J Musculoskel Neuron Interact 2004; 4(2):209-226

Hylonome

Abstracts from the 4th International Workshop on Musculoskeletal and Neuronal Interactions

May 28th - 31st, 2004, Chalkidiki, Greece

Program Chairmen: J.A. Gasser, B. Noble and S. Miller

The presentation of the Workshop on Musculoskeletal and Neuronal Interactions abstracts in two parts: Oral Presentations (OR) and Posters (P).

OR-01

ENOS IS REQUIRED FOR MECHANICALLY-INDUCED BONE FORMATION

Chow JWM, Angotzi AR, Jagger CJ, Chambers TJ
Dept. of Cellular Pathology, St George's Hospital Medical School, London, UK

Introduction: Nitric oxide (NO) is increasingly recognised to play a role in local regulation of bone metabolism and more recently, endothelial nitric oxide synthase (eNOS) gene knockout mice have been found to have impaired bone formation and reduced bone mass. Since the osteogenic response to mechanical stimulation depends on NO, it is possible that the low bone mass in eNOS knockout mice is due to failure of bone to respond to mechanical stimulation in the absence of NO. We tested this hypothesis by studying the bone formation response to mechanical stimulation in these animals.

Materials and methods: We subjected the 8th caudal vertebrae of 16-week-old female C57Bl mice to a single episode of dynamic mechanical loading and measured the bone formation rate thereafter. We then analyzed the mechanical responsiveness in eNOS knockout mice. Wild type (+/+) and homozygote (-/-) eNOS knockout mice (age 16 weeks) were subjected to a single episode of mechanical loading of the 8th caudal vertebrae comprising 100 cycles (1 Hz) using 30N

Results: To validate the model, we found a dose-responsive increase in the cancellous bone formation rate of the 8th caudal vertebra with increasing load magnitude (3N, 10N, 30N) and with increase in number of cycles of loading (30, 100, 300) in C57Bl mice. The maximum load (30N) was calculated to produce 700 microstrain in the caudal vertebrae of these mice. In experiments using wild type (+/+) and homozygote (-/-) eNOS knockouts, we found a 2-fold increase in the bone formation rate of mechanically-loaded 8th caudal vertebrae in eNOS +/+ animals compared with the 6th or 8th caudal vertebrae of non-loaded +/+ animals. While there was no significant difference between the bone formation rate of the non-loaded 6th caudal vertebrae of eNOS +/+ and -/- animals, we found absence of an osteogenic response to mechanical stimulation in the 8th caudal vertebrae of eNOS -/- animals.

Conclusion: Our results show that NO generated by eNOS is required for mechanical responsiveness in mice. The key role that eNOS and NO play in bone formation provides opportunities for novel approaches in the treatment of osteoporosis.

OR-02

THE ROLE OF FAS/CD95 IN OSTEOCYTE DEATH IN BONE

Kogianni G, Mann V, Stevens H, Noble BS
Musculoskeletal Research Unit, University of Edinburgh Medical School, Teviot Place, Edinburgh, UK

Osteocyte apoptosis has been described in both healthy and pathological bone in particular following mechanically-induced microdamage prior to bone resorption, in response to estrogen loss, chronic administration of glucocorticoids and during growth-related modeling of bone. Activation of the Fas death receptor pathway induces apoptosis in several non-bone cell systems both in response to glucocorticoid administration and mechanical loading. Here we investigate the Fas/Fas Ligand (Fas-L) system in osteocytes using *in vivo* and *ex vivo* rat models and *in vitro* MLO-Y4 osteocyte-like cultures, in response to a variety of stimuli, including Fas-L at 6.25-100ng/ml, H_2O_2 at 0.4mM, Dexamethasone at 10^{-6} M and reduced mechanical loading. Apoptosis was determined using Nick Translation, Acridine Orange and Annexin V staining.

RT-PCR studies demonstrated the presence of Fas mRNA in MLO-Y4 osteocytes, while immunohistochemistry revealed the presence of Fas protein in osteocytes and osteoblasts *in situ* rat bone and MLO-Y4 cultures. In contrast to healthy MLO-Y4 cultures, expression of Fas was low in healthy rat calvarial osteocytes. Receptor functionality was demonstrated by Fas-L induced apoptosis of MLO-Y4 osteocytes ($p=0.016$ compared to control) and calvarial osteocytes *in situ* ($p<0.001$ compared to control). Fas expression (%positive cells) was induced in MLO-Y4 cells by 20-fold during H_2O_2 and Dex engendered apoptosis and up to 9-fold in calvarial osteocytes dose dependently after addition of Fas-L and during culture-induced nutrient depletion. In addition, *in vivo* loading of rat ulnae resulted in 30% depression of Fas expression in osteocytes at the mid-shaft, which was associated with reduced osteocyte apoptosis and resorption. Local production of Fas-L was found to occur in calvarial osteoblasts but not osteocytes in healthy bone. Upon nutrient depletion both calvarial osteoblasts and osteocytes were shown to produce Fas-L while by contrast, PCR and immunocytochemical studies demonstrated a lack of Fas-L production by the MLO-Y4 cell line. We have shown that the Fas/Fas-L system is present, functional and inducible in osteocytes in response to a variety of stimuli. These data point to the importance of the Fas system in the maintenance of the osteocyte population in bone. This information might lead to the development of novel compounds that could control osteocyte viability and targeted resorption activity in bone.

OR-03

THE OSTEOLYTIC RESPONSE RESULTING FROM THE INOCULATION OF ^{4T1}luc 2000 MOUSE MAMMARY CARCINOMA CELLS INTO THE TIBIA OF ATHYMIC NUDE MICE IS COMPLETELY BLOCKED BY ZOLEDRONIC ACID

Gasser JA, Stumm M, Ingold P, Wenger F, Tinetto W, Martinuzzi-Duboc L, Grosios K
Novartis Institutes for Biomedical Research, Basel, Switzerland

Bone is a favored location for several cancer metastases, especially breast, prostate, kidney and myeloma. Bony pathology in cancer patients represents a significant source of morbidity and mortality. In this report, we have examined

the ability of the ^{4T1}luc 2000 mouse mammary carcinoma to colonize the tibia of athymic nude mice and the effects of treatment with zoledronic acid (ZA) (0.1mg/kg, s.c., 2x / week). 1x105 cells were injected into the tibia of mice. Cell growth was followed by means of IVIS Xenogen imaging. The osteolytic response was monitored non-invasively at baseline and after 3 weeks by peripheral quantitative computed tomography (STRATEC pQCT, XCT Research SA+) and visualized by *in vivo* micro computed tomography (SCANCO MicroCT, VivaCT40). In addition, DEXA measurements were performed *ex vivo* on excised tibias (Hologic QDR1000). *Ex vivo* measurements of BMD in the proximal tibia by the DEXA indicated a 27.7% ($p<0.05$) decrease as a result of osteolytic activity of the tumor, which was completely prevented in the ZA-treated animals, where BMD actionally increased to 7% above the level of the control group. pQCT measurements carried out at a distance of 3 mm from the proximal end of the tibia indicated that the bisphosphonate completely blocked the decrease in bone mineral density of 5.5% observed in vehicle–treated animals ($p<0.01$). MicroCT images clearly showed that in vehicle-treated mice, osteolysis occurred in the cancellous and cortical compartment and in many cases the tumor broke through the cortical shell invading the tibia-fibular space. Bi-weekly s.c.-administration of ZA did not only protect animals from cancellous and cortical bone erosion but prevented the tumor to break through the cortical shell in all 6 mice. In contrast to the impressive anti-osteolytic effect of ZA, no effect of the bisphosphonate on ^{4T1}luc 2000 tumor growth was observed. Data suggest that ZA is highly effective in preventing tumor osteolysis occurring after inoculation of ^{4T1}luc 2000 mouse mammary carcinoma cells into the proximal tibia metaphysis of athymic nude mice and should ideally be combined with an anti-cancer drug acting directly on tumor growth. *In vivo* microCT measurements now allow for the first time to visualize tumor growth and the anti-osteolytic effect of therapies non-invasively.

OR-04

Mechanical Factors in the Emergence of Cartilage Morphology

Eckstein F[1,2], Gratzke C[1], Grams A[1], Siedek V[1], Hudelmaier M[1,2]
[1]Musculoskeletal Research Group, Institute of Anatomy, LMU München, Germany
[2]Institute of Anatomy, Paracelsus Private Medical University, Salzburg, Austria

Introduction: Healthy articular cartilage has the ability to transfer enormous loads across synovial articulations without suffering damage. It is tempting to hypothesize that, as in other tissues with mechanical function, the emergence and maintenance of articular cartilage morphology is guided by a process of functional adaptation to mechanical stimuli and is not fully pre-programmed in the genome.

Materials and methods: We studied cartilage morphology in quadrupeds and chimpanzees with different body weights (2.5 kg rabbit to 1200 kg rhinoceros) and 125 normal healthy subjects, 14 top athletes (weightlifters and bobsled sprinters) versus 14 non-athletic volunteers, 13 monozygotic twin pairs, and in one patient in which the knee had been transplanted to the site of the knee 5 years earlier because of a tibial osteosarcoma (van Nees rotation plasty).

Results: We show that humans possess the greatest known knee joint cartilage thickness among species, even when compared with quadrupeds 15 times heavier. We observed a relatively constant ratio between body weight and joint surface area across species, but not between body weight and cartilage thickness. There was no relevant difference in cartilage morphology between top athletes and non-athletic volunteers, despite the 30% higher extensor forces and muscle cross sectional areas in weight lifters and bob-slade sprinters. There was, however, a remarkable similarity amongst monozygotic twins. After amputation, transplantation of the ankle to the knee site (in which the cartilage is commonly thicker), no increase in cartilage thickness was seen in comparison with the contralateral ankle

Conclusions: These findings suggest that differences in cartilage form between species, subjects, and anatomical sites are not explained by differences in mechanical stimulation. Contrary to other tissues with mechanical function, articular cartilage morphology and competence appears to be determined by specific genetic programs, and not to have the ability to adapt to changes in the mechanical environment in adolescence (where enchondral ossification occurs) and adulthood.

OR-05

DEXA Assessment of Muscle-Bone Relationships in Humans - III. Application Study: Differential Diagnosis Between 'Systemic' and 'Mechanical' Osteopenias in Fractured Pre- and Post-Menopausal Women

Capozza RF[1], Cointry GR[1], Cure-Ramírez P[2], Ferretti JL[1], Cure-Cure C[2]
[1]CEMFoC, Natl Univ of Rosario, Argentina
[2]Metropolitan Univ of Barranquilla, Colombia

DEXA could analyze the bone/muscle (BMC/lean mass, LM) proportionality and distinguish between osteopenias of "mechanical" (disuse) and "systemic" (primary or secondary) etiologies based on z-scores of the corresponding correlations. To validate this hypothesis we compared whole-body and lower-limb (WB, LL) data of 623 pre- and post-MP Hispanic women fractured in specific locations (hip, spine, wrist, arm, leg; Type-I Fx, n=396) or other sites (Type-II Fx; n=227). Individual z-scores for the BMC-vs.-LM correlations were calculated with respect to reference BMC-vs.-LM curves previously determined in 814 pre- and 1,656 post-MP healthy Hispanic women of comparable ages. The BMC-LM z-scores were similar to controls in the whole Type-II-Fx group and significantly lower than that in the Type-I-Fx group. The BMC-vs.-LM curves for pre-MP women with Type-I or Type-II Fx and for post-MP women with Type-II Fx were linear and similar to controls in WB and LL, showing a high prevalence of normal BMC-LM z-scores. Instead, post-MP women with Type-I Fx showed non-linear BMC-vs.-LM relationships, with BMC and BMC-LM z-scores rapidly decreasing toward low LM values. Variance of the data was lower in LL than in WB. This indicates that 1. 'systemic' osteopenia (low BMC-LM z-scores) predominated in women with Type-I Fx while 'mechanical' osteopenia (normal BMC-LM z-scores) did in those with Type-II Fx; 2. Post-MP women had more fractures associated to 'systemic' than 'mechanical' osteopenias compared with pre-MP women, in which the former were practically absent; 3. in post-MP women, "systemic" etiology tended to predominate as LM decreased, perhaps because the lack of estrogen reduced the sensitivity of bone cells to mechanical stimuli, and 4. determinations in LL are more reliable than those in WB for this purpose. Results show that DEXA could differentiate osteopenias with different treatments (physical intervention in 'mechanical' cases, pharmacological resources in 'systemic' cases), thus allowing monitoring therapeutic effects according to biomechanical criteria with no additional costs over the standard WB determinations. Nevertheless, the BMC/LM proportionality can not distinguish by itself between individuals more or less exposed to fractures in any instance. Neither DEXA could be used as a single resource to diagnose osteoporoses (defined as an 'osteopenic fragility' by the NIH).

OR-06

The Effect of Mechanical Stimulation on Osteocyte Viability in Bone

Mann V[1], Kratz M[2], Jones DB[2], Noble BS[1]
[1]The Scottish Mechanotransduction Consortium, University of Edinburgh Medical School, Edinburgh, UK
[2]Dept. Experimental Orthopaedics and Biomechanics, Philipps University, Marburg, Germany

Physical activity is a key determinant of bone mass and health. It has been proposed that it contributes to the viability of bone cells through enhanced supply of nutrients and oxygen via the osteocyte lacuna/canilicular system. Physical activity is required for the full perfusion of cortical bone and during sustained unloading *in vivo* osteocytes become hypoxic and may die. We have studied the effect of chronic mechanical stimulation on osteocyte viability in bovine trabecular bone maintained in a bioreactor.

The Zetos™ bioreactor is capable of maintaining bone biopsies *ex vivo* for up to 45 days. Trabecular bone samples from the proximal head of bovine femurs where prepared and transferred to bioreactor chambers. Samples were loaded for 5 minutes a day using either a walking (3000 μstrain), jumping (3000 μstrain) or control (no load) waveform repeated at 1 Hz for 28 days. Hydrogen peroxide treated negative control groups where

loaded in a similar mode. After the experimental period total cell numbers and cell viability were determined in unfixed cryostat sections by nuclear staining (DAPI) and lactate dehydrogenase activity (LDH), respectively. Cells where categorized as residing up to 1.5mm (outer zone) or further than 1.5mm (inner Zone) from the biopsy surface perimeter. Osteocyte numbers where not significantly different between treatments and controls in any zone indicating a lack of clearance of dead cells from the bone. Osteocyte viability in control samples was lower in the outer zone than the inner zone. In the inner zone a higher percentage of live cells was observed in the walking (46.49 ± 8.9 SE n=3, p=0.05) and jumping (54 ± 4.2 SE, p= 0.006) samples compared to control (28.14 ± 8.3 SE). In the outer (less viable) zone jumping but not walking stimulation had a dramatic positive effect on osteocyte viability such that it reached similar levels to those in the more healthy inner zone (15.5 % control vs. 50% jumping).

These data indicate that in this model system, mechanical stimuli are capable of maintaining osteocyte viability. In addition, they would suggest that vigorous mechanical stimulation is capable of cell rescue from the death associated with drilling trauma at the biopsy surface.

OR-07

A Selective Estrogen Receptor Modulator (SERM) Inhibits Osteocytes Apoptosis During Estrogen Loss Implications for Bone Quality Maintenance

Collishaw S[1], Mosley J[1], Taylor C[1], Reeve J[2], Noble BS[1]
[1]Edinburgh University, Edinburgh, UK
[2]Cambridge University, Cambridge, UK

Ideally, Selective Estrogen Receptor Modulators should demonstrate all the positive bone-associated effects of estrogens without their soft tissue side effects. While SERMs are known to alter bone formation and resorption in favour of bone mass, their full range of activities in bone are unknown. Estrogens are associated with positive effects not only on the quantity of bone, but also its quality including the maintenance of osteocyte populations through the inhibition of their apoptosis. Here we have used a rat model of ovariectomy (OVX) to determine whether the osteocyte sparing effect of estradiol can be mimicked by the SERM LY117018. Sixteen 12-week-old rats (weight: 263) were divided into 4 treatments groups: sham operated (SHAM) (n=4), OVX (n=4), and OVX + 17b-estradoil (E2) (0.125mg/kg/day) (n=4), OVX + SERM (3mg/kg/day) (n=4). After 7 days treatment, radius and ulna were removed and the percentage of aopototic osteocytes determined using an *in situ* nick-translation method. The success of ovariectomy was assessed by measurement of uterine weight. The proportion of apoptotic osteocytes present was five times higher in the OVX compared with the SHAM groups in both radius (1.09% vs. 0.21%, respectively; p<0.01) and ulnae (1.40% vs. 0.36%, respectively; p=0.001). Addition of estradiol to the OVX animals completely abrogated the increase in osteocyte apoptosis in both the radius (0.38%) and ulna (0.26%). Addition of the SERM to the OVX animals abrogated the increase in apoptosis to the same extent as estradiol in both the radius (0.35%) and ulna (0.38%). Nuclear morphology and electrophoresis of DNA confirmed the presence of apoptotic cells in the samples. In conclusion, estradiol and SERM are equally good at preventing the increase in osteocyte apoptosis engendered by OVX. These data point to the potential benefits of SERMs in the maintenance of both the quantity and the quality of bone in the postmenopausal individual. We will discuss the relevance of these data to our understanding of SERM function and the design of future disease intervention strategies.

