follows: 0.0% in group 1, 1.2% in group 2, 32,4% in group 3, 42,4% in group 4, 44.4% in group 5 and, 54.9% in group 6. Differences in amount of bone tissue between group 3 and group 2 were statistically significant, the difference was also statistically different between groups 3 and 1 and between groups 1 and 2 ($p=0.0013$, $p=0.0004$ and $p=0.0525$ respectively). In the other cases, the differences between BMP-2 affected implants and implants without BMP-2 were even greater. It was notable, that there was more hyaline cartilage in the implants located close to the opened femoral canal.

The results show clearly that introduction of BMP-2 is a crucial part in cell transformation to bone cell and formation of the bone tissue. We concluded that presence of osteoconductive matrix and introduction of an osteoinductive agent (e.g. BMP-2) are the main components of designing of bone tissue and introduction of exogenous bone cells is not as important as the first two. However, it was notable that some bone formation can be achieved also by introducing multipotent femoral canal cells exogenously.

# Collagen network orientation in hamster articular cartilage and effect of exercise

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**Introduction**
Objective was to investigate the properties of collagen network in hamster articular cartilage in young and adult animals and whether voluntary running wheel exercise affects collagen network structure.

**Methods**
Syrian Golden Hamsters were divided into five groups: 1-, 3- and 6-month-old control groups and 3- and 6-month-old runner groups which had free acces to running wheels. The collagen network was studied with a novel polarized light microscopic technique.

**Results**
Runner animals were heavier than the controls (21% for 3-month-old and 13% for 6-month-old groups, $p<0.001$). Running distance was approximately 17km per day at the start decreasing gradually to 10km per day at the ages of 3 and 6 months. Cartilage thickness decreased (25%) from 1 to 3 months of age and after that remained the same. Incidence of osteoarthrotic changes increased with age. More healthy cartilage was found in the runner groups.

The collagen fibril orientation had a similar kind of profile in each group. Collagen orientation angle increased from the surface towards deep zones of articular cartilage. Average orientation angles in articular cartilage zones were 15 – 36 – 51 – 48 degrees in superficial, intermediate, upper deep and lower deep zones, respectively. 6-month-old controls, however, had collagen orientaton profile more parallel to the surface compared to the other groups ($p<0.05$).

**Conclusions**
Voluntary joint loading in the young hamsters seems to have decreasing effect on osteoarthrosis. Collagen orientation does not change much during maturation in hamsters but exercise makes collagen fibrils run more parallel to the surface in the intermediate and upper deep zones of cartilage.

# Sex-dependent effect of dexamethasone administered prenatally on femur development in piglets

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The study was performed to determine whether dexamethasone administered during 45 last days of pregnancy to sows had different effect on the development of femur in male and female piglets.

The study was carried out on 5 control sows and 5 experimental sows administered with Dex i.m. at the dosage of 3 mg/sow/48h during the last 45 days of pregnancy. Piglets chosen randomly just after the birth from the control sows were assigned to the control group. Piglets born by sows treated with Dex were assigned to Dex group. Each group of piglets consisted of 10 males and 10 females and was hold with their mother up to 35 day of postnatal life. Bone mineral density (BMD),

bone mineral content (BMC) and the mechanical properties were determined.

BMD of femur in female piglets from Dex group (0.439±0.02 g/cm2) was higher when compared with males from Dex group (0.231±0.003 g/cm2). BMD of femur in control males and females were 0.376±0.002 g/cm2 and 0.360±0.02 g/cm2, respectively. The value of ultimate strength of femur obtained from females belonging to Dex group reached 1553±98 N and was higher when compared with male piglets from Dex (817±64 N). The value of ultimate strength of femur from females and males in control group reached 1346±75 N and 1463±66 N, respectively.

Our results indicate that prenatal exposure of piglets to excess of glucocorticoid such as dexamethasone during the last 45 days of pregnancy in sows disturbed metabolic processes in skeletal system and influenced the processes involved in programming of skeletal development which may persist and appear in later life. Effects of dexamethasone prenatal exposure are sex-dependent and induce stronger consequences in males than in females.

## Biochemical bone resorption and formation markers in children and adolescents with phenylketonuria

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Phenylketonuria (PKU) is a disorder in which dietary phenylalanine restriction is the only known therapy for the prevention of mental impairment in patients. However, this form of diet may influence bone metabolism, especially in childhood and adolescence when growth and bone turnover are most intensive. Some authors have described decreased bone mineral density and osteopenia in patients suffering from PKU. Measurements of bone mineral density (BMD) reflect bone mineral status but not the dynamics of bone turnover. Non-invasive biochemical markers which show global skeletal activity have lately been developed and validated for the assessment of bone formation and bone resorption processes. We measured osteocalcin (OC) and bone alkaline phosphatase (BALP) as bone formation markers and collagen type I crosslinked C-telopeptide (CTX) as a bone resorption marker in 32 prepubertal children and 46 adolescents with PKU treated at the Department of Pediatrics of the Institute of Mother and Child in Warsaw. Healthy prepubertal (n=40) and pubertal (n=38) subjects sent to our laboratory for routine analytical control were the reference group. Serum bone turnover markers were determined by an immunoenzymatic assay. In prepubertal children with PKU we observed significantly lower serum OC (83.4;34.2 versus 104.9;20.0 ng/mL, $p<0.01$) and CTX (1562;486 versus 2116;298, $p<0.001$) concentrations than in the control group. Activity of BALP was similar in both studied groups. In adolescent patients serum OC, BALP and CTX were slightly lower (by about 5-10%) compared to their healthy counterparts. Our results concerning the formation as well as resorption markers suggest a decrease in bone turnover rate in prepubertal children with PKU and seem to be normalized in adolescents. Further studies of bone metabolism markers in patients with PKU in different age-groups are needed in order to prevent bone abnormalities in adult.

## A novel cytokine Fam3C induces formation of osteoclast-like cells in vitro

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To identify new genes that are regulators of differentiation and function of osteoclasts we performed a suppressive subtraction hybridization comparing cDNAs prepared from rat osteoclasts

cultured either on bone or plastic. Cells were isolated from newborn rat bone marrow using magnetic beads covered with monoclonal anti-b3 integrin. mRNAs were isolated and reverse transcribed to cDNAs for amplification. One of the messages which was differentially expressed being elevated in cells cultured on bone was found to be a rat homologue of recently described human Fam3C gene (1). We cloned the full length cDNA and the rat sequence was deposited in GenBank (2) QPCR data show uniform low level expression in tissues studied. We prepared recombinant protein of rat Fam3C in Pichia pastoris with N-terminal 6-His tag. To study the role of this novel cytokine, we cultured primary spleen cells and incubated them in the presence of recombinant Fam3C protein. We noticed a facilitation of growth, which was dependent on the starting cell concentration. This indicates indirect effect on cell proliferation. In bone marrow cultures, there was a Fam3C dose dependent increase of in the number of large tartrate resistant acid phosphatase-positive multinucleated cells, if M-CSF and RANKL were also present. Waerner et al. (3) recently identified Fam3C, using microarray strategy, to be a regulator of epithelial - mesenchymal transition and named it accordingly as ILEI (Interleukin Like Emt Inducer). With data presented here, we show that Fam3C/ILEI affects haemopoietic cells, too, not just epithelial cells. We are in the process of knocking out this gene in mice.

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## Autophagic vacuoles associate with the transcytotic route in osteoclasts

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Bone degradation products in the resorption lacuna are transcytosed from the ruffled border to the functional secretory domain (FSD) via vesicles containing cathepsin K (CatK) and tartrate-resistant acid phosphatase (TRACP). Ruffled border has both late endosomal and lysosomal characteristics, but it does not clearly fit into any known endosomal compartment classification. Less is known about the endosomal nature of the post-lysosomal transcytotic route. We studied the association of transcytotic vesicles with autophagy, which is one of the major pathways for degradation of intracellular macromolecules in lysosomes. Rat osteoclasts were cultured on fluorescein-coated bovine bone slices and autophagic vacuoles were detected with monodancylcadaverine (MDC). Cells were then fixed and labeled with antibodies against CatK and TRACP, and analyzed by fluorescence microscopy. The results indicate a partial colocalization of MDC-containing vesicles with the fluorescein labeled bone and CatK at all levels of osteoclasts. Colocalization of MDC with TRACP is observed only at the middle and superior levels of the cells. This finding suggests that the autophagic vacuoles fuse with the transcytotic vesicles during the process of bone degradation.

## Bone turnover markers in children and adolescents with galactosemia

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Classical galactosemia is an inherited metabolic disorder results from galactose-1-phosphate uridyltransferase (GALT) deficiency. Dietary lactose exclusion reverses many clinical manifestations of acute phase of the disease. Unfortunately, most of the patients despite dietary treatment develop long-term complications among them disturbances of bone mineralization lead to decrease of bone mineral density (BMD). The aim of presented study was to assess of bone formation and resorption processes with bone turnover markers in children and adolescents with galactosemia. We studied 62 galactosemic children (age range 2-20 years). The clinical diagnosis had been confirmed by absence of GALT activity in erythrocytes. All patients were diagnosed in the neonatal period and had good dietary control. Healthy children (n=70) were the reference group. Serum osteocalcin (OC), bone alkaline phosphatase (BALP), collagen type I crosslinked C-telopeptide (CTX), 25OH-D vitamin were determined by immunoenzymatic ELISA assays. We observed similar mean values of bone formation markers in prepubertal patients (age range 2-10 years) as compared to the age-matched controls. The level of bone resorption marker CTX was lower by about 20% ($p<0.001$) in galactosemic children (12435±4456 pmol/L) than in healthy group (15778±2464 pmol/L). On the contrary we obtained similar mean value of CTX in pubertal and postpubertal patients (age range 10-20 years) in comparison to the age-matched controls. The values of OC and BALP in these patients were significantly higher than in healthy children (111.84±52.07;g/L versus 82,28±42.99 ;g/L, $p<0.02$; 95.40±45.69 U/L versus 72.60±40.63 U/L, $p<0.05$ respectively). Our results suggest that bone turnover abnormalities in children and adolescents with galactosemia may concern imbalance between bone resorption and bone formation processes.

