ABSTRACTS

PLENARY LECTURES

IN2

C-FMS SIGNALING IN OSTEOCLAST FORMATION AND FUNCTION

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Binding of Macrophage colony stimulating factor (M-CSF) to c-Fms, its auto-phosphorylating tyrosine kinase receptor, regulates osteoclast (OC) differentiation and function. To dissect c-Fms signaling we transduced M-CSF-dependent bone marrow macrophages (MDBMs) with a puromycin-selectable retrovirus that contains a cDNA coding for the external domain of the erythropoietin (Epo) receptor linked to the intracellular domain of c-Fms. Treatment of transduced cells cultured on tissue plastic or whale dentin with RANKL and either M-CSF or Epo, yields comparable OC formation and bone resorption, showing that chimeric receptor and endogenous c-Fms transmit functionally-equivalent signals. To examine the role of individual tyrosine (Y) residues in the c-Fms tail, we transduced MDBMs with chimeric receptors in which specific cytoplasmic Y residues were mutated to phenylalanine (F), incapacitating the signal transduction capacity of each Y. Transduced cells were cultured with combinations of RANKL, M-CSF and Epo, using various aspects of OC differentiation and function as readouts. Previous studies, performed by c-Fms over-expression in transformed cell lines, ascribed important signaling functions to Y residues 697, 706 and 721, putative binding sites for Grb-2, STATs and PI3-Kinase (PI3K), respectively. In contrast, we find that MDBM proliferation and differentiation, OC apoptosis and bone resorption and activation of the Akt and ERK pathways in MDBMs and OCs are controlled by Y559 and Y807. While Y559, which binds c-Src family members, regulates all phases of OC differentiation, Y807, whose binding partners are unknown, is involved only at the earlier stages. Confirming functionality, a site-specific phosphotyrosine antibody documents rapid, transient phosphorylation of Y807. Surprisingly, Y706F reverses the suppressed OC formation mediated by Y559F. The use of c-Src family- and PI3K-specific inhibitors reveals that Y559-derived signals regulate PI3K activation, and that PI3K stimulates the ERK pathway. Activation of ERKs in OCs occurs in two temporally-distinct waves, mediated separately by Y559 and Y807. In summary, we have documented for the first time components of the c-Fms cytoplasmic tail which signal in OCs and their precursors following M-CSF treatment. Our data reveal key roles for the Y residues 559 and 807.

IN3

MOLECULAR MECHANISMS REGULATING LIFE AND DEATH OF THE OSTEOCLAST

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Osteoclasts are terminally differentiated cells with a short life span, and undergo rapid apoptosis in the absence of trophic cytokines such as macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor-kB ligand (RANKL). Members of the Rho family of small GTP-binding proteins mediate a large number of biological processes that are stimulated by growth factors, cytokines, and adhesion molecules, and regulate the organization of the actin cytoskeleton and the regulation of lipid metabolism, gene transcription, and vesicle trafficking. Recent studies have also elucidated the implication of Rac1 in the regulation of cell survival, but its precise role in survival signaling remains highly controversial. We found that Rac1 plays a role in regulating osteoclast survival as well as cytoskeletal organization. Rapid activation of Rac1 was observed in osteoclasts in response to M-CSF stimulation, and overexpression of constitutively active mutant of Rac1 (Rac^{CA}) via the adenovirus vector system promoted survival of osteoclasts in the absence of survival cytokines, while dominant negative Rac1 (Rac^{DN}) expression suppressed M-CSF-induced prolongation of their survival. RacDN also suppressed membrane ruffling and spreading of the cells induced by the cytokine. The prolongation of osteoclast survival induced by Rac^{CA}expression was completely abrogated by treating the cells with PI3K inhibitors such as wortmannin or LY294002. These results suggest that Rac1 is involved in the survival signaling downstream of M-CSF receptor via PI3K pathways. We also found an important role of proapoptotic Bcl-2 family member Bim in osteoclast apoptosis. Bim, was first identified as a Bcl-2 interacting protein, and induces apoptosis by blocking the function of anti-apoptotic Bcl-2 family members. Bim has been shown to play essential roles in apoptosis of T- & B-lymphocytes and neurons, and its activity is regulated both transcriptionally and post-translationally. We found that apoptotic stimulation induced rapid upregulation of Bim in osteoclasts post-translationally, and this upregulation is strongly suppressed by M-CSF treatment. Overexpression of Bim in osteoclasts promoted their apoptosis, which is recovered by co-expression with Bcl-xL. Osteoclasts from bim-/- mice showed marked prolongation of survival in the absence of survival cytokines, suggesting that Bim plays a central role in osteoclast apoptosis.

IN4

PTH AND THE FUTURE OF ANABOLIC TREATMENTS FOR OSTEOPOROSIS

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Osteoporosis cures must stimulate formation of normal bone, increase trabecular, cortical, and total bone, increase in vitro strength of vertebrae, proximal femur, etc. in animals and eliminate fragility fractures in humans, permit normal bone remodeling and repair, and maintain normal bone shape. PGE1, PGE2, IGFI, PDGF-BB, PTH, and PTH analogues have many of these properties; other bone anabolic agents will be developed.

Human PTH-(1-34) reconstitutes trabecular bone mass and architecture, increases cortical bone mass, and increases vertebral, metaphyseal and diaphyseal bone strength in animals. It improves trabecular BMD and internal architecture in osteoporotic men and women, and decreases vertebral and non-vertebral fractures in osteoporotic women, more than anti-resorptive drugs do in similar patients. It does not routinely restore BMD to youthful levels or eliminate fragility fractures in osteoporotic humans; its effects are more dramatic in animals. Combining PTH with drugs that block bone resorption might reverse osteoporosis more completely. Because tests of this hypothesis in animals gave mixed results, we organized a prospective, randomized 30 month comparison of daily alendronate 10 mg, hPTH-(1-34) 40 mcg, or both in 93 postmenopausal osteoporotic women, and a parallel study in 83 osteoporotic men. PTH treatment started at month 6. In the 54 women and 63 men who have reached study month 18, alendronate prevents the decrease in forearm cortical BMD this PTH dose causes in women, accelerates the PTH-induced increase in total body bone mineral in men, and reduces the incidence of PTH-induced mild, transient, hypercalcemia in each sex. In each sex alendronate blunts PTH-induced increases in spine and vertebral body BMD and serum alkaline phosphatase (Ptase), and to date does not clearly alter PTH's effects on hip BMD. PTH+alendronate increases spine BMD and Ptase more than alendronate in each sex.

The blunted increases in Ptase and the lack of additive increases in BMD, suggest that PTH's effect on bone formation depends, at least partly, on its ability to increase bone resorption. If so, other PTH-anti-resorptive combinations will have similar effects. Alternately, alendronate may specifically inhibit bone formation, or PTH-induced bone formation. Discrimination among these possibilities affects short-term prospects for curing human osteoporosis.

IN5

ABSOLUTE FRACTURE RISK AS AN INDICATION FOR THERAPY IN OSTEOPOROSIS I

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Osteoporosis is a significant cause of morbidity and lowered quality of life, particularly in elderly women. Hip fractures are the most important consequences of osteoporosis. Over the last 15 years, we have made great strides in our ability to accurately and efficiently diagnose osteoporosis and assess fracture risk. Bone mineral density (BMD) as assessed by dual energy x-ray absorptiometry (DXA) is accepted as a strong predictor of fracture risk. In particular, BMD at hip is the best predictor of hip fracture risk with a relative risk of about 2.5 to 3 per standard deviation decrease in BMD. Diagnostic categories using fixed BMD cut points (based on T-scores) were first proposed by the WHO in 1992 and have been widely adopted by other groups.

However, risk factors other than BMD have an important impact on risk of fracture. For example, age is a very strong risk factor for fracture, especially hip and vertebral fractures. In women, risk of hip fracture rises exponentially with age doubling approximately every 5 years. Non-BMD risk factors are independent of BMD: for example, even at the same BMD value, a 60 year old woman has about 5 times the risk of an 80 year old woman. Other independent risk factors for hip fracture include weight, history of osteoporotic fracture and family history of hip fracture. Incident vertebral fractures are similarly related to age and are strongly related to existing vertebral fractures, particularly those that have recently occurred.

Clearly incorporation of non-BMD risk factors can improve fracture prediction. Use of non-BMD risk factors, together with BMD, can allow us to more efficiently determine individuals to whom we can direct interventions. This suggests that perhaps we should abandon fixed t-score cut points for diagnosis and treatment thresholds and instead use risk levels to determine diagnostic and treatment decisions. In order to make this conversion, it will be necessary to first develop algorithms for fracture risk prediction and secondly to use cost-effectiveness analysis to determine optimal risk levels for various interventions. These risk levels will likely vary in different countries depending on locally available resources.

Several groups have developed algorithms for risk factor prediction. The Study of Osteoporotic Fractures group has developed a simple scoring system to estimate 5 year risk of hip fractures with and without BMD. Kanis, Johnell and colleagues are developing comprehensive systems for prediction of 10 year risk of fracture which accounts for non-hip fractures by calculating a "hip fracture equivilent" based on morbidity and costs of various types of fractures.

Important questions remain to be answered including how well we can predict fractures other than those at the hip and spine, whether treatments can lower risk of non-spine fracture in those with BMD above osteoporotic levels and how best to predict fracture in men and non-caucasian women. In addition, cost-effectiveness analysis of treatment requires continual reevaluation as new treatments become available (e.g. PTH) and we come to understand more about existing treatments (e.g. HRT).

Risk of clinical outcomes is becoming more commonly used for treatment decisions in other disease areas such as cardiovascular disease, breast cancer, etc. It is likely that fracture risk prediction will eventually lead us away from the use of fixed T-scores to some form of risk-based diagnostic and treatment decision-making.

IN6

ABSOLUTE FRACTURE RISK AS AN INDICATION FOR THERAPY IN OSTEOPOROSIS II

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The diagnosis of osteoporosis is defined by a WHO group as BMD value (T score of minus 2.5 SD or less) at hip, spine, wrist with DXA technique. T scores have different prognostic significance in different ages and also for different measuring sites. Thus diagnostic thresholds are not equivalent to intervention threshold due to the fact that the risk varies markedly at any given BMD. There are many risk factors in addition to age that can provide information on fracture risk over and above that provided by BMD alone. The absolute fracture risk that a patient has should be the guideline for intervention. The 10 year absolute fracture risk is probably the best estimate for creating intervention thresholds. Another problem is which fractures should be included? Besides hip fractures we have also to include other osteoporotic fractures in our absolute fracture risk assessment like spine, wrist, pelvis, humerus and more fractures. Clinical risk factors for fracture prediction should be validated in multiple populations, readily assessable by primary care physicians and be amenable to therapeutic manipulations. There are several candidates for risk factors apart from BMD in this assessment of the patients absolute fracture risk, some strong risk factors like age, gender, prior fracture, use of glucocorticoids etc. The intervention thresholds should partly be based on cost effectiveness and thresholds should be internationally applicable. Ideally intervention thresholds should be based on three different decisions: no further assessment or treatment required, further assessment indicated, e.g. diagnostic assessment, treatment indicated irrespective of any diagnostic assessment.

IN7

FGF SIGNALING IN SKELETAL DEVELOPMENT

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Human chondrodysplasia and craniosynostosis syndromes, result from activating or neomorphic mutations in fibroblast growth factor receptors (FGFR) 1-3. These mutations underscore the essential role for FGF signaling in skeletal development. Fgfrs 1-3 are differentially expressed throughout skeletal development. Fgfr1 and 2 are expressed in the perichondrium and periosteum, Fgfr3 is expressed in proliferating chondrocytes, and Fgfr1 is expressed in prehypertrophic and hypertrophic chondrocytes. These unique expression patterns suggest unique function in skeletal development.

Inactivation of FGFR3 results in skeletal overgrowth and gain of function in FGFR3 results in achondroplasia. These phenotypes define FGFR3 as a negative regulator of bone growth.

Embryos harboring homozygous null mutations in Fgfr1 or Fgfr2 die prior to skelatogenesis. To address the role of FGFR1 and FGFR2 in normal bone development, a conditional gene deletion approach was adopted. Homologous introduction of cre recombinase into the Dermo1 gene locus allowed robust expression of CRE in mesenchymal condensations giving rise to the osteoblast and chondrocyte lineage. Inactivation of a floxed Fgfr2 allele with Dermo1-cre resulted in mice with skeletal dwarfism and decreased bone density. Although the osteoblast lineage appeared to develop normally, the mature osteoblast was atrophic and produced less bone matrix. These studies demonstrate that FGFR2 signaling is not required for osteoblast differentiation but rather for the normal anabolic function of the osteoblast. Inactivation of FGFR1 in hypertrophic chondrocytes and in osteoblasts results in an expanded hypertrophic zone but decreased bone formation. These observations suggest that FGFR1 has both an anabolic affect on osteoblasts, possibly redundant with FGFR2, and an affect on terminal the differentiation of hypertrophic chondrocytes.

We have identified Fgf18 expression in the perichondrium. Mice homozygous for a targeted disruption of Fgf18 exhibit a growth plate phenotype similar to that observed in mice lacking Fgfr3. These data suggest that FGF18 acts as a physiological ligand for FGFR3. In addition, mice lacking Fgf18 display delayed ossification and decreased expression of osteogenic markers, phenotypes not seen in mice lacking Fgfr3. These data demonstrate that FGF18 signals through another FGFR (most likely FGFR1 and FGFR2) to regulate osteoblast anabolic function. Signaling to multiple FGFRs positions FGF18 to coordinate chondrogenesis in the growth plate with osteogenesis in cortical and trabecular bone.

IN8

HYPOXIA AND HIF 1α IN ENDOCHONDRAL BONE DEVELOPMENT

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Adaptation to hypoxia is an important aspect of both tumor progression and survival of tissues in which blood flow has been suddenly interrupted. The transcription factor HIF-1alpha (or Hypoxia Inducible Factor-1 alpha) is a major regulator of the transcriptional response to hypoxia. We have found that the mammalian fetal growth plate, which is an avascular structure, is hypoxic with a typical out-in gradient of oxygenation. Conditional inactivation of HIF-1 alpha in growth plate chondrocytes have demonstrated that this transcription factor is critical for chondrocyte survival and growth arrest, and that it also regulates cartilage matrix formation. In the center of the growth plate chondrocytes lacking HIF-1 alpha undergo massive cell death, which is very likely secondary to impairment of metabolic pathways such as anaerobic glycolysis. Conversely, viable chondrocytes at the periphery display a significant increase of their proliferation rate, which is probably caused by a decreased expression of CDK inhibitor p57kip2. Furthermore, collagen type II production by growth plate chondrocytes appears to be augmented in hypoxic conditions and in presence of HIF-1alpha. Interestingly, expression of VEGF, which in other tissues is one of the main targets of HIF-1alpha activity, is only partially regulated by this transcription factor in the growth plate. The ubiquitously expressed tumor suppressor gene VHL (or Von Hippel Lindau), a novel E3 ubiquitin ligase, negatively regulates HIF-1alpha levels. Consistent with this finding, conditional inactivation of VHL in the growth plate causes up regulation of HIF-1alpha transcriptional activity. Lack of VHL in cartilage leads to dramatic decrease of chondrocyte proliferation, severe hypocellularity, appearance of atypical chondrocytes with a high cytoplasm-to -nucleus ratio in both the resting zone and in the columnar layer, and increased accumulation of cartilage matrix. Therefore, our data strongly suggest that the tumor suppressor gene VHL paradoxically is a positive regulator of chondrocyte proliferation, and appears to uncouple cell size from cell proliferation in the developing growth plate. Further investigations will be now needed to establish whether this complex growth plate phenotype is solely due to up regulation of HIF-1alpha transcriptional activity.

Taken together, our findings show that adaptation to hypoxia is likely to play a major role in endochondral bone development.

IN9

ASSESSING BONE DENSITY

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The incidence of osteoporosis is increasing along with a rapid increase in the elderly population. Therefore, it is important to detect, prevent, and treat osteoporosis. Since low bone mass and microarchitectural deterioration of bone tissue lead to bone fragility and a consequent increase in fractures, it is essential to measure bone mass accurately, as bone quality cannot be properly assessed in vivo.

Recently, many bone densitometric techniques have been developed, have become available, and have contributed to a better understanding of osteoporosis both in research and clinically.

Each method has its peculiar features; e.g., precision error, measurement site, data acquisition time, and radiation dose.

Since age-related bone losses and the response to therapeutic interventions are often small, it is necessary to maintain high quality assurance (QA) and high quality control (QC).

The cutoff value of lumbar and femoral neck BMD for osteoporosis has been defined as -2.5 SD of the T score according to the diagnostic criteria of WHO, and its absolute value should be determined from the databases of individual race.

The rate of bone loss after menopause varies. Individuals who lose BMD at faster rates are more likely to reach the threshold of fracture risk more quickly. Therefore, the annual rates of BMD need to be documented throughout the pre- and postmenopausal life of normal women.

Among the bone desitometric techniques, QCT and pQCT have the potential to measure true volumetric BMD (g/cm3), separating cortical and trabecular BMD. Peripheral QCT can evaluate cortical thickness, calculated using the thresholding algorithm and a circular ring model assumption, and BMD in the radius by thin-slice and high-resolution. More than 80% of age-related bone loss in the radius is caused by decreased thickness of the cortical bone.

Bone microarchitecture is another determinant of bone strength. The trabecular microarchitecture of small bones can be assessed by micro CT. Micro CT using X-ray or synchrotron radiation can image trabecular microarchitecture three dimensionally. The structural analysis of these in vitro data would contribute to understanding the bone quality of individuals at risk for osteoporotic fracture.

IN10

OSTEOPOROSIS TREATMENT: WHY, WHO, WHEN

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The purpose of treatment is not to restore bone mass, prevent bone loss or fractures; it is to affordably prevent fractures causing morbidity and mortality. Evidence of a drug's anti-fracture efficacy does not justify treatment. This decision requires knowledge of baseline absolute fracture risk (AFR), and morbidity and mortality accompanying fx. This information is not provided by clinical trials. Few women have the same baseline AFR as trial participants, while the sample size and duration of trials preclude morbidity and mortality assessment. Why treat if AFR, fx morbidity and mortality are trivial, if morbidity or mortality are high but not reduced by fx prevention? If a drug halves fx risk, why treat if the AFR is 2/1000/yr; one event is prevented, one woman sustains a fx, 998 dont fx so 999 women/yr are treated needlessly. The cost of treating many to avert one event is higher than the cost of fx. If AFR is 2/100 women/yr, halving the risk prevents one event also, but only 100/yr are treated and 99/yr needlessly. Treating fewer at high risk later, rather than many at low risk sooner benefits most. Identifying women with many risk factors identifies fewer and fewer without solving the population burden; ~55% of all fxs occur in women without osteoporosis - we cannot afford to treat the large population source of these fx. The 15% of all fxs in <65 yr-olds are the most costly to avert because 90% occur in non-osteoporotic women. The largest fx burden comes from >65 year olds. Highest risk groups are men and women with (i) osteoporosis with or without fxs, (ii) recent fx, (iii) osteopenia and fxs. Patients at risk for hip fx have the highest morbidity and mortality; preventing the fx is likely to modify morbidity, whether survival is prolonged is unclear. Over 80 yr-olds are a growing high risk population; more fx can be averted per dollar in this group with lowest longevity. Reducing fxs requires (i) treatment based on the heterogeneous patho-physiological and structural basis of fragility as this may increase anti-fx efficacy, (ii) better identification of moderate and high risk groups, (iii) knowledge of the fxs that reduce remaining quality days, and (iv) shifting the population's bone strength to a higher level without drugs.

IN11

OSTEOPOROSIS TREATMENT: WHAT, HOW, WHERE

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The treatments for osteoporosis may be classified into antiresorptive, anabolic and mixed antiresorptive and anabolic. The antiresorptive drugs include the bisphosphonates, hormone replacement therapy, selective estrogen receptor modulators and calcitonin. The drugs are usually given along with calcium and vitamin D supplementation. There is evidence that all of these drugs groups result in decrease in the risk of vertebral fracture, and most also decrease the risk of non-vertebral fractures. They work by decreasing the rate of bone turnover (thus preserving bone microarchitecture) and increasing bone mineral density. The anabolic drugs include parathyroid hormone and this has its maximal effect over 18 months and the increases in bone density are even greater than those with the most potent bisphosphonates and there are decreases in the risk of vertebral and non-vertebral fractures. Bone turnover increases. The mixed antiresorptive and anabolic agents include strontium ranelate and possible the statins and calcitriol. They result in small decreases in bone resorption and small increases in bone formation. The routes by which these drugs are given differ and the dose frequency also differs. It is likely that we will see further developments in giving drugs by new routes and less frequently, e.g. annual intravenous injections of bisphosphonates. Osteoporosis is managed by many specialists, e.g. endocrinologists, rheumatologists, geriatricians, but there is an expanding role for the general practitioner in the management of this common disease.

IN12

OSTEOPOROSIS IN CHINA 'RISK FACTOR, PREVENTION AND TREATMENT'

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Osteoporosis is a major public health problem in aging Asian women. Osteoporosis results in fracture of the hip, vetebra and forearm. In elderly Hong Kong Chinese women, 3 per 1000 subjects fracture their hip in a year, and 30% have osteoporotic vertebral fracture. By WHO definition, 50% of women are osteoporotic. Results from the Asian Osteoporosis Study (A multi-center study in 5 Asian countries) suggested that osteoporosis is also a major health problem in Singapore, Malaysia, Thailand and the Philippines.

Osteoporosis may be preventable. Results from the Asian Osteoporosis Studies showed that a lack of load-bearing activities (Relative Risk, RR=1.5; 95% Cl=1.1-2.1); and a low dietary calcium intake (RR=1.4; 95% Cl=1.0-2.0 for lowest quartile) were risk factors for hip fracture. In addition, subjects with a history of other fratcures and liable to falls were at higher risk. As osteoprosis and fractures are irreversible events, prevention is the only cost-effective means to deal with this epidemic.

The population-based approach seeks to move the bone mineral density of Asian women upwards. Theoretically, this is feasible by increasing physical activity and dietary calcium intake. However, there are practical problem with compliance. The high-risk approach seeks to identify women at high risk of osteoporosis and treat them by drugs or hormonal replacement therapy. Theoretically, this is feasible, by measuring bone mineral density of subjects followed by drug treatment. Practical problems are unprovened cost-effectiveness and poor compliance to drug treatment.

Despite the practical problems, the prevention of osteoporosis should be a high priority area, due to the ever increasing size of the problem in aging Asian women. All Asian governments and health authorities should put this high up on their health agenda.

IN13

Abstract not available.

IN14

THE PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL ROLES OF FGF-23 IN PHOSPHATE AND BONE METABOLISM

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X-linked hypophosphatemic rickets/osteomalacia (XLH), autosomal dominant hypophosphatemic rickets/osteomalacia (ADHR) and tumor-induced osteomalacia (TIO) share common clinical features including hypophosphatemia due to renal phosphate wasting and inappropriately low serum 1,25-dihydroxyvitamin (1,25D) level for hypophosphatemia. The responsible gene for XLH was named PHEX (phosphate-regulating gene with homologies to endopeptidases on the X chromosome). In addition, ADHR was recently shown to be caused by missense mutations in FGF-23 gene. We have cloned FGF-23 as a responsible factor for TIO. FGF-23 was overexpressed in tumors causing TIO. Implantation of CHO cells stably expressing FGF-23 into nude mice reproduced biochemical and histological features of TIO. In addition, recombinant FGF-23 decreased serum 1,25D level at least in part by reducing expression of 25-hydroxyvitamin D-1alpha-hydroxylase. Injection of FGF-23 also caused hypophosphatemia by lowering expression of sodium-phosphate cotransporter type 2a in the renal brush border membrane. FGF-23 protein had a proteolytic processing site and this processing abolished the activity of full-length FGF-23 to cause hypophosphatemia. Mutant FGF-23 proteins found in patients with ADHR were resistant to this processing. However, these mutant proteins retained the activity to induce hypophosphatemia. Therefore, missense mutations responsible for ADHR were considered to increase the biologically active full-length FGF-23. Establishment of sandwich enzyme-linked immunosorbent assay for full-length FGF-23 indicated that circulatory level of FGF-23 was actually high in patients with TIO and rapidly decreased after surgical removal of the responsible tumors. Moreover, serum level of FGF-23 was high in most patients with XLH. These results indicate that FGF-23 is deeply involved in the development of several hypophosphatemic rickets/osteomalacia. Furthermore, full-length FGF-23 was present in normal circulation suggesting that FGF-23 has some physiological roles. Knockout mice for FGF-23 were viable, but showed growth retardation and had short life span from unknown reason. They also exhibited hypercalcemia, hyperphoshatemia and high serum level of 1,25D. Therefore, FGF-23 is also considered to be a physiologically indispensable factor for normal bone and mineral metabolism. Elucidation of mechanism of actions of FGF-23 may lead to the development of novel therapeutic approaches to several diseases with abnormal bone and mineral metabolism.

IN15

TWO MAJOR PATHWAYS IN TGF-BETA/BMP SIGNALING

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Members of the TGF-beta superfamily bind to two different serine/threonine kinase receptors, i.e. type I and type II receptors. Upon ligand binding, type I receptors activate intracellular Smad proteins. R-Smads are direct substrates of type I receptors; Smads 1, 5, and 8 are specifically activated by BMP type I receptors, ALK-2, ALK-3, and ALK-6. In addition, these BMP-specific R-Smads are activated by ALK-1. In contrast, Smads 2 and 3 are specifically activated by activin/nodal and TGF-beta type I receptors, ALK-4, ALK-5, and ALK-7. More than 30 proteins have been identified as members of the TGF-beta superfamily, and can be classified based on whether they activate BMP-specific R-Smads or TGF-beta/activin-specific R-Smads. R-Smads form complexes with Co-Smads and translocate into the nucleus, where they regulate transcription of target genes. TGF-beta/activin-specific R-Smads bind to various transcription factors and transcriptional co-activaotrs/co-repressors,

whereas BMP-specific R-Smads interact with only a limited number of DNA-binding proteins. Moreover, many genes have been identified as targets of TGF-beta and activin signaling, whereas not many have been found as BMP-specific targets. Among those, Id (inhibiter of differentiation) proteins, which bind to basic helix-loop-helix transcription factors, appear to play important roles in osteogenesis, neuroenesis, and angiogenesis. Balance between TGF-beta/activin and BMPs appear to play important roles in differentiation of embryonic stem cells into more differentiated cells, including vascular endothelial cells and osteoblasts. Understanding the mechanisms of regulation of TGF-beta superfamily signaling is thus important for development of new ways to treat various clinical diseases in which TGF-beta superfamily signaling is involved.

IN16

MOLECULAR GENETICS OF HYPOPARATHYROIDISM

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Hypoparathyroidism is characterised by hypocalcaemia and hyperphosphataemia that result from a deficiency of parathyroid hormone (PTH) secretion or action. There are a variety of causes of hypoparathyroidism and in particular hypoparathyroidism may occur as a congenital abnormality either as part of a complex disorder, eg. the Di George syndrome, or as an isolated endocrinopathy. Molecular genetic studies of such hypoparathyroid disorders have helped in our understanding of the pathways underlying parathyroid gland development. The four parathyroid glands (2 superior and 2 inferior) develop from the third and fourth branchial pouches. The 2 inferior parathyroids develop from the third branchial pouch, which also gives rise to the thymus, whilst the 2 superior parathyroids develop from the fourth branchial pouch. Recent molecular genetic studies have identified some of the genes (eg. GATA3, Gcm2 and Hoxa3) involved in these developmental pathways of the branchial pouches and parathyroids, and these will be reviewed. GATA3 haploinsufficiency has been shown to cause the human hypoparathyroidism, deafness and renal dysplasia (HDR) syndrome, which may be inherited as an autosomal dominant syndrome. GATA3 belongs to a family of zinc-finger transcriptional factors that are involved in vertebrate embryonic development, and the HDR phenotype is consistent with the expression pattern of GATA3 during human and mouse embryogenesis in the developing kidney, otic vesicle and parathyroids. The homeobox gene, Hoxa3, has also been shown to be of importance in this pathway, as homozygous mutant knockout mice (-/-) lacking Hoxa3 have an absence of the thymus and parathyroids as well as a reduction in thyroid size, heart defects and craniofacial abnormalities. Homozygous mutant mice (-/-) that are deleted for Gcm2 (glial cells missing 2), which is the mouse homologue of the Drosphilia gene, Gcm, also lack parathyroids. However, these Gcm2 deficient mice had PTH concentrations identical to those of normal mice, indicating an auxillary source of PTH. This auxiliary source of PTH was found to be a cluster of PTH - expressing cells under the thymic capsule. Gcm2 has a role in human parathyroid development as a deletion in a patient has been reported to lead to hypoparathyroidism. These studies of hypoparathyroid patients and mouse models have helped to elucidate some of the genes involved in the embryological development of the parathyroids.

IN17

ANGIOGENESIS AND BONE METASTASIS

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Tumor progression and metastasis is highly dependent on the ability of cancer cells to induce the formation of new blood vessels from pre-existing ones (angiogenesis or neovascularization). It is becoming increasingly clear that metastasis of a variety of osteotropic carcinomas (e.g. breast and prostate) occurs primarily through the lymphatic system and that the extent of lymph node involvement is also strong prognostic indicator.

Tumor-stroma interactions are of primary importance in determining the pathogenesis of metastasis. Gene expression patterns of tumor cells in metastatic lesions in the skeleton or soft tissues were investigated and compared in a mouse model of bone metastasis.

In all metastatic lesions, MDA-MB-231 cells express a variety of angiogenic factors including vascular endothelial growth factors (VEGF-A, -B, and -C). Strikingly, steady-state mRNA levels of VEGF-A and -B by tumor cells were elevated significantly in bone metastases when compared to visceral metastases, indicating tissue-restricted expression of these tumor progression factors.

Several of these angiogenic factor were found to be up-regulated by TGFbeta, that is stored in large amounts in bone matrix and is released during bone turnover.

In conclusion, MDA-MB-231 breast cancer cells express a variety of angiogenic factors in vivo that have been implicated in metastatic bone disease and that correlate with poor survival of patients with breast cancer. The observed up-regulated expression of angiogenic factors by the breast cancer cells in the skeleton may, therefore, underlie the clinically observed osteotropism of breast cancer cells and pathogenesis of bone metastases. Some data from transcriptional profiling studies will be presented and the putative involvement of certain gene families in tumor progression and metastatic bone disease will be discussed.

IN18

THE CENTRAL CONTROL OF BONE MASS

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We previously showed that leptin inhibits bone formation by an undefined mechanism. Here, we show that hypothalamic leptin-dependent antiosteogenic and anorexigenic networks differ, and that the peripheral mediators of leptin antiosteogenic function appear to be neuronal. Neuropeptides mediating leptin anorexigenic function do not affect bone formation. Leptin deficiency results in low sympathetic tone, and genetic or pharmacological ablation of adrenergic signaling leads to a leptin-resistant high bone mass. b-adrenergic antagonist increases bone mass in wild-type and ovariectomized mice. None of these manipulations affects body weight. This study demonstrates a leptin-dependent neuronal regulation of bone formation with potential therapeutic implications for osteoporosis.

IN19

RETHINKING HRT RISKS AND BENEFITS AFTER WHI

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Despite a great deal of observational and laboratory data, most of it compatible with a cardioprotective effect of HRT, a reduction in cardiovascular events has not been shown in randomized clinical trials conducted in postmenopausal women with or without known cardiovascular disease. In fact, several clinical trials have found an early increased risk of CHD and stroke. The most definitive results come from the Women's Health Initiative where there is no evidence of cardiovascular benefit among healthy postmenopausal women in any age group.

Nevertheless, the estrogen hypothesis is not dead, as evidenced by the apparent cardioprotective effects of raloxifene, a selective estrogen receptor modulator. Post hoc analyses of data from an osteoporosis trial suggest about a 40% reduced risk of all cardiovascular disease and a 60% reduced risk of stroke over 4 years.

Thus, although no currently available estrogen therapy seems likely to improve unstabile plaque or prevent a cardiovascular event, current data do not exclude a possible protective effect of *endogenous* estrogen on atherosclerosis. It remains to be seen whether divergent estrogen-receptor mediated or nongenomic effects of raloxifene versus estrogen can explain their apparent divergent effects on clinical disease.

ABSTRACTS

ORAL PRESENTATIONS AND SHORT COMMUNICATIONS

Short Communications will also be exhibited as posters throughout the conference in the Exhibition Hall, 3rd floor. The suffix after the abstract number indicates the poster session in which the poster will be attended (W - Wednesday, F- Friday, S - Saturday).

OR1

TGFBETA IS A PHYSIOLOGICAL REGULATOR OF BONE RESORPTION AND BONE MASS

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The role of TGFbeta in regulation of bone resorption has been difficult to discern from in vitro studies, since in some systems it stimulates osteoclast formation and in others it inhibits. To determine the effects of endogenous TGFbeta on bone resorption in vivo, we generated transgenic mice which express a dominant negative type II TGFbeta receptor (dnTbRII) targeted to bone cells. The expression of dnTbRII transgene was targeted to osteoblasts at early and late stages of differentiation by using the 3.2 kb type I collagen promoter (Col1a1-dnTbRII) and the 1.8 kb osteocalcin promoter (OC-dnTbRII). We analyzed bone mineral density (BMD) and bone histomorphometry in 2, 4 and 6-mo-old OC-dnTbRII and Col1a1-dnTbRII mice. BMD in 4 and 6-mo-old OC-dnTbRII mice was increased 4-5% and BMD in 2, 4 and 6-mo-old Col1a1-dnTbRII mice was increased 6-10% compared with their wild-type (wt) littermates. Bone volume in 4 and 6-mo-old OC-dnTbRII mice was increased 21 and 29%, respectively and bone volume in 2, 4 and 6-mo-old Col1a1-dnTbRII mice was increased 23, 39 and 58% compared with their wt littermates. Osteoclast numbers in 4 and 6-mo-old OC-dnTbRII mice and 2, 4, and 6-mo-old Col1a1-dnTbRII mice were significantly reduced. In contrast, osteoblast numbers and bone formation rates in these mice were not significantly different from wt littermates. In bone marrow cell cultures, TGFbeta induced osteoclast formation in the presence of M-CSF and sRANKL in 5-wk-old wt mice but its activity was significantly reduced in OC-dnTbRII or Col1a1-dnTbRII transgenic mice. In co-culture experiments, osteoblasts isolated from calvariae of OC-dnTbRII or Col1a1-dnTbRII mice had 5-8 fold less activity to support 1,25-(OH)2 D3-induced differentiation of spleen cells (from wt mice) into TRAP-positive multinucleated osteoclasts compared with osteoblasts isolated from wt mice. In OC-dnTbRII and Col1a1-dnTbRII mice, the mRNA expression (RT-PCR) of OPG but not RANKL in bone tissue was enhanced. These results demonstrate that endogenous TGFbeta signaling in osteoblasts at both early and late stages of differentiation is required for normal osteoclast formation in vivo. Since bone volume is increased in the basal state, these results also show that TGFbeta is a physiological regulator of bone mass in vivo.

OR2

PARTNERSHIP BETWEEN NFAT AND C-JUN/C-FOS IS CRITICAL TO RANKL-INDUCED OSTEOCLASTOGENESIS

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Activation of JNK/c-Jun, c-Fos and NF-kappaB upon treatment with RANKL which plays a central role in osteoclastogenesis and genetic studies using transgenic or knockout mice strongly suggest that these signaling molecules are critical to the promotion of osteoclast formation. However, overexpression of JNK/c-Jun/AP-1 and/or NF-kappaB in the hemopoietic osteoclast progenitor cells failed to induce the osteoclastic differentiation, suggesting that activation of these signaling molecules is not sufficient and that participation of an additional interacting molecule is required in the induction of osteoclastogenesis. As a candidate for such a molecule, we studied NFAT. NFAT is a transcription factor known to serve as a partner of AP-1 (c-Jun/c-Fos) in activated T cells and promotes their differentiation. Of note, overexpression of NFAT1 or NFAT2 using adenovirus system induced TRAP-positive osteoclast-like cell (OC) formation in the absence of RANKL in RAW264 cells, demonstrating that NFAT1 is sufficient for induction of osteoclastogenesis. In conjunction, NFAT1 increased the TRAP promoter activity. Moreover, a constitutive active NFAT1, which lacked the negative-regulatory domain, showed greater stimulation of TRAP promoter activity than the wild type NFAT1, while a dominant negative (DN) NFAT1 without the transactivation domain showed no effects. We next examined whether NFAT was associated with RANKL signaling activation along with the osteoclastic differentiation in RAW cells. RANKL increased NFAT expression and enhanced the NFAT-induced TRAP-positive OC formation. Of interest, cyclosporin A, an inhibitor of NFAT activation, suppressed RANKL-promoted TRAP-positive OC formation. Western analysis using the RANKL-treated cell lystaes demonstrated that NFAT1 was activated and translocated to the nucleus. Transcriptional activity of NFAT was up-regulated by RANKL treatment. In addition, overexpression of TRAF6, which is an important mediator of RANKL signaling, also dramatically increased NFAT transcription activity. The effect on NFAT1 on the TRAP promoter activity was enhanced when JNK/c-Jun pathway was activated by overexpressing MKK7-JNK1 and suppressed when AP-1 activation was inhibited by introducing DN-c-Jun or DN-c-Fos. Finally, NFAT-induced osteoclastogenesis was suppressed by overexpression of DN-c-Jun or DN-c-Fos. In conclusion, our results suggest that NFAT alone can promote osteoclastic differentiation and that the osteoclastogenesis was augmented under the cooperation of NFAT with c-Jun/c-Fos.

OR3

TSH NEGATIVELY REGULATES BOTH OSTEOCLAST AND OSTEOBLAST FORMATION AND SURVIVAL: EFFECTS ON JNK SIGNALING AND C-*JUN* TRANSLOCATION

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We report for the first time, that TSH receptors (TSHRs) negatively regulate bone remodeling. TSH null mice generated by disrupting exon 1 of the TSHR gene including the euthyroid heterozygotes were profoundly osteoporotic with a marked reduction in bone mineral density at the femur, tibia and lumbar spine. Histological analysis revealed focal sclerosis, woven bone, and disorganized collagen: hallmarks of rapid bone turnover that are reminiscent of pagetic bone. Tetracycline double labeling showed an increased bone formation rate. Osteoblast progenitors in bone marrow cell cultures, CFU-F and CFU-OB, were also markedly elevated. Likewise, hematopoietic stem cell cultures showed enhanced TRAP-positive osteoclast formation. TSH inhibited both the formation and survival of TRAP-positive osteoclasts. Together, the results argue strongly for the TSHR as a negative regulator of osteoblast and osteoclast formation, function and survival. We next explored the localization of TSHRs to bone cells by following the expression of GFP integrated at the TSHR deletion site. Both CFU-Fs and osteoclast precursors in bone marrow cell cultures derived from TSHR deficient mice showed intense GFP fluorescence. Dual photon confocal scanning microscopy of the inner table of the skull likewise confirmed GFP localization to osteoblasts in situ. That TSHRs were expressed in wild type bone tissue and marrow cell cultures was further confirmed by real time RT-PCR and (or) immunoblotting. Expression of the TSHR coincided with RANK and calcitonin receptor expression at day 2, and preceded TRAP expression. The functional significance of the TSHR in negatively regulating osteoclast formation was further confirmed by demonstrating a marked inhibition of TRAP-positive osteoclast formation from TSHR expressing RAW264-C3 cells. In these cells, TSH inhibited JNK signaling and c-*jun* nuclear translocation achieved by exposure to RANK-ligand. Overall, therefore, the localization of the TSHR to bone cells, its direct regulation of osteoclast and osteoblast formation and function, and the significant impact on bone of its absence reveal a *hitherto* yet uncharacterized role for the TSHR and its ligand, TSH, in the negative regulation of bone remodeling.

OR4

SECRETED FRIZZLED-RELATED PROTEIN INHIBITS TNFALPHA- OR RANKL-DEPENDENT OSTEOCLAST FORMATION

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Wnt signaling is crucial for osteoblast differentiation, apoptosis and the development of bone mass, exemplified by the genetic loss of LRP5, a co-receptor for Frizzled (Fz) family of proteins. Fz proteins can be antagonised by secreted frizzled-related proteins of which there are five members. The sFRPs can bind to Wnt proteins to modulate their activity.

We have found that sFRPs are expressed by the osteoblast and that these influence osteoclast formation. Neutralizing antibodies against sFRP-1 enhanced TRAP positive mononuclear and multinuclear osteoclast formation in cocultures of murine osteoblasts with spleen cells treated with PGE2 (10^{-7}M) and 1,25 $(OH)_2$ vitamin D_3 (10^{-8}M). Recombinant sFRP-1, or the CRD of sFRP-1was able to dose dependently inhibit RANKL-dependent osteoclast formation in either osteoblast/spleen cocultures, RANKL+M-CSF-treated splenic or bone marrow cultures, or in RANKL-treated RAW264.7 cell cultures. In RANKL-independent osteoclast formation assays using RAW264.7 cells treated with TNFalpha+TGFbeta, sFRP-1 also blocked osteoclast formation while OPG did not affect osteoclast formation. The action of sFRP-1 to limit RANKL- or TNFalpha-induced osteoclast formation may be explained in part by the observation that recombinant sFRP-1 binds specifically to RANKL in an ELISA format. This finding raised the possibility that sFRP-1 limits osteoclast formation by binding directly to these TNF family members, and suggested that sFRP-1 may act as a decoy receptor with dual specificity for RANKL and TNFalpha. However other mechanism are also likely. In bone marrow cultures in which TGFbeta was present during the first three days and RANKL present only during the last three days of culture, sFRP-1 and OPG had different actions when added with TGFbeta. sFRP-1 inhibited osteoclast formation, while OPG did not effect osteoclast formation. Further the potential for involvement of the Wnt pathway in osteoclast formation was also explored. RAW264.7 cells as well as adherent splenic cells expressed mRNA for Wnts, Fz, sFRPs, LRP5 and GSK3beta, demonstrating that the Wnt pathway may also impinge upon osteoclast formation. Combined, these findings suggest that sFRP-1 may affect osteoclast formation by binding to TNFalpha or RANKL as well as through direct action upon hematopoietic cells, presumably interrupting Wnt signaling.

OR5 W

OSTEOPROTEGERIN REVERSES SPACEFLIGHT-INDUCED OSTEOPENIA IN FEMALE C57BL/6J MICE

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This experiment characterized the effects of spaceflight (SF) on the skeleton of mice treated with or without OPG, a protein that blocks osteoclast activity. This study represents the first time the effect of microgravity has been examined in mice. 10-week-old female C57BL/6J mice (n=12/group) received a single injection (SC, 24 h pre-launch) of either OPG (20mg/kg) or vehicle (VEH) and spent 12-days in orbit on Space Shuttle flight STS-108. Mass and age-matched ground control (GC) mice had similar treatments.

Upon landing, SF reduced elastic strength at the femoral midshaft in VEH mice, while OPG increased elastic strength in SF mice. From a structural perspective, femur mid-diaphysis cross-sectional area was reduced for SF/VEH compared to GC/VEH, though changes in principle moments of inertia were not significant. Bone mass and composition were altered to a greater extent: SF/VEH femur dry mass was lower compared to GC/VEH mice. OPG significantly increased femoral dry mass in both SF and GC conditions. Femur whole bone mineral mass (Min-M) and percent mineral composition (%Min) were lower in SF/VEH mice compared to GC/VEH. SF/OPG had a greater Min-M and %Min than SF/VEH.

Micro CT analysis of the femoral neck and mid-diaphysis revealed a deficit in BMD of SF/VEH mice compared to GC/VEH. OPG blocked these SF-induced BMD changes. pQCT analysis of the proximal tibia and lumbar spine (L5) demonstrated similar BMD effects of both SF and OPG treatment, indicating a systemic effect.

Serum and mRNA (humeral diaphysis) analyses suggest changes in both bone formation and resorption contributed to the SF-induced osteopenia. A significant decline in mRNA expression of osteocalcin combined with a decline in serum alkaline phosphotase levels indicate a reduction in bone formation. Additionally, SF reduced periosteal and endocortical bone formation rates in the femoral diaphysis. Increased bone resorption in SF mice was suggested by a trend towards increased mRNA expression of the pro-resorptive cytokine RANK ligand and significantly elevated serum TRAP levels. OPG treatment reduced osteoclast surfaces in the proximal tibia by >95% in all groups. . This experiment demonstrates that the mouse is an appropriate model for SF-induced osteopenia, and that OPG is an effective countermeasure.

OR6 W

EXPERIMENTAL STUDY OF CELL DIFFERENTIATION INDUCED BY MECHANICALLY DAMAGED OSTEOCYTES UTILIZING THREE-DIMENSIONAL GEL-EMBEDDED CULTURE SYSTEM

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Bone is continuously subjected to repetitive loading, which leads to microdamages even if the strain level is within physiological range. The damaged site must be promptly removed by a remodeling mechanism that is targeted to microdamages; otherwise, the accumulation of microdamages can lead to clinical bone fractures. Osteocytes have been considered to be a promising candidate to provide a cellular basis for mechanosensing and bone remodeling. However, very little is known about their role in the initiation of remodeling process, i.e. osteoclastic resorption of the damaged bone area. The aims of this study were, therefore, to develop a relevant in vitro model of targeted bone remodeling, and to demonstrate that damaged osteocytes can induce the initiation of bone resorption. In order to apply a mechanical damage to the osteocytes locally, we established a new device in which osteocyte-like cells are cultured inside the collagen gel. Damage of osteocytes is caused by local scratching with a thin stainless steel pick, and can be restricted to only few cells. Bone marrow cells were cultured on the gel containing MLO-Y4 osteocytes. This allowed us to examine the effects of damaged osteocytes to the differentiation of bone marrow cells. When MLO-Y4 cells were cultured inside the gel, a double fluorescent labeling technique demonstrated that gap junctions were joined between the cells. Bone marrow cells cultured on the gel revealed that the existence of MLO-Y4 had an inhibitory effect on the differentiation of TRAP-positive cells. When mechanical scratching induced local damage to osteocytes, enhanced formation of TRAP-positive cells was observed along the scratching path. This demonstrates that damaged osteocytes could locally promote osteoclastic differentiation, and further suggests that the local death of osteocytes provides an important mechanism to target remodeling to microfractures.

OR7 W

OSTEOPETROSIS IN TRANSGENIC MICE CARRYING A DOMINANT-NEGATIVE C-JUN DRIVEN BY THE TRAP PROMOTER

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RANKL is a central cytokine that stimulates osteoclastogenesis. Upon RANKL binding to RANK, cytoplasmic molecules involving JNK, c-Jun, which is a partner of c-Fos in the AP-1 complex, c-Fos, NF-kappaB and p38 are activated, suggesting that these molecules relay RANKL signaling that ultimately leads to the promotion of osteoclast formation. In support of this notion, genetic studies demonstrated that c-Fos and NF-kappaB knockout mice exhibited osteopetrosis due to impaired osteoclastogenesis. On the other hand, since both JNK1/JNK2- and c-Jun-deficient mice are embryonic lethal, the role of JNK and c-Jun in RANKL-induced osteoclastogenesis still needs to be elucidated. In the present study, we generated transgenic mice (DN-c-Jun Tg) carrying dominant-negative (DN) c-Jun driven by the TRAP promoter. The DN-c-Jun Tg manifested severe phenotype of osteopetrosis including a defect of tooth eruption, reduced body size and increased radiodensity in bones. Histological examination showed that the bone marrow cavity was markedly reduced and the number of TRAP-positive osteoclasts (TRAP+OC) was also profoundly diminished. To examine whether the osteopetrotic phenotype seen in DN-c-Jun Tg was due to abnormal osteoclastogenesis and/or increased apoptosis in osteoclasts, we isolated spleen cells from DN-c-Jun Tg or wild type mice and examined the capacity of these cells to form TRAP+OC in the presence of RANKL and M-CSF. The results showed that number of TRAP+ OC formed in spleen cells of DN-c-Jun Tg was dramatically reduced compared with that of wild type mice. Consistent with these in vivo observations, a specific inhibitor of JNK, SP600125, significantly inhibited TRAP+OC-like cell formation in mouse bone marrow cultures. To further prove the importance of JNK/c-Jun in osteoclastogenesis, we generated dominant-negative (DN)-JNK1 and DN-c-Jun and then overexpressed these mutants into mouse monocytic leukemia cell line, RAW264, using adenovirus system. Overexpression of DN-JNK1 or DN-c-Jun markedly suppressed sRANKL-induced c-Jun activation and TRAP+OC-like cell formation. In conclusion, these results collectively suggest that activation of JNK/c-Jun signaling pathway is essential to RANKL-promoted osteoclastogenesis in vitro and in vivo and deepen our insights into the molecular mechanisms by which RANKL regulates the osteoclast differentiation.

OR8 W

PIVOTAL ROLE OF PROAPOPTOTIC BH3-ONLY PROTEIN BIM IN OSTEOCLAST APOPTOSIS AND FUNCTION

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Bcl-2 protein family plays a central role in the signaling events leading to apoptosis in various types of cells. The BH3-only subfamily members are pro-apoptotic molecules, which trigger apoptosis via interaction with anti-apoptotic Bcl-2 members. In an attempt to identify a key initiator of osteoclast apoptosis, we examined the expression of BH3-only proteins in osteoclasts. Osteoclast-like cells (OCLs) generated in co-cultures of mouse osteoblasts and bone marrow cells are subjected to rapid apoptosis in the absence of supporting cells or stimulatory cytokines. Interestingly, the apoptosis stimuli caused rapid increase in the protein level of Bim in the cells as determined by Western blotting, while the expression of other members did not show such dramatic changes. This increase appears to be due to the posttranscriptional regulation because Bim mRNA level did not change after the stimuli as determined by RNase protection assay and RT-PCR. Immunohistochemical analysis showed the time-dependent translocation of Bim to the mitochondrial membrane, which corresponded to the cytochrome c release to the cytosol. To further elucidate the role of Bim in osteoclast apoptosis, we utilized adenovirus vector expression system and Bim -/- mice. Adenovirus vector-induced overexpression of Bim induced rapid apoptosis in OCLs, which was inhibited by co-expression of the anti-apoptotic Bcl-2 family protein, Bcl-XL or Bcl-2. OCLs differentiated from Bim -/- bone marrow cells showed remarkable resistance to the apoptosis stimuli, and almost 100% cells survived after 48hrs of cytokine withdrawal compared to 0% survival of wild type OCLs. Although Bim-/- OCLs showed marked resistance to apoptosis, their pit forming activity was significantly reduced, consistent with the observation that Bim-/- mice showed mild osteopetrosis in spite of the reduced number of apoptotic osteoclasts. Taken together, proapoptotic BH3-only protein Bim is the key regulator of both apoptosis and survival of osteoclasts.

OR9 W

REGULATION OF MOUSE OSTEOPROTEGERIN GENE EXPRESSION BY STEROID HORMONES

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Glucocorticoid-induced osteoporosis is a serious complication of systemic glucocorticoid use. It is generally accepted that glucocorticoids increase bone resorption in vitro as well as in vivo. In cocultures of ST2 cells and bone marrow macrophages, 1,25 dihydroxyvitamin D_3 supports osteoclastogenesis, and dexamethasone (Dex) administration synergistically stimulates it. To explore the precise mechanism whereby Dex stimulates osteoclastogenesis, we analysed the effect of Dex on cis-acting elements of the mouse osteoprotegerin (OPG) and the receptor activator of NF-kappaB ligand (RANKL) gene. The 5'-flanking region of the mouse OPG gene was cloned, and a series of deletion constructs ligated with a pGL3-Basic vector plasmid (Luc -116, Luc -697, Luc -1125, Luc -1487) were transfected into ST2 cells and subjected to luciferase assay. Transfected cells were treated with Dex (10^{-7}M) for 48h to assess its effect on the OPG promoter activity. Mirroring Northern blot analysis, Dex reduced the promoter activity of Luc -1487 to 50%. Electrophoretic gel motility shift assay (EMSA) carried out to determine protein-DNA binding on the putative AP-1 binding site (-293/-287, TGACTGA) showed specific binding to the putative AP-1 site and the supershift with an anti c-Jun antibody; the mutation of the putative AP-1 site (TGACTGA to CTCCCTC) nullified the protein-DNA binding. A construct containing the mutated AP-1 binding site (Luc -1487m) showed reduced promoter activity. Moreover, the Luc -1487m construct was resistant to Dex-driven suppression. To assess the effect of Dex on the OPG at the protein level, the amount of OPG protein secreted by ST2 cells into the culture medium was measured by sandwich enzyme-linked immunosorbent assay (ELISA). Dex downregulated the OPG gene both at mRNA and protein levels. Dex may negatively regulate OPG mainly by transrepressing OPG promoter activity through the AP-1 site. On the other hand, Dex slightly increased the expression of RANKL mRNA probably through the GRE half sites (-642/-628) in the RANKL promoter. We speculate therefore that glucocorticoids per se promote osteoclastogenesis mostly by inhibiting OPG and partly by concurrently stimulating RANKL reciprocally, thereby enhancing bone resorption.

OR10 W

NEMO (NF-KB ESSENTIAL MODULATOR) BINDING DOMAIN (NBD) PEPTIDE PREVENTS OSTEOCLAST DIFFERENTIATION IN VITRO AND IN VIVO

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Inflammatory bone destruction that accompanies diseases such as periodontitis and arthritis occurs because of an increase in the number of osteoclasts responsible for bone resorption. Osteoclast differentiation requires the activity of the inducible transcription factor NF-kB as gene targeting studies have demonstrated that mice lacking the p50 and p52 NF-kB subunits are deficient for osteoclasts. Consequently it is possible that selective inhibition of NF-kB activation in osteoclast precursors would prevent osteoclast differentiation and provide the basis for therapeutically effective drugs for the treatment of inflammatory bone disease.

We have recently generated a cell permeable peptide that disrupts the IkB kinase (IKK) complex, a critical upstream signaling component required for pro-inflammatory NF-kB activation. This peptide blocks the interaction of the regulatory subunit named NEMO (NF-kB essential modulator) with the catalytic components of the IKK complex and we have named this the NEMO binding domain (NBD) peptide. We therefore investigated the effects of the NBD peptide on osteoclast differentiation and found that it inhibited osteoclastogenesis in RANK (receptor activator of NF-kB) ligand-stimulated bone marrow macrophages (BMMs) in vitro and more importantly, prevented LPS-induced osteoclastogenesis in vivo by decreasing the number of osteoclast precursors. Furthermore, treatment with the NBD peptide significantly reduced the incidence and severity of arthritis in an experimental model of collagen-induced arthritis in which we observed abrogation of joint swelling and reduced destruction of bone and cartilage. These results therefore strongly suggest that inhibition of NF-kB by the NBD peptide offers an effective novel strategy for therapeutically inhibiting inflammatory bone resorption.

OR11 W

REGULATION OF C-SRC ACTIVITY, ACTIN RING FORMATION AND BONE RESORPTION BY THE CSK FAMILY TYROSINE KINASE, CHK

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C-Src is essential for osteoclasts to resorb bone. It has been shown that c-Src activity is negatively regulated by the tyrosine kinase Csk, which selectively phosphorylates a tyrosine residue in the c-terminal portion of c-Src. Since Csk is ubiquitously expressed and thus unlikely to specifically regulate c-Src activity in osteoclasts,we attempted to identify an osteoclast-specific Csk-like kinase in a human osteoclast cDNA library. Using the PCR nested cloning technique, we isolated a Csk-like kinase Chk from this library. Biochemical studies showed that Chk phosphorylated the tyrosine527 in the c-terminus of c-Src and inhibited c-Src kinase activity. Western analysis demonstrated that Chk was restrictedly expressed in osteoclasts and brain, whereas Csk expression was observed in most tissues. Immunohistochemical examination using human bones showed that Chk was specifically expressed in the ruffled borders in bone-resorbing osteoclasts. To further explore the interactions between Chk and c-Src in osteoclasts, we determined the subcellular localization of Chk and c-Src in TRAP-positive osteoclast-like cells that formed in bone marrow cells isolated from either wild-type (WT) or c-Src-deficient (src-/-) mice. In WT osteoclasts, Chk was co-localized with c-Src in the actin rings. In contrast, src-/- osteoclasts exhibited disrupted actin ring formation and diffuse Chk expression in the cytoplasm. Introduction of c-Src into src-/- osteoclasts using adenovirus system restored the actin ring formation and bone resorption. To investigate the effects of Chk in actin ring formation, c-Src activity and bone resorption, we overexpressed Chk into bone marrow cells before actin ring formation took place. We found that Chk overexpression profoundly disrupted actin ring formation and reduced c-Src activity in these cells. Moreover, a c-Src substrate cortactin which co-localized with c-Src in the actin ring dispersed in the cytoplasm following Chk overexpression. Most importantly, these cells demonstrated abrogated bone resorption as determined by the pit assay. Infection of control virus had no effects on actin ring formation and bone resorption. In conclusion, our results suggest that Chk modulates the actin organization and bone-resorbing activity in osteoclasts by negatively regulating c-Src activity and that the interactions between Chk and c-Src are critical in the regulation of bone resorption.

OR12 W

RANKL STIMULATED OSTEOCLAST-LIKE CELL FORMATION IN VITRO IS PARTIALLY DEPENDENT ON ENDOGENOUS INTERLEUKIN-1 PRODUCTION

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Receptor activator of NF-kB ligand (RANKL) and interleukin-1 (IL-1) individually play a critical role in differentiation and activation of osteoclasts in bone. In addition, both RANKL and IL-1 activate similar signal transduction pathway(s) including P38 MAP kinase and c-Jun NH_2 terminal kinase (JNK). We examined if endogenously produced IL-1 influenced osteoclast-like cell (OCL) formation in murine bone marrow and bone marrow monocyte cultures (BMM) that were stimulated with M-CSF and RANKL. RANKL stimulated OCL formation in a dose dependent manner in bone marrow cultures and this response was significantly inhibited by IL-1 RA (100 ng/ml), a specific IL-1 antagonist. Interleukin-1 further increased OCL formation in BMM cultures that were treated with M-CSF (30 ng/ml) and RANKL (1, 3, 10 and 30 ng/ml). In addition, BMM cultures from IL-1 type I receptor deficient mice, which do not respond to IL-1, showed a significant decrease in OCL formation in all groups. To investigate if IL-1 enhanced OCL formation by inhibiting apoptosis, we studied mice that globally overexpress BCL-2. BMM from BCL-2 overexpressing mice showed increased OCL formation compared to wild-type mice with M-CSF and RANKL. However, addition of IL-1 produced no added effect on OCL number.

We examined the time course and dose response of IL-1alpha protein expression by ELISA in BMM cultures that were treated with or without M-CSF and RANKL (both at 30 ng/ml). RANKL stimulated IL-1alpha protein significantly (46%) only in 6-day cultures. RANKL also dose dependently stimulated IL-1alpha protein levels with significant effects at 10 and 30 ng/ml in 6-day cultures. The combination of RANKL and IL-1 showed synergistic effects on JNK activity (872 unit/ml/mg protein) compared to RANKL (435 unit/ml/mg protein) or IL-1 (107 unit/ml/mg protein) alone. RANKL and IL-1 together increased phospho-P38 levels additively (26 fold) compared to RANKL (23 fold) or IL-1 (9 fold) alone.

These results demonstrate that endogenously produced IL-1 augments the response of BMM cells to RANKL by mechanisms that appear dependent on inhibition of apoptosis and may involve MAP kinases.

OR13 W

ROLES OF CBL PROTEINS IN OSTEOCLAST FUNCTION

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The mammalian members of Cbl family proteins c-Cbl and Cbl-b are predominantly expressed in hematopoietic cells and function as adaptors and /or ubiquitin ligases mediating down regulation of both receptor and non-receptor tyrosine kinases. Even though c-Cbl and Cbl-b are homologous in their phosphotyrosine binding domain and RING finger, there are significant differences between the two proteins in their C-terminal proline-rich and acidic regions. These structural variations result in interactions with different sets of signaling molecules, contributing to protein-specific roles in signaling and regulation of cell function. One such difference is the mechanism of interaction of the p85 subunit of PI3 kinase with Cbl proteins. The SH2 domain of p85 binds to c-Cbl via the Src-dependent phosphorylation of Y731. In contrast, Cbl-b interacts with p85 despite the lack of Y731 via proline rich domain. Unlike c-Cbl, over-expression of kinase-dead Src has no effect on the interaction between p85 and Cbl-b. These differences in binding properties also translate into the functional differences in OCs. Deletion of the c-Cbl gene alters the ability of OCs to migrate in vitro and in vivo during development, delaying bone resorption and cartilage ossification in long bones, although detailed histomorphometric analysis of the adult bones failed to demonstrate any changes in bone volume or bone resorption parameters. In contrast to c-Cbl-/- mice, 12 wks old Cbl-b-/- mice show decreased trabecular bone volume (4.6 ± 1.6 for Cbl-b-/- vs. 7.7 ± 1.1 for wt, mean ± SD, $p < 0.01$), while no changes in osteoblast or OC surface and number are observed. Dynamic parameters of bone formation were also unaffected. In vitro pit assay revealed that the pit area resorbed by Cbl-b-/- OCs was 2.5 fold higher than wt control OCs, in contrast to OCs derived from c-Cbl-/- (wt, 0.044 ± 0.017; c-Cbl-/-, 0.039 ± 0.009; Cbl-b-/-, 0.110 ± 0.02, $p<0.01$ vs. wt; pit area/total area/number of OCs, mean ± SD). Collectively these data suggest that the two Cbl proteins play specific roles in osteoclast function, which cannot be compensated by the other.