OR-08

The Antioxidant Effects of Estrogen and Serms in the Protection of Osteocytes From Oxidative Stress-Induced Cell Death

Huber C, Mann V, Kogianni G, Towell C, Noble BS
The Scottish Mechanotransduction Consortium, University of Edinburgh Medical School, UK

Withdrawal of estrogen promotes postmenopausal bone loss and is associated with the apoptotic death of osteocytes. Selective Estrogen Receptor Modulators (SERMs) are a class of non-steroidal compounds with estrogen agonist and antagonist effects in a number of target tissues. They demonstrate classic estrogen-like bone sparing effects and in addition have been shown to prevent ovariectomy-induced osteocyte apoptosis *in vivo* (see Abstract Collishaw *et al.* SERMs Inhibit Osteocyte Apoptosis in Estrogen Loss). Our recent *in vitro* studies have suggested that estrogens' ability to save osteocytes from oxidant-induced death occurs independently of the estrogens' receptor and may be related to estrogens known activity as an antioxidant. In this study we have sought to determine whether SERMs are capable of saving osteocytes from oxidant-induced death through a non-receptor mediated route. Treatment of the osteocyte-like cell line MLO-Y4 with H_2O_2 induced apoptotic cell death (22.4% ± 2.1 SD vs. control 3% ± 1.3 SD) which was inhibited after pre-treatment with 17-β-estradiol (4.35 ± 1.7 SD) at near physiological concentrations (10nM). The saving effects of 17-β-estradiol were shown to be receptor independent since pre-treatment of cells with estrogen receptor antagonist ICI 182,780 did not block the estradiol inhibition of apoptosis. Pre-treatment of cells with raloxifene and LY 117018 also significantly reduced oxidant-induced apoptosis to 5% ± 0.5 SD p=0.00015 and 4.2% ± 0.5 SD p=0.00013 respectively. The saving effect of the SERMs was shown to be receptor independent since pre-treatment with ICI 182,780 prior to the addition of the SERMs did not block the effect.

Using the free radical indicator 2'7'- dichlorodihydrofluorescein diacetate we have shown that 17-β-estradiol, raloxifene and LY 117018 all significantly reduce (p<0.0002) the number of reactive oxygen species positive cells suggesting that these molecules poses antioxidant activity. It is possible that loss of osteocytes during estrogen insufficiency may occur through a failure to suppress the activity of naturally-occurring or disease associated oxidant molecules. In addition, these data suggest that estrogen and SERMs ability to save osteocytes from oxidant-induced death might occur independently of estrogen receptor activation pointing to novel design criteria for pharmacological interventions.

OR-09

A Trophic Anti-Glucocorticoid Action of HE2200

Orlinska U[1], Miller SC[2], Pappas BA[3], Reading CL[1]
[1]HollisEden Pharmaceuticals, Inc., San Diego, CA
[2]Univ. of Utah, Salt Lake City, UT, USA
[3]Carleton Univ., Ottawa, Canada

Cognition and immune competence are both complex functions that can be acutely impaired following trauma-induced elevations in endogenous glucocorticoid (GC) levels. Here we show that the steroid compound HE2200 (5-androstene-3b,7b,17b-triol or bAET) appears to preserve these vital functions by exerting a trophic or anabolic activity. In an animal model of acute cerebral ischemic stroke consecutive to bilateral carotid occlusion in gerbils, bAET treatment significantly improved cognitive abilities when compared to stroke alone (p=0.03). Thus, the measured food-searching latency period in each group was 6.9 ± 0.9 sec for sham, 46.9 ± 13.6 sec for stroke alone and 14.8 ± 4.8 sec for stroke treated with bAET. Concomitantly, the stroke-induced loss in CA1 hippocampal neuron count was markedly abrogated by bAET (sham = 362,247 ± 6,839; stroke = 152,354 ± 11,575; and stroke + bAET = 207,854 ± 47,334). In bone, bAET seemed to act upon the principal bone structures, i.e., cortical and trabecular layers, and growth plate. In thermally-injured mice (20% total body surface area) treated with bAET, loss of cortical (femur) and trabecular/cancellous (tibia) bone mass, as well as suppression of chondrocyte proliferation in proximal tibial epiphyseal growth plate, were all significantly (p<0.01) prevented by bAET. Histomorphometry of the femur cortical bone suggested an increase in bone formation rate. We observed partial protection against loss of bone mineral content as measured by dual X-ray absorptiometry. The femur ash weight was significantly (p<0.01) greater than that in the vehicle-treated burned mice, suggesting that bAET preserves bone mineral content. Recent studies (Dinkel K et al., J Neurochem 2003; 84:705) demonstrated pro-inflammatory effects of chronically high GC levels in brain, suggesting that GC may worsen the outcome of neurological insults. The adrenal steroid DHEA (5-androstene-3b-hydroxy-17one), an upstream metabolic precursor of bAET, has been demonstrated to prevent dexamethasone-induced thymic involution in mice (Blauer KL et al., Endocrinology, 1991; 129:3174). Taken together, these findings suggest that

bAET may suppress GC-induced loss of functional tissue, thereby preserving bone structure upon thermal injury or improving cognition following stroke through a possible anti-glucocorticoid mechanism.

OR-10

The Novel *In Vivo* Microct in Combination With pQCT is a Powerful Tool to Monitor Inflammation-Induced Bony Lesions in a Rat Model of Adjuvant Arthritis

Gasser JA[1], Ingold P[1], Hiestand P[1], Koller B[2]
[1]Novartis Institutes for Biomedical Research, Basel, Switzerland
[2]SCANCO Medical, Bassersdorf, Switzerland

Rheumatoid arthritis is a systemic chronic inflammatory disease, the most important and most debilitating aspect of which is the erosive, inflammatory joint disease. The etiology of the disease is unknown, but may be due to a combination of genetic and environmental factors including infectious agents. The cellular pathology of the disease is characterized by synovitis followed by the formation of fibrous pannus. The latter forms at the junction of cartilage and synovium and is responsible for the marginal erosion of cartilage and subchondral bone which ultimately destroys the normal joint architecture. Adjuvant arthritis was induced in Lewis rats by intra dermal injection of a suspension of 6 mg mycobacterium tuberculosis H37 RA. Arthritic lesions developed in the tarsal and metatarsal joints 12 – 15 days later. Ten rats did not receive the antigen and served as healthy controls to monitor age-related changes. Antigen inoculated rats were treated with 0.5mg/kg dexamethasone qd or vehicle (1% CMC qd by gavage). Joint swelling was monitored at baseline, day 14 and day 21 by a micro caliper along with *in vivo* measurements of the cancellous bone structures on the SCANCO vivaCT 40 and of bone mass and cortical geometry on the STRATEC pQCT XCT960A. Inflammation-induced bone loss was significant at 2 weeks after induction of adjuvant arthritis in vehicle treated controls. pQCT-measurements indicated a decrease in Cs.BMD of 3.8% ($p<0.01$), of Cn.BMD of 2.3% ($p<0.01$) and a decrease in Ct.Th. of 7.8% ($p<0.01$). Cortical thinning resulted from endocortical resorption as indicated by the increase in Ec.Pm. of 10.8% ($p<0.01$) but animals also showed some periosteal woven bone formation of 2.2%. No significant changes in volumetric cortical BMD were seen. MicroCT-measurements carried out *in vivo* indicate a 13.7% drop in the trabecular bone volume ($p<0.01$ vs. negative control) which resulted entirely from trabecular thinning (-6.3%, $p<0.01$ vs. negative control) without any change in the number of trabecular elements (+1.0%, ns). All detrimental effects of adjuvant arthritis were fully prevented by dexamethasone. The drop in Cs.BMD (-3.8%) observed in the vehicle control was reverted into a bone gain of 1.5% in dexamethasone-treated rats ($p<0.01$). Dexamethasone fully blocked cortical thinning from -7.8% to result in an increase in Ct.Th. of 2.2% ($p<0.01$), which is close to the age-related gain of healthy control animals (+2.6%). The most striking effect with dexamethasone was seen at the endocortical surface, where the increase in Ec.Pm. of 10.8% was turned into a bone gain (Ec.Pm. -2.6%, $p<0.01$ vs positive control). At the Ps.Pm., no significant change was observed under dexamethasone treatment but cortical BMD increased ($p<0.05$ vs. positive control). Taken together, dexamethasone prevents the inflammation-induced periosteal woven bone formation and fully blocks endocortical as well as cancellous bone resorption. A combination of *in vivo* micro CT and pQCT are powerful tools to describe architectural changes over time and to image inflammation induced bony erosions in arthritic joints.

OR-11

Tomographic and Mechanical Evaluation of Muscle-Bone Interactions in Mice Artificially Selected for Body Conformation

Cointry GR[1], Di Masso RJ[2], Capozza RF[1], Ferretti JL[1], Font MT[2]
[1]Ctr for P-Ca Metab Studies (CEMFoC)
[2]Expl Genetics Inst (IGE), Natl Univ of Rosario, Argentina

Antagonistic artificial selection of adult male and female mice with wide variation in body conformation produced animals with light body/long skeleton (CBi/L) or heavy body/short skeleton (CBi/C) from a parental line CBi. On changing the natural proportions between body and skeletal size/shape, this procedure provided an interesting model for analyzing correlations between the body and gastrocnemius weight and indicators of material, geometric and mechanical properties of cortical bone of femur diaphyses (as assessed by pQCT and bending tests) avoiding the natural, allometric associations which normally blunt the biomechanical interrelationships between muscles and bones. As expected, the selection procedure determined a wide inter-strain variation of the natural proportions between gastrocnemius mass, body weight (bw) and femur length, and between femur length and diaphyseal cross-sectional moment of inertia (CSMI). Analyzing all the animals as a whole, it was observed that a. the CSMI correlated closer with gastrocnemius weight than it did with bw; b. diaphyseal strength correlated significantly with CSMI, gastrocnemius weight and bw, and c. correlation of CSMI with gastrocnemius weight was closer than with bw and was the only graph describing the studied association as a single (linear) function for all the 3 strains as a single group. Results suggest that 1. muscle mass would not depend alometrically on bw in any circumstance; 2. geometric proportions between long-bone length and cross-sectional properties would not be independent determinants of bone structure or strength; 3. muscle development would not depend on bone development; 4. the diaphyseal design would be adapted to muscle ability to directionally deform the skeleton rather than to the weight of the supported biomass, and 5. the biomechanical adaptation of bone strength to customary mechanical usage as allowed by the biochemical and microstructural constitution of the skeleton would be determined more closely by the dynamic influence of muscle contractions than by the static, gravitational load of the bw. Those relationships, difficult to assess in natural conditions, are crucial for interpreting the biomechanical homeostasis of the skeletal structure and the etiopathogenesis of all osteopenias and osteoporoses. This knowledge could be extrapolated to the pathogenetic analysis of many human bone-weakening diseases.

OR-12

Necessity of Immune Recognition and Control of a Human Organ Stem Cell Circulating in Blood as a Monocyte, in Order to Avoid Proliferating Diseases

Labat ML[1], Mouthon G[2], Pouchelet M[3], Gouhier N[3], Escaig F[2], Boireau P[1]
[1]UMR 956 Ecole Nationale Veterinaire d'Alfort (ENVA)
[2]France; Chimie et Physique Biologiques et Medicales, ENVA,
[3]Audiovisuel, INSERM, Le Vesinet, France

Aim: Study of the regulation of an organ stem cell present in blood. Dysregulation of stem cells has been reported to contribute to neoplasia. Organ stem cells present in blood might represent one single population of stem cells in homeostatic equilibrium with the reserve stem cells present in the different organs. These circulating stem cells are normally almost quiescent. Under precise circumstances such as wound healing, they may proliferate and migrate in order to participate in repair of the damaged tissue. Indeed, such a powerful cell has to be tightly controlled.

Methods: The organ stem cells are characterized by immunofluorescence and their destruction by phage T lymphocytes is studied by time-lapse microcinematography.

Results: Organ stem cells circulate under the appearance of monocytes. However, spontaneous expression of neuronal markers including nestin, suggest a neural crest origin. Time-lapse microcinematography shows how a subpopulation of CD4+ T lymphocytes, called phagic T lymphocytes, destroy the organ stem cells as soon as they start differentiating *in vitro*. These stem cells that constitutively express HLA-DR molecules are both the activators and the targets of phagic T lymphocytes that penetrate and circulate inside them until the stem cells explode. This mode of killing differs from necrosis and apoptosis. It is a beneficial exception to self-tolerance, restricted to normal organ stem cells, in order to avoid their accumulation out of a repair purpose. In disorders such as fibrosis and chondrosarcoma, these circulating stem cells proliferate indefinitely, escape destruction by phagic T lymphocytes and as a result accumulate, giving rise to a tissue that evokes the lesion of the patient. This process observed *in vitro* may mimic what happens *in vivo* at the capillary-tissue interface.

Conclusions: Failure in the regulation of circulating stem cells might be

involved in the common early steps leading to fibrosis and malignancy such as chondrosarcoma. Any attempt to stop the proliferation of organ stem-cells present in blood and to activate phagic T lymphocytes might be beneficial in terms of cancer prevention.

Acknowledgment: This work was supported by Foundation Jerome LEJEUNE

OR-13

Spatial Distribution of Bone Strength, Cancellous and Cortical Bone Parameters Along the Length of the Radius in Osteoporosis and Rheumatoid Arthritis in Comparison to Normals. A Mathematical Model Multislice Analysis as Assessed by pQCT

Raptou P[1], Dimitriou P[2], Skarantavos Gr[1], Koulouris I[1], Trovas G[1], Galanos [A], Lyritis GP[1]
[1]Laboratory for the Research of Musculoskeletal System, University of Athens, KAT Hospital
[2]1st Department of Radiology, University of Athens, Greece

Introduction: The purpose of this study was to assess a mathematical description of cancellous and cortical bone turnover as well as of strength strain index in a proximal direction along the radius in normals and how this model is differentiated in the presence of osteoporosis and of rheumatoid arthritis.

Materials and methods: The study population consisted of 52 healthy (NO) females aged 43-60 yrs, (mean:52 yrs), 55 postmenopausal (5 yrs) osteoporotic (OP) females (t-score $_{L1-L4}$ ≥-2.5) aged 43-60 yrs (mean: 51.3 yrs) and 42 females suffered from rheumatoid arthritis (RA, ARA criteria) aged 40-62 yrs (mean: 52 yrs). All patients were measured in a pQCT XCT-960 Stratec machine at the non-dominant forearm, taking multislice data at the distal radius site (7 symmetric slices from 4% to 10%) and one proximally adjacent slice at 30%. Analysing each slice, volumetric BMD (total, trabecular, subcortical, cortical) and corresponding BM Content (mg) and Cross-Sectional Area (mm^2) of each bone section were estimated as well as cortical thickness (mm) and Stress/Strain Index in torsion ($SSIp:mm^3$). Data statistical analyses were performed using SPSS software package (version 8). General Linear Model (GLM) Repeated Measurements was applied by using two factors: 1) patient group and 2) slice along radius. For multiple comparisons between groups, tests Tukey were used. For the description of bone parameter spatial differentiation along radius, regression curve estimation used and the best fitted function was considered ($r^2>0.9$). Throughout, $p<0.05$ was considered significant. Results: Concerning all groups and moving proximally along the radius, total BMD increases linearly with the exponential logarithm of the slice location ($y=alnx+b$, $r^2=0.99$), whereas trabecular BMD at the distal radius decreases linearly ($y=-ax+b$, $r^2=0.95$). Even the values of BMDtot in OP and RA groups are significantly decreased from the normals (NO) at all sites, the rate of BMD increment (ΔBMD/mm) decreases exponentially moving proximally, significantly faster in normals than in OP and RA. Concerning only BMDtrab, that of the OP group decreases significantly faster from ultradistal to proximal direction in comparison to normals ($p=0.01$) and even more in comparison to RA ($p=0.000$). Bone strength index in torsion (SSIp) decreases linearly from UD to proximal direction ($r^2>0.92$) at all groups and concerning OP group, is significantly decreased in comparison to normals until site 8% and beyond site 7%, it becomes significantly decreased from RA. The difference between SSI_{ud}-SSI_{shaft} is significantly higher ($p=0.05$) in normals than in RA and OP groups.