## Comparison of neuromuscular performance characteristics in patients with bilateral and unilateral knee osteoarthritis before total knee replacement

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This study compared neuromuscular performance characteristics in female patients with bilateral (BL) and unilateral (UL) knee osteoarthritis (OA) before total knee replacement. Fifteen female patients with BL knee OA (with mean±SE age of 66.8±1.9 years), 16 female patients with UL knee OA (with mean age of 63.4±1.8 years), and 14 age- and gender-matched asymptomatic subjects as controls (with mean age of 65.1±2.4 years) participated in this study. The patients with BL knee OA were measured before the second and the patients with UL knee OA were measured before the first total knee replacement. The isometric dynamometry was used to measure the isometric maximal

voluntary contraction (MVC) force and rate of force development at 50% of MVC (RFD50) of the knee extensor (KE) muscles. Contractile properties of KE muscles were assessed by submaximal isometric tetanic contraction (with the duration of 1 s) at 25%MVC, evoked by percutaneous electrical stimulation using square-wave impulses with the duration of 1 ms at frequency of 50 Hz. The rate of force development at 50% of maximal tetanic force (RFD50, TET) and half-relaxation time of tetanic contraction (HRTTET) were measured. The voluntary (MVC force, RFD50) and electrically evoked tetanic contraction (RFD50, ES) force production characteristics of KE muscles were lower ($p<0.05$) in patients with BL and UL knee OA for both legs compared to controls. In patients with UL knee OA, isometric MVC force, RFD50, MVC and RFD50, ES of the knee extensor muscles for involved leg were lower ($p<0.05$) compared to uninvolved leg. However, these force production characteristics in patients with UL knee OA for uninvolved leg were greater ($p<0.05$) compared to patients with BL knee OA for both legs. In patients with BL knee OA, HRTTET for involved (preoperative) leg was longer ($p<0.05$) compared to uninvolved leg of patients with UL knee OA and controls.

## Clinical Results from Latvia with Targon H

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### Background

The fracture of humerus is quite widespread in the population. Most fractures should be fixed with the implants, from which almost all shaft fractures of the humerus can be fixed with the intramedullar nail using Targon H system. However, it should be noticed that in some cases when the proximal third shaft fractures of the humerus is encountered for the sake of stability it should be better used Targon PH implant. The intramedullar nailing of humerus shaft fractures becomes more and more popular in our country. The reason for this is that it provides with good results in the process of recovering taking into account a good range of motion. Furthermore, the risk of impingement in the shoulder is reduced using intramedullar nail.

### Methods

During 2006 in three largest hospitals of Latvia 101 humerus fractures were fixed using intramedullar Targon H and PH nails.Targon H system is a good solution in case of fixing the humeral shaft fracture. From the two possible surgical techniques, as far as it concerns my own practice, the retrograde technique is more popular. This is due to the fact that the rotator cuff is staying intact during the surgical procedure, which additionally reduces pain in the shoulder after surgery.The parts of the fracture can be fixed with interlocking distal screws. Then, if the construction is stable, the proximal fixation of the nail is not always needed.The antegrade technique is useful for proximal third multifragmentar shaft fractures. Thanks to it, it is possible to change the above-mentioned construction to Targon PH system in order to improve the fixation of the fracture.

### Results

Targon H intramedullar nail is a universal method of humeral shaft fracture fixation. It provides with good stability of the fracture and the process of recovering is much faster.

# Glycosaminoglycan content in hamster articular cartilage and effect of running wheel exercise

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**Introduction**
Objective was to investigate the glycosaminoglycan content in hamster articular cartilage in young and adult animals and whether voluntary running wheel exercise affects glycosaminoglycan content.

**Methods**
Syrian Golden Hamsters were divided into five groups: 1-, 3- and 6-month-old control groups and 3- and 6-month-old runner groups which had free acces to running wheels. The glycosaminoglycan content was assessed from safranin-O stained sections using digital densitometry.

**Results**
Runner animals were heavier than the controls (21% for 3-month-old and 13% for 6-month-old groups, $p<0.001$). Running distance was approximately 17km per day at the start gradually decreasing to 10km per day at the age of 3 and 6 months. Cartilage thickness decreased (25%) from 1 to 3 months of age and after that remained the same. Incidence of osteoarthrotic changes increased with age. More healthy cartilage was found in the runner groups.

The glycosaminoglycan content increased from surface to deep zones and in each articular cartilage zone during maturation. The glycosaminoglycan content was lower in the runner groups and especially in the superficial zone when compared to the controls ($p<0.001$-$0.05$). Decrease of glycosaminoglycan content in the deepest zone of articular cartilage was found after three months of age ($p<0.05$). Exercise had no effect on glycosaminoglycan content in the deepest zone.

**Conclusions**
Runner animals had significantly lower glycosaminoglycan content in the superficial part of articular cartilage. Voluntary joint loading in the young hamsters seemed to have decreasing effect on osteoarthrosis development, however, a decrease in the superficial glycosaminoglycans has previously been thought to be an early sign of osteoarthrosis.

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# Pamidronate treatment of osteogenesis imperfecta type III – case report

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Osteogenesis imperfecta (OI) is a disease of „brittle bones" that is primarily caused by genetic defects in the production of collagen type I. Clinical symptoms of type III of OI are: bones fracture, often present at birth, X-ray may reveal healed fractures that occurred before birth. Children have triangular face, blue scleras, bone deformity, often sever ones and other. In last years the new possibilities of therapy of OI are implemented – bisphosphonates. The aim of the study was to

present history of 6-years old boy with severe OI type III. The boy from Ist pregnancy, Ist delivery (cesarean section- hydramnion, pelvic lie of the fetus), born on term with weight 3200 g, Apgar score 7/8. After the birth we found shortness of the body, shortness and deformity of the lower limbs, little, chicken thorax, disproportionate, big caput, triangular face, blue scleras, decreased muscle tone of the limbs, reduction of activity. In the second year of life in our Department pamidronate cyclic treatment was implemented with the literature data. Till now boy was given 16 doses of pamidronate. He is monitored with calcium-phosphate metabolism indices, leucocytosis and clinical symptoms. He tolerates therapy well, sporadically had non-symptomatic hypocalcaemia. After first cycle he had high fever.

Results of the treatment: lack of bone pains, decreased frequency of bones fracture (the last fracture was observed in the 3rd years of life), improved quality of life. Results of the disease: short stature, deformities of limbs and trunk, abnormal dentition, restricted mobility, retarded somatic development, disability of locomotor system.

Pamidronate treatment is safe, well-tolerated and improves quality of life in children with OI.

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## Novel characteristic of mesenchymal stem cells: induction of endothelial cell differentiation and vasculogenesis

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In the field of tissue engineering, vascularization has been shown to be a major problem to overcome. Blood mononuclear cells (BMCs) are potential endothelial cell source, and they are easy to collect and isolate. Hematopoietic stem cell-originated CD14+ monocytes, which are the major cell population in BMCs can be induced to differentiate into osteoclasts, dendritic cells, macrophages and endothelial cells. In addition, mesenchymal stem cells (MSCs) form an interesting cell population that can be used in tissue engineering for their wide renewal capacity. Mesenchymal stem cells have an important role in the regulation of the proliferation, differentiation and maintenance of hematopoietic cells in the bone marrow by forming structures called "stem cell niche". In our study, we noticed that a co-culture of BMCs and MSCs lead to accelerated endothelial cell differentiation and activation and finally to vessel-like structure formation in the absence of exogenously supplied growth factors. We co-cultured MSCs and BMCs for 4 weeks and studied the expression of two endothelial cell markers, PECAM-1 and Endoglin, using immunohistochemistry. Expression of vascular endothelial growth factor receptors 1 and 2 was studied using RT-PCR at different time points during differentiation and vessel-like structure formation. Endothelial cell differentiation was seen already after 7 days of culture. The co-culture system could be a novel method to vascularize an implant in vitro before placing the graft into target tissue.

## The Impact of Liquid Crystals on Bone Strength

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W.L. Hukins et al(1977) demonstrated that the collagen fibres found in bone tissue have liquid-crystal properties. This view is not supported by S. Lees (1985) who in his work cites the water between the collagen fibres as a substance which could also behave like liquid crystals. Nevertheless, later research has offered more proof of the liquid crystal properties of collagen fibres in bones: M-M Giraud-Guille et al (1997, 1992, 2002).

The aim, method of the research: considering the research results described above, the aim was to determine the existence of liquid crystals in human femur. The research was carried out using the optical microscope MICROPHOT FX and polarised light. Decalcified micro-samples (coloured with haematoxylin-eosin) were studied. The samples were placed under the polarisation microscope so that the polarisation planes were perpendicular. This resulted in three types of polarised light phenomena with varying optical activity: I – bright, optically active, double-refracting; II – intermediate, less active, with a double-refracting phenomenon; III – dark, a type with no optical activity whatsoever - polarised light coming from the sample in conditions of resonance – total absorption.