OR14 W

STRUCTURE AND FUNCTIONAL ELEMENTS OF HUMAN AND MOUSE RANKL GENE PROMOTER

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Receptor activator of NF-kappa B ligand (RANKL) has been identified as requisite for osteoclastogenesis. To elucidate the molecular mechanism controlling RANKL gene expression, human and mouse RANKL promoters were cloned and characterized. The basic promoter structure of both mouse and human gene is well preserved and composed of inverted TATA- and CAAT-boxes and a consensus binding site of RUNX2, indicating the importance of these structures in the gene regulation. Vitamin D, one of the major calcium regulators, accelerated osteoclastogenesis by upregulating RANKL gene expression at the transcriptional level in both human and mouse osteoblastic cells. In the human promoter, VDR-RXR alpha heterodimers bound to the direct repeat of the steroid response elements and conveyed 1alpha,25(OH)2D3 signaling. In the mouse RANKL promoter, VDRE was also located in the about 1 kb upstream of the basic promoter as an inexact direct repeat of steroid response elements; not RXR alpha but RXR beta formed heterodimers with VDR to bind to the VDRE. RANKL gene was thus demonstrated to be a Vitamin D-responsive gene. PTH/PTHrP has also been recognized to promote osteoclastogenesis through RANKL induction on osteoblastic cells, and we further analyzed its effect on RANKL promoters. Transfection studies with the human and the mouse promoter constructs showed that 6-12 hours treatment with PTH/PTHrP increased in the RANKL transcriptional activity. Forskolin enhanced the promoter activity; furthermore, H89 abolished the inductive effect of PTHrP and forskolin, indicating that PTH/PTHrP promotes RANKL transcription through the PKA pathway. By EMSA and transfection studies with the deletion constructs, a cAMP-response element (CRE)-like sequence was identified in the human (-1650) and the mouse (-940) RANKL promoters: a CRE-like site of the mouse promoter was overlapped by VDRE. Both human and mouse CRE-like sequences showed specific binding to the nuclear extract from PTHrP-treated osteoblastic cells and the supershifted complexes with anti-CREB-1 and ATF-2 antibodies. A functional CRE was preserved in human and mouse RANKL promoters. Thus we characterized the molecular mechanism by which major calcium regulators act on RANKL gene expression.

OR15 W

SMALL GTP-BINDING RAB3D IS INVOLVED IN POST-TGN SECRETORY VESICLE TRANSPORT NECESSARY FOR BONE RESORPTION

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Osteoclasts are terminally differentiated multinucleate cells responsible for the physiological and pathological degradation of mineralised bone. Osteoclastic bone resorption is a highly dynamic process that requires the tight ordering of intracellular trafficking events in order to maintain the structural and functional polarization of the ruffled border and basolateral domains. Here, in search of novel molecules related to the control of exocytosis in osteoclasts we have identified the small Rab GTP-binding protein Rab3D as a putative regulator of secretory vesicle transport by a degenerative PCR-based approach. Using a combination confocal immunofluorescence and time-lapse microscopy, Rab3D was found to localize and direct post-TGN vesicular transport and fusion events. This subcellular localisation was redistributed in the presence of increasing doses of pamidronate indicating that the anti-resorptive inhibited the isoprenylation and membrane attachment of Rab3D. Moreover, in an attempt to delineate the biological function of Rab3D we have generated a number of RAW.264.7/osteoclast precursor stable cell lines over-expressing EYFP-tagged wild-type Rab3D and Rab3D mutants deficient in GTP/GDP hydrolysis and prenylation and examined their effects during osteoclastic bone resorption. Over expression of 'constitutively activate' Rab3DQ81L resulted in the enlargement and aggregation of Rab3D compartments. In contrast, expression of 'dominant-negative' Rab3DN1351 restricted Rab3D trafficking at the TGN in osteoclasts and their mononuclear precursors indicating that the TGN serves as a site of Rab3D recruitment and activation. Finally, osteoclasts over expressing Rab3DN1351 displayed impaired resorption capacity. Our results implicate a functional role for Rab3D in the early stages of post-TGN secretory vesicle transport in osteoclasts during bone resorption.

OR16 W

THE SMALL GTP-BINDING PROTEIN RAC IS INVOLVED IN SIGNAL TRANSDUCTION OF OSTEOCLAST SURVIVAL

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Although recent findings suggest that osteoclast survival is regulated through interactions of various cytokines and molecules, the precise molecular mechanism is not fully understood. We previously reported that small GTP-binding protein (G-protein) Ras plays an important role in osteoclast survival by regulating ERK activation. Rac is a member of Rho family small G-proteins, which is known to be involved in the cytoskeletal organization. Recent studies, however, have revealed that Rac also mediates anti-apoptotic signals in some types of cells. In the present study, we examined the role of Rac in osteoclast survival using adenovirus vector expression system. We constructed the adenovirus vector carrying either cDNA of fusion protein of EGFP and dominant negative Rac (Rac^{DN}) or constitutively active Rac (Rac^{CA}) gene, and osteoclast-like cells (OCLs) generated in mouse co-culture system were infected with these viruses as well as the control virus (EGFP virus). Clear induction of these molecules in OCLs was demonstrated by green fluorescence and Western blot

analysis. To examine the role of Rac in osteoclast survival, OCLs were purified by removing the osteoblasts 24 hrs after infection. After 24 hrs of osteoblast removal, almost 100% of control virus-infected OCLs became apoptotic in the absence of stimulatory cytokines. Overexpression of Rac^{CA} enhanced OCL survival in the absence of survival cytokines, and more than 30% of the cells survived after 24 hrs. Macrophage colony-stimulating factor (M-CSF), which has strong anti-apoptotic effects on OCLs, activated Rac as early as 1 minute after application to OCLs. Anti-apoptotic effects of M-CSF on OCLs was suppressed by overexpression of Rac^{DN}. The pro-survival effect of Rac^{CA} was completely abrogated by the treatment with phosphatidylinositol 3-kinase (PI3-kinase) inhibitor, LY294002 or wortmannin. Rapamycin, mTOR inhibitor also had a potent inhibitory effect, while MEK inhibitor PD98059 showed only partial inhibition. Consistent with these results, phosphorylation of Akt by M-CSF treatment was completely blocked in Rac^{DN}-expressed OCLs. These data suggest that Rac lies downstream of M-CSF receptor signaling, and mediates survival signal of OCLs mainly via PI-3 kinase pathways.

OR17

HIP PROTECTORS FOR PREVENTION OF HIP FRACTURE: RESULTS OF TWO AUSTRALIAN RANDOMIZED TRIALS

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The value of external hip protectors for prevention of hip fractures remains uncertain, partly because of flaws in study methodologies and also because all studies to date have involved older people living in residential care facilities, not the community. We have recently completed two randomized trials of external hip protectors in Sydney, Australia. One trial involved women living in residential care facilities and the other women living in the community.

There were 174 women (mean age 85 years) in the residential care study, from 32 different aged care facilities. Women were individually randomized and then followed for 18 months. There were 15 hip fractures during follow-up. Adherence with use of hip protectors was about 60%. Hip protectors did not reduce the risk of hip fracture in this study: relative risk (RR) 1.46, 95% confidence interval (CI) 0.53-4.51 (intention-to-treat analysis). One hip fracture occurred while a hip protector was being worn, but the protector was being worn incorrectly.

The community study involved 600 women (mean age 83 years) who had had two or more falls or a fall requiring hospital admission in the past year. Women were individually randomized and followed for two years. There were 43 hip fractures during follow-up. Adherence was just above 50%. Hip protectors did not reduce the risk of hip fracture in an intention-to-treat analysis (RR 0.92, 95% CI 0.51-1.68). However, the risk of hip fracture was much lower in falls that occurred while wearing hip protectors than in unprotected falls (RR 0.24, 95% CI 0.08-0.72). Four women suffered hip fractures while wearing properly applied hip protectors.

The community study, and biomechanical data, show that hip protectors can prevent most hip fractures if they are worn at the time of a fall on the hip. However, adherence needs to be markedly improved if these devices are to have a major impact on the hip fracture epidemic.

OR18

EFFECTIVENESS OF HUMANIZED ANTI-INTERLEUKIN-6 RECEPTOR MONOCLONAL ANTIBODY (MRA) IN SECONDARY OSTEOPOROSIS COMPLICATED WITH RHEUMATOID ARTHRITIS

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Interleukin-6 (IL-6) is a representative proinflammatory cytokine and plays a central role in the pathogenesis of synovitis, periarticular and systemic osteoporosis in rheumatoid arthritis (RA). We evaluate the efficacy of humanized anti-IL-6 receptor monoclonal antibody MRA on bone metabolism in active RA. After six patients with active RA were randomized to an intravenous infusion of 0, 4, 8 mg/kg of MRA every 4 weeks for 3 months, all patients received 8 mg/kg of MRA every 4 weeks for 36 weeks. All of 6 patients were allowed to continue taking prednisolone (PSL, <10 mg/day). The treatment with MRA was well tolerated and no adverse effect was observed. The mean percent reduction in the tender and swollen joint counts at 48 weeks was 89 and 85% respectively. CRP (3.1 to 0.0 mg/dl, $P<0.01$) and ESR (60 to 6 mm/hr, $P<0.01$) and serum matrix metalloprotease-3 (MMP-3, 281 to 48.8 ng/ml, $P<0.05$) were markedly reduced at 48 weeks. Surprisingly, bone mineral density, which was evaluated by DEXA of L_{2-4}, increased in 5 out of 6 patients and 1 patient showed more than 10% improvement from a base line value, despite the patient was taking PSL with highly active RA. Furthermore, while urine NTx level was not changed, serum osteocalcin (OC) level was significantly increased (6.0 to 11.3 ng/ml, $p<0.05$). Thus, the specific inhibition of IL-6 signaling alleviated arthritis and serum inflammatory markers and improved secondary osteoporosis, implying that IL-6 plays a pivotal role in a complicated cytokine network and cell to cell and/or matrix interaction in RA. Among them, it is noteworthy that the antibody significantly decreased bone formation marker OC and MMP-3, indicating that IL-6 not only induces osteoclastogenesis and matrix metalloprotease synthesis but may inhibit bone matrix production of osteoblasts via unknown mechanisms. These results also suggest therapeutic efficacy of MRA on the improvement of joint and bone destruction as well as secondary osteoporosis in RA.

OR19

BONE MINERAL DENSITY UNDER-REPRESENTS THE SKELETAL EFFICACY OF TERIPARATIDE [RECOMBINANT HUMAN PARATHYROID HORMONE (1-34)] IN OVARIECTOMIZED MONKEYS.

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The functional quality of bone was shown previously to be dependent upon bone mass and geometry, both of which can be measured by dual energy x-ray absorptiometry (DXA) as bone mineral density (BMD), bone mineral content (BMC) and projected area, with BMD = BMC/area. BMD, BMC and projected area were evaluated separately for lumbar vertebrae (L2-4) in a randomized, placebo controlled study of ovariectomized cynomolgus monkeys (Macaca fascicularis) treated with teriparatide (rDNA origin) injection [TPTD, rhPTH (1-34)]. Monkeys were injected subcutaneously with 0 (sham and ovariectomized controls (Ovx)), 1 (TPTD1), or 5 microg/kg/d (TPTD5) teriparatide for 18 months. Lumbar vertebrae 2-4 were analyzed by DXA after 0, 6, 12, 15, and 18 months of treatment. A mixed model repeated measures (MMRM) approach was used to analyze the longitudinal data collected over 18 months, with least squares means compared at each time point via contrast statements. BMC increased dose dependently by 21% relative to baseline for TPTD5, with significance observed for both treatment groups at each time point ($P<0.002$) and compared to Ovx ($P<0.004$). BMD increased dose dependently by 15% relative to baseline for TPTD5, with significance observed at each time point ($P<0.001$) and compared to Ovx ($P<0.001$). Interestingly, TPTD5 projected area increased by 5% relative to baseline with significance observed at each time point ($P<0.005$). Failure testing of excised L 3-4 at study termination showed dose-dependent increase of vertebral strength (yield force, Fy) by 41% ($P<0.001$) over Ovx controls. Excellent correlation of BMC to vertebral strength (Fy) was observed with $R= 0.7$ ($P<0.001$). Therefore, analysis of DXA and biomechanical parameters showed that BMD under-represents the beneficial skeletal effects of teriparatide, because both BMC and projected area increased with treatment, as expected for an agent that stimulates new bone formation. Furthermore, correlation analyses showed that both BMC and projected area are beneficial to bone quality.

OR20

LOW MAGNITUDE MECHANICAL LOADING INCREASES TIBIAL TRABECULAR BONE MINERAL DENSITY IN CHILDREN WITH DISABLING CONDITIONS: ARE BIGGER SIGNALS NECESSARILY BETTER?

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Functional loading of the skeleton has long been known to play a critical role in defining bone mass and morphology. Recent animal evidence of the anabolic potential of extremely low-magnitude, high frequency mechanical stimuli prompted a study to evaluate the ability of such signals to enhance bone density in children.

A heterogeneous group of twenty pre and post pubertal ambulant children with disabling conditions (14 males, 6 females; mean (SD) age 9.1 (4.3), range 4-19 years) were randomised to standing on active (n=10; 0.3G, 90Hz) or placebo (n=10) devices for 10 minutes/day, 5 days/week for 6 months. Pre-&-post trial proximal tibial and spinal (L2) volumetric trabecular bone mineral density (vTBMD) (mg/ml) was measured by 3-D quantitative computer tomography (QCT).

Over the six month trial, the mean change in proximal tibial vTBMD in children who stood on placebo devices was –6.65 mg/ml (–8.4%), while children who stood on active plates was +11.55 mg/ml (+11.6%), representing an 18.2mg/ml benefit of treatment (20.5%) (95% CI: 7.31, 29.09; p=0.0036). The net benefit of treatment, as compared to placebo, in spinal vTBMD was 3.79 mg/ml, (95% CI: –6.35, 13.93; p=0.43). Compliance was 44% (4.4 minutes per day), as determined by mean time on treatment (567.9 minutes) compared to expected time on treatment over the 6 months.

This randomised, double blind, placebo controlled, trial indicates that low magnitude, high frequency mechanical stimuli are anabolic to trabecular bone in humans, perhaps by providing a surrogate for suppressed muscular activity in the disabled. The treatment offers a non-invasive, non-pharmacological and safe approach to improving trabecular vBMD in the lower limbs of children with disabling

conditions. In essence, these data suggest that bigger mechanical signals may not necessarily be 'better' signals in terms of functional influences on the morphology of the skeleton.

OR21

PREVENTION AND TREATMENT OF GLUCOCORTICOID INDUCED OSTEOPOROSIS: A COMPARISON OF CALCITRIOL , VITAMIN D PLUS CALCIUM AND ALENDRONATE

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High dose corticosteroids, used for many medical conditions, are associated with rapid bone loss from sites such as the vertebrae and compression fractures can be observed within months. Recent trials suggest treatment with bisphosphonates or active vitamin D analogues can reduce bone loss and the risk of fracture associated with glucocorticoids but few studies have directly compared such agents. We conducted a randomised, multicentre, open label trial to compare the efficacy of alendronate, calcitriol and simple vitamin D in prevention and treatment of glucocorticoid induced bone loss. 195 subjects (134 females and 61 males) commencing or already taking glucocorticoids. were randomised to one of three groups: calcitriol 0.5 to 0.75mcg/day; simple vitamin D (ergocalciferol 30,000 IU weekly) plus calcium carbonate (600mg daily); or alendronate 10mg/day plus calcium carbonate (600mg daily). Over 2 years mean lumbar BMD change was 5.9% with alendronate, -0.5% with ergocalciferol and -0.7% with calcitriol ($p<0.001$). At the femoral neck, there was no significant difference in BMD change between the treatments over 2 years; alendronate (+0.9%), ergocalciferol (-3.2%) and calcitriol (-2.2%). Lumbar bone loss varied according to whether patients were starting or receiving chronic glucocorticoids and there was a significant treatment x prior glucocorticoid use interaction effect. Six of 66 calcitriol subjects, 1 of 61 ergocalciferol subjects and 0 of 64 alendronate subjects sustained new vertebral fractures. These data do not suggest any difference between simple vitamin D and calcitriol, but demonstrate that alendronate was superior to either treatment for glucocorticoid induced bone loss.

OR22 W

RISEDRONATE PRESERVES TRABECULAR ARCHITECTURE IN EARLY POSTMENOPAUSAL WOMEN IN JUST 1 YEAR: A PAIRED BIOPSY STUDY USING 3-DIMENSIONAL MICROCOMPUTED TOMOGRAPHY

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Risedronate has been shown to reduce vertebral and nonvertebral fractures, including those at the hip, in osteoporotic postmenopausal women. Morphometric vertebral fracture risk is reduced by up to 70% within the first year of therapy. Understanding the relationships between bone mass and bone quality (turnover, architecture and material properties) is fundamental in the study of therapies for osteoporosis. Deterioration of bone quality, which is not measured by BMD, contributes to fractures. Preservation of bone architecture contributes to bone strength and, consequently, to reduced fracture risk. The objective of the present study was to determine the effect of risedronate treatment on trabecular architecture in early postmenopausal women. Paired (baseline and 1 year) iliac crest bone biopsy samples were obtained from women (6-60 months postmenopausal) who were enrolled in a double-blind, placebo-controlled study evaluating the effects of risedronate (5 mg/daily) on lumbar spine (LS) BMD. Trabecular architecture was measured in bone biopsies (n = 12 placebo pairs; n = 14 risedronate pairs) using three-dimensional microcomputed tomography (3-D micro-CT). After 1 year, LS BMD decreased by 3.3% from baseline in the placebo group but increased by 2.1% in the risedronate group. The placebo group showed decreases versus baseline in bone volume, BV/TV, ($P = 0.0342$), and trabecular number

($P = 0.0522$), a trend toward reduced trabecular thickness ($P = 0.0923$), and increases in trabecular separation ($P = 0.0562$) and star volume ($P = 0.0397$). These changes indicate a deterioration of trabecular architecture. In the risedronate group, the bone volume and the architectural parameters did not change significantly from baseline. The pair-wise differences (1 yr-baseline) between risedronate and placebo were significantly different for each of the aforementioned parameters. In summary, risedronate preserves trabecular architecture in postmenopausal women within 1 year, and this may partly explain how treatment with risedronate results in a greater reduction in fracture risk than would be expected based on the increase in BMD alone.

OR23 W

PREDICTION OF THE BIOMECHANICAL STRENGTH OF BONE BY ANALYSIS OF LOCAL 3D-SCALING PROPERTIES EXTRACTED FROM HIGH RESOLUTION MRI OF HUMAN TRABECULAR BONE IN COMPARISON WITH BONE MINERAL DENSITY IN VITRO

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PURPOSE: A novel tool for the diagnosis, prediction of fracture risk and follow-up in the context of osteoporosis is introduced. We apply a non-linear structural measure based on local 3D-scaling-properties to high resolution magnetic resonance images (HR-MRI) of human trabecular bone specimens in order to predict their biomechanical strength in vitro and compare the diagnostic performance with bone mineral density (BMD).

METHOD AND MATERIALS: Axial High Resolution MR images (voxel size 117x156x300microm) of 30 femoral and 11 spinal bone cubes (volume 1.73cm^3) harvested from human cadaveric specimens were obtained at 1.5 T using a 3D-Gradient-Echo sequence. Following measurement of BMD by quantitative computed tomography (QCT) all specimens were tested destructively for maximum compressive strength (MCS). The MR images were analyzed using an algorithm based on the analysis of local 3D-scaling properties to extract the new non-linear texture measure deltaP(alpha). Furthermore, from the image data, two conventional morphological parameters, namely apparent bone fraction (app.BV/TV) and apparent trabecular spacing (app.Tb.sp), were determined.

RESULTS: R^2 for MCS versus DP(a) for the vertebral specimens was 0.85 ($p<0.001$), for the femoral specimens 0.62 ($p<0.001$). Correlation between BMD and MCS was R^2=0.55 for the vertebral and R^2 0.77 ($p<0.001$) for the femoral specimens. In the femoral and vertebral specimens, for app.BV/TV R^2 was 0.34 and 0.54 and for app.Tb.Sp 0.43 and 0.51, respectively. By combining BMD and both morphological measures by means of a multiple correlation analysis, for the femoral samples prediction of MCS could be improved over deltaP(alpha) or BMD alone (R^2=0.86). For the spinal specimens, the combination of two or more parameters did not improve the predictive potential of deltaP(alpha).

CONCLUSIONS: A non-linear structural parameter based on local 3D-scaling properties can effectively predict the biomechanical strength of human trabecular bone specimens in vitro when applied to HR-MR images. The new measure is superior to conventional morphological parameters and - depending on the anatomic site - is equally or better suited to predict strength than BMD - the present gold-standard parameter in clinical routine.

Our results indicate that microstructure - along with the degree of mineralization - contributes substantially to the strength of bone tissue.

OR24 W

MILK SUPPLEMENTATION REDUCED BONE LOSS IN POSTMENOPAUSAL CHINESE WOMEN IN MALAYSIA

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Dietary studies often report low calcium intake amongst post-menopausal Malaysian women and calcium deficiency has been implicated as part of the etiology of age-related bone loss leading to osteoporosis. Therefore, the objective of this study was to examine the effectiveness of high calcium, skimmed milk to reduce bone loss in Chinese postmenopausal women. A total of 173 subjects aged 55 to 65 years and who were more than 5 years postmenopausal were randomized to a control group (n=82) and a milk group (n=91). The milk group consumed 50g of high-calcium, skimmed milk (ANLENE GOLD^{TM}, provided by New Zealand Milk, Wellington, New Zealand) daily, which contained 1200 mg calcium (taken as two glasses of milk a day). The control group continued with their usual diet. The milk supplement was found to significantly reduce the percentage of bone loss at the total body (-0.13%)compared to the control group (-1.04%) at 24 months ($p<0.001$). At the lumbar spine, the percentage of bone loss in the control group was significantly higher (-0.90%) when compared to the milk (-0.13%) supplemented group at 24 months ($p<0.05$). Similarly, milk supplementation reduced the percentage of bone loss at the femoral neck (control 1.21%, milk 0.51%)($p<0.01$) and total hip (control 2.17%, milk

0.50%)($p<0.01$). The control group had significantly ($p<0.05$) higher serum parathyroid hormone levels than the milk group at the end of 24 months. The serum 25-OH vitamin D_3level improved significantly ($p<0.01$) from baseline in the milk

group at the end of the study. In conclusion, ingestion of high calcium, skimmed milk was effective in reducing the rate of bone loss at clinically important lumbar spine and hip sites in these postmenopausal women.

OR25 W

ZOLEDRONIC ACID ADMINISTERED AS A SINGLE INTRAVENOUS DOSE PRESERVES MECHANICAL PROPERTIES AND MAINTAINS CORTICAL- AND CANCELLOUS BONE STRUCTURES IN OVARIECTOMIZED RATS

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The aim of our study was to investigate the duration of bone protective effects in ovariectomized rats of a single intravenous dose of zoledronic acid (ZA). Seven-month-old virgin Wistar rats were ovariectomized and treated with a single intravenous injection of ZA at doses of 0.8, 4, 20, 100 or 500 microg/kg. Changes in cancellous and cortical bone were measured in the proximal tibial metaphysis at 4-weekly intervals for 32 weeks by quantitative computed tomography. Mechanical testing was performed on the 4th lumbar vertebral body, the distal femoral metaphysis and on the femoral shaft.

OVX-rats showed rapid cortical thinning with a loss of 26% at week 32 ($p<0.01$). Cancellous bone mineral density decreased to equilibrate at 55% ($p<0.01$). A single i.v. administration of 0.8 microg/kg ZA offered mild but significant protection against cortical- and cancellous bone loss up to week 4 and 20, respectively. At 4 microg/kg, partial protection against cancellous bone loss was still visible at week 32 while cortical thinning was reduced significantly up to week 12. Full protection of all cancellous and cortical bone parameters was achieved at 20 microg/kg, while higher doses even resulted in a 5% increase in cortical thickness over time compared to sham-operated controls.

Ovariectomy induced significant loss of bone strength at all three sites. Administration of ZA resulted in dose-dependent improvement of both extrinsic and intrinsic strength parameters that reached a plateau at about 100 microg/kg. Statistically significant improvements could be detected at 100 microg/kg of ZA in the distal femoral metaphysis and lumbar vertebral body and at 4-20 microg/kg in the femoral shaft. ZA at 20 microg/kg fully prevented ovariectomy-induced loss of bone strength. At 100 or 500 microg/kg of ZA, strength parameters were at least 10% higher than those observed in sham-operated animals. This effect could be the result of increased secondary mineralization due to reduced bone turnover.

Results indicate that a single i.v.-dose of 20 microg/kg ZA exerts significant protection against cancellous and cortical bone loss and preserves its mechanical properties for up to 32 weeks. Based on the different length of the bone remodeling cycle in rats and humans, these data support the proposed use of an infrequent dosing regimen for ZA for the treatment of osteoporosis (Reid et al. NEJM 2002 346:653-661).

OR26 W

RELATIONSHIP OF VERTEBRAL KYPHOSIS WITH THE ANTERIOR-POSTERIOR RATIO FOR VERTEBRAL DISC HEIGHT AND VERTEBRAL BODY HEIGHT

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Vertebral kyphosis is associated with increased risk of osteoporosis. We previously showed that kyphosis is related to anterior wedge fracture determined by a reduced vertebral anterior-posterior (AP) ratio (- 3SD below the normal range) using Morphometric X-Ray Absorptiometry (MXA). However, it is not clear if vertebral disc morphometry is also related to kyphosis.

We therefore determined the relationship beween kyphosis and the AP ratios for vertebral body and disc heights. Vertebral body and disc morphometry was measured by MXA 6-point placement facility (Hologic)on 435 women >70 years recruited from the Western Australian population. AP ratios were calculated for vertebral bodies and discs. Thoracic curvature was measured using a flexicurve ruler and a kyphosis index (KI) was calculated from the length of the vertebral column and the maximum saggital displacement of the thoracic spine in the posterior direction. The mean age of the subjects was 75 ±3 years, with a mean kyphosis of 14.4 ±3.4. At each vertebra, the AP ratio for disc height was negatively correlated with the corresponding AP ratio for vertebral height (range $r= -0.14$, $p=0.01$ at T4 to $r= -0.33$, $p<0.001$ at T5). Regression analysis demonstrated a significant negative relation between vertebral body AP ratio and the KI at T4, T6, T7, T9 and T10 (R2 value for vertebral AP ratios as a determinant of kyphosis = 0.3). In a separate analysis, disc AP ratios were not correlated with KI except at L1 (R2 value for disc AP ratios as a determinant of kyphosis =0.06). However, a linear regression model that examined both vertebral body and disc AP ratios as deterimants of KI indicated that, after adjustment for the vertebral body AP ratios, the disc AP ratios at T5-T7, T9-T11 and L2 were independent determinants of the KI, such that a decrease in the AP ratio of these discs was associated with increased kyphosis. The combination of vertebral and disc AP ratios resulted in a more predictive linear regression model of kyphosis (R2 =0.44) than vertebral body AP ratios alone.

These data show that vertebral kyphosis is associated with decreases in the AP ratio of vertebral discs in association with wedging of vertebral bodies, suggesting that deterioration of vertebral discs is associated with the pathogenesis of kyphosis.

OR27 W

THE EFFECT OF RISEDRONATE ON FEMORAL NECK AND INTERTROCHANTERIC FRACTURE RISK IN OLDER POSTMENOPAUSAL WOMEN

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Epidemiological data suggest a difference in pathophysiology between femoral neck (FN) and intertrochanteric (IT) fractures. However, little is known about the effects of treatment on these 2 types of fractures.

The Hip Intervention Program (HIP) study was a randomized placebo-controlled trial (N = 9331) designed to assess the effects of risedronate (RIS) on hip fracture risk as a primary endpoint in older postmenopausal women. Patients were enrolled into either group 1: patients 70 to 79 years old with low FN bone mineral density (BMD) consistent with a diagnosis of osteoporosis or group 2: patients >= 80 years old with at least 1 clinical risk factor for hip fracture. BMD was not measured in group 2. As reported, RIS reduced hip fracture risk in older women with established osteoporosis. Since it is recognized that a stronger relationship exists between low BMD and IT fractures than with FN fractures, we conducted a post hoc analysis to assess the effect of RIS on the incidence of FN and IT fractures. These data are presented in the table.

In group 1, RIS reduced both IT and FN fracture risk, with statistical significance being achieved with FN fractures. In group 2, there was a 40% reduction in IT fracture risk, which approached statistical significance, but not in FN fracture risk.

These data are consistent with previous findings of a stronger relationship between low BMD and IT fractures in elderly women (>= 80 years). Risedronate had a similar effect on FN and IT fractures in women aged 70 to 79 years with low BMD, suggesting that BMD measurement remains an important tool for identifying individuals at high risk of fracture who could benefit from treatment with antiresorptive drugs such as risedronate.

Women 70–79 Years of Age with Osteoporosis				
	Control (n=1821)	**RIS (n=3624)**	**RR**	**P value**
IT Fractures	14 (1.0)	15 (0.5)	0.54	0.089
FN Fractures	28 (1.9)	34 (1.2)	0.61	0.048
Women ≥80 Years of Age with ≥1 Clinical Risk Factors for Hip Fracture				
	Control (n=1313)	**RIS (n=2573)**	**RR**	**P value**
IT Fractures	22 (2.2)	26 (1.4)	0.60	0.071
FN Fractures	25 (2.7)	53 (2.7)	1.1	0.772
RR=Relative risk; data are number of patients with fractures (%). Incidence based on Kaplan-Meier estimates.				

OR28 W

INTERMITTENT INTRAVENOUS IBANDRONATE INJECTIONS: A NEW THERAPEUTIC OPTION FOR PREVENTING POSTMENOPAUSAL BONE LOSS

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Introduction: Although current oral bisphosphonates are effective in preventing postmenopausal bone loss, their use as preventive agents is limited by the need for patients to adhere to frequent dosing schedules (daily or weekly) and stringent dosing recommendations. Alternative dosing strategies that enhance patient convenience may help to overcome these limitations. Ibandronate is a highly potent, nitrogen-containing bisphosphonate that can be administered as an intravenous (i.v.) injection in regimens featuring extended between-dose intervals. Here, we present the results of a multicentre study investigating the efficacy, safety and optimal dose of intermittent i.v. ibandronate injections administered once every 3 months in the prevention of postmenopausal osteoporosis (PMO).

Methods: A total of 627 non-osteoporotic postmenopausal women were enrolled into one of four strata on the basis of baseline lumbar spine (L1-L4) bone mineral density (BMD) and time since menopause (TSM) characteristics. Participants were randomised to receive placebo (n=156), ibandronate 0.5mg (n=157), 1mg (n=156) or 2mg (n=158) as an i.v. injection given once every 3 months for 1 year. All participants received daily calcium (500mg) supplementation. The primary endpoint was the relative change from baseline in lumbar spine (L1-L4) BMD.

Results: Intermittent i.v. ibandronate injections produced significant and dose-dependent increases in lumbar spine and proximal femur BMD, relative to baseline. After 1 year, 2mg, 1mg and 0.5mg ibandronate produced overall lumbar spine BMD gains of 2.5%, 1.8% and 1.0%, respectively (relative to baseline), versus a loss of 0.4% in the placebo group ($p<=0.0001$ for each ibandronate dose vs placebo). The greatest increase in spinal BMD was observed in the osteopenic subgroup (2.8% and 2.9% versus placebo in women with TSM <=3 and >3 years, respectively). BMD increases were associated with dose-related reductions in biochemical markers of bone turnover (serum CTX, urinary CTX/creatinine and osteocalcin). Furthermore, intermittent i.v. ibandronate injections were well tolerated.

Conclusions: This is the first study to demonstrate the efficacy and tolerability of a bisphosphonate when administered as an intermittent i.v. injection in the prevention of postmenopausal bone loss. These findings demonstrate that intermittent i.v. ibandronate injections provide an effective and convenient alternative to current therapies in the prevention of PMO.

OR29 W

EVIDENCE THAT STRONTIUM RANELATE INCREASES BONE QUALITY IN RATS BY IMPROVING BONE STRENGTH AND ARCHITECTURE

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Strontium ranelate (SR) is a new antiosteoporotic agent, with a dual mode of action, simultaneously increasing bone formation and decreasing bone resorption. SR was recently shown to reduce both vertebral and peripheral fracture risks in osteoporotic post-menopausal women. In vivo studies were performed in various animal models in order to better understand the mechanisms underlying this effect.

It was found that, in non-OVX rats, SR improved bone micro-architecture at the cortical and trabecular bone levels, as measured by histomorphometry. This was associated with an improvement in bone strength, measured by a 3-point bending test of the midshaft femur and a compression test of the lumbar vertebra (LV4). This indicates that the increased bone strength by SR treatment could be related to an improvement in bone architecture, and this was further tested in ovariectomized (OVX) rats. In an initial study, a 60-day treatment with SR (77, 154 and 308mg/kg/d) was shown to increase trabecular bone volume (BV/TV) at the tibia level. In a second study, a 52-week preventive treatment with SR (125, 250 and 625mg/kg/d) was initiated in 6-month old OVX Sprague Dawley rats. Lumbar vertebra (LV5) was sampled and tested for its mechanical properties. At this level, SR increased the maximum load by 25% ($P<0.05$), ultimate strength by 26% ($P<0.01$), and energy to failure by 75% ($P<0.01$) in OVX rats treated with SR (625mg/kg/d) compared to the OVX control group, without alteration of bone stiffness. Bone histomorphometry in tibia and lumbar vertebra (LV3) showed that SR induced a marked and dose-dependent increase in BV/TV (73-115% in tibia and 36-49% in LV3) and in trabecular number (47-64% in tibia and 19-28% in LV3) compared to the OVX control group. Furthermore, the bone formation rate in SR-treated OVX rats was maintained at high levels similar to those observed in control OVX rats (i.e., approximately 180% and 160% greater than SHAM values for tibia and vertebra, respectively). These beneficial effects of SR occurred without modifications in bone mineralization.

These results demonstrate for the first time that SR increases bone quality in rats by improving bone strength as well as trabecular and cortical bone micro-architecture.

OR30 W

DO CERVICAL AND TROCHANTERIC HIP FRACTURES HAVE DIFFERENT RISK FACTOR PATTERNS?

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Hip fracture is the most feared osteoporotic fracture. Some papers have suggested that cervical and trochanteric hip fractures may have different aetiologies and therefore different risk factor patterns. These studies have been made concerning mainly women and investigated subjects who have already suffered a hip fracture.

In our study we had 22444 men, mean age 44, and 10902 women mean age 50 participating in a cardiovascular prevention study. At screening the participants answered a large questionnaire concerning life style, illnesses, heredity, social status, underwent height and weight measuring, blood sampling and pulmonary function tests.

The subjects were followed prospectively for 16 years (men) and 11 years (women) concerning death, malignancy, and fractures.

In this group we found 178 hip fractures among the men, 89 cervical and 89 trochanteric, and 133 hip fractures among the women, 93 cervical and 40 trochanteric.

Among all the available data we chose some relevant factors and analysed them with multiple age adjusted logistical regression.

In women significant risk factors for both fracture types were only height and earlier fracture. For trochanteric fractures smoking and diabetes were significant and for cervical fractures elevated pulse rate and earlier stomach ulcers.

BMI and weight were significant protective factors for trochanteric fractures only while high FVC(Forced Respiratory Capacity) was protective for cervical fractures.

In men diabetes, height, elevated serum- glutamyl transferase, high diastolic blood pressure, height and to be single were significant risk factors for both fracture types. BMI and weight were protective factors for both fracture types while to feel healthy and have high FVC were protective only for cervical fractures. Smoking, poor appetite, manual occupation, excess alcohol consumption, and a high pulse rate were risk factors for trochanteric fractures only.

We conclude that even as early as in middle age a different risk factor pattern can be seen both in men and women when comparing trochanteric and cervical hip fractures. These facts supports earlier papers suggesting different aetiology for these 2 fracture types and more research is needed in this area.

OR31 W

PREDICTION OF THE STRENGTH AND FRACTURE LOCATION OF THE FEMORAL NECK BY MEANS OF THE CT BASED FINITE ELEMENT METHOD: A PRELIMINARY STUDY ON THE PATIENTS WITH HIP FRACTURE

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Conventional approaches for predicting hip fracture risk in elderly people have been bone densitometry and diagnostic imaging. These methods cannot provide information on the structural strength of the bone. The aim of this study was to predict fracture load and fracture location of the femora by means of the CT based finite element method utilizing elastic analyses based on the maximum principal stress failure theory. The femora of 15 patients with contralateral hip fracture were analyzed to estimate fracture strength and to investigate whether the predicted fracture locations were similar to those of the contralateral hip fractures. The femora of five normal volunteers were also analyzed to compare with those of the patients. Axial CT images of the whole femora with were obtained with a calibration phantom using a GE Light Speed QXi scanner. 3D finite element models with 7mm tetrahedron elements for a cancellous bone and 3 nodal-points shell elements with a thickness of 0.5 mm for a cortical bone, both of which contained heterogeneous linear isotropic mechanical properties, were constructed. The relationship between the Hounsfield units and ash densities were given by the regression equation determined from the Hounsfield units of the phantom rods. Loading and boundary conditions were applied to represent two load configurations, one approximating joint loading during single limb stance, and the other simulating impact from an oblique fall backward and to the side. The analyses of hip fracture load and failure locations were performed using the finite element analysis software MECHANICAL FINDER. The predicted strength of patients tended to be less than one half of those of normal volunteers, and in all patients, the predicted fracture sites existed at the subcapital or the proximal cervical region for either the stance or the fall loading configuration. The current method might be useful for predicting hip fractures in elderly people.

Result: The predicted fracture loads (Kg)		
	Stance loading condition	**Fall loading condition**
Patients with hip fracture	Mean 265Kg SD 67.1Kg	Mean 207.5Kg SD 32.3Kg
Normal Volunteers	Mean 575Kg SD 81.5Kg	Mean 498.8Kg SD 64.6K
Patients with hip fracture (age range 51-90, mean 72.1; 15 females) Normal Volunteers (age range 25-60, mean 56.0; 4 females, 1 male) The elastic modulus and strength of each element were calculated from the data according to Keyak et al (1994). And the Poisson's ratio was also determined from the data according to Minamisawa (1981)		

OR32 W

POSITIVE EFFECTS OF SHORT-TERM GROWTH HORMONE TREATMENT ON LEAN BODY MASS AND BONE MINERAL CONTENT AFTER A HIP FRACTURE.
A DOUBLE-BLIND PLACEBO CONTROLLED PILOT STUDY

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Patients operated on for a hip fracture lose muscle and bone mineral mass (BMC) postoperatively; a catabolic situation develops. The aim with the present study was to investigate if GH given postoperatively could increase IGF-I and reduce the loss of lean body mass and BMC postoperatively.

Patients

Twenty patients operated on for a hip fracture were randomised to a double-blind randomised study with daily s.c. injections of recombinant human growth hormone (GH, Genotropin, (n=11), or placebo (n=9).

The mean GH dose administered was 5.8 IU/day. The duration of treatment was 21-28 days, depending on the length of the hospital stay. The study comprised 15 women and 5 men, all over 65 years of age, previously ambulant, with a femoral neck or trochanteric fracture.

Methods

The total body composition, was determined by DXA on admission, after treatment and 2 months after termination of treatment. BMC was measured at three locations in both legs by QCT, on admission and 2 months after termination of treatment. Serum IGF-I and IGFBP-1 concentrations were measured on admission, 7 days after the start of treatment, on the last day of treatment and 2 months after termination of treatment by a specific RIA technique.

Results

The mean S-IGF-I increased from an initial value of 59 nanog/milliL to 206 nanog/milliL after 1 week of GH treatment, while in the placebo group corresponding values were 63 and 80 nanog/milliL.

The placebo group showed a significant loss of total body BMC 2 months after termination of treatment, while the value was unchanged in the GH group (DXA). The group treated with GH lost significantly less body weight and lean body mass during treatment compared to the placebo group.

Conclusions

GH treatment increased serum IGF-I levels and had positive effects on BMC and lean body mass. The treatment was well tolerated with few adverse events.

OR33

TARGETED OVEREXPRESSION OF C-TYPE NATRIURETIC PEPTIDE IN CHONDROCYTES RESCUES DWARFISM IN ACHONDROPLASIA

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Achondroplasia is the most common genetic form of dwarfism of which no efficient therapy has been established so far. Recently, we have demonstrated that C-type natriuretic peptide (CNP) is a novel skeletal growth factor regulating endochondral ossification. Here we report the therapeutic efficacy of CNP for achondroplasia using mice model of achondroplasia with targeted overexpression of the activated FGFR3 in cartilage (FGFR3ach-Tg), which develop dwarfism with shortening of bones formed through endochondral ossification. To investigate the direct effect of CNP on longitudinal bone growth of FGFR3ach-Tg mice, we performed organ culture experiments using tibiae from 16.5-G.D. fetal FGFR3ach-Tg mice. Fetal FGFR3ach-Tg tibiae were significantly shorter than non-Tg tibiae throughout the 4-day culture period, and 10-7M CNP elongated the cultured FGFR3ach-Tg tibiae to the level of vehicle treated non-Tg tibiae at the end of the culture period. Histological analysis revealed that CNP elongated the short cartilagenous primordia of FGFR3ach-Tg tibiae, both in the proliferative and hypertrophic chondrocyte layers, with a characteristic picture of the enlarged extracellular space there. The decreased extracellular matrix synthesis of FGFR3ach-Tg tibiae estimated by 35SO4 incorporation was recovered by 10-7M CNP up to just the same extent to that of non-Tg tibiae treated with vehicle. Then we achieved targeted overexpression of CNP using typeII collagen promoter in cartilage of FGFR3ach-Tg mice to observe the effect of CNP for achondroplasia in vivo, and exhibited that CNP substantially rescued the shortening of bones formed through endochondral ossification. Histological analysis showed that CNP recovered the narrowed growth plate of FGFR3ach-Tg mice with increased extracellular space, as was observed in organ culture experiments. On the other hand, decreased proliferation of the growth plate chondrocytes in FGFR3ach-Tg mice shown by the BrdU staining was not recovered. CNP overexpressed in cartilage did not alter the intensity of type II and X collagens shown by in situ hybridization, or the delayed formation of the secondary ossification center in FGFR3ach-Tg mice. These results exhibit that the mechanism by which CNP could rescue the shortening of bones of FGFR3ach-Tg mice is to increase the extracellular matrix with little alteration in the proliferation and the differentiation of the growth plate chondrocytes, followed by the substantial recovery in the bone length, indicating the efficacy of CNP for the treatment of human achondroplasia.

OR34

REGULATION OF BMP SIGNALING BY SMURF1

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Bone morphogenetic proteins (BMPs) are multifunctional growth and differentiation factors belong to the transforming growth factor (TGF)-beta superfamily. Signals of BMP ligands are propagated to the nucleus through specific interaction of transmembrane receptors and intracellular Smad proteins. Smurf1 was originally identified as a HECT type E3 ubiquitin ligase that binds to receptor-regulated Smads (R-Smads) for BMPs, Smad1 and Smad5, and promotes their degradation in a signal-independent manner. Here we show that, in addition to direct binding to BR-Smads, Smurf1 also binds to inhibitory Smads (I-Smads), Smad6 and Smad7, to form E3 ubiquitin ligase complexes to target BMP type I receptors (BMPR-Is) and BR-Smads for degradation. Smurf1 interacts with nuclear I-Smads and induces their translocation to the cytoplasm. Smurf1 then associates with BMPR-Is, and enhances turnover of the receptors. Furthermore, I-Smads-Smurf1 complex binds to activated BR-Smads, and induces their ubiquitination and degradation. Consistence with these data, Smurf1 synergies with Smad6 to inhibit BMP signaling in Xenopus embryos. These data elucidate novel mechanisms whereby I-Smads-Smurf1 complexes inhibit BMP signaling both in vitro and in vivo, by mediating downregulation of activated BMPR-Is and activated BR-Smads.

OR35

PROTEASOME INHIBITORS STIMULATE BMP-2 GENE TRANSCRIPTION THROUGH TRANSCRIPTION FACTOR GLI3 VIA SPECIFIC RESPONSE ELEMENTS IN THE BMP-2 PROMOTER

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Recently, we have shown that proteasome inhibitors increase bone formation in vitro and in vivo. This effect is BMP-2 dependent, since these compounds increase BMP-2 expression in osteoblasts and their effects on bone are reversed by noggin. To determine the mechanisms by which proteasome inhibitors increase BMP-2 expression, we examined the role of Gli3, the mammalian homolog of cubitus interruptus (ci) in Drosophila, on BMP-2 gene transcription based on the following reasons: (1) ci is proteolytically processed to a truncated form that represses dpp (the drosophila homolog of BMP-2/4) gene expression, and (2) the cleavage of ci is proteasome-dependent. We generated a trGli3 expression construct and transfection of this construct into calvarial bone tissue impaired the capacity of proteasome inhibitors to stimulate bone formation. Transfection of trGli3 inhibited BMP-2 promoter activity in a dose-dependent manner up to 70% in C2C12 cells. To define the response regions in the BMP-2 promoter to trGli3, a series of deletion constructs were examined. We found that the transcriptional activities of BMP-2 promoter constructs comprising -2712/+165, -1997/+165, -838/+165 and -310/+165 were inhibited by trGli3 (up to 70%). In contrast, trGli3 had no significant effect on -150/+165 promoter construct, suggesting that the Gli3 responsive region is located in the -310/-150 region of the BMP-2 promoter. Sequence analysis revealed three putative Gli binding sites in this region. Mutation of each of these three response elements resulted in a significant attenuation of trGli3-induced inhibition of the BMP-2 promoter. Western blot analysis showed that beta-TrCP, the E3 ligase for Gli3, induced the degradation of Gli3 in C2C12 cells. The proteasome inhibitors PS1 and epoxomicin prevented the degradation of Gli3 mediated by beta-TrCP. trGli3 blocked the capacity of PS1 and epoxomicin to induce BMP-2 promoter activity, suggesting that the effects of proteasome inhibitors on BMP-2 gene transcription are Gli3-dependent. In summary, our results demonstrate that proteasome inhibitors stimulate BMP-2 gene transcription at least in part through inhibition of the proteolytic processing of Gli3. The product of this proteolytic processing, trGli3, represses BMP-2 gene transcription through specific response elements in the BMP-2 promoter.

OR36

PTH-SMAD3 AXIS EXERTS ANTI-APOPTOTIC EFFECT AND AUGMENTS AN ANABOLIC ACTION OF TGF-B IN OSTEOBLASTS

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Although anabolic action of PTH on bone is partly explained by PTH-induced antiapoptotic signals in osteoblasts, its precise mechanism has been unknown. On the other hand, TGF-b, abundantly stored in bone matrix, stimulates bone formation with

a local injection in rodents. The previous study revealed that mice with the target disruption of Smad3, a TGF-b-signaling molecule, exhibited the decreased bone formation. We recently demonstrated that Smad3 promotes the expression of type I collagen, ALP activity and mineralization in mouse osteoblastic MC3T3-E1 cells (JBMR, JBC. 2002). These findings suggest that Smad3 is an important molecule for the stimulation of bone formation. However, no reports have been available about the effects of PTH on Smad3 activity. We now show the effect of PTH on Smad3 and its physiological significance in osteoblasts.

PTH promoted the Smad3mRNA expression within 10 minutes and the protein synthesis in a dose-dependent manner in MC3T3-E1 cells and rat osteoblastic UMR106 cells. PKC activator, PMA as well as PKA activators, forskolin, dbcAMP and Sp-cAMPs promoted Smad3 synthesis, and inhibitors of both PKA and PKC antagonized PTH-induced Smad3 synthesis, which indicated that PTH promotes the production of Smad3 through PKA and PKC pathways. Next, we examined anti-apoptotic effects of PTH and Smad3 in these cells, employing Trypan blue, Tunel and Hoechst stains. Pretreatment with PTH or overexpression of Smad3 decreased the number of apoptotic cells induced by dexamethasone and etoposide. Moreover, a dominant negative mutant, Smad3DC, abrogated PTH-induced anti-apoptotic effects. On the other hand, PTH augmented TGF-b-induced transcriptional activity in the luciferase assay with 3TP-lux containing Smad3-responsive element. Furthermore, PTH enhanced TGF-b-induced expression of type I procollagen mRNA. These observations implicated that PTH amplified the anabolic effects of TGF-b by accelerating the transcriptional activity of Smad3. In conclusion, we first demonstrated that PTH-Smad3 axis exerts anti-apoptotic effects in osteoblasts and reinforces the anabolic action by TGF-b. Hence, PTH-Smad3 axis would be involved in the bone anabolic action of PTH.

OR37

DYSFUNCTION OF CGMP-DEPENDENT PROTEIN KINASE 2 CAUSES DISSOCIATION BETWEEN PROLIFERATION AND DIFFERENTIATION OF GROWTH PLATE CHONDROCYTES IN MINIATURE RAT ISHIKAWA

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The start of chondrocyte hypertrophy is precisely synchronized with the termination of proliferation in the growth plate. However, the molecular mechanism that tightly couples proliferation and differentiation of chondrocytes remains an enigma. The Miniature rat Ishikawa (MRI) is a naturally occurring mutant caused by an autosomal recessive mutation (*mri*), which exhibits longitudinal growth retardation without other organ abnormalities. The *mri* locus was mapped to a genomic region between D14rat76 and D14rat6, in which several candidate genes were identified by comparative mapping. By sequencing them, we found a 220-bp deletion in the cGMP-dependent protein kinase 2 (cGK2) transcript in MRI, which caused a frame shift and a premature stop codon, predicting a truncated product that lacks the kinase domain. We further investigated the mechanism of growth retardation by analyzing the growth plate. Height of the MRI growth plate was about 2.5-fold that of wild-type (WT) with a thick layer of small and flattened chondrocytes between the proliferative and hypertrophic zones. BrdU labeling and immunohistochemistry / in situ hybridization for differentiation markers (PTH/PTHrP receptor, Ihh, ALP and COL X) revealed that these chondrocytes were abnormal cells that had stopped proliferation but had not started hypertrophic differentiation. Similar impairment of endochondral bone formation was observed during the fracture healing process after osteotomy in the tibiae. Proliferation of cultured chondrocytes isolated from the growth plate was similar between MRI and WT; however, differentiation of MRI chondrocytes determined by alcian blue and ALP staining was markedly suppressed. Differentiation of WT chondrocytes was impaired when cocultured with MRI chondrocytes in a double chamber dish separated by a porous membrane, although that of MRI cells was not rescued by WT cells. This suggests that cGK2 controls differentiation of chondrocytes by suppressing humoral anti-differentiation factor(s). We conclude that a mutation in the *cGK2* gene causes growth retardation in MRI by impairing coupling between proliferation and differentiation of growth plate chondrocytes.

OR38

OSTEOBLAST SPECIFIC CONNEXIN43 (CX43) GENE DELETION IN MICE LEADS TO REDUCED PEAK BONE MASS AND OSTEOBLAST DYSFUNCTION

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Osteoblasts are highly coupled by gap junctions formed primarily by Cx43, and genetic deficiency of Cx43 *in vivo* causes skeletal developmental defects and delays osteoblast differentiation and function. To determine the role of Cx43 in the adult skeleton, and overcome the lethality of the conventional 'knockout' mice, we developed two models of osteoblast-specific Cx43 gene deletion using Cre mediated recombination of a 'floxed' Cx43 allele, which results in replacement of the Cx43 reading frame with the LacZ reporter cassette. Homozygous $Cx43^{fl/fl}$ mice were crossed with mice expressing Cre driven by the OG2 promoter in a heterozygous Cx43-null background (OG2-Cre;$Cx43^{+/-}$), yielding OG2-Cre;$Cx43^{-/fl}$ mice, in which osteoblast specific Cx43 gene deletion occurs. A similar strategy was used with transgenic mice expressing Cre under the control of a 2.3 kb fragment of the Col1a1 promoter to obtain 2.3col1a1-Cre;$Cx43^{-/fl}$ animals. In either case, the recombination event was demonstrated exclusively in osteoblasts by X-gal staining of tibia sections and calvarial cell cultures from OG2-Cre;$Cx43^{-/fl}$ and 2.3colIa1-Cre;$Cx43^{-/fl}$ mice, and by the progressive disappearance of Cx43 mRNA and protein abundance in OG2(2.3col1a1)-Cre;$Cx43^{-/fl}$ calvaria cells, relative to $Cx43^{+/fl}$, OG2(2.3colIa1)-Cre;$Cx43^{+/fl}$ and $Cx43^{-/fl}$ controls. This decline was associated with reduced alkaline phosphatase activity, Cbfa1, Col1a1 and osteopontin mRNA abundance, and delayed matrix mineralization *in vitro* in conditionally deleted calvarial cells compared to controls.

Total body bone mineral density *in vivo* by DEXA was consistently lower in OG2-Cre;$Cx43^{-/fl}$ mice compared to controls up to 6 months of life (OG2-Cre;$Cx43^{-/fl}$: 59±1.6mg/cm^2, $Cx43^{+/fl}$: 64.3±3.6mg/cm^2, $Cx43^{-/fl}$: 63.3±4.1mg/cm^2, $p<0.05$, at 5 months). Col1a1 mediated Cx43 gene inactivation led to a more pronounced and earlier reduction in total body bone density, primarily in females, compared to their control littermates, with significant differences detectable as early as 2 months of age (2.3col1a1-Cre;$Cx43^{-/fl}$: 44.66±1.6mg/cm^2, $Cx43^{+/fl}$: 46.92±1.4mg/cm^2, $Cx43^{-/fl}$: 46.05±1.9mg/cm^2, $p<0.05$), and increasing with time.

Thus, Cx43 gene deletion in vivo in osteoblasts leads to delayed osteoblast differentiation and reduced bone mass during the period of bone mass accumulation. Cx43 gap junctional communication is critically important for adult bone homeostasis, contributing to the achievement of peak bone mass.

OR39

IN VIVO BONE FORMATION INDUCED BY BMP INJECTIONS ONTO CALVARIA AND ANGIOGENESIS DURING FRACTURE HEALING ARE IMPAIRED IN ID1/ID3 DOUBLE GENE KNOCKOUT MICE

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Id proteins suppress activities of helix-loop-helix transcription factors such as E proteins and expression of Id genes in osteoblasts is under the control of calciotropic agents such as BMP and vitaminD. Angiogenesis plays an important role during bone development and regeneration. Id1 and Id3 have been shown to be required for tumor angiogenesis. However, the function of Id1 and Id3 during in vivo bone formation and fracture healing has not yet been elucidated. The purpose of this paper is to understand the role of Id1 and Id3 in regulation of bone metabolism. We first examined fracture healing which requires angiogenesis. The levels of PCNA positive chondrocytes in the callus of fractures made in the ribs were similar between wild type and Id1-/+Id3-/- double knockout mice. However. Id1-/+Id3-/- double knockout mice revealed suppression of angiogenesis in the callus. Quantification of CD31 positive vessels in the tissue sections revealed more than two-fold reduction in the number of vasculatures ($p<0.05$). This was associated with delayed remodeling process in callus including new bone formation. We further examined calvarial sutures as another site of interaction between angiogenesis and bone formation in vivo. The calvarial sutures were clearly observed on the soft X-ray films in wild type mice. In contrast, such sutures in Id1-/+Id3-/-double knockout mice were unclear and almost fused in part compared with those in wild type. Quantification of the suture gap revealed more than 50% reduction in Id1-/+Id3-/- double knockout mice compared to wild type. To obtain the cellular basis for the mechanism of such defects, we examined proliferation of the primary calvaria osteoblasts. Proliferation was significantly reduced in Id1-/+Id3-/- double knockout osteoblasts. Finally, we examined in vivo bone formation in response to BMP injections onto calvariae. BMP injections induced ectopic bone formation. However, its levels were less in Id1-/+Id3-/- double knockout. Quantification of the ectopically-formed bone based on micro-CT revealed about 50% reduction in Id1-/+Id3-/- double knockout mice compared to wild type. These results indicated that Id1 and Id3 are required for angiogenesis during fracture healing and the bone formation in response to BMP in vivo.

OR40 F

IDENTIFICATION OF OSTEOPONTIN PROMOTER REGION WHICH DIRECTS CELL-SPECIFIC AND DEVELOPMENTAL REGULATION IN BONE, KIDNEY, PLACENTA AND MAMMARY GLAND: AN ANALYSIS OF TRANSGENIC MICE

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Osteopontin (OPN) is one of the major noncollagenous bone matrix proteins. OPN is considered to plays important roles for controlling physiological and pathological calcification. OPN-expressing cell types in bone tissues are identified as Osteoblasts, hypertrophic chondrocytes and osteoclasts. OPN expression is also detectable in a variety of cells such as epithelial cells in the distal tubes of developing kidney and renal stone-forming kidney, granulated metrial gland cells (GMG cells) of maternal origin in placenta, glandular epithelial cells in mammary gland, macrophages in atherosclerotic plaques, and calcifying foci in breast cancer. Identification of the osteopontin promoter region controlling cell-specific expression in vivo provide us valuable information for the elucidation of the molecular mechanism in the calcification of the tissues. The transgenic mice carrying green fluorescent protein (GFP) gene under the control of 5.5-kb (Op5.5GFP), 2.5-kb (Op2.5GFP), 1.5kb Op1.5GFP) and 0.9-kb (Op0.9GFP) upstream region of mouse osteopontin gene were generated. Expression of GFP was investigated by means of Northern blotting, Western blotting, immunohistochemistry, in situ hybridization and the GFP-derived fluorescence. Localization of GFP-expressing cells was compared with those of OPN-expressing cells. GFP-expressing cell types in Op5.5GFP were identical to that of OPN-expressing ones. However, GFP expression was detected not only in hypertrophic chondrocytes but in proliferating and resting chondrocytes in Op2.5GFP, and fibroblastic cells expressed GFP in Op1.5GFP. No significant expression of GFP in bone tissue was detected in Op0.9GFP mice. Furthermore, the promoter region between 2.5kb and 1.5kb upstream was essential for the GFP expression in the kidney and placenta. The expression patterns obtained by the analysis of transgenic lines indicated the different promoter regions are involved in cell type specific expression of OPN gene.

OR41 F

ALENDRONATE INHIBITS OSTEOPHYTE FORMATION AND PARTIALLY PROTECTS CARTILAGE DETERIORATION IN THE RAT ANTERIOR CRUCIATE LIGAMENT TRANSECTION MODEL OF OSTEOARTHRITIS

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Osteoarthritis (OA) is a degenerative joint disease characterized by articular cartilage degradation, subchondral bone sclerosis and osteophyte formation. To investigate the potential contribution of bone turnover to OA progression, we evaluated the effect of Alendronate (ALN), a potent bone resorption inhibitor, on cartilage degradation and periarticular bone changes in the rat anterior cruciate ligament transection (ACLT) model of OA. Male rats underwent ACLT or sham-operation in the right knee. The animals were then treated with either vehicle or ALN (0.03 and 0.24 mg/kg/wk, s.c., 2X per wk) and sacrificed at 2-or 10-wk post-surgery. ALN had significant chondroprotective effects on the ACLT-joints. ALN partially reduced the histological Mankin score ($p<0.05$, at 0.24 mg/kg/wk) of cartilage damage during OA progression, and suppressed the elevated levels of two specific markers associated with cartilage damage, serum cartilage oligomeric matrix protein ($p<0.05$) and urinary Col2CTx ($p<0.001$). Subchondral bone resorption was markedly increased 2-wks post-surgery and formation was higher 10-wks post-surgery in the tibial plateau of non-treated ACLT, by comparison to sham-operated joints. ALN at both doses effectively inhibited the ACLT-induced subchondral bone remodeling, as determined by histomorphometry. ALN also markedly reduced the levels of urinary deoxypyridinoline (D-Pyr) and Col1CTx in both Sham-operated and ACLT-groups, indicating that ALN inhibited the increased rate of bone turnover in these animals. Lastly, we observed that ALN dose-dependently inhibited osteophyte formation as determined by osteophyte scoring ($p<0.05$) and osteophyte area. Taken together, these results strongly suggest that subchondral bone remodeling plays an important role in OA pathogenesis. The demonstration that treatment with ALN can slow down the progression of OA in ACLT-joints raises the possibility that antiresorptive agents may have therapeutic benefits for OA in humans.