Conclusions: In postmenopausal osteoporosis (OP), rapid decrease of cancellous bone mass occurs from UD site to proximal direction, accompanied by endocortical porosity (subcortical decrease) and subsequently thinning of the cortex, without significant change of total bone area. In the case of rheumatoid arthritis (RA), significant expansion of total bone area occurs with cancellous bone density being decreased at each site, because of geometrical changes at distal radius. This increment of radius moment of inertia going proximally, decreases the difference between SSI_{ud}-SSI_{shaft} and seems to be a defense mechanism having the time to "protect" radius from fracture at the weakest site (3 cm proximally from articular surface).

OR-14

Image Analysis of Radiographs of Cadaver Proximal Femora Yield Values for Trabecular Structural Parameters That Correlate Well With 3-D μCT Measurements and With Measurements of Bone Strength

Arnaud C[1], Liew S-W[1], Steines D[1], Voracek RV[1], Nazarian A[2], Muller R[3], Linder B[1], Lang P[1]
[1]Imaging Therapeutics, San Mateo, CA, USA
[2]Orthopedic Biomechanics Laboratory, Harvard Medical School, Boston MA, USA
[3]Swiss Federal Institute of Technology, Zurich, Switzerland

Bone mineral density measurements (BMD) successfully identify subjects at risk for fracture and can help physicians select those individuals who will derive greatest benefit from therapy. However, overlap exists in the distribution of BMD of patients with and without osteoporotic fracture and, BMD does not accurately predict the *presence* of fractures. Thus factors other than BMD contribute to fracture risk. Key among those factors are alterations and disruptions of trabecular structure. We developed automated hip radiographic imaging technology that measures all of the trabecular parameters used in bone histomorphometry. In general it involves X-ray digitization, identification of regions of interest, trabecular extraction, background subtraction to obtain an image of trabecular structures and binarization and skeletonization of those structures. Parameters of the geometry and connectivity of trabecular structure are measured using algorithms. 2-D measurements of trabecular structure in ordinary radiographs of cores of proximal cadaveric femora correlated with similar measurements we made with 3-D μCT. Those 2-D measurements also correlated with biomechanical failure loads applied to cores and with bone stiffness. We also determined if 2-D measurements of bone structure could be measured in radiographs of whole proximal cadaveric femora. BMD values and radiographs were obtained from the proximal region of fifteen intact cadaveric femora. They were tested biomechanically at 15 degrees of tilt and 8 degrees of external rotation to generate a load vector at the femoral head simulating single-legged stance (Cheal et al. 1992) with an Instron instrument. Radiographs were analysed to yield indices of trabecular structure and macrostructural indices (such as cortical width). There was a weak positive correlation of BMD with intact femoral neck failure load ($r=0.34$). Intact femoral neck failure load was more highly correlated with 2-D indices of proximal femoral trabecular structure such as trabecular area ratio, interconnectivity index, trabecular length, trabecular thickness, normalized number of nodes and normalized number of end nodes. Multivariate linear regression analyses of combined structural and macro-anatomical parameters provided estimates of predicted fracture loads that correlated highly ($r=0.81$) with actual fracture load. We conclude that 2-D measurements of bone structure derived from radiographs of cadaver proximal femoral bone cores correlate highly with corresponding 3-D μCT measurements of those cores. Furthermore, those same 2-D measurements correlate well with biomechanical failure loads of whole cadaver proximal femora. These results suggest that inexpensive and non-invasive proximal femoral trabecular micro-structural analysis from hip radiographs may yield improved diagnostic assessment of osteoporosis and estimation of fracture risk as compared with BMD.

OR-15

Impact of Eccentric Exercice on Areal BMD in Female Runners

Wulff Helge E, Kanstrup EIL
Institute of Exercise & Sport Sciences, University of Copenhagen, Denmark and Department of Clinical Physiology & Nuclear Medicine, Herlev Hospital, Denmark

From animal studies it is well known that strain rate and magnitude resulting from muscle contractions are important stimuli in mechanotransduction leading to bone formation. It is also known that force development is higher in maximal eccentric than in maximal concentric contractions.

Purpose: Thus, we wanted to investigate the impact of eccentric training on areal bone mineral density (aBMD). The main hypothesis was that seven months of maximal eccentric exercise three times a week would change muscle fibre composition and increase aBMD. Secondly, we hypothesized to find an association between increase in maximal eccentric muscle torque (MEMT) and increase in aBMD.

Methods: Subjects were 15 female recreational runners (running > 2 hrs/wk), age 23-40 years, all normal menstruating. Eight participated in the strength training and seven were controls. Training consisted of maximal eccentric knee extension and flexion, forearm supination and pronation, and hip flexion. Regional and whole body aBMD (g/cm^2) were measured by DXA, and MEMT was measured as isokinetic peak torque (Nm). Muscle fibre biopsies were taken in vastus lateralis.

Results: At baseline aBMD values in left forearm were lower than in right forearm ($P<0.05$) in both control and training group, but in the latter this difference disappeared after training. At four and seven months aBMD were increased from baseline in distal ulna (12.4% and 9.7%), in total ulna (4.6% and 2.9%), and in L2-L4 (1.5% and 1.1%), $P<0.05$. In addition, there was a 0.9% increase in aBMD whole body at seven months, $P<0.05$. At four months MEMT was increased (6.0% - 44.8%) in all test exercises ($P<0.05$) except back extension and forearm supination. There was no relationship between increase in MEMT and increase in aBMD, however, data indicated an association between muscle fibre composition and aBMD after training.

Conclusion: Eccentric exercise is a potent stimulus in bone formation in non-weight bearing bones (ulna and lumbar back) that habitually are unexposed to large eccentric muscle contractions. The finding of no association between the increases in aBMD and MEMT indicate that eccentric muscle contractions leading to bone strain not necessarily lead to parallel increases in maximal isokinetic muscle torque. However, muscle fibre composition might have an impact on mechanotransduction.

OR-16

Cartilage Damage and Synovium Changes in Haemophilic Arthropathy: Evaluation With Magnetic Resonance Imaging

Fotiadis NI, Girtovitis FI, Haritanti A, Dimitriadis AS, Makris PE
Radiology Department and Haemostasis and Thrombosis Unit A Propedeutic Clinic of Aristotle University of Thessaloniki, Greece

Haemophilia is an X chromosome linked disease characterized by an increased tendency to hemorrhage mainly into joints. Due to recurrent haemarthroses specific changes occur in synovium and cartilage. This process is called haemophilic arthropathy. The aim of our study was to determine the value of magnetic resonance imaging (MRI) in the evaluation of early pathologic changes of haemophilic arthropathy.

We investigated 53 joints (27 knees, 14 elbows, 10 ankles and 2 shoulders) in 18 patients with Haemophilia A aged 5-57 years old (median age 21) which all had at least one episode of previous acute haemarthroses. In all patients plain film radiographs (anteroposterior and lateral) and MRI evaluation was performed when there was no clinical signs of acute haemarthroses. MRI protocol consisted of T1 SE, T2*GRE, T2 FSE and whenever necessary STIR in three perpendicular planes. We used intravenous contrast medium (Gadolinium DTPA) in only 3 cases to better delineate the hypertrophy of the synovium.

Synovial hypertrophy and hemosidirin deposition was detected with MRI in 36 patients, a finding with a very important role for the pathogenesis and evolution of the disease, and not detected with plain X-rays. Current concepts about the pathogenesis of haemophilic arthropathy hold that the synovium becomes catabolically active because of the exposure to blood components and as a result induces cartilage destruction. Focal or diffuse destruction of the cartilage was observed in 27 joints with MRI while plain radiographs gave only indirect signs of cartilage destruction in 17 joints. Bony changes were equally well estimated with both methods.

In conclusion, we believe that MRI imaging, with specific sequences, is a non-invasive tool for the assessment of early joint cartilage and synovium pathological changes still undetectable by clinical examination or conventional X-rays in the haemophilic setting.

OR-17

Bone Mineral Density After Induction Chemotherapy in Children With Acute Lymphoblastic Leukemia

Tragiannidis A[1], Athanassiadou F[1], Rousso I[1], Katsos G[2], Sidi V[3], Tsitouridis I[4], Koliouskas D[3]
[1]2nd Pediatric Department, Hematology Oncology Unit, Aristotles University of Thessaloniki AHEPA Hospital
[2]2nd Pediatric Department, Aristotles University of Thessaloniki, Hippokration Hospital
[3]Pediatric Oncology Department, Hippokration Hospital, Thessaloniki
[4]Radiology Department, Papageorgiou Hospital, Thessaloniki, Greece

The aim of our study was to evaluate bone metabolism in children with glucocorticoid-induced osteoporosis/osteopenia after induction chemotherapy for acute lymphoblastic leukemia.

Materials and methods: Fifteen children with acute lymphoblastic leukemia who completed induction chemotherapy protocol ALL BFM '95 and were treated with cumulative dose >2g of prednisone equivalent. Lumbar spine (L1-L4) bone mineral density (BMD; g/cm^2) was measured by dual energy X-ray absorptiometry (DEXA) using a Norland unit. In addition, for each child sex, age, and protocol (standard/median or high risk) were evaluated. Bone mineral density values were expressed as z-scores, which express deviation from the mean BMD for age, weight and sex. Results were compared with those of 15 healthy controls.

Results: Of the 15 children with acute lymphoblastic leukemia, 10 were grouped in standard risk and 5 in high risk. The z-scores values demonstrated that 7 children presented z-scores <1 SD, 7 between 1 and 2.5 SD (osteopenia) and one >2.5 SD (osteoporosis). Statistical evaluation using t-test for paired samples demonstrated mean z-score:-1.25 for children with acute lymphoblastic leukemia and –0.52 for healthy controls ($p=0.012$). Mean bone mineral density between the two groups (0.611 g/cm^2 and 0.670 g/cm^2, respectively) presented also a significant difference ($p=0.011$). No statistical difference was found between BMD and risk group, age and sex. The analysis of biochemical markers did not demonstrate a significant difference between patients and controls.

In conclusion, our data indicate that children with acute lymphoblastic leukemia are osteopenic-osteoporotic after induction chemotherapy. The mechanism is probably multifactorial but is mainly related to chemotherapy and in a minor grade to the limited exercise capacity and the underlying disease. In addition, we conclude that a follow-up of BMD could facilitate the identification of those children who may require specific therapeutic interventions to prevent any further decrease in skeletal mass.

OR-18

Spine Stress State Under Brace Effect and Scoliosis Treatment Computer Simulation

Culik J
Institute of Biomedicine Engineering, Czech Technical University of Prague, Czech Republic

Purpose of the Study: Corrective braces are used for the treatment of spine scoliosis of children (deformation of chest curve). The brace pushes on child trunk and after a long time using it corrects pathologic spine curve. The inter-vertebrae discs and ligaments remodelled to be the spine deformation corrected. The brace is worked at this manner: it is made a plaster negative and then a positive form of child trunk. The orthotic according to his and orthopaedist experience deeps the plaster positive form at the place where the brace has to push on the child trunk. The plastic brace is then made according to this plaster form. The brace after its application pushes at the places, where the form has been deepen (the small shoe principle). The brace force effect is result of orthopaedist experiences only. The research has searched algorithms and computer programs, which are able to determine the stress state at vertebras and inter-vertebrae discs for a concrete brace using. The spine curvature remodelling depends on stress state at inter-vertebrae discs and ligaments and time of the brace application. The treatment was simulated on a computer and the results ware verified with real treatment courses.

Materials and Methods: The finite elements method (deformation variant according to the Lagrange principle) was used for the stress state solving. It was supposed that the vertebrates have no deformation. The potential energy was calculated for the intervertebrae discs and ligaments volume and for the

pressed soft tissue region of the trunk. Because it is no deformation between vertebrae centre and intervertebrae disc bounder, the central spine line is linear at vertebrae parts and curvilinear between vertebrae. The pressed soft tissue can be considered as an elastic grunt. The brace pushes at a child trunk at the place, where the plaster positive form has been deepen; it means that the trunk surface (soft tissue) has at these places the non-zero prescribed displacements. The prescribed displacement is supposed above for lying patient. The two algorithms ware implemented. The 1st algorithm has as input the prescribed trunk surface displacements and the results are spine deformations, spine inner forces and forces between spine and trunk soft tissue. The 2nd one has as input spine deformation. The spine deformation is measured as differences between the spinal curves without and with a brace on the X-rays as a brace force effect. The results are spine inner forces, forces between spine and trunk soft tissue and need trunk surface displacement as causal agent. The algorithms ware applied for the frontal and sagital plane.

Results: If the brace is put of the child trunk after some time of application, then the spine does not return to previous position but the pathologic spine form is partly corrected. The permanent part of deformation depends on spinal curve type according to King, on stress state and time of brace application. The simulation program considers that the percentage spine correction is constant in time. The algorithm determines correction curve parameters according to King_s curve type.

Conclusion: Many child patients are observed and the computer simulation model and its parameters ware verified to be the behaviour of the model same that the child treatment course and it will be used for cure prognosis and searching of optimal brace form variant.

The research was supported by grant MSM 21-0000012 "Trans-disciplinal Research at Biomedical Engineering Area".

OR-19

DEXA ASSESSMENT OF MUSCLE-BONE RELATIONSHIPS IN HUMANS

I. REFERENCE STUDY OF THE LEAN MASS/ BMC RELATIONSHIPS IN WHOLE BODY AND LIMBS OF 2512 NORMAL MEN AND PRE- AND POST-MENOPAUSAL WOMEN

Capozza RF[1], Cointry GR[1], Cure-Ramírez P[2], Ferretti JL[1], Cure-Cure C[2]
[1]Center for P-Ca Metabolism Studies (CEMFoC), Natl Univ of Rosario, Argentina
[2]Metropolitan Univ of Barranquilla, Colombia

A whole-body (WB) DEXA study of 1,450 normal Caucasian individuals [Bone 22:683,1998] found that mineral mass, either crude (BMC) or statistically adjusted to fat mass (FA-BMC) correlated linearly with lean mass (LM, proportional to muscle mass), showing similar slopes but decreasing intercepts in the order: pre-MP women > men > post-MP women > children. This supported the control of bone status by muscle strength in humans (bone "mechanostat" theory) and the positive interaction of sex hormones with that control. Now we study those relationships in 2,512 normal Hispanic adults (307 men, 753 pre-MP women, 1,452 post-MP women), including determinations in upper and lower limbs (UL, LL). In all studied regions the slopes of the BMC or FA-BMC vs. LM relationships were parallel. However, the intercepts of the curves showed regional differences. In WB, the BMC/LM relationships showed the same intercept differences observed previously. In LL, those differences were smaller but highly significant, showing the order: pre-MP women>men=post-MP women. In UL, the decreasing intercept order was: men>pre-MP women>post-MP women. After fat-adjustment of the BMC, the intercept order in both limbs was men>pre-MP women>post-MP women. Parallelism of the curves was always maintained. A larger independent influence of LM than FM, body weight or age on these results was shown. Parallelism of the curves further supports a common biomechanical control of bones by muscles in humans. Results suggest that the sex-hormone-associated differences in the DXA-assessed muscle-bone proportionality in humans could vary in different regions because of the different weight-bearing nature of the musculoskeletal structures studied. Besides the obvious anthropometric associations, FM would exert a mechanical effect as a component of body weight, evident in the LL, while muscle contractions would induce a dynamic effect in both limbs. Multiple regression tests showed that muscles exert a significantly larger influence than FM, body weight and age on BMC in WB and LL, regardless of gender and reproductive status of the individual. In order to ease the clinical evaluation of these bone-muscle relationships, specific reference charts and a special software utility were developed from normal data, as reported separately.