The results showed that liquid crystals were found in all of the samples we studied. We identified three groups based on optical properties: optically active liquid-crystal properties in groups I and II; in group III, the polarised light resonated towards the axis of the liquid crystal with its wavelength and was absorbed. In case of group III, the bones were much stronger than in groups I and II. Since we are dealing with changes in bone strength brought about by the liquid crystals in collagen, the results indicate that there was no difference in the age of the dead people between these groups; in other words, age-related factors affecting bone strength could not have had a significant impact on bone strength.

## Proliferation and differentiation of osteoblasts on pectin-coated titanium

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Biocompatibility of bone and dental implants determined by interactions between biomaterials and bone cells is a crucial aspect of successful clinical operations. Coating of biomaterials, such as titanium, with appropriate macromolecules may improve biocompatibility. In this study we have assessed the interactions of bone-forming osteoblasts and titanium coated with plant-derived pectin polysaccharides.

Pectins can be isolated and enzymatically treated to rhamnogalacturonan-I fragments called modified hairy regions (MHRs), whose composition, charge, and wettability can be controlled. Titanium discs had been covalently coated with either MHR-A or MHR-B of apple pectin. MHR-B contains shorter side chains and produces a more hydrophobic surface than MHR-A. Pure titanium (TI) was used as a control. Murine MC3T3-E1 preosteoblasts were differentiated to mineralizing cells in 2-week cultures on the discs and then investigated with fluorescing stainings and microscopy.

MC3T3-E1 cells grow better on MHR-B and TI than on MHR-A indicated by cell spreading and attachment. The mineralization capacity of the cells analyzed by the amounts of the deposited tetracycline-stained calcium also revealed differences between sample types: on MHR-A, the calcium quantity was significantly lower than on MHR-B or TI, between which the calcium production did not differ. On MHR-A mineral was seen mainly as small vesicles, whereas on MHR-B and TI calcium formed large and dense aggregates probably reflecting more advanced mineralization.

These results indicate that the functionality of osteoblasts can be affected with pectins, and that MHR-B-like fragments could be considered as candidate molecules for bone implant nanocoatings.

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## Effect of dietary alpha-ketoglutarate (AKG) on bone collagen metabolism in early stages of life in piglets

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### Background

The recent study revealed that enteral treatment with alpha-ketoglutarate (AKG) increases the portal net appearance of proline and may increase the supply of this indispensable amino-acid for bone collagen synthesis, mainly type I, in the developing skeletal system in new-born piglets. The aim of this study was to determine the effect of dietary supplementation with AKG sodium salt, the precursor of proline, on growth rate in relation to bone collagen formation from 1st to 70th days of postnatal life in piglets.

### Methods

The experiment was carried out on 120 piglets divided into two groups, a control, which was administered physiological saline (PhS) per os and experimental, which was administered AKG as an aqueous solution in a dose 0.4 g/kg b.w./day. All the piglets were tested at 6 age periods, namely at 3, 14, 21, 35, 56 and 70 days of life, respectively. The epiphysis was collected from the distal part of each femur for histological examination. The microscopic images were subjected to computerized analysis. The following parameters of bone were determined: area, mean diameter, maximal diameter, perimeter and the orientation of collagen trabeculae. Moreover, using the three-point bending test the bone strain was determined. Concentration of osteocalcin in blood palsma as a bone formation marker was estimated as well.

### Results and conclusion

The obtained results show that dietary AKG supplementation stimulated bone collagen synthesis. The area, diameter and perimeter of collagen trabeculae, their number and plasma osteocalcin levels significantly increased in the AKG treated group. Results indicate the existence of a gut-bone axis mediated by enteral AKG possibly as a proline precursor affecting the collagen synthesis of bone. The exact mechanism by which AKG influences the skeletal system, via the digestive tract, is not known and requires further research.

## Quadriceps femoris muscle function and quality of life in women with early knee osteoarthritis: a two-year study

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The aim of this study was to investigate the quadriceps femoris (QF) muscle function and quality of life in women with early knee osteoarthritis (OA) in the course of two years. Women with OA participated in low-intensity exercise therapy training twice per week, except for 3 summer months. During the two-year monitoring, the 7 female patients with OA (mean±SE age 44.3 ± 2.8 years) were tested twice per year. The control group consisted of 6 age- and gender-matched asymptomatic women, who were tested once. Isometric maximal voluntary contraction (MCV) force and voluntary activation (VA) of the QF muscle and power output (PO) during isokinetic knee extension at angular velocities 60°/s and 180°/s were measured on both legs. The sit-to-stand performance was assessed by the duration of 5 fast sit-to-stand movements. Quality of life, sports and recreational activities were evaluated with the questionnaire of Knee Injury and Osteoarthritis Outcome Score. Isometric MCV and VA of the QF muscle, PO during isokinetic knee extension at both measured angular velocities, and sit-to-stand performance did not change significantly following the two-year period in patients with OA. In patients with OA, isometric MCV and PO during isokinetic knee extension at both measured angular velocities were greater ($p<0.05$) during the first measurements compared with controls, whereas they did not differ significantly between the measured groups during the next measurements. The indicators of quality of life and sports and recreational activities were lower ($p<0.05$) in patients with OA than in asymptomatic control group, whereas these parameters in patients did not change significantly during the two-year period. In conclusion, two years of low-intensity exercise therapy had a positive effect on the motor functions of lower limbs in 34-55-year-old women with early knee OA, maintaining these functions on relative by stable level.

## The effect of cAMP on osteogenesis of embryonic bone cultivated in tissue culture

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The aim of this study was to evaluate the effect of cAMP on DNA synthesis of embryonic alveolar bone in tissue culture. Bone fragments were cultured in the medium which composed of 80% medium 199, 155 horse serum, 4 mg/ml glucose, 100 u penicillin per ml, using the grid method. The explants were cultured 12 days. In the second series the effect of cAMP in a concentration of 10-6M on bone was studied, cAMP assay kit by Amersham, UK was used. DNA synthesis was determined by calculating mitotic labelling indices for 3H-thymidine incorporation into cells within cultured explants at 3 to 12 days. The medium was supplemented with 1 microCyrie/ml 3H-thymidine for 4 hours and processed for autoradiography. The mitotic labelling index was determined in the 5 micron

histological sections. Alveolar bone resorption was first noted at 2-3 days. On 10th day new trabeculae formed in the middle part, the 12th day revealed the presence of new osteoid, which in some region took on the basophilic staining characteristics associated with calcification. Statistical analysis showed that there werte differences in mitotic incidence (MI) of osteogenous cells in control cultures and with cAMP during 12 days in vitro. The mean value of MI was 4,627+_ 1,001 in control and 7,706+_1,188 in the cultures where cAMP was added (P<0,05). Hence we conclude that cAMP is stimulating osteogenesis in organ culture. The study is supported by ESF grant nr. 5692.

## Bone Fractures and changes in Spinal Muscular Atrophy (SMA I)

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Neonatal spinal muscular atrophy (SMA) is a severe autosomal recessive disorder leading to progressive symmetric muscular atrophy. We present an infant girl with severe generalized weakness and multiple bone fractures. No history of musculosceletal disorders. The fetal karyotype was normal (46, XX). Two weeks before delivery mother detected a significant decrease in fetal movements. A girl was born at 38 weeks of gestation by Caesarean section. A cracking sound of fracturing bones was reported during delivery of child. X-rays showed middiaphyseal fractures of both humeri and right femur. No signs of former fractures, rib or skull deformities. X-rays also showed generalized osteopenia – thin cortical bone and the difference between cortical and trabecular bone was not very clear. Due to brittle bones and fractures diagnosis of osteogenesis imperfecta was suspected, but DNA analysis confirmed diagnosis of SMA. The activity of bone specific alkaline phosphatase was 3 times below that normal, alkaline phosphatase isoenzymes ratio was normal. Activity of osteocalcin was 2 times higher, DPD/creatine index in urine 2-3 times higher. Ca and P levels were within the normal limits. Histological analysis of humeral bone showed partly formed cortical bone, present as a compact zone in deeper layers. Superficial bone layers, beneath periosteum, were of trabecular type and in 2/3 of circumference these trabecula extended to central part of bone without forming any compacta. In areas where compacta was forming, only single attenuated osteoblastic cells could be seen. Most of cells present here were osteoclasts, forming small clusters in some areas. These morphological changes represent extensive periosteal new bone formation following fracture. We found low bone formation and mineralization activity markers as well as increased bone resorption activity. The rise in activity of bone resorption markers can be explained by inactivity of child.

# N-acetylgalactosamine (Ga1NAc) glycoconjugates associate with the post-lysosomal vesicular route in bone resorbing osteoclasts

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The expression of the aberrant N-acetylgalactosamine (GalNAc) glycoconjugates, detected by binding of the lectin from Helix pomatia (HPA), is associated with metastatic competence and poor prognosis in a range of human adenocarcinomas. However, their function in the metastatic process remains unknown. We analyzed HPA lectin binding site distribution in physiological tissue penetrating cells – bone resorbing osteoclasts - of human and rat origin, cultured on bovine bone slices with confocal laser scanning microscope. Our results show that fluorescently conjugated HPA lectin labels transcytotic vesicles carrying degraded bone from the ruffled border to the FSD in rat osteoclasts. Some of these vesicles are acidic and also contain TRACP and cathepsin K. However, HPA-lectin does not generally associate with endocytosed transferrin suggesting its association with late endosomal, lysosomal and post-lysosomal compartments, but not with the early endocytotic route. In human osteoclasts, HPA lectin also labels the ruffled border and Golgi membranes. Our results suggest a common vesicular glycoconjugate for penetrative cell types of both pathological and physiological background, whose function, however, in penetration to tissues remains unknown.