OR42 F

TRANSCRIPTIONAL INDUCTION OF FOSB/DELTAFOSB GENE BY FLUID SHEAR STRESS IN OSTEOBLASTS

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DeltafosB, a short splicing isoform of fosB, has been shown to increase bone mass by stimulating bone formation when over-expressed in transgenic mice. We have shown that osteogenic stimuli such as mechanical stress and PTH induces deltafosB mRNA and protein expression, followed by induction of an AP-1 target interleukin-11, which, as we have already reported, also has the capacity of stimulating bone formation and thereby increasing bone mass in vivo. Therefore, deltafosB may play a role in mechanical stress- and PTH-induced bone formation. In the present study, we further explored the mechanism of deltafosB induction. Agents that mimic downstream signaling molecules activated by PTH including cAMP, PKC, intracellular calcium all induced fosB/delta fosB expression. Inhibitor experiments however suggested importance of calcium but not PKC. To analyze transcriptional effects, we cloned the mouse fosB gene promoter region, subcloned into a luciferase reporter vector, transfected into primary osteoblasts and examined fluid shear stree (FSS) effects. We found that the (-1000-+307) and (-603-+307) but not the (-327-+307) fragment responded to FSS with more than two-fold stimulation, indicating that the region -603 to -327 conferred the FSS effects. This region contained upstream AP-1/CRE-like (-469 to -462), SRE (-419 to -410), and downstream AP-1/CRE-like sequences (-404 to -397), which are highly conserved in c-fos gene and have been suggested to play key roles in FSS-induced c-fos gene transcription in vascular cells. EMSA analysis identified a single major specific complex binding to each element. However, the amout of protein binding to each element was not altered by FSS, suggesting a post-translational mechanism such as phosphorylation to increase transactivating function but not DNA-binding activity as in the case of CREB. Further transcriptional analyses using tandem oligonucleotides corresponding to each element suggested a major contribution of SRE, although the other elements may also contribute. Consistently, FSS activated ERK in a calcium-dependent manner, and deltafosB induction was completely abolished by ERK inhibitors U0126 and PD98059. In conclusion, we have demonstrated for the first time that FSS induces fosB expression at the transcriptional level in osteoblasts in a Ca- and ERK-dependent manner and mainly through SRE.

OR43 F

A GENE-TRAP APPROACH REVEALS THE INVOLVEMENT OF TRANSCRIPTION FACTOR NFIB IN CHONDROCYTIC DIFFERENTIATION OF ATDC5 CELLS

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Gene-trapping is a genome-wide approach utilized to clarify the gene function based on an idea that random insertion of a trapping vector may disturb the function of inserted genes. Although this method is usually applied to ES cells to generate trap mice, in the current study we utilized murine chondrogenic precursor ATDC5 cells to identify the molecules involved in chondrocyte differentiation. In the presence of insulin, ATDC5 cells differentiate into mature chondrocytes, which allows us to evaluate the phenotype of the trap clones in vitro. As the trap vector, we used pT1-geo, which lacks its own promoter and enhancer, but contains LacZ-neo fusion gene as the reporter driven by the promoter of the trapped gene. After introducing pT1-geo into ATDC5 cells by electroporation, the neomycin-resistant clones were screened for beta-galactosidase activity, and the selected clones were cultured in the differentiation medium to evaluate the chondrogenic phenotype by Alcian blue and Alizarin red staining. One of the clones, named UT7-57, which exhibited accelerated calcification compared with parental ATDC5 cells, was subjected to further analysis. 5'RACE analysis revealed that pT1-geo was inserted in the intron 6 of the nuclear factor IB(NFIB) gene in the clone. NFIB belongs to the nuclear factor I (NFI) family of site-specific DNA-binding proteins, and has been reported to be expressed around developing cartilage. We confirmed the expression of NFIB in parental ATDC5 cells. As the result of gene-trapping, the trapped allele of NFIB was assumed to express a fusion protein containing the N-terminal region of NFIB and LacZ-neo product instead of the C-terminal functional domain of NFIB, which was confirmed by Western blotting using the antibody against beta-galactosidase. The expression of PTHrP and chondromodulin-I was markedly reduced in the clone compared with the parental ATDC5 cells. The expression of aggrecan was also suppressed. As to the expression of PTH/PTHrP receptor, it started to be detected earlier in the clone than in the parental cells. The expression of osteocalcin gene was increased in the clone. Taken together, these results suggested that NFIB might be involved in chondrocyte differentiation and endochondral ossification.

OR44 F

MSX2 STIMULATES OSTEOBLASTIC DIFFERENTIATION INDEPENDENT OF CBFA1

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Msx2 is a family member of homeobox genes. Mice deficient in Msx2 gene manifested persistent calvarial foramen and defects of skull ossification with marked reduction in bone formation of both cortical and cancellous bones and decreases in osteoblast numbers. In human, heterozygous mutations of Msx2 cause enlarged parietal foramina (PFM) characterized by oval defects of parietal bones. These results demonstrate that Msx2 plays a role in osteogenesis. To understand the mechanism by which Msx2 contributes to osteogenesis, we examined the effects of Msx2 on osteoblastic differentiation using the multipotent mesenchymal cell lines, C3H10T1/2 and C2C12. Upon treatment with BMP2, these cells differentiated into osteoblastic cells with increased alkaline phosphatase activity (ALP). In conjunction with this, Msx2 expression was also induced. Overexpression of Msx2 using adenovirus system promoted the osteoblastic differentiation of C3H10T1/2 and C2C12 cells, which was enhanced in the presence of BMP2. Moreover, overexpression of Cbfa1/Runx2 also enhanced the effects of Msx2, suggesting the functional relationship between Msx2 and Cbfa1. To test this, we examined the effects of Msx2 overexpression in the C2 mesenchymal cells which were established from the calvariae of Cbfa1-deficient mice. BMP2 induced Msx2 expression and promoted the differentiation of C2 cells into ALP-positive osteoblastic cells (ALP(+)OB) and overexpression of Msx2 induced the differentiation of C2 cells into ALP(+)OB. These results suggest that induction of Msx2 is independent of Cbfa1 and that Msx2 stimulates osteoblastic differentiation of mesenchymal cells, in part, in a Cbfa1-independent fashion. We next evaluated whether Msx2 was involved in the control of terminal differentiation of osteoblasts, since the mutations of Msx2 cause impaired ossification in human and mice. To address this, we overexpressed Msx2 into primary osteoblasts isolated from neonatal mice calvariae and examined the capacity of these cells to mineralize by the alizarin red staining. Overexpression of Msx2 increased the calcification of primary calvarial osteoblasts in the presence of BMP2. In addition, overexpression of mutant Msx2, which is shown to causes PFM in human, inhibited BMP2-induced calcification of these cells. In conclusion, our results suggest that Msx2 promotes the early and late stages of osteoblastic differentiation.

OR45 F

NOGGIN CHANGES CELL FATE OF EMBRYONIC CALVARIAL CELLS FROM OSTEOBLASTS INTO ADIPOCYTES

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Calvarial bones are generated through membranous bone formation. While fetal calvarial cells mainly consist of osetoblasts and their precursors, in vitro studies show that the cells give rise to chondrocytes and adipocytes as well at low frequency. In this study, we show that noggin over-expressing fetal primary calvarial cells change their fate from to differentiate into osteoblasts to adipocytes. We infected noggin-expressing adenovirus (Nog-Ad) into the primary calvarial cells derived from 15.5 dpc embryos. We also infected LacZ-expressing adenovirus(LacZ-Ad) into these cells as control. In the presence of beta-glycerophosphate and ascorbic acid in culture, the LacZ-Ad infected control cells showed mature osteoblastic phenotypes that included high alkaline phosphatase activity, osteocalcin mRNA expression and mineralized nodule deposition. In contrast, the Nog-Ad infected cells lost all the osteoblastic phenotypes. The noggin over-expressing cells showed lipid-like deposit stained positive for Oil red staining. Furthermore, mRNA levels of adipocyte markers, PPAR gamma and ap2, were increased compared with controls whereas osteocalcin mRNA expression was suppressed. Thus, noggin inhibits osteogenesis and induces adipogenesis instead in the fetal calvarial cells, raising a possibility that noggin may be a regulator of cell fate decision in mesenchymal cells.

OR46

ALLELIC VARIATION IN THE LDL RECEPTOR-RELATED PROTEIN 5 GENE (LRP5) AND ITS ASSOCIATION WITH BONE MASS, SIZE AND THEIR CHANGES IN CAUCASIAN MALES AND FEMALES

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Loss- and gain-of-function mutations in the LRP5 gene are responsible for osteoporosis pseudoglioma (OPPG) and 'high bone mass' syndromes, respectively. Transgenic mice and in vitro studies have demonstrated the influence of LRP5 on bone mass acquisition and osteoblastic function. In addition, linkage mapping in humans suggests a QTL for bone mineral density (BMD) at 11q12-13, the LRP5 locus. However, the contribution of LRP5 to peak bone mass variation in the general population is unknown.

Association studies were conducted between variants in exons 9, 15 and 18 and introns 4 and 17 and lumbar spine (LS) BMD, BMC and area, femoral neck (FN) BMD, and height in 890 young adults, adolescents and children from both genders. Among these, 405 prepubertal children were followed-up for two years, and their bone mass changes analyzed in relation to LRP5 polymorphisms.

In the whole population, a strong association was detected between a novel missense substitution c.1999 G>A (exon 9, frequency(A)=6%) and LS BMD (p=0.0035 by ANOVA, adjusting and standardizing for age, sex and weight, Z-scores), BMC (p=0.0005) and area (p=0.0017), and height (p=0.002), with the largest differences among genotypes being observed in adult males (0.673 Z-score for LS BMC). Weaker associations (p=0.03-0.07) with these parameters, but also with FN BMD, were found for a missense substitution c.3989C>T (exon 18, frequency(T)=15%) and a silent substitution c.1932 G>A (exon 9, frequency(A)=6%). In turn, LRP5 haplotypes were strongly associated with differences in LS BMD (p=0.0016), BMC (p=0.0083), area (p=0.057) and FN BMD (p=0.0031) Z-scores in adults. Consistent with these observations, the less common alleles in exons 9 and/or 18 were associated with significantly lower gain in LS BMC, area, FN BMD and height over two years in prepubertal children. Altogether, LRP5 polymorphisms accounted for 4% of the total variance in vertebral bone mass and size (independently of age and weight) in adults (p=0.018 and 0.023, respectively), and for up to 15% in men (p=0.0002 and 0.0014, respectively).

These results suggest a significant contribution of allelic variation in the LRP5 gene to peak bone mass and size in the general population and confirm the role of LRP5 on the modeling of bone mass in humans.

OR47

A META-ANALYSIS OF PREVIOUS FRACTURE AND FRACTURE RISK

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Previous fracture is a well documented risk factor for future fracture. The aim of this study was to quantify this risk from an international basis and to explore the relationship of this risk with age, sex and BMD.

We studied 14,959 men and 43,917 women from nine cohorts comprising EVOS/EPOS, OFELY, CaMos, Rochester, Sheffield, Rotterdam, Kuopio and two cohorts from Gothenburg. Cohorts were followed for a total of 230,000 person years. The effect of previous fracture, BMD and age on all fracture risk and hip fracture risk alone was examined using a Poisson model in each cohort. The results of the different studies were merged from the weighted b-coefficients.

Previous fracture was associated with a significantly increased risk of fracture. Risk was marginally downward adjusted when account was taken of BMD and ranged from 1.46 to 1.72 depending on age (Table 1). The contribution of BMD was larger at higher ages than at lower ages. No difference in risk ratio was seen between men and women. For hip fracture risk, prior fracture was a significant risk factor from the age of 60 years. Relative risk (RR) adjusted for BMD was highest at younger ages (RR = 3.38 at age 50 years) and decreased with age (RR = 1.56 at age 85 years).

We conclude that previous fracture confers a risk of fracture of substantial importance beyond that explained by measurement of BMD. Its validation on an international basis permits the use of this risk factor in case finding strategies.

Table. Risk of any fracture following a previous fracture

	RR without BMD		**RR with BMD**	
Age (years)	**Mean**	**95%CI**	**Mean**	**95%CI**
50	1.83	1.58-2.12	1.72	1.42-2.09
55	1.82	1.65-2.01	1.66	1.43-1.94
60	1.82	1.64-2.01	1.69	1.49-1.91
65	1.72	1.59-1.86	1.66	1.61-1.83
70	1.66	1.56-1.78	1.66	1.51-1.82
75	1.66	1.53-1.80	1.59	1.43-1.78
80	1.73	1.56-1.92	1.46	1.33-1.61
85	2.22	2.01-2.46	1.66	1.47-1.87

OR48

GENETIC DETERMINATION OF BONE MINERAL DENSITY: EVIDENCE FOR A MAJOR GENE WITHIN FAMILIES

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Bone mineral density (BMD), the primary determinant of fractures, is largely determined by genetic factors. However, the genes regulating BMD variation in different types of bone have not been well documented. This study was designed to fill

that gap in knowledge by specifically testing the hypothesis of a major major gene influence on the BMD variation within families. To test the hypothesis, BMD at the lumbar spine and femoral neck was measured by DXA (GE LUNAR DPX-L) in 330 men and 413 women, aged between 18 and 90 years, who were participants of the Dubbo Osteoporosis Genetic Study. The participants were from 250 nuclear families, including 5 large pedigrees with 325 individuals, who were identified through an index case with moderately high bone density at the femoral neck (Z score>=1.28). Complex segregation analysis (CSA), based on tests of hypotheses regarding the fit of Mendelian segregation ratios for BMD in families, were performed so that a major gene hypothesis for BMD variation could be examined.

After adjusting for age and body weight, familial factors accounted for up to 72% of the total variation in BMD. There was no significant difference (p=0.57) in the heritability between males and females. A multifactorial (polygenic) model did not fit the data adequately (Chisq=26; df=8; p=0.003). Rather, the best fit model suggested the Mendelian transmission of a major gene locus with significant residual correlations among sibs (Chisq=4.9; df=7; p=0.67). This most parsimonious genetic model also suggested that the proportion of genetic variance of BMD attributable to this major gene effect was up to 42% within families. Further analyses did not provide support for shared familial environmental effects but suggested the existence of residual polygenic effects after adjusting for sex, body weight and age.

These findings support the hypothesis that a large component of the variance of BMD is under genetic control, and provide evidence for a major gene locus influencing BMD within family groups. These results suggest that study of large pedigrees identified via high or low probands may increase the probability of finding individual genes for BMD variation.

OR49

CHROMOSOME X AFFECTS THE INVOLUTIONAL BONE LOSS IN SAM (SENESCENCE ACCELERATED MOUSE) MICE

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[Purpose] We identified two significant and one suggestive quantitative trait loci (QTLs) specifying the bone mass on chromosome (Chrs) 11,13 and X by interval mapping in two mouse strains, SAMP2 and SAMP6. The latter strain is an established murine model of senile osteoporosis and shows a lower relative bone mass than that of SAMP2. The locus on Chr X was associated oppositely with a lower relative bone mass to SAMP2 allele. In the current study, we find the association of involutional bone loss with the locus on Chr X. [Materials and methods] By six successive backcrosses with microsatellite marker selections, we recently constructed a congenic strain, which carried a single genomic interval from the Chr X of SAMP6 on a SAMP2-derived background. We measured femurs and whole body BMD in female SAMP2 and congenic strains at 4 and 8 months of age with two methods, radiographic absorptiometry and dual energy X-ray absorptiometry (DXA) (Hologic QDR-2000), respectively. [Results] No contamination of unselected P6 genomic elements was found in the N7F3 generation using more than 60 markers throughout the whole genome except Chr X. Finally, the P6-derived 45.6 centiMorgan-long interval was carried intact, including an unselected flanking interval carried along with the selected interval. The congenic strain shows slower bone mineral decrease than SAMP2 mice. [Discussion] R.J.Shmookler Reis et al. reported a QTL on Chr X regulating involutional bone loss (ASBMR in 2001). Our data of the congenic strains support their report. In addition, the P6 allele at the locus on Chr X is associated oppositely with a higher bone density in the present study, as well as our past linkage analysis.

OR50

PTH, FALLS RISK AND MORTALITY IN THE FRAIL ELDERLY

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Very frail older people constitute an increasing proportion of ageing populations and frequently fall and sustain osteoporotic fractures. Vitamin D status, which is often subnormal in this population, is considered a risk factor for falls and fractures but the exact mechanisms of this relationship remains unclear. We studied serum 25OH vitamin D, PTH and objective measures of falls risk in 854 subjects living in institutional aged care facilities at baseline as well falls and deaths prospectively over 6 months. The study sample comprised 184 men (mean age 81.8) and 670 women (mean age 86.3). Over 6 months, 68 men and 254 women fell one or more times. Serum 25OHD but not PTH was associated with static balance (p<0.001), but not quadriceps muscle strength in either sex. However serum PTH levels were higher in fallers compared to non-fallers (for men: 55 vs 73, p<0.006). Over 6 months, 16/184 men and 50/670 women died. Serum PTH was significantly associated with mortality at 6 months (OR 1.74, p<0.02) independently of age and gender. Serum PTH may be more important than 25OHD in determining falls risk in the frail elderly and mortality.

OR51

B-LYMPHOCYTES ARE ACCUMULATED IN BONE MARROW OF OVX MICE AND INDUCE RANKL EXPRESSION IN OSTEOBLASTS BY THE SIGNAL VIA CELL-TO-CELL CONTACT

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We have reported that the deficiency of sex steroid caused by ovariectomy (OVX) or orchidectomy (ORX) selectively stimulates B-lymphopoiesis, resulting in a marked accumulation of pre-B cells in mouse bone marrow. Accumulation of pre-B cells in bone marrow by the treatment with IL-7 could induce marked bone loss in mice with intact ovarian function, suggesting that accumulated B cells is closely related to the increase in bone resorption due to sex steroid deficiency. In this study, we examined the influence of B cells in the expression of RANKL in osteoblasts.

Both OVX mice and ORX mice showed the elevation of osteoclastic bone resorption and the accumulation of B cells in bone marrow at 2 weeks after operation. When total RNA was extracted from trabecular bone and bone marrow of tibiae at 2-4 weeks after operation, the expression levels of RANKL mRNAs were elevated in OVX and ORX mice compared with respective sham mice. Using B220-coated magnetic micro-beads, B cells were isolated from bone marrow, and more than 98 % of the isolated cells were B220-positive B cells. Removal of the B cells from bone marrow cells markedly suppressed osteoclast formation in coculture of bone marrow cells and osteoblasts, and the addition of B cells recovered the osteoclast formation induced by IL-1 or PGE. Therefore, B cells are essential for osteoclast formation. In FACS and RT-PCR, the isolated B cells weakly express RANKL on their surface. On the other hand, co-culture of B cells and osteoblasts stimulated RANKL expression in osteoblasts. The expression of RANKL in osteoblasts was also induced by the contact with the fixed B cells, and the induction rate of RANKL was correlated with the number of fixed B cells added. Treatment with PD98059 (an inhibitor of ERK 1/2) and SB203580 (an inhibitor of p38 MAPK kinase) suppressed the B cell-induced RANKL expression in osteoblasts, suggesting the involvement of ERK 1/2 and p38 MAP kinase in the signals via cell-to-cell contact between B cells and osteoblats. These results suggest the roles of B-lymphocytes in RANKL-induced osteoclastogenesis and in pathogenesis of osteoporosis.

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GENETICS OF HIP FRACTURE: INDEPENDENT EFFECTS OF THE POLYMORPHISMS OF THE VITAMIN D RECEPTOR AND COLLAGEN 1 ALPHA 1 GENES

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Previous studies have shown that hip fracture is partially genetically determined. Polymorphisms of the Vitamin D Receptor (VDR) and Collagen I Alpha 1 (COLIA1) genes have been associated with bone mineral density (BMD), and with fracture risk. However, it is not clear whether the two genes have additive effects on hip fracture risk.

To examine the contribution of VDR and COLIA1 genotypes to the liability to hip fracture in postmenopausal women, VDR genotypes (TT, Tt, and tt) and COLIA1 genotypes (SS, Ss, and ss) were determined in 677 (69 hip fracture and 608 non-fracture) women of Caucasian background, aged 70 ± 7 (mean ± SD), who were participants in the Dubbo Osteoporosis Epidemiology Study. Atraumatic hip fractures were prospectively identified through radiologists' reports. BMD (g/cm^2) at the hip and lumbar spine was measured by dual energy x-ray absorptiometry.

When analyzed simulateously with age, weight, height and BMD in a multiple logistic regression model, carriers of the tt genotype (16% in the sample) had a 2.6-fold (95% CI: 1.2 to 5.3), and women with ss genotype (5% prevalence) were associated with a 3.8-fold (95% CI: 1.3 to 10.8), increase risk of hip fracture after adjusted for femoral neck BMD (odds ratio [OR]: 3.4; 95% CI: 2.3 to 5.0) and age (OR: 1.4; 95% CI: 1.1 to 1.7). Approximately 33% of the liability to hip fracture was attributable to the presence of the tt genotype of the VDR gene and ss genotype. It is concluded that Caucasian women with VDR tt genotype and COLIA1 ss genotype independently contributed to the risk of hip fracture even after adjustment for age and BMD.

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GROWTH-RELATED CHANGES IN BONE STRENGTH AND SITE-SPECIFIC GEOMETRY AT THE MID-FEMUR DETERMINED BY MRI

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Background: Nature demands the development of stronger, not necessarily heavier bones during growth. In contrast to bone mass, there is little information on the development of bone strength, partly due to the lack of appropriate imaging techniques and software. The biomechanical measures associated with fracture load in animals may also provide useful information on bone strength in humans. Subjects and Methods: In this study on 145 healthy subjects (51 males) aged 6 to 25 years, we examined growth-related changes in bone strength and bone geometry at the proximal (66%), middle (50%) and distal (33%) mid-femoral sites using magnetic resonance imaging (MRI) and dedicated software. Cross-sectional (CSMI) and polar (PMI) moments of inertia as well as the bone geometry measures total (TA), cortical (CA) and medullary areas (MA) were measured. The bone strength index (BSI) of the mid-slice femur (cortical density principal CSMI) was calculated and predictors of BSI and site-specific CSMI were identified. Results: CSMI, PMI and BSI increased significantly with age ($p<0.001$), with percentage increases exceeding by two to four fold those for geometric measures. At the 33% site, MA and TA were largest and CA smallest. At the 50% site, CSMI and PMI were largest and TA smallest. Males had statistically greater CSMI and PMI than females. The CA/total muscle area ratio decreased significantly with age in males, but was constant across ages for females. CSMI and BSI were significantly correlated with geometric and anthropometric variables during growth, but not in adults. In conclusion, the biomechanical resistance of the femur to bending and torsional forces increases with age more than geometric bone measures in both sexes. The observed site-differences in bone geometry and strength within the mid-femur are attributed to either genetic influences and/or site-specific adaptations of bone strength to mechanical loading.

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AGE-RELATED TRENDS IN HIP CROSS-SECTIONAL GEOMETRY IN ASIAN WOMEN

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Independent of bone mineral density (BMD), indices of hip geometry are predictive of hip fractures. This study examined the hip geometry of 203, randomly selected women from Southern Sri Lanka.

Hip DEXA scan pictures (Norland Eclipse XR) of first 206 women, who participated in an ongoing community-based osteoporosis study, were further analyzed for strength related measurements. Hip axis length (HAL) was measured manually using the ruler function, while the neck-shaft angle (NSA) was measured by the software automatically. Strength related indices in the femoral neck were obtained by standard formulae using femoral neck BMD and neck diameter. All women were free of bone active diseases and were not taking bone active drugs. All measurements were adjusted for height and weight and mean values are given for five age groups (39 or below, 40-49, 50-59, 60-69 and 70 years or above). Numbers of subjects in each group were 20, 54, 53, 38 and 38 respectively.

The average age, weight and height of women were 55.4 (12.9) years, 48.3 (9.9) kg and 1.48 (0.06) m respectively. Femoral neck BMDs of age groups were 0.793, 0.808, 0.719, 0.654 and 0.611 gm/ cm2 ($p=<0.001$). Cross-sectional areas (CSA) were 2.27, 2.23, 2.05, 1.91 and 1.80 cm2 ($p<0.001$) while estimated mean cortical thickness (EMCT) were 0.152, 0.155, 0.137, 0.124 and 0.116 cm ($p<0.001$). Femoral neck widths (FNW) in five groups were 3.02, 2.91, 2.99, 3.06 and 3.07 cm ($p=0.049$) while HAL and NSA did not change in a significant way. Although no significant change in cross-sectional moment of inertia (CSMI) with age was observed, the section modulus showed a gradual decline (1.22, 1.13, 1.09. 1.06 and 1.02, $p=0.079$) with age. The buckling ratios of respective age groups were 10.23, 9.76, 11.13, 12.65 and 14.07 ($p<0.001$).

The age-related decline in BMD and bone mass (CSA) at the femoral neck is a universal phenomenon and seen in our women too. Widening of neck with advancing age probably compensates for progressive loss of mass and cortical thickness and partly maintains the CSMI and SM in the old age as seen in other populations.

OR55 F

GENETIC AND ENVIRONMENTAL DETERMINANTS OF BONE MINERAL DENSITY IN SOUTHERN CHINESE WOMEN

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Introduction: Bone mass, an important determinant of osteoporosis, is regulated by multiple genetic and environment factors. The interaction between these factors is unclear.

Objective: To determine the association between bone mineral density (BMD) and genetic as well as environment factors in southern Chinese women.

Method: Polymorphisms of estrogen receptor (ER) alpha and beta, calcium sensing receptor, transforming growth factor beta1 gene and dietary as well as life style factors were analyzed in 118 pre- and 188 postmenopausal women using stepwise multiple regression analysis.

Results: In premenopausal women, body weight was the strongest predictor of BMD, accounting for 9.7% of the variance at the spine, 10.9% at femoral neck, 12.2% at trochanter, 16.3% at the total hip region and 5% at Ward's triangle. Other significant predictors were the dinucleotide CA repeats polymorphism of ERbeta genotype, accounting for 6.5% of the variance at L1-4 BMD, 2.7% at femoral neck BMD, 5.1% at trochanteric BMD and 5.2% at total hip BMD; and also the ERbeta AluI genotype at L1-4 BMD (2.7%). As for postmenopausal women, body weight was the strongest predictor of BMD, accounting for 28.7% of the variance at the spine, 28.4% at trochanter and 31% at total hip. Age was the other strong predictor, accounting for 24.5% of the variance at femoral neck and 34.2% at ward's triangle. Other significant predictors were height at femoral neck (1.4%) and trochanter (1.4%), weight bearing time at femoral neck (1.1%) and trochanter (1.6%), calcium intake at total hip (1.5%). None of the studied candidate genes was a significant predictor for BMD at any site in the postmenopausal women in this model.

Conclusion: Body weight together with ERbeta gene polymorphisms explained ~18% of the variance of bone mass in premenopausal women, whereas age, weight and environmental factors explained ~47% of the variance of bone mass in postmenopausal women.

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GREEN TEA DRINKING IS ASSOCIATED WITH INCREASED BONE MINERAL DENSITY AMONG ELDERLY WOMEN

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Tea drinking is associated with increased bone mineral density (BMD), as demonstrated by previous studies, suggesting that flavonoids, contained in tea, may partly account for it. However, to our knowledge, this study serves as the first to investigate the relationship between consumption of green tea, which also contains flavonoids, and BMD. The aim of this study is to determine whether green tea drinking is associated with increased BMD.

This study included six hundred and fifty-five women aged 60 years and above (mean age 71.6 ±7.6 years) visiting the Osteoporosis Outpatient Clinic in Tokyo Metropolitan Geriatric Medical Center. At entry to the study, body height and weight were measured, and body mass index (BMI) was calculated. Subjects were interviewed their lifestyles by means of a questionnaire which includes consumption of 10 dietary items such as green tea, milk, cheese, yogurt, fish, vegetable, tofu, natto, meat and coffee, smoking, consumption of alcohol, physical activity and use of drug for osteoporosis. For each dietary item, subjects were divided into two groups: consuming five or more days per week, and less than five days per week. BMD of lumbar spine, serum calcium, serum phosphorus, serum parathyroid hormone, serum alkaline phosphatase, serum osteocalcin, serum 1,25-dihydroxyvitamin D and urine deoxypyridinoline were measured. Statistical analysis was performed using a non-paired t test and multiple regression analysis.

Among six hundred and fifty-five subjects, six hundred (91.6%) consume green tea five days or more per week and their mean BMD was 0.808 ±0.199 g/cm^2, and fifty-five (8.4%) consume green tea less than five days per week and their mean BMD was 0.738 ±0.174 g/cm^2. The BMD of the former subjects was significantly higher than the latter. The same result was achieved after adjustment for age, BMI, other dietary items, smoking, alcohol, physical activity and use of drug for osteoporosis. No significant correlation was observed between green tea drinking and any serum or urinary levels. Estrogenic effect induced by flavonoids or apoptosis of osteoclasts induced by (-)-epigallocatechin-3-gallate, one of flavonoids, may account for the significant increase of the BMD. We conclude that green tea drinking is associated with increased BMD among elderly women.

OR57 F

POSSIBLE PATHOGENETIC ROLE OF INTERLEUKIN- 8 AND 10 IN POSTMENOPAUSAL OSTEOPOROSIS AND CHANGES DURING CALCITONIN AND ALENDRONATE THERAPY

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IL-8 is a chemokine of importance in inflammatory processes, and causes an increase in the levels of parathyroid hormone (PTH) mRNA. Additionally, IL-8 may play an important role in the occurrence of pain in osteoporosis. IL-10 has been shown to suppress the levels of the inflammatory cytokines. Moreover, the specific neutralization of IL-10 resulted in an increase in the production of IL-1 and TNF-alpha .

The present study was designed to determine if levels of serum interleukin (IL) -8 and 10 are different in osteoporotic and non-osteoporotic postmenopausal women, and to evaluate the effects of calcitonin and alendronate therapies over a six month period on these cytokine levels in postmenopausal osteoporotic women.

Serum levels of IL-8 were found to be significantly higher ($p<0.05$), and serum IL-10 significantly lower in the calcitonin (N=68) and the alendronate (N=67) treatment groups than in the control group (N=55) ($p<0.05$). But, no significant difference was apparent between the calcitonin and alendronate treated groups before treatment.

Statistically significant changes occurred in patients, with respect to the levels of serum IL-8 after one month ($p<0.05$), IL-8 and IL-10 after three and six months of calcitonin therapy ($p<0.05$). No significant difference was found in IL-8 or IL-10 after three months between the calcitonin and control groups, whereas these parameters were significantly different from the baseline values. In the alendronate treated group, statistically significant changes did not occur in the levels of serum IL-8 and IL-10 during therapy ($p>0.05$). No significant difference was observed in IL-10 after three months between the alendronate group and the control group, whereas these parameters were significantly different at baseline.

In conclusion, we suggest that; 1) IL-8 and IL-10 may have roles in the etiopathogenesis of osteoporosis, 2) calcitonin therapy have a more distinct influence on serum levels of IL-8 and IL-10 and have an earlier effect than alendronate therapy, 3) calcitonin may effect on pain by reducing IL-8 levels. Further longitudinal studies are needed to identify the cytokines involved in the pathogenesis of postmenopausal osteoporosis and to evaluate the influence of different treatments on these cytokines.

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EARLY POSTMENOPAUSAL BONE LOSS - 10.6-YEAR FOLLOW-UP OF OSTPRE COHORT

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We studied the natural developoment of postmenopausal osteoporosis on 398 women from the random sample of the population-based OStPRE cohort. Bone mineral density (BMD) was measured with DXA (Lunar DPX) at the spine (L2-L4) and hip (femoral neck thrice: at baseline, 5.7-year and at 10.5-year follow-up. The study population was formed by excluding women who were premenopausal at 5.7-year follow-up, women without information about menopause, women who had used HRT or bisphosphonates or calcitonin during follow-up and women who lacked a comlete series of valid spine and hip BMD results.

The mean follow-up times were 5.7 years for the first and 4.8 years for the second study period. We divided the study population into three groups: PERI=menopause during the first study period (N=101), EARLY=postmenopausal<=5 years at baseline (N=177) and LATE= postmenopausal>5 years at baseline (N=120). The mean baseline age was 53.8 (SD 2.8) years for the total study population, and 51.2 (1.9), 53.7 (2.5) and 56.1 (1.8) years for the PERI, EARLY and LATE groups, respectively.

Baseline BMDs and annual BMD changes (%) during two study periods are presented in Table 1.

It appears that femoral bone loss rate is quite stable (though PERI lost more than EARLY during the first period ($p<0.001$)), while the rapid perimenopausal spinal bone loss (1 % per year) stops totally in late menopause.

Table 1. Baseline BMDs and annual BMD changes (%) during two study periods

Site& **Group (n)**	**Baseline BMD, g/cm^2**	**Annual BMD change (%) during two study periods**		
		1st Period (5.7 years)		**2nd Period (4.8 years)**
Spine				
PERI (101)	1.19 (0.12)	-1.11	***	-0.19
EARLY (177)	1.12 (0.14)	-0.63	***	+0.07
LATE (120)	1.05 (0.15)	-0.13	*	+0.17
Total (398)	1.12 (0.15)	-0.60	***	+0.03
Hip				
PERI (101)	0.99 (0.13)	-0.96	ns	-0.93
EARLY (177)	0.93 (0.11)	-0.68	ns	-0.81
LATE (120)	0.90 (0.11	-0.51	ns	-0.73
Total (398)	0.93 (0.12)	-0.70	ns	-0.81
Paired t-tests between study periods: ***p<0.001, *p<0.05				

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GP130 REGULATES BONE TURNOVER AND BONE SIZE BY DISTINCT DOWNSTREAM SIGNALING PATHWAYS

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IL-11, IL-6 and LIF signal by interaction of their specific receptors with the gp130 co-receptor subunit. gp130 signaling is itself mediated through at least two intracellular pathways including the SHP2/ras/MAPK and STAT1/3 pathways.

To further define the pathways through which gp130 regulates bone mass, we have analyzed the bone phenotypes of two gp130 mutant mice. The first mutant ($gp130^{DELTASTAT}$) harbors a C-terminal truncation deleting all STAT1/3 signaling (Ernst 2001) . The second mutant ($gp130^{Y757F}$) contains a point mutation to abolish SHP2/ras/MAPK signaling (Tebbutt 2002).

Bone turnover and trabecular bone volume were normal in $gp130^{DELTASTAT}$ mice. However, tibial and femoral lengths were significantly lower than controls, and growth plate closure was observed as early as 12 weeks of age. This indicates that while gp130 signaling through STAT1/3 is not required for normal bone turnover, it is required for continued longitudinal bone growth.

$gp130^{Y757F}$ bones were of normal size, but trabecular bone volume was reduced by approximately 50%. This was associated with high bone turnover; osteoclast surface (OcS/BS) was 70% greater, osteoblast surface (ObS/BS) was doubled and osteoid volume (OV/BV) was three times that of control mice. Similarly, in vitro RANKL-driven osteoclastogenesis was elevated in cultures of bone marrow cells from $gp130^{Y757F}$ mice compared to controls, indicating that this effect does not relate to alterations in systemic hormones. The high bone turnover in these mice indicates that gp130 signaling through SHP2/ras/MAPK is required for normal bone turnover. This is comparable with elevated osteoclastogenesis in the gp130 knockout previously described, and suggests that gp130-mediated effects on osteoclastogenesis and trabecular bone volume are mediated through the SHP2/ras/MAPK pathway.

Although male $IL\text{-}6^{-/-}$ mice have no bone abnormality, in male compound $gp130^{Y757F}$; $IL\text{-}6^{-/-}$ mice ObS/BS was rescued to levels of wild type-mice, while OcS/BS remained high, causing severe osteopenia. Thus, IL-6 selectively mediates the moderating effect of the SHP2/ras/MAPK pathway on bone formation but not on osteoclastogenesis.

In conclusion, gp130 is required for normal longitudinal bone growth and regulation of trabecular bone mass, but these activities are mediated by distinct downstream signaling pathways.

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FEMALE TRANSGENIC MICE EXPRESSING HUMAN INTERLEUKIN-11 WITH OR WITHOUT OVARIECTOMY DEMONSTRATE HIGHER BONE MASS THAN CONTROL LITTERMATES

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Cytokines in interleukin (IL) -11 subfamily participate in the regulation of bone cell proliferation and differentiation. We have reported positive effects of IL-11 on osteoblasts and bone formation. Overexpression of human IL-11 gene in male transgenic mice results in the stimulation of bone formation to increase cortical thickness and strength of long bones, and in the prevention of cortical bone loss with advancing age. Bone resorption and osteoclastogenesis are not affected in IL-11 transgenic mice. In experiments in vitro, IL-11 stimulates transcription of the target gene for bone morphogenetic protein (BMP) via STAT3, leading to osteoblastic differentiation in the presence of BMP-2, but inhibits adipogenesis in bone marrow stromal cells. These results indicate that IL-11 is a stimulatory factor for osteoblastogenesis and bone formation in male mice to conserve cortical bone, possibly by enhancing BMP actions in bone. It is, however, yet uncertain whether IL-11 has the same effects on bone in female mice as those in male mice, in particular after estrogen withdrawal. To address this issue, the present study was undertaken to clarify effects of ovariectomy on bone metabolism in 10-week-old female mice

overexpressing human IL-11 gene in comparison with their control littermates. Static and dynamic histomorphometry of proximal tibiae was analyzed 4 weeks after ovariectomy. Bone mineral density of femurs was determined with dual energy X-ray absorptiometry. Bone marrow cells obtained from long bones were also examined. Similar to male transgenic mice, bone formation and osteoblastogenesis was higher in female transgenic mice than control littermates with or without ovariectomy. Bone resorption was increased after ovariectomy, but was not different between transgenic and control mice with or without ovariectomy. As a result, bone mass and bone mineral density decreased with ovariectomy, while they were higher in transgenic mice than control littermates with or without ovariectomy. These results indicate that IL-11 does not enhance bone resorption after estrogen withdrawal and that it is a stimulatory factor for bone formation in female mice as in male mice. Thus, IL-11 may be a new therapeutic target for osteoporosis.

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ELEVATED CIRCULATORY AND EXPRESSION LEVEL OF FIBROBLAST GROWTH FACTOR (FGF)-23 IN HYPOPHOSPHATEMIC MICE

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The presence of an unidentified phosphaturic factor, sometimes called phosphatonin, has been implicated in the pathogenesis of both X-linked hypophosphatemic rickets (XLH) with mutations in PHEX gene and tumor-induced osteomalacia (TIO). Hyp mice are murine homologues of XLH and the presence of the humoral phosphaturic factor has also been demonstrated in this model mice. Fibroblast growth factor (FGF)-23 has been identified as a causative factor of TIO and demonstrated to induce hypophosphatemia with increased phosphate excretion and decrease of serum 1,25-dihydroxyvitamin D (1,25D) level. In addition, we have recently shown that circulatory FGF-23 level is elevated in patients with XLH by establishing ELISA for human FGF-23. These results strongly suggest that FGF-23 is a circulatory phosphaturic factor in patients with XLH and Hyp mice. To address this issue, we investigated the changes in serum level and expression profile of FGF-23 in Hyp mice (female, Hyp/+) compared to those of control mice. We first confirmed that mouse FGF-23 can be measured by our ELISA. Serum concentration of FGF-23 in Hyp mice was significantly higher than that of age-matched control mice (1436.6 ± 98.7 vs 112.2 ± 7.6 pg/mL at 10 weeks of age). To identify tissues producing FGF-23, we analyzed the expression of FGF-23 by RT-PCR in calvaria, femurs containing bone marrow, heart, thymus, spleen, liver, lung, kidney, jejunum and brain. Although amplified signal was detected only in spleen in control mice, weak signals were also observed in thymus, heart and femur in Hyp mice. In addition, prominent expression of FGF-23 was found in calvaria of Hyp mice. Furthermore, immunohistochemical staining of parafin embedded tissue sections for FGF-23 demonstrated strong staining in the region surrounding osteocytes in femoral cortical bone of Hyp mice. Similar pattern was also observed in calvaria. Specific staining could not be observed in any other organs. These findings suggest that abnormal overproduction of FGF-23 in bone causes elevation of circulatory FGF-23 level and consequently hypophosphatemia with inappropriately low 1,25D level in Hyp mice. It is also implicated that PHEX gene product is involved in the regulatory mechanism of FGF-23 expression in bone cells.

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FGF23 RECEPTOR RESPONSIBLE FOR INHIBITING TYPE IIA NA-PI COTRANSPORTER (NAPI COTRANSPORTER) APPEARS TO BE DIFFERENT FROM FGF RECEPTORS (FGFRS) 1-4

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Autosomal dominant hypophosphatemic rickets (ADHR) is caused by mutations of FGF23, that make the molecule less susceptible to proteolytic cleavage, whereas overproduction of FGF23 is the cause of tumor-induced osteomalacia. In both disorders, FGF23 inhibits phosphate reabsorption through NaPi cotransporter at renal proximal tubules. In the previous study (ASBMR 24th annual meeting, Abstract F137) using OK cells which represent proximal tubular cells, we examined the expression of FGFRs 1-4, their phosphorylation after stimulation by FGF23[R179Q-HA-His], recombinant FGF23 with one of the ADHR mutations and an HA-His epitope at the C-terminus, and their interaction with NaPi cotransporter. OK cells expressed all FGFRs 1-4. Only FGFR2 was phosphorylated among the four receptors when they were stably transfected, and FGFR2 was further phosphorylated when stimulated by FGF23[R179Q-HA-His]. Extracellular domain of FGFR2-IIIb or -IIIc fused to IgG-Fc fragment interacted with FGF23[R179Q-HA-His]. Furthermore, only FGFR2 interacted with NaPi cotransporter. In the current study, we examined if expression of FGFR2 wild type (WT) or dominant negative type (DN) affects the expression of NaPi in OK cells. When increasing doses of FGFR2 cDNA and a fixed dose of NaPi cDNA are cotransfected into OK cells, FGFR2-WT increased NaPi expression in a dose-dependent fashion. In OK cells cotransfected with FGFR2-DN and NaPi, the expression level of NaPi did not changed as compared to the cells transfected with NaPi alone, but incubation with FGF23[R179Q-HA-His] for 3 hours decreased NaPi expression significantly in the cotransfected cells. These results suggest that FGFR2 activation increases NaPi expression post-transcriptionally, and that FGF23 inhibits NaPi cotransporter activity by decreasing the cotransporter expression by binding to a receptor distinct from FGFR2. The distinct FGF23 receptor is likely to be different from all known FGFRs (1, 2, 3 or 4), since FGFRs can heterodimerize each other and can be inhibited by FGFR2-DN.

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VITAMIN D SUPPRESSES PERIOSTEAL BONE FORMATION THROUGH SUPPRESSING RUNX2 GENE EXPRESSION

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1,25-Dihydroxyvitamin D3 ($1,25(OH)_2D_3$) is a principal regulator of calcium and phosphorus homeostasis through actions on intestine, kidney, and bone. $1,25(OH)_2D_3$ is not considered to play a significant role in bone formation, except for its role in supporting mineralization. Actually newborn vitamin D receptor null mutant mice (VDRKO) show no abnormalities in bone until weaning. However, its role in bone formation under normal mineral circumstance has not been clarified. To clarify this issue, we analyzed VDRKO bone under normal mineral circumstance by using ectopic bone transplantation system.

Dissected femurs or calvariae from 2 week old wild or VDRKO mice were transplanted into the back muscle of wild mice. As expected, wild bone in VDRKO showed marked bone resorption. However, the VDRKO bone in wild mouse (KOW) showed dramatic increase in bone density compared with wild bone in wild mouse (WW). Bone volume was 0.175 mm^3 in KOW and 0.105 mm^3 in WW at 4 weeks after transplantation. Similarly, bone thickness of calvaria was 180 microm, which was 2.7 fold compared to WW. To exclude the possible contamination of the host cells, we next transplanted the bones wrapped with membrane filter. Even in the absence of the wild type host cells, KO calvarie showed increased bone thickness and KO femoral diaphysis showed increased cortical thickness. Histological examination with calcein labeling clearly demonstrated that the increases in calvarial thickness and in cortical thickness of femoral diaphysis were not due to the decreased osteoclastic bone resorption but due to the increased bone formation. To explore the mechanism, we next examined mRNA expression of the genes, which may play key roles in bone metabolism, by quantitative RT-PCR in the transplanted bone. The results demonstrated increased expression of *runx2* and *opg* in KO bone.

From these results, we conclude that vitamin D may play an important role in normal bone by protecting it from excessive periosteal bone formation through suppressing the expression of *runx2*.

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LACTOFERRIN, A POTENT ANABOLIC FACTOR IN BONE, SIGNALS THROUGH THE LOW-DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN (LRP) 1

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Lactoferrin (Lf) is an 80 kDa glycoprotein that is present in breast milk and epithelial secretions, and is released by inflammatory cells during the immune response. It circulates at 2-7microg/ml and is believed to be involved in regulation of iron metabolism, immune function and embryonic development.

We recently identified Lf as an osteoblast growth factor present in fractionated bovine milk. Lf dose-dependently and potently stimulates proliferation of cultured osteoblastic cells of rat, mouse and human origin, and inhibits apoptosis induced by serum withdrawal in primary rat osteoblasts. Lf strongly inhibits osteoclastogenesis in a murine bone marrow culture assay, but did not affect the bone-resorbing activity of mature osteoclasts. The ability of Lf to induce osteoblast anabolism and inhibition of osteoclast development *in vitro* suggested that it might be anabolic to bone *in vivo*. Indeed, when Lf was administered to adult mice by unilateral local hemicalvarial injection, there were dose-dependent and substantial increases in indices of bone formation and bone area.

Lf has been reported to bind to two endocytic members of the family of low density lipoprotein receptors, LRP1 and LRP2/megalin. We found that LRP1 and LRP2 are both expressed in rodent osteoblastic cells, but only LRP1 is expressed in SaOS2 cells. Using confocal microscopy, we observed that Lf is endocytosed by primary rat osteoblastic cells, and that the LRP1/2-specific inhibitor, receptor-associated protein (RAP), abrogates this process. Lf activates p42/44 MAP kinase signaling in osteoblastic cells, and Lf-induced osteoblast proliferation is inhibited by specific inhibitors of MAP kinase kinase. Lf-induced osteoblast proliferation and MAP kinase phosphorylation are also blocked by RAP, implicating LRP1/2 as mediators of the mitogenic effects of Lf in osteoblasts. Lf induces proliferation in SaOS2 cells, which express LRP1 but not LRP2, and Lf-induced proliferation in LRP1-null fibroblasts is substantially less marked than that observed in $LRP1^{+/+}$ cells. Taken together, these data demonstrate that Lf is anabolic to bone, and that its growth factor-like effects on

osteoblastic cells are mediated in large part by LRP1. This work provides further evidence for an important role of the LRP receptor family in regulation of bone growth.

OR65

HYPOTHALAMIC NEURONS SENSITIVE TO MONOSODIUM GLUTAMATE CONTRIBUTE TO THE REGULATION OF BONE FORMATION

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We showed earlier that hypothalamic neurons control bone formation. In particular goldthioglucose-sensitive neurons predominantly located in the ventromedial hypothalamic nuclei are required for leptin inhibitory effect on bone mass. In the course of these studies we observed that neurons sensitive to monosodium glutamate (MSG) did not affect bone mass. This observation was surprising as MSG-sensitive neurons are known to control many homeostatic functions. Moreover, MSG treatment leads to hypogonadism, a condition that should increase bone resorption and lead to a low bone mass over time. To analyze in a more systematic manner the role of MSG-sensitive neurons in the control of bone mass we assessed bone resorption and bone formation parameters in mice treated by MSG. We show here that MSG-treated mice as expected display an increase in bone resorption, but also display an increase in bone formation. The extent of the increase in bone formation is such that it neutralizes the effects of the elevated bone resorption thus explaining the normal bone mass of MSG-treated mice. Correction of MSG-induced hypogonadism by estradiol treatment corrected the abnormal resorptive function of these mice and uncovered their high bone mass phenotype. Thus, this study demonstrates that MSG-sensitive neurons do play a role in the control of bone formation albeit more modest than GTG-sensitive neurons and reveals that distinct populations of hypothalamic neurons are required for the control of bone mass. These results are consistent with the notion that additional molecules besides leptin control bone formation via a central relay.

OR66 S

VITAMIN D RECEPTOR NULL BONE SHOWS HIGHER PERIOSTEAL BONE FORMATION

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Although vitamin D has roles in many biological phenomena such as bone metabolism and mineral homeostasis, the direct action of vitamin D in bone formation has not been clarified. It has been reported that mice lacking the vitamin D receptor (VDR) show indistinguishable bone phenotype at birth, and after weaning they develops rickets. Moreover, this rickets may be rescued by calcium supplementation or phosphate restriction. However, these findings could not exclude the direct action of vitamin D, because of the hyperparathyroidism and unstable levels of the serum calcium and phosphorus.

To clarify the direct action of vitamin D on bone, we compared bone metabolism between wild type (WT) bone and VDR null (VDRKO) bone using organ culture.

The quantitative data on VDRKO bone have not been available so far. Thus we first analyzed the femur of VDRKO at birth with microfocus X-ray, microfocus CT, pQCT and histology. All these morphological analyses clearly indicated that VDRKO bone showed increased cortical bone at diaphysis and was indistinguishable at metaphysis.

The total bone mineral density (BMD) of VDRKO femur was 1.14 fold, the cortical bone volume of VDRKO was 1.8 fold, the cortical BMD was 1.15 fold, and the cortical thickness was 2.44 fold higher than those of WT.

Seven days organ culture of the femur without serum exaggerated the differences. The total bone mineral density (BMD) of VDRKO femur was 1.28 fold, the cortical BMD was 1.24 fold, and the cortical thickness was 7.25 fold higher than those of WT after 7days culture.

Moreover, in the presence of 10-8 M 1,25 dihydroxycholecalciferol, VDRKO femur did not show any responses but the cortical BMD of the WT femoral diaphysis decreased by 54% of the cortical BMD of vehicle control.

To explore cellular mechanism, osteoclast number was counted on the longitudinal section. However, we could not demonstrate any differences between VDRKO and WT either at birth or after 7 days culture.

These results suggest that VDRKO bone has higher periosteal bone forming potential and that vitamin D may play a role to regulate periosteal bone formation negatively.

OR67 S

THE EFFECT OF DELETION OF INDUCIBLE CYCLOOXYGENASE (COX-2), PROSTAGLANDIN RECEPTOR EP2 OR EP4 ON OSTEOCLASTOGENESIS INDUCED BY MOUSE MAMMARY CANCER CELL LINES

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We previously reported that induction of osteoclast (OC) formation by two mouse mammary cancer cell lines, MMT060562 (MMT) and BALB/c-MC (BALB), was dependent on production of PGE2 through COX-2 in co-culture of these cells with bone marrow cells. However MMT was able to express COX-2 and produce PGE2 by itself, while BALB acted by inducing COX-2 in marrow cells. To confirm and extend these studies we have examined the response to MMT and BALB in co-cultures with marrow cells from mice in which COX-2, EP2 or EP4, which is associated with osteoclastogenesis, has been deleted. Co-cultures of MMT with marrow cells from COX-2 wild-type (WT) or knockout (KO) mice showed similar increases of the number of OC (45 ±17/well vs 42 ±8/well). In contrast OC were not formed and PGE2 concentration was not increased in co-cultures of BALB with COX-2 KO marrow cells (OC: 40 ±14/well vs 1 ±1/well, PGE2: 2.3 ±0.6 ng/ml vs 0.2 ±0.1 ng/ml; COX-2 WT vs COX-2 KO, respectively). The induction of OC by cancer cells was not decreased when the marrow cells were derived from EP2 KO mice (MMT: 38 ±11/well vs 33 ±8/well, BALB: 51 ±13/well vs 42 ±12/well; EP2 WT vs EP2 KO, respectively). In co-cultures of cancer cell lines and EP4 KO marrow cells the number of OC was markedly diminished (MMT: 156 ±26 vs 6 ±2/well, BALB: 50 ±15 vs 2 ±1/well; EP4 WT vs EP4 KO, respectively). We conclude that two mammary cancer cell lines, MMT and BALB, induce OC formation via different mechanisms with respect to COX-2. MMT can produce sufficient PGE2 to induce osteoclastogenesis by itself, while BALB stimulate COX-2 in marrow cells and hence are ineffective when the bone marrow cultures are from COX-2 KO animals. The critical receptor for OC formation in this co-culture system appears to be EP4 and not EP2.

OR68 S

SPROUTY2 INDUCED BY CONSTITUTIVELY ACTIVE FGF RECEPTOR 3 SUPPRESSES THE CHONDROCYTE PROLIFERATION THROUGH INHIBITION OF IGF-1 SIGNALING

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Achondroplasia, the most common form of short-limb dwarfism, and its severe type, thanatophoric dysplasia (TD) are caused by constitutively activated mutations in FGFR3. The excessively activated FGFR3 appears to suppress the proliferation of chondrocytes, resulting in disturbing the growth of long bones. However, little is known how these mutations suppress proliferation of chondrocytes. To address this, we determined whether Sprouty2, which mediates FGFR3 signals, is involved in this process, using a chondrogenic cell line, ATDC5. BrdU incorporation assay demonstrated that the cells expressing TDII-mutated FGFR3 (TDII cells) showed the decreased DNA synthesis by 40%, compared with wild-type FGFR3 expressing cells (WT cells). In contrast, the expression of Sprouty2 mRNA is significantly increased in TDII cells. GH-IGF axis is an important system in bone growth. Especially, IGF-1 has a potent stimulator of chondrocyte proliferation. Of note, overexpression of Sprouty2 in WT cells markedly decreased IGF-1 dependent-DNA synthesis by 30% of WT cells. Furthermore, overexpression of dominant-negative Sprouty2 recovered DNA synthesis in TDII cells. These data suggest that Sprouty2 negatively regulates the mitogenic effects of IGF-1. To further investigate the mechanism, by which TDII FGFR3 mutation suppressed the IGF-1-dependent proliferation, we examined the effect of Sprouty2 on the MAP kinase since IGF-1 exhibits the mitogenic activity through MAP kinase pathway. In WT cells, IGF-1 increased MAP kinase activity as well as proliferation. Overexpression of Sprouty2 suppressed the IGF-1-induced MAP kinase activity. In conclusion, these results collectively suggest that excessive activation of FGFR3 by TDII mutation suppressed MAP kinase by up-regulating the Sprouty2 expression, consequently inhibiting the proliferation of chondrocytes. Our data suggest that excessive expression of Sprouty2 may account for the disturbance of endochondral bone formation in FGFR3-related skeletal dysplasia.

OR69 S

OVER-EXPRESSION OF OPG BY BREAST CANCER CELLS ENHANCES TUMOR GROWTH

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Osteoprotegerin (OPG) is a natural decoy receptor for RANKL (receptor activator of NF-kB ligand), and inhibits osteoclast formation and activation. We have examined the expression of OPG in breast cancer cell lines and breast tumors and established

that its expression varied. Since the local production of OPG by breast tumor cells may influence their growth in bone, we have studied the effects of OPG expression in MCF-7 parental cells and MCF-7 cells over-expressing PTHrP following transfection with full-length cDNA for OPG (1-401 aa) in pCEP4. In vitro analysis demonstrated that over-expression of OPG in MCF-7 cells resulted in increased cell growth, in the presence or absence of PTHrP.

Parental, vector control and MCF-7 cells over-expressing PTHrP, OPG or PTHrP plus OPG were injected into the proximal tibiae of athymic nude mice. The animals were monitored for 2.5 weeks, after which they were sacrificed and their limbs were assessed by radiology and histology. No osteolysis was observed radiologically following inoculation of the MCF-7 parental cells or the MCF-7 cells over-expressing OPG, although small intramedullary tumors were evident histologically. Surprisingly, the MCF-7 cells over-expressing PTHrP and OPG developed larger tibial tumors than the MCF-7 cells over-expressing PTHrP alone (18.6 ± 3.1 % osteolysis as determined by radiology, compared with 9.0 ± 2.2 %, $p < 0.05$). The tumors over-expressing OPG also exhibited a change in histology, which was reflective of a less differentiated phenotype compared with MCF-7 cells over-expressing PTHrP. This increased tumor growth afforded by the over-expression of OPG was abrogated by treatment with OPG-Fc (2.5 mg/kg/day, subcutaneous) resulting in inhibition of tumor growth (2.5 ± 0.3 %, $p < 0.001$ compared with control).

The same cell lines were injected into the mammary fat pad, and growth assessed for up to 8 weeks. Similar to the in vitro findings, OPG over-expression conferred enhanced growth to either MCF-7 or MCF-7 and PTHrP expressing cells in the mammary fat pad.

These results indicate that full-length native OPG exerts dramatically different actions on tumor behavior than OPG-Fc. These differences may relate to the death domains of native OPG or its intracellular expression.

OR70 S

OSTEOCLASTS ENHANCE MYELOMA GROWTH AND SURVIVAL: A VICIOUS CYCLE BETWEEN BONE DESTRUCTION AND MYELOMA EXPANSION

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Multiple myeloma (MM) is a malignancy of plasma cells. It almost exclusively develops in the bone marrow, and generates devastating bone destruction by osteoclasts (OCs) induced by MM cells in their close vicinity. We and others have identified and reported that CC chemokines macrophage inflammatory protein (MIP)-1 alpha and beta are among major OC activating factors secreted by MM cells. Since MM cells almost exclusively expand in the bone marrow and preferentially grow in bone destructive lesions, close interactions of MM cells with bone cells may be critical to the tumor expansion and the develoment of the bone disease. Accordingly, we have reported that human PBMC-derived OCs enhanced survival and growth of MM cells in a partially IL-6-dependent but strongly contact-dependent manner. In the present study, we further investigated a role as well as a mechanism of OC-mediated MM cell expansion. Even though bone marrow stromal cells also supported MM cell expansion, the stimulatory effect of OCs on MM growth was much stronger than that of stromal cells. When RPMI8226 cells were exposed to doxorubicin at 2.0 microM for 2 hours, washed and co-cultured with PBMC-OCs, the MM cells resumed to proliferate. Fold increase in MM cell number enhanced by OCs was more prominent in surviving cells after doxorubicin treatment than in non-treated cells (6- vs. 3-fold at day 4), suggesting that OCs counteract cytotoxic effects of doxorubicin. We next turned to osteopontin (OPN), a noncollagenous matrix protein, because it was found to be produced by OCs at a large amount, and MM cells abundantly expressed OPN receptors on their surface including alphavbeta3 integrin, VLA-4, and CD44 which have been shown to be involved in growth and survival signals. Co-cultures with MM cells also increased OC production of OPN as well as IL-6 in a contact-dependent manner. Consistently, anti-OPN antibody significantly, although still partially, suppressed the OC effect in concert with anti-IL-6. OC-derived unknown factor(s) may be involved in addition to IL-6 and OPN. In conclusion, OCs augment growth and activity of MM, thereby forming a vicious cycle that leads to extensive bone destruction and MM expansion.

OR71 S

ACTIVATION OF ESTROGEN RECEPTOR IS MEDIATED BY STIMULATION OF THE CALCIUM-SENSING RECEPTOR IN MCF-7 BREAST CANCER CELLS

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Bone is the most common site affected by breast cancer cells, even more when they are estrogen receptor (ER)-positive. Metastatic cells can stimulate directly or indirectly osteoclast-mediated bone resorption. Tumor-induced osteolysis is often extensive and leads to the release of large quantities of calcium. Metastatic cancer cells can be thus exposed to high calcium concentrations (40 mM has been reported at the resorption site). Using the MCF-7 cell line, we have previously shown (Endocrine Society 2002 Meeting, abstract #P1-653) that 20 mM Ca^{++} down-regulated ER protein and caused ER-mediated transactivation of a reporter gene by 55 ±10% (mean ±SD) in MVLN cells (MCF-7 cells stably transfected with the ERE cloned upstream the luciferase reporter gene). Moreover, 3 mM Ca^{++} enhanced progesterone receptor (PgR) expression by 81 ±10% as determined by EIA. These data suggest that Ca^{++} may exert a weak estrogenic effect by acting at the cell membrane level since a Ca^{++} influx caused by the ionophore A23187 did not induce any ER activation. We have further examined the mechanisms of Ca^{++} actions and investigated the involvement of calcium-sensing receptor (CaR) expressed on MCF-7 cells (Sanders et al., Endocrinology 2000). For this purpose, we tested the effects of the calcimimetic CaR activator NPS R-467, and its less active stereoisomer NPS S-467, used at 0.001 and 0.01 mM, in the presence of 1 mM Ca^{++}. We detected by Western blot a marked decrease in ER protein when MCF-7 cells were exposed to 0.01 mM NPS R-467. This R-isomer increased PgR expression by 41 ±4% in MCF-7 cells and increased the transcriptional activity of ER by 67 ±11% in MVLN cells. The S-isomer was less effective, indicating that the effects were indeed mediated by the CaR. In summary, our results suggest that calcium released during the process of metastatic bone destruction could modulate ER, a key receptor involved in breast cancer cells growth and function, and thus play a role in the pathogenesis of tumor-induced osteolysis. These data also indicate that Ca^{++}-induced ER activation is mediated by the CaR on breast cancer cells.