OR-20

DEXA ASSESSMENT OF MUSCLE-BONE RELATIONSHIPS IN HUMANS

II. VALIDATION STUDY FOR A NOVEL DIFFERENTIAL DIAGNOSIS BETWEEN "DISUSE" AND "SYSTEMIC" OSTEOPENIAS

Capozza RF[1], Cointry GR[1], Cure-Ramírez P[2], Ferretti JL[1], Cure-Cure C[2]
[1]CEMFoC, Natl Univ of Rosario, Argentina
[2]Metropolitan Univ of Barranquilla, Colombia

DEXA assessment of bone mass (BMC) can be complemented by measuring *lean mass* (LM, proportional to *muscle mass*) in whole body (WB) or limbs. This little-profited resource may provide a differential diagnosis between "disuse" and "systemic" osteopenias. We have showed linear, parallel BMC(y)-*vs*-LM(x) relationships in WB and limbs of 1,450 Argentine and 3,000 Colombian normal individuals. Parallelism of the curves would reflect the biological control of bones by muscles by bone *mechanostat*. However, the intercepts of the graphs differed between groups, in the order: boys/girls<post-MP women<men<pre-MP women, suggesting a positive modulation of that control by sex hormones.

These results allowed performing *z-scored* graphs, specific for gender, reproductive status, region studied, race, and DEXA equipment employed, suitable for evaluating the bone/muscle mass proportionality. They may allow evaluating whether an eventually low BMC value is or not adequately proportionate to individual's WB or regional muscle mass. "Disuse-related" osteopenias (as well as small or lean, normally active individuals) should show a normal z-score for the BMC-*vs*-LM relationship. "Systemic" osteopenias caused by alterations of bone cells (either primary or secondary to endocrine-metabolic changes) should show low BMC-*vs*-LM z-scores. Such cases should be further studied employing other technologies to determine whether bone strength is or not affected; i.e., for diagnosing an *osteoporosis* as a metabolic or systemic "osteopenic fragility" (NIH criterion), which is outside the scope of DEXA.

This report shows some studies in which we tested the ability of our WB or lower-limb BMC-*vs*-LM percentile or z-score reference charts and application software to detect "metabolic" osteopenias in a. haemodialysed men (37) and pre- and post-MP women (71), in which the validation was achieved by showing that z-scores decayed as time on dialysis or serum PTH increased; b. obese hyperinsulinemic euglycemic men (30) and pre- and post-MP women (110), in which z-scores diminished as BMI or fasting plasma insulin increased or insulin sensitivity decreased; c. professional young adult female ballet dancers (20), in which z-scores decayed as calciuria increased, presumably because of a disturbed estrogen metabolism, and d. hypopituitary men (14) and women (15) before and after treatment with rhGH, in which z-scores improved as serum IGF-I levels increased.

P-21

DEXA ASSESSMENT OF MUSCLE-BONE RELATIONSHIPS IN HUMANS

IV. IMPACT OF PARATHYROID STATUS AND Ca AND VITAMIN-D SUPPLEMENTATION ON MUSCLE-BONE RELATIONSHIPS IN 208 BELARUSSIAN CHILDREN AFTER THYROIDECTOMY BECAUSE OF THYROID CARCINOMA

Schneider P[1], Biko J[1], Reiners C[1], Demidchik YE[2], Drozd VM[2], Capozza RF[3], Cointry GR[3], Ferretti JL[3]
[1]Clinic for Nuclear Medicine, Univ of Würzburg, Germany
[2]Ctr of Thyroid Cancer and Inst of Radiation Med & Endocrinology, Minsk, Belarus
[3]Ctr for P-Ca Metab Studies (CEMFoC), Natl Univ of Rosario, Argentina

This observational study analyses Ca-P metabolism and its impact on bone mass accrual and density and the muscle-bone mass/mass relationships in male and female children and adolescents who were parathyroidectomized because of thyroid carcinoma. Two hundred and eight children and adolescents (119 girls and 89 boys) from Gomel city (Belarus) and its rural surroundings were referred to the Würzburg Clinic after having undergone

total thyroidectomy for the treatment of advanced papillary thyroid cancer. A subgroup of children with demonstrated primary hypoparathyroidism received dihydrotachysterol (AT-10) and/or Ca supplementation. Among routine procedures over a maximum follow-up period of 5 years (average 3.7 years, maximum 8 visits), whole-body DXA scans were taken at each visit in order to determine whole-body bone mineral content (TBMC), projected 'areal' bone mineral density (TBMD), total lean mass (TLM) and total fat mass (TFM). The average serum Ca, P and AP concentrations over the whole observation period were significantly different between the groups; however, TBMC z-scores for all studied children were statistically similar in all visits. In girls, supplementation exerted no effect on height- and weight-adjusted TBMC and TBMD or the TBMC/TLM ratio, suggesting that the total bone mass accrual was not impaired by PTH deficiency in the studied conditions. However, non-supplemented boys showed lower values of the TBMC/TLM ratio than girls, and supplementation normalized these values in direct correlation with the induced improvement in serum P availability to bone as assessed by serum P concentration. Results indicate that the primary impairment in parathyroid function and bone metabolism indicators in the thyroidectomized children was unrelated to any measurable change in crude bone mass values. However, in boys this condition impaired the TBMC/TLM ratio in such a way that the administered supplementation could normalize it as a function of improved P availability. Girls' skeleton seemed to have been naturally protected against the negative metabolic effect of the studied condition. An estrogen-induced enhancement of the biomechanical impact of muscle contractions on bone mass and structure could not be excluded in this group.

P-22

The Presence of Glucocorticoid and Thyroid Hormone Receptors in Mitochondria of Animal Cells: Possible Role in Regulation of Energy Production

Solakidi S[1], Psarra A-M[1,2] and Sekeris CE[1]
[1]Laboratory of Molecular Endocrinology, Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, Athens, Greece
[2]Laboratory of Biochemistry and Cell Biology, Centre of Basic Research, Foundation of Biomedical Research of the Academy of Athens, Greece

ATP production in mitochondria depends on an intact respiratory chain whose protein components (OXPHOS) are encoded partly by nuclear and partly by mitochondrial genes. This necessitates a coordination of the availability of OXPHOS subunits, gene transcription being a major site of such coordination. Steroid and thyroid hormones are known regulators of transcription of OXPHOS genes, the prevailing concept being a nuclear receptor mediated activation of nuclear OXPHOS genes and of genes generating regulatory signals of mitochondrial OXPHOS gene transcription. A second possibility would be a parallel direct action of the hormones, by way of their cognate receptors, on the nucleus and on the mitochondria, which would be based on the availability of such receptors in mitochondria. Indeed, glucocorticoid receptors have been found to be present in mitochondria of animal cells (HeLa, Müller cells of salamander retina, rat brain and C6 glioma cells). Furthermore, thyroid hormone receptors have been detected in mitochondria of HeLa cells. As a continuation of these studies we proceeded to assess the presence of glucocorticoid receptors in mitochondria of human hepatocellular carcinoma cells (HepG2) and C2C12 mouse myoblasts and myotubes. Using immunofluorescence labeling and confocal LASER scanning microscopy, as well as immunogold electron microscopy, we showed that the glucocorticoid receptor is specifically enriched at the sites of mitochondria of HepG2 cells, which were visualized by labeling with the vital dye CMX. Western blotting experiments also revealed the presence of glucocorticoid receptor in mitochondria isolated in these cells In addition, in these mitochondria other regulatory signals, such as the mitochondrial transcription factor A (mtTFA) and the p50 subunit of the NF-κB transcription factor were detected, by *in situ* and biochemical experiments. These results, our previous data and those from other laboratories, working in *in organello* transcription systems, support the concept of a receptor-mediated direct action of steroid and thyroid hormones on mitochondrial gene transcription. In mitochondria of C2C12 myoblasts no glucocorticoid receptor could be demonstrated by *in situ* or biochemical methods, suggesting that the known effect of glucocorticoids on mitochondrial OXPHOS gene transcription in myocytes is indirect by way of nuclear effects of the hormones. We are currently investigating the possible localization of glucocorticoid receptors in C2C12 mouse myotubes.

P-23

Static and Dynamic Osteogenesis in Bone Histogenesis and Healing

Marotti G[1], Palumbo C[1], Ferretti M[1], Cavani F[1], Botti P[2], Cadossi M[1], Canè V[1]
[1]Dept. of Anatomy and Histology, University of Modena and Reggio Emilia
[2]Dept. of Animal Health, University of Parma, Italy

Introduction: It is known that bone healing initially involves three main consecutive stages: 1) hematoma formation; 2) inflammatory phase; 3) osteoblast differentiation and bone formation. It has also been suggested that postnatal bone repair recapitulates under many aspects the sequence of events occurring in bone histogenesis during fetal life. Because of this, we investigated whether static (SO) and dynamic osteogenesis (DO), we recently showed to occur during normal intramembranous ossification (Ferretti et al., Anat Embryol 206: 21-29, 2002; Palumbo et al., J Anat 2003; 203-589, 598), also takes place in bone healing.

Materials and methods, The process of bone repair was analyzed by light (LM) and transmission electron (TEM) microscopes inside transcortical holes (4.5 mm diameter) drilled at the mid-shaft level of the 3rd metacarpal bone in adult horses.

Results and conclusion: After the hematoma and inflammatory stages, all the holes studied were filled with a highly cellular and vascularized fibrous connective tissue. Afterward, cords of plum cells, displaying the typical osteoblastic ultrastructure, differentiate in between the blood capillaries. These osteoblasts appear to be stationary since they do not move, but transform into osteocytes in the same site where they differentiate, thus giving origin to a trabecular bony framework laid down by SO. Soon after, typical movable osteoblastic laminae differentiate along the surface of these SO-trabeculae and thicken them by DO. Briefly, it appears from this preliminary investigation that, at least under the morphological viewpoint, the process of bone healing recapitulates the same sequence of events occurring during bone histogenesis. At the present moment, we believe that different factors and signals should be involved in triggering and driving the two types of osteogenesis in both normal and pathological conditions: SO probably depends on inductive factors (such as cytokines and growth factors), whereas DO more likely is mainly driven by mechanical signals.

P-24

The Basic Regulators of Bone Reconstruction

Petrtyl M, Danesova J
Czech Technical University in Prague, Faculty of Civil Engineering, Laboratory of Biomechanic and Biomaterial Engineering, Prague, Czech Republic

Aim of study: The bone tissue remodeling is a cyclic process involving the replacement of an old tissue by the new one (in the volume micro/mezo tissue unit–BMU). Each remodeling limit cycle consists of five principal intensive biochemical reactions (intensive metabolic processes), which are defined by five stoichiometric equations, and periods of weakly steady states (i.e., periods in which the biochemical processes are relatively calm). Determination of speeds of bone remodeling processes has the fundamentals importance for the exact understanding of reconstruction processes. The purpose of our study was to determine the basic components of resultant speed (of j^{-th} biochemical reaction).

Methods: The limit cycles are regulated (governed) genetically and biomechanically. The genetic (internal) control and biomechanical (external) control initiate biochemical (metabolic) processes. The genetic factors (under normal physiological conditions) not only establish the precise time rhythm of the intensive biological activities and the rhythm of the weakly steady states, but they also "start" the remodeling limit cycles.

The overall volume changes in molecular mixtures in the BMU are generally given by the sum of volume changes in molecular mixtures, which are the product of biochemical processes (genetically initiated), and the volume changes in molecular mixtures, which are the product of biochemical reactions as well, being, however, initiated by mechanical/biomechanical effects. The general equation is as follows: $\eta_j =$

$\eta_{j,g} + \eta_{j,m}$, in which $\eta_{j,g}$ is the volume change resulting from biochemical reactions (metabolic processes) initiated genetically; $\eta_{j,m}$ is the volume change of molecular mixtures resulting from biochemical reactions initiated mechanically. The volume changes (in four primary molecular mixtures) have the dominant and absolutely decisive influence on the process of remodeling limit cycle.

Results and conclusions: The speed of biochemical reactions can be generally expressed as follows: $k_j = C_j \cdot e^{-\eta j(p-pe)}$ in which η^j (j = 1, 3, 4, 5) are volume changes of the determined components of remodeling processes in the bone tissue and $(p-p_e)$ are the stress changes. Each volume change η_j equals the sum of the volume changes resulting from the proceeding biochemical reactions, and the volume changes resulting from the proceeding biochemical reactions initiated mechanically. Thus, $\eta_j = \eta_{j,g} + \eta_{j,m}$. For the speeds of biochemical reactions, according to the previous expression, we can write:

$k_j = C_j \cdot e^{-(hj,g + hj,m)(p-pe)}$, respectively: $k_j = C_j \cdot e^{-hj,g\,\Delta p} \cdot e^{-hj,m\,\Delta p}$

For the speed of the j-th biochemical reaction, the following general expression is obtained:

$k_j = C_j \cdot k_{j,g} \cdot k_{j,m}$ .Then, it is obvious from the expression that the resultant speed of the j-th biochemical reaction, which forms part of biochemical (metabolic) processes in the bone tissue (in the remodeling limit cycle) is dependent on the product of speeds of the biochemical reaction *initiated genetically* and on the speed of chemical reaction *initiated biomechanically*. Thus, the j-th biochemical reaction is influenced by the internal – genetic effects and the external – biomechanical effects. The external effects and the internal influence on the speed of remodeling processes in the bone tissue are completely coordinated by the live environment.

Acknowledgments: This research has been supported by a grant of GACR No. 106/03/0255

P-25

Measuring Lumbar Spine Trabecular Bone Elastic Modulus by Nanoindentation

Antonarakos P[1], Kapetanos G[2], Bouzakis K-D[3], Michailidis N[3], Tsougas M[4], Pournaras J[1]
[1]1st Department of Orthopedic Surgery
[2]Laboratory for Machine Tools and Manufacturing Engineering
[3]3rd Department of Orthopedic Surgery
[4]Department of Forensic Medicine, Aristotle's University of Salonika, Greece

Purpose: The purpose of our study was to measure the intrinsic elastic properties of human vertebral trabecular bone.

Materials and methods: Specimens from 5 lumbar vertebrae (L-4) were obtained from fresh, unembalmed human cadavers (2 males and 3 females), aged from 16 to 90 years. After mounting in epoxy resin, nanoindentation tests were conducted to measure Young's modulus and the hardness of individual trabeculae. Measurements were made in both longitudinal and transverse directions.

Results: After 719 nanoindentations in 103 trabeculae both in longitudinal and transverse direction the average Young's modulus was found to be 13.7(2.5) Gpa, which is higher than the one obtained by classic micromechanical tests.

Conclusion: Nanoindentation is a very promising technique for evaluating intrinsic mechanical properties of bone at sub-micro level of organization.

P-26

Novel Effects on Material's Properties and Pre- and Post -Yield Behavior of Rat Bones - I. Effects of Hypophysectomy and Recombinant Human Growth Hormone

Capozza RF[1], Feldman S[1], Cointry GR[1], Leite Duarte ME[2], Sarrió L[1], Ferretti JL[1]
[1]Center for P-Ca Metabolism Studies (CEMFoC), Natl Univ of Rosario, Argentina
[2]Univ Fluminense, Rio de Janeiro, Brazil

Aiming to analyze the musculoskeletal effects of hypophysectomy (Hx) and a partial substitutive treatment with recombinant human growth hormone (rhGH), we determined the intrinsic stiffness (elastic modulus, E) and volumetric BMD (vBMD) of cortical bone; the periosteal and endosteal perimeters, area and moment of inertia (CSMI) of the cross-sections, and the structural stiffness and pre- and post-yield strength of femur diaphyses (pQCT and mechanical tests), and the gastrocnemius weight of rats, either intact (n=9) or Hx at 15 days of age (20), otherwise untreated (Hx controls, 4) or given 0.4 (8) or 2.0 (8) IU/kg/d sc of rhGH since 15 days after surgery during 45 days. The Hx delayed the musculoskeletal development (gastrocnemius weight, bone geometric properties), thus affecting the diaphyseal stiffness and strength. It also reduced the cortical vBMD through an undefined mechanism, paradoxically increasing E. The Hx also affected the correlation between bone geometric and material properties (CSMI vs. E), suggesting an anti-anabolic interaction with the biomechanical control of bone modeling. As an integrated result, Hx reduced the stiffness and the post-yield and ultimate strength of the diaphyses. These effects should reflect changes in bone tissue microstructure associated with crack generation and progress, unrelated to bone mineral mass. Results are compatible with a delayed collagen turnover with associated increases in fibers' diameter and crystals' size, resulting from the suppression of other hormones, presumably thyroid. The assayed doses of rhGH tended incompletely to prevent the negative Hx effects on bone and muscle development correlatively. However, rhGH treatment failed to prevent the curious, demineralizing/stiffening effect of Hx on bone tissue and the unusual effects observed on the post-yield strength (less clearly related to muscle development than the former). The effects of larger rhGH doses and the interaction of other hormones with the described effects remain to be investigated. These findings point out a novel feature in rhGH effects on bones and challenge the prevailing view that in endocrine-metabolic bone-weakening diseases the bone matrix always shows a normal composition.