# Risk factors for the enthesopathy and juvenile osteochondrosis as the overuse injuries in young athletes

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Sports participation starting already from the childhood and adolescence periods is known to be a powerful health promotion tool. Though, in parallel to the lower general morbidity in a sports population, there is a trend to increased injury incidence among the active athletes. Enthesopathy and juvenile osteochondrosis are among the most often seen musculoskeletal diseases classes in the young athletes, which fit into the definition of an overuse injury. There are only few reports available on the importance and possible interrelation of the different overuse injury predisposing factors in the young athletes.

Aim of the present study was to analyze the incidence and predisposing (risk) factors of enthesopathy's and juvenile osteochondrosis of the lower extremity's in the young athletes. We have calculated and followed over the five years time period the both, internal (age, gender, body composition, body height's dynamics, body posture, length of the muscles, flexibility) and external (kind of sport, duration of participation, training load, technique) predisposing factors. All the reported lower extremities musculoskeletal conditions, classified as enthesopathy or juvenile osteochondrosis (E, JO), as well as the acute injuries, were analyzed in relation to the above mentioned variables. 395 athletes of both genders (113 females and 282 males), 6 to 18 years old

were under control during the five years time period.

The incidence of E and JO was calculated as one case per 16956 training/competition hours (41 case in the respective group of athletes). Typical risk factors were: 1) age15 years, 2)males prevailing over females, 3) gain of body height during the last year over 10cm, 4) lower level of flexibility of the lower trunk and legs, muscular dysbalanss, 5) kinds of sport – basketball, athletics, sports dance.

It can be noted that along with the age dependent onset of enthesopathy and juvenile osteochondrosis, there are strong sports related risk factors.

## Osteoma miliaria disseminatum faciei: A model of ectopic intramembranous ossification

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### Introduction

Osteoma cutis is a condition where bone is deposited in the skin. There are different forms of osteoma cutis. In multiple miliary osteoma of the face tiny bone fragments are developed in the dermis and subcutis. Pathogenesis of the ossification is unknown. In the primary forms occurring in the childhood there is a shown genetic background.

### Patients

One female patient (58 years) and two male patients (55 and 66 years) have been studied. They have small, hard granules in the skin of their face and/or upper chest. Dermatologists have tried different pharmaceutical treatments to get rid of these disturbing granules without results. At the moment best treatment is needle microincision, but it does not work with dozens of granules.

### Histopathological analysis

Histologically the granules are true lamellar bone nodules in the dermis or subcutis, diameter 0,5-5mm. There are no signs of bone remodeling and the bone formation is very slow, since no active osteoblasts are present. The lesions are BMP4, FGF8b and estrogen receptor negative.

### Discussion

This condition has been reported earlier (e.g. Thielen et al. JEADV 2006, Altman et al. J Am Acad Dermatol 2001) with only minor suggestions for the mechanism. There are many putative mediators for ectopic ossification. BMP-4 overexpression has been found from progressive heterotopic ossifications in fibrodysplasia ossificans progressiva. FGF-8b predetermines bone marrow cells to differentiate to osteoblasts. Human bone marrow osteoprogenitors express estrogen receptor-alpha, BMP-2 and BMP-4 mRNA during osteoblastic differentiation. The osteomas appear to area where fetal osteogenesis is intramembranous. Subcutaneously implanted BMP-4/7 heterodimer can lead to intramembranous ossification in mouse. The lesions locate at previous acne areas, which indicate inflammation background. Our approach is to develop in vitro model for skin ossification in order to test various cytokines and growth factors in this ectopic ossification. In vitro model will be described.

## Regulation of osteocyte-specific genes in vitro and in vivo by glucocorticoids

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The action of native or pharmacological glucocorticoid hormones, such as dexametasone (Dex), is mediated via glucocorticoid receptors. Dex is recognized by multiple effects on a wide range of tissues and physiological conditions in the body, including bone metabolism. A low dose of Dex promotes osteogenesis in vitro, and induces the expression of osteogenic markers in mesenchymal stem cells. However, excess glucocorticoids can lead to osteoblastic dysfunction, enhanced bone resorption by osteoclasts, and induce osteocyte apoptosis. In this study we aimed to investigate how glucocorticoids directly affect osteocytes. We used the MLO-Y4 cell line to study the effect of Dex treatment on osteocyte-specific gene expression. Another approach was to study the effects of a short intermittent dose of glucocorticoids in vivo on the expression of gene expression in bone. For comparison we used data from a mesenchymal stem cell culture grown under osteoinductive conditions, consisting of alpha-MEM-medium supplemented with beta-glycerophosphate and ascorbic acid. In all experiments, we measured the osteocyte-specific gene expression of pleiotrophin (PTN), phosphate-regulating endopeptidase homolog, x-linked (PHEX), dentin matrix protein-1 (DMP1), sclerostin (SOST), matrix extracellular phosphoprotein (MEPE), fibroblast growth factor-23 (FGF23), polycystin-1 and -2 (PC1, PC2), polaris, mouse homolog of intraflagellar protein 88, (TG737) and osteocalcin (OC), using mRNA-specific primers and real-time semi-quantitative RT-PCR. Our data is indicating the up-regulation of genes believed to be important for bone-formation, for example DMP1 and PHEX in vivo and OC in vitro. Similarly, we report the down-regulation of genes, like SOST and FGF23 in vivo and FGF23, SOST and MEPE in vitro. However, OC-expression is indicating the opposite effect in vivo compared to in vitro. In conclusion, our present results give novel quantitative data concerning the effect of glucocorticoids on osteocytic gene expression under different in vitro and in vivo conditions.

## Instrumental gait and motor investigation of idiopatic scoliosis subjects before and after spinal fusion

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**Summary**
The aim of this prospective study is to find common changes in function of lower limbs and keeping of body balance in the group of idiopathic scoliosis (IS) patients, going to surgical treatment. This prospective study will continue with investigation of the some patient group one year after surgery and comparing to previous gait pattern in quest of common changes depending from fusion levels and body balance. The results of first study stage are investment of arguments in discussion about scoliosis etiology. The results of second stage would be interesting for spine surgeons, shoving benefits and disadvantage of fusion operations.

**Conclusions**
We have opinion that the origin of IS coming from disease of passive spine motion structures. We are

looking forward to investigate gait and muscle work changes in post fusion session.

**Patients/materials and methods**

The study analyzing two groups: healthy adolescents and IS patients in pre op. and after fusion session. The time between sessions is one year. Instant number of included ISP is 18 patients. (We are going to collect 30-40 IS patients) Age range is 12-18 y. All of them were evaluated with the some gait lab fitted with 6 Proflex 240hz cameras, AMTI force plate, Delsys electromyograph (EMG) interconnected with C-motion software. Muscles selected for EMG were m.errector spine; gluteus medius and maximus, rectus, lateral and medial hamstrings, lateral head of gastrocnemius, tibialis anterior.

**Results**

The preliminary results allow to declare, that in the pre op. patient group was no statistically significant (P<0, 05) signs of asymmetrical gait parameters and also EMG signal was symmetrical what indirectly indicate that vestibular and body balance mechanism is intact.

## Effectiveness of glucosamin and chondroitin for treatment of pain syndrome under knee osteoarthritis

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The research was aimed at evaluating the effectiveness of glucosamin and chondroitin and its combination with ibuprofen of pain syndrome under knee osteoarthritis.

The first group included 16 patients (aged 64,2±1,9 years) with knee osteoarthritis of II-III stages, according to Kellgren-Lourenz who took Theraflex-Advance (TA), (250 mg glucosamin sulphate, 200 mg chondroitin sulphate and 100 mg ibuprofen). The control group included 16 patients with the same diagnosis (aged 63,9±1,7 years), who took Theraflex (T) (500 mg glucosamin hydrochloride and 400 mg chondroitin sulphate). The following methods of study were used: VAS, Leken index, Womac scales, determination of life quality by EuroQol 5D scale.

After two weeks of treatment, patients taking TA observed a reliable decrease of pain syndrome by Womac scale (before treatment 58,5 ± 5,5; after two weeks 40,7 ± 5,9; t=2,38; p=0,037), decrease of constraint in movements (index before treatment 57,8 ± 6,5; after two weeks 36,7 ± 6,3; t=2,65; p=0,022), improvement of index of everyday activity (from 64,6 ± 4,1 to 44,0 ± 6,1; t=2,82; p=0,017). In the group taking T, intensity of pain syndrome certainly decreased after two months of treatment, according to VAS scale (before treatment - 50,9 ± 3,9; after two months- 42,7 ± 4,6; t=3,1; p=0,011), Womac pain scale (before treatment - 47,0 ± 5,3; after two months- 30,4 ± 6,5; t=2,89; p=0,016).

Hence, Theraflex-Advance is instrumental in rapid decrease of intensity of the pain syndrome (after two weeks) in patients with knee osteoarthritis. Over a month after cessation of preparation taking, positive effect remains: knee pain is significantly lower in comparison with indexes before treatment. The analgesic effect after taking Teraflex becomes noticeable after two months when it is followed by the essential decrease of constraint index, improvement of index of everyday activity.