OR72 S

REGULATORY EFFECT OF 1,25-DIHYDROXYVITAMIN D ON GENE EXPRESSION OF EPITHELIAL CALCIUM CHANNELS 1 AND 2 IN INTESTINE AND KIDNEY OF MICE

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Calcium transport in intestine and kidney is stimulated by 1alpha,25-dihydroxyvitamin D_3 (1,25-D_3). Calcium regulating proteins, such as calbindin-D, Ca-Mg ATPase(PMCA1b) and sodium calcium exchanger (NCX1), are involved in this process and gene expression of these proteins is thought to be regulated by 1,25-D_3. Recently, epithelial calcium channels 1 and 2 (ECaC1 and ECaC2) have been cloned from the intestine and kidney of mammals, and it is expected that they may serve as a gatekeeper of 1,25-D_3-dependent active transepithelial calcium transport. However, regulatory mechanisms of the proteins remained unclear. In this study, to clarify the regulatory effect of 1,25-D_3 on the gene expression of ECaC, ECaC mRNA expression in the intestine and kidney were examined in wild and vitamin D receptor null mutant (VDRKO) mice. Furthermore, gene expression in primary renal tubular cells (PRTC) of mice was evaluated. Calbindin-D_{9k} mRNA was also measured as a 1,25-D-dependent positive control in both tissues. Expression of ECaC2 mRNA was observed in both tissues, while ECaC1 mRNA was observed only in the kidney. In a time-course study, intestinal ECaC2 mRNA began to increase from 3h after intravenous injection of 1,25-D_3 (6.25 microg/kg) and reached to the maximum at 6h. Similarly, renal ECaC1 and ECaC2 mRNAs began to increase from 3h and continuously increased by 9h. In a dose-dependent study, ECaC1 and ECaC2 mRNA in both tissues increased in response to 1,25-D_3 injection (0.1-10 microg/kg). Intestinal ECaC2 and calbindin-D_{9k} mRNA of VDRKO mice were significantly lower than those of wild mice. However, no significant difference was observed in the mRNA levels of renal ECaC1 and ECaC2 between wild and VDRKO mice. Renal calbindin-D_{9k} mRNA of VDRKO mice was extremely lower than that of wild mice. In the PRTC of wild mice, ECaC1 and calbindin-D_{9k} mRNA increased in response to 1,25-D_3 treatment (10^{-9} - 10^{-7} M). However, in the PRTC of VDRKO mice, ECaCs and calbindin-D_{9k} mRNA did not change by treatment with 1,25-D_3. These results suggest that gene expression of ECaC1 and ECaC2 is regulated by 1,25-D_3. However, other factors except 1,25-D_3 would be involved in the regulation of renal ECaCs mRNA expression in hypocalcemic condition.

OR73 S

INTERLEUKIN-11 IS AN OSTEOGENIC CYTOKINE THAT INHIBITS OSTEOBLAST APOPTOSIS: UP-REGULATION OF BCL-2 AND SYNERGISM WITH ESTROGEN

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Impaired bone formation plays a role in glucocorticoid (GC)-induced and senile osteoporosis. We have shown that marrow stromal cell expression of an AP-1 taget interleukin-11 is reduced by aging and GC treatment. Conversely, osteogenic stimuli such as PTH and mechanical stress induces IL-11 expression by the osteoblast lineage cells both in vitro and in vivo. Moreover, we have demonstrated that transgenic mice over-expressing IL-11 exhibit high bone mass due to enhanced bone formation and are protected from aging-associated bone loss. These results suggest that IL-11 may play a role in physiological and pathological regulation of bone formation. As for the mechanism of IL-11 action, we have found that IL-11 not only stimulates

osteoblastogenesis while inhibiting adipogenesis but also strongly suppresses osteoblast apoptosis. Therefore, in the present study, we investigated the mechanisms by which IL-11 couteracts osteoblast apoptosis. We found that IL-11 inducers such as mechanical stress and PTH, IL-11 itself, and both estradiol and tamoxifen, all blocked dexamethasone and etoposide-induced apoptosis assessed by DNA ladder formation and trypan blue exclusion assays. The effect of PTH was partially reversed by an anti-IL-11 neutralizing antibody. The anti-apototic effect of IL-11 was shared by at least some other gp130 family cytokines such as LIF. Interestingly, we observed that all the anti-apototic agents above increased expression of bcl-2 mRNA and protein in osteoblasts while GC reduced it. This GC effect was reversed by PTH or IL-11. IL-11 induced phosphorylation of Stat3, ERK and PKB/Akt in osteoblasts, but only inhibition of ERK, but not of JAK or PI-3 kinase, clearly blocked the bcl-2 induction by IL-11. Inhibition of transcription resulted in loss of the effects of PTH and estrogen, but not of IL-11, suggesting a distinct mechanism of the IL-11 action. Consistently, we found that the maximal effect of IL-11 was further enhanced by co-treatment with estrogen. These observations suggest a divergent role of IL-11 in the regulation osteoblast apoptosis and thereby bone formation under both physiological and pathological conditions. Our results further imply that IL-11 itself or its inducers may serve as an effective therapeutic intervention, acting cooperatively with estrogen or SERM.

OR74 S

ASSOCIATION BETWEEN HYPOPHOSPHATEMIA AND IMPAIRED OSTEOCLASTOGENESIS AND BONE RESORPTION IN HYP MICE

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X-linked hypophosphatemic rickets (XLH) is characterized by hypophosphatemia and impaired calcification. Studies using Hyp mice, which are a murine homologue of XLH, suggest that autonomous dysfunction of osteoblasts is the primary cause of the skeletal abnormalities. On the other hand, the involvement of osteoclasts in the pathogenesis of these skeletal abnormalities has not been explored yet. In the present study, we examined whether osteoclasts take a part in the pathophysiology of the skeletal phenotype in Hyp mice. Histomorphometrical examination of Hyp mouse bones demonstrated marked decrease in TRAP-positive osteoclasts and disoriented trabecular bones in addition to increased osteoid and disturbed endochondral ossification compared with wild-type (WT) mouse bones. These in vivo observations suggest that reduced number of osteoclasts is associated with the skeletal phenotype of Hyp mice. We, therefore, examined whether osteoclastogenesis was diminished in Hyp mice. Contrary to our expectation, TRAP-positive osteoclast-like cell formation in Hyp mice-derived bone marrow cells was not reduced. Furthermore, bone-resorbing activity of Hyp osteoclasts determined by pit formation on dentin slices was not diminished compared with WT osteoclasts, either. These results led us to hypothesize that the reduced osteoclast number and activity in Hyp mice were due to changes in host milieu rather than autonomy in cells of osteoclast lineage. Accordingly, we examined whether the extracellular phosphate levels affected osteoclast differentiation and function. Reduction of phosphate levels in the culture medium dose-dependently decreased TRAP-positive osteoclasts-like cell formation and bone-resorbing activity in both Hyp and WT bone marrow cells. In conjunction with this, RT-PCR analysis showed that mRNA expression of RANKL in marrow stromal cells was decreased, whereas OPG mRNA expression was not altered. As another host factor that may affect osteoclastogenesis in Hyp mice, we tested matrix extracellular phosphoglycoprotein (MEPE). MEPE is a candidate for phosphatonin and its expression is increased in Hyp osteoblasts. Our data showed that MEPE inhibited TRAP-positive osteoclast-like cell formation, suggesting that MEPE may play a role in impaired osteoclastogenesis in Hyp mice. In conclusion, these results suggest that impaired osteoclastogenesis and bone resorption due to hypophosphatemia contribute to the pathogenesis of skeletal abnormalities in Hyp mice.

OR75 S

HIGH BONE MASS IN HUMAN AND MURINE LIPODYSTROPHY, STUDY OF THE ROLE OF LEPTIN

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We previously showed that, in mice, leptin regulates bone formation through a hypothalamic relay independently of its regulation of body weight. Consequently, leptin signaling deficient as well as adipocytes-deficient mice display a high bone mass phenotpye. Here we report the existence of high bone mass in a lipodystrophic patient, indicating that leptin antiosteogenic function may have been conserved through evolution. To demonstrate more directly that leptin was the gene expressed by adipocytes whose absence causes high bone mass in lipodystrophy, we attempted a rescue of this phenotype in an animal model of lipodystrophy by restoring leptin expression. Leptin expression normalizes the bone mass of these lipodystrophic animals, demonstrating that the absence of leptin is the cause of the high bone mass phenotype in lipodystrophy.

OR76

CADHERIN-11-MEDIATED HOMOPHILIC INTERACTIONS BETWEEN CANCER CELLS AND MARROW STROMAL/OSTEOBLASTIC CELLS ENHANCE BONE METASTASES

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Bone is one of the most preferential sites of cancer metastasis. Although the mechanism underlying the selective spread of cancer cells to bone is poorly understood, involvement of cell adhesion molecules including integrins and cadherins has been proposed. Cadherin-11 is one of the classical type-2 cadherin family members and has been reported to be expressed in human breast and prostate cancers, which show a strong predilection for spreading to bone. However, the role of cadehrin-11 in cancer metastasis to bone is unknown. Since cadherin-11 is specifically and constitutively expressed in marrow stromal cells/osteoblasts, we hypothesized that the interactions between cancer cells and stromal cells via cadherin-11 played a role in the pathophysiology of bone metastasis. To study this, the 293T human embryonic kidney cells which exhibited little expression of cadherin-11 were stably transfected with cadherin-11 cDNA (293T/cad-11) and examined for the capacity to develop bone metastases following heart inoculation in nude mice. Radiographic and histomorphometric examinations revealed that the number and size of osteolytic lesions and tumor burden in bone were markedly increased in 293T/cad-11 compared with 293T parental cells (293T/pa). Subsequently, we examined whether 293T/Cad-11 cells exhibited increased homing to bone. FACS analysis using a long-term tracing fluorescent dye showed that the number of cells arrested in bone marrow shortly after the heart inoculation was greater in 293T/cad-11 than 293/pa cells. Cell motility assay using Boyden chambers directly put on the monolayer of ST2 stromal cells or MC3T3-E1 osteoblastic cells, both of which constitutively express substantial levels of cadherin-11, demonstrated that the directed migration of 293T/Cad-11 was significantly increased compared with 293T/pa cells. In contrast, NIH-3T3 fibroblasts which expressed little cadherin-11 showed no effects. Moreover, the conditioned medium obtained from 293T/cad-11 and MC3T3-E1 cell co-cultures stimulated TRAP-positive osteoclast-like cell formation in mouse marrow cultures, whereas the conditioned medium of 293T/pa and MC3T3-E1 cell co-cultures showed much less effects. In conclusion, our results suggest that cadherin-11 expression in cancer cells enhances homing, migration and osteoclastogenesis via cadherin-11-mediated interactions with stromal/osteoblastic cells, thereby stimulates bone metastases. Cadherin-11 might contribute to the preferential spread of cancer cells to bone.

OR77

TRANSCRIPTIONAL REGULATION OF OSTEOPONTIN PRODUCTION BY TGFBETA IN BREAST CANCER CELLS: IMPLICATION IN BONE METASTASIS

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A secreted phosphoglycoprotein osteopontin (OPN) is expressed in majority of human breast cancers. Since OPN has been implicated in distant metastasis in breast cancer, which has a strong predilection for spreading to bone, regulation of OPN production in breast cancer cells colonized in bone is of great importance for our understandings of the pathophysiology of bone metastasis. Here we studied the effects of TGFBeta, one of the most abundant growth factors in bone, in 4T1 mouse mammary cancer cells which constitutively produced plenty of OPN. Semi-quantitative RT-PCR and Western analysis showed that TGFBeta increased OPN mRNA and secretion in 4T1 cells, respectively. TGFBeta also stimulated OPN promoter activity determined by the reporter assay and caused Smad3 binding to the Smad-binding element (SBE) in the OPN promoter on electrophoretic mobility shift assay (EMSA). TGFBeta-stimulated OPN promoter activity was enhanced by overexpression of Smad3/Smad4. Of note, this enhancement was completely abolished when the SBE was mutated. Smad3 failed to bind to the mutated SBE. The homeobox gene Hoxa-9, which functions as a repressor through binding to the Hox-binding element (HBE) in the OPN promoter, inhibited TGFBeta-stimulated OPN promoter activity. The inhibition was not observed when the HBE was mutated. EMSA demonstrated that the hetero-complex of Smad3/Smad4 inhibited the Hoxa9 binding to the HBE. These results suggest that TGFBeta up-regulates OPN gene transcription through promoting Smad3/SBE interactions and suppressing Hoxa-9/HBE interactions. 4T1 cells stably transfected with a luciferase reporter and an anti-sense OPN cDNA (4T1/luc/AS cells) showed diminished OPN production and anchorage-independent growth in soft agar. More importantly, histological examination revealed that metastatic tumor burden of 4T1/luc/AS cells in bone was markedly decreased compared with the empty-vector-transfected cells following orthotopic cell inoculation in female balb/c mice. Lung metastases of 4T1/luc/AS cells assessed by luciferase activity were also reduced but to a much lesser extent than bone metastases. In conclusion, our results suggest that OPN is involved in distant metastasis of breast cancer with more critical role in metastases to bone than non-bone sites. Inhibition of OPN production may be beneficial in the treatment of metastases, especially bone metastases, in breast cancer.

OR78

GS-ALPHA EXPRESSION IS BIALLELIC IN NORMAL AND FIBROUS DYSPLASTIC BONE

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Fibrous Dysplasia (FD) is a skeletal disease caused by activating mutations of GNAS1 and characterized by variable severity of clinical expression, which is based on the postzygotic occurrence of GNAS1 mutations. However the potential contribution of epigenetic factors has never been investigated. Imprinting is an epigenetic phenomenon that establishes the monoparental expression of a gene, usually by methylation of its promoter. In humans, parental specific methylation has been shown for the GNAS1 promoters that drive expression of NESP55, Xlas and, recently, 1A, whereas the promoter that drives Gs-alpha seems to be unmethylated at both alleles. However, clinical evidence have long suggested that Gs-alpha has a predominantly maternal expression in some tissues, recently demonstrated in human pituitary. Furthermore, it has recently been shown that loss of imprinting (relaxation) of GNAS1 promoters is associated with some endocrine diseases such as somatotroph adenomas (Gs-alpha promoter) and PHPIB (exon1A). We asked whether Gs-alpha imprinting in bone marrow stromal cells (which generate FD lesions) contributes to the phenotypic expression of the mutated genotype. To address this issue, we took advantage of the polymorphism (ACT/ATT) within exon 5 of GNAS1, which allows recognition of the allelic origin of the Gs-alpha transcript in heterozygous patients. FD patients (N=16) were analyzed by PCR amplification of the relevant gDNA fragment, followed by digestion with the restriction enzyme, FokI (that recognizes the ACT allele), and DNA sequencing. Gs-alpha mRNA was analyzed in stromal cells isolated in vitro from 3 informative (heterozygous) patients. Cells were grown as pure populations of normal and mutated cells in order to separately analyze the imprinting status of Gs-alpha in the normal and disease genotype. Using FokI digestion followed by subcloning and DNA sequencing, we demonstrated the biparental origin of Gs-alpha mRNA in all the samples analyzed with no difference between mutated and non-mutated cells. Our work shows for the first time the lack of imprinting of Gs-alpha in the stromal component of the bone microenvironment, which gives rise to bone, and excludes any major role for Gs-alpha imprinting in the clinical expression of FD.

OR79

PATHOGENESIS AND TREATMENT OF OSTEOARTHRITIS CAUSED BY EXTRACELLULAR INORGANIC PYROPHOSPHATE DEFICIENCIES

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Inorganic pyrophosphate (PPi) is a potent inhibitor of bone mineral deposition and a variety of human skeletal disorders develop due to abnormal levels of extracellular PPi. The nucleosidetriphosphate pyrophosphohydrolase activity of PC-1 (Enpp1) and the transmembrane PPi-channeling protein ANK are responsible for supplying the larger amount of PPi to the extracellular spaces. Deficiencies in PC-1 and ANK activities lead to soft-tissue ossification and osteoarthritis. The hydrolysis of extracellular PPi is mainly accomplished by tissue-nonspecific alkaline phosphatase (TNAP). Deficiencies in TNAP cause hypophosphatasia, which is characterized by elevated extracellular PPi levels accompanied with poor bone mineralization and calcium pyrophosphate dihydrate (CPPD) crystals in articular cartilage.

Despite the different manners by which PC-1 and ANK supply PPi to bone matrix, PC-1 and ANK-deficiencies appear to cause similar hypermineralization abnormalities. We found, however, that Enpp1 knockout (KO) mice exhibit a more severe soft-tissue ossification phenotype than ank/ank mice. Attempts to rescue the ank/ank abnormalities by crossbreeding to TNAP deficient (Akp2 KO) mice produced only a partial correction of the hypermineralization abnormalities in contrast to the full correction previously observed in Akp2/Enpp1 double KO mice (Hessle et al., 2002). Intra- and extracellular levels of PPi were significantly improved in TNAP/ANK double-deficient osteoblasts compared to ank/ank osteoblasts.

Since a genetic deficiency in TNAP improves the hyperossification and elevates the extracellular PPi levels in both Enpp1 KO and ank/ank mice, we assessed the effectiveness of inhibiting TNAP activity as a potential treatment for osteoarthritis using the Enpp1 KO and ank/ank mice as paradigms. In vitro studies on primary osteoblasts suggested that tetramisole was the inhibitor of choice for these experiments. In vivo we found that osteoarthritic mice treated with tetramisole for 3 months were significantly more flexible compared to control untreated Enpp1 KO or ank/ank mice, which suggests an improvement in their osteoarthitic condition. Our results point to TNAP, PC-1 and ANK as key molecules controlling in vivo bone mineralization through the regulation of extracellular PPi levels.

OR80

ADENOVIRAL TRANSFER AND EXPRESSION OF COLLAGEN TYPE 1 PRO-ALPHA 2 CDNA IN THE BONES OF A MURINE MODEL OF HUMAN OSTEOGENESIS IMPERFECTA

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Osteogenesis imperfecta (OI) is a connective tissue disorder whose hallmark is bone fragility. Most forms of OI result from mutations that affect the structure of the genes that encode the pro-alpha 1 and pro-alpha 2 polypeptide chains of type I collagen, the major protein of bone. Since the disease is of genetic origin, there is no cure available. Cell and gene therapy are being investigated as potential treatments for the disease. As an attempt to evaluate the potential of gene therapy for OI, an adenovirus carrying the murine type I collagen pro-alpha 2 chain was constructed and tested for its expression in the bones of a mouse model of human OI. This mouse has deficient synthesis of the collagen pro-alpha 2 chains and this results in the accumulation of alpha1(I) collagen homotrimers in the bones and other tissues leading to frequent fracturing. The murine pro-alpha 2 cDNA was inserted in the adenovirus and was expressed off the CMV early promoter. Transduction of the bone marrow stromal cells from the OI mouse model that are deficient in pro-alpha 2 chain collagen synthesis, demonstrated efficient synthesis of type I collagen comprised of alpha 1 and alpha 2 heterotrimers in a correct ratio of 2:1 in vitro. Direct injection of the viral vector in the bones of the OI mice followed by biochemical analysis of the collagens present in the bones of the recipient mice, demonstrated presence of type I collagen comprised of alpha1and alpha 2 heterotrimers. The collagen expression was detected up to 21 days after viral vector injection, the last day at which collagen analysis was performed. Presently, the bones of the recipient mice are being evaluated histologically and biomechanically to assess changes in structural and material properties of the viral vector recipient bones. These data suggest that collagen gene transfer and expression in bone is possible and that gene replacement in conjunction with antisense gene therapy may offer potential for OI treatment and warrants further investigation.

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FR167653, A POTENT P38 MITOGEN-ACTIVATED PROTEIN KINASE INHIBITOR, PREVENTS THE ONSET AND PROGRESSION OF COLLAGEN-INDUCED ARTHRITIS IN RATS

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Objective: FR167653 is a potent inhibitor of p38 mitogen-activated protein kinase (MAPK) and inhibits tumor necrosis factor-alpha (TNF-a) and interleukin-1beta (IL-1b) production in monocytes and lymphocytes. Collagen-induced arthritis (CIA) is a widely used experimental model of polyarthritis that has many histopathologic features in common with Rheumatoid Arthritis (RA). In this study we investigated the effect of FR167653 on CIA.

Methods: CIA rats were subcutaneously injected with FR167653 at a dose of 32 mg/kg/day starting on the day of the booster injection (day 7) and after the onset of arthritis (day 21) in the prophylactic and therapeutic treatment groups, respectively. The hind paw swelling, body weight, and radiologic and histologic arthritis scores were evaluated. The plasma concentration of TNF-a and IL-1b were measured with commercial ELISA kits. Flow cytometric analysis of T-lymphocytes from bone marrow was performed using anti-CD4 and anti-CD8a antibodies. The effect of FR167653 on *in vitro* osteoclast formation induced by macrophage colony-stimulating factor (M-CSF) and soluble receptor activator of NF-kappaB ligand (sRANKL) or TNF-a was also tested. In addition, calcified matrix resorption activity of the osteoclast-like cells was tested using BD BioCoat osteologic calcium hydroxyapatite coated slides.

Results: Hind paw swelling and weight loss occurred in CIA rats but not in the prophylactic treatment group. Therapeutic treatment also significantly reduced the paw swelling, but failed to recover body weight. The mean radiographic and histologic scores of the treatment group were significantly lower than those of CIA rats without treatment. FR167653 treatment reduced serum TNF-a and IL-1b levels assessed by ELISA and the CD4-CD8a+ T-cell population in bone marrow. Furthermore, FR167653 inhibited the osteoclast-like cell differentiation induced by both sRANKL and TNF-a *in vitro*. The calcified matrix resorption induced by sRANKL was also suppressed in the presence of 10^{-6}M FR167653.

Conclusions: FR167653, a potent p38 MAPK inhibitor, prevents not only the onset of arthritis in prophylactic treatment but also suppresses the progression of joint destruction in therapeutic treatment of rats with CIA. The putative mechanisms include inhibition of TNF-a and IL-1b production, CD4-CD8a+ T-cells recruitment, and osteoclastic bone resorption. These findings suggest that p38 MAPK is a potential therapeutic target for RA.

OR82

STUDY ON THE COUPLING MECHANISM BETWEEN BONE RESORPTION AND BONE FORMATION IN OPG-DEFICIENT MICE

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The discovery of RANKL elucidates the mechanism of osteoclast differentiation and function regulated by osteoblasts. Osteoprotegerin (OPG), a soluble decoy receptor of RANKL, inhibits both differentiation and function of osteoclasts. OPG-deficient (OPG -/-) mice exhibited severe osteoporosis caused by enhanced osteoclastic bone resorption. Deficiency of OPG in human has been shown to result in juvenile Paget's disease. The previous morphological study showed that osteoblastic bone formation was activated in OPG-/- mice. Blood alkaline phosphatase activity of OPG -/- mice was about four times as high as that of wild-type mice. These results suggest that osteoclastic bone resorption coincidentally induces osteoblastic bone formation by an unknown factor (called coupling factor). Using a bone morphometry technique, we explored whether such a coupling factor is present in bone in OPG -/- mice. When bisphosphonate (risedronate) was injected into OPG -/- and wild-type mice every day for 30 days (0.01 mg/Kg), bone resorption-related parameters were sharply decreased in both OPG -/- and wild-type mice. Treatment of OPG -/- mice with bisphosphonate induced complete disappearance of activated cuboidal osteoblasts which was often observed in untreated OPG -/- mice. All histomorphometric bone formation parameters as well as bone resorption parameters were significantly decreased in bisphosphonate-treated OPG -/- mice. Medication of bisphosphonate in OPG -/- mice decreased serum alkaline phosphatase activity to the level lower than that of wild-type mice. These results indicate that bone resorption is accurately coupled with bone formation in OPG -/- mice. We next investigated whether ectopic bone formation induced by BMP is also accelerated in OPG -/- mice. Collagen sponge disks containing rhBMP-2 were implanted into the dorsal muscle pouches in OPG -/- mice and wild-type mice, and bone mineral density (BMD) of the collagen sponge disks was determined every week for 3 weeks. No significant difference in BMD of the ectopic bone was observed between OPG -/- mice and wild-type mice. These results suggest that coupling between osteoclastic bone resorption and osteoblastic bone formation occurs at the site in bone, and that coupling factor is a local factor but not a systemic one.

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SEXUAL DIMORPHISM IN SKELETAL GROWTH AND IGF-I LEVELS IN YOUNG AROMATASE DEFICIENT (ARKO) MICE

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Aromatase catalyzes the biosynthesis of estrogens from androgen precursors. We have shown previously that 5 month old adult aromatase deficient (ArKO) female mice have significantly decreased trabecular bone volume, high bone turnover, and normal femur length compared with wildtype female mice. On the other hand, male ArKO mice also have significantly lower trabecular bone volume but low turnover, and significantly shortened femurs compared with normal male mice. In the present study, we followed and compared the growth of female and male ArKO mice to their wildtype littermates from age 3 weeks to 7 weeks. Compared to wild-type littermates, female ArKO mice had significantly greater nasal anal lengths, body weight, longer femurs and greater mid-shaft diameter of the femurs. Estradiol treatment reversed these changes. On the other hand, ArKO males have shorter nasal anal lengths, lower body weight, and shorter femur lengths compared with wild-type littermates. Compared to wild-type females, serum IGF-I levels at the time of sacrifice were higher in female ArKO mice compared with normal controls. On the other hand, IGF-I serum levels in male ArKO mice were lower than wildtype males. We have initiated a trial of IGF-I therapy in the ArKO males. The data obtained to date shows that IGF-I treatment restores body growth (weight and nasal anal length) to normal. Femur length is also normalized with treatment. Since the study was conducted during the pubertal period in mice, these data suggests that estrogens regulate body growth during puberty through IGF-I. The effects of estrogen deficiency on serum IGF-I is sexually dimorphic and presently the molecular mechanisms are unknown. Histomorphometric analysis is underway to determine if the IGF-I treatment also rescues the low turnover osteopenia phenotype in the males.

OR84

LASOFOXIFENE IS SUPERIOR TO RALOXIFENE IN THE PREVENTION OF BONE LOSS IN POSTMENOPAUSAL WOMEN: 1-YEAR RESULTS

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BACKGROUND: A prospective, randomized, double-blind, placebo- and active treatment controlled trial was performed to compare the safety, toleration and efficacy of lasofoxifene at 2 dose levels versus raloxifene or placebo on bone mineral density (BMD) of the lumbar spine (L1-L4). Biochemical markers of bone turnover and indices of lipid metabolism were also determined. A total of 410 postmenopausal women, 50 to 74 years of age, within plus2.0 SD and minus2.5 SD of age-and sex-matched BMD (Z-score) were randomized to one of the four treatments: 0.25 millig/d lasofoxifene (N = 82), 1.0 millig/d lasofoxifene (N = 82), 60 millig/d raloxifene (N = 163), or placebo (N = 83). The primary efficacy endpoint was lumbar spine BMD (average of L1-L4). Secondary endpoints included biochemical markers of bone turnover and indices of lipid metabolism.

RESULTS: At 1-year, lasofoxifene (0.25 millig/d) treated subjects showed statistically significant increases in lumbar spine BMD compared to raloxifene (60 millig/d) and placebo ($P<0.05$). The lasofoxifene group (0.25 millig/d) demonstrated a 2% increase in lumbar spine BMD compared to a 0.8 % and minus0.9% for the raloxifene (60 millig/d) and the placebo groups respectively. At 6 months, lasofoxifene showed consistently greater mean reductions in biochemical markers of bone turnover (N-telopeptide, osteocalcin, deoxypyridinoline, and bone specific alkaline phosphatase) compared to raloxifene and placebo. At 1-year, low density lipoprotein-cholesterol (LDL-C) reduction was significantly greater with 0.25 millig/d lasofoxifene (minus20%), compared to 60 mg/d raloxifene (minus9%) and placebo (minus1%) ($p < 0.001$).

CONCLUSION: At 1-year, lasofoxifene (0.25 millig/d) has superior effects on lumbar spine BMD and LDL-C reduction when compared to raloxifene (60 millig/d).

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eNOS IS REQUIRED FOR MECHANICALLY-INDUCED BONE FORMATION

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Mechanical forces are known to be a major determinant of bone mass. 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 knock out 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. To this end, 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. 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 these mice. The maximum load (30N) was calculated to produce 700 microstrain in the 8th caudal vertebra of these mice. We then analysed 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. 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 no osteogenic response whatsoever to mechanical stimulation in the 8th caudal vertebrae of eNOS -/- animals. 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.

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INDEPENDENT CONTRIBUTIONS OF ANDROGEN AND ESTROGEN SIGNALINGS IN THE MAINTENANCE OF BONE MASS IN MALES - ANALYSIS OF ANDROGEN RECEPTOR DEFICIENT MICE -

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Bone mass of males is known to be higher than that of females in both humans and mice, implying the importance of androgen signaling. We recently established an androgen receptor (AR) deficient mouse (ARKO) line by using a Cre/loxP system. Radiologic and histomorphometric analyses revealed that ARKO males exhibited a marked decrease in both trabecular and cortical bone volumes with high bone turnover as compared to male and female wild-type (WT) littermates. However, this abnormality is not ascribalble solely to the AR deficiency since ARKO males showed severe genital organ atrophy and a marked decrease in testosterone, the main androgen, that activates not only AR, but also estrogen receptors (ER) through aromatization into estradiol. To investigate the contributions of AR and ER impairments to the bone loss of ARKO males, 3-week-old WT and ARKO males were orchidectomized and implanted with a slow releasing pellet of placebo, testosterone, or its metabolite dihydrotestosterone (DHT), and were analyzed at 8 weeks. Bone mass of ARKO and orchidectomized WT reduced to similar levels, indicating that testis is the principal source of bone regulatory androgen. In DHT-implanted groups, a half restoration was seen in orchidectomized WT as compared to sham-operated WT, although orchidectomized ARKO showed no restoration. This indicates the contribution of AR signaling to the maintenance of bone mass because DHT cannot be converted to estradiol. In testosterone-implanted groups, bone mass of orchidectomized ARKO showed approximately a half restoration, while that of orchidectomized WT showed full restoration to the sham-operated WT levels. The half restoration in the ARKO can be regarded as the effect of estradiol converted from the implanted testosterone, whereas the full restoration in the WT is due to the sum effects of the converted estradiol through ER and the direct effect of androgen through AR. We conclude that the impairment of both AR and ER signalings contributes to osteopenia in ARKO males. Bone mass of WT males is maintained by both androgen and estrogen signalings that act similarly but independently, and is higher than that of females which is maintained mainly by ER signaling since androgen levels are quite low.

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CYSTATIN 10, A NOVEL CHONDROCYTE-SPECIFIC PROTEIN, IS INVOLVED IN CALCIFICATION OF HYPERTROPHIC CHONDROCYTES OF THE GROWTH PLATE

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In efforts to elucidate the molecular mechanism of endochondral ossification, we recently identified a novel gene, cystatin 10 (Cst10), which was upregulated in association with ectopic calcification of mouse auricular cartilage. Expression of Cst10 was specific to cartilage, especially to prehypertrophic and hypertrophic chondrocytes of the growth plate. In a mouse chondrogenic cell line, ATDC5, Cst10 expression preceded the type X collagen expression and increased in synchrony with maturation. Overexpression of *Cst10* cDNA in ATDC5 cells accelerated hypertrophic differentiation and calcification determined by type X collagen expression and Alizarin red staining, and induced apoptosis with an increased sub-G1 population of the cell cycle, the fragmentation and condensation of nuclei, and the activation of caspases. To further investigate the physiological role of Cst10 *in vivo*, we created mice lacking the Cst10 gene ($Cst10^{-/-}$ mice) by homologous recombination in mouse embryonic stem cells. $Cst10^{-/-}$ mice developed normally without abnormalities of major organs, and showed normal levels of serum and urinary markers (Ca, P, ALP, osteocalcin, deoxypyridinoline). In long bones, however, a significant decrease in bone volume was observed solely at the metaphysis by bone densitometry and 3D-microCT analyses as compared with wild-type (WT) littermates at 8 weeks of age. Bone histomorphometric analysis in the proximal tibiae revealed that the volume of primary spongiosa was decreased in $Cst10^{-/-}$ mice. In histological analyses of the growth plate, the columnar architecture and the expression patterns of type II and type X collagen were preserved, indicating that the early and the middle stages of cartilage differentiation are not affected by Cst10 deficiency. However, at the hypertrophic layer, a marked decrease in calcification was seen in $Cst10^{-/-}$ mice; the ratio of the height of the calcified layer to that of the whole growth plate was only 65% of that in WT, and the average number of chondrocytes with calcified matrix per column was 60% of that in WT. In addition, the number of TRAP-positive chondroclasts was significantly decreased at the $Cst10^{-/-}$ hypertrophic layer. We therefore conclude that Cst10 plays an important role in calcification of hypertrophic chondrocytes and the subsequent formation of primary spongiosa.

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STRONTIUM RANELATE: A NEW EFFECTIVE ANTIOSTEOPOROTIC TREATMENT REDUCING THE INCIDENCE OF VERTEBRAL AND NON VERTEBRAL FRACTURES IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS

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Strontium ranelate (SR) is a new antiosteoporotic agent demonstrated to increase in bone formation and decrease in bone resorption in preclinical and clinical studies. A phase III study, SOTI has showed that SR 2g/d reduced the risk of new vertebral fracture by 41%, during 3 years. An early effect was also observed from the first year with 49% (RR = 0.51, 95% CI [0.35;0.73]) risk reduction of new vertebral fracture ($p<0.001$).

The TROPOS study (TReatment Of Peripheral OSteoprosis) was a randomised, double-blind, placebo controlled, phase III study involving 5091 post menopausal women from 75 centres in 12 countries (Europe and Australia). The main objective was to assess the efficacy of SR 2g/d taken orally during 3 years or more on new non vertebral fractures. A secondary endpoint was the femoral neck BMD change.

The patients were included in two parallel groups [age:76.8 years ±5; femoral neck BMD: 0.552 g/cm^2 ±0.066, mean (SD)], 38.6% of them having at least one previous non vertebral fracture at inclusion. Mean treatment duration was 885 days ±484.

SR significantly reduced the incidence of patients with new non vertebral fracture, in the intent-to-treat (ITT) population (relative risk (RR)=0.84, 95% CI [0.71;1.00], $p=0.05$).

The reduction of the risk by 33% (RR=0.67, 95% CI [0.54;0.85]) was also significant ($p<0.001$) on the incidence of patients experiencing a new non vertebral fracture in the minimal exposed data set (minimal exposure to SR during the first 18 months). A 41% ($p=0.025$) reduction in the relative risk of experiencing a hip fracture was also demonstrated in the minimal exposed data set.

Femoral neck BMD was also ($p<0.001$) increased in the SR group with a 6.54% relative change from baseline as compared to placebo. Bone specific alkaline phosphatase increased while Urinary CTX decreased. SR was well tolerated.

SR is a new effective and safe orally administrated treatment of vertebral and non vertebral fractures, in osteoporotic postmenopausal women.

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ENHANCED TRABECULAR BONE MASS AND ARCHITECTURE IN TRANSGENIC MICE CONSTITUTIVELY OVEREXPRESSING HISTONE H4 DERIVED OSTEOGENIC GROWTH PEPTIDE

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Histone H4 genes encode at least two peptides: the 103 amino acid histone H4 protein and a circulating mitogen, Osteogenic Growth Peptide (OGP), which is identical to the 14 carboxy terminal residues of H4. OGP is synthesized de novo from H4 mRNA following leaky ribosomal scanning through the imperfect H4 AUG initiator. Consequently, alternative translation initiates at codon 85, a perfect AUG initiator, ultimately giving rise to OGP. Here we engineered transgenic mice, expressing a mutant H4 mRNA, H4tTG1, which encodes OGP but not H4. The transgene is controlled by the chicken ß-actin promoter, the human CMV enhancer and the rabbit ß-globin 3'UTR. In adult mice, H4tTG1 was expressed in all tissues tested, including skeletal muscle, liver, spleen, kidney and brain. Muscle H4tTG1 mRNA levels were particularly high, reaching the level of spleen endogenous H4 mRNA. The effect of the OGP-encoding H4tTG1 transgene on bone was evaluated by quantitative micro-computed tomography analysis of femora from 8, 17 and 34 week-

old mice. The results demonstrate a marked increase in trabecular, but not cortical, bone volume density at all ages particularly in females, which exhibited a 2-fold increase in trabecular bone density compared to wild-type controls. The enhancement of trabecular bone density was accompanied by increased trabecular number and connectivity. No adverse effect of OGP over-expression was noticed in transgenic mice up to 18 months of age. Thus, continuous OGP over-expression throughout life results in a specific augmentation of trabecular bone without negative effects on cortical bone or extra-skeletal tissues. These results imply a role for OGP in peak bone mass accrual and maintenance, and point at OGP as a realistic approach for the development of effective and safe bone anabolic therapy.

OR90

NEUROPEPTIDE Y Y2Y4 RECEPTOR DOUBLE KNOCKOUT: EVIDENCE FOR SEX HORMONE INTERACTION IN THE NEUROREGULATION OF BONE MASS

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Neuropeptide Y (NPY) Y2 receptor has previously been shown to profoundly affect cancellous bone formation in the mouse by a centrally mediated mechanism. Pancreatic polypeptide is the major ligand for NPY Y4 receptor and its elevated circulating levels in the Y2 knockout (KO) suggest that the Y4 receptor may be involved in the anabolic bone phenotype. The present study therefore compared the outcomes of NPY Y2 KO, Y4 KO and Y2/Y4 double KO on regulation of bone mass in femora from 4-month old knockout & wildtype mice.

The Y2 KO had greater cancellous bone mass (BV/TV) than wildtype mice 12.4 ± 1.7% vs 6.6 ± 1.4%, mean ± SEM. The Y4 KO mice did not differ from wild type in cancellous 8.2 ± 1.2% or cortical bone mass. Cortical area did not differ between lines; wildtype 1.1 ± 0.1 vs Y2KO 1.2 ± 0.1 vs Y4 KO mice 1.1 ± 0.1 mm^2.

In contrast, to the lack of effect in Y4KO mice, Y2/Y4 KO in male mice had a synergistic effect on cancellous bone. BV/TV in Y2/Y4 KO mice 17.4 ± 2.1% was elevated compared to Y4 KO 8.2 ± 1.2% and Y2 KO 12.4 ± 1.7% ($p < 0.06$). However, cortical area was reduced; Y2/Y4 KO 0.92 ± 0.03 vs Y4 KO 1.12 ± 0.04 vsY2 KO mice 1.09 ± 0.05 mm^2.

Interestingly, in female Y2/Y4 KO mice, there was no synergistic increase in BV/TV, with Y2/Y4 mice 10.6 ± 0.6% not different from Y2 KO females 10.8 ± 2.1%, but with both greater than wildtype 5.9 ± 0.3%. Moreover, cortical area 0.91 ± 0.2 vs 0.92 ± 0.03 mm^2, femoral length 16.0 ± 0.1 vs 16.0 ± 0.1 mm and mid-femoral circumference 5.2 ± 0.1 vs 5.2 ± 0.1 mm were unchanged between female and male Y2Y4 KO mice respectively, despite significant sex differences in Y2KO and Y4KO mice. Although the Y4 receptor pathway does not independently regulate bone mass, there is a synergistic interaction between the Y2 and Y4 pathways to reduce cancellous bone volume and increase cortical bone mass in males. These data suggest an interaction between the NPY pathways and sex hormone effects on bone mass.

ABSTRACTS

POSTER PRESENTATIONS

All poster presentations will be displayed throughout the conference in the Exhibition Hall, 3rd floor. The suffix after the abstract number indicates the poster session in which the poster will be attended (W - Wednesday, F- Friday, S - Saturday).

Bone, Cartilage and Connective Tissue Matrix

P1 W

ESTIMATION OF CORTICAL BONE RESORPTION RATE FOR MEN AND WOMEN BASED ON *IN VIVO* MEASUREMENTS OF ^{90}SR INCORPORATED IN SKELETON DUE TO RADIOACTIVE ACCIDENT

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This paper presents an assessment of age- and gender dependent rate of cortical bone resorption evaluated assuming ^{90}Sr as a tracer to study bone metabolism. Radioactive traces were used to estimate the bone remodeling rate in the sixties after the global fallout of radionuclides. Current methods of bone remodeling assessment, such as biochemical markers and histological methods, have their own disadvantages: they can not provide information separately for the cortical and trabecular parts of the skeleton, and can not provide sufficient quantitative information on the age- and gender- specific turnover processes in cortical bones. Such information can be derived from the study of Techa river population affected by radioactive wastes discharged from the Mayak Production Association in the early fifties. Residents ingested an average of about 3,000kBq of ^{90}Sr. The affected people have been followed for many years by scientists at the Urals Research Center for Radiation Medicine. The whole body content of ^{90}Sr of about 15,000 individuals has been measured over a period of 24 y (1974 - 1997) using a special whole body counter (WBC). Almost the entire amount of ^{90}Sr had deposited in the cortical part of the skeleton (built into crystals of hydroxyapatite) by 25 y following intake, which is confirmed by direct measurements of strontium concentration in different types of bones. Therefore ^{90}Sr elimination from the skeleton occurs as a result of cortical bone resorption, and individual values of Sr elimination rate reflect the rate of bone resorption. WBC data on persons who had been measured 5 or more times were selected for study. Individual- measurement results were fit to an exponential function and individual strontium- elimination rates were estimated. In order to derive the cortical bone resorption rate the individual values of strontium elimination rate were corrected for rate of strontium recycling. The rate of recycling was estimated on the basis of strontium biokinetic model as 0.18% per year. For men, a significant increase (from 2.8 % y^{-1} to 3.3 % y^{-1}) in resorption rate after age 55 is seen. For women, the increase in elimination rate was significant at age 45, and reached 6.1 % y^{-1} after the age of 60.

P2 F

METHODS AND APPLICATION OF ACCELERATOR MASS SPECTROMETRY FOR HIGHLY ACCURATE BONE RESORPTION DETERMINATION UTILIZING 41CA

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Many biochemical markers used to monitor human bone turnover suffer from relatively large natural fluctuations, making small but significant differences in bone resorption impossible to resolve. The commonly-used calcium radioisotopes ^{45}Ca and ^{47}Ca have relatively short half-lives, prohibiting long-term studies; stable isotopes are generally too expensive for widespread application to long-term bone turnover. ^{41}Ca has a long half-life (100 ka) and a single 5-100nCi dose will remain in the skeletal calcium pool at quantifiable levels for many months to years, allowing determination of long- and short-term bone resorption parameters. The decay mode of pure electron capture deposits almost no ionizing radiation, and in combination with the low initial dose and long half-life, a typical lifetime radiological dose in such an experiment is below that of a brief airplane trip.

We will report some improvements to the commonly-used calcium fluoride preparation technique (Freeman *et al.*, 1995), limiting handling time and maximizing throughput. We have sought to alleviate the difficulties of ^{41}Ca/Ca measurement through use of a high-intensity ion source and efficient beam transport - allowing us to collect accurate data at <5% precision in several minutes. Analysis at high ion energy (tens of MeV) allows separation from interferences, although the total ion current extracted from individual samples imposes a ^{41}Ca/Ca=10^{-13} limit of quantitation - which is 1/100th that expected one year after a single 5nCi dose.

We have also prepared new ^{41}Ca dose materials for oral and intravenous administration. This ^{41}Ca was provided from a 1% ^{41}Ca solution used in preparation of our primary standard, laboriously purified through selective precipitation and ion exchange (Nishiizumi *et al.*, 2000). Radiological activity was measured for beta and gamma radiation - both were at typical background levels. The trace elemental content was also measured, assuring the overall purity of the dose material for human use.

Several projects are underway, and we will also report on their status.

Freeman SFPH, Serfass RE, King JC, Southon JR, Fang Y, Woodhouse LR, Bench GS and McAninch. Nucl. Instr. Meth. Phys. Res. B 99 (1995) 557.

Nishiizumi K, Caffee MW and DePaolo DJ. Nucl. Instr. Meth. Phys. Res. B 172 (2000) 399.

P3 S

DEVELOPMENT OF THREE-DIMENSIONAL GEL-EMBEDDED CULTURE SYSTEM FOR STUDYING MECHANICAL RESPONSES OF OSTEOCYTES

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Osteocytes are thought to act as mechanosensors in bone and thus be important regulators of remodeling. They are embedded deep inside mineralized bone matrix and are connected to each other via gap junctions. This structure is ideal to sense changes in the local mechanical environment. The aim of this study was to develop a relevant in vitro model to study the effects of mechanical loading to osteocytes. Mimicking the in vivo situation, a three-dimensional (3-D) culture system was established for mouse osteocyte-like cell line MLO-Y4. In this model, the cells were embedded in collagen gel matrix where they formed several cell-to-cell contacts. Furthermore, elastic culture well and loading apparatus was newly designed to apply mechanical stretch to the gel-embedded cells. Osteocytes were subjected to the cyclic tensile strain up to 6.8% (68000 micro-strain), which covers physiological and over-physiological strain ranges. The culture system has several advantages. (1) Cells surrounded by collagen gel can sustain their original organization and functions during the culture. (2) Gel-embedded osteocytes can form 3-D cell-to-cell contacts via gap junctions as seen in vivo. This was confirmed by a double-fluorescent labeling technique, which demonstrated calcein dye is transferred from labeled MLO-Y4 cells to the neighbors. (3) Gap junctional communication from the cells on the gel surface to the ones underneath the gel was also observed after placing the double-labeled cells onto the gel for 1 hour. This observation indicates a possibility that cells on the gel can communicate with the others under the gel. (4) Our loading apparatus can apply the calculated strain to the gel-embedded cells and widely cover the strain range above physiological level. In conclusion, our 3-D gel-embedded culture system accompanying the loading apparatus could provide an interesting model to further understand the role of osteocytes as mechanosensors and their participation to the regulation of bone remodeling.

P4 W

STAT3 IS ACTIVATED DURING IL-6/IL-6SR INHIBITION OF INORGANIC PYROPHOSPHATE ELABORATION BY ARTICULAR CHONDROCYTES AND STIMULATION OF CHONDROCYTES VESICLE MINERALIZATION

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Signal transduction pathway of articular chondrocytes in physiologic or pathologic mineralization has not been fully understood. Production of inorganic pyrophosphate (PPi), a natural inhibitor of apatite formation, is an important phenotypic feature of

chondrocytes to maintain integrity of articular cartilage. Proinflammatory cytokines such as IL-1beta and TNFalpha downregulate PPi elaboration from chondrocytes. IL-6 is one of the major proinflammatory cytokines, and IL-6 and its soluble receptor of IL-6 (IL-6sR) are elevated in synovial fluids from patients with both inflammatory and degenerative arthritis. Although IL-6 contributes to the destructive changes of cartilage accompanying joint diseases, its mechanism has not been well understood. We studied the effects of IL-6 and/or IL-6sR on chondrocytes PPi elaboration, vesicle mineralization and examined downstream signal transduction pathways.

Chondrocytes were released from adult porcine articular cartilage and cultured at high density in DMEM supplemented with 10% fetal bovine serum (FBS) and antibiotics. IL-6 and/or IL-6sR was added to confluent culture in DMEM supplemented with 25mM HEPES, heat-inactivated 0.5% FBS, and antibiotics, after cells were serum-starved for overnight. Cell lysate was isolated after 15-min stimulation with IL-6 and/or IL-6sR in DMEM and subjected to Western blotting for signal transduction analysis. Extracellular PPi in media was measured at 48 hours. Vesicles from chondrocytes were isolated by sequential ultracentrifugation after 90-min digestion with 0.2% collagenase. ^{45}Ca biomineralization assay with isolated vesicles was performed.

IL-6 inhibited PPi elaboration from chondrocytes with increased dose at 48 hours incubation. Additional IL-6sR (50ng/ml) enhanced inhibition of PPi elaboration. Vesicle mineralization was increased with IL-6 and further augmented by addition of IL-6sR. IL-6 dramatically induced STAT3 activation in a dose-dependent manner. The addition of IL-6sR didn't alter STAT3 signals in 15 min. ERK1/2 and Akt were constitutively activated in these chondrocytes and IL-6 and/or IL-6sR did not affect their activation.

IL-6 inhibited chondrocyte PPi elaboration, increased vesicle mineralization and may lead to loss of chondrocyte specific phenotype and loss of integrity of cartilage. STAT3 was activated during this modulation.

P5 F

A NEW METHOD OF EVALUATION OF THE WATER CONTENTS IN ARTICULAR CARTILAGE USING NEAR INFRARED SPECTROSCOPY

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Introduction: Besides of histological investigation, there is no method to evaluate biomaterial properties of the degenerated articural cartilage. A specific characteristic of near infrared spectroscopy to measure the internal water content of specimens can be utilized to evaluate the properties of articular cartilage. In this basic study, the possibility of new arthroscopic technique is presented.

Materials and Methods: By the self-developed indicator and sensor of near infrared spectroscopy, specimens were irradiated with 0.00146 mm. After partial absorption by hydroxyl, reflected spectroscopy was measured. The relation between the intensity of incident and reflected light is defined as; Absorbance: A=log10 (Ii/Ir), where Ii is intensity of incident light and Ir is that of reflected light. The absorbance means the index of the internal water content in the specimen.

The following were examined 1) Relation between thickness and absorbance of porcine articurlar cartilage. 2) Change of absorbance of porcine costal and patellar cartilages, before and after indentation.

Results & Discussion: 1) More than1.0mm in thickness, no change of absorbance was measured. 2) Recovery of absorbance was much higher in a costal cartilage than in a patellar cartilage. Maximum recovery rate of absorbance in a costal cartilage is on 10 minutes after indentation, and on34 minutes in a patellar cartilage.

The measurement of absorbance of near infrared spectroscopy can detect the change of water content in a cartilage. A next device is designed to mesure the water content of joint cartilage with indentation at the same time of. the irradiation of near infrared rays.

Conclusion: This method can be utilized arthroscopically to evaluate the biomechanical property of the cartilage.

P6 S

RELATIVE AMOUNT OF MINERAL AND MATRIX CONTENT IN RAT FEMURS DETERMINED BY FOURIER TRANSFORM INFRARED SPECTROSCOPY AS AN INDEX OF BONE MATURATION

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We examined changes in the mineral to matrix ratio in rat femurs during development by Fourier transform infrared spectroscopy. Twenty-five male Wistar-derived albino rats were divided into 5 groups, 3 week-old group (n=5), 6 week-old group (n=5), 9 week-old group (n=5), 12 week-old group (n=5),and 15 week-old group (n=5). Infrared absorption spectra were obtained from the diaphysis of femurs in each group. The spectra bands of interest are both mineral bands and organic bands. Peak positions near 1020 cm^{-1} and 1650 cm^{-1} were assigned to the PO_4^{3-} stretching vibrations of apatites and the C=O (amide 1) stretching vibration of bone organic matrix, respectively. The mineral / matrix ratio was calculated from the ratio of absorbance of the phosphate band at 1020 cm^{-1} to that of the amide 1 band at 1650 cm^{-1}. The mineral to matrix ratio was 0.71 ±0.09 in the 3 week-old group, 0.97 ±0.10 in the 6 week-old group, 1.11 ±0.10 in the 9 week-old group, 1.23 ±0.11 in the 12 week-old group, and 1.31 ±0.14 in the ratio of 15 week-old rat. Our previous studies have shown that bone mineral density and bone strength of the rat femoral shaft increase as rats grow and develop. The results suggest that the mineral to matrix ratio is an important index of maturation and strength of bone.

P7 W

WITHDRAWN

P8 F

CALCINEURIN AND NFAT4 INDUCE CHONDROGENESIS

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Elevation of intracellular calcium triggers cell growth and differentiation in many cell types. Calcium signals lead to the activation of the phosphatase calcineurin. Calcineurin, in turn, dephosphorylates the transcription factor family, nuclear factor activated T cells (NFATs). Dephosphorylation of NFATs results in translocation from cytoplasm to nucleus and subsequently regulation gene expression. We report here that elevated intracellular calcium induces chondrogenesis through a calcineurin/NFAT signaling axis that activates bone morphogenetic protein (BMP) expression. In the chondroblast clone RCJ 3.1C5.18 (C5), the calcium ionophore, ionomycin, induced aggrecan gene expression. Additionally, induction of aggrecan expression was inhibited following the addition of the calcineurin inhibitor, cyclosporine A. A physiological inhibitor of calcineurin, CAIN, also inhibited the induction of aggrecan by ionomycin. Using mouse limb bud culture assay, we showed that ionomycin augments chondrogenesis as demonstrated by increased numbers of alcian blue nodules. Furthermore, cyclosporin A blocked the differentiation of limb bud mesenchyme. These data indicate that ionomycin induces chondrogenesis and aggrecan expression through a calcineurin-dependent pathway. The calcineurin substrate, NFAT4, also induced chondrogenesis and aggrecan gene expression. Furthermore, the BMP antagonist, noggin, or dominant negative BMP receptors blocked the effect of elevated intracellular calcium on chondrogenesis. This result suggests that calcineurin/NFAT4 activate chondrogenesis via BMP expression. Consistent with this, BMP2 gene expression was increased by ionomycin and suppressed by cyclosporine A. Furthermore, activated NFAT4 induced BMP2 gene expression. Thus, we conclude that calcineurin/NFAT4 and BMP2 signal axis play an important role in chondrogenesis.

P9 S

IMMUNOHISTOCHEMISTRY OF PTHRP, IHH AND PTC ON TIBIAL GROWTH PLATE FROM RAT WITH NUTRITIONALLY INDUCED GROWTH RETARDATION OR HYPOPHOSPHATEMIC RICKETS

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Adequate dietary intake of vitamin D (D) and calcium (Ca) are essential to ensure normal bone formation and mineralization during growth. The impact of diets modified in Ca and D contents were studied on the expression of parathyroid hormone related peptide (PTHrP), Indian Hedgehog (Ihh) and its receptor Patched (Ptc)which are known to be involved in the proliferation and the differentiation of growth plate chondrocytes during endochondral ossification. The expression of these proteins were revealed by immunohistochemistry performed on tibial growth plate (GP) taken from young male rats fed a normal diet (N), a diet depleted in Ca and D (Ca-D-) inducing growth retardation or with a high Ca and D depleted diet (Ca+D-) leading to the development of hypophosphatemic rickets. In N rats, PTHrP was localized at the proliferative zone (PZ) and its presence gradually diminished through the pre-hypertrophic (PHZ) and hypertrophic (HZ) zones. The Ihh immunostaning, was present at the PZ and less intense at the PHZ. In Ca-D-, expression of PTHrP and Ihh were similar to N but the immunostaining was less intense. These results are in accordance with growth retardation observed in these rats. Ca+D- rats are rachitic with a widening of their GP at the HZ. In these rats, localization of PTHrP was similar to N and Ca-D- but the immunostaining was much more intense. Strong staining was also observed for Ihh at PZ and PHZ. Ptc in Ca+D- was present through all GP zones and its localization was no more restricted to PZ as N and Ca-D- groups. The over expression of these proteins observed in Ca+D- disturbed endochondral ossification

which probably led to the development of rickets. Taken together, these results suggest that the expression of PTHrP, Ihh and Ptc are modulated by dietary Ca and D and could play a role in the development of diseases such as rickets.

P10 W

THE EFFECT OF THE RECOMBINANT HUMAN INTERLEUKIN-1 RECEPTOR ANTAGONIST PROTEIN ON THE GLYCOSAMINOGLYCAN AND PROSTAGLANDIN E2 SYNTHESIS IN CHONDROCYTES

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Objective: The purpose of this study was to clone human interleukin-1 receptor antagonist gene (IL-1RN) and to evaluate the effects of this recombinant human interleukin-1 receptor antagonist (rhIL-1Ra) on the glycosaminoglycan (GAG) and prostaglandin E2 (PGE2) synthesis in vitro chondrocyte culture model.

Method: The complementary DNA of the secreted form of IL-1RN (534bp) was cloned and expressed in E. coli expression system and purified IL-1Ra by metal-chelate affinity chromatography. The human chondrosarcoma cell line SW1353 was cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and cultured and incubated. Media were then removed and replaced either with fresh media containing lipopolysaccharide (10microg/ml); control group or new media containing lipopolysaccharide (10microg/ml) and rhIL-1Ra in the concentration range 1-100 (1, 10, 25, 50, 100) nanog/ml; experimental group. PGE2 production was measured by EIA. GAG content was measeured using 1,9-dimethyl methylene blue dye on day 5.

Results: All experimental groups showed significantly lower levels of PGE2 production and greater GAG amount than controls. The higher concentration of rhIL-1Ra produced lesser production of PGE2 and greater synthesis of GAG.

Conclusion: These results suggest that rhIL-1Ra have inhibitory action on PGE2 synthesis and anabolic effect on GAG synthesis in vitro arthritis model of chondrocytes.

P11 F

TRANSCRIPTIONAL INDUCTION OF CONNECTIVE TISSUE GROWTH FACTOR/ HYPERTROPHIC CHONDROCYTE-SPECIFIC 24 GENE BY DEXAMETHASONE IN HUMAN CHONDROCYTIC CELLS

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Connective tissue growth factor/hypertrophic chondrocyte-specific gene product 24 (CTGF/Hcs24) is a critical growth factor for chondrocytic growth and differentiation. In this report, we describe for the first time glucocorticoid-mediated induction of the CTGF/Hcs24 gene in a chondrocytic cell line, HCS-2/8. Steady-state mRNA levels of CTGF/Hcs24 were drastically increased after treatment with 50 nM dexamethasone, as confirmed by Northern blotting and quantitative real-time polymerase chain reaction (PCR) analysis. Corresponding to the increase in mRNA, production of CTGF/HCS24 protein was remarkably enhanced, following a time course up to 6 h. The observed increase in mRNA can be ascribed to transcriptional enhancement, since the stability of *ctgf/hcs24* mRNA was not affected by the same concentration of dexamethasone, as determined by an mRNA degradation assay. However, unexpectedly, the prototypic *ctgf/hcs24* promoter was totally nonresponsible to the dexamethasone stimulation, suggesting the glucocorticoid receptor binding site(s) to be elsewhere in the *ctgf/hcs24* gene. Also, repressive effect of the 3'-untranslated region of the *ctgf/hcs24* gene was not affected by dexamethasone treatment. However, enhancement of the prototypic promoter activity by dexamethasone was observed in murine fibroblastic cells, demonstrating the complexity of the regulatory mechanism of *ctgf/hcs24* gene expression. Of importance, dexamethasone at the same concentration significantly stimulated proteoglycan synthesis in HCS-2/8 cells up to the same levels as exogenously-added CTGF/Hcs24 did. These findings represent a novel effect of glucocorticoid on the production of CTGF/Hcs24 by chondrocytic cells, and indicate that CTGF/Hcs24 may mediate the stimulative effect of dexamethasone on chondrocytic phenotypes. Also, our results shed light on the complex mechanism of CTGF/Hcs24 induction by glucocorticoids.

P12 S

ROLES OF MITOGEN-ACTIVATED PROTEIN KINASES IN THE CHONDROGENIC DIFFERENTIATION OF ATDC5 CELLS

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The embryonal carcinoma-derived cell line, ATDC5 differentiates into chondrocytes in response to insulin or insulin-like growth factor-I stimulation. In this study, we investigated the roles of mitogen-activated protein kinases (MAPKs) in insulin-induced proliferation and chondrogenic differentiation of ATDC5 cells. The MEK-1/2 inhibitor U0126 inhibited insulin-induced phosphorylation of both p44 and p42 MAP kinases in ATDC5 cells. Insulin-induced proliferation of ATDC5 cells in culture was inhibited in a concentration-dependent manner by U0126, and the p38 MAPK inhibitor SB203580, whereas JNK inhibition by SP600125 was not affect it. U0126 and SB203580 inhibited insulin-induced synthesis of glycosaminoglycan and accumulation of markers of chondrogenic differentiation, collagen type II, collagen type X, and aggrecan mRNA. On the other hand, SP600125 enhanced the synthesis of glycosaminoglycan. We have previously clarified that the induction of the cyclin-dependent kinase inhibitor, p21 (WAF1/Cip1) was essential for the chondrogenic differentiation of ATDC5 cells. To assess the relationship between the induction of p21 and MAPK activity, we investigated the effect of U0126 and SB203580 on the insulin-induced expression of p21 in ATDC5 cells. Insulin-induced accumulation of p21 mRNA and protein were completely inhibited by the addition of U0126 and SB203580. These results indicate that p44/42 MAPKs and p38 MAPK act as positive regulators of chondrogenesis, and JNK acts as a negative regulator of chondrogenesis, respectively.

P13 W

COORDINATED GENE INDUCTION AND REPRESSION OF TWO CCN FAMILY MEMBERS, CTGF AND CYR61, IN CHONDROCYTIC CELLS

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[Objective] Cyr61 and CTGF are both encoded by immediate early genes of the ccn family members and are reported to promote chondrocytic differentiation. During the development of mouse embryo, Cyr61 and CTGF mRNAs were shown to be expressed, following different time courses. These findings suggest different roles of these 2 genes in embryonic development. However, little is known about the expression response and functional significance of Cyr61 and CTGF gene expression in differentiated chondrocytes in maintaining phenotype under the stimulation of various exogenous factors. Therefore, our present study was performed to assess the gene regulation profile of Cyr61 and CTGF in chondrocytic cell line, HCS-2/8, in response to the stimulation by various factors.

[Methods] To analyze the expression response of Cyr61 and CTGF genes to exogenous stimuli, we stimulated the chondrocytic HCS-2/8 with various factors, such as TGFbeta, estrogen, TNFalpha and dexamethasons. Subsequently, after we isolated total RNA from the cells and obtained cDNAs by reverse transcription using MuLV reverse transcriptase, we analyzed the mRNA expression of Cyr61 and CTGF genes by a real-time PCR quantification method using the LightCycler system (Roche).