P-27

Novel Effects on Material's Properties and Pre- and Post-Yield Behavior of Rat Bones - II. Effects of Recombinant Human Growth Hormone on Bones and Muscles After Ovariectomy

Cointry GR, Ferretti JL, Capozza RF, Reina P, Fracalossi NM, Feldman S
Center for P-Ca Metabolism Studies (CEMFoC), Natl Univ of Rosario, Argentina

Doses of 150 IU/kg/d of human recombinant growth hormone (rhGH, BioSidus, Buenos Aires) were sc given during 3 months to 3-month-old rats, either intact or OX at that age. At the end of the study their diaphyses were scanned by pQCT and tested in bending. The fresh gastrocnemius muscles were weighed. OX reduced bone tissue's mineralization and stiffness. A significant enhancement of bone growth in width improved significantly the diaphyseal architecture (cross-sectional moment of inertia, CSMI). This geometric improvement overcompensated the negative impact of the OX-induced impairment on bone material's mineralization and stiffness, thus the diaphyseal strength was increased. The assayed rhGH dose rhGH was little effective in intact rats. However, it prevented the OX-induced impairment in bone tissue's mineralization (not stiffness) and improved additively the OX-enhanced geometric variables. These effects of OX and rhGH were correlative with additive increases in muscle mass. Simple regression analyses showed that the impact of muscular improvement was more evident on bone architecture than on bone strength. The positive OX and rhGH effects on cortical bone mass and architecture may have derived from the induction of an "anabolic" shift of bone mechanostat threshold for triggering bone modeling during growth, with a positive biomechanical impact on the diaphyses (larger CSMI and fracture load than controls). The apparent incongruity between the repercussion of the additive improvement in muscle mass induced by OX and rhGH on bone geometry (large impact) and strength (relatively low impact) can be explained because rhGH did not prevent the OX-induced impairment in bone material's stiffness because rhGH did not act on the microstructure of mineralized tissue. Based on original arguments, this evidence suggests the ability of rhGH to improve human post-menopausal osteopenias in which a relatively large impairment in cortical bone mass and/or distribution occurs. However, the actual benefit of the positive rhGH effects on bone mass and architecture in any

species would remain uncertain as long as the nature of rhGH effects on the OX-impaired bone's material stiffness is unknown. These results are interesting because they defy the prevailing view that the remaining bone tissue in metabolic osteopenias is normal.

P-28

Novel Effects on Material's Properties and Pre- and Post -Yield Behavior of Rat Bones - III. New Insights on the Effects of Bisphosphonate (Olpadronate) Administration

Cointry GR[1], Mondelo N[2], Ferretti JL[1], Capozza RF[1]
[1]Center for P-Ca Metabolism Studies (CEMFoC), Natl Univ of Rosario
[2]Gador SA, Buenos Aires, Argentina

Doses of 45-90 mg/kg/d of olpadronate (OPD, IG-8801, Gador, Buenos Aires, carcinogenicity dose-range finding study) were orally given during 3 months to 20 male and 24 female rats 4-5 weeks old (7 & 9 controls). The cortical vBMD, cross-sectional perimeters (PM), area (CSA) and moments of inertia (MI) of femur diaphyses and their structural stiffness (load/deformation ratio) and strength (ultimate load) during the successive "elastic", reversible (pre-yield, no microcracks) and "plastic", irreversible (post-yield, microcrack accumulation) deformation periods were determined by pQCT and bending tests. The pre-yield stiffness of cortical tissue (elastic modulus, E) and a Bone Strength Index, BSI = vBMD*MI (which can predict ultimate strength but does not capture any microstructural indicator of cortical tissue) were calculated from those data. No effects on growth were observed. Treatment improved significantly CSA and MI by increasing both endosteal and periosteal PMs, more evidently in male than female rats (probably a size-related difference), with no effects on cortical vBMD and E. As a result, mild increases in diaphyseal stiffness and strength at yield (only significant in males) were observed. Diaphyseal ultimate strength was substantially enhanced (males, +68.1%, $p<0.001$; females, +21.7%, $p<0.01$) chiefly because of a large increase in the post-yield fraction of ultimate load (a correlate of bone "toughness"; males, +344%, $p<0.001$; females, +101%, $p<0.05$). The BSI failed to predict ultimate load in treated animals. The positive effects of the assayed OPD doses on pre-yield bone behavior would reflect an anabolic improvement in diaphyseal geometry induced independently of bone material's mineralization and elastic stiffness (i.e., beyond the homeostatic control of bone structure as predicted by bone mechanostat theory). The large effects on bones' post-yield behavior and ultimate strength should be assigned to changes in some "creeping" factors not determined in the study, affecting crack progress within cortical tissue ("plastic" deformation period) previously to fracture. Failure of BSI to predict ultimate strength suggests that the observed bone strengthening would have been determined chiefly through changes in some mineralization-unrelated, microstructural factors. These results point out some novel bisphosphonate effects on bone strength and mechanism of fracture with no apparent involvement of bone mineralization.

P-29

Novel Effects on Material's Properties and Pre- and Post -Yield Behavior of Rat Bones - IV. Novel Effects of Ovariectomy and Alendronate

Chiappe MA[1], Capozza RF[2], Iorio G[1], Alvarez E[1], Feldman S[2], Ferretti JL[2], Cointry GR[2]
[1]Fac of Veterinary, Univ of Buenos Aires
[2]Centre for P-Ca Metabolism Studies (CEMFoC), Natl Univ of Rosario, Argentina

Effects of bisphosphonates on bone mineralization and strength are not necessarily correlative. To analyze ALN effects on bone strength, forty 3-month-old rats were OX and given immediately 0 (OX-C, n=13), 5 (OX-5, n=13) or 25 ug/kg sc (OX-25, n=14) 2/wk for 6 months, keeping a further 15 as sham controls. Their femurs were scanned by pQCT and tested in bending. Despite not affecting bone mineralization (cortical vBMD) and cross-sectional diaphyseal geometry (diameters, moment of inertia -MI-), OX impaired the intrinsic stiffness of cortical tissue (elastic modulus, E) and the structural stiffness of femur shafts (load/deformation ratio), and reduced yield and fracture loads (Wy, Wf). The post-yield fraction of Wf (Wp = Wf -Wy) was significantly enhanced by OX, perhaps because of the naturally inverse relationship between the tissue's ability to prevent crack generation (impaired) and progress (improved). Effects of ALN were dose-dependent. The highest ALN dose prevented all negative effects of OX and improved Wf over sham values. No changes in Wy were observed in treated rats (no effect on crack generation). However, Wp (bone toughness) was enhanced in a similar proportion than it was in OX rats. The naturally negative "distribution/quality" curves (correlations between cortical architecture, MI and intrinsic stiffness, E) shifted to the "anti-anabolic" region (lower-left) in the graphs for OX rats and to the "anti-catabolic" region (upper-right) for ALN-treated rats with respect to sham controls. This would indicate negative or positive interactions of OX and ALN, respectively, with the feedback control of bone architecture as a function of bone stiffness and mechanical usage of the skeleton (bone mechanostat theory). In agreement with previous observations in intact rats treated with Olpadronate, lack of effects on bone mineralization and geometry in this study suggests that both OX and ALN treatment would have improved Wp (and additionally ALN would have improved Wf) by affecting some microstructural determinant(s) of bone material's stiffness and toughness (creeping factors) independently of bone mineralization. These novel effects of bisphosphonates may explain the striking dissociation observed between induced improvements in BMD and fracture incidence in large studies with post-menopausal osteoporotic women.

P-30

Novel Effects on Material's Properties and Pre- and Post -Yield Behavior of Rat Bones - V. Chronic Effects of Aluminum Accumulation on Cortical Bone

Cointry GR[1], Negri AL[2], Ferretti JL[1]
[1]Center for P-Ca Metabolism Studies (CEMFoC), Natl Univ of Rosario
[2]Research Department, Del Salvador University, Buenos Aires, Argentina

In order to analyze the effects of Al accumulation on pre- and post-yield behavior of cortical bone, 14 rats aged 90 days received ip doses of 27 mg/d of elemental Al as Al(OH)3 during 26 weeks while another 14 remained as controls. Their femur diaphyses were studied tomographically (pQCT) and tested in bending. The load/deformation curves obtained showed the successive, linearly elastic (Hookean, pre-yield) and non-linear, "plastic" (non-Hookean, post-yield) deformation periods of bones, separated by the yield point. No effects on body weight were observed. Aluminemia and bone histological and ash data confirmed Al accumulation. Treatment reduced cortical bone mineralization (volumetric cortical BMD, $p<0.01$) with a negative impact on the intrinsic stiffness of cortical tissue (Young's elastic modulus, $p<0.05$). Despite the absence of any cortical mass increase (cross-sectional area), an improvement of the spatial distribution of the available cortical tissue (cross-sectional moment of inertia, MI, $p<0.05$) occurred through a directional modulation of the modeling drifts during growth. Up to the yield point, neither the strength, strain, nor structural stiffness (load/deformation ratio) of the diaphyses was affected by treatment. However, Al intoxication reduced significantly the ultimate load, Wmax and the "post-yield" fraction Wp of that load (an estimation of bone "toughness", $p<0.01$). A positive correlation between Wmax and Wp for all the studied animals as a whole was observed. The presumably adaptive response of bone modeling (as assessed by the MI) to the induced impairment of the intrinsic stiffness of bone tissue should have resulted adequate for maintaining a normal structural stiffness (load/deformation ratio) of femur diaphyses according to the bone "mechanostat" theory, but not so to provide a complete neutralization of the impaired diaphyseal strength (Wmax). Although a relative inhibition of bone formation could not be discarded, an Al-induced impairment of bone "toughness" (Wp) should have caused the striking disruption observed between effects on bone stiffness and strength. In addition to describe an unusual finding, these

results suggest that the microstructural elements affecting the post-yield behavior of cortical bone in these conditions ("creeping factors") ought to be further investigated as a promising field in skeletal research.

P-31

Estrogen Deficiency: A Negative Influence on Spinal Fusion With Recombinant Human Bone Morphogenetic Protein 7

Moazzaz P, Gupta MC, Gilotra MM, Gilotra MN, Maitra S, Theerajunyaporn T, Chen J, Martin RB, Reddi AH
University of California, Davis, Department of Orthopaedic Surgery, Sacramento, CA, USA

Introduction: Intertransverse process spinal fusion using recombinant human bone morphogenetic protein-7 (rhBMP-7) was performed in intact and ovariectomized (OVX) female rats, a well-established animal model of osteoporosis. The purpose of our study was to examine fusion rates in intact and OVX female rats using rhBMP-7 to determine if spine fusion is dependent on estrogen status. Rat spinal fusion has been established as a consistent, efficient model for posterolateral intertransverse process fusions. Previous experiments have confirmed the efficacy of pellets containing the carrier, Insoluble Collagen Bone Matrix (ICBM), and rhBMP-7 to augment intertransverse process single level fusion in a rat model. Recent clinical trials have demonstrated the success of both anterior and posterolateral spinal fusion utilizing bone morphogenetic proteins. Studying these implications in an osteoporosis model is of clinical value as there are many patients undergoing spinal fusion surgery that exhibit osteoporotic bone disease, and there is a steady rise in this group of patients.

Material and methods: Fifteen OVX and fifteen intact Sprague-Dawley female rats were randomly assigned to groups receiving 25mg ICBM alone, 25mg ICBM+10ˣgrams rhBMP-7, and 25mg ICBM+30ˣgrams rhBMP-7. Spinal fusion was evaluated by manual motion testing at each lumbar segment, radiographic evaluation using the Lenke grading system, and histology.

Results: OVX and intact rats receiving 25mg carrier ICBM alone did not demonstrate spinal fusion. With 25mg ICBM+10×grams rhBMP-7, there was not a significant difference in fusion rates between intact and OVX rats ($p=0.63$). OVX rats receiving 25mg ICBM+30×grams rhBMP-7 demonstrated significantly lower fusion rates than intact rats ($p=0.013$).

Conclusion: This data suggests that spinal fusion is estrogen-dependent in rats. At the dosages used, rhBMP-7 was unable to overcome the inhibitory effects of estrogen deficiency on spinal fusion.

P-32

The Effect of Ovariectomy-Induced Osteoporosis on Orthodontic Tooth Movement in th Rat

Tsolakis AI, Dontas I, Alexandridis K, Chrysomalli E, Chaldi L, Lyritis GP, Spyropoulos MN
University of Athens, Athens, Greece and University Hospital, Larisa, Greece

The influence of experimental osteoporosis on both the rate and the quality of orthodontic tooth movement was investigated. Twenty-four eight-month-old female rats were divided into two equal groups, one surgery and one control. Bilateral ovariectomies were performed to all surgery group rats the first experimental day. An orthodontic appliance delivering a mesial force of sixty grs* was placed on the maxillary right first molar of all animals the 60th experimental day and the force lasted for 14 days. Direct inspections of the upper jaws, measurements of orthodontic movement of the upper right first molars as well as histologic examinations of the alveolar bone in the upper right and left first molar regions of both groups were performed. As a result a greater orthodontic movement of the upper right first molar for the surgery group animals was revealed. Also, disturbed lamellar structure of the alveolar bone and the presence of large areas of marrow cavities were observed in the surgery group. In conclusion, the results of the study indicate that rats subjected to ovariectomy undergo faster orthodontic movement and they present differentiated osteoclastic activity and a different type of calcified alveolar bone tissue.

P-33

Effect of Different Types of Inulin and OligofructoseE From Chicory on Bone Mineral Density in Rats

Nzeusseu A, Manicourt DH, Hallot M-C, Depresseux G, Dienst D, Devogelaer JP
Department of Rheumatology, Saint-Luc University Hospital, Université catholique de Louvain, B-1200 Brussels, Belgium

Intestinal calcium absorption can be increased by the addition of oligosaccharides to the diet of rats. The aim of this study was to assess the effect of inulin (IN) and oligofructose (OF) from chicory on BMD in growing rats. 52 Whistar male rats, aged 6 weeks and weighting 150-175 g on average were fed with AO4 diet containing 1 % calcium and 0.6 % in order to provide sufficient alimentary calcium. In, OF dosage in diet amounted to 5 %. Group 1 served as controls. Group 2 received OF with a degree of polymerization (Dp) between 2 and 20. Group 3 received IN with Dp of minimum 8. Group 4 received IN with a DP of minimum 20. Treatment duration was 3 months. BMC of Whole Body was measured by DXA (Hologic 1000W, Bedford, Ma) at the start and after 3 months. Animal pQCT of L_3, of left mid-femur and of left tibia was used to measure BMD after 3 months (research XCT, Norland, Fort Atkinson, Wi). The weight gain was higher after 3 months in group 2 as compared to group 1 ($p<0.05$), group 3 ($p<0.001$) and group 4 ($p<0.001$). WBBMC increased significantly in group 2 as compared to other groups. pQCT measurements showed a WB BMD, trabecular BMD and cortical + subcortical BMD of L_3, significantly higher in group 3 as compared to controls. The BMD of total femur was also highest in group 3. At mid-tibia and its metaphyseal proximal region, BMD was higher in group 3, as compared to other groups. In conclusion, there was a positive effect of IN-feeding on BMD of peripheral bones in growing rats.

Disclosure: this study was performed with the aid of a grant from Cosucra SA, B-7740 Warcoing, Belgium.