## Bone loss in men of different age

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This research was aimed at studying the age dependent peculiarities the state of bone mineral density and bone mineral consents in men.

Research object: The total of 210 men 20–89 years old (54,6 ± 1,2) were examined and divided in to following age-dependent groups: 20- 29 years old, 30-39, 40-49, 50-59, 60-69, 70-79, 80-89.

### Research methods

The mineral density and mineral consent of bone was determined with the use of Dual X-ray absorbciometry by apparatus "Prodigy" (GE Medical systems, Lunar, model 8743, 2005).

### Research results

The analysis of variance did not expose reliable influence of age on the indexes BMD and BMC of lumbar spine. Bone mineral density was significant higher in group 80-89 years in comparence with other group that may indicates on some "selectivity"of this group. BMD hip in group 70-79 years (0,95 ± 0,05) was significant lower in comparence with other groups: 20-29 year (1,08 ± 0,05; p = 0,012), 40-49 year (1,04 ± 0,02; p = 0,036), 60-69 year (1,04 ± 0,03; p = 0,039). It was established the correlation of BMD lumbar spine with height (r = 0,16; p = 0,021), weight (r = 0,27; p < 0,0001), BMI (r = 0,22; p = 0,001), BMD hip (r = 0,62; p < 0,001); BMD hip with age (r = - 0,15; p = 0,024), height (r = 0,30; p < 0,0001), weight (r = 0,35; p < 0,0001), BMI (r = 0,23; p < 0,001).

### Conclusions

Age in men has a substantial influence on hip BMD: the lowest indexes were observed in group of 70-79 years. The osteoporosis of lumbar spine was observed in 19,2%, hip osteoporosis in 7,6 % of patients in this group.

## Bone tissue structure and functioning in postmenopausal women engaged in various physical exercises

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This research was aimed at influence of physical exercises on bone state in postmenopausal women.

### Material and methods

52 women of postmenopausal age were divided into five groups: 1) attending yoga class no less than 5 years, n=8; 2) jogging no less than 5 years, n=12; 3) former gymnastic champions with nearly 10-20-year sporting life, n=10; 4) unengaged in any sports (control, n=13); and 5) postmenopausal women (14.0±2.2 years, n=10) at around 65 years of age, suffering from osteoporosis and doing physical exercises with loads (1 kg weight) during 6 months. All women were standardized by age, body weight and post menopause duration. Bone tissue measurements of speed of sound (SOS), broadband ultrasound attenuation (BUA), Stiffness index (STF) and Z (SD) were performed by ultrasonic densitometry ("Achilles+").

**Results**

The SOS parameter values were (1526±12.8; 1529±5.1; 1534±9.3; 1520±8.4) in groups 1, 2, 3 and 4, respectively, and had no significant differences among study groups. The BUA value was higher in gr. 3 (113.7±3.1) in comparison with gr. 4 (106±1.4; $p<0.05$). Its value did not have significant differences in gr. 1 and gr. 2 (106.5±3.4 and 110.4±2.8) compared to control group value (106±1.4). The STF values in gr. 1, gr. 2 and gr. 3 made respectively 78.3±5.6; 81.7±3.1 and 85.2±4.5 and did not differ significantly from the control (76.1±3.1). The parameter Z (SD) registered in gr. 3 group versus gr. 4 had the following meanings: 0.96±0.2 and 0.02±0.2 ($p> 0.05$). In gr. 1 and gr. 2, this parameter was 0.0±0.5 and 0.4±0.3 ($p> 0.05$).

**Conclusion**

Sports at young age and performance of physical exercises with loads lead to the increase of bone tissue indices at the postmenopausal age, as has been proved by ultrasonic densitometry data.

## Back pain and quality of life in the women with osteoporotic vertebral fracture

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Research was aimed at studying the peculiarities of vertebral pain syndrome and its influence on life quality in women with postmenopausal osteoporosis in relation to localization and vertebral bodies' deformation types.

**Object**

353 women in postmenopausal period aged from 50 to 89 years were examined and divided into groups depending on localization and type of vertebral deformations.

**Methods**

The questionnaire, X-ray of pectoral and lumbar spine in two projections, morphometry of vertebral analysis were used.

**Results**

Intensity of vertebral pain syndrome in women with osteoporosis and its complications depends on localization of deformed vertebrae. In pectoral spine intensity of pain syndrome (VAS) was higher in women with fractures of pectoral vertebrae (3,9±0,6 points, $p<0,05$) and vertebral fractures (pectoral and lumbar spine) of combined localization (3,7±1,1 points, $p<0,05$) compared with fractures present only in lumbar spine (2,7±0,7 points). In lumbar spine intensity of pain syndrome (VAS) was higher in women with fractures of combined localization (6,5±0,4 points, $p<0,05$) compared with fractures of vertebral bodies only in pectoral (4,8±0,6 points) or only in lumbar spine (5,1±0,6 points). According to questionnaire EvroQol-5D, Life Quality of women with fractures of only pectoral vertebrae was significantly lower (4,7±0,6 points, $p<0,05$) in contrast to women with fractures of lumbar spine vertebrae (5,9±0,4 points) or combined localization fractures (6,4±0,5 points). Life Quality and general condition, related to back pain caused by the osteoporosis, according to the questionnaire ECOS-16, in women with vertebral fractures of (52,3±6,2 and 53,8±2,4 points respectively, $p>0,05$) only pectoral or only lumbar spine did not differ significantly, while women with fractures of combined localization had a considerably lower life quality index (60,6±2,2

points, p<0,05). The life quality indexes and daily activity were certainly higher in patients with fractures of three vertebral bodies in comparison with fractures present in 1-2 bodies or plural deformations.

## Identification of risk groups of osteoporosis using IOF's one-minute osteoporosis risk test

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**Background**
This research was aimed at evaluating the relation between structural-functional state of bone according to the ultrasound densitometry and results of IOF's One-Minute Osteoporosis Risk Test.

**Materials and methods**
We've examined 147 postmenopausal women aged 50-69 years (mean age 59,8 ±0,7). Structural-functional state of bone was evaluated by means of an ultrasound bone densitometer ("Achilles+"). The speed of sound (SOS, m/s), broadband ultrasound attenuation (BUA, dB/MHz) and a calculated "Stiffness" index (SI, %), T and Z-range were measured. IOF's One-Minute Osteoporosis Risk Test was translated into Ukrainian.

**Results**
Significant correlation was found between positive answer to question 2 ("Have you broken a bone after a minor bump or fall?") and SOS (r = -0,17; p = 0,042), BUA ( r = -0,28; p = 0,0005), SI (r = - 0,25; p = 0,002), Z- range (r = -0,26; p = 0,015); between positive answer to question 3 ("Have you taken corticosteroid tablets for more than 3 month") and SOS (r = -0,16; p = 0,047), BUA ( r = -0,29; p = 0,0003), SI (r = - 0,21; p < 0,000001), and between positive answer to question 4 ("Have you lost more than 3 cm (just over 1 inch) in height?") and the following indexes of structural-functional state of bone: SOS (r = -0,32; p < 0,00001), BUA ( r = -0,27; p = 0,00096), SI (r = - 0,36; p < 0,000001), Z- range (r = -0,27; p = 0,0015).

**Conclusion**
Application of IOF's One-Minute Osteoporosis Risk Test gives an opportunity to determine structural-functional changes of bone. Among the test questions, the most reliable and informative as for postmenopausal women proved to be questions 2 ("Have you broken a bone after a minor bump or fall "), 3 ("Have you taken corticosteroid tablets for more than 3 month ") and 4 ("Have you lost more than 3 cm (just over 1 inch) in height?).

# The orphan nuclear receptor ERRalpha regulates proliferation and osteoblastic differentiation of mouse mesenchymal stem cells

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Estrogen-related receptor alpha (ERRalpha) is an orphan nuclear receptor expressed in several adult and embryonic tissues e.g. in the osteoblasts of bone. It has been suggested that ERRalpha plays a role in bone formation: it is expressed in ossification zones of the mouse embryo, it up-regulates the expression of the osteopontin gene and overexpression of ERRalpha in rat calvarial osteoblasts increases their proliferation and mineralization. However, the function of ERRalpha in osteoblastogenesis has not been studied in mesenchymal stem cells, the multipotent osteoblastic progenitor cells in the bone marrow. Therefore, we used mouse mesenchymal stem cells isolated from the bone marrow of ERRalpha knockout (ERRalpha -/-) and wildtype (ERRalpha +/+) male mice to study how ERRalpha influences the proliferation and osteoblastic differentiation of mouse mesenchymal stem cells.

The expression of ERRalpha was studied by RT-PCR. The proliferation of mesenchymal stem cells was studied by 3H-thymidine incorporation and cell cycle analysis. The stage of differentiation induced by ascorbic acid and sodium beta-glycerophosphate was studied by defining the expression of osteoblastic marker genes (osteopontin, type I collagen, osteocalcin, bone sialoprotein, Runx2 and alkaline phosphatase) by RT-PCR and the mineralization by von Kossa and alizarin red S staining methods.

The expression of ERRalpha was fairly constant throughout the osteoblastogenesis of mouse mesenchymal stem cells. ERRalpha influenced both proliferation and mineralization of mesenchymal stem cells: ERRalpha -/- cells proliferated less, mineralized slower and showed diminished mRNA expression of osteopontin and bone sialoprotein compared to the wild type. Therefore, ERRalpha seems to have a positive impact on mouse bone formation in vitro.