[Results] First of all, we confirmed the identity of quantitative PCR amplification products by agarose gel electrophoresis and direct DNA sequencing analysis. Also, quantitative reliability of real-time PCR quantification was confirmed by comparing the results with those of Northern blot analysis. Thereafter, it was found that both Cyr61 and CTGF mRNA expression was upregulated by TGFbeta and dexamethason treatment, respectively. Downregulation of gene expression was observed by TNFalpha stimulation also in both genes. No significant change was observed by estrogen treatment either in Cyr61, or CTGF mRNA level.

[Discussion] In this study, we demonstrated that Cyr61 and CTGF genes exhibited almost the same pattern of response against the stimulation of various factors on chondrocytic cells. These results suggest that those two genes are performing their missions coordinately or synergistically in differentiated chondrocyte under changing biological conditions. In the future, comparative analysis of Cyr61 and CTGF expression in mesenchymal cells during embryogenic and chondrogenic stages is going to be performed.

P14 F

THE KOSMOL INFLUENCE ON HISTOLOGIC STRUCTURE OF EPIPHYSARY CARTILAGES ON OLD WHITE RATS

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The experiment had been carried out on 56 old white rats with initial weight of 300-320 grams. The animals of the 1-st group received intrastomach biologically active food products 'Êîñìîë' in dosage according to weight 3 grams/kilogram every day. The intact animals had been used as control. All animals lived in standard conditions of vivarium.

At the end of the experiments (7, 15, 30 and 60 days) all the animals had been killed by ester narcosis and took away for tibial bones (TB) research. The fragments of the TB proximal epiphysis fixed in 5 % solution of neutral formalin, demineralized, dehydrated and filled in paraffin. The histologic cuts of 10-12 microns were painted by hematoxylin-eosin and researched according to standard technology.

Results. The injection of the experimental animals by Kosmol was accompanied by the tendency of bones sizes prevalence over control ones in late terms of observevation. The greatest length of TB by 30 and 60 days of experiment was more controlled on 2,38 % and 4,19 %, and cross-section of its sizes diaphysis on 4,14-6,4 %. Similar changes have been revealed and in osteometria and third lumbar vertebrae.

Histomorphometric research of proximal epiphyseal TB had demonstrated, that in group of the animals receiving Kosmol, its zone structure in comparison with intact animals had not essentially changed. Nevertheless, the tendency to optimization of osteogenic functions of cartilage had been observed because the volumetric contents initial spongiosa prevailed over control during the period from 15 till 60 days in 10,13 (p was traced<0,05), 7,31 % and 3,39 % accordingly.

According to data of T.Fujita (1990), the deficiency of calcium is a initial factor that in future leads to increasing in secretion of parathyroid hormone, and as result to mobilization of calcium from depot and loss of bone weight. In this case, the supply of calcium in organism with biologically active food. Kosmol normalizes the secretion of parathyroid hormone and levels the phenomenon of initial age according to bone osteoporosis (Teegarden D., Weaver C., 1994).

Thus, application of biologically active food Kosmol in dosage according to weight 3 grams/kilogram in old rats was accompanied by levelling of the age phenomena according to bone rarefication that is demonstrated by the tendency to bone formation functions optimization of epiphyseal cartilages of tibial bones.

P15 S

IN SITU TISSUE ENGINEERING FOR BIO-RESURFACING OF SYNOVIAL JOINTS WITH ARTICULAR CARTILAGE

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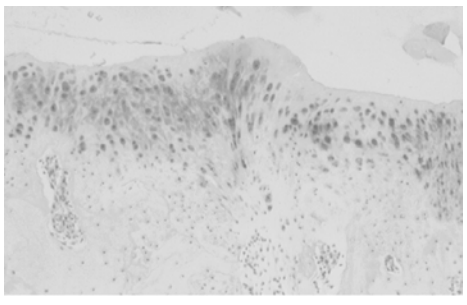
The aim of this study is to develope a novel approach to tissue engineering *in vivo*, in which the adaptive response of skeletal tissues to the imposed mechanical environment will be utilised to induce a cartilaginous resurfacing of the acetabular articulation in a hemi-arthroplasty model of hip replacement. Our hypothesis was that a cartilaginous resurfacing of subchondral bone can be induced by applying 0 to 3 MPa stress characteristics to the articular surface of the acetabulum.

Three groups of six sheep received unilateral hip hemi-arthroplasties were sacrificed 24 weeks post-operatively to harvest the acetabula. At operation, acetabular cartilage was removed completely and the subchondral bone was reamed to bleed. Three femoral head sizes, 25-, 28-, and 32-mm, were used to induce different contact stress levels. Vertical ground reaction force (GRF) data were measured and normalised by body weight for both limbs pre-operatively and every 4 weeks post-operatively. Five Specimens each from the 25- and the 28-mm group and eight un-operated controls were processed and stained with Safranin O and Sirius Red. Cartilage proteoglycans in the regenerated tissues from four specimens in 25-mm group were detected by immunoblotting using specific monoclonal antibodies.

The operated limbs were subjected to an average of 80 to 90% pre-operative GRF after the eighth post-operative week and maintained till the end of the study. No significant difference was noted in the period among all three groups. A layer of regenerated tissue was noted on all specimens processed and was Sirius positive. Four operated specimens processed in the 25-mm group and three in the 28-mm group were Safranin O positive. Cartilage proteoglycans (chondroitin sulphate, keratan sulphate, biglycan, decorin, and link protein) were detected by immunoblotting.

We conclude that a cartilaginous resurfacing of acetabulum can be induced *in vivo* under the mechanical environment imposed by our hemi-arthroplasty model.



P16 W

DEC1 AND DEC2 ARE REGULATORS FOR DIFFERENTIATION OF CARTILAGE, ADAPTATION TO HYPOXIA, AND CIRCADIAN RHYTHM

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Recently we cloned cDNA for a novel basic helix-loop-helix (bHLH) transcription factor DEC1, which was expressed at higher level in human primary chondrocytes than in fibroblastic cells. The amino acid sequences of DEC1 and its related protein DEC2 have highest similarities to those of Drosophila Hairy and Enhancer of split, and mammalian HESs. Overexpression of DEC1 in ATDC5 cells accelerated chondrogenic differentiation of the cells. Further, forced expression of DEC1 in mesenchymal stem cells induced chondrogenic differentiation. Expression profiles of DEC1 in mesenchymal stem cells differentiating into a chondrogenic lineage are similar to that of Runx2, which is a crucial factor for ossification, suggesting their co-operative functions in chondrogenic differentiation.

Part of cartilage is in hypoxic condition and hypoxia-inducible factor-1alpha (HIF-1alpha) is crucial for cartilage development. It is interesting that the expression of DEC1 and DEC2 was induced by hypoxia in chondrogenic ATDC5 cells as well as 293T and HeLa cells. Luciferase assay showed that the promoter activity of DEC1 and DEC2 genes was enhanced by hypoxia. The hypoxia response elements (HREs) in these promoters were responsible for the induction by hypoxia or HIF-1. DEC1 HRE contains additional elements, CRE-like and CACAG sequences, which were also important for induction by hypoxia.

Circadian rhythm may be an important factor for skeletal development, because a growth rate of bone varies depending on the time of day. Expression of DEC1 and DEC2 showed a circadian rhythm in various tissues of rats as well as in the suprachiasmatic nucleus, a central biological clock. The gel retardation assay showed that DEC1 and DEC2 bound to the E-box (CACGTG), which is responsive to CLOCK/BMAL1 heterodimer, the essential positive components in molecular clock. Luciferase assay demonstrated that CLOCK/BMAL1 heterodimer enhanced the DEC1 promoter activity, and that DEC1 and DEC2 proteins suppressed the enhanced activity. These lines of evidence established that DEC1 and DEC2 are novel regulators of the mammalian molecular clock.

Various factors such as hypoxia, retinoic acid, cAMP, and biological clock modulate the expression of DEC1 and DEC2 genes, which may lead to the regulation of the cartilage differentiation.

P17 F

REGULATION OF CHONDROCYTE MATURATION IN THE GROWTH PLATE: THE ROLE OF LEPTIN

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Bone growth in the developing limb originates in the growth plate. Cartilage tissue is first formed and is eventually replaced by mineralized skeletal elements. Chondrocytes involved in the process of endochondral ossification display several biochemical changes.

Leptin is a 16-kDa protein encoded by the obese (ob) gene and is a circulating hormone involved in feeding behavior and energy homeostasis. In addition, leptin has been reported various physiological actions on skeletogenesis.

As has been reported previously, the femoral lengths of the ob/ob mice, that had no signaling of leptin, were shorter than those of the wild mice. We also found that the growth plates of ob/ob mice were more fragile than those of wild mice by mechanical test and easy to break at hypertrophic zone. Next, the localizaion of leptin receptor (Ob-Rb) in mouse growth plate was examined immunohistochemically. Expression of Ob-Rb was identified in hypertrophic chondrocyte. To obtain a better understanding of the role of leptin in enchondral ossification, we investigated the effect of leptin on murine ATDC5 cells undergo chondrogenesis in vitro by quantitative assay using a real-time polymerase chain reaction (PCR) for parathyroid hormone-related protein

(PTHrP) and type X collagen mRNA. Leptin, at a concentration of 1-100 ng/ml, increased PTHrP mRNA expression in ATDC5 at three weeks. However, in cultured cell without leptin, PTHrP mRNA expression was low. Type X collagen mRNA expression and the content of the calcium extracellular matrix were suppressed when cells were cultured with leptin at a concentration of 1-100 ng/ml. Moreover, the suppression of matrix calcification was partially blocked by anti-PTHrP neutralizing anti body.

These results indicate that leptin affects chondrocyte maturation in growth plate, probably through local regulation of PTHrP expression, which is a novel role of leptin in endochondral ossification.

P18 S

EFFECTS OF OVEREXPRESSION OF MEMBRANE-BOUND TRANSFERRIN-LIKE PROTEIN (MTF) ON CHONDROGENIC DIFFERENTIATION

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[Introduction] Membrane-bound transferrin-like protein (MTf) was originally identified as a human tumor-associated antigen of 97 kDa. MTf is present at high or moderate levels in melanomas and some other tumors. In addition, MTf is expressed in many fetal tissues and several adult tissues. MTf is expressed in parallel with the expression of cartilage-characteristic genes during differentiation of chondrocytes, and the MTf level is much higher in cartilage than in other tissues. However, the role of MTf on chondrogenic differentiation remains unknown.

[Methods and results] We examined the effects of MTf overexpression on differentiation of ATDC5 and mouse pluripotent mesenchymal C3H10T1/2 cells. Rabbit MTf cDNA (full length) was subcloned into pcDNA3.1/Zeo (+). MTf was expressed under the control of CMV promoter. Undifferentiated ATDC5 cells and C3H10T1/2 cells were transfected with the expression vector containing rabbit MTf cDNA. Forced expression of rabbit MTf in ATDC5 cells induced aggrecan, type II collagen, matrilin-1 and type X collagen mRNAs, and cell-shape changes from fibroblastic cells to spherical chondrocytes. Acccordingly, the synthesis and accumulation of proteoglycans were higher in MTf-expressing cultures than in control cultures. These effects of MTf overexpression correlated with the MTf protein level on the cell surface, and decreased in the presence of anti-MTf antibody. MTf overexpression in C3H10T1/2 cells also induced aggrecan and/or type II collagen mRNA, but not the spherical phenotype.

[Conclusion] The expression of MTf on the cell surface facilitates the differentiation of prechondrogenic cells, although MTf overexpression alone seems to be insufficient to commit pluripotent mesenchymal cells to the chondrocyte lineage. We are now examining the effect of MTf RNAi in chondrogenic differentiation of mesenchymal cells.

P19 W

UP-REGULATION OF CHONDROGENIC GENES IN SYNOVIAL FIBROBLASTS IS INDUCED ADENOVIRUS VECTOR-MEDIATED ALK 3, 6 GENES

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Background: Articular cartilage is known to have a limited intrinsic healing potential. Recently, transplantation of autologous chondrocytes, mesenchymal progenitor cells or periosteum have been used for repair of focal osteochondral defects. However, it is difficult to obtain enough materials. Synovium is a thin tissue, which lines the nonarticular surfaces of diarthrodial joints. There is evidence that synovium contains cells with chondrogenic potential, and it was previously reported that synovial fibroblasts (SFs) demonstrated chondrogenesis when treated with TGF beta 1. Chondrogenic differentiation of ATDC5 cells was induced by the receptors of the BMPR-I group but not by those of the activin receptor-like kinase (ALK) -1 group. In the present study, we demonstrated that adenovirus vector-mediated ALK-3 and ALK-6 gene expression induced chondrogenic differentiation of SFs.

Methods: Synovial tissues were obtained from synovial tissues of the knee joints of adult Japanese white rabbits or rheumatoid arthritis patients at the time of total knee arthroplasty under written informed consents. Synovial cells were isolated from the tissues by enzymatic digestion. After 3-5 passages, subcultured synovial cells are mainly composed of SFs with fibroblastic morphology and free from T cell or macrophage markers. SFs were then infected with adenovirus vectors carrying LacZ (control), hemagglutinin (HA)-tagged constitutively active forms of ALK-3, 5, or 6 genes for 2 hours. Chondrogenic phenotypes of SFs were examined by Northern blotting or RT-PCR of type II collagen, aggrecan, and type X collagen genes as well as Alcian blue staining.

Results: Expression of type I receptors in SFs was confirmed by Westen blotting with anti-HA antibody. After 3 days of infection, dramatic induction of type II and aggrecan genes were observed in rabbit and human SFs infected with ALK-3 or ALK-6 virus, while no chondrogenic phenotypes were observed in LacZ or ALK-5-infected cells. Type X collagen expression or calcification was not observed in any types of cultures.

Conclusions: These results suggest that adenovirus vector mediated ALK-3 or 6 gene expression can induce chondrogenic differentiation of SFs, and that they are promising candidates for developing novel cell-based therapeutic approaches for postnatal articular cartilage repair.

P20 F

DOWN-REGULATION OF PTHRP AND PTH/PTHRP RECEPTOR BY FGFR3 SIGNALING IN CHONDROCYTES

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Parathyroid hormone (PTH)-related peptide (PTHrP), PTH/PTHrP receptor (PTH-R) and fibroblast growth factor receptor type III (FGFR3) play an important role in chondrocyte proliferation and differentiation. PTHrP/PTH-R signaling is controlled by negative feedback mediated by indian hedgehog (Ihh) that is expressed in hypertrophic chondrocytes. FGFR3 has been reported to inhibit chondrocyte proliferation by signal transduction of JAK/stat (signal transducers and activators of transcription). In this study, we have examined the possibility of gene regulation among PTHrP, PTH-R and FGFR3 without mediating PTHrP/Ihh negative feedback in chondrocytes. Epiphyseal cartilage of newborn mice revealed Ihh, which is normally expressed in the hypertrophic zone, whereas a chondrocytic cell line, CFK2 cell in subconfluent culture, expressed PTHrP, PTH-R and FGFR3, but not Ihh. Therefore, gene regulation among PTHrP, PTH-R and FGFR3 in CFK2 cells could be elucidated without participation of Ihh. CFK2 cells were transfected with cDNAs encoding PTHrP, PTH-R or FGFR3. PTH-R and FGFR3 proteins were observed on the cell surface of the transfected CFK2 cells, in correspondence with authentic localization of these receptors. RT-PCR revealed slightly reduced expression of PTHrP and markedly diminished PTH-R mRNA in CFK2 cells transfected with FGFR3 cDNA. Quantitative analysis with real-time PCR showed reduced PTHrP and PTH-R mRNAs to one half and to one fifth of the amount of the control, respectively, in the transfected cells. Coincident with previous reports, CFK2 cells with transfection of cDNA encoding FGFR3 displayed inhibited cell proliferation. Stat 1 was accumulated in the nuclei of the transfected cells, while faint immunoreactivity was seen throughout the cytoplasm of intact CFK2 cells, thereby, indicating JAK/stat1 signaling in the transfected CFK2 cells. Treatment with inhibitors of JAK2 (AG490) and JAK3 (WHI-P131) on CFK2 cells with transfection of FGFR3 cDNA induced a similar expression level of PTH-R mRNA when compared with the control CFK2 cells. Therefore, the downregulation of PTH-R in CFK2 cells transfected with FGFR3 cDNA appears to be the result of signaling mediated by JAK/stat. Thus, the expression of PTHrP and PTH-R appears to be suppressed by FGFR3 signaling without mediating Ihh negative feedback.

P21 S

P300/CBP ACTS AS A COACTIVATOR TO CARTILAGE HOMEOPROTEIN-1(CART1), PAIRED-LIKE HOMEOPROTEIN, VIA ACETYLATION OF THE CONSERVED LYSINE RESIDUE ADJACENT TO THE HOMEODOMAIN

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Cartilage homeoprotein-1 (Cart1) encodes a paired-like homeoprotein that expressed selectively in the chondrocyte lineage during embryonic development. Although its target gene remains unknown, gene disruption studies have revealed that Cart1 plays an important role for craniofacial bone formation as well as limb development by cooperating with other homeoprotein Alx4. In this report, we study the functional involvement of p300/CBP, coactivators with intrinsic histone acetyltransferase (HAT) activity, in the transcriptional control of Cart1. In luciferase assay using a putative Cart1 binding site, p300 and CBP stimulate Cart1- dependent transcriptional activity, and this transactivation is inhibited by E1A and Tax oncoproteins which are known to suppress the activity of p300/CBP. Cart1 binds to

p300 in vivo and in vitro, and this interaction requires the homeodomain of Cart1 and N-terminal 139 amino acids of p300. Confocal microscopy analysis shows that Cart1 recruits overexpressed and endogenous p300 to a Cart1- specific subnuclear compartment. We further demonstrate that Cart1 is acetylated in vivo, and that sodium butyrate and trichostatin A, histone deacetylase inhibitors, markedly enhance the transcription activity of Cart1. In addition, the affinity of Cart1 binding to p300 is augmented by HAT activity in the presence of acetyl-CoA. Deletion and mutagenesis analysis identifies the 131st lysine as a target of p300-HAT. This lysine locates immediately adjacent to the homeodomain and is highly conserved in paired-like homeoproteins. Furthermore, a point mutation to K131R attenuates the binding affinity to p300 as well as p300-dependent transcriptional activity. Together, these results indicate that p300/CBP acts as a cotransactivator to Cart1 through a direct interaction and specific lysine acetylation.

P22 W

THE ROLE OF PROGRAMMED CELL DEATH ON MORPHOGENESIS OF CARTILAGE CANAL

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In order to study the role of cell death in cartilage canal morphogenesis, the eggs were incubated in 38 centigraded. At the 13th, 15th and 18th days of incubation the proximal tibial epiphysis of the embryos were studied using histological methods (LM and TEM).

The results were as follows:

1 - The cells around canals reacted positively to acid phosphatase.

2 - Cartilage canals were found in the regions of the cell death.

3 - The cells differing from chondroblast are seen around of the canals and adjacent prichondrium.

These cells have special featurs such as condensed nucleus and vacuolated cytoplasm. Examination of ultrathin sections show that vessels in canals are capilarytype and their endathelial cells are fenestrated type and have pinocytotic vesicles. The evidence indicated that vacuolated cells have features of non-apoptotic programmed cell death. Probably these cells are died and open canals for growing of blood vessel. Vacuolated cells are apear to secretion of enzymes, in the way of merocrine type and removed matrix. The feature of endothelial cells of capilary showed that these vessels have nutritional role of cartilage tissue.

P23 F

FGF INDUCES A RAPID DEPHOSPHORYLATION OF THE RETINOBLASTOMA PROTEIN P107 IN CHONDROCYTES: IN SEARCH OF A PHOSPHATASE

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Fibroblast growth factors are important regulators of skeletogenesis. Activating FGF receptor

mutations are involved in chondrodysplasic disorders such as achondroplasia, the major form

of human dwarfism. One major function of FGF during chondrogenesis is to negatively regulate chondrocyte proliferation. We recently showed that the retinoblastoma (Rb) protein family p107 and p130 cell cycle regulators are involved in the growth inhibitory pathway triggered by FGF in chondrocytes (Laplantine et al. J Cell Biol (2002) 158:741-750). In this previous study, we noticed a rapid dephosphorylation of p107 protein following FGF treatment of a chondrocyte cell line, suggesting that p107 dephosphorylation could be an initiator in the cascade of events leading to chondrocyte growth arrest. We now found that FGF-induced dephosphorylation of p107 is not associated with a reduction in cyclin/CDK kinase activity nor it does require transcriptional events, since dephosphorylation of p107 occurs in presence of Actinomycin D. To test the hypothesis that FGF activate a p107-targeted phosphatase, we performed p107 immunoprecipitations using an anti-p107 specific antibody followed by serine/threonine (S/T) phosphatase assays. Indeed, we found that FGF induces an increase in p107 associated S/T phosphatase activity. Using S/T phosphatase inhibitors, we show that okadaic acid or calyculin A (at a concentration which inactivate both PP2A and PP1 classes of phosphatases) abrogated FGF-induced p107 dephosphorylation in cultured chondrocytes. Interestingly, inhibition of various signaling pathways further revealed a requirement for MAPK signaling. Together, these data indicate that FGF leads to the recruitment/activation of a p107 phosphatase that could be critically involved in the signal transduction cascade leading to cell cycle arrest in chondrocytes. The results of our ongoing efforts to identify this phosphatase will be presented.

P24 S

SPATIAL AND TEMPORAL EXPRESSION OF PERIOSTIN, A NOVEL GENE DETECTED BY COMPLEMENTARY DNA MICROARRAY DURING FRACTURE HEALING

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To comprehensively evaluate gene expression in the early stage of fracture healing, we used a cDNA microarray with 2304 cDNA clones derived from an oligo-capped mouse embryo library. Closed mid-diaphyseal fractures were created in mouse tibiae and expression profiles were analyzed three days after fracture. While the expression levels of eleven genes were down-regulated, six genes were up-regulated in comparison to those in unfractured bones and these included three novel genes previously identified but never shown to be present in fractures, periostin, calumenin, and FHL-1. Cloning of these novel genes has been completed but their function during fracture healing and bone formation remains to be elucidated. Up-regulation of the six genes was reconfirmed by semi-quantitative RT-PCR analysis. Because it displayed the highest up-regulation ratio, spatial and temporal expression of one of the novel genes, periostin, was performed using in situ hybridization. A strong signal for periostin was detected in undifferentiated mesenchymal cells and immature osteoblasts in the periosteum between days 3 and 7 after fracture, but rapidly decreased by day 14. This finding was confirmed by Northern analysis which showed a peak in expression on day 3 and a decline to baseline levels by day 14. These findings suggest that periostin is a specific marker for preosteoblasts and may play an important role in callus formation during the early stage of fracture healing.

P25 W

REGENERATION OF DEFECTS IN THE ARTICULAR CARTILAGE IN RAT KNEE JOINTS BY CONNECTIVE TISSUE GROWTH FACTOR/HYPERTROPHIC CHONDROCYTE-SPECIFIC GENE PRODUCT 24 (CTGF/HCS24)

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Connective tissue growth factor/hypertrophic chondrocyte specific gene product 24 (CTGF/Hcs24) is a unique growth factor to stimulate proliferation and differentiation but not hypertrophy of articular chondrocytes *in vitro* . To objective of this study is to investigate the therapeutic use of recombinant CTGF/Hcs24 (rCTGF/Hcs24) in both monoiodoacetic acid (MIA)-induced experimental rat osteoarthritis (OA), and full-thickness defects of rat articular cartilage. Firstly, we detected CTGF/Hcs24 in the clustering of chondrocytes, indicating an attempt to repair the damaged cartilage, formed in OA lesions induced by MIA by using immunohistochemical techniques. These findings suggest that it plays an important role in the repair of articular cartilage. In fact, an injection of 1.0 or 1.5 microg of rCTGF/Hcs24-incorporated gelatin hydrogel (rCTGF/Hcs24-hydrogel) into the joint cavity of MIA-induced OA was repaired articular cartilage 7 days after injection, and the histological findings were similar to those of normal cartilage. Secondly, in order to examine the direct effect of rCTGF/Hcs24 on the repair in articular cartilage, defects (2 mm in diameter) were created in the surface of the articular cartilage, lyophilized 1.0 microg rCTGF/Hcs24-hydrogel or PBS-hydrogel with collagen was implanted in the defects. In the implantation with rCTGF/Hcs24-hydrogel-collagen group, new cartilage had filled the defect 4 weeks postoperatively. In contrast, there was either soft tissue repair only or no evidence of tissue repair in the implantation with the PBS-hydrogel-collagen group. These findings suggest that CTGF/Hcs24 could stimulate the repair of defects in articular cartilage during 4 weeks. Although further studies for longer time periods are needed on the use of rCTGF/Hcs24 in joint disease, the present study suggests that CTGF/Hcs24 may be useful in regeneration of articular cartilage.

P26 F

IDENTIFICATION OF GROWTH-RELATED RENAL NA/PI COTRANSPORTERS IN WEANING ANIMALS

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Inorganic phosphate (Pi) is of critical importance to body functions, particularly during periods of growth. The kidneys contribute to the maintenance of the positive Pi balance required for growth by reabsorbing a high fraction of the filtered Pi. The capacity for Na^+-dependent phosphate cotransport across the luminal brush border membrane of renal proximal tubular cells is higher in juveniles than in adults. The bulk of filtered Pi is reabsorbed in the proximal tubule where type IIa sodium-

dependent Pi transporters in the brush-border membrane mediate the rate-limiting step in the overall Pi reabsorptive process. Furthermore, a specific type IIa-related Na/Pi cotransporter (growth-related) was postulated to account for high Pi transport rates in weaning animals. In the present study, we isolated a cDNA from the human and rat kidney that encodes a growth-related Na^+-dependent inorganic phosphate (Pi) cotransporter (type IIc). Microinjection of type IIc cRNA into Xenopus oocytes demonstrated sodium-dependent Pi cotransport activity. Affinity for Pi was 0.07 mM in 100 mM Na^+. The transport activity was dependent on extracellular pH. In electrophysiological studies, type IIc Na/Pi cotransport was electroneutral, while type IIa was highly electrogenic. In Northern blotting analysis, the type IIc transcript was only expressed in the kidney and highly in weaning animals. In immunohistochemical analysis, the type IIc protein was shown to be localized at the apical membrane of the proximal tubular cells in superficial and midcortical nephrons of weaning rat kidney. Hybrid depletion experiments suggested that type IIc could function as a Na/Pi cotransporter in weaning animals, but its role is reduced in adults. In thyroparathyroidectomized (TPTX) rats maintained on normal rat chow, the levels of type IIc immunoreactive protein (75-80 kDa) were significantly increased in the BBMV from the kidney compared with those in the control (sham-ope). PTH leaded to the rapid-endocytosis and the lysosomal degradation of type IIa transporter. In contrast, the type IIc immunoreactive signals were gradually decreased in the apical membrane and existed in subapical domains of proximal tubule cells. The finding of the present study suggest that the type IIc is a growth-related renal Na/Pi cotransporter, which has a high affinity for Pi and is electroneutral, and that PTH in involved in the regulation of the type IIc Na/Pi cotransporter.

P27 S

PHOSPHO1 - A NOVEL PHOSPHATASE SPECIFICALLY EXPRESSED AT SITES OF MINERALISATION IN BONE AND CARTILAGE

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Mineralisation of bone and cartilage is essential for skeletal development and function. The importance of alkaline phosphatase (ALP) for the generation of inorganic phosphate is well recognized but data from ALP knockout mice suggests the existence of other phosphatases involved in skeletal mineralisation. We have previously reported a novel gene (PHOSPHO1) whose expression is upregulated in mineralising chondrocytes and has an active site structure that suggests it is a phosphatase. The present aims were to confirm that PHOSPHO1 is indeed a phosphatase and to determine its localisation in skeletal tissues.

Recombinant derived PHOSPHO1 protein was produced by amplifying the protein coding sequence of chick PHOSPHO1 by PCR followed by cloning into bacterial expression vector pBAD-TOPO-TA. It catalysed the hydrolysis of p-nitrophenyl phosphate (pNPP) at pH 6.5 and the reaction followed Michealis-Menton kinetics with a Km of 33mM for pNPP. Affinity purified PHOSPHO1 antiserum was generated and used to stain cryostat sections of skeletal (tibiae and calvaria) and soft (liver, muscle and kidney) tissues. The periosteum of cortical bone, forming surfaces of primary osteons and osteocytes situated within the periosteal area displayed clear PHOSPHO1 immunostaining. The endosteum was negative. In growth plate cartilage immunoreactivity was limited to the early mineralising chondrocytes and the ossification groove of Ranvier. Proliferating and terminally differentiated chondrocytes were negative. Cartilage remnants and trabecular bone within the primary spongiosa exhibited strong immunoreactivity on their mineralizing surfaces. In embryonic tissue, the intramembranous and periosteal bone surfaces of calvaria stained positively for PHOSPHO1 as did hypertrophic chondrocytes and the bony trabeculae surfaces. All soft tissues were negative which confirmed previous RT-PCR data. Further confirmation that PHOSPHO1 is involved in mineralisation was obtained from SaOS-2 cells, which mineralize their matrix and express PHOSPHO1 and ALP mRNA. In contrast, MG-63 cells do not produce a mineralized matrix and showed no PHOSPHO1 or ALP mRNA expression. PHOSPHO1 gene expression was unchanged by dexamethasone, estradiol, 1,25-dihydroxyvatamin D or PTHrP treatment.

These results confirm that PHOSPHO1 is a phosphatase capable of the generation of inorganic phosphate and that its protein expression is specific to mineralising cells. Its importance in skeletal mineralisation is unknown.

P28 W

CHONDROCYTE AND PERIOSTEUM EXPRESS EPHRIN-B2 AND EPHB4 MRNAS DURING FRACTURE HEALING

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Ephrin-B2 is trans-membrane ligand that is important regulator in morphogenic processes such as angiogenesis and axon guidance. One of the receptor of ephrin-B2 is Eph (Erythropoietin-producing hepatoma amplified sequence) B4 which has tyrosine kinase activity. In vascular remodeling, ephrin-B2 is expressed on arterial endothelial cells, however, EphB4 is identified on venous endothelial cells. Ephrin-B2 and EphB4 are believed to mediate reciprocal interactions between arterial and venous endothelial cells and surrounding cells to form each characteristic vessel. Recently, ephrin-B2 is revealed to co-localize with chondromodulin-I, a potent endothelial cell growth inhibitor, in epiphysis during skeletal development. We hypothesized that ephrin-B2 and EphB4 were expressed in endochondral ossification to establish boundary between non-vascular matrix cartilage and vascular rich matrix bone. The aim of this study was to analyze expression and distribution of Ephrin-B2 and EphB4 mRNAs in bone regeneration by using of mouse rib fracture healing model. A total of 36 male ICR mice were used. A transverse rib fracture was made on each mouse. Total RNA was extracted from the fracture sites on days 1, 3, 5, 7, 10, 14, 18, 21, and 28. Both ephrin-B2 and EphB4 mRNAs were expressed during whole these days detected by RT-PCR and in situ hybridization. In the early phase, intense signals were detected on the periosteum, however, scattered signal was identified in the bone marrow. In the middle phase, ephrin-B2 and EphB4 mRNAs were expressed on the proliferative, prehypertrophic, and hypertrophic chondrocytes, as well as the periosteum. The positive signals peaked on the prehypertrophic chondrocytes, and it gradually attenuated on the hypertrophic chondrocytes. These data suggest that ephrin-B2 and EphB4 are important for proliferation of chondrocytes in endochondral ossification, providing the boundary of cartilage and bone matrices. This ligand/receptor complex in the periosteum may also play a critical role for membranous ossification.

P29 F

PERIODONTAL LIGAMENT FIBROBLASTS RESPOND TO MECHANICAL STRESS IN A WAY DISTINCT FROM THAT OF OSTEOBLASTIC CELLS

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The periodontal ligament (PDL) is a connective tissue located between the cementum of teeth and alveolar bone. Previous studies demonstrated that the PDL cells are capable of inducing matrix mineralization. Some studies even suggested that mechanical stress (MS) promotes in vitro differentiation of PDL cells into hard tissue-forming cells similar to osteoblasts. However, the PDL never calcifies and maintain its width constant in vivo despite constant exposure to intermittent MS, i.e., occlusal forces. These discrepancies between in vivo and in vitro observations may have resulted from the fact that no cell line for the PDL is available and that those in vitro data were obtained using mixed population of cells taken from the PDL possibly contaminated with adjacent tissues, although the majority of PDL composites are fibroblastic cells. We have recently established 19 cell lines from the PDL. Of these, 11 clones including PDL-L2 show exactly the same pattern of gene expression with in vivo PDL fibroblastic cells. PDL-L2 cells do not produce mineralized nodules in differentiation medium unless they are exposed to strong stimulators such as rhBMP-2. These results indicate that PDL-L2 cells represent the PDL fibroblasts. We used therefore this cell line to examine MS effect by means of Flexorcells. When osteoblastic cell line MC3T3-E1 was subjected to tensile strain, mineralized nodule formation was markedly induced with a concomitant increase of osteocalcin expression. In contrast, neither nodule formation nor osteocalcin expression was induced in PDL-L2 by the same stimuli. Recent studies suggested that MS causes osteoblastic differentiation by enhancing transcriptional activity of Runx2 possibly through a pathway involving stimulation of integrin receptors, followed by sequential phosphorylation of Erk 1/2 and Runx2. In our system, we also demonstrated that MS enhances phosphorylation of Erk1/2 and transcriptional activity of Runx2 in MC3T3-E1. In PDL-L2, however, no such changes were observed. These results indicate presence of mechanisms that are unique to the PDL, possibly involving a specific combination of integrin receptor and its downstream molecules. Such mechanisms may enable the PDL to maintain its morphology and function, and to keep itself free from being mineralized under constant exposure to MS.

P30 S

CLONING AND CHARACTERIZATION OF HUMAN PERIODONTAL LIGAMENT FIBROBLASTS

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Human periodontal ligament (PDL) has important roles such as holding the teeth, maintaining homeostasis, and regenerating periodontal tissue. PDL consists of heterogeneous cell populations, and therefore, human PDL cells used in many previous studies were heterogeneous, containing various proliferative and differentiated stages of cells, and the results of previous studies with these cells lacked consistency. Clonal analysis is consequently essential for characterizing the composition of PDL cell populations. We cloned human PDL cells in the primary culture, and analyzed their cellular types in terms of their morphology, proliferations, and osteoblastic characteristics. Human PDL was obtained from the middle of the roots of two maxillary first premolars extracted from 23-year-old adult female with

clinically healthy periodontium. The PDL was digested with collagenase and trypsin, and the isolated cells were plated sparsely. One or two day after culture, single cells were marked. Fourteen days after culture, colonies derived from a single cell were cloned. Seven cloned PDL cells were analyzed within the culture period at which characteristics of all clones were maintained. All clones demonstrated a fibroblastic spindle morphology, and became proliferative senescence at 12th passage. Clones showed various cell proliferations, and basal alkaline phosphatase (ALP) activities. Basal ALP activities in all clones were enhanced by dexamethasone (DEX). For mineralization analysis, clones were cultured in mineralizing medium containing ascorbic acid, beta-glycerophosphate and DEX for 28 days, and stained by Von Kossa method. Expressions of messenger RNAs (mRNAs) for bone-matrix proteins, osteopontin (OP), bone sialoprotein (BSP), and osteocalcin (OC), were also detected by RT-PCR when cultured in mineralizing medium for 28 days. Von Kossa staining showed three different patterns; 1) clones stained extensively in almost area, 2) clones partially stained, and 3) clones not stained at all. However, all clones expressed mRNAs for OP, BSP, and OC. These results suggest that human PDL consists of heterogeneous cell populations with each own property. PDL might perform many functions due to the heterogeneity of cell populations based on proliferation and osteoblastic characteristics.

P31 W

SUBSTITUTING THE UPPER JAW DEFECTS BY THE MATERIAL 'LITAR'

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Reconstructing the defective parts of the bone tissue in the jaws is a topical problem in maxillofacial surgery. The bone defects can be birth as well as they can results from traumatic injuries and after performing surgical interventions on the facial skeleton bones. It is advantageous to use biodegradable osteoplastic materials for reconstructing the defective parts because these materials lyse in the organism in the shortest time. One of the representatives of this group is material 'LitAr'. The material is a composition containing hydroxyapatite or hydroxyfluorapatite and collagen. The synthesis of the implant was achieved according to an original method which could ensure a high degree of structural integraty of the components in the composite. The biodegradation of the material has been achieved by a high porosity accounting for 75%. The composite material 'LitAr' doesn't stimulate a return immunoreaction, can be easily formed in the defect zone. The composite material was used for substituting the defective bone tissue parts of the upper jaw, for correcting the alveolar appendix in the case of birth punctured cleft palate, of the defects which appeared as a result of removing benign neoplasms, of the jaw fracture. We have performed compensating for trepanized holes of the front wall of the maxillary sinus which was formed in the course of surgical treatment the chronic sinusitis and the sinus cysts. Checking the biotransformation of the material was performed with the use of computed tomography over a period of 2, 30, 60, 90, 120 days postoperative. The reconstruction of the bone tissue in the defective parts after removing neoplasms and fractures was seen approximately in 30 days. In the course of reconstructing the front wall of the maxillary sinus it took 90 days. In the case of the alveolar appendix cleft the outset of the process of reparative osteogenesis accounted for the 120th days. Thus, the application of the composite material 'LitAr' promotes reconstructing the full-value native bone tissue in the region of the upper jaw defect and provides the highest biotransformation rate from among the known materials of this type.

P32 F

PERIODONTIUM OF THE GROWING VITAMIN C DEFICIENT RAT

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The purpose of the present study was to investigate histologically and immunohistochemically the changes of the periodontium in growing vitamin C deficient od/od rats which have a hereditary osteogenic disorder caused by a deficiency of L-gulonolactone oxidase. These rats become scorbutic without a supply of ascorbic acid. The trait is autosomal recessive. Normal +/+ rats which can synthesize ascorbic acid were also used as control animals. Fifteen +/+ rats (Group I) and 15 od/od rats (Group II) were used in this study. The animals of both groups which were kept on an ascorbic-deficent lab chow diet (CL-2 : Clea Japan Inc.) after birth were killed at 1, 2 and 3 weeks. The histological sections were stained with hematoxylin-eosin, azan-mallory and oxytalan fiber. The sections were also stained with type I collagen. All sections were examined with a light microscope. Histologically, in Group I, at 1 week, the formation of the enamel matrix and dentin was observed, but the periodontium was not found. At 2 weeks, the formation of the root, periodontal ligament and alveolar bone was observed. At 3 weeks, the teeth erupted into the oral cavity and the roots were elongated. The teeth and the periodontium appeared normal. The oxytalan fibers type I collagen were observed. In Group II, at 1 week, the formation of the enamel matrix and dentin was slightly decreased in amount as compared with Group I. At 2 weeks, the formation of the periodontal ligament and alveolar bone was slightly decreased in amount as compared with Group I. At 3 weeks, the formation of the periodontal ligament and alveolar bone was markedly decreased in amount as compared with Group I. In addition, the arrangement of the periodontal fibers was very irregular as compared with Group I, and type I collagen was also reduced in amount. But the amount of oxytalan fibers was almost the same as compared with Group I. The results of the present study indicate that vitamin C deficiency produces marked structural changes of the periodontium in growing rats.

P33 S

A NOVEL ANTI-RHEUMATIC DRUG, T-614, STIMULATES OSTEOBLASTIC DIFFERENTIATION IN VITRO AND BONE MORPHOGENETIC PROTEIN-2 INDUCED ECTOPIC BONE FORMATION IN VIVO

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T-614 (N-[3-(formylamino)-4-oxo-6-phenoxy-4H-chromen-7-yl] methanesulfonamide), a newly developed anti-rheumatic drug under clinical trial, is an anti-inflammatory agent that has been reported to show the inhibitory effect of bone destruction in vivo arthritis model. We found that T-614 stimulated osteoblastic differentiation of stromal cell line (ST2) and preosteoblastic cell line (MC3T3-E1) in the presence or absence of recombinant human bone morphogenetic protein-2 (rhBMP-2). When cultured in osteogenic medium containing beta- glicerophosphate and ascorbic acid, calcium content of mineralized nodules was 14-fold elevated by the addition of T-614 in the presence of rhBMP-2 in ST2 but not MC3T3-E1. Oral administration of T-614 to mice also promoted rhBMP-2 induced bone formation in vivo. In order to elucidate the mechanisms of T-614 effects on osteoblastic differentiation, we examined the phosphorylation levels of Smad 5 and the expression levels of Id1, which were direct targets of BMP signaling. T-614 neither affected the phosphorylation levels of Smad 5 nor expression levels of Id1. The transcription factors, Cbfa1/Runx2 and osterix, both play essential roles in osteoblast differentiation. In Northern blotting analysis, the expression levels of Cbfa1/Runx2 were not stimulated by T-614, on the other hand, the mRNA levels of osterix were 3-fold increased by T-614 in the presence of rhBMP-2 in ST2. Taken together, we speculated that T-614 was not involved in the early signal transduction pathway of BMP-2 but a stimulator of osterix. These results suggested that T-614 possessed anabolic effects on bone metabolism, besides suppressor of bone resorption, by increased expression of osterix.

P34 W

DO SKELETONS PLAY AN IMPORTANT ROLE IN CALCIUM RESERVOIR IN FROGS?

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Vertebrate skeletons have changed their structure depending on environment. There will be several differences in skeletal structure between land animals and aquatic animals. Buoyancy helped behavior of aquatic animals, but land animals should acquire strong skeletons to behave actively with gravity. In addition, skeletons in land animals acquired an important function as a calcium reservoir. These backgrounds prompted us to explore the morphological differences in skeletons between land animals and aquatic animals. We previously demonstrated that femurs of various growing frogs including water frogs, land frogs and tree frogs lack endochondral ossification, while femurs of the matured frogs exhibited various amounts of irregularly arranged endochondral ossification. Since these morphological features in growing frogs are different from those in mammals, we compared bone structure among several kinds of animals including frogs, turtles, lizards and mice. Enzyme histochemical studies demonstrated that osteoblasts of *Xenopus laevis*(water frog) and *Rana catesbeiana*(land frog) had week ALP activity, compared to those of turtles, lizards and mice. Mineral apposition rate (MAR) as judged by bone histomorphometric analyses after injection of calsein was extremely low (0.352 ±0.166 microm/day) in *Xenopus laevis*, compared to that in mice (4.172 ±1.000 microm/day). Turtles and lizards have higher MAR than frogs. Osteoclasts in turtles, lizards and mice showed TRAP activity, whereas those in *Xenopus laevis* and *Rana catesbeiana* lacked its activity. Three-dimensional analyses by scanning electron microscope (SEM) and micro-CT demonstrated that trabeculae observed in endochondral ossification of matured frogs retained well connectivity of trabeculae, compared to those observed in mice. SEM observation also revealed that the number and size of resorption pits on trabecular and endocortical surfaces of frogs are apparently smaller than those observed in mice. These results indicate that bone turnover in frogs is very low, compared to those in land living animals. Taken together, bones in frogs may not acquire well-developed function as a calcium reservoir. Land

and tree frogs have well-mineralized endolymphatic sacs along vertebrates, which are composed of carcium carbonate. Since frogs can use calcium carbonate physiologically, these endolymphatic sacs might be important organs as a calcium reservoir in some frogs.

P35 F

PREGNANCY AND THE INCIDENCE OF 'REAL' TRABECULAR TERMINI

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Placental transfer of calcium and phosphorus from mother to foetus is a central event in the mineral metabolism of mammalian gestation. In consequence there prevails an increased requirement for these inorganic elements to meet the developmental demands of the foetus while maintaining maternal serum calcium homeostasis. Using iliac crest bone biopsies we have previously 1 reported that pregnancy produces a fluctuation in the maternal cancellous bone volume, such that early bone loss is restored by term, in the process becoming a more complex system of increased but thinner trabeculae. In a minority of women pregnancy is apparently associated with an increased fracture predisposition, for reasons which are not clear. With the aim of examining further the microarchitectural modulation, the previous iliac crest bone biopsies were analysed using a novel method 2 based upon the superficial staining of thick (300 mm) embedded bone slices to demonstrate the combined 2- and 3-D microstructure, thereby identifying ' real ' trabecular termini (ReTm) as loci of weakness and separating them from apparent i.e. planar artifactual termini.

Using specimens from age-matched, non-pregnant and pregnant women, results confirmed that ReTm were rare in biopsies from such normal healthy subjects. Application of the S-Plus 2-sample test for equality of proportions found no significant difference ($p<0.05$) between the groups whether non-pregnancy (ReTm 2 hits per 14 samples), early pregnancy (ReTm 2 hits per 12 samples) or late pregnancy (ReTm 0 hits per 14 samples). It was concluded that despite the initial decline in the mean relative bone volume from 23% to 17% and its restitution by the end of gestation, the maternal skeleton is not left weakened by trabecular disconnection. It was also confirmed that the number of Re Tm is independent of the bone mass, unlike some indirect measures of trabecular interconnection.

1.Shahtaheri et al, Brit J Obs Gynaecol 106, 432-438, 1999.
2.Aaron et al, Bone 27, 277-282, 2000.

P36 S

IMMUNOHISTOCHEMICAL LOCALIZATION OF BONE MATRIX PROTEINS IN FROZEN AND UNDECALCIFIED SECTIONS

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The immunolocalizations of bone matrix proteins were investigated in frozen and undecalcified sections. Furthermore, in order to evaluate the effect of decalcification, some sections were immunostained after decalcification with EDTA.

Ten-day-old rat femurs were used in this experiment. After rapidly freezing, femurs were embedded in 5% carboxymethyl cellulose (CMC). These frozen specimens were attached to the sample stage of the cryomicrotome (CM 3500; Leica Instruments, Germany) and sectioned sagitally, at a thickness of 5µm. These sections were fixed with 4% paraformaldehyde in 0.1M phosphate buffer at room temperature for 5 minutes, and investigated immunohistochemically using antibodies to bone sialoprotein (BSP), osteocalcin (OCN), and type I collagen. Some sections were decalcified with 5% EDTA for 30 minutes after fixation, and immunostained in the same way.

In the undecalcified sections, intense, highly specific immunostainings for BSP were observed in the cement lines on the bone surface, while bone matrix was lightly stained or negative. Immunodetectable OCN was demonstrated in cement lines and some osteocytes in bone matrix. But the inner bone matrix was essentially devoid of immunostain.

On the other hand, after decalcification of these sections, BSP and OCN localization was discerned not only in cement line on the bone surface but also throughout bone matrix. It was considered that the immunoreactivity of bone matrix proteins may be masked by minerals, and that after decalcification, they will be exposed and become immunodetectable. To confirm this hypothesis, we examined immunohistochemical distribution of type I collagen in the frozen sections with or without decalcification. In decalcified sections, bone matrix distinctly positive for type I collagen. However, in undecalcified sections, immunodetectable type I collagen was restricted to osteoid, and no immunostaining could be found in mineralized bone matrix.

These observations suggest that bone matrix proteins are embedded in calcified matrix which is separated from aqueous environment and that provide additional insight for interaction between calcification and bone matrix.

P37 W

OCCURRENCE OF ELECTRON DENSE SEGMENTS IN OSTEOIDAL COLLAGEN FIBRILS IMMEDIATELY ADJACENT TO THE MINERALIZATION FRONT OF BONE IN RATS

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Aim: To elucidate precise mechanisms of appositional mineralization of bone, structural features of mineralizing collagen fibrils of osteoid in normal and hypocalcemic rats were examined in detail. Methods: Postnatal 24-day-old Wistar rats were fed either with calcium-free diet (0% Ca, 0.35% P) or normal calcium diet (0.5% Ca, 0.35% P), each supplemented with 10 IU vitamin D_3, for 25 days with free access to deionized water. These animals and young rats grown under regular diet were perfused with 4%paraformaldehyde (PFA), further fixed by immersion in a mixture or PFA and glutaraldehyde. Undecalcified or EDTA-decalcified specimens were embedded in Epon 812 for light and electron microscopic examination. Results: In the rats fed with regular- or normal calcium diet, collagen fibrils in the osteoid of various types of bone often displayed a segment of high electron density immediately adjacent to the mineralization front. The electron dense segments appeared only after Ur-Pb staining, and were more distinct in undecalcified sections than in decalcified sections. The electron dense segments were slightly thicker than the other portions of the fibrils but showed no sign of deposition of crystalline figures. In hypocalcemic animals, no electron dense segments of collagen fibrils could be identified in the widened osteoid. Discussion: Our study has provided the first evidence of the presence of electron dense segments in the collagen fibrils of osteoid near the mineralization front, in various types of bone under normal conditions. Since such electron density does not appear without Ur-Pb staining, a deposition of certain organic substance is suggested to occur in the dense segments of collagen fibrils as a requisite for its mineralization. The deposition of putative substance in the segments of collagen fibrils does not occur all at once along the mineralization front, but proceeds in groups of collagen fibrils at different time intervals in different micro domains of the osteoid, thus facilitating appositional mineralization of bone. Absence of electron dense segments of collagen fibrils in the osteoid of hypocalcemic animals may imply co-existence of mineral phase in the dense segments of collagen fibrils, coupled with the deposition of organic substance under physiological conditions.

P38 F

MORPHO-FUNCTIONAL CONDITION OF EPIPHYSEAL CARTILAGES IN CONDITIONS OF IRRADIATION OF PREADOLESCENT WHITE RATS BY NON-IONISING ELECTROMAGNETIC RADIATIONS

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Research is carried out on 280 preadolescent white rats with initial weight of 65-70 grams. Animals were exposed to the influence of 61,5 Gig Hertz in modes of continuous and impulse generation with frequency of following of impulses irradiated with electromagnetic waves frequency of 5 Hertz and 45 Hertz; the helium - neon laser in modes of 0,5 milliwatts/cm ^{2} during 10 minutes and 15 milliwatts/cm ^{2} during 2 minutes, and also volume-combinant electromagnetic impulse fields with amplitude 0,04/0,01 Tesla (a 'direct' configuration) and 0,04/0,05 Tesla (a 'cross-section' configuration). After terms of experiment animals were hammered under a aether narcosis, tibial bones were removed intact, soft tissues were gently dissected and the tibial epiphyseal cartilages undergone complex histomorphometric analysis due to morpho-functional classification by V.Koveshnikov (1980).

We have established, that in the case of long-term (30 and 90 days) irradiation by the electromagnetic waves with extremely-high frequency there is a greatest expansion of the epiphysial cartilago,including all its zones, and the reduction of its osteogenesis function.

Influence of the helium - neon laser, since 30 days of an irradiation, also was accompanied by expansion epiphyseal cartilage and all its zones with infringement osteogenesis functions. During the period of readaptation after a 30 days irradiation the revealed deviations appreciably smoothed out. In this case the maximal amplitude of deviations was observed in group with the greater capacity of radiation.

At last, in the groups after volume-combinant electromagnetic impulse fields iradiation also had been revealed expansion epiphyseal cartilage and all its zones with reduction in the volumetric contents of primary spongiosa, levelled in the period of readaptation. Thus in group with a 'direct' configuration of impulses expressiveness of deviations during the period of the readaptation was the greater.

The received data allow to assume, that researched low - intensive non-ionising radiations possess the similar mechanism of action on morpho-functional properties of the epiphyseal cartilages of long bones preadolescent rats.

P39 S

MODELS OF ENDOCHONDRAL BONE FORMATION ALLOW IDENTIFICATION OF SELECTIVE ANTI-ANGIOGENIC COMPOUNDS WITH IMPROVED PHARMACOLOGICAL PROFILES

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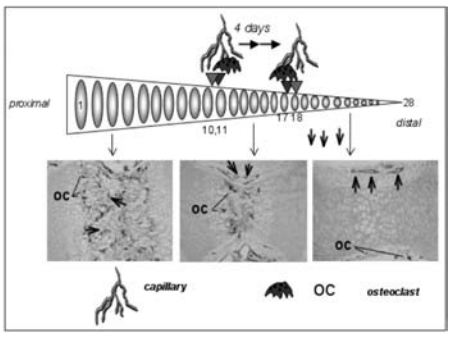
Angiogenesis and bone (re)modeling are absolute requirements for the development and maintenance of the vertebral skeleton. Disturbances in angiogenesis and bone turnover, therefore, have been implicated in the pathogenesis of various inherited, metabolic and malignant bone disorders (e.g. skeletal dysplasia, osteoporosis and metastatic bone disease).

We describe here convenient, cost-saving, and reproducible in vitro and in vivo assays for angiogenesis and osteoclastogenesis that 1. provide a rapid screening procedure for putative pharmacological agents (drug discovery), 2. allow identification of their mechanisms of action and 3. allow identification of in vivo toxicity. The advantages of the in vivo assay, that is based endochondral bone formation in tails of young growing mice, is highlighted by the fact that newly developed matrix-metalloproteinase inhibitors (MMPIs) were well-tolerated and displayed either simultaneous inhibition angiogenesis and osteoclastogenesis (CH5902) or selective inhibition of angiogenesis without affecting osteoclast-formation (CH3921). In contrast, the broad-spectrum MMPI marimastat was poorly tolerated and cytotoxic in vivo, which supports the observed side effects of this compound in clinical trials.



P40 W

TISSUE TRANSGLUTAMINASE LOCALIZATION, ACTIVITY AND SUBSTRATES IN TEETH

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The extracellular matrix (ECM) of calcified tissues contains abundant noncollagenous proteins (NCPs) that participate in cell attachment and signaling of osteogenic and odontogenic cells, and in the regulation of ECM mineralization. We have recently shown that some of the NCPs in bone, namely osteopontin, bone sialoprotein and alpha$_2$HS-glycoprotein, serve as substrates for the enzyme tissue transglutaminase (tTG) which creates protein multimers by forming covalent isopeptide bonds between substrate proteins in a calcium-dependent reaction. Such high-molecular weight forms of the proteins are abundant in bone. tTG participates in cell adhesion and tissue stabilization and is localized to the osteoid and cell-matrix interfaces in bone. In this study, we have analyzed TG enzyme activity, and tTG localization and its substrates in teeth. TG enzyme activity was assayed in EDTA-extracts (demineralized) of teeth using microplate- and Western blot-based activity and substrate assays; both utilizing incorporation of biotinylated primary amine into TG-substrate proteins. Substantial TG activity was found in tooth extracts where TG catalyzed the incorporation of primary amine into two proteins having molecular weights of 90 and 100 kDa. Avidin-affinity chromatography allowed identification and purification of both biotin-labeled substrates which were identified by N-terminal sequencing as variants of dentin phosphoprotein (DPP) (also known as phosphophoryn). The tTG isoform and isopeptide bonds were localized immunohistochemically in predentin, odontoblast processes/tubules and the pericellular matrix of cementocytes in cellular cementum, an immunostaining pattern resembling that for osteoid and the pericellular matrix of osteocytes in bone. DPP is expressed by pre-ameloblasts and odontoblasts, constitutes the majority of the noncollagenous protein in the dentin matrix, and is potentially involved in the regulation of dentin matrix mineralization. DPP is known to undergo a calcium- and magnesium-dependent, ionic self-association which produces large protein complexes measuring up to 25 nm in size. Based on our results, we hypothesize that these large complexes exist in teeth and could be further stabilized by covalent cross-links created by the tTG enzyme, and that this configuration may function in the regulation of dentin mineralization. Supported by the CIHR, Academy of Finland, FRSQ and Chinese Scholarship Council.

P41 F

LUMICAN IS ASSOCIATED WITH OSTEOID FORMATION IN HUMAN NEONATAL ENDOCHONDRAL OSSIFICTATION PROCESS

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Lumican belongs to small leucin-rich keratan sulfate proteoglycan, which was found in corneal transpracency. Recently, lumican has been reported to be expressed in cartilage, cancer cells, or endocrine tissues. To invastigate which lumican is associated with endochondral ossification process, we examined 10 cases of human neonatal vertebral tissues comparing with collagen deposition pattern. Lumican is deposited in the osteoid matrix of new bones, perichondrium and periostium in endochandral ossification tissues. It also expressed in cytoplasm of proliferating chondrocytes. Type I collagen was deposited in the new bonematrix, osteoid matrix, perichondrium and periostium, whereas tyep III colagen was depositedin the periostium, perichodrium and partly in the stroma around the blood vessels. In conclusion, lumican is associated with osteoid formation to co-localize with type I collagen. Ist cytoplasmic expression was also related to proliferation of chondrocytes in endochondral ossification process.

P42 S

BONE MORPHOGENETIC PROTEIN-2 RESTORES MINERALIZATION IN GLUCOCORTICOID-INHIBITED MC3T3-E1 OSTEOBLAST CULTURES

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Pharmacological glucocorticoids (GC) inhibit osteoblast function and induce osteoporosis. Bone morphogenetic proteins (BMPs) stimulate osteoblast differentiation and bone formation. In MC3T3-E1 osteoblasts, 1 μM dexamethasone (DEX) strongly inhibited differentiation-related cell cycle, nodule formation, osteocalcin and BMP-2 gene expression as well as mineralization. Replenishment of GC-inhibited cultures with 10 or 100 ng/ml recombinant human BMP-2 (rhBMP-2) dramatically rescued mineral deposition. The rhBMP-2-rescued mineral was bone-like apatite nearly identical to the mineral of control cultures, as determined by Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction. The rhBMP-2 rescue was associated with increased mRNA levels for a1(I) collagen, osteocalcin and Cbfa1 types I and II, as well as alkaline phosphatase (ALP) activity. However, rhBMP-2 did not rescue the GC-inhibited differentiation-related cell cycle, nodule formation or collagen accumulation. When administered alone, rhBMP-2 also increased the mRNA levels for a1(I) collagen, osteocalcin and Cbfa1 types I and II, as well as ALP activity. However, treatment with rhBMP-2 alone inhibited cell cycle progression, nodule formation and extracellular collagen accumulation. Surprisingly, in contrast to its rescue of mineralization in DEX-treated cultures, rhBMP-2 inhibited mineralization in the absence of DEX. In parallel to its bi-modal effect on mineralization, rhBMP-2 stimulated endogenous BMP-2 mRNA in the presence of DEX, but inhibited endogenous BMP-2 mRNA in the absence of DEX. We conclude that suppression of BMP-2 gene expression plays a pivotal role in GC-inhibition of osteoblast differentiation; and, rhBMP-2 exerts both positive and negative effects on osteoblasts that may depend on the differentiation stage and/or the existing BMP signaling.

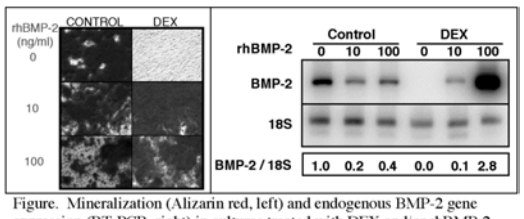

Figure. Mineralization (Alizarin red, left) and endogenous BMP-2 gene expression (RT-PCR, right) in cultures treated with DEX and/or rhBMP-2.

Abstracts

P43 W

THE EXTRACELLULAR MATRIX 1(ECM1) GENE IS ESSENTIAL FOR MOUSE EMBRYOGENESIS

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The Extracellular matrix gene 1 encodes for a glycoprotein of 559 amino acid residues with a typical cystein CC-(X7-10)-C distribution. This cystein arrangement forms 'double loop' domains similar to those of the serum albumin protein family (1). During embryogenesis Ecm1 mRNA was detected in close association with specific sets of developing blood vessels at different stages, particularly during midgestation. Its distribution there is very similar to that of flk-1. However,unlike flk-1, Ecm1 mRNA expression was downregulated before birth.

The biological function of Ecm1 encoded protein(s) is still unknown. A role for Ecm1 in processes such as epidermal differentiation (2), angiogenesis (3) and endochondral bone development (4) has been suggested. Recently, ECM1 was linked to the rare human autosomal recessive disorder Lipoid proteinosis (5). To understand the function of Ecm1, we used homologous recombination in mouse embryonic stem cells to produce Ecm1 null mice by deleting the first 2 exons of the mouse Ecm1 gene thus deleting the start of transcription and of translation. Mice heterozygous for the Ecm1 null mutation (Ecm1 +/-) are fertile and grossly indistinguishable from wild type. Mice homozygous for the Ecm1 null mutation (Ecm1 -/-) are not viable and the mutants die around gastrulation. Analysis of the embryo's of heterozygous matings revealed that the embryonic lethality occurs between 4.5 dpc and 6.5 dpc. Further characterization is currently being performed. The phenotype demonstrates a crucial role for Ecm1 in the early stages of embryogenesis that cannot be compensated for at that time.

In order to assess the putative role of Ecm1 during endochondral bone formation transgenic mice overexpressing Ecm1 in cartilage by the chondrocyte-specific cis-element of the alfa1(II) procollagen gene have been generated. The impact of Ecm1 on terminal chondrocyte differentiation and growth plate tissues is currently under investigation in these animals.