P-34

Effects of Vitamin K2 on Osteoporosis

Iwamoto J and Takeda T
Keio University, Japan

Vitamin K2 is a cofactor of γ-carboxylase, which converts the glutamic acid (Glu) residue in osteocalcin molecules to γ-carboxyglutamic acid (Gla), and is, therefore, essential for γ-carboxylation of osteocalcin. Available evidence suggests that vitamin K2 also enhances osteocalcin accumulation in the extra cellular matrix of osteoblasts *in vitro*. Osteocalcin-knockout mice develop hyperostosis, suggesting that the Gla-containing osteocalcin promotes normal bone mineralization. Although the precise role of osteocalcin in bone mineralization remains obscure, it probably regulates the growth of hydroxyapatite crystals. Furthermore, vitamin K2 also inhibits the expression of the osteoclast differentiation factor (ODF)/RANK ligand, tartrate-resistant acid phosphatase activity, and mononuclear cell formation, and induces osteoclast apoptosis *in vitro*. There is some evidence indicating that vitamin K2 prevents bone resorption in ovariectomized rats, retards the increase in bone turnover in orchidectomized rats, ameliorates the increase in bone resorption and decrease in bone formation in sciatic neurectomized rats, and prevents the decrease in bone formation in glucocorticoid-treated rats. These findings suggest that vitamin K2 may not only stimulate bone formation but also suppress bone resorption *in vivo*. Clinically, vitamin K2 sustains the lumbar bone mineral density (BMD) and prevents osteoporotic fractures in patients with age-related osteoporosis, prevents vertebral fractures in patients with glucocorticoid-induced osteoporosis, increases the metacarpal BMD in the paralytic upper extremities of patients with cerebrovascular disease, and sustains the lumbar BMD in patients with liver-dysfunction-induced osteoporosis. Vitamin K deficiency, as indicated by an increased circulating level of undercarboxylated osteocalcin, may contribute to osteoporotic fractures. Vitamin K2 also reduces total cholesterol levels. Even though the effect of vitamin K2 on the BMD is quite modest, this vitamin may have the potential to regulate bone metabolism and play a role in reducing the risk of osteoporotic fractures. No randomized well-

controlled prospective studies conducted on a sufficiently large number of patients have been reported yet, therefore, further studies are needed to confirm the efficacy of vitamin K2 in the treatment of osteoporosis.

P-35

The Effect of a Synthetic Molecule (T1) on Peripheral Quantitative Computerized Tomography (pQCT) Parameters of Ovariectomised Mature Rats

Xeinis S[1], Dontas I[1], Kasiotis K[2], Raptou P[1], Galanos A[1], Haroutounian S[2], Lyritis GP[1]
[1]Laboratory for the Research of Musculoskeletal System, School of Medicine, University of Athens
[2]Chemistry Laboratory, Agricultural University of Athens, Greece

Introduction: the purpose of this experimental study was to determine the effect of a newly synthesized molecule (T1), which belongs to the category of Selective Estrogen Receptor Modulators, on bone mass of ovariectomised mature rats with established osteoporosis.

Materials and methods: thirty female Wistar rats 7 months old were used. The animals were divided into three groups: A, B and C consisting of eight, ten and twelve rats respectively. All animals underwent bilateral ovariectomy. One month after ovariectomy, administration of the following drugs to the rats began: group A received placebo, group B estradiol 20μg/kg/day and group C the molecule T1 1mg/kg/day. The drugs were administered subcutaneously for 6 months. Measurements of the rats right tibial bone mass were carried out prior to ovariectomy and 1, 3 and 6 months after it by the non-invasive method of pQCT on the proximal right tibia 4 mm from the articular surface.

Results: measurements of the total and trabecular density showed significant differences between groups A and C the 3rd month after ovariectomy ($p=0.019$ and $p< 0.0005$ respectively). This difference was no longer present at 6 months. There were no significant differences between groups concerning cortical density and cortical area at the same time measurements. Trabecular area was significantly higher in group C than in B ($p=0,028$) the 3rd month, but was borderline ($p=0.08$) the 6th month. There was also a significant difference of total area between groups A and B ($p < 0.0005$), A and C ($p=0.014$), but no difference between B and C at 6 months. Conclusion: it appears that the synthesised molecule T1 has a positive effect on total density, trabecular density and trabecular area compared to non-treated rats the first 3 months of its administration, which is later minimised. In general, it has no negative effect on all the above parameters at the end, in comparison to placebo or estradiol.

P-36

Elderly People's Quality of Life

Bakas E, Kouloulas E, Tsemperlidou E, Kyritsis G, Grentzelos T, Maragakis N, Tzanos G
Physical Medicine and Rehabilitation Clinic, KAT Hospital, Athens, Greece

Objective: To evaluate elderly people's quality of life by recording their prospect for their social life, activities of daily living (ADLs), sexual behavior and communication with relatives and friends.

Subjects and methods: A specially constructed questionnaire was used in 318 elderly subjects who were at Community Centers for Elderly People (n = 247) or were individuals (n = 71). There were 167 women and 151 men and their mean age was 69.7 ± 5.9 years. The elderly's aspect about direct mobility problems, ADLs, emotional status and other parameters were recorded. A SPSS spread sheet was used and correlation was made with the chi square.

Results: The 85.5% stated that they had no difficulty in daily hygiene and dressing. The 49.4% carry out home services without any difficulty while only the 6.3% mentioned they need help. The 75.5% of them accomplish shopping (goods and clothes) without any difficulty. Long trips and transportations once monthly were declared by 33%, once every six months by 28.9% and never by 10.4%. Recreation activities were reported by 27.7% once monthly, while entertainment with hobbies once weekly were mentioned by 61.9% and sports activities and dancing by 42.1%. The 48.7% of the subjects go to church regularly and to other similar social events. The 33% stated that they swim more than 30 times every summer. The 54.1% declared that they still have an active sexual life and the 17.6% are interested in a new date. The 70% of them mention visits at relatives and friends at least once weekly, while the 66.7% report daily visits to their children and the 31.1% to their friends. The 17.6% states absolutely satisfied with the support of their friends and relatives.

Conclusions: Three out of 4 elderly declare completion of ADLs without any difficulty while 1 out of 3 participates in travels, long transportations and recreation activities. Half of them state maintenance of active sexual life and 1 out of 5 was interested in a new date. Three out of 4 were keeping a very good touch and communication with their family and friendly environment.

P-37

Elderly People'e Activities of Daily Living and Correlation

Bakas E, Kyritsis G, Tzani E, Kotsifi K, Moschou D, Stamoulis N, Armakola F
Physical Medicine and Rehabilitation Clinic, KAT Hospital, Athens, Greece

Objective: To investigate elderly people's prospect about the completion of their Activities of Daily Living (ADLs) and their correlation with their gender, educational level, family status and area of residence. Subjects and methods: A specially constructed questionnaire was used in 318 elderly subjects who were at Community Centers for Elderly People (n = 247) or were individuals (n = 71). There were 167 women and 151 men and their mean age was 69.7 ± 5.9 years. The elderly's aspect about direct mobility problems, ADLs, emotional status and other parameters were recorded. A SPSS spread sheet was used and correlation was made with the chi-square.

Results: The 89.3% of the elderly who lived in urban centers stated no difficulty in their daily hygiene while small or moderate difficulty was reported by 90.7% and 34.4% of the dwellers of the small urban and rural centers respectively ($p<0.0001$). A percentage of 69% of the elderly who lived in urban centers reported no difficulty with household activities and 57.1% of those who lived in small urban centers, while small or moderate difficulty was stated by the 49.3% of the rural centers dwellers ($p<0.0001$). The 74.7% of the urban and the 69.1% of the small urban centers declare no difficulty in shopping for daily needs in contrast to the 39.7% of the rural centers ($p<0.0003$). There was no correlation with gender as far as daily hygiene was concerned ($p<0.5913$), or dressing ability ($p<0.7251$). The correlation was borderline concerning food shopping. Statistical significance was found for men concerning their home services ($p<0.045$), shopping for clothes and the rest of necessary goods ($p<0.077$). Educational level and family status presented no statistically significance in daily hygiene ($p<0.9075$ and $p<0.9271$ respectively), home works ($p<0.6457$ and $p<0.5805$), food shopping ($p<0.1152$ and $p<0.8427$), shopping for clothes and the rest of goods ($p<0.9299$ and $p<0.5734$)

Conclusions: Men dwellers of urban and small urban centers mentioned no difficulty in their ADLs, home works and shopping of daily needs. The educational level and the family status do not seem to influence the above parameters.

P-38

DISTAL RADIUS BONE DENSITY IN THALASSAEMIC PATIENTS: A p-QCT STUDY

Gandaifis N[1,4], Boscainos P[1], Ladis V[2], Spiliopoulou Ch[3], Korres DS[1]
[1]1st Orthopaedic Department, KAT Trauma Hospital, University of Athens
[2]1stPaediatric Department, Thalassaemia Unit, "Agia Sofia" Children's Hospital, University of Athens
[3]Department of Forensic Medicine and Toxicology, University of Athens
[4]National Hellenic Center for Emergency Medical Care, Athens, Greece

Introduction: Bone metabolic disease and consequence osteoporosis is emerging today as an important cause of morbidity in adult patients with beta-thalassaemia major and intermediate, taking as a fact their improved survivorship.

The aim of the study is to investigate the occurrence of osteoporosis in thalassaemic patients with the use of peripheral quantitative computed tomography (pQCT) which has the ability to improve the diagnostic utility of densitometry because it measures the exact volumetric bone mineral density (vBMD).

Materials and Methods: We performed pQCT (XCT-2000 scanner, Stratec. Inc.) at the distal radius in 68 thalassaemia patients with beta-thalassaemia, major (57) and intermediate (11) (32 males and 36 females,

ages 44-21 years, mean 30.3 +/- 5.1). Total vBMD, trabecular, "cortical + subcortical" vBMD, Stress-Strain Index (SSI) as well as cross-sectional area (CSA) were determined at the classic "4%" reference site of the distal radius.

Results: The values of volumetric bone mineral densities (total, trabecular and "cortical + subcortical" vBMDs) and SSI in thalassaemia patients was significant lower than in healthy adults, in relation with the reference values of healthy adults. In thalassaemia patients, male patients also had significant higher values of vBMD, SSI and CSA than female patients.

Conclusions: The data confirms the significant reduction in BMD in the Greek thalassaemic population, especially in the female patients. Thalassaemic bone metabolic disease leads to osteoporosis and possible associated morbidity (increased risk of fracture). In conclusion, in patients with thalassaemia-induced osteoporosis, the administration of anti-osteoporotic agents maybe have to be started early in order to prevent the reduction in BMD (by means of fracture prevention), a medical directive confirmed by data of the associated literature.

P-39

Orthoses With Bending Pre-Stressing as a Metod of Choice for Leg Deformities Treatment in Children With Bone Dysplasias

Marík I, Kuklík M, Maríková A, Zemková D
Ambulant Centre for Defects of Locomotor Apparatus affiliated to Dept. of Anthropology and Human Genetics, The Faculty of Science, Charles University, Prague, Czech Republic

Purpose of the study: There is a possibility to treat a significant (severe) valgus and/or varus deformities of legs (in various levels of shank and distal part of femur) by the new limb orthoses with high bending pre-stressing that were developed by authors. The encouraging treatment results of the group containing 30 children with so-called "idiopathic" valgus or varus deformities were presented and *published in 2002* and 2003***.

The aim of communication is to present 8-years experience with a new type of orthosis with dynamic (elastic) pre-stressing that is suitable for correction of varus or valgus deformities of legs in various shank level and distal part of femur (supracondylar region). Step by step correction of bone deformities is based on remodelling of growth epiphyses and bones due to bending forces of orthosis acting on the basis of the three points principal. In the course of growth, the epiphyseal plates considerably affect the remodelling. At the oblique loading according to Hüter-Volkmann law, orthosis acts on epiphyseal plate and regulate the growth of long bones into the direction of the pressure resultant.

Material and Methods: The orthotic treatment (over night, 10 hours or more) was introduced in 16 children suffering from bone dysplasias and severe varus deformities (diagnosis of pseudoachondroplasia, achondroplasia, hypophosphatemic and oncogenic rickets and epi-metaphyseal dysplasia was proved by geneticist and osteologist). Step by step correction of bone deformities by orthoses is based on viscoelastic properties of bones. Limb orthoses are made according to plaster patient leg forms (negative and positive). The orthosis has two parts connected at definite level by joint and screw with spring. The screw operates with known force and the orthosis pushes on leg for a sufficient time (during night). Efficacy of orthotic treatment was evaluated according to correction of tibio-femoral angle measured at X-rays of legs in standing patient. In the most cases, the results of treatment were followed by measurement of intercondylar distance in standing and/or lying child. The bow legs in three children suffering from achondroplasia underwent combined treatment – a resection of a part of fibula due to its "overgrowth" and fitting with the new orthoses.

Results: The treatment was started in preschool age and lasted from 2 to 5 years. The clinical and X-ray results of orthotic treatment of severe leg varosity of both groups with use of the new developed limb orthoses with bending (elastic) pre-stressing are encouraging. There are a few case reports introduced in the lecture.

Conclusion: The study proved high biomechanical efficacy of the new developed limb orthoses with high bending pre-stressing and delineated conditions that are necessary for successful conservative treatment of severe varus deformities in children (preschool age) suffering not only from acquired but congenital bone deformities, too.

**Marík I, Culík J, Cerny P, Zemková D, Zubina P, Hyánková E. Neue Orthesen mit hoher Biegevorspannung. Orthopädie–Technik, 11, 2002, p. 856-861.*

*** Marík I, Culík J, Cerny P, Zemková D, Zubina P, Hyánková E. New Limb Orthoses with High Bending Pre-Stressing. Orthopädie-Technik Quarterly, English edition III/2003, p. 7 - 12.*

P-40

Coexistence of X-Linked Hypophosphatemic Rickets and Hyperparathyroidism

Giannikou P, Paspati I, Katsalira A, Tournis S, Lyritis GP
LRMS Laboratory, Athens Greece

X-linked hypophosphatemic rickets (XLH), a genetic disorder caused by mutations in the PHEX gene, inherited as a dominant trait and characterized by hypophosphatemia, rarely coexists with hyperparathyroidism. Normal or slightly elevated levels of PTH are the rule in XLH. Greatly increased PTH levels as a result of hypocalcaemia caused by large doses of phosphorus are encountered as a complication of phosphorus supplementation therapy. Our patient, a 56-year old male, with symptomatic XLH since childhood, presents the typical physical and radiological findings of the disease and has never received supplemental phosphorus. His presenting complaint is knee pain that begun in his early twenties and has become increasingly troublesome with age. Anteroposterior and lateral X-ray views of the knees and spine performed initially reveal bilateral knee joint destruction, more severe on the right side, and diffuse osteopenia. As a consequence of the latter unexpected finding, he is referred for BMD measurements of the hip and spine plus a biochemical evaluation. At the hip, total BMD is 0.719g/cm^2 (T-score -2.7), while at the spine a total BMD of 0.874 g/cm^2 (T-score -2.0) confirm the X-ray findings. Laboratory testing of bone turnover indices reveals an increase of the following in the serum: calcium, ALP, osteocalcin, BAP, TRAP 5-β and PTH. Serum phosphate is low, as expected. Urine bone indices are increased as well. A scintigram, using Tc 99m M.I.B.I (sestamibi), reveals a focus of uptake at the lower pole of the left thyroid lobe, suspicious for an adenoma or hyperplasia of the left lower parathyroid gland. Surgical treatment is advised. After surgery, histological examination of the removed parathyroid gland (left lower) reveals hyperplastic parathyroid tissue. Treatment with calcium (1g/day) and alphacalcidol (1μg/day) is initiated to counteract the usual postsurgical hypocalcemia. Improvement of joint mobility and subsiding of knee pain are noted. Three months after the surgery serum and urine bone indices are normalized. PTH levels are still in the upper normal range. The coexistence of XLH and hyperparathyroidism is very rarely referred in the literature. However, this is a possibility we should take in consideration in case of XLH with high PTH levels, especially in patients they aren't treated with phosphorus.