# The use of molecular markers of bone turnover for prognostication of the 12-months effect of risedronate therapy on bone mineral density in postmenopausal osteoporosis

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Bone markers describe some specific aspects of bone status. Most frequently they are used for monitoring antiresorptive therapy in patients with postmenopausal osteoporosis. In positive cases collagen resorption decreases during the first months of therapy (1-3. months), whereafter (3-6. months) the synthesis decreases. If the values of bone markers do not change substantially, it is questionable whether bone mineral density (BMD) increases after 12-24 months therapy. So, there is

a possibility to characterize separately the intensity of both resorption and synthesis. Nevertheless, such an approach is not routinely used.

**The aim**

To investigate the changes of bone markers (during the first months) as a tool to prognosticate the increase of lumbal BMD after 12 months of risedronate therapy.

Material: The study group consisted of 18 patients with postmenopausal osteoporosis (mean BMD 0.855, T-score L2-L4 from -2,5 to -4,1) aged 49-65 (mean 59) years, who had received risedronate therapy for 12 months. 27 healthy women aged 28-48 years with normal lumbal DEXA finding served as controls.

**Methods**

Bone resorption was assessed by S-CTx-I and formation by S-P1NP (Roche, Elecsys). Analyses were repeated at 1, 3, 6 and 12 months after initiation of therapy.

Index of bone turnover (BT%) in every month was calculated as P1NP% from baseline + CTx% from baseline.

**Results**

Before the therapy - based on bone markers - the patients fell into two groups: 10 had increased and 8 had normal (although above the median value of the control group) bone turnover. After the 3 months of risedronate therapy BT% was significantly lower in women whose lumbal BMD increased >3% after 12 months (p=0.006).

**Conclusions**

1. Bone metabolism in women with postmenopausal osteoporosis is heterogeneous.
2. Total bone turnover at 3rd month of risedronate therapy offers some prognostic value for changes of lumbal BMD after 12 months.

## Endostatin inhibits endochondral ossification

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**Background**

Angiogenesis is essential for the replacement of cartilage by bone during skeletal growth and regeneration. Vascular endothelial growth factor-A (VEGF-A) is a key regulator of angiogenesis whereas endostatin, a potent inhibitor endothelial cell proliferation and migration, is a natural antagonists of VEGF-A. Regulatory role of these peptides in angiogenesis and bone formation was investigated using adenoviral gene delivery of VEGF-A and endostatin in mouse ectopic ossification model.

**Methods**

Bone formation was induced in the hamstring muscles of adult mice with native bone morphogenetic protein extract (BMP) implemented in gelatine gel together with VEGF-A and endostatin recombinant adenoviral vectors. The mice were sacrificed 1, 2, and 3 weeks after the operation and ectopic bone formation was followed radiographically and histologically.

**Results**

Significant bone formation was induced by BMP extract in all treatment groups. VEGF-A stimulated

and endostatin prevented the formation of FVIII related antigen positive vessels as well as the number of cartilage resorbing chondroclasts / osteoclasts. Endostatin alone or in conjugation with VEGF-A reduced bone formation. Excess of VEGF-A stimulated and endostatin reduced bone formation, respectively, at three weeks time point.

**Conclusion**

Our findings indicate that endostatin retards the cartilage phase in endochondral ossification which subsequently reduces bone formation in our experimental model. We conclude that bone growth and healing, which share features with ectopic bone formation, may be regulated by endostatin.

## Effect of AKG on postnatal skeletal development in piglets after prenatal chronic treatment with high doses of dexamethasone using model of ribs

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The study was performed to determine whether postnatal administration of AKG to piglets had an effect on the development of bones assessed on the model of ribs after prenatal influence of dexamethasone.

The study was carried out on control and experimental sows administered with Dex i.m. at the dosage of 8 mg/sow/48h during the last 25 days of pregnancy. Piglets chosen randomly just after the birth from the control sows were assigned to the control group. Piglets born by sows treated with Dex were assigned to Dex/PhS group treated with saline and to Dex/AKG group treated per os with AKG at the dosage of 0.4 mg/kg/bw/daily up to 35th day of postnatal life. Every group consisted of 10 males piglets. Bone mineral density (BMD), bone mineral content (BMC), fractal dimension of trabecular bone, trabecular bone volume (BV/TV %) and trabeculae border line length were determined.

BMD from Dex/AKG group (0.123±0.0025 g/cm2) was significantly higher when compared with Dex/PhS group (0.099±0.0027 g/cm2) and control group (0.102±0.0028 g/cm2) with P=0.0078. Fractal dimension was higher in Dex/AKG group (D=1.3043±0.01695) than in Dex/PhS (D=1.2354±0.01428) and control group (D=1.2294±0.01197) with P=0.0116 and P=0.0065 respectively. The trabeculae border line length was significantly longer in Dex/AKG than in Dex/PhS group.

Our results indicate that postnatal AKG administration diminish negative effect maternal dexamethasone treatment on the maturation of bone tissue persisting in later life in piglets and improve development of skeletal system in animals being under prenatal action of dexamethasone.

## The three dimentional unilateral instrumentation of sacrum fractures

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The number of sacrum fractures has increased at the last time. The unilateral isolated fractures of sacrum are not find often and sometimes not recognized, asspecialy in politrauma cases.

The 14 patients with unilateral isolate fractures were treated by threedimentinal instrumentation and included at least two unilateral transpedicular screws, other one pelvic, another one transfracture with good or satisfactory results in period of time 2002 – 2006. The poor results were recognized in two cases (nonuning and loosening of screws).

This method of threedimentional unilateral instrumentation of unilateral sacrum fractures provides stable fixation, allows to reduce operation time and tissue damages and to start early mobilization and rehabilitation.

## Characterisation of micro-RNA expression profiles of mouse bone and cartilage cells

**S. Suomi[1], H. Taipaleenmäki[2], K. Väänänen[1], A-M. Säämänen[2], T. Hentunen[1], K. Büki[1], T. Laitala-Leinonen[1]**
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MicroRNAs (miRNAs) are small single-stranded RNA molecules that suppress translation of target genes by binding to their mRNA and thus have a central role in gene regulation in health and disease. It has been reported that miRNAs regulate processes as diverse as early development, cell proliferation and cell death, apoptosis and fat metabolism, cell differentiation, and brain development. Like mRNAs, miRNA expression profiles vary from tissue to tissue but may be similar in hard tissues. Several studies indicate that tissues in developing and mature organisms are characterised by unique profiles of miRNA expression.

It has been reported that microRNAs modulate the differentiation of hematopoietic stem cells and we hypothesise that the same applies for mesenchymal stem cells. Our main focus in the Bone Biology Research Consortium is skeletal tissue biology, and we are therefore interested in miRNA expression in bone and cartilage cells. Osteoblasts and chondrocytes both differentiate from mesenchymal stem cells and our aim is to study and compare the expression profiles of miRNAs in these cell types during the differentiation.

We studied miRNA expression profiles using real-time quantitative PCR. Stem cells of mesenchymal and hematopoietic origin were enriched from mouse bone marrow. Using specific culture conditions, mesenchymal stem cells were induced to differentiate into osteoblasts and chondrocytes and hematopoietic cells into osteoclasts. Total RNAs from these different lineages were analysed with real-time quantitative PCR and the expression profiles of 29 selected miRNAs were compared between these cell types. The results showed marked differences in individual miRNA expression levels between cells of hematopoietic and mesenchymal origin, and between cells from osteogenic and chondrogenic lineages.

# Expression and localisation of BMP2 and different TGF beta isoforms in early heterotopic ossification using a rat model

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Our study focused on the localization and expression of bone morphogenetic protein 2 (BMP-2) and different isoforms of transforming growth factor beta (TGF beta 1, TGF beta 2 and TGF beta 3) in early stages of heterotopic ossification (HO). Bone growth was induced in rats by using implants immersed either in rhBMP-2 solution or in normal saline at the site where the HO is usually expected after total hip replacement. Implants were removed either 3 or 21 days after the operation. Implants were dissected following principles of stereology. Expression of the all growth factors in different types of tissues was determined by non-radioactive in situ hybridization. The presence of these growth factors on protein level was determined by immunohistochemistry.

**Results**

BMP-2 was mostly expressed in formed bone and cartilage. There were no significant differences in the expression of TGF beta 1, TGF beta 2 and TGF beta 3 in the control groups. After three days TGF beta 2 was more expressed in the undifferentiated cells as compared to the control group. After 21 days the connective tissue near the forming bone expressed significantly more of TGF beta 3 and has tendency to express more of TGF beta 2, compared to TGF beta 1 - 6.58% of the forming HO consisted of connective tissue expressing TGF beta 3 vs. 3.86% connective tissue expressing TGF beta 1 ($p=0.046$) and 5.27% of the forming HO consisted of connective tissue expressing TGF beta 2 vs. 3.86% connective tissue expressing TGF beta 1; ($p=0.09$).

**Conclusions**

Intramembranous ossification is an important part of the formation of HO. It is also evident that TGF beta 2 and 3 are activated at the sites of intramembranous HO development and their expression can be related to the formation of HO.