P44 F

THE LEVELS OF VITAMIN K1 AND K2 ANALOGUES IN HUMAN AND RAT BONE

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To investigate the physiological and pathophysiological roles of vitamin K1 and K2 (MK-n) analogues in the bone metabolism, especially in the osteoporosis, osteoarthritis and osteopenia, we investigated the levels of vitamin K1 and K2 (MK-n) analogues in human (osteoporosis and osteoarthritis) and rat (osteopenia) bone samples using an originally developed HPLC-electrochemical detection (HPLC-ECD) method. We determined the contents of vitamin K1 and K2 (MK-n) analogues in the bone obtained from the patients with osteoporosis or osteoarthritis. High levels of vitamin K1 and MK-4 were found in both cortical and trabecular bone of patients with osteoarthritis. While, the levels of all vitamin K analogues in trabecular bone taken from tibia of the osteoporotic patients were lower. We also investigated the levels of vitamin K1 and K2 (MK-n) analogues in the femur (head, diaphysis, metaphysis and distal epiphysis) of streptozotocin-treated diabetic rat. Vitamin K1 and K2 (MK-4, MK-5 and MK-6) were found in each femoral parts (head, diaphysis, metaphysis and distal epiphysis). The levels of vitamin K1, MK-4, MK-5 and MK-6 in the femoral head of streptozotocin-treated diabetic rats were higher than in normal rats. While, the levels of vitamin K1, MK-4, MK-5 and MK-6 in the femoral epiphysis of streptozotocin-treated diabetic rats were significantly lower than in controls. These changes may be suggested that vitamin K1 and K2 analogues plays a different role in each bone parts.

P45 S

ESTABLISHMENT OF NON-UNION MODEL OF RABBIT TIBIA WITH EXTERNAL FIXATOR

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Although modern orthopaedic treatment is advanced greatly, the rate of delayed union or non-union of all fractures remains about 10%.

To study the biology of non-union, we compare two methods of development of non-union model of rabbit tibia. 6 adult New Zealand White rabbits were divided into two groups: 1) salistic tubing group, after open osteotomy and insertion of external fixator of tibia, 5mm long of bone fragment was removed from mid-shaft and put the salistic rubber tubing to connect both ends of fracture site. Periosteum and bone marrow was removed . The rubber tubing then was removed 6 weeks post-operation. 2). For the 3mm-gap group, after open osteotomy, 3mm long of bone fragment was removed from mid shaft. The periosteum from the pins to pins was removed. All the bone marrow was removed. Serial X-ray radiographs were taken for radiographical absorptiometrical analysis. And the rabbits were held by 12 weeks post-operation.

Both methods showed the fracture site was not united at 12 weeks and the end of both fracture segment was sclerotic. Although the area of radiotranslucent region of salistic tube was more than 3mm gap at 12-Week post-operation. During development of non-union, periosteal callus also was formed from proximal and distal fragments and tended to approach each other.

In salistic tube group, the radiotranslucent region was large. The large periosteal callus formed outside the salistic tube. After removal tube, callus continued to grow. Finally the callus was irregular in shape. In 3mm gap, the periosteal callus started to grow from two fragments and approached each other. But at 12 Week, clear the radio-translucent region in rectangular shape was found.

For radiographical absorptiometry, the % change of BMD of the fracture gap increased gradually up to maximum (80%) on week 8 and then leveled off for further 12 week post-operation in 3mm gap method. However % change of BMD increased slowly but has tendency to increase in salistic tube method.

Both methods developed un-united fracture gap. The 3mm gap was more resemble to clinical situation and could be act as animal model for further intervention treatment.

P46 W

THE EFFECTS OF ALPHA-KETOGLUTARATE (AKG) ON THE BONE MINERALISATION, GEOMETRICAL AND MECHANICAL PROPERTIES AFTER ITS EXPERIMENTAL FRACTURE AND DENERVATION

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The propose of this study was to investigated the effects of AKG administration on the bone development, mineralisation and its repair processes. Another reason was to support hypothesis about digestive system and skeletal system axis.

Materials and methods: All experiment was performed on turkeys, divided into 4 main groups (A, B, C, D). Turkeys from the main groups were divided into 2 subgroups, the first one with AKG and the second one with physiological saline (PhS) administration. Group A contained turkeys with the experimental fracture and denervation of the ulna, group B with fractured ulna, in group C were turkeys with denervated ulna. Shame operated turkeys were in group D. All surgical procedure was performed at the age of 21 days. After 14 weeks of experimental lasting (AKG and PhS daily administration in the dosage of 0,4 g/kg b.w.) the ulnae bones were isolated to calculate geometrical properties, like cross sectional area (A), second moment of inertia (Ix) and mean relative wall thickness (MRWT). The maximal elastic strength (Wy) and ultimate strength (Wf) of the ulna was determined too. Bone mineral density (BMD) was measured by DEXA method. The heterogeneity of the ulna was estimated by scanning electron microscopy. The trabecular bone volume was calculated by confocal microscopy technique. Using ELISA the osteocalcin and CTX was also assayed.

Results: The geometrical and mechanical properties of the ulna reached higher values after AKG administration, if compared to PhS subgroup. BMD, calcium percentage content and Ca:P ratio increased also in AKG subgroup. The higher percentage trabecular bone volume was obtained in the AKG subgroup too. Similar changes was observed considering osteocalcin and CTX.

Conclusion: It was proved the positive effects of AKG on the bone formation, mineralisation, geometrical and mechanical properties. It suggest possibility of existence of the digestive system-skeletal system axis mediated by exogenous, enteral AKG. Elaborated method of the ulna fracture and denervation in the turkey might serve as model for further experiments.

This investigation was sponsored by Gramineer Int AB, Lund, Sweden

P47 F

USING OF CERAMIC HYDROXYAPATITE FOR BONE DEFECTS FILLING

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Introduction: The research of the materials for bone defects filling has a long history. In modern orthopaedics hydroxyapatite and tricalciumphosphate based materials are used most often for bone tissue substitution. This report informs about the utilisation of Ukrainian domestic material: ceramic hydroxyapatite (CERHAP).

Materials and Methods: CERHAP is a completely similar with the natural bone tissue. It features absolute biocompatibility and osteogenesis stimulation. CERHAP is used in a form of powder, granules, dense and porous ceramics. During February 1999 to November 2002 there were 53 patients aged from 4 to 18 years (mean 13,4 years). They underwent surgery with CERHAP. Most of the patients had benign bone tumours and tumour-like diseases, one - malignant giant cell tumour, one patient had chronic osteomyelitis.

Results: Follow-up results of one month to 44 months showed good effect and bone shape recover in all patients. No complications were noted.

Conclusions: Our positive results of CERHAP utilisation confirm its usage efficacy as a plastic material for bone defects filling.

P48 S

LOW INTENSITY PULSED ULTRASOUND ENHANCES BONE MINERALIZATION DURING DISTRACTION IN CALLOTASIS

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Distraction osteogenesis is known as bone regeneration by a rhythmic and progressive axial lengthening of osseous tissue. However, the period of bone consolidation takes very long duration. Low intensity pulsed ultrasound was reported to enhance fracture healing and consolidation stage of distraction osteogenesis. The effect of low intensity pulsed ultrasound on the bone mineralisation of distraction gap pre-consolidation (latent and distraction) stage in rabbit tibial model was studied.

After open osteotomy of tibia of adult New Zealand female white rabbits had been performed, an external fixator was applied on medial side. Distraction started at the 7th day after osteotomy with 1mm, continued for 1 week to obtain 6mm lengthening. Low intensity pulsed ultrasound was applied on anterior side. The rabbits were divided into three groups: 1) ultrasound applied on distraction stage (group US-D), 2) ultrasound applied latent and distraction stage (group US-LD) and 3) sham group. The X-ray radiography was taken 14 day post operation before euthanasia. The areal BMC was measured by DEXA and volumetric BMD and BMC were measured by pQCT.

In the X-ray radiography, the more radio-opaque region was found on both ultrasound treated group as compared with sham group. The radio-opaque region was the mineralization of periosteum.

For DEXA result, the areal BMC of group US-D was more than the sham group. Both ultrasound groups were more or less the same.

For pQCT result, the volumetric BMC in distraction gap of group US-D (0.0547g) was greater than the sham group (0.0249g) while the BMC of group US-LD (0.0343g) was slightly greater than sham group. For volumetric BMD, both group US-D and group US-LD were greater than sham group by 70.3% and 28.0% respectively.

Low intensity pulsed ultrasound induced periosteum so as to enhance bone mineralization in X-ray radiography. Thus bone mineral acquisition of both treatment groups was higher than sham group. Moreover, LIPUS also increased the BMD of distraction gap of both treatment groups. The BMD of US-LD group was less than that of US-D. The result showed that application of LIPUS on latent period did not enhance but reduced the bone acquisition.

Low intensity pulsed ultrasound augmented bone acquisition during distraction stage of distraction osteogenesis. The application of ultrasound was suggested to start after latent period.

P49 W

LASER IRRADIATION EFFECTS ON HISTOMORPHOMETRICAL PARAMETERS AND BONE MATRIX ORGANIZATION DURING TIBIA WOUND HEALING IN RATS

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The influence of daily energy doses of 0.03, 0.3 and 0.9 J of He-Ne laser irradiation on the repair of surgically produced tibia damage was investigated in Wistar rats. Laser treatment was initiated 24 h after the trauma and continued daily for 7 or 14 days in two groups of nine rats (n = 3 per laser dose and period). Two control groups (n = 9 each) with injured tibias were used. The course of healing was monitored using morphometrical analysis of the trabecular area. The organization of collagen fibers in the bone matrix and the histology of the tissue were evaluated using Picrosirius-polarization method and Masson's trichrome. After 7 days, there was a significant increase in the area of neoformed trabeculae in tibias irradiated with 0.3 J and 0.9 J compared to the controls. At a daily dose of 0.9 J the 7-day group showed a significant increase in trabecular bone growth compared to14-day group. However, the laser irradiation at the daily dose of 0.3 J produced no significant decrease in the trabecular area of the 14-day group compared to the 7-day group, but there was significant increase in the trabecular area of the 15-day controls compared to the 8-day controls. Irradiation increased the number of hypertrophic osteoclasts compared to non-irradiated injured tibias (controls) on days 8 and 15. The Picrosirius-polarization method revealed bands of parallel collagen fibers (parallel-fibered bone) at the repair site of 14-day-irradiated tibias, regardless of the dose. This organization improved when compared to 7-day-irradiated tibias and control tibias. These results show that low-laser level therapy stimulated the growth of the trabecular area and the concomitant invasion of osteoclasts during the first week, and hastened the organization of matrix collagen (parallel alignment of the fibers) in a second phase not seen in control, non-irradiated tibias at the same period. The active osteoclasts that invaded the regenerating site were probably responsible for the decrease in trabecular area by the fourteenth day of irradiation.

P50 F

EFFECTS OF PENTADECAPEPTIDE BPC-157 ON RAT CALVARIAL BICORTICAL DEFECTS

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Gastric pentadecapeptide BPC-157 (currently in clinical phase II for inflammatory bowel disease) was tested in 7 mm diameter surgically created defects of rat calvaria. Insufficient or absence of bone healing is a frequent problem within all surgical fields. This often necessitates treatment by various bone grafting procedures, utilization of osteopromotive membrane techniques, or local delivery of growth-stimulatory factors. Based on the previously recognized positive osteogenic results of gastric pentadecapeptide BPC-157 on non-union fracture, segmental osteoperiosteal bone defect, its pure peptidergic effect with no carrier interference, together with gastric epithelial cells induced osteogenesis, and a hypothetical gastric hormone opposing bone disturbances development (Bone 24 195, 1999), the aims of the present study were to further develop a possibility of osteopromotion by various routes. Rats received agents locally, BPC 157 2 microg, 2 ng/ml or saline, 1 ml bath, at the injury or intraperitoneally once daily BPC 157 10 microg, 10 ng or 10 pg/kg or saline 5.0 ml/kg , first application immediately following injury, last 24 h before sacrifice (3, 7, 14, 21 days post-injury). Both microscopical and densitometry investigation reveal gastric pentadecapeptide BPC-157 improvement of healing of rat calvarial bicortical defect at all of the tested intervals ($p<0.01$). In conclusion, these results indicate along with our previous result in rabbit pseudoarthoris this suitably stable gastric pentadecapeptide BPC-157 given either system or by local application may be beneficial also calvarial defect healing.

P51 S

EFFECTS OF FIBROBLAST GROWTH FACTOR-2 ON THE METAPHYSEAL FRACTURE REPAIR IN RABBIT TIBIAE

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Fibroblast growth factor-2 (FGF-2) has been found to have stimulatory effects on fracture repair at diaphysis, while its effect on the metaphyseal fracture repair, where spongiosal bone is dominant, has not been studied. This study was conducted to

investigate the effect of FGF-2 on the metaphyseal fracture healing in a rabbit proximal tibial metaphyseal model. The proximal tibial metaphysis of 6-month old Japanese white rabbit was osteotomized bilaterally. 250microg of FGF-2 mixed with gelatin hydrogel and gelatin hydrogel alone used as the control were injected to each osteotomy site of the rabbit proximal tibiae, and the osteotomies were fixed with staples. One and two weeks after surgery, osteoid area in the repairing spongiosal bone at the fracture site was significantly larger in the FGF-2 group than in the control group ($p<0.05$). Using immunohistochemistry, the proliferating cell nuclear antigen had a tendency of larger positive cell number in the FGF-2 group. After four and eight weeks, bone mineral density and the cancellous bone area in the healing region of the fracture site were significantly larger in the FGF-2 group ($p<0.05$). These data suggest that local application of FGF-2 may have accelerating effect on the repair of the metaphyseal fracture.

P52 W

DEVELOPMENT OF A STANDARDIZED CLOSED FRACTURE INSTRUMENT IN THE RAT TIBIA

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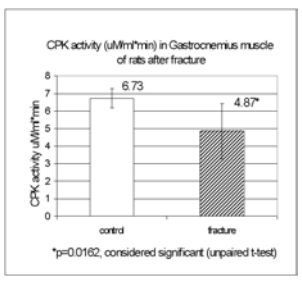
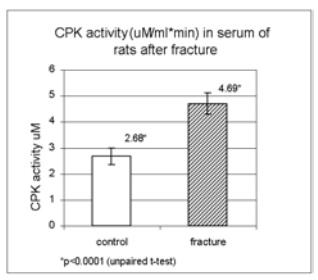
We describe a device that produces a closed fracture with reproducible location and structure. Fractures were produced in tibiae of Wistar female rats by a pendulum-like instrument applying an external blow directly to the tibia. The mass of the blow and angulation, thus the velocity can be controlled and modulated. Unlike generally used instruments, the uniqueness of our device is in taking advantage of the elasticity and plasticity nature of the long bone, which is held by two rotating holders.

Clinically, minimal soft tissue damage was observed around the fracture site. Radiographically this method resulted in a highly reproducible bicortical transverse fracture. Fracture bridging by hard callus was noticed by five weeks post fracture. In order to asses the soft tissue damage, CPK activity in the serum and in the posterior compartment muscles of the calf were analyzed and compared to control groups. The results demonstrated reproducibility of these parameters.

We suggest that our device is very simple to build and to operate and can be modulated and employed for different laboratory animals.





P53 F

CITED-2-MEDIATED REGULATION OF MATRIX METALLOPROTEINASES UNDER SHEAR STRESS IN CHONDROCYTES

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CITED (CBP/p300-Interacting Transactivator with ED-rich tail)-2 is a founding member of a new family of transcriptional activators inducible by cytokines, serum, lipopolysaccharide, hypoxia, etc. Our recent studies revealed that CITED-2 was responsive to mechanical stimuli with its highest mRNA level under shear stress at 5 dyn/cm^2. It is well known that mechanical stimuli at appropriate intensity are essential for growth and maintenance of bone and articular cartilage, and our previous studies indicated that gentle mechanical shear at 5 dyn/cm^2 reduced expressions and activities of many matrix metalloproteinases (MMPs) in synoviocytes and chondrocytes. These observations allowed us to hypothesize that CITED-2 would be a mediator in regulation of MMPs under mechanical stimuli. To test our hypothesis, we transfected the wild type and dominant negative plasmids of CITED-2 to C28/I2 human chondrocytes and examined expression and activity of MMPs. The results showed that overexpression of CITED-2 repressed expression and activity of MMP-1 and MMP-13, and the dominant negative CITED-2 abolished the shear-induced downregulation of MMP-1 and MMP-13. Since interleukin (IL)-1 was known to upregulate expression of MMP-1 and MMP-13, and mechanical shear at < 5 dyn/cm^2 reduced IL-1-stimulated MMP expression, we investigated whether CITED-2 expression would be affected by IL-1. The results indicated that the CITED-2-mediated mechanical responses of MMPs were independent of the signaling of IL-1. Taking together, we provided strong evidence for CITED-2-mediated regulation of MMPs under mechanical stimuli.

P54 S

SHOCK WAVE ACCELERATES FRACTURE HEALING AND BONE STRENGTH ACCOMPANIED WITH INCREASE IN TGF-BETA 1 AND OPG EXPRESSIONS IN AGED OVARIECTOMIZED RATS

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Osteoporotic fractures commonly occur in the elderly and menopause women. Study of physical intervention for accelerating osteoporotic fracture healing is limited. We have previously demonstrated extracorporeal shock wave (ESW) could effectively promote fracture healing associated with increase in TGF-beta1 expression. This study was further elucidated the influence of physical ESW on fracture healing, bone mass, and biomechanical properties in aged ovariectomized rats. Fractured femurs treated with and without 500 impulses of ESW at 0.16 mJ/mm^2 were subjected to determination of bone mineral density (BMD), bone strength, and histological changes in calluses. Results showed that ESW-treated healing femurs displayed 25.3 % increase in BMD, and twofold increase in energy required to break the bone 6 weeks after ESW treatment. Interestingly, ESW-augmented elevation of BMD and bone strength was persistent for 24 weeks. Histomorphometric results demonstrated ESW treatment could accelerate progressively osteogenesis leading to large area of bony tissue in callus. Immunohistomorphmetric finding also implicated ESW treatment could enhance bone formation as determined by osteoblast PCNA expression and reduced bone resorption as demonstrated by osteoclast apotosis/TUNEL expression. Fracture callus received ESW treatment displayed intensive TGF-beta1 expression. Furthermore, there were increases in osteoproteogerin (OPG) expressions of osteoblasts and osteoclasts adjunct to new-developed bone after ESW treatment. These results suggest that physical ESW treatment is an effective non-invasive alternative to enhance healing of osteoporotic fracture and reduce bone loss.

P55 W

SHOCK WAVE THERAPY ENHANCED THE BONE MASS AND BONE STRENGTH AFTER FRACTURES OF THE FEMURS IN RABBITS

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Purpose: To investigate the effect of shock wave therapy on bone mass and bone strength after fracture of the femur in rabbit.

Materials and Methods: This study was performed in 24 New Zealand white rabbits which were randomly divided into three groups. A closed fracture of the right femur was created with three-point bending method. The first group (the control) received no shock wave therapy. The second group received low-energy shock wave therapy, and the third group received high-energy shock wave therapy. Shock wave therapy was applied in one week after fracture. The measurements of bone mass included bone mineral density (BMD), callus formation, ash content and calcium content; and the bone strength tests included peak load, peak stress and modulus of elasticity of the femur.

Results: At 12 and 24 weeks, the BMD values of high-energy shock wave group were significantly higher than the control group, whereas the results of low-energy shock wave group were comparable when compared with the control group. High-energy shock wave group showed significantly more callus formation, higher ash content and calcium content than the control and low-energy shock wave groups. In bone strength tests, high-energy shock wave group showed significantly higher peak load, peak stress and modulus of elasticity than the control and low-energy shock wave groups.

Conclusion: High-energy shock wave therapy produced significantly higher bone mass and better bone strength than the control group after fractures of the femurs in rabbits. The effects of low-energy shock wave therapy were less prevailing with comparable results as compared with the control group. The effect of shock wave therapy on bone mass and bone strength appeared dose-dependent.

P56 F

EXPERIMENTAL EVALUATION OF FRACTURE MECHANICS PARAMETERS OF BONE AT THE BODY TEMPERATURE.

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Three point bending test to failure was done on 18 cortical specimens that had been cut from ten bovine femoral bones which had range in age from 18 to 20 months at the time of massacre. After harvesting the femoral bones 18 specimens with the dimension 56 mm long and 14 mm wide and 6.7 mm thick were machined in accordance with ASTM geometric criteria for single edge notched bend

{SENB}specimens. A 2.7 mm narrow slit were cut into the specimens with the depth of 5.4 mm at the middle point of one side. All cuts ended in a sharp 60 degrees notch. The length dimension of each specimen was in the longitudinal direction of the femoral bone. The specimens were kept moist with saline solution during machining and also stored wet at temperature range in 0°C to 4°C.

24 hours before testing the specimens were placed in saline solution at room temprature. The specimens were tested in a bath of saline solution at 37°C in an Instron testing machine (Model 8503) at a cross head rate of 0.051 cm/min. In each test the displacements were plotted versus loads. K_C for each specimen was calculated from the critical load at the time of failure due to crack propagation and the measured dimensions of the specimen.

The results of 13 successful tests produced a mean critical stress intensity factor of release 7.055 ±SD 0.83 $MNm^{minus3/2}$ and a critical strain energy G_C of 5091 J/m^2 .

P57 S

INCREASED TEMPOROMANDIBULAR JOINT LOADING INDUCES ACTIVATION OF THE JNK/AP-1 SIGNALING CASCADE AND PROMOTES CHONDROBLASTIC DIFFERENTIATION

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Mechanical loading has been long recognized as an important regulatory factor in bone and cartilage homoestasis and a determinant of skeletal morphology. However, the molecular mechanisms that govern the response of chondroblasts to mechanical stimulation remain elusive.

The transcription factor c-Jun together with members of the Fos family proteins (c-Fos, FosB, Fra1/2) are major components of the AP-1 (activator protein-1) transcription complex. Recent in vitro studies have indicated that AP-1 plays an important role in chondrogenic gene regulation as well as chondroblastic differentiation and proliferation. The aim of the present study was to explore the effect of increased temporomandibular joint loading on the proliferation and differentiation of condylar cartilage chondrocytes, in vivo.

To this end, 100 rats were assigned to two groups: the first group was fed soft diet (which simulates normal masticatory movements), while the second group was fed hard diet that causes increased temporomandibular joint loading. Biopsy sections from temporomandibular joint of both groups were obtained at 2, 6, 12, 24, and 48 hours after the experiment initiation. We employed immunohistochemical staining analysis to investigate the in situ expression of c-Fos, in correlation to cellular levels of pc-Jun, the phosphorylated (hence activated) form of c-Jun. Moreover, the expression and activation profile of JNK2 (c-Jun N-terminal kinase 2), the principal kinase targeting c-Jun, was examined.

The articular cartilage of the first group displayed nuclear immunoreactivity for c-Fos, that was gradually increased as the experimental procedure evolved. The expression levels of c-Fos were accompanied by a co-localized enhancement of pc-Jun. Augmented levels of JNK2 and its phosphorylated/activated form, p-JNK, were observed at 24 and 48 hours after experiment initiation. In the second group, immunoexpression of the aforementioned proteins was also increased as the experiment progressed. However, expression levels of the examined proteins were significantly higher in the second, compared to the first group, at 12, 24, and 48 hours after the experiment initiation.

Our results suggest that mechanical loading potentiates the JNK/AP-1 signaling pathway and pose a novel mechanism that might be implicated in the chondroblastic differentiation process.

P58 W

ENHANCEMENT OF ALBUMIN EXPRESSION IN BONE TISSUES WITH HEALING RAT FRACTURE

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Fracture healing is a complex physiological cascade. The mechanism of fracture healing has not been clarified. The characterization of 66 kDa protein molecule, a major protein component which is produced from femoral-diaphyseal tissues with fracture healing [Igarashi A and Yamaguchi M, Int J Mol Med, 9:503-508, 2002], was investigated.

Weanling rats were killed at 7 and 14 days after femoral fracture. When the femoral-diaphyseal tissues with fracture healing were cultured for 48 hours in a serum-free medium, many proteins in the bone tissues were released into the medium. Analysis with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed that a protein molecule of approximately 66 kDa was markedly increased in culture medium with fracture-healing bone tissues. N-terminal sequencing of 66 kDa protein indicated that its N-terminus was identical to that of rat albumin. Western blot analysis of medium 66 kDa protein showed expression of albumin. This expression was significantly enhanced by fracture healing. The production of albumin was seen in the diaphyseal (cortical bone) and metaphyseal (trabecular bone) tissues of rat femur. When the femoral-diaphyseal tissues obtained at 7 days after femoral fracture were cultured in a serum-free medium containing either vehicle, parathyroid hormone, insulin-like growth factor-I or zinc acexamate, medium albumin was significantly increased in the presence of those bone-stimulating factors. The addition of albumin (0.5 or l.0 mg/ml of medium) caused a significant increase in calcium and deoxyribonucleic acid (DNA) contents in the femoral-diaphyseal and -metaphyseal tissues obtained from normal rats in vitro.

The present study demonstrates that fracture healing increases a remarkable of production of albumin which is a major protein component produced from femoral-diaphyseal tissues of rats, and that albumin has an anabolic effect on bone components in vitro.

P59 F

THE REPAIR OF CRANIAL BONE DEFECT BY USING OF ENDOCHONDRAL BONE MATRIX GELATIN (EC BMG) IN RAT

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Introduction: Many investigators have used bone matrix gelatin (BMG) for bone induction intramuscularly and subcutaneously. A literature review revealed only a few studies about the process and type of ossification by BMG in skull bone defects.

Aims of investigation: Evaluation of Ec BMG effects on repair of cranial bone defects.

Materials and Methods: Ec BMG was prepared as previously described by Urist. The defects were produced with 5-mm diameter in parietal bones and filled by BMG particles. No BMG was used in control groups. For evaluation of new bone formation and repair, the specimens were harvested on days 7, 14, 21 and 28 after operation. The samples were processed histologically, stained by H&E, Alizarin red s staining and Alcian blue and studied by a light microscopy.

Results: In control groups: Twenty-eight days after operation a narrow rim of new bone was detectable attached to the edge of defect. In BMG groups: At day 7th after operation young chondroblast cells appeared in whole area of defect. At day 14th after operation hypertrophic chondrocytes showed by Alcian blue staining and calcified cartilage were detectable by Alizarin red s staining. The numerous trabeculae spicules, early adult osteocyte and highly proliferated red bone marrow well developed on day 21. Finally, typical bone trabeculae with regulated osteoblast cells and some osteoclast cells were detectable at day 28 after operation.

Discussion: Ec BMG could stimulate bone induction in cranial bone defects by the way of endochondral ossification. Although, some of researchers haven't believed this idea.

Conclusion: According to results of this research, Ec BMG could be a good biomaterial substance for new bone induction in bone defects.

P60 S

POSSIBLE INVOLVEMENT OF PTHRP AND MECHANICAL STRESS IN CELL-TO-MATRIX INTERACTION OF CHONDROCYTES

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Chondrocyte proliferation and differentiation appears to be controlled by cell-to-matrix interaction as well as systemic and local growth factors. Mechanical stress including hypergravity and microgravity may affect the ultrastructure at the site of cell-to-matrix contacts of chondrocytes, therefore, we have examined the morphological changes of epiphyseal chondrocytes under similar conditions to hypergravity and microgravity. Mouse tibial epiphyseal cartilage of 17-day-old fetuse was cultured in continuous centrifuge (5 G) or in rotation at 15 rpm for 16 hours, simulating the condition of hypergravity and microgravity, respectively. Stationary culture of the epiphyseal cartilage was employed for the control experiment. Centrifugal force and rotation for 16 hours had no effect on the size of the epiphyseal cartilage. Although actin filaments were localized evenly along the cell membrane of proliferative chondrocytes in the control cartilage, chondrocytes affected by centrifugal force developed a thick layer of actin filaments and well-formed focal contacts. Alternatively, in rotational culture, chondrocytes with a flattened cell shape and ruffling of the cytoplasmic processes revealed less-developed focal contacts and uneven distribution of actin filaments along the cell membrane, thereby, indicating less cell-to-matrix association. In the hypertrophic zone of the rotational culture, non-hypertrophic cells were found interspersed amongst hypertrophic cells, which were similar to abnormalities seen in PTHrP-deficient mice in our previous reports. Under an electron microscope, non-hypertrophic chondrocytes in the PTHrP deficient mice displayed less-developed focal contacts underlain with attenuate actin filaments. Instead, they were often attached to each other, forming cell nests. The chondrocytic cell line, CFK2, transfected with PTHrP cDNA showed partial co-localization of actin filaments and this molecule, especially in the region close to the cell membrane. Therefore, non-secretory PTHrP may have an affinity to the site of cell-to-matrix interaction associated with actin filaments. Thus, non-hypertrophic chondrocytes in

the hypertrophic zone in the PTHrP deficient mice and mice with rotational culture consistently showed poor cell-to-matrix interaction. We concluded that PTHrP may participate in the cell-to-matrix interaction of chondrocytes.

P61 W

ROLE OF SMAD6 DURING CARTILAGE FORMATION

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Bone Morphogenetic Proteins (BMPs) are known to play an important role during cartilage development. We previously showed that BMPs are essential molecules accelarating cartilage formation as well as chondrocyte differentiation through analysis of transgenic mice expressing BMPs and Noggin, an antagonist for BMPs. Smad1 and Smad5 are intracellular transducers of BMP signals and Smad6 is an inhibitory Smad preventing them from activation. Various BMPs exist in cartilage, probably exerting their effects through complex pathways which involve Smads and other molecules. To clarify a role of Smad6 during cartilage formation, transgenic mice expressing Smad6 in cartilage were generated. To obtain cartilage-specific expression, we used promoter / enhancer sequences from the a2(XI) collagen gene. No overt abnormalities were detected in Smad6 transgenic mice at birth. Then, the mice gradually showed obvious chondrodysplasia-like phenotype with dwarfism and short snout. Close examination of F0 transgenic embryo revealed limited abnormalities in chondrocyte differentiation. The severity of skeletal abnormalities seen in Smad6 transgenic mice was much lower than those in Noggin transgenic mice previously reported, althouhg both transgenic mice bear identical promoter / enhancer sequences. Expressin analysis showed that amounts of transgene mRNA was 3-700 times as large as those of endogenous Smad6 mRNA. These results suggest existence of mechanism which Smad6 alone could not inhibit completely during the transduction of BMP signals.

P62 F

EFFECT OF GASTRIC ADMINISTRATION OF LECTIN ISOLATED FROM RED KIDNEY BEAN (PHASEOLUS VULGARIS) ON GEOMETRIC AND MECHANICAL PARAMETERS OF FEMUR AND HUMERUS DURING THE POSTNATAL LIFE IN THE PIG

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Aims: The aim of the experiment was to determine the effects of intragastric lectin administration on the development of the structural and mechanical parameters of the skeletal system analysed on the model of humerus and femur during the postnatal life in the pig.

Materials and methods: Experiment was carried out on 36 piglets of the Polish Large White breed divided to one control and five experimental groups. The piglets from the control group were administrated physiological saline on the 11th day of life in the dose of 2 ml/kg b.w. The piglets from the experimental groups were administrated lectin intragastrically in the dose of 100 mg/kg b.w. on the 7th , 11th, 15th, 21st and 28th day of postnatal life. All the piglets were weaned at 35th day of life and fed on food without antibiotics and zinc oxide (only Lutamix BASF as Premix was added). At 38th day of life the piglets were sacrificed and then humera and femora were isolated for three-point bending test estimation using INSTRON 4302 apparatus.

Results: Ultimate strength of the humera and femora was the highest in the piglets that were administrated lectin on 7th day of life and amounted 1346,1 N and 1512,5 N respectively, and in piglets that received lectin on the 21st day of life (1378.4 N and 1483,6 N respectively) is comparison with values from control piglets (621,6 N 1180.33 N respectively). The highest cross sectional area of the humerus was found in the piglets which were administrated lectin in 21st day of life (78.6 mm2) and at 28th day of life (74.33 mm2) in comparison with control value (56,06 mm2).

Conclusion: Intragastric administration of lectin on the 7th and 21st day of postnatal life in piglets effected the highest mechanical and geometrical parameters in the humerus while in femur the highest values of these parameters were observed when lectin was administrated at 15th and 21st day of life. Lectin from Phaseolus vulgaris during the first weeks of postnatal life effects positive mechanical and geometric structure of the skeletal system analyzed of femur and humerus in the piglets.

Diseases of Bone

P63 S

ENHANCEMENT OF BONE FORMATION BY DOUBLE GENE TRANSFER OF BMP4 AND SONIC HEDGEHOG

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Introduction: We reported that electroporatic gene transfer of BMP4 could induce ectopic bone formation in vivo [Bone 31(2) Aug 2002:340-347]. The mass of induced bone in muscle of mice was tiny. For the clinical application of this method, enhancement of bone formation must be achieved. Co-expression of other factors could be a major candidate for enhancement. We employed gene transfer of Sonic hedgehog (Shh). Previous work indicated that Shh signal from floor plate and notochord promoted chondrogenesis of the somatic mesoderm in vertebrate development. Shh also increased the commitment of mesenchymal cells into osteoblastic lineage in vitro. In this study we tested the enhancement of bone formation in co-expression of Shh and BMP4.

Methods: full length of mouse BMP4 cDNA and N-terminal Shh were inserted into the cloning site of expression vector CAGGS and MiwII (CAGGS-BMP4, Miw-Shh), respectively. LacZ containing vector MiwZ was also used. One hundred micrograms of each plasmid was intramuscularly injected with insulin syringe, and in vivo electroporation was performed with 6 electric pulses (100V, 50ms) via paired needle electrodes inserted in the gastrocunemius muscle. In Shh-BMP group, CAGGS-BMP4 was electroporated 7days after Miw-Shh electroporation. In LacZ-BMP group, CAGGS-BMP4 was electroporated 7 days after MiwZ electroporation. Mice of these groups were sacrificed 14 days after CAGGS-BMP4 electroporation. Osteoinductivity of Shh was checked after 14 days after single electroporation of Miw-Shh. Bone formation was evaluated by the area of ossification on the soft X-ray. The differences in the area of ossification were statistically evaluated using the Mann-Whitney's U-test.

Results: Single electroporation of Miw-Shh did not show osteoinductivity. The area of induced bone in Shh-BMP group was significantly larger than in LacZ-BMP group (3.58 ± 2.52 vs 0.86 ± 0.59 mm^2, $p=0.05$).

Conclusion: Bone formation by electroporatic transfer of BMP4 gene was enhanced by co-transfer with Shh.

P64 W

TRANSPLANTATION OF OSTEOBLASTIC CELLS ENHANCED FUNCTIONALLY BY ADENOVIRAL BMP-2 IS EFFICIENT FOR REPAIRING CRITICAL-SIZED BONE DEFECTS

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It is important to trace the fate of the cells transplanted in bone regeneration study, many kinds of treatments are adopted. We employed cell transplantation systems using GFP (Green fluorescent protein)-expressing immortalized cells, which were isolated from GFP transgenic mice (provided by Dr. Masaru Okabe, Osaka University). Osteoblastic cells were isolated from calvariae of newborn GFP transgenic mice. These cells were passaged until 26th, then two kinds of single cell-derived clonal cell lines (GFP-C1, GFP-C3) were established. Characterization of these cell lines was investigated in vitro. In addition, we transplanted C3 cells into back subfascia or critical sized bone defects on calvaria in wild type mice (C57 Black6) carried by PGS (poly-D,L-lactic-co-glycolic acid/gelatin sponge), after treatment with rhBMP-2 or infection with adenovirus vectors encoding Cbfa1(AdCbfa1) or BMP-2(AdBMP-2). Also we created bone defects on diaphysis of femur in wild type mice (C57 Black6) without any treatment, and monitored the fate of these transplanted cells by detecting GFP fluorescence. Incubation of these cells with rhBMP-2 in culture increased alkaline phosphatase activity with a concomitant expression of mRNAs for osteocalcin and osterix by a dose- and time- dependent manner, though the expression levels of these markers in the cells without rhBMP-2 were under detectable levels. These stimulatory effects of rhBMP-2 on osteoblast phenotypes were more prominent in GFP-C3 than GFP-C1 cells, so we employed C3 cells for following studies in vivo. In vivo experiments demonstrated that many rhBMP-2-induced GFP-positive cells surrounded mineralized bones and some of them were embedded into mineralized bone matrices which also retained alkaline phosphatase activity, indicating that the transplanted cells differentiated into osteoblasts in vivo. Furthermore, when AdBMP-2-infected cells were transplanted into critical-sized bone defects on calvaria, these cells induced newly bone formation from 2weeks later, and kept their new bone tissue 4 weeks later, whereas scarcely any C3 cells without any treatment or infected with AdCbfa1 could differentiate into even

osteoblasts. These results indicate that our cell lines are capable of differentiating into bone forming osteoblasts and terminally differentiated osteocytes in vivo without exclusion from the regenerative process in the recipient mice. Thus, transplantation of osteoblastic cell infected with AdBMP-2 is efficient for bone regeneration.

P65 F

PHOSPHATE STARVATION ENHANCES BONE MORPHOGENETIC PROTEIN GENE EXPRESSION IN A CULTURED MOUSE MARROW STROMAL CELL LINE ST2

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Alkaline phosphatases (ALPs) are glycosylated, membrane-bound enzymes that hydrolyze various monophosphate esters at an optimum high pH, and are present in nearly all living organisms. In Escherichia coli, extracellular phosphate (Pi) limitation induces the ALP gene, indicating a role of extracellular Pi in ALP gene regulation. However, little is known about similar mechanisms in mammalian cells. Previously, we reported that Pi starvation increased the tissue-nonspecific ALP (TNSALP) activity and regulated its expression in the mouse stromal cell line ST2, derived from mouse bone marrow. In the present study, we further examined the effects of Pi starvation on the mechanism of TNSALP induction. The specific activity of TNSALP increased markedly after treatment by Pi starvation for 5 days, and RT-PCR analysis revealed that the mRNA of the bone morphogenetic protein-4 (BMP-4) gene was highly stimulated. The combination of Pi depletion and mouse BMP-4 receptor IA/Fc chimera down-regulated the TNSALP activity. These results indicated that Pi depletion stimulates the TNSALP activity for the Pi supplementation, and that this system may involve the signaling pathway of the BMP-4 gene at the transcription level.

P66 S

IDENTIFICATION OF EARLY RESPONSE GENES TO BONE MORPHOGENETIC PROTEIN AND PARATHYROID HORMONE USING A CDNA MICROARRAY TECHNIQUE

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Bone formation *in vivo* is a highly complicated process regulated by numerous factors, such as cytokines, growth factors, hormones, extracellular matrix and adhesion molecules. Therefore, our knowledge about this process is very limited, although we have accumulated considerable findings using simple model systems. In order to approach the bone formation process, we screened early response genes to bone morphogenetic protein (BMP)-2 and parathyroid hormone (PTH), which are potent stimulators of bone formation, using a cDNA microarray technique, SupperArray Mouse Signal Transduction Pathway Finder.

We treated mouse osteoblastic MC3T3-E1 cells with 100 ng/ml BMP-2, and 10 nM PTH for 0.5 and 3 hours and collected RNA. Among 96 genes screened, MC3T3-E1 cells constitutively expressed BMP-4, cyclin-dependent kinase inhibitor p57Kip2, cathepsin D, epidermal growth factor receptor, engrailed1, fibronectin, NFkappaB1, Stra8, VCAM-1, ELAM-1, small inducible cytokine A2 (Scya2), MMP7, and IGFBP3.

BMP-2 induced only early growth response (egr) -1 after 0.5 hours. PTH induced Scya2 after 0.5 and 3 hours, egr-1 after 0.5 and 3 hours, ornithine decarboxylase after 3 hours, cyclin D1 weakly after 3 hours. Inductions of ornithine decarboxylase and cyclin D1 by PTH have been already known.

The expression of the zinc finger transcription factor egr-1 was induced by both BMP-2 and PTH. Although physiological role of egr-1 in osteoblasts is unknown, it is highly expressed in bone and cartilage during embryogenesis, and is, thus far, implicated in the response of osteoblasts to mechanical stress, EGF, FGF-2, prostaglandins, and TGF-beta. Therefore, our results suggest that egr-1 could play an essential role in osteoblasts.

Scya2, or monocyte chemotactic protein-1, has been shown to play a role in the recruitment of monocytes, memory T lymphocytes, and natural killer cells to sites of injury and infection. Our result implies a novel role of Scya2 in PTH action on osteoblasts.

Out of only 96 genes studied, we could have identified at least 2 genes whose functions in osteoblasts might be essential but not well understood, indicating the usefulness of this method to unravel the complicated process of bone formation.

P67 W

EFFECT OF BMPR-IA/FC CHIMERA ON TOOTH MORPHOGENESIS

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Epithelial-mesenchymal interaction regulates tooth development. Bone morphogenetic proteins (BMPs), especially BMP4 are thought to be important molecules for the interaction. However, few studies have been assessed the role of BMP4 on tooth development in vivo, because the knocked-out mice of the Bmp4 gene are lethal at early embryonic stages.

In this study, to clarify the role of BMPs on murine tooth development, we assessed the expression of BMPs, and their type I receptor in each stages of tooth development. BMP2 and BMP4 were expressed in all stages of tooth development, but only the Bmpr1a of type I receptors was expressed in all stages. BMPR-IA/Fc chimera protein binds BMP4 with high-affinity, and is a potent BMP4 antagonist in vitro. Therefore, we inhibited all of signaling via BMPs by addition of BMPR-IA/Fc in the tooth culture with molars at the bud stage (E13.5) of tooth development. After 2 weeks, molars treated with BMPR-IA/Fc formed smaller cusps than human IgG-treated controls, while, odontoblasts and ameloblasts normally differentiate, and produce dentine and enamel matrix, respectively.

Next, to assess which stages of tooth development require the BMP signaling, we performed the culture of molars at the bud stage (E13.5) for 2 weeks, and BMPR-IA/Fc were added in either the first one week or last one week. Abnormal cusp formations were observed in both of cultures. Furthermore, to assess the effect of BMPR-IA/Fc on the terminal differentiation of odontoblast, 0.5-day old tooth germs of Dsp-LacZ mice which specifically express LacZ on odontoblasts, are cultured for 1 week. The expression of LacZ was significantly decreased in the incisior treated with BMPR-IA/Fc compared with a control.

These results suggest that the BMP signaling is critical for tooth morphogenesis, especially, the cusp formation rather than the differentiation of odontoblasts and ameloblasts from the bud stage to bell stage. Moreover, the reduction of Dsp gene expression in ododntoblasts, indicates BMPs signaling may be related with the terminal differentiation of odontoblasts.

P68 F

HEPARIN ENHANCES THE BMP ACTIVITY BY PROLONGING THE BMP ACTION THROUGH DIRECT INTERACTION

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Bone morphogenetic proteins (BMPs) induce osteoblast differentiation in mesenchymal cells. Originally, the BMP activity was purified from demineralized bone matrix using heparin-affinity chromatography. BMP activity is regulated by a number of molecules at the outside and the inside of the target cells. In the present study, we examined the effects of heparin on the BMP-induced osteoblast differentiation in C2C12 myoblasts. ALP activity was slightly induced at 100 ng/ml of BMP-2 and increased at more than 300 ng/ml. Heparin enhanced the ALP activity induced by 100 ng/ml of BMP-2 in a dose-dependent manner. However, chemically desulfated heparin-derivatives have lost this stimulatory capacity. Heparin enhanced the ALP activity induced by transient transfecting with a BMP-2-expression vector, but not by with the constitutively active BMP type I receptor (BMPR-IA) and Smad1 without adding BMP-2. We constructed an expression vector carrying FLAG-BMP-2 cDNA, and transfected it into COS7 cells. Treatment with the conditioned media containing FLAG-BMP-2 induced the ALP activity in C2C12 cells, and it was further enhanced by heparin. Surprisingly, affinity cross-link experiments showed that heparin decreased the amounts of FLAG-BMP-2 bound to the cell layer or BMPR-IA-His receptor. The amounts of FLAG-BMP-2 in the culture media were decreased without heparin, but it was kept with heparin. Soluble BMPR-IA-His was precipitated by heparin-agarose only in the presence of FLAG-BMP-2. We examined the effect of heparin on the BMP signaling using a destabilized EGFP reporter construct, which was driven under the control of the Id1 promoter. Treatment with BMP-2 in the absence of heparin induced the EGFP(+) cells within 4 h, but they disappeared at 30 h. Similar number of the EGFP(+) cells was observed in the cultures treated with BMP-2 in combination with heparin at 4 h. Moreover, the number of EGFP(+) cells was kept even at 30 h in the presence of heparin. Taking together, these results suggest that heparin enhances the BMP activity by keeping the BMP action up through direct interaction with the ligands at the outside of cells. The heparin specific structures, including sulfated glycosaminoglycans, would be essential for this stimulatory capacity on the BMP activity.

P69 S

EFFECTS OF EXCESS AND DEFICIENCY OF ENDOGENOUS PTH ON THE VOLUMETRIC BONE MINERAL DENSITY AND BONE GEOMETRY DETERMINED BY PQCT IN FEMALE SUBJECTS

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Peripheral quantitative computed tomography (pQCT) is useful to evaluate volumetric bone mineral density (vBMD) as well as BMD of cortical and trabecular bones separately. Although PTH affects cortical and trabecular bones differently, no reports have been available about the effects of endogenous PTH on vBMD and bone geometry by pQCT. We, therefore, investigated the effects of excess and deficiency of endogenous PTH on bone by employing pQCT in 36 female patients with primary hyperparathyroidism (hyper), 9 female patients with idiopathic or postoperative hypoparathyroidism (hypo), and one 100 normal controls matched to age and gender (cont). Then we compared BMD and area of both lumbar and radius-1/3 by DXA as well as vBMD of trabecular and cortical bones, bone geometry and bone strength index at both 4% and 20% from distal site by pQCT. Lumbar BMD by DXA was higher in order of hypo>hyper=cont, and that of radius-1/3 was significantly higher in order of hypo>cont>hyper. Area of radius-1/3 was significantly higher in hyper, compared to that of cont. As for pQCT, trabecular vBMD was significantly higher in order of hypo>cont>hyper at 4% site. Cortical vBMD was higher in order of hypo>cont>hyper at 20% site. Total bone area as well as endosteal and periosteal circumferences were significantly higher in hyper, compared to those of cont and hypo. Cortical area and thickness were higher in order of hypo>cont>hyper. Bone strength indices such as polar moment of inertia and polar strength strain index were not significantly different. These results were similar with those in postmenopausal female. Single regression analysis of relationships between intact-PTH and various parameters revealed that the differences of vBMD as well as bone geometry among three groups were partly associated with endogenous PTH levels. In conclusion, vBMD evaluation revealed that endogenous PTH excess was catabolic for both cortical and trabecular bones, and that the deficiency of endogenous PTH was anabolic mainly for trabecular bone. Excess of endogenous PTH stimulated the periosteal bone formation which might partly compensate a decrease in bone strength induced by low BMD.

P70 W

ARTERIAL BLOOD PRESSURE, SERUM CALCIUM AND PARATHYROID HORMONE LEVELS IN PRE- AND POSTMENOPAUSAL WOMEN WITH PRIMARY HYPERPARATHYROIDISM

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The purpose of this study was to analyze whether a correlation exists between BP and the main clinical and biochemical parameters in pre- and postmenopausal women with primary HPT.

Patients and Methods: A series of 241 consecutive patients (median age 56 years, range 18-82 years) with confirmed primary HPT was reviewed. There were 84 (34.8%) premenopausal (Group A, median age 45 years, range 18-52 years) and 157 (65.2%) postmenopausal (Group B, median age 62 years, range 47-82 years) women. All patients underwent successful parathyroidectomy and subsequent measurement of the removed parathyroid (PT) glands.

Results: Both systolic (133.6±16.4 vs. 150.1±17.8 mm Hg) and diastolic (82.5±12.0 vs. 91.2±10.7 mm Hg) BP differed significantly (p=0.00001) between Groups A and B, as well the mean serum calcium levels (3.10±0.41 vs. 2.95±0.30 mmol/L, p=0.001). Serum creatinine (79.5±23.7 vs. 83.6±31.9 micromol/L; p=0.30), alkaline phosphatase (162.4±139.7 vs. 156.8±111.7 U/L; p=0.74), and intact PTH levels (191.6±155.7 vs. 174.9±125.2 ng/L; p=0.37) did not differ between groups. There was no relationship between age and serum calcium (Group A: R=0.037, p=0.73; Group B: R=0.087, p=0.28), and between age and serum PTH (Group A: R=0.081, p=0.46; Group B: R=0.073, p=0.36) in each Group. Age significantly correlated with BP, both systolic (Group A: beta=0.606, p=0.000001; Group B: beta=0.43, p=0.000001), and diastolic (Group A: beta=0.65, p=0.00001; Group B: beta=0.31, p=0.00008), while serum creatinine correlated with BP only in premenopausal patients (systolic: beta=0.33, R2=0.11, p=0.002; diastolic: beta=0.23, p=0.04). No correlation was found between systolic BP and both serum calcium (Group A: beta=0.08, p=0.45; Group B: beta=0.11, p=0.17) and PTH (Group A: beta=0.06, p=0.58; Group B: beta=0.01, p=0.89). There was no correlation between diastolic BP and both serum calcium (Group A: beta=0.02, p=0.84; Group B: beta=0.10, p=0.19) and PTH (Group A: beta=0.07, p=0.55; Group B: beta=0.06, p=0.49) in each Group.

Conclusions: Both in pre- and in postmenopausal women with primary HPT, no relationship between BP, serum calcium, and serum PTH levels was found. Although the prevalence of hypertension among such patients could be higher than in the general population, BP should be considered related exclusively to age and serum creatinine levels.

P71 F

QUANTITATIVE ULTRASOUND TECHNOLOGY AND DUAL ENERGY X-RAY DENSITOMETRY IN PATIENTS WITH PRIMARY HYPERPARATHYROIDISM

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Fifty one patients with surgically proven primary hyperparatyroidism (PHPT), 11 males and 40 females, with a mean age ± SD: 55.9±14.1 years, and 58 age- and sex-matched normal subjects were studied. The femoral and lumbar bone mineral density (BMD), as well as quantitative ultrasonometry (QUS) of the phalanges of both hands were measured in the patients and the controls. Patients with PHPT showed DXA values and QUS parameters significantly lower than those of the controls. In the male patients, BMD values measured on the lumbar spine and femoral regions were similar to those found in male controls, while QUS values were significantly lower. Women with PHPT were further divided into two subgroups: pre-menopause (n=13) and post-menopause (n=32). The pre-menopausal women with PHPT showed significantly lower DXA values than those of the pre-menopausal control women, but similar QUS parameters. The post-menopausal women with PHPT showed both DXA and QUS parameters significantly lower than those found in the post-menopausal controls. The relative risk of osteopenia was significantly increased in PHPT patients at several measurement sites. There was a highly significant correlation between spine and femoral BMD and QUS parameters, while PTH serum levels did not correlate with any of the densitometric variables. In conclusion, QUS parameters would seem to be able to distinguish patients with PHPT from normal controls in male subjects and in post-menopausal women, but not in pre-menopausal women. This would suggest that the higher estrogen levels in pre-menopausal patients might preserve the bone from significant structural changes. This may also suggest that the hyperparathyroidism, in addition to the reduction of bone mineral content, should cause an alteration of bone structure with a further increase in fracture risk in postmenopausal women. Furthermore, the alterations of QUS parameters in patients who do not show significant changes in DXA measurements, may suggest an involvement of bone that is independent of mineral content and may be helpful for selecting patient candidates for surgery, according to NIH criteria.

P72 S

BONE DISEASE IN PRIMARY HYPERPARATHYROIDISM: PATHOGENESIS AND RESPONSE TO TREATMENT

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Unlike the contemporary presentation of primary hyperparathyroidism (PHPT) in the West, bone disease continues to be the dominant clinical feature of this disease in India. In this series of 71 consecutive PHPT patients, 64 had osteitis fibrosa cystica (OFC), 39 presented with fracture/brown tumors and 24 patients were bedridden (parathyroid cripples). Fourteen had palpable parathyroid tumors.

Mean serum calcium was 12.3 mg/dl, and mean total alkaline phosphatase and PTH level was more than 10 times the upper limit of normal (741pg/ml and 1274 IU/L respectively). Mean serum 25 OHD level was 11.3 ng/ml. Mean parathyroid gland weight at surgery was 5.85 gm, with a range from 100 mg to 36.5 gm. There was an inverse relationship between serum 25 OHD level and parathyroid gland weight. When compared to patients from the US (Detroit), the 25 OHD was significantly lower and the parathyroid gland weight, PTH level and prevalence of OFC was significantly higher, suggesting a pivotal role of vitamin D (and perhaps calcium) nutrition in determining presence of these manifestations. The prevalence of parathyroid cancer was also much higher in Indian patients (6%).

Following parathyroidectomy, there was rapid and marked recovery of skeletal features. Bone pains improved within a week, and fractures healed by 3 months. There was a dramatic rise in bone density, seen as early as one week post operatively, with rates of recovery at 1 year being 133.4%, 106.3%, and 21.0% at total hip, lumbar spine and distal forearm respectively. Bone lesions, including some brown tumors filled up rapidly, often becoming hyperdense, but overall contour defects persisted. Bone turnover markers (serum osteocalcin and urinary crosslaps) declined slowly and continued to be elevated in some patients 1 year after surgery.

Our data suggest that a) Indian patients have severe bone disease and larger parathyroid tumours as compared to the west, and this can be related to the low 25 OHD levels (and probably poor calcium intakes) seen in these patients b) there is rapid and dramatic recovery of BMD following parathyroidectomy, although deformities and brown tumors persist.

P73 W

ELEVATED LEVELS OF PTHRP IN PLASMA OF WOMEN WITH UTERINE LEIOMYOMAS

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Uterine leiomyomas (fibroids or myomas), benign tumours of the human uterus, are the single most common indication for hysterectomy. They are clinically apparent in up to 25% of women and cause significant morbidity, including prolonged or heavy menstrual bleeding, pelvic pressure or pain, and, in rare cases, reproductive dysfunction. It has been demonstrated that parathyroid hormone-related protein (PTHrP) acts as a local cell growth modifier in an autocrine/paracrine fashion on the uterus and may have a similar role on uterine leiomyomas. Humoral hypercalcemia of benignancy is a recently described syndrome characterized by hypercalcemia induced by PTHrP produced by a benign tumor. Five cases have been reported, one each caused by ovarian dermoid cysts, intestinal leiomyoma, mammary hyperplasia, pheochromocytoma, and uterine leiomyoma. The purpose of this study was to investigate the level of PTHrP in the plasma of women with uterine leiomyomas (n=36), endometriosis (n=29), adenomyosis (n=25) and controls (n=52). Real-time quantitative RT-PCR was also performed from excised leimyoma tissue from the leiomyoma group. In the leimyoma group 17% of patients had an elevated PTHrP level, levels of which correlated with the size of the leimyoma(s). Two women of the leiomyoma group that had highly elevated plasma levels of PTHrP had evidence of hypercalcemia. None of the women in the other groups had elevated plasma PTHrP concentration or evidence of hypercalcemia. Real-time quantitative RT-PCR showed significant overexpression of PTHrP in all leimyoma tissue compared to surrounding myometrial tissue. These findings suggest that womem that present with uterine leimyomas should be examined for humoral hypercalcemia of benignancy.

P74 F

MALIGNANT TRANSFORMATION AND PTHRP INDUCTION OF UTERINE SMOOTH MUSCLE CELLS BY CLINICAL ISOLATED OF MYCOPLASMA SPP. FROM HUMAN LEIOMYOMAS

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Recent findings show that there is an association between chronic persistant infection of cells with Mycoplasma spp. and oncogenesis. We investigated the role of PTHrP in chronic persitant infection of human uterine smooth muscle cells (hUSMC) with Mycoplasma spp. hUSMC underwent malignant transformation while chronically infected with clinical isolates of Mycoplasma fermentans, Mycoplasma hominis and Ureaplasma strains from uterine leiomyomas tissue. Mycoplasma-mediated oncogenic process had long latency and showed multistage progression characterised by reversibility and irreversibility of malignant properties upon removal of Mycoplasma spp from culture and inhibition by polyclonal antibodies to outer membrane mycoplasma lipoprotein (MALP). Marked expression of PTHrP and PTHrP receptor mRNA was found in the mycoplasma-transformed hUSMC cells that exhibited characteristic malignant properties of morphological changes and uncontrolled cell growth. However, at least up to the ninth week of persistent mycoplasma infection the marked expression of PTHrP mRNA in hUSMC cells depended on continued presence of the mycoplasma in culture. PTHrP mRNA rapidly declined to the levels of nontransformed parental hUSMC, and all malignant properties of the once-fully-transformed hUSMC quickly reversed, if the mycoplasma was eradicated from culture. Furthermore, the addition of 17b-estradiol to hUMSC cultures markedly decreased the time of onset of the mycoplasma-induced morphological changes of malignant properties. This correlated with further increase of expression of PTHrP mRNA. In comparison between species of mycoplasma, chronic persistant infection with M. fermentans and then M. hominis showed most evident signs of malignant transformation. No evidence of maligant transformation was seen with Ureaplasma spp. and no increase in PTHrP and PTHrP receptor mRNA was observed. Moreover, after further prolonged (15 weeks) infection with either M. fermentans or M. penetrans, hUSMC cells revealed prominent chromosomal changes and became permanently transformed. These findings may shed new light into the understanding of the formation of leiomyomas.

P75 S

PROGRESSIVE DEFORMITY OF THE THORACIC CAGE DURING 6 YEARS IN A YOUNG ADULT WITH PRIMARY HYPERPARATHYROIDISM

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A thirty-year-old Sri Lankan woman, who came to Japan recently, visited us for evaluation of hypercalcemia and bone deformity. Except for mild headache and morning nausea, she had few symptoms attributable to hypercalcemia. Her height was 155.5cm, weight 56kg and blood pressure 110/76. Pertinent physical findings were thoracic deformity and slight bowing of the extremities. Bone pain or muscle weakness was absent. Past history revealed no nutritional problems. Laboratory data indicated severe primary hyperparathyroidism (serum calcium 14.4mg/dl, phosphate 1.6mg/dl, iPTH >1000pg/ml and 1,25-dihydroxyvitamin D 89.5pg/ml) with extremely enhanced bone turnover (Al-P 4341 U/l, osteocalcin 200ng/ml, 1-CTP 81.5ng/ml). Serum creatinine and BUN were 0.7 and 12mg/dl, respectively. X-ray examinations revealed marked deformity of the thoracic cage, rugger-jersey appearance of the vertebrae, irregular cortical opacities of long bones. On abdominal CT, bilateral hydronephrosis and urolithiasis were demonstrated. Cervical ultrasonogram and MIBI scintigram located a probable parathyroid adenoma behind the left thyroid lobe. After successful resection of parathyroid adenoma, she was treated with 1,25-dihydroxyvitamin D3 and supplemental calcium. Her serum calcium was maintained around the normal level without severe hungry bone syndrome. Severe skeletal involvement of primary hyperparathyroidism is rarely seen in Japan nowadays. This patient from abroad gave us a unique opportunity to follow the natural course of hyperparathyroid bone disease. Her medical records showed the followings. She was diagnosed as primary hyperparathyroidism 6 years earlier, when she had a traumatic fracture in her right humerus. Serum calcium was 11.3mg/dl at that time. Iliac bone biopsy was compatible with primary hyperparathyroidism. Right hemi-thyroidectomy was performed based on the CT findings, but no adenoma was found in the removed tissue. Postoperatively, serum calcium and iPTH concentrations were 10.5mg/dl and 161pg/ml. They increased to 14.2mg/dl and 319pg/ml, respectively, 4 years later. During these years she noticed progressive thoracic deformity. However, she did not seek any treatment because she had been otherwise symptom-free. Comparison of bone X-ray films taken 6 years apart clearly showed the progression of skeletal deformities. Interestingly, however, subperiosteal bone resorption and osteopenia seemed to have ameliorated. These findings indicate the complexity of hyperparathyroid bone disease.

P76 W

PRIMARY HYPERPARATHYROIDISM IN MALE AND FEMALE PATIENTS: CLINICAL, BIOCHEMICAL AND PATHOLOGICAL FEATURES IN A POPULATION OF 278 PATIENTS WHO UNDERWENT SURGERY

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Background: The overall incidence of primary hyperparathyrodism (HPT) is approximately 1 in 2000, and the disease is two to three times as common in women as in men. The aim of this study was to analyze whether differences exist between men and women with regard to clinical and biochemical features in a population of patients with confirmed primary HPT who underwent successful parathyroidectomy.

Patients and Methods: A database of information (age, main clinical and biochemical features, and final histopathology) was recorded, and charts of 278 consecutive patients (median age 58 years, range 13-82 years) with primary HPT was reviewed. There were 212 (76.3%) women (Group A), and 66 (13.5%) men (Group B).