P-41

VDR Polymorphism is Related to Increased Risk of Vertebral Fractures in Postmenopausal Osteoporotic Women

Katsalira E, Dedoussis G, Stathopoulou M, Giannikou P, Galanos A, Lyritis GP
Laboratory for the Research of the Musculoskeletal System, Athens, Greece

VDR polymorphism has been reported to associate with differences in BMD of spine and other skeletal regions. From the existing literature there is a controversy which VDR allele is related to low bone mass and moreover if there is an association between VDR polymorphism and increased risk of vertebral fractures. In the present study 159 women suffering from postmenopausal osteoporosis with T-score below –2.5 were classified according to BsmI polymorphism of the vitD receptor (VDR). We found 28 patients with BB (17.61%), 87 with Bb (54.71%) and 42 with bb (26.41%). There was not found any significant difference in BMD, in both spine and hip, among all groups. Lateral X-rays of the thoracic and lumbar spine revealed 4 patients with vertebral fractures in group BB (14.3%), 20 patients in Bb (22.5%) and 14 patients in bb (33.3%). bb group was found to have a statistically significant prevalence in vertebral fractures ($p=0.005$) than other groups. There was not found any significant difference in reported fractures of the peripheral skeleton between groups.

Conclusion: A possible increase of vertebral fractures in osteoporosis patients with bb polymorphism cannot be attributed to a decreased BMD and seems to be an independent genetic factor of bone fragility.

Bibliography. [1]The association of bone mineral density with vitamin D

receptor gene polymorphisms (Gong G. Osteoporosis Int.9:55-64 1999). [2]Interaction between the vitamin D receptor gene and collagen type Ialpha1 gene susceptibility for fracture (Uitterlinden AG, JBMR 2001 16(2(:379-85).

P-42

The Response to Nasal Calcitonin and Alphacalcidol Combined Therapy is not Related to VDR Polymorphism in Women With Postmenopausal Osteoporosis

Dedoussis G, Katsalira E, Stathopoulou M, Louizou E, Galanos A, Lyritis GP
Laboratory for the Research of the Musculoskeletal System (LRMS), Athens, Greece

A combination therapeutic scheme with intermittent nasal salmon calcitonin (200 IU daily one month on / one month off) and 500 mg calcium carbonate plus 0.25 μg alphacalcidol daily for a period of 12 months, was administered in 157 postmenopausal osteoporotic women. Cases with secondary osteoporosis and/or other metabolic bone diseases were excluded. All patients were divided in three groups according to the VDR polymorphism (28 BB (17.61%), 87 Bb (54.71%), 42 bb (26.41%). BMD of spine and hip were measured initially and at the end of the study. A beneficial effect of the combination therapy was found in the spine in all groups but no statistical difference was shown between the VDR allele subgroups. On the other hand, BMD of the hip had no statistical difference before and at the end of the treatment. In conclusion VDR polymorphism does not seem to affect the response to a combination therapy with nasal calcitonin and alphacalcidol while all VDR polymorphisms had a similar beneficial effect on the spine BMD but not on the BMD of the hip.

P-43

Peripheral Neuropathy in Scleroderma

Zacharof AK, Koutri R, Chartzoulakis G, Papamichail K, Kanakis K, Deliousis A, Flevaris C, Petrogiannopoulos K, Karahalios G
2nd Department of Internal Medicine, Hellenic Red Cross Hospital Athens, Greece

Objective: Because patients with scleroderma report frequently symptoms as numbness, paresthesias, or dysesthesias, we assessed a study of peripheral nerve function in such patients.

Material and Methods: During the last 8 years in our Department hospitalized 13 patients with scleroderma. All these patients underwent complete neurologic examination, nerve conduction studies (NCS) and quantitative sensory testing (QST).

Results: Detailed neurologic examination revealed reduced vibration in 5 patients in other 4 pinprick sensation in the upper or lower extremities, focal atrophy or proximal weakness in 2, and decreased deep tendon reflexes in 2. NCS showed reduced sensory nerve action potentials in 1 patient and carpal tunnel syndrome in another 1. QST of the upper and lower extremity revealed increased cold or vibration detection thresholds in 8 of 13 patients.

Conclusions: Our findings suggest that in our scleroderma patients peripheral neuropathy occurs at a higher frequency than previously noted. These findings cannot be ascribed to compression neuropathies, but rather involve large and small fibres in a non-length-dependent fashion. Larger, prospective studies using the more sensitive QST as well as pathologic studies of nerve, including cutaneous innervations, are needed to further assess the characteristics and aetiology of the neuropathy.

P-44

Secular Trends of Hip Fracture Epidemiology in Greece During 1977-2002

Charopoulos J, Paspati I, Galanos A, Lyritis GP
Laboratory for the Research of Musculoskeletal System (LRMS), University of Athens, Kifissia 145 61, Greece

In the present study we investigated hip fractures occurred in Greece in year 2002 and simultaneously we compared our results with that of a previous similar study. The aim was to identify and assess the hip fracture's age- and sex- specific incidence, the increase rate and the differences in several epidemiological parameters within a period of 25 years. Data collection was achieved with the contribution of orthopedic departments all over Greece. We are recording only the subcapital and intertrochanteric fractures in men and women over 50. Greek population details, concerning the total population and the age groups separately were taken from the National Census Department. Absolute numbers of hip fractures in Greece showed 154,92% increase during the interval 1977-2002 greater than expected due to population aging (1977: 5100, 1982: 6900, 1987: 9250, 1992: 10953, 1997:12106, 2002: 13501). The most affected age-group was that of 80 and over, which is the mainly responsible age-group for the increase of hip fracture incidence. Approximately 48% of the patients with hip fracture in 2002 were 80 and over, while this age-group was only 22.49% of patients in 1977. Men and women aged 50-69 did not show any change in hip fracture incidence during the 25 years of observation. On the other hand the initial increment of hip fracture incidence for the age-group 70-79 that was observed during the period of 1977-1987, was not verified in the interval of 1987-2002. In stead of this we observed that the rate of increase appears to decline and this is valid also for 2002. Finally, in the group of patients aged above 80 there is a continuous increase of hip fracture incidence throughout the 25 years of observation, mainly in women than in men.

P-45

Profane - Prevention of Falls Network Europe - A Four Year Thematic Network

Dontas I[1], Papakitsou E[1], Lyritis GP[1], Skelton D[2], Todd C[2], on behalf of the ProFaNE Group
[1]Laboratory for the Research of Musculoskeletal System (LRMS) School of Medicine, University of Athens, Greece
[2]School of Nursing, Midwifery & Health Visiting, University of Manchester, UK

Introduction: ProFaNE, Prevention of Falls Network Europe, is a four year thematic network coordinated by the University of Manchester, UK, with 25 partners across Europe and funded by the European Community Framework 6. There are also Network Associates from a number of EU and non-EU countries who give their advice and experience at steering meetings, seminars and conferences. The aim is to bring together workers from around Europe with the objective of developing multi-factorial prevention programs to reduce the incidence of falls and fractures amongst elderly people.

Methods: The work of ProFaNE is practical, both in terms of developing the evidence base for implementation of effective interventions and encouraging best practice across Europe. The task of each work package is to convene workshops, undertake personnel exchanges and set up collaborative studies and data sharing in order to develop evidence-based protocols and publications which can be used to implement change. These are: Work Package 1 - Fall prevention trials - Taxonomy of interventions and agreed set of outcomes. A Consensus taxonomy and outcome measures statement, trial design statement, meta-analysis protocol and self help materials will be produced. Work Package 2 - Clinical Assessment and Outcomes. Aims to gain an understanding of the current issues surrounding falls prevention across Europe translated into working models of practice. It will establish a robust network of key members and derive a consensus approach to assessment and management of older people at risk of falling in a variety of clinical settings using the existing evidence base as well as inviting expert opinions in the field. Work Package 3 - Assessment of balance function and prediction of falls. It will combine the expertise of different disciplines for the development of balance assessment tools that predict the risk of falling, give objective assessment of balance function needed for daily life performance and meet the requirements for large-scale intervention studies and routine use in clinical settings. Work Package 4 - Psychological aspects of falling. This work package will investigate the psychosocial factors which are associated with falls, the benefit of falling prevention programs for older people, will form guidelines for the design of interventions and will develop self-test falling indices.

Conclusions: the prevention of falls in the older population is of major importance because falls are associated with considerable suffering, mortality, morbidity and costs for older people, their families and society. The Network will determine successful multifactorial falls prevention strategies to implement throughout Europe.

P-46

Peak Bone Mass and Related Factors in Young Men

Kyriazopoulos P, Charopoulos J, Antonogiannakis EM, Trovas G, Chiotis J, Galanos AN, Lyritis GP
University of Athens, Laboratory for the Research of Muskuloskeletal System (LRMS) KAT Hospital, Kifisia, Greece

Objective: The objective of this study was to evaluate the contribution to peak bone mass of dietary factors (calcium, proteins, alcohol, coffee intake), exercise, smoking, sun exposure and period of immobilization in young men.

Methods: Three hundred healthy young men aged 18-30 (mean 22) participated in this study. Weight was 80.1 kg (53-125 kg range) and height was 179 cm (160-195 cm range). Forearm BMD and BMC at distal and ultradistal sites were measured by single X-ray absorptiometry (osteometer DTX 100). The correlation between BMD, BMC and these factors was evaluated using a one-way analysis of variance ANOVA model. Subjects were divided in four groups of calcium intake (<400 mg/d, 400-800 mg/d, 800-1200 mg/d, >200mg/d), four groups of protein and alcohol intake. There also were five groups of exercise level, six groups of sun exposure, and two groups of duration of immobilization.

Results: calcium intake, exercise level and sun exposure were statistically significant correlated with distal forearm BMC and BMD. In the group with the lowest levels of calcium intake (<400 mg/day) distal and ultradistal BMD was lower than in the other groups of calcium intake (p= 0.03). Distal BMC and ultradistal BMD were lower (p= 0.36 and 0.09 respectively) in subjects with low physical activity (<1 hour/week), while distal BMC was higher (p=0.02) in subjects with very often sun exposure. There was not found any significant correlation between the other examined factors and BMD or BMC.

Conclusion: Calcium intake, exercise level and sun exposure are significant correlated with distal BMD and BMC in young men adults.

P-47

Is Slipped Capital Femoral Epiphysis Hormonally Induced?

Papavasiliou KA[1], Kapetanos GA[1], Kirkos JM[1], Pournaras J[2]
[1]Aristotle University of Thessaloniki – Medical School, 3rd Orthopaedic Department, "Papageorgiou" General Hospital, Thessaloniki, Greece
[2]Aristotle University of Thessaloniki – Medical School, 1st Orthopaedic Department, "G. Papanikolaou" General Hospital, Thessaloniki, Greece

Aim of the study: Epiphysiolysis of the upper femoral epiphysis or Slipped Capital Femoral Epiphysis (SCFE) is a relatively rare type of fracture that occurs during the early stages of adolescence. This prospective clinical study aims at assessing the potential pathologic influence of any hormonal disorders or fluctuations on the development of SCFE.

Materials and methods: Fourteen patients, seven boys and seven girls suffering from SCFE were included in the study. We measured the levels of Thyroid Hormones [3.5.3'-Triiodothyronine (T_3), Thyroxine (T_4) & Thyroid Stimulating Hormone], Testosterone, Estradiol, Dehydroepiandrosterone Sulfate (DHEA'S), Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), I-Parathyroid Hormone (I-PTH), human Growth Hormone (hGH), Adrenal Cortex Hormone (ACTH) and Cortisol, along with serum Calcium (Ca), Phosphorus (P) and Alkaline Phosphatase (ALP) levels. Every other necessary anthropometric (age, height, weight & sexual development according to the Tanner classification) or clinical (degree and location of slipping) data was also taken into account.

Results: An increased incidence of pathological values was detected. Fifty two out of a total of 168 hormonal determinations (31%) were pathological. The serum determinations of LH (10 pathological out of 14 determinations), I-PTH (9 pathological out of 14 determinations), FSH (9 pathological out of 14 determinations) and testosterone (6 pathological out of 14 determinations) revealed the bulk of the pathological values. None of the patients had any clinical signs and/or symptoms whatsoever that suggested the existence of an endocrinopathy.

Conclusions: The recent developments in the understanding of the homeostasis of the growth plate together with the increased incidence of hormonal disorders that were detected in our patients shed new light on the etiology of SCFE and actually rejuvenate the "theory of hormonal intervention" as a contributing factor in the pathogenesis and development of this multi-factorial disease. It is possible that a temporary hormonal disorder (and not necessarily an endocrinopathy) during the early years of adolescence, may play a potentially significant role (along with others etiologic factors: e.g. obesity, minor or major trauma, growth plate's planarity and inclination angle, insufficiency of hydrostatic and tensile growth plate's components) in the development of SCFE.

P-48

Prediction of Failure Loads at the Hip, Spine and Distal Radius

Lochmüller EM[1], Kuhn V[1,2], Priemel M[3], Müller R[4], Eckstein F[2,5]
[1]Gynecology Hospital, LMU München, Germany
[2]Musculoskeletal Research Group, Institute of Anatomy, LMU München, Germany
[3]Department of Trauma Surgery, Hamburg University, Germany
[4]Biomedical Engineering, ETH and University Zürich, Switzerland
[5]Institute of Anatomy, Paracelsus Private Medical University, Salzburg, Austria

Introduction: Osteoporotic fractures represent a major health care problem around the world. Because treatment is available, accurate prediction of bone strength and fracture risk represent an important goal in medical diagnostics. In this study we evaluate the ability of various techniques to predict mechanical bone failure loads at the hip, spine, and radius.

Materials and methods: 126 formalin fixed cadavers were examined (age 80 ± 10 yrs.). DXA of the proximal femur, lumbar spine, and distal radius was performed (DPX-L, Lunar) and pQCT at the distal radius was performed with intact soft tissues (XCT 2000, Stratec). QCT (Somatom Plus 4, Siemens) and QUS of the calcaneus (Achilles+, Lunar) were measured were acquired in degassed specimens ex situ. Iliac crest biopsies were subjected to quantitative histomorphometry. Femora were tested by simulating a fall, three vertebral bodies as functional segments (axial compression) and the distal radius by simulating a fall.

Results: DXA of the femur provided the highest correlation with mechanical strength of the femur (r^2 = 49%). DXA at other sites, QCT (spine), pQCT (radius), and QUS (heel) displayed significantly ($p < 0.05$) lower correlation coefficients. In the spine, lumbar QCT and DXA displayed similar ability in predicting thoraco-lumbar failure loads (r^2 = 62%), whereas other techniques and /or sites displayed significantly ($p< 0.01$) lower coefficients. Forearm DXA and pQCT displayed significantly higher correlations with failure of the radius (r^2 = 49%) than non-site-specific DXA, QCT, and QUS. Most techniques showed similar capability in predicting a combined index of strength (r^2 50-60%), with only trochanteric DXA and QUS at the heel showing significantly ($p < 0.05$) lower associations. Structural parameters from histomorphometry and QUS were unable to add significant independent information in multiple regression models.

Discussion: The results of this study show that site-specific analysis is superior to non-site-specific analysis in predicting bone strength, that pQCT is equivalent to DXA at the radius and for predicting the combined failure index, and that QUS and structural parameters from histomorphometry are unable to contribute independent information to predicting bone strength in multiple regression models.

P-49

Do Postmenopausal Women With Degenerative Osteoarthritic Changes of the Lumbar Spine Have Higher Bone Mineral Density of the Hip?

Triantafillopoulos D, Papakitsou E, Katsiri M, Galanos A, Lyritis GP
Laboratory for the Research of Musculoskeletal System (LRMS) University of Athens, KAT Hospital, Kifisia, Greece

Several studies based on densitometric measurements in osteoarthritic subjects indicate an inverse relationship between osteoporosis and osteoarthritis.

Aim of the study was to evaluate the effect of degenerative osteoarthritic changes of the lumbar spine on densitometric measurements of the lumbar

spine and the hip.

For this purpose we examined 193 postmenopausal women, aged 50 to 85 years old with densitometric measurements in the lumbar spine and the hip. Patients with scoliosis and lumbar fractures were excluded. According to the presence of degenerative spondylosis, the women were classified in group S, or controls.

Results: 42,5% of the women had degenerative spondylosis (group S) and 37 % included the controls.