# BMP-2, BMP-6 and BMP-7 enhanced TGF-BETA3 induced chondrogenesis of mouse bone marrow stromal cells

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Bone morphogenetic proteins (BMPs) are members of TGF-beta superfamily. They are essential differentiation factors for embryonic development affecting limb morphogenesis and skeletal development. The aim of this study was to compare the expression and distribution of BMP-2, -6 and -7 in developing mouse limb cartilage and to characterize their effect on chondrogenic differentiation of mouse bone marrow derived mesenchymal stem cells (MSCs).

Gene expression profiles of BMPs during in vivo chondrogenesis were extracted from Affymetrix microarray data covering embryonal development of hind limbs. BMPs had distinct spatial and temp-

oral distribution patterns in vivo in the developing limb cartilage. BMP-7 mRNA was stably expressed at a low level throughout the limb development while expression of BMP-2 and BMP-6 continuously increased after E12.5.

In vitro experiments were conducted by inducing chondrogenic differentiation of MSCs in pellet culture. Differentiation medium was supplied with TGF-beta3 with or without different BMPs. All BMPs promoted the progression of in vitro chondrogenesis, evaluated by increased size of the pellet, changes in cell morphology and deposition of proteoglycans and type II collagen in the extracellular matrix. Phenotypic observations were confirmed by determining the expressions of cartilage specific transcripts. Induction with any of the BMPs increased the expression Agc1 and Col2a1. According to the phenotype and gene expression analysis the progression of chondrogenesis was enhanced in the order: BMP-2  BMP-6 > BMP-7.

BMB-2 and BMP-6 were clearly better inducers of in vitro chondogenesis than BMP-7. The tissue distribution in differentiating chondrocyte zones and increased expression during late embryonic development seem to correlate with the higher in vitro chondrogenic potential of at least these three BMPs. The timing of exposure to different BMPs might also be an important factor to consider when inducing chondrogenesis in vitro, as suggested by the changes in expression levels during embryonic development.

## The effect of VEGF-A gene transfer on trabecular bone in rats

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Changes in human skeleton in postmenopausal osteoporosis have similarities with development of ovarectomy induced osteoporosis in rats. Bone turnover, especially osteoclast activation and bone resorption are high after ovarectomy. Vascular endothelial growth factor (VEGF-A) can stimulate in vitro osteoblast abelingiation and proliferation. Under the influence of VEGF-A, callus formation and bone fracture healing occur faster. The intention was to study the effects of VEGF-A transfer on trabecular bone using lacZ gene transfer and saline as references.

Rats aged 12-weeks (n=105) were ovarectomized. VEGF-A (n=30) and lacZ (n=30) gene transfer was made locally to proximal tibia marrow cavity a month after ovarectomy by using adenovirus as a gene transfer vector. Contralateral side was used as a reference by injecting saline which was also used for controls (n=45). Baseline controls were killed at the time of gene transfer and other groups either at three-week or at three-month time points. Tibial biopsies were obtained and undecalcified sections were prepared using hard tissue technique. Double fluorochrome abeling was performed before the biopsy. Histomorphometric measurements were done using semiautomatic analysis software with a magnification of 200X. In each group, three to seven specimen were adequate for analysis. Side-to-side differences were analysed using nonparametric tests.

There was no significant difference between VEGF-A intervention group and controls in bone volume density, erosion surface, amount of osteoblasts, osteoid thickness and wall thickness. Osteoid volume was significantly higher in VEGF-A intervention group than in saline control group

at three-week time point. Dynamic indices did not show any remarkable changes between the groups.

Local VEGF-A gene transfer did not stimulate either bone formation or increase bone volume in rats tibia. The gene transfer had no effect on resorption parameters either. The number of cases in this study was small.

## Reproducibility of Bone Histomorphometry

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Bone histomorphometry is the method of choice for quantifying bone metabolism and remodellation. It enables two-dimensional quantitative histological analysis of formation, resorption and bone cells. However, the method is demanding and requires expertise and training. The aim of the study was to assess reproducibility of the histomorphometric analysis.

Horizontal iliac crest biopsies (n=5) were obtained and undecalcified Masson-Goldner (3um) and unstained (10um, UV) sections were prepared using hard tissue technique. Double fluorochrome labelling was performed before the biopsy. Histomorphometric measurements of trabecular bone (20-30 fields per sample) were done without any background information of the case, using semiautomatic analysis software (Bioquant Osteo, Bioquant Image Analysis Corporation, Nashville, TN) with a magnification of 200 X. Both intraindividual and interindividual (n=3) variability were assessed in five specimens. The duration between the different measurements was at least one week.

For structural parameters (bone volume, BV/TV) intraindividual reproducibility (coefficient of variation, CV%) was good (0-10%) and interindividual CV% moderate (0-15,4%). In contrast, for erosion variables (ES/BS) the repeatability was poorer (intraindividual 41,3-47,7%, interindividual 25,7-25,9%). The results for formation parameters (OV/BV, OS/BS, O.Th) and dynamic indices (MAR, MS/BS) were between these values. CV% for cell indices (Ob.S/BS, Oc.S/BS) was 25,3-47,8% intraindividually and 17,3-25,4% between the researchers. Reproducibility was also studied between two bone laboratories (collaboration with Addenbrook's Hospital, Cambridge, UK - J. Compston and S. Vedi). CV% was 9.9% for BV/TV, 29.6% for OS/BS and 25.5% for O.Th.

In conclusion, the structural and formation parameters showed the highest reproducibility. Interpretation of the erosion surfaces is influenced by the quality of bone samples. The reference values have a wide range and even with these results the histomorphometric diagnosis for a patient did not change in any case between the measurements. According to our knowledge, there are no earlier studies on the repeatability between laboratories.

# Acute exercise does not influence the level of serum markers of bone turnover in boys

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**Background**
Regular physical activity, particularly weight-bearing exercise, in puberty is an important determinant of peak bone mass. Bone formation increases markedly, when bone is subjected to strain. Pubertal growth spurt is characterized by increased bone turnpver.

**Aim**
To investigate the response of N-terminal propeptide of type I procollagen (PINP), crosslinked telopeptide of type I collagen (ICTP) and growth hormone/ insulin-like growth factor (GH-IGF) axis to acute aerobic exercise in boys at different pubertal stages.

**Methods**
60 healthy boys (group I - Tanner stage 1, n=20; group II - Tanner stages 2 and 3, n=20; group III - Tanner stages 4 and 5, n=20) exercised 30 minutes at constant load on cycle ergometer at the level of ~ 95% of their individual ventilatory threshold. Venous blood samples were obtained before, immediately after and after 30 minutes of recovery for the measurement of serum PINP, ICTP, GH, IGF-I, IGF binding protein-3 (IGFBP-3) and testosterone.

**Results**
Acute exercise did not affect significantly serum PINP, ICTP, IGF-I and IGFBP-3 concentrations in any of study groups. The rise in GH concentration during exercise was highest in group III (62.3±41.7 mU/l vs 15.5±11.4 in group I and 41.8±20.0 in group II). The increment in serum GH level during exercise was positively correlated ($r=0.64$; $p<0.001$) to basal serum testosterone concentration.

**Conclusions**
Acute exercise did not affect significantly serum markers of bone turnover, either testosterone, IGF-I or IGFBP-3 levels. GH response to exercise was directly dependent on serum testosterone concentration.

# Use of ex vivo cells to enhance bone repair

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University of Tartu, Estonia, Quattromed Cell Factory Ltd., Estonia

Modern tissue engineering methods have proposed ex vivo cell growing technique as a promising solution for treatment of bone fractures or diseases leading to the local deficiency of bone. This experiment was designed to assess the effect of ex vivo-cultured cells in ectopic bone induction model with normaly functioning connective tissue cells.

**Material and methods**
Bone marrow cells, harvested via puncture of tibial canal of male Wistar line rats, were cultured using traditional method, and differented into osteogenic lineage using chemical stimulus (ascorbic acid, beta-glycerophosphate and dexamethasone).

After differentiation osteoprogenitor cells were

**Table. Average relative volumes of bone and connective tissues and cross-charts of statistical differences between the groups**

| Group | Ratio of formed bone | 2 | 3 | 4 | 5 | 6 | Ratio of connective tissue | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0% | Ns | Ns | * | * | * | 95% | Ns | Ns | * | ** | * |
| 2 | 0% | | Ns | * | * | * | 99% | | Ns | * | * | * |
| 3 | 0% | | | * | * | * | 97% | | | * | * | * |
| 4 | 40% | | | | Ns | Ns | 53% | | | | Ns | Ns |
| 5 | 28% | | | | | Ns | 64% | | | | | Ns |
| 6 | 34% | | | | | | 58% | | | | | |

* p<0.001, **p<0.01, ns – statistically insignificant

transferred into beta-tricalcium phosphate scaffolds using either centrifugation or simple diffusion. Six types of implants (beta-tricalcium phosphate matrixes) were implanted into subcutaneous pouches. In the control-group saline-immersed implants were used; in the group 2 the ex vivo cells were transferred into the implant by diffusion and in the group 3 by centrifuging; in the groups 4, 5 and 6 the implants were processed as in first three groups, respectively, but 12.5 microgram of rhBMP-2 was added to the each implant.

After 21 days the implants were removed and dissected systematically. Hematoxylin-eosin histochemical staining was used. Histomorphometry analysis was performed following the principles of stereology.

### Results and discussion

Main results are present in Table.

Bone formation was found only in the implants where rhBMP-2 was introduced. The other implants consisted mostly of connective tissue and in lesser extent of the unchanged scaffold. However, no distinctive differences were found between the implants where rhBMP-2 only or implants where differentiated cells and rhBMP-2 were introduced. The results show clearly that osteoinduction is crucial in ectopic bone formation if there is no cellular dysfunction present. The inductive effect of rhBMP-2 cannot be compensated by the abundance of the pre-differentiated osteogenic cells as shown by the absence of bone induction in the groups 2 and 3 in this model.