Results: Women were significantly older than men (58.9±12.1 vs. 49.4±14.9 years, p=0.000001, t-test). The clinical presentation did not differ significantly (p>0.05) between Groups (hyperparathyroid renal disease=12.7% vs. 21.2%, p=0.21, chi-square test; hyperparathyroid bone disease=40.1% vs. 22.7%, p=0.09; gastrointestinal symptoms=11.3% vs. 18.2%, p=0.29; weakness, lethargy, depression 14.1% vs. 10.6%, p=0.65; no symptoms or signs 21.7% vs. 27.3%, p=0.56). Most laboratory findings were similar in Groups A and B (alkaline phosphatase=132.0±76.4 vs. 163.0±212.0 U/L, p=0.07; serum creatinine=78.4±23.0 vs. 80.9±32.6 micromol/L, p=0.49; intact-PTH=175.0±139.5 vs. 207.0.9±193.0 ng/L, p=0.14), while the mean serum calcium levels were significantly higher in men (3.04±0.40 vs. 2.89±0.27, p=0.000001). There was a direct correlation between calcium and PTH serum levels both in Group A (R=0.196, F=8.383, t=102.010, p=0.004) and Group B (R=0.560, F=29.271, t=45.014, p=0.000001), while a relationship between age and serum calcium was found only among women (Group A: R=0.187, F=7.559, T=36.310, p=0.006; Group B: R=0.095, F=0.577, t=18.043, p=0.45). Final pathology showed

228 (82.0%) solitary parathyroid (PT) adenomas, 8 (2.9%) PT carcinomas, and 33 patients (11.9%) with multiglandular disease. PT cancer was more frequent in Group B (7.6% vs. 1.4%, p=0.03).

Conclusions: Primary HPT presents similar clinical and biochemical findings in men and women. However, in our experience, serum calcium did not correlate (p=NS) with the age of the male patients, although they had higher mean preoperative calcium levels. A role of sex hormones in protecting bone mass against the destructive effects of PTH could be hypothesized.

P77 F

DIAGNOSIS OF NORMOCALCEMIC HYPERPARATHYROIDISM BY ORAL CALCIUM LOADING TEST

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The oral calcium loading test (OCLT) was evaluated in diagnosing normocalcemic primary hyperparathyroidism. Calcium and PTH levels were measured before, 60,120 and 180 min after oral 1 gr. of calcium gluconolactate administration in 102 consecutive women with high circulating PTH levels, and 25 controls. Patients were classified as follows:Group A, patients with a parathyroid adenoma identified by two imaging modalities. Sub-Group AO, hyperparathyroid patients[n=13, mean age(±SD)59 years(10)]evaluated prior to parathyroidectomy. Sub-Group AH, non-operated hypercalcemic patients[n=29, age 63 yr.(11)]. Sub-Group AN, normocalcemic non-operated women[n=14, age 59 yr.(8)]. Group B, normocalcemic individuals[n=46, age 58 yr.(11)]with negative parathyroid imaging. Group C, control patients[n=25, age 56 yr.(12)]. Product P, defined as circulating PTH nadir (pg/ml) multiplicated by peak calcium concentration (mg/dl), better discriminated Sub-Group AN from Group B, Area Under the Curve=0.98(95% Confidence Interval 0.95,1.00), than did Ratio R, defined as relative PTH decline divided by relative calcium increment, AUC=0.86(95% CI 0.73,0.99). Assuming normal threshold of Product P and Ratio R at 260 and 17 respectively, the combined parameters diagnose normocalcemic hyperparathyroid patients with 100% sensitivity and 87% specificity.

P78 S

ENHANCED OSTEOCLASTOGENESIS IN CONGENITAL PSEUDARTHROSIS OF THE TIBIA AND ITS TREATMENT WITH BISPHOSPHONATE

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The purpose of this study was to examine the possible pathophysiology of congenital pseudarthrosis of the tibia and explore the clue of treatment for this disease, one of the most controversial pediatric entities in terms of etiopathogenesis, pathology, and treatment. Eight patients with this disease and seven adult patients with post-traumatic pseudarthrosis as a control were reviewed histologically, using pathologic materials. The area of congenital pseudarthrosis was divided into three parts with different morphological features; a highly cellular, fibromatous area, a cartilagenous area and an osseous area. Interestingly, a marked number of osteoclasts were detected on the surface of the bone and cartilage, and even in the fibrous area apart from bone surfaces. Bone histomorphometric analysis revealed that in congenital pseudarthrosis, osteoclast number (N.Oc/BS) and osteoclast surface (Oc.S/BS) were 2.66±0.92 [/mm](mean±SD) and 10.67±4.86%, respectively, while in the case of adult pseudarthrosis, N.Oc/BS and Oc.S/BS were 0.62±0.33 [/mm] and 2.28±1.20%, respectively. Furthermore, immunohistochemical study showed that RANK ligand, an essential factor for osteoclastogenesis, was highly expressed not only in the fibroblastic cells but also osteoclasts themselves in congenital pseudarthrosis. RT-PCR analysis also revealed the higher expression of RANK ligand in congenital pseudarthrosis of the tibia than in post-traumatic pseudoarthrosis. Because the increase in osteoclast differentiation and activity seems prominent in congenital pseudarthrosis of the tibia, we treated a patient with pamidronate. After the unsuccessful initial reconstructive surgery at age 5 in 1999, the patient was infused with pamidronate as a monthly regimen of 30 mg/m^2 for 4 months, which was followed by the subsequent surgery involving resection of the pseudarthrosis in the distal tibia and distraction osteogenesis at the proximal tibia by Ilizarov external fixation system. The distal tibia successfully united in 3 months. Serial DXA showed marked improvement in bone mineral content (BMC) in the entire tibia. BMC increased from 46% of the contralateral leg in August 2000 to 72% in August 2001. The patient is now ambulatory in a brace with no pain. In conclusion, based on the possible pathophysiology of this disease, inhibitory agents such as bisphoshonate and osteoprotegerin might be potent therapeutic candidates for this refractory disease.

P79 W

ALTERED CELLULAR KINETICS IN THE GROWTH PLATE IN AN ANIMAL MODEL OF AVASCULAR NECROSIS OF THE FEMORAL HEAD

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Although maturation of growth plate of the femoral head is assumed to be disturbed in avascular necrosis of the femoral head (Legg-Perthes disease), there were no studies regarding the characteristics of cellular kinetics in the capital femoral physis. This study was designed to investigate any histopathological changes in the physis using the spontaneously hypertensive rats (SHRs). 42 SHRs and 20 Wister Kyoto rats (WKRs) were killed at each of 6, 9, 12, 15, and 18 weeks of age. The epiphyseal growth plate with metaphyseal and epiphyseal bones was obtained from the middle of the femoral head of each rat. Bromodeoxyuridine (BrdU) was used to label cells in the S phase of mitosis as an indicator of chondrocytes with proliferative potential. Paraffin sections after decalcification were prepared and stained with Hematoxylin and Eosin. Localization of incorporated BrdU was done with a monoclonal antibody using a biotin/avidin system with a peroxidase-labeled secondary antibody. For the investigation of cellular homeostasis and study of apoptotic kinetics within the physis, we performed terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL). The pathological changes in the physis of SHRs started from the age of 9 weeks and the thickness of the growth plate decreased thereafter. Chondrocytes lost their organized pattern in the proliferating and hypertrophic zone and the most affected zone was proliferative, which was demonstrated by decreased height ratio of proliferating/hypertrophic zone with aging. BrdU incorporation in the proliferating zone decreased in SHRs as compared to WKRs, and TUNEL staining showed apoptosis occurred mostly in the proliferating and hypertrophic zone in SHRs, while apoptosis occurred predominantly in the hypertrophic zone in WKRs. In conclusion, we demonstrated altered cellular kinetics in the capital femoral physis in an animal model of avascular necrosis of the femoral head. Both the proliferating and hypertrophic zones were affected, but the proliferative zone was more severely disturbed. This finding suggest that both the physis and secondary ossification center are the targets in Legg-Perthes disease, and may explain why the femoral neck is shortened regardless of the disease process of spontaneous remission.

P80 F

BONE STRUCTURE AND STRENGTH AND MUSCLE-BONE INTERACTIONS IN 15 CHILDREN WITH OSTEOGENESIS IMPERFECTA (O.I.): A PQCT STUDY

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This study analyzes the skeletal status and muscle-bone relationships in 5 boys and 10 girls aged 2-16 years with untreated type I (5), III (5) and IV O.I. (5) and 31 3-21-year controls. The cross-sectional area and vBMD (attenuation threshold = .710 cm-1) of tibial cortical bone (CtCSA, vCtBMD) and the whole- and fat-free CSA's of the calf muscles (WmCSA, FFmCSA) were measured by pQCT (XCT-3000, Stratec) at the standard, 66% site of the leg.

The vCtBMD, increasing slowly with age in controls, showed great dispersion in O.I. children, with very low values for type IV. The CtCSA increased with age in controls and more slowly in type I O.I., remaining constantly low in types III-IV. The CtCSA also increased with a mobility score, a variable affected by the O.I. type. Both CtCSA and vCtBMD increased in all groups with the ffMCSA, more so in control than in O.I. children. The whole O.I. group showed single slopes for both variables with increasing values for types IV < III < I. The vCtBMD of all O.I. children increased asyntotically with the FFmCSA, reaching normal values at 600 mm3 of FFmCSA regardless of O.I. type. The FFmCSA / WmCSA ratio decreased in the order: controls > type I O.I. > types III-IV O.I.. Fracture incidence rate decreased exponentially with CtCSA and mobility score in O.I. children of all types together, but it was not affected by the vCtBMD.

This shows that untreated O.I. children (specially types III and IV) are unable to improve the cortical vBMD, mass and strength with age, presumably because of defects in both, mass / architecture and mineralization (low bone formation, enhanced intracortical porosity by disuse-mode remodeling) in proportion with muscle weakness. These effects could be associated with shifts in the mechanostat setpoints for triggering bone modeling and disuse-mode remodeling. Despite that pQCT determinations neglect the mineralization-unrelated, microstructural factors affected in O.I., the CtCSA predicted adequately the fracture incidence in O.I. children. Results indicate that 1. bone could respond to mechanical stimuli, and 2. pQCT indicators can describe the effects of physical and pharmacological treatments in these children.

P81 S

TREATMENT OF OSTEOGENESIS IMPERFECTA CHILDREN WITH PAMIDRONATE

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Nine Osteogenesis imperfecta(OI) children, mean age was 8.5 years-old, were treated with cyclic administration of pamidronate intravenously(mean dose, 6.5mg/kg/year). Observation period was twenty months in average. The incidence of fracture was decreased from 2.0 to 1.8 per year. In general, BMD of lumber spine were increased, D-Pyr of urine and serum ALP were decreased after administration. Mobility and quality of life of seven children were improved. As side, effect body temperatures were increased in all cases, nausea were in two and leukocytepenia were in two after first administration. All symptoms were mild and recovered soon. After second administration there were no side effect. So we concluded that pamidronate reduced bone metabolic turn-over and the treatment was useful for OI children and safe.

P82 W

TREATMENT OF OSTEOGENESIS IMPERFECTA WITH PAMIDRONATE FROM EARLY INFANT

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Osteogenesis imperfecta is characterized by osteopenia, frequent fracture and growth retardation. We report here the effect of a bisphosphonate, pamidronate on bone mineral density (BMD) and bone strength in four infants with osteogenesis imperfecta (Table).

Pamidronate (Aredia, Novartis) was administered iv in cycles of 3 consecutive days. Total dose of pamidronate was 9 mg/kg /year. The drug was diluted in normal saline to a final concentration of at most 0.1 mg/ml and was administered over 4 h. The cycles were given every 2 months under 1 years old and every 3 month under 3 years old. Clinical status, BMD of lumbar spine, biochemical markers of the bone metabolism and radiographic changes were assessed regularly during the treatment.

The lumbar BMD of Patient 1, 2, 3 has increased from 0.180 g/cm2 to 0.317 g/cm2, from 0.309 g/cm2 to 0.392 g/cm2, from 0.188 g/cm2 to 0.320 g/cm2, respectively. The height of Patient 1 has improved from minus 4.29SD to minus 2.18SD. Bone fracture was observed only once in both patient 1 and 2. The side effect of pamidronate was similar to that in elder patients. Three patients developed fever and mild hypocalcemia in first cycle of treatment; however these side effect tended to disappear after the second cycle. Other developmental index showed similar significant catch up.

In conclusion, cyclic administration of pamidronate in infants with osteogenesis imperfecta showed beneficial effects on BMD and bone strength without causing serious side effects.

Patient	Age	Sex	Sillence	Bone deformity	Cycles
1	2M	F	III	+	10
2	9M	M	I	-	4
3	2M	F	III	+	3
4	3M	M	I	+	1

P83 F

LONG-TERM TREATMENT OF A CRANIOMETAPHYSEAL DYSPLASIA PATIENT WITH A LOW CALCIUM DIET COMBINED WITH ORAL 1,25$(OH)_2D_3$ PULSE THERAPY

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Craniometaphyseal dysplasia(CMD) is a rare craniotubular dysplasia transmitted in autosomal dominant (AD) or recessive form. This disease is characterized by hyperostosis of cranial bone and deformity of the metaphysis of long bones. Recent investigation revealed that a mutation of ANK gene in CMD patients with AD form(1,2). We reported a patient with CMD treated by a low calcium diet combined with oral 1,25$(OH)_2D_3$ pulse therapy for about 10 years. At 3 years old, the patient visited Osaka University Hospital complaining of the skull enlargement and was diagnosed as CMD due to bone XP. He showed facial nerve palsy in the right at 4 years old. We started a low calcium diet (300 to 400mg/day) combined with 1.5 micro g of oral 1,25$(OH)_2D_3$ pulse therapy twice a week. In a year, facial nerve palsy improved gradually and disappeared completely when he was 12 years old. He complained no visual problems. The dose of oral 1,25$(OH)_2D_3$ was reduced to 0.5 micro g twice a week to avoid hypercalciuria. By the therapy, total body BMD was normalized and lumbar BMD remained in the normal range. Also recent gene analysis revealed that he had a mutation in ANK gene, which resulted in the impaired intracellular pyrophosphate transport into the bone matrix. Since pyrophosphate works to inhibit calcification, ANK gene mutation is thought to result in the hyperostosis of the CMD patients. In our case, we speculated that 1,25$(OH)_2D_3$ was useful for the increase of pyrophosphate levels in the bone matrix, because 1,25$(OH)_2D_3$ was reported to enhance osteoblastic ecto-nucleoside triphosphate pyrophosphatase activity (3), which produced pyrophosphate from ATP. Also, pyrophosphate was reported to inhibit osteoclastic proton transport(4), which is possibly linked to our previous observation of the impaired osteoclastic proton pump expression in the same patient(5). On the basis of these data, we propose that the low calcium diet combined with oral 1,25$(OH)_2D_3$ pulse therapy is a useful long-term therapy for CMD patient.

References: 1)Nature Genet 28:37-41,2001 2)Am J Hum Genet 68: 1321-1326, 2001 3) J Bone Mineral Res 9 : 1259-1266,1994 4) J Bone Miner Res 11:1498-1507,1996 5)J Clin Invest 91:362-367,1993

P84 S

THE COMPARISON OF RESPONSE TO PAMIDRONATE TREATMENT AMONG CHILDREN WITH OSTEOGENESIS IMPERFECTA TYPE I AND III

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Objective: To evaluate whether the difference in type I or III of osteogenesis imperfecta (OI) may influence the response to cyclical pamidronate (APD) treatment.

Methods: Eight patients were studied; group A included four boys with OI type I; group B included 3 boys and a girl with OI type III. Intravenous administrations of APD (1 mg/kg/dose) for 3 consecutive days were repeated in 4 months-cycle. Serum calcium, phosphate, alkaline phosphatase (ALP), osteocalcin (OC), urinary calcium creatinine ratio (u-Ca/Cr) and urinary deoxypyridinoline (u-DPD) were studied. Samples were collected at three points in each cycle of administration; i.e. the first and the third day of current series of infusions and at the day one month after. Statistical comparisons of two groups were made among corresponding points. Mean values demonstrated hereafter are calculated for each point. Lumbar BMD was examined by DEXA at the beginning of entire treatment and about a year later and expressed by Z score.

Results: The ages when treatment started were 5.5 years in group A and 1.2 in group B though the difference was not significant. Only group A improved Z scores significantly (-5.0 to -3.3, $p<0.05$ in Group A vs. -6.4 to -5.7, N.S. in group B). Both groups presented transient falls on the third day with serum calcium (10.3 mg/dl to 9.6, $p<0.001$ vs. 10.4 to 9.8, $p<0.001$) and phosphate (5.0 mg/dl to 4.1, $p<0.001$ vs. 5.2 to 4.5, $p<0.001$) as well as u-Ca/Cr (0.26 to 0.08, $p<0.05$ vs. 0.86 to 0.38, $p<0.001$). They recovered to the former levels after a month. The shift of serum calcium and phosphate was similar in both groups. The first day level of OC was significantly lower in group B than group A. (15.2 ng/ml vs.8.0, $p<0.001$), while that of u-DPD was higher (29.8 nmol/mmol Cr vs. 41.4, $p<0.001$).

Discussions: Patients of OI type III failed to increase BMD. OC and u-DPD suggested that insufficient formation and undersuppressed absorption of bone persisted in these patients despite APD treatment. Since transient fall in calcium supports APD does affect, the modification of administration protocol may be necessary and effective.

P85 W

EVIDENCE FOR DIRECT EFFECT OF AMINOBISPHOSPHONATE ON HISTIOCYTES - SUCCESSFUL TREATMENT OF MULTICENTRIC RETICULOHISTIOCYTOSIS WITH ALENDRONATE

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Multicentric reticulohistiocytosis (MR) is an extremely rare disease characterized by nodular erythematous skin lesions accompanied by destructive arthritis. Although a number of treatments for this disease have met with some success, the appropriate drug regimen on this disease is controversial. Histological study of skin and synovial biopsy specimen from a 44-year-old female Japanese admitted to Osaka City University Hospital for further investigation of severe nodular erythematous skin lesions and arthritis mutilans revealed heavy infiltration of skin and synovia by histiocytes and multinucleated giant cells which supported a diagnosis of MR. These cells were positive to tartrate-resistant acid phosphatase staining, indicating them to have the characteristics of osteoclasts. Moreover, immunohistochemical analysis of RANKL showed positive in some of the mononuclear cells in the skin, suggesting that multinucleated giant cells may differentiate locally in the skin from infiltrating histiocytes with the help of RANKL-positive stromal cells in MR patients. These

findings indicated the use of bisphosphonate, which is known to suppress osteoclast function. Alendronate, a second-generation aminobisphosphonate, was given at 10 mg once a week intravenously. The patient showed a dramatic response, with decrease in the number of painful joints followed by disappearance of skin nodules one month later. MRI study showed decrease in the volume of synovia in the swollen knee joint. After two months of treatment, alendronate was reduced to 10 mg intravenously once a month. The patient has remained in remission for two years. Except for fervesence, alendronate has had none of the adverse effects of conventional therapy with immunosuppressant. These findings suggest that bisphosphonates may be added to the list of drugs for treatment of this rare but potentially disabling disease. This also clearly demonstrate the presence of a direct effect of the alendronate on cells of macrophage/monocyte lineage not mediated by the release of bone mineral-buried bisphosphonate.

P86 F

CORRELATION REVISED CRITERIA FOR MARFAN AND EHLERS-DANLOS SYNDROMES AND OSTEOGENESIS IMPERFECTA WITH MOLECULAR GENETICS RESULTS

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450 patients with inherited connective tissue diseases were examined by means of a differential diagnostic system based on revised criteria for Marfan and Ehlers-Danlos syndromes as well as for osteogenesis imperfecta, benign joint hyperelasticity and juvenile osteoporosis of the youth. In 250 patients all data necessary for differential diagnostics were obtained: 57 patients met the criteria for Marfan syndrome / % of examined population/, 49 patients Ehlers Danlos syndrome and 30 osteogenesis imperfecta, 45 patients had benign joint hyperelasticity and in the remaining 25 patients another diagnosis was demonstrated. 44 individuals did not meet distinct diagnosis by means of revised criteria . Examined biochemical parameters e.g. OC was significantly increased in patients with type IV of OI when compared with type I and significantly lower level of PICP in patients with OI than corresponding to age and sex. Crosslinks were significantly higher in patients with OI as well as in patients with Marfan syndrome up to 13 years, but in older children no difference was found. The highly specific marker for Marfan syndrome was bird chest and the test of thumb. On the other hand in osteogenesis imperfecta drum chest and larger head circumference are typical. Decreased vital pulmonary capacity was found in all severe chest deformities and in scolioses greater than 25 degrees. Patients with Marfan syndrome were tall and had long extremities owing to trunk and also had longer anteroposterior bulbus length measured by ultrasonography besides the highest incidence of hernias. Acetabulum protrusion or spondylolisthesis also occur only in that group of patients.

Recurrent luxations, varicose veins and chronic pains occurred only in Ehlers-Danlos syndrome. The differences were also recorded in joint hyperelasticity measured according to Beighton and they were changing in individual diseases with age. Complex of laboratory, biochemical, densitometric, radiologic and ultrasonic seems to be adequate for differential diagnosis.

The final diagnosis was verified by genetic examination or using specific proteins. An extremely high correlation was found between the results of conclusive diagnosis based on revised criteria and following verification based on molecular genetics.

P87 S

LOCAL EXPRESSION OF OXYGEN METABOLIC ENZYMES AND SKELETAL MASS: POSITIVE CORRELATIONS IN OSTEOPETROTIC MICE

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Superoxide and free radicals and and are involved in the metabolism and transport in cells and tissues. Immunohistochemical evaluation of the related enzymes in some cells has been reported. However, the kinetics of these enzymes in osteopetrotic osteoblasts are unknown. In this study, we evaluated Mn-SOD, i-NOS and e-NOS mRNAs expression in normal and osteopetrotic (op/op) mouse calvarial osteoblasts by performing experiments to understand the relationship between bone formation and superoxide, free radical related enzymes. Normal and op/op mice(3,5,18,40 weeks) were anesthetized, then perfusion fixation and paraffin embedding were performed. We evaluated mRNA expression by in situ hybridization, protein expression by western blot and immunohistochemistry by nitrotyrosine antibody. Mn-SOD mRNA expression was weakly positive in all normal osteoblasts, whereas they were strongly positive in op/op osteoblasts(3w) especially those near bone marrow spaces. i-NOS mRNA expression was negative in all the normal mouse osteoblasts, whereas they were positive in op/op mouse osteoblasts(3w) on both periosteal and endosteal surfaces and strongly positive in osteoblasts near bone marrow, as observed with Mn-SOD. e-NOS mRNA signals were negative in all normal mouse osteoblasts, whereas they were positive in op/op mouse osteoblasts on both periosteal and endosteal surfaces. These results suggest that possibility that active oxygen and free radicals are involved in the remodeling of the bone acting on calvarial osteoblasts in op/op mice.

P88 W

FGF-23 IN PATIENTS WITH END-STAGE RENAL DISEASE ON HEMODIALYSIS

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Fibroblast growth factor (FGF)-23 is a recently identified circulating factor which causes renal phosphate wasting disorders. Although the mechanism of regulation of FGF-23 secretion is unknown, plasma FGF-23 level may be regulated or affected by serum phosphate levels because of its hypophosphatemic effect. We tested the hypothesis that plasma FGF-23 levels may be increased in hyperphosphatemia in patients with end-stage renal disease on maintenance hemodialysis. We measured plasma FGF-23 levels in 158 male uremic patients on maintenance hemodialysis by ELISA. Plasma FGF-23 level exhibited significant and positive correlations with inorganic phosphate, intact PTH, corrected calcium, and duration of hemodialysis on simple regression analyses. All these associations remained significant in multiple regression analyses. Serum phosphate, calcium, and intact PTH could be regulators of FGF-23 levels in uremic patients on maintenance hemodialysis. Our results may provide new insights into the pathophysiological effects of FGF-23 on calcium-phosphate homeostasis.

P89 F

CLINICAL USEFULNESS OF THE SERUM N-TERMINAL PROPEPTIDE OF TYPE I COLLAGEN AS A MARKER OF BONE FORMATION IN HEMODIALYSIS PATIENTS

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The aim of the this study was to investigate the clinical usefulness of serum intact procollagen I amino-terminal peptide (PINP) as a bone formation marker in hemodialysis (HD) patients. To examine whether HD session may affect serum markers for bone formation, 14 female patients were measured for their serum bone alkaline phosphatase (BAP), intact osteocalcin (OC), and PINP just before and after a single HD session. Futhermore we mesured serum PINP, BAP, OC, intact parathyroid hormone (PTH) and a number of bone resorption markers [deoxypyridinoline (DPD), pyridinoline (PYD), beta-crossLaps (beta-CTx)] and examined the correlation with annual BMD change at distal radius 1/3. Serum PINP, in contrast to BAP and OC, did not show any significant change between before and after a HD session. Correlations of serum PINP with bone resorption markers were significantly stronger than those of serum BAP. Bone formation markers and bone resorption markers had significant and negative correlations with annual BMD change at distal radius 1/3. The levels of PINP decreased significantly with decreasing tertiles of bone loss at distal radius 1/3. PINP/PYD, PINP/DPD, and PINP/beta-CTx correlated significantly in a positive manner with annual bone loss at the distal radius 1/3. In conclusion, serum PINP may be useful for prediction of radius bone loss as well as other serum bone formation markers.

P90 S

IS IT USEFUL TO MEASURE OSTEODENSITOMETRY IN HEMODIALYSIS PATIENTS?

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Background: Bone mass loss is one of the major problems in hemodialysis (HD) patients, caused by renal osteodystrophy (in Europe up to 60 % and in North America up to 40 % by high-turnover osteopathy) and / or by osteoporosis due to the increased age of the patients on HD. In these patients various investigations demonstrated considerable differences in osteopenia, osteoporosis, and risk of fracture. Diagnostic methods, such as the determination of bone markers in serum and urine as well as osteodensitometry, are discussed, because the gold standard bone biopsy will frequently refused by the patient.

Methods: In this prospective study dual energy x-ray absorptiometry (DEXA) on lumbar spine (LS) and on left femur neck (LFN) was performed in 17 HD patients, aged 61 ±15 years, at start and one year after intermittant HD therapy. Results were

compared to serum and urine bone markers including intact parathyroid hormone (iPTH), osteocalcin, bone alkaline phosphatase, pyridinoline, desoxypyridinoline, vitamin D (25-/1.25- vit. D3), aluminium, calcium (Ca), and phosphorus.

Results: At start of intermittant HD therapy bone density was reduced on LS in 1 patient (6 %) and on LFN in 5 patients (29 %) below fracture threshold (T-Score < -2.5). Additionally, osteopenia (T-Score < -1.0) was detected in 9 patients (53 %) on LS and in 10 patients (59 %) on LFN, respectively. The patients with osteopenia on LS and LFN (n = 10) showed vitamin D deficiency (1.25-vit. D3 < 20 ng/l: 100 %, 25-vit D3 < 23 nmol/l: 60 %), hypocalcaemia (Ca < 2,20 mmol/l: 50 %), and hyperparathyroidism (iPTH > 250 pg/ml: 40 %). In 3 patients bone density increased significantly due to decline of PTH to normal levels.

Conclusion: Osteodensitometry by DEXA is appropriate for evaluation of osteopenia in HD patients with risk factors, especially in vitamin D deficiency, but also in hypocalcaemia and / or hyperparathyroidism.

P91 W

POSSIBLE ROLES OF UREMIC TOXINS ON THE PATHOGENESIS OF ADYNAMIC BONE DISEASE IN CHRONIC RENAL FAILURE

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Adynamic bone disease (ABD) is emerging as a major type of bone abnormality in chronic dialysis patients. Although its prevalence is increasing, the pathophysiological mechanisms underlying ABD are not fully elucidated yet.

In order to clarify the pathogenesis of ABD, we have developed ABD model rats and analyzed bone turnover and examined the effect of PTH administration. Male SD rats, 10 weeks old, underwent thyro-parathyroidectomized (TPTx) and either 1/2, 3/4, 5/6 nepherectomy (Nx) or sham operation. Rats were fed with a special chow containing Ca 2.0% and P 1.0 %. These rats were continuously infused with rat PTH (0.1mcg/kg/hr) with osmotic mini pump and injected L-thyroxine (0.4 mcg/100g BW) subcutaneously three times per week. TPTx-5/6Nx rats at 6 weeks after Nx were further divided into four groups and treated either with rat [1-34] PTH 10, 30, 90 mcg/kg BW, or vehicle given subcutaneously three times per week.

By bone histomorphometry, bone turnover in TPTx-Nx group was suppressed compared with that in TPTx-Sham group at 6 weeks after Nx. The decline of bone formation rate was dependent on the degree of renal dysfunction. Intermittent PTH treatment ameliorated the suppression of osteoblast function (mineral appositional rate), and bone formation (bone formation rate) in a dose dependent manner.

Serum levels of indoxyl sulfate, one of identified uremic toxins, increased depending upon the degree of decline of renal function. In primary culture system, indoxyl sulfate treatment significantly suppressed osteoblast proliferation.

These data suggest that renal dysfunction plays a major role independent of low PTH in the development of ABD in chronic renal failure. Furthermore, some of the accumulated uremic toxins, such as indoxyl sulfate, may be responsible for such a suppression of osteoblastic function.

P92 F

LOW PHOSPHATE DIET EVOKES A GENOMIC RESPONSE IN THE KIDNEY WHICH IS ALTERED IN *HYP* MICE

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Low phosphate diets lead to increased renal tubular reabsorption of phosphate and increased renal synthesis of 1,25-dihydroxyvitamin D3. Whether this response is abolished in mutations of the *Phex* gene, such as in the X-linked hypophosphatemic, *Hyp*, mice, is unclear. To investigate this, 5-week-old mice, normal and *Hyp*, male and female, were fed a control (1.0% P) or low phosphate (0.03% P) diet for 3 to 5 days. The kidneys were collected, and RNA was extracted. RNA was pooled between 3 mice of the same genotype and diet. cRNA was prepared and hybridized to 20 Affymetrix U74Av2 GeneChip microarrays with probes for 12,473 genes (5 arrays per group). An average of 5,178 genes were scored as present on each array. Of this number 388 genes were significantly ($P < 0.01$) altered in their RNA expression on low phosphate diet in normal mice. In contrast, only 55 genes were altered in *Hyp* mice fed the low phosphate diet ($P < 0.01$). In addition, 367 genes differed between normal and *Hyp* mice on the control diet, of which 284 had no significant response to low phosphate diet. These genes include klotho (AB005141) and carbonic anhydrase XIV (AB005450) which both decrease significantly ($P < 0.001$) in the *Hyp* mice. Only the former responded to low phosphate diet in normal mice ($P < 0.01$), but not *Hyp* mice. All members of the serine protease inhibitor family 1 (e.g., M75718) were increased in *Hyp* mice ($P < 0.01$) with no significant response to low phosphate diet. These changes suggests roles for the *Phex* gene outside of phosphate homeostasis. In addition, of the 388 genes altered in normal mice by low phosphate diets, 11 genes were significantly altered in the opposite direction in *Hyp* mice on the control diet. In conclusion, low phosphate diet evokes a genomic response in the kidneys of normal mice that is reduced, but not abolished in *Hyp* mice. In addition, there are changes in gene expression in the kidneys of *Hyp* mice that seem unrelated to phosphate homeostasis.

P93 S

MOLECULAR ANALYSIS OF JAPANESE PATIENTS WITH HYPOPHOSPHATEMIC RICKETS

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Several hereditary disorders of isolated phosphate wasting have been described including X-linked hypophosphatemic rickets (XLH) and autosomal dominant hypophosphatemic rickets (ADHR), hypoppohsotamic rickets with hypercalciuria (HHRH). Inactivating mutations of the PHEX gene are responsible for XLH. Mutations of the FGF 23 gene cause ADHR. In this study, we analyze the PHEX, FGF23, and NPT2 genes in 12 patients with hypophosphatemic rickets (2 familial patients, 4 sporadic patients). Two splicing junction mutations (849+1 g->a and 2071-1 g->a) and one missense mutation (G579R) of the PHEX gene were identified in 4 patients. In the remaining 8 patients, there was no mutations of the FGF 23 gene. We are now analyzing NPT1 and NPT2 genes.

One patient, whose mother was affected, could be definitively diagnosed as having XLH at one week after birth by the identification of the PHEX gene mutation. Treatment with 1a-hydroxyvitamin D3 and phosphate supplementation could be begun at 1.5 months of age. At 24 months of age his rachitic changes have improved, so that early initiation of treatment is effective.

In conclusion, the PHEX gene mutations are heterogeneous and genetic analysis of the PHEX gene is useful for both early definitive diagnosis and treatment. Regarding the molecular basis of hypophopatemic rickets, further studies are required.

P94 W

HEREDITARY HYPOPHOSPHATEMIC RICKETS WITH HYPERCALCIURIA: ANALYSES OF PHOSPHATE TRANSPORTER GENE AND OSTEOBLASTIC FUNCTION

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Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) is a rare inherited disorder characterized by hypophosphatemia, short stature, rickets and/or osteomalacia and secondary absorptive hypercalciuria. We reported here two cases of hereditary hypophosphatemic rickets with hypercalciuria (HHRH). They were the Japanese female siblings. No consanguinity was found in the family history. Both of them manifested short stature and bowing legs, and the biochemical examinations revealed hypophosphatemia, phosphaturia and hypercalciuria. The serum concentrations of $1,25(OH)_2D$ were elevated. In oral phosphate loading test, the serum phosphate levels were markedly increased in the HHRH patients, and the elevation was much higher than that in both patients affected with X-linked hypophosphatemic rickets (XLH) and normal control, suggesting the increased gastrointestinal absorption of phosphate in HHRH. Bone histology studies showed the increased osteoid surface and width in HHRH, which was compatible with osteomalacia. In one of the HHRH patients, phosphate administration alone almost completely cured the osteomalatic change within a year, which was proved by bone biopsy. These patients showed no hypomineralized periosteocytic lesions (HPL), which is a hallmark of XLH in bone histology. We also investigated the phosphate transporter gene of NPTII and its three isoforms, however we found no gene abnormality. In osteoblasts isolated from a HHRH patient, the responsiveness to $1,25(OH)_2D_3$ to produce osteocalcin is not dependent upon medium phosphate concentration. In contrast, in normal and XLH osteoblasts, the osteocalcin synthesis by $1,25(OH)_2D_3$is dependent on medium phosphate concentration; 2 mM for normal and 4 mM for XLH osteoblasts to increase osteocalcin synthesis by $1,25(OH)_2D_3$in both osteoblasts. However, ALP activities of osteoblasts from normal, XLH and HHRH patients were increased in response to medium phosphate concentrations in the same way. These lines of evidences suggest that the cause of HHRH is related to abnormal $1,25(OH)_2D_3$ actions on osteoblast dependinig on extracellular levels of phosphate, which might be linked to abnormal phosphate metabolism of osteoblast in HHRH patients.

P95 F

TARGETED ABLATION OF FIBROBLAST GROWTH FACTOR 23 CAUSES HYPERPHOSPHATEMIA, INCREASED 1,25-DIHYDROXYVITAMIN D LEVEL AND SEVERE GROWTH RETARDATION

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Fibroblast growth factor (FGF) -23 has been identified as a humoral factor that plays important pathophysiological roles in the development of tumor-induced osteomalacia and autosomal dominant hypophosphatemic rickets. In addition, we showed that administration of recombinant FGF-23 into normal mice resulted in hypophosphatemia, low serum 1,25-dihydroxyvitamin D level and rachitic bone phenotype. Therefore, it is now evident that excess actions of FGF-23 can disturb mineral homeostasis and cause hypophosphatemic disorders. To further address physiological roles of FGF-23, we generated FGF-23 knockout mice lacking a part of exon 1 of FGF-23 gene. Heterozygous founders did not show significant differences from wild type mice in general appearance. By mating heterozygotes, homozygous founders were born at the expected Mendelian frequency, indicating that ablation of FGF-23 did not cause embryonic lethality. Although body size of homozygotes was not different from that of wild type or heterozygous mice at birth, the gain in body weight of homozygotes ceased after 13 days of age. Moreover, the life span of homozygous mice was shorter than that of wild type and none of them survived more than 13 weeks. Biochemical analysis revealed that serum phosphate and 1,25-dihydroxyvitamin D levels were significantly increased from 10 days of age and thereafter in homozygous mice. Sodium dependent phosphate uptake of kidney brush border membrane vesicles from FGF-23 null mice was elevated compared to that of wild type indicating that hyperphosphatemia in homozygotes was at least partly mediated by enhanced renal phosphate reabsorption. In addition, homozygous mice demonstrated remarkable increase of renal 1alpha-hydroxylase mRNA levels from 10 days and thereafter. This enhanced 1alpha-hydroxylase activity probably contributed to high serum 1,25-dihydroxyvitamin D in FGF-23 null mice. None of such changes were observed in heterozygous mice. Taking account of the fact that these changes in phosphate and vitamin D regulations observed in FGF-23 null mice are mirror images of those seen in animals received recombinant FGF-23, it is concluded that FGF-23 is an essential factor to maintain bone and phosphate homeostasis. Furthermore, it is possible that FGF-23 is also involved in the regulation of growth and life span after birth.

P96 S

TARTRATE-RESISTANT PHOSPHATASE 5B(TRAP5B), OSTEOCALSIN (OC), INTACT PARATHYROID HORMONE (IPTH), AND VITAMIN D METABOLITES IN THE ASSESSMENT OF BONE TURNOVER IN ELDERLY PATIENTS WITH HIP FRACTURES

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BACKGROUND: Hip fractures increase with age, reaching epidemic levels among elderly in many developed countries. In the elderly, hip fractures are associated with significant increased mortality and morbidity. Osteomalacia and osteoporosis are amongst the most important contributing factors to hip fracture in the elderly. Evaluation of the risk of bone loss and osteoporotic fractures is a vital step in the primary prevention of osteoporosis and osteoporotic fractures

The present study was undertaken to elucidate osteoblast and osteoclast activity with regards to vitamin D status in elderly patients with acute hip fractures.

METHODS: Tartrate-resistant phosphatase 5b(TRAP), osteocalsin (Oc), intact Parathyroid Hormone (iPTH) vitamin D metabolites 25OH)D3 and 1,25(OH)2D3, were measured acutely in 60 consecutive patients after a hip fracture in the period May/June (n=30) and November/December (n=30)

Vitamin D metabolites were measured by radioimmuno assay after purification and extraction. Oc was measured by luminescence immunoassay and TRAP 5b by a solid phase immunofixed enzyme activity assay. iPTH was measured by immunometric assay.

RESULTS: During the summer months 18 (60 percent) patients had low values for either one or both of Vitamin D metabolites, whilst all patients but 1 had decreased vitamin D values in the winter months. 20 patients (30 percent) had elevated values of iPTH

A significant correlation was seen between iPTH and Oc ($p<0.05$) whilst no correlation was found between TRAP5b and Oc values. This is in contrast to findings in patients with osteoarthritis and healthy blood- donors in whom we have observed significant correlation between TRAP 5b and Oc and normal vitamin D values.

CONCLUSION: Vitamin D deficiency is widespread in the elderly acute fracture population and more pronounced in the winter months in temperate climates. This may induce secondary hyperparathyroidism and may promote increase in bone remodelling rates leading to fractures. In the acute phase of hip fractures, there is no correlation between the measured osteoblast and osteoclast markers.

The situation in the stable postoperative phase is unknown.

P97 W

SERUM HUMAN FGF-23 LEVEL IN X-LINKED HYPOPHOSPHATEMIC RICKETS

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Fibroblast growth factor (FGF) -23 was identified as a common cause of two different hypophosphatemia, Tumor-induced osteomalacia (TIO) and autosomal dominant hypophosphatemic rickets (ADHR). Though investigators soon hypothesized this novel family of FGF can be the major phosphate regulating factor 'phosphatonin', the role of FGF-23 in phosphate metabolism and in the pathogenesis of X linked hypophsphatemic rickets (XLH) has not been clarified.

We measured the serum level of FGF-23 in patients with hypophosphatemia using enzyme-linked immunosorbent assay (ELISA) kit detecting carboxyl terminal (C-term) epitopes of human FGF-23 (Immutopics Inc. San Clemente, CA). Serum FGF-23 level of hypophosphatemic rickets showed remarkable patient to patient variation and were distributed from 96 to 1221 U/ml. FGF-23 level of three XLH patients whose mutation in PHEX gene were confirmed showed remarkable contrast. A female case with frame shift mutation in exon 3 showed remarkable increase of 709 U/ml. On the other hand, two male cases with the same point mutation in the splice acceptor site of exon 14 showed only high normal value. In three patients, the effect of the aging and the therapy for approximately 3 years were also tested. Our results showed that the serum level of FGF-23 might be stable and not affected by the age or by the therapy.

Using ELISA recognizing for C-term epitope of FGF-23, remarkable increase in serum level of FGF-23 in TIO patients had been reported (Miyazaki et al,2002). Miyazaki and his colleagues also reported the elevation of serum FGF-23 in XLH patients.

Our results also indicate the possibility that the over production or the accumulation of FGF-23 can be involved in the pathogenesis of some hypophosphatemic rickets. However, there has been remarkable variation in the serum levels of FGF-23. Moreover, we could not find statistical significant correlation between the serum level of FGF-23 and serum phosphorus level. These results suggest that FGF-23 may not be a direct phosphate regulator, and that there may be other factors.

P98 F

STATE OF BONE TISSUE IN MYELOSUPPRESSION

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AIM: The cells of bone tissue are the main part of hemopoietic microenvironment. Earlier we demonstrated the importance of bone cells in regulation development hemopoietic precursors in normal hemopoiesis. The aim of this work was study of bone tissue in hypoplasia hemopoiesis.

METHODS: Functional and structural features cells of iliac bone have been characterized in 50 patients with hypoplasia hemopoiesis connected with defects of hemopoietic microenvironment. Cultural, morphometric and ultracytochemical methods were used.

RESULTS: The organ culture of bone tissue showed high proliferative activity of cells precursors of bone marrow stroma. The morphometric investigation of the whole bone fragments showed the increase of value of bone trabeculs in hypoplastic conditions 1.4 volume increase, in aplasia 1.7 volume increase. The number of osteogenic cells per unit of square in histological preparation of iliac bone in hypoplasia was 1.5 volume increase, and in aplasia was 2.0 volume increase. The ultracytochemical analysis demonstrated the increase functional activity of the intramedullar and endosteal cells of bone fragments. In osteogenic and stromal cells were found pathological intranuclear bodies.

CONCLUSIONS: Our results indicates about close relationship bone tissue with stromal cells of bone marrow and hemopoietic precursors. Close relationship between bone cells and hemopoietic cells confirms the thesis about the existence of a common cell progenitor of the hemopoietic and bone tissue in adult. Our data can be used for the establish of cause of bone formation disorders and creation new preparations for treatment diseases of bone tissue.

P99 S

AN EXTREMELY LOW BIRTHWEIGHT INFANT WITH METAPHYSEAL OSTEOPENIA POSSIBLY DUE TO MATERNAL ADMINISTRATION OF MAGNESIUM SULPHATE

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Maternal magnesium sulphate treatment is frequently used during pregnancy for inhibition of premature uterine contractions. However, little information is available about the effects of antenatal magnesium sulphate exposure on fetal and neonatal bone mineral status. We report a male infant presenting with hypermagnesemia and generalized metaphyseal osteopenia born to a mother who was treated with magnesium sulfate for 5 weeks. Our case supports the existence of a causal relationship between prolonged intravenous magnesium tocolysis and abnormal fetal bone mineralization. Further extensive studies may be needed to elucidate the clinical significance of these bone changes and abnormal mineral status.

P100 W

BONE GRAFT SUBSTITUTION USING OPTEFORM FOR TUMORS AND INFECTED NONUNIONS

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This study evaluates the effectiveness of Opteform bone grafting as applied to several bone defects. The progression of healing was followed in sixty-seven patients who underwent Opteform bone graft procedures over a seven-year period (1995-2002). This group consisted of forty-one bone tumors, including giant cell tumors, non-ossifying fibromas, and oseonecrosis, and twenty-six chronic infected nonunions (8 feet/ankles; 4 hips/femurs; 9 tibiae; 4 humeri; 1 radius). Healing time was determined based on incorporation of the graft as evaluated by radiograph at two weeks, three months, six months, and one year. Incorporation of the Opteform graft was achieved in all patients, with an average healing time of 6.3 months. Complications impeding healing included two fractures, two post-op infections, and two non-healing wounds. All patients achieved functional healing. In addition to being readily available and simple to use, Opteform is acceptable for use with a variety of bone defects, including difficult infected nonunions. With short recovery times and low incidence of complications, it is a convenient alternative to traditional grafting materials and appears clinically superior to other bone graft substitutes.

P101 F

PROMOTION OF SPINAL FUSION BY SURFACE INTERFERENTIAL STIMULATION DEVICE

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The current study evaluated the efficacy of a clinically available cutaneously applied stimulation device using interferential current to enhance the spine fusion process. This aparatus enhances patient compliance by providing pain relief and improves on current technology in that there are no leads implanted in the bony fusion mass.

Fifty-six (56) skeletally matured New Zealand White (NZW) rabbits had a bilateral intertransverse process fusion surgery using morselized bone from the iliac crest. Two pairs of surface electrodes were adhered to the skin approximately one inch apart on four corners of the fusion site. Each of the 4 groups had 14 rabbits, calculated by sample size determination. The control animals, which underwent the fusion were handled daily. Electrodes were applied to their backs without stimulation. Groups 2 (RS 4i device with a 241 co-processor) and 3 (RS 4i device with a 2406 co-processor) underwent one hour of stimulation per day. Group 4 underwent stimulation 24 hours per day with a conventional device (60 K Hz Sine Wave at an ouput of 5 volts). The animals were sacrificed at 8 weeks post-surgery, and the fusion mass recovered for biomechanical strength testing. Uniaxial tensile testing at a displacement rate of 0.5 cm/min was done on a modified Instron system. Load/displacement data were generated for assessing the peak load to failure (MaxLoad, N) and the stiffness (at 50 and 75% of the peak load, N/m2), and for calculating the energy (mJ). The data from the unfused third-fourth lumbar vertebral segments and the fused fourth-fifth segments were used to calculate a ratio for internal control and to minimize variation among animals. Statistical significance was analyzed on the ratios using ANOVA and Dunnett's procedure.

The fusion mass was strongest in rabbits receiving stimulation from the RS 4i device with a 241 co-processor (Group 2), as compared to the control, non-stimulated group, in all three parameters. The p values were 0.0028 (MaxLoad), 0.0042 (Stiffness) and 0.0145 (Energy). The data suggests that surface interferential stimulation can be an attractive alternative to interbody metal cages and osteogenic factors for spine fusion, in addition to the relief of back pain.

P102 S

ANALYSIS OF EXPRESSION AND CIRCULATING LEVEL OF FGF-23 IN NON-MCCUNE-ALBRIGHT SYNDROME FIBROUS DYSPLASIA

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There have been increasing evidences for the involvement of FGF-23 as a common pathogenetic mechanism in oncogenic osteomalacia (OOM), autosomal dominant hypophosphatemic rickets (ADHR) and X-linked hypophosphatemia (XLH). Fibrous dysplasia (FD), a focal and benign fibrous bone lesion, is associated with McCune-Albright syndrome (MAS) in a small minority of patients,, but most patients affected by this order show no features of MAS and are therefore referred to as non-MAS FD. Some of non-MAS FD patients display renal phosphate wasting, similar to that observed in OOM. However, there has been no report about the possible involvement of FGF-23 in non-MAS. Here we report a case of non-MAS FD presenting with hypophosphatemia due to increased renal phosphate excretion, who showed high serum FGF-23 levels (232.4 RU/ml) as assessed by a recently developed assay which detects the carboxyl-terminal portion of this molecule. A mosaic R201C mutation of the GNASI gene was identified in the resected lesion of the right femur but not in DNA from peripheral blood leukocytes. RT-PCR of the resected specimen indicated the expression of FGF-23. These findings suggest a possibility that FGF-23 is expressed in the non-MAS FD as well as tumors with OOM, although it remains to be elucidated whether FGF-23 plays a role in the pathogenesis of phosphate wasting disorder associated with non-MAS FD, since its plasma level was relatively low compared to that in OOM, and was not decreased after the operation, and the expression of FGF-23 in the lesion was not remarked.

P103 W

REDUCED SYTHESIS OF VITAMIN D IN SKIN: A POSSIBLE CONTRIBUTOR TO POST-BURN VITAMIN D DEFICIENCY

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Children develop progressive vitamin D deficiency following severe burns (*J Trauma* 2002; 52:346) with a direct relationship between serum 25-hydroxyvitamin D and lumbar spine bone mineral density. While lack of vitamin D supplementation likely contributes to D deficiency, post-burn sun exposure is variable. Failure of skin to synthesize vitamin D normally may also figure in D deficiency in burn patients. Our aim was to study cutaneous pre-vitamin D synthesis from its precursor 7-dehydrocholesterol (7DHC)by exposure to ultraviolet B (UVB)light.

Methods: We obtained 0.5 cm^2 skin biopsies from burn scar and adjacent normal-looking tissue in 6 children, ages 4-14 years, burned at least 40% body surface 14±1.4 (SEM)months post-burn and 4 normal controls. Biopsies were analyzed by straight-phase high performance liquid chromatography for 7DHC, exposed to UVB irradiation for 4 minutes in a standard irradiation box, and then assayed for pre-vitamin D. Percent conversion of 7DHC to pre-vitamin D was calculated for burn and control subjects.

Results: Control skin (n=4)converted 20.2±1.6%(SEM) of 7 DHC to pre-vitamin D while burn scar (n=6) converted 5.1±1.1%, and adjacent normal-looking skin converted 6.2±1.1%, p=0.01 for both versus controls.

Conclusions: These data suggest that vitamin D synthesis is abnormal not only in burn scar but also in adjacent normal-appearing tissue and suggests that normal-appearing skin near a burn scar may not be biochemically normal. Vitamin D supplements are needed to prevent deficiency post-burn and further studies must investigate why vitamin D synthesis is impaired following UVB exposure.

P104 F

BPC-157, A STABLE GASTRIC PENTADECAPEPTIDE BENEFICIAL FOR HEALING OF CONTUSIONED MUSCLE, REVERSES DELAYED CORTICOSTEROID-IMPAIRMENT IN RAT

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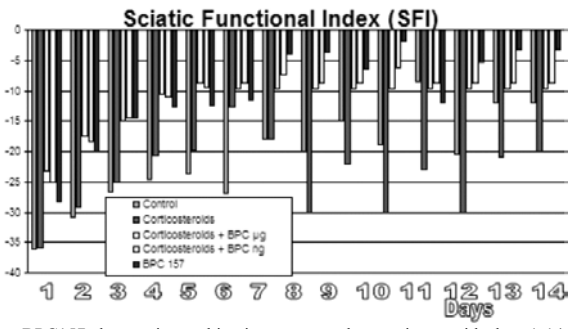
Gastric pentadecapeptide BPC 157 is stable peptide currently in clinical phase II for inflammatory bowel disease (PL-10, PLD-116, PL-14736, Pliva) that heals internal and external wounds (i.e., Burns 27 817, 2001, Bone 24 195, 1999), effective also in corticosteroid impaired burned animals (Burns, 2002, in press). We investigate in corticosteroid treated rats the improvement of healing and function recovery of rat contusioned right gastrocnemius complex muscles (GCC) (the impact of a blunt nonpenetrating object (drop-mass technique (150 g, height 49 cm, spherical radius 8 mm)). Wistar male rats received agents (once daily, intraperitoneally, /kg) 6-alpha-methylprednisolone (Depo-medrol, Pharmacia&Upjohn) (1.0 mg) and/or BPC 157 10 microg/kg or 10 ng/kg or saline (5.0 ml), first application immediately following injury, last 24 h before sacrifice (at 2 hr, 1, 2, 4, 7 and 14 days post-injury). Full assessment (walking track analysis computing sciatic function index (Plast Reconst Surg 83 129, 1989), new motor recovery test, the extensor postural thrust (Exp Neurol 168 192, 2001), macroscopic assessment of injured site (haematoma+edema+hyperaemia), haematoma on the surface of the GCC, GCC maximum circumference, GCC weight, and microscopy (vimentin, desmin)) reveals gastric pentadecapeptide BPC-157 healing and function recovery at all tested intervals (Figs. 1, 2). Relative to controls, corticosteroid presents a dual effect, positive at early intervals (till 4 post-injury day), negative at latter (7-14 post-injury days). Corticosteroid-aggravation is abolished by co-administration of BPC 157. In conclusion, these results indicate this stable gastric pentadecapeptide BPC-157 beneficial for healing muscle, able to reverse delayed corticosteroid-impairment.



BPC157 alone or in combination vs. control or corticosteroids days 1-14
Corticoids significant vs. control days 7-14

P105 S

IS BONE MATRIX STILL IMPORTANT SUBSTITUTE FOR BONE GRAFT SURGERY-FROM POINT OF VIEW OF HEAT AND BMP ACTIVITY

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One approach to opimizing an osteoinductive preparation is to focus on isolating or synthesizing BMPs and combining them with suitable carriers. Another apporoach is to make available human demineralized bone matrix.

For autogenous and allogeneic bone grafts,heat treatment has been thought to kill malignant cells and viruses such as human immunodeficiency virus. It is unclear whether heat treatment could preserve bone-inductive activity.

Materials and Methods: Cortical bones from 6-week-old rat were heated in a water bath at a temperature from 50 centigrade to 100 centigrade for periods of 15 minutes to 10 hours After decalcified,each sample was transplanted into muscle of rats. Eleven days after tansplantation, mRNAs were determined for alkaline phosphatase and collagens in the transplants. Twenty-one days after transplantation actual bone formation was studied.

Results: Heat treatment at 60 centigrade for 10 hours and at 70 centigrade for 1 hour preseved bone-inductive activity, as indicated by the induction of mRNAs for alkalne phosphatase and Type1 and Type2 collagens. Significant decreased in Type2 collagen mRNA and calcium content were observed at 70 centigrade when the transplants were heated for 10 hours, suggesting the importantce of evaluating of heat treatment.

Discussion: The authors reported that the activity of BMP was preseved by heat treatment from 50 centigrade to 70 centigrade decreased at temperatures more than 80 centigrade and was lost conmpletely at 100 centigrade.

For allogeneic bone grafis, infectious agents such as human immunodeficiency virus and hepatitis viruses must be inactivated. Human immunodeficiency virus can be inactivated in human plasma by heat treatment at 60 centigrade for 10 hours. The current results support the idea that heat tretment at 60 centigrade and possibly at 70 centigrade for 10 hours is useful for allogeneic bone grafts and does not impair bone-inductive activity.

P106 W

ROLE OF FIBROBLASTS IN OSTEOLYSIS ASSOCIATED WITH ASEPTIC LOOSENING

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Aseptic loosening is generally associated with the presence of wear particle-associated macrophages in the pseudomembrane commonly formed around failed prosthetic implants. The extent of the macrophage response evoked by the wear particles has been shown to correlate with the amount of periprosthetic osteolysis. Numerous studies have shown that wear particle-associated macrophages contribute to osteolysis by (i) releasing inflammatory cytokines and/or (ii) differentiating into bone resorbing osteoclasts. Although macrophages are the main inflammatory cells found in periprosthetic tissues, numerous fibroblasts are also present in the connective tissue pseudomembrane. RANKL has been shown to play a central role in the osteoclast formation and bone resorption observed in aseptic loosening. We have shown that arthroplasty macrophages are capable of osteoclast differentiation; this process is inhibited by OPG, the soluble decoy receptor for RANKL. As fibroblasts are known to express RANKL, the aim of the present study was to determine whether fibroblasts isolated from periprosthetic tissues could induce the generation of bone resorbing osteoclasts which would contribute to the osteolysis commonly seen in the periprosthetic loosening. Fibroblast-like cells were isolated from pseudomembrane from patients undergoing hip revision due to aseptic loosening. Generated fibroblast-like cells were then co-cultured with normal human peripheral blood monocytes in the presence of (i) no added factors, (ii) M-CSF, (iii) M-CSF + OPG/RANK:Fc and (iv) M-CSF + anti-TNF receptor p55 & p75 antibodies. The extent of osteoclast differentiation was then determined by the expression of specific osteoclast markers (e.g. TRAP and VNR) and evidence of lacunar resorption. In the absence of M-CSF, no osteoclast formation was noted in fibroblast/monocyte cultures. However, in the presence of M-CSF alone, large numbers of TRAP+ and VNR+ multinucleated cells capable of lacunar resorption were noted. The addition of OPG or RANK:Fc, significantly reduced the extent of osteoclast formation and lacunar resorption. Similarly, the addition of antibodies directed against p55 and p75 receptor subunits markedly reduced the osteoclast formation. These results indicate that one means whereby periprosthetic osteolysis may occur is by fibroblasts in the arthroplasty pseudomembrane inducing macrophage-osteoclast differentiation. These findings indicate that suppression of osteoclast formation by a combination of OPG/RANK:Fc and anti TNF drugs may be a possible form of therapy for reducing prosthetic loosening.

P107 F

EFFECTS OF PENTADECAPEPTIDE BPC-157 ON TRANSOSSEOUS RAT MANDIBULAR DEFECTS HEALING IN VIVO

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The efficacy of local and systemic delivery of pentadecapeptide BPC-157 (currently in clinical phase II for inflammatory bowel disease) to promote bone healing was evaluated in transosseous rat mandibular drill defects. Insufficient or absence of bone healing is a frequent problem within all surgical fields. This often necessitates treatment by various bone grafting procedures, utilization of osteopromotive membrane techniques, local delivery of growth-stimulatory factors, or compression-distraction procedures. Based on the previously recognized positive osteogenic results of gastric pentadecapeptide BPC-157 on non-union fracture, segmental osteoperiosteal bone defect, its pure peptidergic effect with no carrier interference, together with gastric epithelial cells induced osteogenesis, and a hypothetical gastric hormone opposing bone disturbances development, the aims of the present study were to further develop a possibility of osteopromotion by various routes (Bone 24 195, 1999). Transosteal defects were performed proximal to the entry of the inferior alveolar artery in the left rat mandibular ramus using extraoral approach. Rats received agents (i) BPC 157 10 microg, 10 ng/kg intraperitoneally immediately after the injury, or (ii) BPC-157 2 microg, 2 ng/ml (1 ml bath) locally at the injury site. The effects were assessed at 3 or 10 days post injury using

densitometry assessment. Results indicate that gastric pentadecapeptide BPC-157 given either systemically or by local application significantly improves transosseous mandibular defect healing (Table 1).

Table 1. Gastric pentadecapeptide BPC-157 given either systemically or by local application significantly improves mandibular defect healing assessed by densitometry at 10th post-injury day (median/minimum/maximum) (relative to standard density marker).

Transosseous rat mandibular defects	**Intraperitoneal application**	**Local application**
Control 0.9%NaCl	0.42 (0.41-0.42)	0.42 (0.41-0.42)
BPC 157 ng	0.47 (0.44-0.50) *	0.46 (0.44-0.51)*
BPC 157 microg	0.56 (0.54-0.60) *	0.57 (0.54-0.61) *
* p<0.01 vs. control		

P108 S

GASTRIC PENTADECAPEPTIDE BPC 157 IMPROVES HEALING AND FUNCTION RECOVERY OF GASTROCNEMIUS COMPLEX MUSCLES (GASTROCNEMIUS, SOLEUS AND PLANTARIS) (GCC) AFTER SINGLE CONTUSION IN RAT

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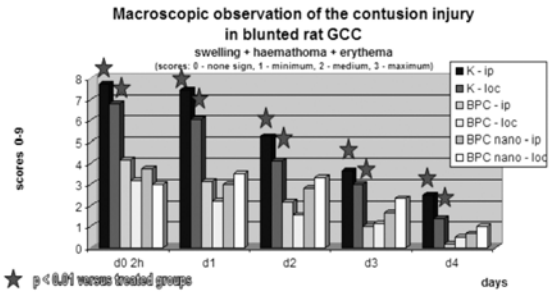
Gastric pentadecapeptide BPC 157 is stable peptide currently in clinical phase II for inflammatory bowel disease (PL-10, PLD-116, PL-14736, Pliva, Croatia) that heals internal and external wounds (i.e., Burns 27 817, 2001, Bone 24 195, 1999). We investigate whether this peptide improves healing and function recovery of rat right gastrocnemius complex muscles (gastrocnemius, soleus and plantaris) (GCC) after single contusion, produced by the impact of a blunt nonpenetrating object (drop-mass technique (150 g, height 49 cm, spherical radius 8 mm). Wistar male rats received agents (once daily, (i) systemically BPC 157 10 μ/kg or 10 ng/kg intraperitoneally or (ii) topically, 1, 0.1 or 0.01 μ/g neutral cream, saline (5.0 ml/kg i.p.), neutral cream (Belobaza, Belupo, Croatia), or nothing (controls)) first application immediately following injury, last 24 h before sacrifice (at 2 hr, 1, 2, 4, 7 and 14 days post-injury). Full assessment (walking track analysis computing sciatic function index (SFI) (Plast Reconst Surg 83 129, 1989), more quantitative recent test of motor recovery, the extensor postural thrust (EPT) (Exp Neurol 168 192, 2001), macroscopic assessment of injured site (haematoma+edema+hyperaemia), haematoma on the surface of the GCC, GCC maximum circumference, GCC weight, and microscopy (vimentin, desmin) reveals gastric pentadecapeptide BPC-157 healing and function recovery at all of the tested intervals (Figs.. In conclusion, these results indicate that in animal blunt injury model this suitably stable gastric pentadecapeptide BPC-157 given either system or by local application may be beneficial to healing muscle.