Women in group S had similar age (58,8±0,87SEM vs 60,9±0,89SEM, P=0,094) and lower body mass index (25,18±0,43SEM vs 28,7±0,53 SEM, P<0,0001) compared to controls. They demonstrated 9% to 16% (95% CI) higher bone mineral density in lumbar spine (BMDL1-4) than controls (P= 0,008). The volumetric density in lumbar spine was also significantly higher in the osteoarthritic group (94,35±5,26SEM vs 77,2±3,52SEM, t-test P= 0,008). There was no significant difference in mean BMD in any anatomic site of the hip (P > 0,2), between the two groups. However when BMI was used as co-variant, the BMI-adjusted mean BMD in the femoral neck was estimated to be significantly higher in women of group S (table).

	pts with degenerative spondylosis	controls	P value
	(mean +/-SEM)		
age *(years)*	58,8 ± 0,87	60,9 +/- 0,89	0,094
BMI *(kgr/m²)*	25,18 +/- 0,43	28,7 +/- 0,53	< 0,0001
$BMDL_{1-4}$ *(gr/cm²)*	0,973 +/- 0,025	0,892 +/- 0,017	0,009
BMDFN *(gr/cm²)*	0,748 +/- 0,017	0,734+/- 0,014	> 0,2
BMDFN*BMI adj *(gr/cm²)*	0,778 +/- 0,014	0,701 +/- 0,013	< 0,0001

The proportion of women that had T-score > -2,5SD in the lumbar spine or the femoral neck was similar among the spondylosis patients and the controls (61% vs 51%, chi² P=0,12 or 63% vs 58%, chi² P= 0,73).

In conclusion, the proportion of women with osteoporosis is the same among postmenopausal women with degenerative osteoarthritic changes of the lumbar spine and controls with similar age. Osteoarthritic sclerotic changes are obviously responsible for the increased bone density of the lumbar spine. However, after adjustment for body mass index, women with degenerative spondylosis present to have also higher bone density in the femoral neck. These results are in accordance with existing literature, but doubt the protective role of increased body weight against osteoporosis in osteoarthritic patients.

P-50

Elastic Properties of Bone Tissue Measured With Nanoindentation and How They are Related to its Macroscopic Elastic Response

Misirlis Y and Stavridi M

Biomedical Engineering Laboratory, Dept. of Mechanical Engineering & Aeronautics, University of Patras, Patras 26500, Greece

The aim of this study is to validate a novel approach to estimate the local anisotropic elastic constants of the bone tissue using nanoindentation. We combine Atomic Force Microscopy with nanoindentation. This instrument allows for two measurement modes. It provides a surface topography of constant contact force in AFM mode and a force displacement curve in nanoindentation mode using the same tip. A nanoindentation curve consists of a loading phase where the tip is pressed into the bone, up to a maximal force, a holding period where the tip creeps into the bone and an unloading phase where the force on the material is released. The loading and holding phases result in both plastic and elastic deformation that cannot be distinguished. During indenter withdrawal, only the elastic portion of the displacement is recovered, which facilitates the use of an elastic solution in modeling the contact process (1,2). The two mechanical properties measured most frequently using indentation techniques are the hardness H , and the elastic modulus, E. Hardness is the mean pressure that a material can support under load. The elastic modulus of the indented sample can be inferred from the initial unloading contact stiffness, the slope of the initial portion of the unloading curve (3). One of the significant improvements in nanoindentation testing is the continuous stiffness measurement (CSM) technique. It offers a direct measure of dynamic contact stiffness during the loading portion of an indentation test. The CSM is accomplished by imposing a harmonic force, which is added to the nominally increasing load on the indenter. The displacement response of the indenter at the excitation frequency and the phase angle between the two are measured continuously as a function of depth. Solving the in phase and out of phase portions of the response results in an explicit determination of the contact stiffness as a continuous function of depth (1).

Bone specimens are characterized by nanoindentation on a transverse and longitudinal plane. A longitudinal Young's modulus for the bone is derived using a numerical scheme (4,5). These values are compared with elastic modulus measured with macroscopic tensile tests. Preliminary results will be presented that show that modulus of bone tissue is related to its macroscopic elastic response. Nanoindentation can be used as a novel approach to estimate the local elastic constants of the bone tissue.

References

1. Oliver WC, Pharr GM: J. Mater Res 1992; 7:1564-83.
2. Phar GM: Mater Sci Eng, A 1998; 253:151-9.
3. Sneddon IN: Int J Eng Sci 1965; 3:47-56.
4. Swadener JG, Pharr GM: Philosophical Magazine A 2001; 81(2):447-466.
5. Zysset PK, Curnier A: Mechanics of Materials 1995; 21: 243-250.

P51

Quantitative Calcaneal Ultrasound Densitometry in Chronic ACL Insufficiency

Yiannakopoulos CK[1,2], Antonogiannakis E[2], Giannikou P[1], Dontas I[1], Lyritis GP[1]

[1]Laboratory for the Research of the Musculoskeletal System (LRMS) University of Athens, Athens, Greece

[2]2nd Orthopedic Department, Athens General Army Hospital, Athens, Greece

The purpose of this paper was to evaluate the effect of chronic, unilateral knee instability due to Anterior Cruciate Ligament (ACL) tear on the bone structure of the calcaneus implementing quantitative ultrasound densitometry. In a group of 15 male patients the speed of sound (SOS) and the broadband ultrasound attenuation (BUA) on both calcanei were measured. In all cases the instability was chronic, affecting significantly the functional performance of the knee according to the Lysholm, Tegner and IKDC scales. The SOS in the affected and the healthy calcaneus was 1534,208±39.411 m/sec and 1563.126±12.284 m/sec respectively, while the BUA was 51.407±3.665 dD/mHZ and 58.13±4.55 dD/mHZ respectively. The difference in both parameters between the healthy and the affected side were statistically significant. There was no significant correlation between the functional performance and the activity level with the ultrasound measurements. The time period between the injury and the measurement did not show correlation. Our results support the notion that a serious ligament injury in a major joint may negatively affect the bone mass of the injured extremity, especially in chronic cases. This is an example of the interaction between the skeletal, the muscular and the neuronal system.

P-52

Thoracolumbar Curve Irregularity: A Measure of Structural Failure

Djoumessi Zebaze RM[1], Maalouf G[2], Maalouf N[3], Seeman E[1]

[1]Department of Endocrinology, Austin and Repatriation Medical Centre, University of Melbourne, Melbourne, Australia

[2]Orthopaedic Department, Saint George Hospital, Beirut, Balamand University, Lebanon

[3]University of Texas Southwestern Medical Center, Dallas, Texas, USA

Deviation from regularity in spinal curvature, assessed in 697 Lebanese women, correlated with numbers of fractures, deficits in height and bone mineral density (BMD), and reliably identifies women with vertebral fractures without the need for controls.

Introduction: Differences in anterior and posterior vertebral heights

(VHs) within a vertebra and between adjacent vertebrae are used to identify vertebral fracture but they are essential for there to be spinal curvatures. These curvatures are adaptations selected for during the evolution of the upright posture because they confer stability in bipedal stance and gait. As VH ratios vary greatly from individual to individual, the distinction between anatomical variability and fracture can be difficult. However, in an individual, adjacent vertebrae resemble each other so they can articulate to form the gently curving spinal structural unit. We hypothesized that the regularity of the spinal curvature is selected for in an individual and structural failure is detectable as a departure from regularity.

Material and Methods: Spine BMD and VHs were measured using DEXA in 697 Lebanese women aged 20-87 yrs. Deformities were assessed by quantitative vertebral morphometry (QVM). Regularity in the spinal curvature is present when the anterior VHs of two adjacent vertebrae lie on the perimeter of one circle and the posterior VHs lie on the perimeter of another. Deviation from unity in the product of anterior/posterior VH of one vertebra with the posterior/anterior VH of the adjacent vertebra is a measure of deviation from regularity, the Spinal Curvature Irregularity Index (SCII).

Results: In premenopausal women, the SCII was independent of age, height and weight, had a mean of 8.5% (range 4 to 15) and was >17% in only 0.8% of women. In postmenopausal women SCII had a mean of 10% (range 4 to 36%) and correlated with age ($r = 0.25$), height ($r = -0.21$), BMD ($r = -0.13$) all ($p < 0.001$) and was >17% in 5.4 % of women. The SCII correlated with the number of deformities ($r = 0.31$-0.60). The 5.5% of women with SCII > 17% had, 3 to 9 fold more deformities than women with SCII < 17%, reduced lumbar spine BMD (-1.01 SD), 2-4 fold greater height deficits (-0.5 SD) than women with deformities.

Conclusions: The SCII is a robust method of identifying structural failure that is easy to compute and does not require controls.

P-53

Structural Measures of Complexity in pQCT and Helical-CT-Imaging

Beller G, Saparin P, Gowin W
Center of Muscle and Bone Research, Dept. of Radiology and Nuclear Medicine, CBF, Charité University Medicine Berlin, Berlin, Germany

Quantitative investigations of the bone in the spinal column *in vivo* are limited so far to dual x-ray absorptometry (DXA) and quantitative computed tomography (QCT). Here we receive information over the bone mineral content (BMC) and the apparent bone density (BMD). Statements about the status and changes of the structural composition of bone tissue within the vertebrae cannot be made. The objective of this study was to verify the possibility to evaluate structural information from high resolution helical-CT images of the lumbar spine. 10 human lumbar vertebral bodies (L3 and L4) with a different degree of osteoporosis were examined. The BMD varied from 5 mg/ccm to 221 mg/ccm. Axial slices were acquired by high-resolution pQCT (pixel size 250 µm, slice thickness 2 mm; XCT 3000, STRATEC, Germany) as well as by high-resolution helical-CT (slice thickness 1 mm, collimation 0.5 mm, feed/rotation 1 mm, field of view 228, reconstruction slice thickness 2 mm; Sensation 4 - VolumeZoom, Siemens AG, Germany). Measurements of BMD and structural measures of complexity were calculated from each set of images and the outcome was compared against each other. The set of complexity measures is based on symbolic and non-linear dynamics. It quantifies the bone structure. Distinct aspects of the bone architecture can be assessed: its complexity and homogeneity, the distribution of local trabecular numbers, the degree of disorder within the architecture, the richness of the trabecular interactions, the bone replacement by marrow tissue, and the total dynamics of the assembly of structural elements. We received high rank order correlations between structural measures of complexity acquired by pQCT and helical-CT of 0.8 and better. We conclude that high-resolution helical-CT-images depict the structural composition of bone very well. Measures of complexity can be used to assess the trabecular bone structure from helical-CT-images and may help to monitor changes of the bone architecture of humans in the future.

P-54

Anthropometrics Biochemical Markers and BMD in Men

Fagarasanu OS, Battie MC, Videman T
University of Alberta, Canada, Faculty of Rehabilitation Medicine

Introduction: It has been theorized that weight and muscle mass influence BMD through associated loading, and fat through hormonal effects. The relation between anthropometrics and biochemical markers has not been studied. The objective was to examine the relation of body weight, fat and fat-free measures with biochemical markers of bone synthesis (PINP) and degradation (NTx) to gain insights into the mechanism through which body size and composition influence BMD.

Materials and methods: 145 randomly selected subjects from 40 to 70 years of age (mean 50.2, SD 7.4) from the population-based Finnish Twin Cohort were studied. Bioelectrical impedance has been used for measuring percent of body fat. Serum and urine specimens were collected in the morning and subsequently stored at -20˚C to await analysis. PINP was determined from serum by radioimmunoassay (Orion Diagnostica, Finland), with intra- assay CV 4.6-10.3% and inter-assay CV 3.1-10.8%. NTx was measured in urine using an ELISA resorption assay (Osteomark™; Ostex International) and was normalized to urinary creatinine (analytic intra-assay CV < 5%, the analytic inter-assay < 8.0%). Bone mineral density was measured with DXA (Lunar DPX, Madison, WI), at the L1-L4 vertebrae (CV 0.9%) and femoral neck (CV1.5%).

Results: Weight, BMI and fat-free measures were similarly associated with BMD ($r = 0.36$-0.46, $p < 0.01$), and were more highly associated with NTx ($r = -0.25$- -0.37, $p < 0.01$) than was fat percentage($r = -0.17$, $p < 0.05$). Fat percentage did not statistically significant correlate with BMD or PINP, and did not add to the variance in BMD or in NTx explained by fat-free BMI. Fat free BMI was a stronger determinant for spine BMD and NTx than weight.

Conclusion: Fat-free BMI was the strongest determinant for NTx, PINP and spine BMD supporting our hypothesis that fat-free parameters as a measure of bone and muscle mass play a more important role in bone metabolism in men than body fat. The underlying mechanism of the fat-free BMI to enhance BMD is related to decreased resorption as assessed by NTx marker.

P-55

The Inflammatory Rheumatic Diseases and Osteoporosis

Ruci D, Tafaj A
Clinic of Rheumatology, "Mother Tereza" University Hospital, Tirana, Albania

Osteoporosis is a systemic skeletal disease in which bone mass is diminished and bone architecture is severely distorted with increased bone fragility and risk of fractures. Osteoporosis is a common finding in rheumatic diseases and it nowadays is a well-known clinical entity.

Objective: This study aims to find osteoporosis in inflammatory rheumatic diseases compared to another group of patients with non-inflammatory diseases.

Material and methods: Were studied 98 patients with inflammatory rheumatic diseases, not receiving cortisone in their therapy. Mean age 26.4 years (18-38) .The control group were 50 patients with the same age suffering from infective dermatological diseases not receiving even locally any kind of cortisone and with normal hepatic, renal and thyroid functions. The bone density was evaluated using an ultrasound bone densitometer (Pegasus type).

Results: Of 98 patients with inflammatory rheumatic diseases 40 (40.9 %) were with Rheumatoid Arthritis, 22 (22.4%) with SLE, 13 (13.3%) with scleroderma, 16 (16.1%) with poliomyelitis and dermatomyositis 7 (7.1%), vasculitis 7 or 7 %.

Osteoporosis was found in 24 patients (24.5%) with systemic rheumatic diseases, more specifically in 13 of 40 with RA or 32.5%, in 6 of 22 with SLE or 27.3%, in 3 of 13 with scleroderma or 18.8%, in 2 of 16 with polymyositis and dermatomyositis or 12.5 %. The mean T-score was -3.2 SD. Osteopenia was found in 19 of 98 patients (19.4 %). From 50 patients with infective dermatological diseases osteoporosis was found in only 4 or 8%.

Conclusions: Osteoporosis is more common in systemic rheumatic diseases than in non systemic diseases. Corticotherapy is not the only factor increasing bone loss in inflammatory rheumatic disease. The interactions with sexual-hormonal causes can not be studied in this series (our patients are 18-38 years old).

P-56

Participation of the Elderly in Social Events and Correlation With Their Residence Area, Family

Bakas E, Tzanos G, Kouloulas E, Tzani E, Sapalidou K, Loizidis Th
Physical Medicine and Rehabilitation Clinic, KAT Hospital, Athens, Greece

Objective: To investigate elderly people's prospect about their participation in social activities and their correlation with their gender, educational level, family status and area of residence. Subjects and methods: A specially constructed questionnaire was used in 318 elderly subjects who were at Community Centers for Elderly People (n = 247) or were individuals (n = 71). There were 167 women and 151 men and their mean age was 69.7 ± 5.9 years. The elderly's aspect about direct mobility problems, ADLs, emotional status and other parameters was recorded. A SPSS spread sheet was used and correlation had been made with the chi-square. Results: Positive correlation with the area of residence, with higher frequency in small urban centers, was showed concerning the realization of trips and distal transportations ($p<0.0001$), the participation in recreational activities ($p<0.0001$), hobbies performance ($p<0.0001$), participation in sports activities ($p<0.0001$), dancing ($p<0.0001$) and going to church ($p<0.0001$). In the above parameters, the correlation with gender was negative. Family status had a positive correlation, with higher frequency in widowers, in travels and transportations ($p<0.0014$) and recreational activities ($p<0.014$). According to the educational level there was no correlation with hobbies and sports activities ($p<0.8329$ and $p<0.1561$ respectively) while there was a positive correlation in the recreational activities ($p<0.01655$), trips and transportations ($p<0.0037$) and dancing ($p<0.0023$) in persons with a higher educational level. Conclusions: Persons who lived in small urban centers participated more in travels, recreation activities, and continued their occupation with hobbies, sports activities, dancing and going to church. Persons who had lost their life partner and persons with a higher educational level participated more often in travels and recreational activities. No difference was recorded among men and women.