## X-ray diagnosis of heterotopic ossification. Sources of the errors and amendment of the assessment system

**A. Toom, A. Arend, T. Haviko, A. Märtson**
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Heterotopic ossification (HO) is a frequent pathological phenomenon after total hip arthroplasty. Incidence of HO after the total hip arthroplasty is in average 43%. Most often classification from Brooker and co-authors has been used to assess the HO. Overall 47% of all studies published until the 1999 have used Brooker's system, but there has been reported a "fair" reproducibility (Cohen's kappa 0.5) of this system.

The aim of the present study was to estimate the influence of used classification system to the evidence of HO, to assess the reliability of the more often used classification systems, to determine the sources of errors in the assessment-process of HO and, to compose a new classification system with higher reliability.

Four investigators assessed HO in 111 patients applying most often used classification systems and the new system. Six investigators measured dimensionality of HO in 28 patients applying method of digital planimetry. Kappa statistics of all the compared classification systems were

calculated. Main sources of errors were detected by dispersion model.

Average evidence of HO differed up to 1.4 times if the different systems were applied to assess HO. Therefore, using literature data, always the criteria of a system used should be taken into consideration.

Most important source of errors in the HO assessment was the error of diagnosing process. This source consisted of two components: inter-observer variation that formed 25.5% (+/-8.0%; p=0.0015) of total error and intra-observer variation that formed 60.9% (+/-7.3%; p<0.0001). Technical performing error had less contribution in total error, namely 8,0% (+/-0.6%; p<0.0001) and subspecialisation of the investigators did not cause any systematic bias having a proportion of 5.7% (+/-4.9%; p=0.2457).

Reliability of the Brooker's system was lowest among the compared systems. Combining the Della Valle's system which had high reliability with the Brooker's system, we composed a new classification preserving high reliability. The results obtained using the new classification allow comparison with the results obtained using each of the parent classifications.

## Results of surgical treatment of C1 subluxation in rheumatoid arthritis patients

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### Introduction

The aim of the presentation is to compare different fixation techniques in patients with severe C1 subluxation with neurologic impairment.

### Material/Method

16 patients with C1 subluxation were operated during 2003-2007. 12 of them suffered from severe neck pain (group A). In 4 patients myelopathy (RANAWAT IIIA) due to the narrowing of the posterior atlanto-axial interval or invagination of C2 odontoid process was diagnosed (2 patients) (group B). All patients demonstrated high grade of rheumatoid arthritis activity. Patients with neck pain underwent C1-C2 posterior fixation with elastic cables (5 cases) and APOFIX hooks (7 cases). 4 patients with spinal cord compression underwent posterior decompression by resection of C1 arch and craniocervical fixation.

### Results

Reliefe of neck pain in the group A was achieved in 10 patients. In one patient (fixation with cables) secondary dislocation of C1 occured with following tetraplegy. One patient complained for persistance of neck pain. In the group B in 3 patients the neurologic deficit regressed. In one case the implant must be removed bacause of wound infection and after wound healing the fixation was performed again.

### Conclusions

1. Surgical treatment of rheumatoid arthritis patients with C1 subluxation provides pain reliefe and regress of neurologic deficit.
2. In patients with neck pain without neurologic deficit posterior C1-C2 fixation with hooks is safer as fixation with cables.
3. In patients with narrowing of posterior atlanto-axial interval with or without invagination of odontoid process, resection of posterior arch of C1 and craniocervical fixation is required.

# Using The Mobility™ Total Ankle System In West–Tallinn Central Hospital

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## Introduction

There are 2 surgical options for the severely affected ankle, either ankle arthrodesis or total ankle replacement.

The mobile bearing ankle concept is the latest step in total ankle replacement surgery. This is cementless, unconstrained, 3-component prosthesis.

The main indication for surgery is pain restricting the patients ability to walk – due to rheumatoidarthritis, osteoarthritis or posttraumatic arthrosis.

The contraindications are latent/prior ankle infection, excessive avascular necrosis, peripheral neuropathy (incl. diabetic), poor skin/vascular condition and severe deformity of the ankle (> 20° valgus or varus).

## Materials and methods

8 Mobility™ Total Ankle Systems have been implanted through an anterior surgical approach for osteoarthritis (5) and rheumatoid arthritis (3) patients aged from 53 and 76 years (average 61) during 2006 and 2007. Maximum follow-up period has been 1 year. The assessment of results continues using ankle evaluating-grading system, which includes preoperative and postoperative questionnaires, physical examination and radiographs (EFAS).

Postoperatively patient should wear a rigid boot or cast for 4 weeks allowing increased weight bearing as tolerated. On the fourth week postoperatively physiotherapy should start.

## Conclusions

During an ankle fusion the joint dorsal and plantar flexion is limited and development of progressive degenerative arthritis in adjacent joints is common. The total ankle replacement is an alternative method to ankle arthrodesis. In our opinion this method should be preferred for patients with polyarticular involvement.

## Results

The follow-up period has been from 3 months to 1 year.

7 patients were satisfied with the results - they had no pain and had significant improvement of ROM. Two patients had delayed wound healing ( about three weeks).We have not seen any loosening or joint instability during the follow-up. Only 1 patient suffered from medial malleolar impingement and localized osteotomy was required to relive symptoms.

# NURSENING

## ENDO Clinic: observer impressions

**M. Haavik, M. Järveots, H-K. Rääk**
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Since 2003 the nurses and physiotherapists of the Clinic of Traumatology and Orthopaedics of Tartu University Hospital have had the opportunity to attend advanced training courses in Hamburg, Germany, staying as an observer in ENDO Clinic. ENDO Clinic is a centre specialising in bone and joint surgery, where all large human joints are replaced. The number of hip, knee, shoulder, elbory and ankle prostheses implanted per year is about 5000. The Clinic can receive 250 patients at a time. It comprises the admission unit, unit of clinical physiology, surgical unit, ICU, unit of infections and general unit, as well as the x-ray unit and the blood bank and the bone bank. The keyword characterising the organisation of work at the Clinic is patient-centredness. The patient is provided with a set of services including necessary preoperative investigations and tests, surgical treatment, preparation for coping with life with an artificialjoint and, finally, upon reguest, the opportunity to continne recovery in one of the eight rehabilitation centres. Printed information is available as well. The whole activity of the Clinic is based on the notion that everybody, either a patient or a staff member, is unigue and valuable. The impression gained from ENDO Clinic is that of a well functioning whole where work is planned and the activity of team members is coordinated. EWverybody knows his/her task and fulfils it correctly. The working atmosphere is peaceful and stress-free.

## Patient guideline on wound care at home

**M. Mikksaar**
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In 2006 I passed special training in Clinical nursing for obtaining the gualification of nurse-specialist. In the tramevork of this training course I compiled a guideline for nurses which included also patient education. The guideline is evidence based a has passed reviewing. For comiling the guideline, I used various literature sources on nursing, mostly in English but also in Estonian. The guideline developed within the training course served as the basis for a patient guideline on wound care at home. Patients too have a number of questions about post-trauma or post-operative treatment and wound care. In connection with this, there arose the need for compiling a patient guideline on wound care at home, focusing on the basic issues related to home care. As an example, I used the guideline on treatment with plaster cast, compiled by nurses of the outpatient department of traumatology two years ago. The patient guideline on wound care at home contains relevant information for patients: care of the surgical wound, problems related to oedema and pain and removal of sutures, wound care after removal of sutures, as well as recommendations regarding the circumstances when a doctor should

be consulted. The guideline is convenient to use for the patient: when he/she has some problem it is not necessary to consult a specialist. The patient can use the guideline and, when needed, obtain additional information from nurses. The guideline is in Estonian and Russian. The completion of the guideline was possible owing to assistance from a number of colleagues to all of whom I would like to express my sincere gratitude.

## Estonian Nurses Association for Orthopeadics

**I. Liimand, M. Järveots**
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Estonian Nurses Association for Orthopeadics (ENAO) was created at the beginning of 2005. Association has confirmed its statues. Main activities of the Association are as follows:to represent orthopeadics nurses in Estonia and outside the country, to develop nursery in order to improve the quality of health care services, to develop nursery research in the speciality of orthopeadics, to give contribution into professional expertise exchage between members, to give importance to professional work of the Association members in our society, to co-operate with all health care associations. The Association has 86 members from 6 different hospital allover the Estonia. Members of the Association are nurses who work in the field of traumatolgy and orthopeadics, surgery nurses and fysioterapeutics. Representatives of other specialities can olso enter into the Association and get member status. Two leading hospitals The North Estonian Hospital and Tartu University Hospital are the majority among our members. In order to implement Association objectives the development plan for the period 2006-2010 has been workd out. According to the development plan our priority actions are: start to develop worldwide contact network with similar organisations, to increase qualifications, evidence-based nursery implementation, to share expertise and practical skills with colleagues publishing articles and participating in different training programmes, to demonstrate our activities through website www.eoos.ee. As a result of the work of Association the members has got good chances to make presentations at different conferences and publish articles in leading Estonian scientific editions and make poster presentations of practical work. Association co-operate very closely with Estonian Traumatology and Orthopaedics Association and Estonian Nurses Union. We wish to create contact network with similar associations in Baltic States.