P109 W

GASTRIC PENTADECAPEPTIDE BPC 157 ANTAGONIZES FUNCTIONAL DISARRANGEMENT AND PRESERVES FUNCTION IN OSTEOARTHROTIC RATS FOLLOWING TEMPOROMANDIBULAR JOINT INJURY

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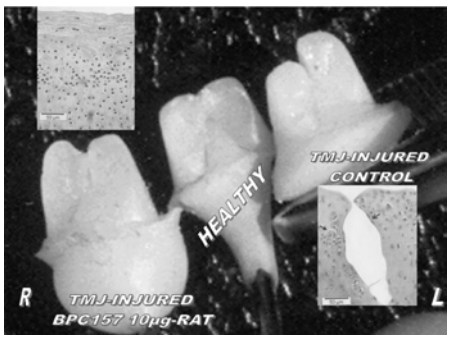
Gastric pentadecapeptide BPC 157 (PL-10, PLD-116, PLD-14736, Pliva, Croatia, in clinical phase II for inflammatory bowel disease) prevents osteoarthrosis in rat after temporomandibular joint injury (Bone 2001;28(5):S107). Now, we demonstrate fully preserved function at the end of the experiment (at 6 months) presents with BPC 157 (10, 1.0, 0.1, 0.01, 0.001 microg /kg i.p.) (unlike saline 5.0 ml/kg i.p.) given immediately after surgery (Figure 1). Impressions of upper and lower incisors were taken, using monophase, single jaw impression technique from controls and pentadecapeptide BPC 157 rats and normal, non-operated rats. Individual trays from polymetyl methacrilate (PK Tray, Hereus-Kulzer, Germany) were made, and filled with low viscosity (injection type), addition curing polyvinyl siloxane impression material (Silagum - AV Quick Light, DMG, Germany). Casts were made from liquid, light curing, micro hybrid composite (Tetric Flow, with Heliolux GTE Ivoclar,Vivadent, Liechtenstein), thickness of each layer 2mm, curing time 20 sec per layer, oriented to each other using wax (Beauty Pink Wax, GC, Japan) bite records. Assessed were (i) rats (16 per group) with upper incisors abnormalities (16 control rats present upper incisors with asymmetrical contour, uneven and heavier abrasion with shorter right incisor, rough incisal edges with sharp proximal angles, palatal surface roughness, scratches and cuts, and incisal edge initial contact, features fully avoided ($p<0.001$) in BPC 157 10-0.01 micrograms-groups; congruency with incisal third of vestibular surface of lower incisors or initial contact on incisal rim of palatal surface are not present in controls, while commonly noted ($p<0.001$) in BPC 157 10-0.01 microg-groups); (ii) uneven upper incisors loss of hard dental tissue (25.5/21/30 (median/minimum/maximum) (% of healthy)) in controls is fully abolished ($p<0.001$) in BPC 157 10-0.1 micrograms groups; (iii) diasthema on lower incisors 599/585/620 in controls is completely reversed ($p<0.001$) in BPC 157 10-0.001 micrograms groups.



P110 F

GASTRIC PENTADECAPEPTIDE BPC 157 IMPROVES ANATOMICAL AND FUNCTIONAL RECOVERY IN THE MODEL OF RAT HINDLIMB ISCHEMIA

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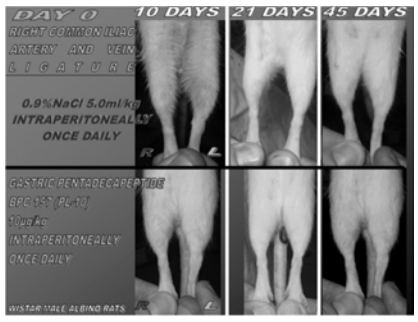
Gastric pentadecapeptide BPC 157 is stable peptide currently in clinical phase II for inflammatory bowel disease (PL-10, PLD-116, PL-14736, Pliva, Croatia) that heals internal and external wounds (i.e., Burns 27 817, 2001, Bone 24 195, 1999). We investigate this peptide in improved healing and function recovery of rat hind leg using ligation of right common iliac artery and vein just below aortic bifurcation. Wistar male received agents (once daily, intraperitoneally, /kg) (BPC 157 (10 microg, 10 ng, 10 pg), saline (5 ml)), first application 1 hr after ligation, last 24 hrs before assessment. Full assessment (walking track analysis (modified sciatic function index (SFI) (Plast Reconst Surg 83 129, 1989)), new test of motor recovery, the extensor postural thrust (EPT) (Exp Neurol 168 192, 2001), macroscopic assessment of injured site, transcutaneous pulse oxygen saturation, on both hind legs and X-ray evaluation

of femurs growth retardation) revealed gastric pentadecapeptide BPC-157 healing and function recovery at all of the tested intervals. For instance, assessment of injured site, walking analysis, muscular strength, pulse oxygen saturation were better in all BPC 157 groups ($p<0.01$). Ligated leg atrophy and femoral bones growth retardation were fully avoided in pentadecapeptide BPC 157 groups ($p<0.01$).



P111 S

STABLE GASTRIC PENTADECAPEPTIDE BPC157 AND EARLY TENDON HEALING TO BONE INTERFACE IN RAT

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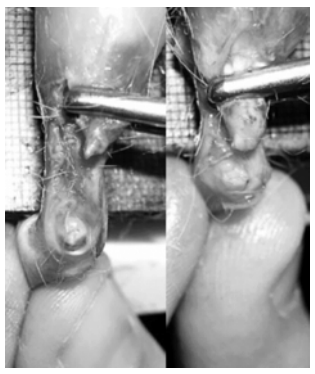
Recently, a stable gastric pentadecapeptide BPC157 (GEPPPGKPADDAGLV, M.W. 1419) (currently in clinical trials for inflammatory bowel disease (PLD-116, Pliva, Croatia)), improves healing of transected Achilles tendon in rat (Bone 28 S107, 2001). To this end the tendon healing to bone is further investigated, particularly with respect to critical early period (1-4 days following Achilles tendon is dissected carefully from calcaneal insertion), and these findings outline early tendon healing to bone interface. Rats received agents (/kg, intraperitoneally, once time daily) (BPC 157 (dissolved in saline, with no carrier addition) (10 microg, 10 ng or 10 pg) or saline (5.0 ml)), first application at 30 min post-surgery, last at 24 h before autopsy. The defect between Achilles tendon and calcaneal bone was measured at day 1 and 4 respectively. In BPC 157 rats present with significantly shorter gap in day 4. At the first post-operative day, the tendon edge was adherent to adjacent muscle with early granulation tissue formed. Furthermore, the gastrocnemial muscle circumference was significantly lower in non treated rats that can be a sign of muscle atrophy. The morphological characteristics of the healing tendon to calcaneal insertion were evaluated at 1, 4, 7, 10 and 14 days following Achilles tendon dissection. BPC 157-treated rats have more mononuclears and less granulocytes ($p<0.01$), subsequently presenting with reticulin fibers denser and more regular distribution, higher fibroblasts number, denser and a more regular distribution of collagen fibers following the distribution of reticulin fibers. Unlike BPC 157 rats, also perpendiculary transected gastrocnemial muscle in non treated rats showed muscle filament atrophy. Together, pentadecapeptide BPC157, ascertaining suitable delivery, favors routine application in future Achilles tendon therapy.

Figure 1. Presentation at 4 post-operative day: 6mm defect between tendon edge and calcanear insertion (control, left); 3mm defect with early granulation tissue at tendon edge (BPC 157 microg, right).



P112 W

STABLE GASTRIC PENTADECAPEPTIDE BPC-157 HEALS TRANSECTED MUSCLE IN RAT

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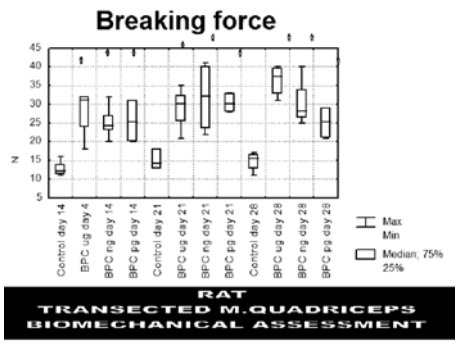
Gastric pentadecapeptide BPC 157 is stable peptide currently in clinical phase II for inflammatory bowel disease (PL-10, PLD-116, PL-14736, Pliva, Croatia) that heals internal and external wounds (i.e., Burns 27 817, 2001, Bone 24 195, 1999). Recently, it improves healing of transected Achilles tendon in rat (Bone 28 S107, 2001). We investigate whether this peptide improves healing and function recovery of rat right quadriceps muscle following complete transection. Wistar male rats received agents once daily, intraperitoneally, BPC 157 10 microg/kg or 10 ng/kg or 10 pg/kg or saline (5.0 ml/kg) (controls), first application 30 min following injury, last 24 h before sacrifice (at 4, 7, 14, 21, 28, 72 days post-injury). Full assessment (i.e., walking track analysis biomechanical analysis, macroscopic assessment of bridging fragment between cut muscle ends, atrophy of distal part of transected muscle and microscopy (HE, Gomori silver stain,Van Gieson staining, vimentin, desmin)) reveals gastric pentadecapeptide BPC-157 healing and function recovery at all of the tested intervals (Fig.). In conclusion, these results indicate that this suitably stable gastric pentadecapeptide BPC-157 heals transected muscle.



P113 F

THE PRODUCTION OF CATHEPSIN K, RANKL AND OSTEOPROTEGERIN IN PRIMARY INTERFACE TISSUE FIBROBLASTS OF ASEPTIC LOOSENED TOTAL HIP REPLACEMENT PROSTHESES

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Aseptic loosening of the prosthesis is the most common complication in total hip replacement surgery. Bone resorption around implant leads to prosthesis loosening. Osteolysis is most probably caused by acidification and cathepsin K production in interface tissue between the implant and bone. These are also the two most important steps in normal osteoclast bone resorption cycle. The reason for cathepsin K production and giant cell formation might be RANKL production in interface tissue. RANKL-RANK interaction then stimulates osteoclast formation. We show by immunohistochemical and quantitative RT-PCR method that both RANKL and RANK are produced in interface tissue. They can interact without interference of OPG, which is present only in the endothelial cells of the interface tissue. Immunofluorescence, immunoprecipitation, ELISA and quantitative RT-PCR show that fibroblasts derived from interface tissue can produce RANKL *in vitro*. In this respect they are as capable of stimulating osteoclast formation as osteoblasts. We did not find any statistical difference in RANKL production after several cytokine stimulations. In contrast, OPG release is regulated by different cytokines. TNF-alpha and parathyroid hormone (PTH) stimulations lead to the highest OPG production in both interface tissue fibroblasts and in osteoblasts but only in osteoblasts is the increase statistically significant.

We also demonstrate that interface tissue fibroblasts secrete active cathepsin K. The reason for cathepsin K production is unclear and it seems that the regulation is quite weak. No marked changes are detected after different stimulations. It has been thought that only osteoclasts are a source of cathepsin K. Recently it has been shown that fibroblasts from rheumatoid and osteoarthritic synovium are capable of cathepsin K production. Osteoblasts might be able to produce cathepsin K since they can resorb bone to some extent. Osteoblasts and fibroblasts originate from same stem cell.

Therefore it is reasonable that fibroblasts are also capable of cathepsin K production. In interface the role of fibroblast cathepsin K is clear: in acidic pH it can directly degrade type I collagen in demineralized bone. Thus, interface tissue fibroblasts have both indirect and direct effect on bone destruction in aseptic loosening.

P114 S

ZOLEDRONIC ACID IMPROVES FEMORAL HEAD SPHERICITY IN A RAT MODEL OF PERTHES DISEASE

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An optimal treatment strategy for Perthes disease currently remains controversial. We hypothesized that zoledronic acid (ZA), a potent bisphosphonate, could maintain femoral head sphericity in Perthes disease by changing the balance between bone resorption and new bone formation. The aim of this investigation is to test the effect of ZA in an established model of Perthes disease - the spontaneously hypertensive rat (SHR).

Male, 4-week old rats were divided into 3 groups of 40: saline monthly, 3 doses of 0.05 mg/kg ZA monthly, or 10 doses of 0.015 mg/kg ZA weekly. Following euthanasia at 15 weeks of age, high-resolution Faxitron radiographs were taken. A modified epiphyseal quotient (height/width) was measured by a blinded observer. Specimens were processed for histology.

Radiographs revealed less osteopenia in the femoral heads in treated groups. DXA measurements documented treated femoral head BMD was increased 20% over controls ($p<0.01$). Epiphyseal Quotient (EQ) was significantly improved in the treated group ($p<0.01$). The proportion of 'flat' heads (EQ < 0.40) was also significantly reduced from 45% to 16% ($p<0.01$).

Histological classification revealed that some femoral heads were affected by osteonecrosis, some by ossification delay, and others by both. There was no difference in the prevalence of osteonecrosis as assessed by histological analysis for control and ZA treated groups. Further analysis of EQ revealed that there was a significant improvement in sphericity with ZA treatment in femoral heads affected by osteonecrosis. ($p<0.01$) The prevalence of ossification delay was also significantly reduced in ZA treated animals ($p<0.01$).

Bone volume/total volume (BV/TV) was higher in ZA treated on the basis of significantly higher trabecular number (TbN) ($p<0.01$). Trabecular thickness (TbTh) was significantly higher in controls ($p<0.05$), it is possible that remaining trabeculae hypertrophy but not enough to prevent deformation. Bone formation rate (BFR) was significantly higher in controls, however not enough to maintain shape ($p<0.01$)

Zoledronic acid favourably altered femoral head shape in this spontaneous model of osteonecrosis in growing rats. Translation of these results to Perthes disease could mean that deformity of the femoral head may be modified in many children and thus reduce the need for surgical intervention in childhood and adult life.

P115 W

DELAYED ERUPTION OF THE PERMANENT TEETH IN A 57-YEAR OLD MAN WITH HYPOTHYROIDISM

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Delayed dentition is one of the symptoms observed in patients with congenital or juvenile hypothyroidism. We report here a 57-year-old man with untreated hypothyroidism in whom many deciduous teeth had been retained until middle age. On admission, he had typical myxoedematous appearance with height of 138cm (less than minus 3SD) and weight of 58.5kg. There was no pitting edema on his extremities. His only complaint was inability to open his eyes due to profound lid edema (blepharoptosis). According to his elder brother, his birth and early development was normal except that he was the shortest in his class when entered an elementary school at age 6. He was not mentally retarded. He had no medical problems and stayed healthy until age 45, after which he became edematous. Although the facial and lid edema worsened with time, he never sought medical attention. Family history was negative for consaguinous marriage, short stature, or hypothyroidism. Laboratory data (fT4 0.07 ng/ml, fT3 0.85 pg/ml, TSH 251 mU/l) established the diagnosis of primary hypothyroidism. Anti-thyroid autoantibodies were negative. Serum calcium, phosphate and PTH were within normal range. X-ray examination revealed retention of deciduous teeth and many impacted permanent teeth. MRI examination indicated atrophic or hypoplastic thyroid gland. Radioiodine uptake in the neck was low. After treatment with L-thyroxine, thyroid hormone levels were normalized and serum biochemical markers reflecting bone turnover increased gradually. However, none of his deciduous teeth fell off during 5 years of observation in the euthyroid state. This case is unusual in that his hypothyroidism remained undiagnosed and untreated such a long time. From the history taking, it was difficult to determine when his hypothyroidism started and how severe had it been. However, his abnormalities in dentition provided useful information concerning the onset of hypothyroidism. Although spontaneous eruption of the permanent teeth was reported in patients with juvenile hypothyroidism and delayed dentition when properly treated in their teens or twenties, it was not the case with our patient in his late fifties.

P116 F

EFFECTS OF WEIGHT REDUCTION ON LEPTIN AND BONE MASS IN MORBIDLY OBESE ADOLESCENTS

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Background: Obesity in childhood and adolescence is a major public health concern in many western countries. In obese adults, weight loss accompanying a bone mass loss. Adolescence is a spurt growth period of bone mass. Little known about the effects of weight loss on bone mass in obese adolescents. Leptin secreted by adipose tissue and play an important role in obese and bone formation.

Objective: To determine the effects of a marked weight loss on primary morbidly obese adolescents.

Methods: Thirty three girls and 22 boys, aged 13.4 ±3.6 y with BMI 34.6 ±3.6 kg/m^2 were included in a 9 ±3 months multidisciplinary weight reduction programme including a slight caloric restriction and regular submaximal physical training. Total and regional bone mass (assessed by DEXA) and biological parameters (calcium, phosphorus, leptin, vitamin D) were measured before and after weight loss.

Results: Mean weight loss is 23.5 ±8.9 kg ($p<0.0001$), growth is 2.7 ±1.6 cm ($p<0.01$). Total BMC decreases (253 ±131 g, $p<0.001$) whereas spinal BMC increases (6.5 ±4.0 g, $p<0.01$), and femoral neck BMC does not vary significantly. Total and femoral neck BMD do not vary whereas spinal BMD increases. Leptin is higher in girls than in boys and decreases dramatically after weight loss (27.2 ±12.1 and 17.4 ±7.8 micromol/l in girls and boys, respectively). The variation of plamastic and urinary calcium and phosphorus is not significant. There is any correlation between BMC and BMD with leptin before and after weight loss. There is an inverse correlation between total BMC and 25 OHD before weight loss ($r = 0.45$, $p<0.001$).

Conclusion: Total and regional BMD and regional BMC maintain whereas total BMC decreases suggest the limitation of interpretation of DEXA in assessing bone mass during a marked weight loss. Leptin is not correlated with any bone mass variables in our morbidly obese adolescents.

P117 S

USE OF VEIN GRAFT AS A TENDON SHEATH SUBSTITUTE FOLLOWING TENDON REPAIR: AN INNOVATIVE TECHNIQUE IN TENDON SURGERY

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OBJECTIVES: This is a new technique for managing tendon repair which can improve the results of existing methods.

METHODS: 105 patient with new or old tendon injuries or complications of previous repair underwent tendon repair by modified Kessler method and a portion of the saphenous veins was used to cover the repaired tendon. 90 patient had flexor tendon injuries which involved zone 1 to 5, and 15 patient had extansor tendon injuries (zone 5 t0 7). A modified Kessler technique with 3-0 prolene was used for the core suture. Afterwards, a running 6-0 nylon or prolene epitendinous suture was used to even the repair site. After the tendon repair, a segment of vein which the tendon has been passed through prior to the repair was used as a tendon sheath substitue. A 6-0 prolene was used for anastomosis of the proximal and distal ends of the sheath defect to an interposed segment of autogenous or frozen vein.

RESULTS: Our preliminary results appear encouraging when compared with outcomes achieved by conventional tendon repair techniques.

CONCLUSIONS: Because this technique reduce the adhesion formation, and also improve tendon nurishment, and also decrease the need of intensive physiotherapy, it can be a standard choice in the future.

P118 W

ORIGINAL PREPARATION FOR TREATMENT OF BONE FRACTURES

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Objective: Today hypothesa about existense over life persist mezenchimal cells has many confirmations. Previously experiments on rats with traumatic fractures of long bones (analog bamp-fracturae) showed near twice improving bone (osseal and marrow) regeneration by the cortilage tissue with next terminal differing and formation bone marrow chanal and periostal muff after using xenogenic immunoglobulin (XIG) with activity to mathering cells (proposed persist mezenchim cells).

Aim: The immunoglobulin preparation (IP) for regulation human pregenitor cells, directed common to osseal tissues and marrow cells.

Methods. Immunological, clinical, rentgenological.

Results: 4 middle age patients, after small effective 3-4 years traditional therapy by posttraumatic osseal damages, one case was complicated by subacute osteomyelitis, were treated XIG. Course treatment included 3 intravenous injections during one

week in dosage 0.05-0.1 mg/kg. Disappearance of inflammation, stoppage of purulence and sclerostenosis of fistulas where obtained during 1.5-2 month. Both restoration supportive function and tissue defects of extremities was found to 4-6 month after injections XIG. X-ray monitoring shown normal bone structure with forming cortical plate. There were no marked any complications and reactivations of illness from curing to present time (1-1.5 years). Any additive drugs were not applicaed.

Conclusion: We proposed that XIG can to treat bont fractures both as stimulator of immunity (by activation bone marrow cells) and as means to restauration of normal structure osseal tissues in locuses of ald fractures.

P119 F

SERUM OSTEOPROTEGERIN (OPG) LEVELS IN CHILDREN WITH JUVENILE IDIOPATHIC ARTHRITIS (JIA)

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Bone erosion is a common and most devastating manifestation in children with JIA. Since the pathophysiology of the disease is not well understood, the processes underlying bone loss in these patients have not been clarified yet. However, the identification of OPG and receptor activator of NF-kappa B ligand (RANKL) as the central regulator of osteoclast recruitment and activation has shed light on the pathogenesis of bone and joint damage in rheumatic diseases. The osteoclast is now recognized as one of the pivotal effector cells in rheumatoid arthritis (RA). Moreover, studies in adult RA patients show that serum OPG levels are elevated compared to normal controls. This paradigm may provide insight into the skeletal pathology of JIA as well.

We therefore evaluated serum OPG levels in JIA patients and sex and age matched controls by ELISA. Relation to disease activity by clinical and radiologic assessment (radiometacarpal length/second metacarpal length) was also evaluated. All patients were under steroid and/or methotrexate therapy.

Contrary to the RA data, serum OPG levels in JIA patients did not differ from that of controls. However, when the patients were evaluated according to onset type, polyarticular RF positive patients showed significantly lower levels of OPG compared to sex and age matched controls (46.0 ±2.8 vs. 99.0 ±12.7 pg/ml; $p<0.05$) while systemic patients did not show any significant difference compared to their counterparts. Systemic patients were further evaluated according to their course. Patients with progressive bone erosion despite intensive therapy exhibited significantly higher OPG levels compared to those with oligoarthritis, well controlled polyarthritis, or controls (102.0 ±16.9 vs. 54.0 ±8.5 vs. 62.0 ±5.3 vs. 76.3 ±16.0 pg/ml; $p<0.05$). No significant relation was found between OPG levels and disease activity within each individual, though in those with systemic onset, levels of this cytokine tended to be higher when arthritis was well controlled.

These results suggest that in systemic patients, OPG may be a compensatory response to increased osteoclast activity, while in polyarticular patients, lack of adequate feedback may lead to refractory bone erosions.

P120 S

FRESH FEMORAL NECK FRACTURES TREATED BY OSTEOSYNTHESIS AND FIBULAR AUTOGRAFT

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Introduction: Femoral neck fractures in younger adults have a poor prognosis because of high incidence of non-union and aseptic necrosis. Prosthetic replacement of the femoral head is reserved for the older patients. Osteosynthesis is the treatment of choice for displaced fracture neck femur in younger adults. Various types of bone graft supplementation have been advocated to reduce the incidence of nonunion and avascular necrosis. We tried cannulated cancellous screw fixation supplemented by primary fibular autografting in femoral neck fractures to overcome the problems of nonunion and avascular necrosis.

Materials and methods: Thirty-four (M:17, F:17) skeletally mature patients (mean age 49.38 years) of fresh femoral neck fracture were treated by closed reduction and one/two cancellous screw fixation along with ipsilateral fibular autograft between January1999 to September 2002. Weight bearing was allowed only after 3 months, or later if the radiological signs of union was not seen.

Results: Twenty-two patients who had completed a follow-up of two years (mean-26.7 months) were analysed. Twenty of the twenty-two patients showed union. In two patients the fracture did not unite. Only two cases showed asymptomatic avascular necrosis. Fibular graft was broken in three cases, screw back out with collapse was seen in two cases. Post-operative radiographs showed penetration of the articular surface by screw and graft in one cases each .

Conclusion: Cancellous screw fixation and primary fibular autografting is a safe, effective and reliable surgical technique for treating fresh femoral neck fractures in younger patients.

P121 W

ABNORMAL DIFFERENTIAL GROWTH - ASYCHRONOUS ENDOCHONDRAL AND MEMBRANOUS OSSIFICATION OF THE SPINE IN ADOLESCENT IDIOPATHIC SCOLIOSIS? A MRI STUDY

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Introduction: During adolescent ages, the longitudinal growth of the anterior column is contributed by the growth plates of the vertebral bodies through endochondral ossification and continues until 16 to 18 years. In contrast, the endochondral ossification of the posterior elements terminates by the end of the first decade. After that the posterior elements grows only circumferentially through membranous ossification. The aim of this study was to investigate the role of synchronous coupling of endochondral and membranous bone formation in the normal growth of the spine during adolescent age, and whether loss of the synchronous growth might be associated with developmental deformities of the spine, such as in idiopathic scoliosis.

Methods: A comparative study of magnetic resonance imaging (MRI) vertebral morphometry of thoracic vertebrae between adolescent girls with idiopathic thoracic scoliosis and age and gender matched normal subjects was conducted. Whole Spine MRI was performed on 83 adolescent girls (12-14 years of age) with idiopathic scoliosis (Cobb's angle 20-90 degrees) and 22 age matched normal subjects. Multiple measurements of each thoracic vertebra were obtained from the best sagittal and axial MRI cuts.

Results: In comparison with normal subjects, scoliotic thoracic spine showed consistently longer vertebral bodies from T1 to T12 in the anterior column and significantly shorter pedicles with larger interpedicular distance in the posterior column. The differential growth between the anterior and the posterior elements of each thoracic vertebra in scoliosis patients was significantly different from that in normal subjects ($p < 0.01$). Significant positive correlation between scoliosis severity score and the ratio of differential growth of anterior to posterior spinal column for each thoracic vertebra was also demonstrated ($p < 0.01$).

Conclusion: Synchronous coupling of endochondral and membranous bone formation plays an important role in the normal growth of the spine during adolescent age. Developmental deformity of the spine, such as idiopathic scoliosis is associated with loss of synchronous endochondral and membranous ossification. Comparing with age matched normal subjects, patients with idiopathic scoliosis have disproportional faster longitudinal growth of vertebral body, which occurs mainly through endochondral ossification. In contrast, the circumferential growth via membranous ossification is retarded in both the vertebral bodies and pedicles.

P122 F

MICE WITH A TARGETED DELETION OF THE TETRANECTIN GENE EXBIT A SPINAL DEFORMITY

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Tetranectin is a plasminogen-binding, homotrimeric protein belonging to the C-type lectin family of proteins. Tetranectin has been suggested to play a role in tissue remodeling, due to its ability to stimulate plasminogen activation and its expression in developing tissues such as developing bone and muscle. To test the functional role of tetranectin directly, we have generated mice with a targeted disruption of the gene. We report that the tetranectin-deficient mice exhibit kyphosis, a type of spinal deformity characterized by an increased curvature of the thoracic spine. The kyphosis angles were measured on radiographs. In 6-month-old normal mice ($n = 27$), the thoracic angle was 73 degrees, while in tetranectin-deficient 6-month-old mice ($n = 35$), it was 93 degrees ($P < 0.0001$). In approximately one-third of the mutant mice, X-ray analysis revealed structural changes in the morphology of the vertebrae. Histological analysis of the spines of these mice revealed an apparently asymmetric development of the growth plates appeared disorganized and irregular, with the disk material protruding through the growth plate. Tetranectin-null mice had a normal peak bone mass density and were not more susceptible to ovariectomy-induced osteoporosis than were their littermates as determined by dual-emission X-ray absorptiometry scanning. These results demonstrate that tetranectin plays a role in tissue growth remodeling. The tetrancectin-deficient mouse is the first mouse model that resembles common human kyphotic disorders, which affect up to 8% of the population.

P123 S

PTH MAY RESCUE THE DISTURBED BONE GROWTH IN FIBROBLAST GROWTH FACTOR RECEPTOR 3-RELATED DISORDERS

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Achondroplasia (ACH) and its severe type, thanatophoric dysplasia (TD), are caused by constitutively activated mutations in FGFR3. The excessively activated FGFR3 is known to inhibit the proliferation of chondrocytes, resulting in disturbing the growth of long bones. Recently, we have reported that an excessive activation of FGFR3 by the mutations accelerated differentiation and increased apoptosis of chondrocytes, and that exogenous addition or endogenously controlled expression of PTH or PTHrP rescued cells from these conditions. To explore further mechanisms and new therapeutic applications, we performed organ culture experiments.

Methods: By using an in vitro organ culture system and adenoviral vectors carrying FGFR3 mutation, K650E (Ad-TD) and wild type (Ad-WT), we examined bone growth and the influence of PTH on bone growth. 1. The fetal mouse femurs which were dissected from ICR mice embryo at d.p.c.15 were infected by incubating with vehicle, Ad-WT or Ad-TD at MOI 18 for 2.5 days. After infection, they were transferred into serum-free medium. Before and after the 3d- and 6d-culture, we measured each longitudinal length and performed histological analysis on the infected femurs. 2. We added PTH on Ad-WT infected femurs, and compared the length of femur cultured with PTH to femur length without PTH at 3day, and 6day after culture.

Results: 1.There were significant differences ($p<0.05$) in an increase in bone length at 3d and 6d after culture between Ad-TD and Ad-WT infected femurs (32.9±10.5% at 3day and 66.9±14.4% at 6day of the growth rate of the Ad-WT infected femur). Ad-TD infected femurs were significantly disturbed in longitudinal bone growth compared with Ad-WT infected ones. 2. Axial growth rates of the Ad-WT infected femurs cultured with 10^{-8}M PTH were 1.2 times at 3day ($p=0.036$), 1.16 times at 6day ($p<0.01$) higher than those of the Ad-WT infected femurs without PTH.

Conclusion: We confirmed that constitutive active FGFR3 mutation suppressed bone growth in organ culture experiments and that PTH partially rescued bone from the growth disturbance. Our results suggest that PTH may be effective for bone growth disturbance of chondrodysplasias such as ACH and TD.

P124 W

BISPHOSPHONATES AND PROSTATE CANCER CELLS: CYTOSTATIC AND APOPTOTIC EFFECTS

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The bisphosphonate zoledronate has recently been shown to reduce the morbidity rate of metastatic bone disease from prostate cancer. As for breast cancer and myeloma, these effects are attributed to their powerful inhibitory activity on osteoclasts. In osteoclasts, clodronate (Clod) can be metabolized to a cytotoxic nonhydrolyzable analog of ATP, whereas aminobisphosponates such as pamidronate (Pam), ibandronate (Iban), and zoledronate (Zole) inhibit the mevalonate pathway. We and others have shown that bisphosphonates can also induce human breast cancer cell death in vitro (Fromigué at al., JBMR 2000). We have investigated the biological effects of these four bisphosphonates on PC-3 prostate cancer cells after 1 to 6 days of incubation. Cell survival, evaluated by MTT assay, was not affected by Clod, whereas MTT values decreased in a time and dose-dependent manner with Pam, Iban and Zole. The largest effect was observed at day 6 with Zole at 10^{-4}M. Inhibition of cell growth was confirmed by measurement of total DNA content. The percentages of cells in G0/G1 and S,G2/M phases of the cell cycle were determined by FACS analysis after 1, 2 and 4 days. Clod and Pam had no significant effects. By contrast, 10^{-4}M Zole and Iban produced cell accumulation in G0/G1 phases after 1 and 4 days of exposure, respectively. The percentages of annexin-positive cells (apoptotic cells) were also determined in cultures treated with 10^{-4}M bisphosphonates for 1 to 6 days. Clod had no effect, Iban induced a 1.3-fold increase in the percentage of annexin-positive cells, while Pam and Zole doubled this percentage, indicating a larger apoptotic effect of these two compounds. In summary, Clod had neither cytostatic nor cytotoxic effects in PC-3 cells. By contrast, aminobisphosphonates, especially Zole, induced cell apoptosis. Moreover, Iban and Zole also exerted cytostatic effects. Our observations suggest that bisphosphonates act differently on neoplastic cells, depending on tumor type. Moreover, such effects could contribute to the beneficial effects of bisphosphonates in patients with bone metastases.

P125 F

PSA-PEAK FOLLOWING I.V. BISPHOSPHONATE IN PROSTATIC CANCER METASTAZISING TO THE BONE

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Introduction and Objective: An increase in total prostate-specific antigen in serum (tPSA) is not only seen in progressive disease but also following treatment of prostatic cancer (PCa) with radiation (PSA-bounce). We observed a tPSA raise in patients with metastatic PCa to the bone following a single dose of i.v. bisphosphonate (zoledronic acid). To substantiate this observation we started a prospective study.

Methods: In 7 consecutive patients with non-progressive prostate cancer with metastasis to the bone the tPSA level in serum was measured before and after i.v. application of 4 mg zoledronic acid.

Results: In 4 out of 7 patients tPSA raise from 0,2, ,5, 3,5 and 2,4 ng/ml to 0,8, 2,0, 5,7 and 4,8 ng/ml respectively within 4 weeks. In a prospective study this observation will be further analysed by measuring the tPSA level the day before and 1 and 4 weeks after the first application of 4 mg of zoledronic acid.

Conclusions: I believe that for the first time a peak of tPSA following i.v. bisphosphonate therapy in metastatic PCa to the bone was observed. This could confirm the in vitro observation that (amino-)bisphosphonates have a direct effect on tumor cells. A larger number of patients in a prospective study will determine the value of early PSA-monitoring following bisphosphonate therapy (PSA-peak).

P126 S

RENAL TUBULAR FUNCTION AND BONE METABOLISM IN 22 WOMEN WITH CADMIUM-NEPHROPATHY: A 15-YEAR FOLLOW-UP STUDY

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Itai-itai disease is well known as a health hazard induced by cadmium (Cd) in the Cd-polluted Jinzu River basin in Toyama Prefecture, Japan. The main clinical features of Itai-itai disease are osteomalacia accompanied with osteoporosis, and multiple proximal renal tubular dysfunction. To clarify the pathogenesis of Itai-itai disease, renal tubular function and bone metabolism were examined twice with a 15-year interval for 22 female subjects. They were 73-80 years old and had lived in the Cd-polluted Jinzu River basin for more than 50 years at the time of the follow-up study in 2000. Multiple proximal renal tubular dysfunction was detected in all subjects showing increased fractional excretions of beta-2-microglobulin and uric acid, generalized aminoaciduria and renal gulucosuria. The mean creatinine clearance had significantly decreased from 62 ml/min in 1985 to 52 ml/min in 2000. Significantly increased excretions of urinary beta-2-microglobulin from 7.9 to 14.7 mg/g creatinine and urinary glucose from 124 to 636 mg/g creatinine were noted after 15 years. Pseudofracture, which is the characteristic sign of osteomalacia, was noted in four subjects on X-ray examination in 2000. In these three subjects, the glomerular filtration rates and the serum concentrations of phosphorus were more markedly decreased after 15 years compared to the other 18 subjects. Serum levels of 25-hydroxyvitamin D (25OHD), 1,25-dihydroxyvitamin D [1,25(OH) $_2$D] and intact PTH, and several bone biochemical markers in serum and urine were measured at the follow-up study. Significantly increased values were detected in serum levels of intact PTH and bone alkaline phosphatase and urinary excretion of type I collagen cross-linked N-telopeptides (NTx) in the 4 subjects with osteomalacia compared to the 18 subjects without osteomalacia. The mean level of 1,25(OH) $_2$D was lower in the 4 subjects with osteomalacia than in the 18 subjects without osteomalacia (38 vs. 51 pg/ml). The serum levels of 25OHD were not different between the two groups with or without osteomalacia (28 vs. 25 ng/ml). Thus, we speculated that decreases in the glomerular filtration rates and the serum concentration of phosphorus might play principal roles in the development of osteomalacia in Cd-induced nephropathy.

P127 W

BONE MINERAL DENSITY IN ADULT PATIENTS WITH END-STAGE RENAL DISEASE BEFORE THE START OF DIALYSIS

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Bone disease is one of the major causes of morbidity in dialysis patients. Hemodialysis patients are at increased risk for bone loss and osteoporosis, however not much is known about the prevalence of osteopenia in patients with end-stage renal disease before dialysis. We investigated bone mineral density (BMD) using dual-energy absorptiometry at the levels of the forearm, lumbar spine and femoral neck in 64 patients with end-stage renal disease (23 men and 41 women) aged 41,3 ±10,9, before the start of dialysis. Osteoporosis was diagnosed (T score lower than -2,5) in 6 patients (9,4%) at the level of the forearm, in 4 patients (6,3%) at the level of the lumbar spine, and in 4 patients (6,3%) at the femoral neck. No one patient had

fractures before the study. The median Z score (age-related) at these sites were not significantly different from 0 (Z score of the reference population with normal renal funtion) and were 0,25 ±1,3 at the forearm, -0,23 ± at the lumbar spine and -0,25 ± at the femoral neck. Most of the studied patients had normal bone mineral density. The risk factors for reduced bone density were: a history of amenorrhea, prednison therapy, aging, smoking. The findings did not correlate with the form or duration of the underlying renal disease.

We conclude that the end-stage renal failure does not influence significantly bone mineral density in patients before dialysis. The risk factors for reduced bone mineral density and osteoporosis in this group of patients are the same as in general population.

P128 F

INSULIN-LIKE GROWTH FACTOR SYSTEM COMPONENTS AND THEIR CORRELATION TO BONE MARKERS IN HEMODIALYSIS PATIENTS

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Background: In recent years the insulin-like growth factor (IGF) system has been recognized to play a key role in regulating bone metabolism. Different studies demonstrated the influence of IGFs and their binding proteins (IGFBPs) on bone cell functions in a positive, e.g. free IGF-I, IGFBP-3 and -5, and in a negative manner, e.g. IGFBP-4. The role of the IGF system in hemodialysis (HD) patients is still in discussion.

Methods: In a 2 years prospective study IGF system components (free IGF-I, IGF-II, IGFBP-1 to -6) were investigated in the serum of 16 HD patients (age: 62 ±13 years / dialysis duration: 38 ±21 months) treated with low dialysate calcium (LDC, 1.25 mmol/l). Levels of IGF system components were determined regularly every 6 month in comparison to serum concentrations of the bone markers: cyclase activating and inhibiting parathyroid hormone (CAP, CIP), parathyroid hormone (Nichol's assay), bone alkaline phosphatase (BAP), pyridinoline (PYR), desoxypyridinoline (DPD), vitamin D (25-/1.25- vit. D3), aluminium, calcium, and phosphorus.

Results: Levels of IGFBP-1 correlated significant positively with those of PYR (r= 0,38; p<0.01) and DPD (r= 0,46; p<0.001), but IGFBP-4 concentrations showed a negative correlation to 1.25-vit. D3 (r= -0,44; p<0.001) and 25-vit. D3 (r= -0,21; p= 0.11). Free IGF-I levels are declined, if IGFBP-1 (r = -0,51; p<0.001) and IGFBP-4 (r= -0,43; p<0.001) increased. The ratio of CAP and CIP correlated with IGFBP-3 (r= 0,34; p<0.05).

Conclusion: In HD patients IGFBP-1 and -4 could be potential key regulators for osteoclast activity and vitamin D metabolism, respectively. Regarding the role of cyclase activating parathyroid hormone, our study demonstrates that an increased ratio of cyclase activating and inhibiting parathyroid hormone might increase IGFBP-3.

P129 S

EXAMINATION OF MEGALIN IN THE RENAL TUBULAR EPITHELIUM IN PATIENTS WITH DENT'S DISEASE

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Dent's disease is an X-linked renal tubular disorder characterized by low molecular weight proteinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis, and renal failure. This disease is caused by loss-of-function mutations in a renal chloride channel gene, CLCN5, which encodes a 746-amino acid protein (CLC-5) with 12 to 13 transmembrane domains. The Japanese variant of Dent's disease has been observed to be less severe. Recently, experimental results on the model mouse demonstrated that low molecular proteinuria and hypercalciuria in this disease are considered to be associated with metabolic disorder of megalin, which is a member of the LDL-receptor family and mediates the uptake of a wide variety of protein ligands in the renal tubular epithelium. To elucidate the roles of megalin in patients with Dent's disease, we examined megalin immunohistologically in the renal tubular epithelium of the biopsy specimens.

Case1 : Five-year old boy revealed asymptomatic proteinuria at the age of 3 with his urine beta 2-microglobulin of 121544 ng/ml. His DNA analysis showed heterozygous defect of all coding region of CLCN5 gene. Urinary Ca/Cr ratio was 0.23, but nephrocalcinosis was not detected. Renal biopsy specimens showed minimal alterations in light microscopy. The staining of megalin in the proximal tubules with immnofluorescence microscopy was significantly decreased compared to the normal controls.

Case2 : Nineteen-years old male revealed asymptomatic proteinuria at the age of 4 with elevated urinary beta 2-microglobulin. Renal biopsy specimens at the age of 9 years showed minimal alterations in light microscopy and no nephrocalcinosis was detected. His DNA analysis showed heterozygous CLCN5 mutation (frameshift deletion involving codon 728). The staining of megalin in the proximal tubules with immnofluorescence microscopy was similar to the normal controls.

These results indicated that the altered megalin metabolism may be related to the deteriorated renal reabsorption of low molecular weight proteins as well as the abnormal calcium metabolism observed in some patients with Dent's disease.

P130 W

RADIATION DOSIMETRY, ANAESTHESIA AND WELFARE OF RODENTS IN MICRO-CT *IN VIVO* SCANNING

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There is increasing interest in imaging bones in vivo by micro-CT in living rodents, to allow real time "before and after" or sequential analysis of the skeletal effects of an experimental regimen. However the animal's welfare must be ensured by micro-CT scanner and experimental design. Radiation doses must be below levels that would harm the animal. The number of general anaesthetic procedures endured must not cause excess stress and weight loss (Salmon et al. 2001). In vivo scanning of rodents imposes constraints on micro-CT scanner design: (a) rotating fixed source-detector assembly around immobile subject, (b) loss of image contrast due to x-ray absorption in soft tissue surrounding bone, (c) breathing and heart-related movement of the animal during scanning. None-the-less, we demonstrate that adequate resolution of trabecular bone structures can be achieved in rodents in vivo, without excessive periods of anaesthesia or excessive radiation dose. The scanner employed was the Skyscan 1076 (Skyscan 2002). 3D images with pixel size of 8.9 microns (Gaussian resolution of 12.5 microns) were obtained at the rodent hindlimb knee with local absorbed dose of about 0.6 Gray and whole body effective dose equivalent of 10 milliSieverts. This resolution allows structural histomorphometric analysis of rat and mouse trabecular bone. X-ray source shuttering can reduce dose by a further 30-50%. Physiological monitoring of breathing, heart rate and temperature, as well as monitoring the animal's well being, enables synchronization of x-ray image capture with the quasi-regular breathing movements. Sensitivity of micro-CT-measured bone parameters to changes in resolution and contrast is systematically evaluated. Connectivity parameters such as Euler connectivity are especially sensitive to resolution. It emerges that the fixed scanning parameters of the in vivo scanner are partly advantageous in that they impose standardisation on scanned images and calculated structural parameters of trabecular bone.

Genetics

P131 F

ESTROGEN-METABOLIZING GENE POLYMORPHISMS BUT NOT ESTROGEN RECEPTOR ALPHA GENE POLYMORPHISMS ARE ASSOCIATED WITH THE ONSET OF MENARCHE IN HEALTHY POSTMENOPAUSAL JAPANESE WOMEN

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Ages at menarche and/or at menopause and overall years of menstruation, which have major implications for the health of pre- and postmenopausal women, have strong genetic inclination. We aimed to identify genetic factors influencing the onset of menarche and natural menopause in a Japanese population by investigating the polymorphisms of estrogen receptor alpha (ER alpha) and estrogen-metabolizing enzyme genes. Three hundred and seventeen postmenopausal Japanese women aged 46 yr and over were enrolled in this study under informed consent. Genomic DNA was extracted from peripheral leukocytes and PCR-based restriction fragment length polymorphism (RFLP) assays were used to determine ER alpha; PvuII and XbaI, and estrogen metabolizing enzymes, CYP17; estrogen biosynthesis (high activity, A2/A2), CYP1A1; hydroxylation (high inducibility, vt/vt) and COMT; inactivation (low activity, L/L) genotypes. There were no significant differences in ages at menarche and natural menopause, and years of menstruation among each PvuII or XbaI genotype and seven combinations of PvuII and XbaI genotypes. We could find ages at menarche in women with A1/A2 (higher activity of CYP 17) (13.6±1.2 yr) were significantly earlier than in those with A1/A1 (lower activity of CYP 17) (14.1±1.3

yr). There were no significant differences in ages at natural menopause and years of menstruation among each CYP17, CYP1A1 or COMT genotypes. The small sample size of each combination of estrogen-metabolizing genotypes made it impractical to evaluate the effects of the interdependency of each genotype including extreme genotype categories such as A2/A2L/Lvt/vt vs. A1/A1H/Hwt/wt genotypes on ages at menarche and/or natural menopause. The results suggest that estrogen metabolizing CYP17 genotype influences on ages at menarche in healthy postmenopausal Japanese women.

P132 S

EFFECTS OF GENE POLYMORPHISMS ON BONE MINERAL DENSITY IN JAPANESE MALE HEMODIALYSIS PATIENTS

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Polymorphisms of the bone metabolism related genes has been linked to bone mineral density in postmenopausal osteoporosis. We examined the possibility that the bone mineral density in Japanese hemodialysis (HD) patients might be determined by such polymorphisms. The study consisted of 302 male HD patients with a mean age of 55.1 years (range 22 to 95), who are dialyzed three times a week for an average of 7.28 (range 0.2 to 24) years. We analyzed restriction fragment length polymorphisms at the vitamin D receptor (Apa I, Bsm I, Fok I, and Taq I), parathyroid hormone (Bst BI and Dra II), and calcium-sensing receptor (Bse RI) gene loci. Bone mineral density (BMD) was estimated at 1/3 of the radius and lumbar spine (L2-L4 and L3) using dual-energy X-ray absorptiometry (DXA). In addition, calcium, phosphorus, and intact PTH levels were measured.

Among these polymorphisms, the Bsm I-vitamin D receptor gene polymorphism was associated with lumbar spine bone density. BMD of lumbar spine was significantly ($P<0.05$) different between genotype groups (L2-4: BB 1.229±0.115, Bb 0.997±0.018, bb 1.003±0.012; L3: BB 1.229±0.136, Bb 1.002±0.019, bb 1.004±0.012; L3 lateral: BB 0.903±0.084, Bb 0.710±0.022, bb 0.706±0.012 g/cm^2).

The levels of serum intact PTH, calcium, and phosphorus did not differ significantly among male patients with different vitamin D receptor genotypes.

These findings suggest faster bone mineral loss in bb genotype of vitamin D receptor in male hemodialysis patients.

P133 W

ASSOCIATION STUDY OF POLYMORPHISMS OF THE ESTROGEN RECEPTOR (ER) GENE AND PARATHYROID HORMONE(PTH) GENE AND BONE MINERAL DENSITY IN BEIJING HAN WOMEN

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Objective: To study the relationship among XbaI and PvuII polymorphisms of the ER gene and PTH gene and bone mineral density in Beijing Han Women. Methods Bone mineral density(BMD) was measured at lumbar spine, proximal femoral and forearm by dual energy X-ray absorptionetry(DEXA) and ER and PTH gene was determined by polymerase Chain reaction-restriction fragment length polymorphism(PCR-RFLP) in 179 Beijing Han Women. Results The frequency of the ER genotype of XX, Xx, xx respectively was 0.302, 0.464 and 0.234 and PP, Pp, pp respectively 0.184, 0.464 and 0.352 in Beijing Han women. There was signficant difference between premenopausal and postmenopausal women in frequency of the XbaI genotype. The BMD of lumbar spine in postmenopausal women was significally lower than that in premenopausal Women ($P<0.01$) and the incidence of osteoporosis in postmenopausal women was 54.3%. Frequency of PTH genotyp of BB, Bb, bb was 0.074, 0.284 and 0.642 repectively. ANOVA analysis showed that the forearm BMD was associated with PTH gene. The women with bb genotype have higher BMD than Bb and BB ones. Logistic regression analysis indicated that there was a significant difference between normal and osteoporosis women with bb genotype. Conclusion Frequency of XbaI and PvuII genotype of ER gene in Han women was apparently different from that in other races. Menopause effected on the frequency of XbaI genotype. The XbaI and PvuII genotype of ER gene was nonasssociated with BMD and XbaI genotype was associated with weight and BMI. The weight and BMI of xx genotype was significantly higher than that of XX and Xx genotypes. The forearm BMD was associated with bb genotype of PTH gene. The forearm BMD of bb genotype was significantly higher than that of Bb and BB gentypes. This results indicated that b allele might have some protective effects to bone mass.

P134 F

INDEPENDENT GENETIC CONTROL OF BONE MINERAL DENSITY AND BROADBAND ULTRASOUND ATTENUATION IN POSTMENOPAUSAL WOMEN

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Epidemiology of osteoporosis has indicated that postmenopausal bone mass is under strong genetic control. Broadband ultrasound attenuation (BUA) has the potential to provide information on bone microarchitecture and bone structure, whereas DXA (dual energy X-ray absorptiometry) represents a method limited to the measurement of bone mineral density (BMD). The objective of the present study was to analyse whether the mentioned characteristics are determined by specific genes. We therefore compared polymorphic markers within several candidate genes in relation to BMD or BUA in a cohort of 114 postmenopausal women. ANCOVA revealed significant associations between body mass index (BMI)- and years since menopause (YSM)-adjusted BMD and FokI (Vitamin D receptor gene, VDR), AluI (Calcitonin receptor gene, CALCR) polymorphisms and ApoE(apolipoprotein E)4 allele ($p<0.007$, $p<0.02$, $p<0.003$, respectively). The calcaneal BUA, after adjustment to the same confounders, was significantly related to BsmI, ApaI and TaqI genotypes in the VDR gene ($p<0.02$, $p<0.0003$, $p<0.02$ ANCOVA, respectively) . The present data show that postmenopausal BMD and BUA are determined by different genetic markers in our population sample. In other words, bone quality and bone density may not share the common genetic control, but each of them is regulated in a separate way. Further studies are required to confirm this hypothesis.

P135 S

ASSOCIATION OF POLYMORPHISMS OF ESTROGEN RECEPTOR AND VITAMIN D RECEPTOR GENE WITH PEAK BONE MASS IN SHANGHAI WOMEN

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Objective: To investigate the association of estrogen(ER) and vitamin D receptor(VDR) gene polymorphisms with peak bone mass in Shanghai women. Methods: The ER Pvu II and Xba I and VDR Apa I genotypes were determined by PCR-Restriction Fragment Length Polymorphism (RFLP) in 515 unrelated healthy women aged 19-40 years of Han nationality in Shanghai city. BMD was measured by dual-energy X-ray absorptiometry. Results: Frequencies distribution of ER Pvu II genotype were PP for 13.2%, Pp for 49.3% and pp 37.5%. Frequencies distribution of ER Xba I genotype were XX for 4.7%, Xx for 40.4% and xx for 54.9%. Frequencies distribution of VDR Apa I genotype were AA for 5.8%, Aa for 41.9% and aa for 52.3%. Hardy-Weinberg equilibrium was evident for both ER and VDR genes polymorphisms. No associations were found between ER Pvu II and Xba I genotypes and BMD in women. A significant association was found between VDR Apa I genotype and BMD at L1-4 ($P<0.05$). Compared with Aa and aa genotype, women with AA genotype had significant lower BMD at L1-4 ($P<0.05$). However, no association was observed between VDR Apa I genotype and BMD at any sites of proximal femur. When a combined analysis of ER Pvu II and Xba I and VDR Apa I polymorphisms was carried out, cross-genotyping Apa I and neither Pvu II nor Xba I polymorphism was associated with BMD in women. Conclusion: These data suggest that ER gene Pvu II and Xba I polymorphisms do not potentially influence peak bone mass in Shanghai women. The attainment and maintenance of peak bone mass at lumbar vertebra are associated with VDR gene Apa I polymorphism in Shanghai women but independent of ER gene Pvu II and Xba I polymorphisms.

P136 W

ASSOCIATION OF POLYMORPHISMS OF VITAMIN D RECEPTOR GENE POLYMORPHISM WITH BONE MINERAL DENSITY AND BONE SIZE IN CHINESE NUCLEAR FAMILY

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Objective: The risk of osteoporotic fracture in elderly is determined by low peak bone density (PBD) achieved in early adulthood as well as the rate of bone loss with aging. Genetic factors are important in determining PBD. Recent studies indicate that polymorphisms of the vitamin D receptor (VDR) gene may account for much of the genetic contribution to bone density. However, few studies were performed with an aim to identify genes for the variation of bone size, which is also an independent risk factor for osteoporotic fracture. In this study, we employed the analyses of covariance

(ANCOVA) and the quantitative trait locus transmission disequilibrium test(QTDT) approach to simultaneously test the association and/or linkage between Apa I polymorphism in VDR gene and BMD, BMC and AREA of premenopausal women.

Methods: A total of 401 Chinese nuclear families with 1260 subjects with children aged between 20-40 were recruited in the present study. All subjects were genotyped by PCR-RFLP at polymorphic sites of Apa I inside the VDR gene. BMD, BMC and AREA were measured at lumbar spine(L1-4) and proximal femur.

Results: Raw BMD,BMC and AREA values were adjusted by age, height, and weight as covariates. Significant association was found between the ApaI polymorphism and the lumbar spine BMD(p=0.047) and BMC(P=0.015) by ANCOVA. No significant association and linkage between VDR Apa I genotypes and BMD, BMC and AREA at spine or hip were detected using the quantitative trait locus transmission disequilibrium test. Conclusion: This is the first study to test VDR gene as quantitative trait locus(QTL) for BMD and bone size by QTDT in a large sample of Chinese nuclear families. Our results indicate the VDR gene Apa I polymorphism is not as a quantitative trait locus(QTL) underlying spine and hip BMD, bone size variation in our Chinese pre-menopausal women.

P137 F

RELATIONSHIP BETWEEN CIRCULATING PARATHYROID HORMONE AND FOKI POLYMORPHISM OF THE VITAMIN D RECEPTOR GENE IN POSTMENOPAUSAL WOMEN

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The aim of this study was to analyze the interplay between the vitamin D receptor (VDR) gene (FokI polymorphism), serum parathyroid hormone (PTH) levels and bone mineral density (BMD) in 98 euparathyroid postmenopausal women. We statistically checked for covariates such as years since menopause (YSM), serum ionised calcium in PTH or YSM and body mass index (BMI) in BMD. Inter-group differences in serum PTH or BMD at the hip or at the spine between FF, Ff and ff allele combinations of FokI polymorphism were evaluated using one-way ANCOVA and least significant difference (LSD) multiple comparisons test. Significant inter-group differences were found in PTH levels (P<0.035, ANCOVA), which were higher in the ff than in FF genotype (P<0.01, LSD). The significant inter-group differences were also demonstrated in BMD at the hip (P<0.004, ANCOVA), lower values being found in the ff than in Ff genotype (P<0.001, LSD). Borderline inter-group differences were shown in BMD at the spine (P<0.06, ANCOVA), lower values being found in ff than in Ff genotype (P<0.05, LSD). Our findings suggest that FokI polymorphism of the VDR gene is closely related to the magnitude of PTH secretion and/or degradation in postmenopausal women. The causal importance of this phenomenon in the development of osteoporosis remains, however, questionable.

P138 S

SEQUENCE VARIATIONS IN THE VITAMIN D RECEPTOR GENE PROMOTER REGION

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Polymorphisms of vitamin D receptor (VDR) gene have been found to be associated with different diseases including, osteoporosis, osteoarthritis, diabetes, prostate and breast cancer. Most studies have used anonymous polymorphisms at the 3'-end and one in the initial coding region of the gene. However, no functional effect of the polymorphisms has been described. We therefore searched the complete VDR gene for additional polymorphisms. The sequencing results on the coding exons (from 2 to 9) and the 3'-UTR were previously reported. Here we describe results from our analysis of the large 5' promoter region using bio-informatics and sequence analysis. In this study we selected 15 young Caucasian individuals, designed 25 PCR fragments for sequencing, and analysed in total 15 kb of the VDR promoter region from 6 exons (exon 1a-1f). We examined 2.5 kb in the promoter of exon 1f and 1c, determined 10 kb continuous sequence from the promoter of exon 1e to exon 1b, and resolved one 500 bp gap in front of exon 1b. The structure of the VDR promoter region was determined, 36 sequence variations were found, including 31 single nucleotide polymorphisms (SNPs), only 13 of which were contained in the public (2) and Celera (11) data bases. Three areas at strong linkage disequilibrium were observed. Interesting, 11 polymorphisms changed the recognition sequences of transcription factors, and this implicates that they could be potential functional polymorphisms. Several of the SNPs will be selected for association and functional studies. For example, we observed different affinity of the two alleles for a transcription factor, i.e., a GATA binding site before exon 1a. In addition, we observed a correlation between a previously described Cdx-2 SNP and the incidence of hip fracture in different ethnic groups, and this SNP was also found to be associated with the risk of fracture in a large Caucasian elderly population. In conclusion, we have now identified 62 polymorphisms in 22 kb sequenced region across the complete VDR gene (>100 kb). This allows for identification of gene-wide haplotypes to be used in association analysis and for independent functional studies.

P139 W

ANDROGEN RECEPTOR CAG REPEAT POLYMORPHISM AND BONE DENSITY IN MEN

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Androgens are important modulators of bone mass in men. Alterations in androgen receptor (AR) function may affect bone metabolism. CAG trinucleotide repeat length in exon 1 of the AR gene is inversely associated with its transcriptional activity. Previous studies in normal men have yielded conflicting results on the role of AR CAG repeat polymorphisms on bone density (BMD). We therefore investigated the association between the AR CAG repeat length (CAGRL) and BMD in men with idiopathic osteoporosis and their first-degree relatives. We determined the CAGRL by automated DNA sequencing of exon 1 of the AR gene. BMD of the lumbar spine (LS), proximal femur and total body were measured by DXA. Forty- nine pedigrees were recruited and 38 sib-pairs were available for linkage analysis. This study forms a subset of a larger pedigree analysis. Two types of association studies were performed. The first was a case-control study of 81 men with idiopathic osteoporosis (mean age 55.6 years, mean LS t-score -2.53 and mean FN T-score -2.96) and 25 normal men (mean age 44.7 years, mean LS T-score 0.40 and mean FN T-score -0.17). Median AR CAGRL for the osteoporotic group was 20 (SD 3.2) versus 21.0 (SD 2.4) for the control group. Each group was dichotomised with regards to short (<22) or long (>22) CAGRL. No significant difference in the short and long CAGRL groups was found between the osteoporotic and normal men by chi-squared analysis. The second study comprised 157 unrelated men (mean age 54.3 years, mean LS T-score -1.60, mean FN T -score -2.11 and median CAGRL of 20) with varying BMD phenotypes, who were subdivided according to the number of AR CAG repeats (range 6-29 repeats) and to the short and long CAGRL groups as mentioned above. Their bone densitometry parameters were compared using one-way ANOVA. No significant associations were detected between CAGRL and LS or FN T or Z-scores or total body bone mineral content. In conclusion, this preliminary examination of the CAGRL and bone density in men suggests that there is no association in our study population.

Hormones and Cytokines

P140 F

ANALYSIS OF LINEAR GROWTH IN SURVIVORS OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Aim: Therapy for childhood acute lymphoblastic leukemia (ALL) is entering a new era in terms of quality-of-life problems. The purpose of this study is to reveal individual differences of linear growth in response to therapy. We analyzed longitudinal growth data both individually and retrospectively, coupled with endocrinological assessments. Patients and methods: Our study included 21 patients (15 males, 6 females) who had been diagnosed with ALL between 1985 and 1998, and treated at Okayama University Hospital. The 16 patients who received chemotherapy and cranial irradiation were assigned to Group A, which consisted of patients who received therapy during prepuberty and were in pubertal growth at evaluation, or Group B, which consisted of patients who received therapy during puberty and had attained final height at evaluation. Remaining 5 patients who had been given stem cell transplantation or testis irradiation were analyzed separately. Results: Linear growth was uniformly attenuated during therapy in all patients. In contrast, after the cessation of therapy, the growth of each patient varied widely from attenuated to dramatic catch-up growth. In Group A patients, the degree of growth after the cessation of therapy was negatively correlated with changes in height Z-scores during therapy (r=-0.76, p=0.004). One of the factors involved in catch-up growth, urinary N-telopeptide/creatinine (U-NTX/Cr), was significantly higher in patients who decreased their Z-scores after cessation of therapy (p=0.01), despite normal pubertal development and normal endocrinological assessments. In addition, there was a discrepancy of height Z-scores at evaluation between Group A and B; that is lower height Z-scores at final height in Group B. Although these two groups were not comparable directly, this discrepancy suggested that growth attenuation during

puberty could affect final height more seriously. Conclusions: This study revealed individual differences in linear growth after the cessation of therapy, and suggests the importance of catch-up growth during puberty.

P141 S

EFFECT OF BONE SIALOPROTEIN (BSP) IN PROMOTING OSTEOGENIC METASTASIS OF HUMAN BREAST CANCER CELLS

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It has been known that human breast cancer has a tendency to metastasize into bone tissues. However, relatively little is understood about the specific molecular mechanisms that are responsible for colonization and preferential growth of breast carcinoma cells in skeletal tissue. Recent studies have shown that almost 90% of human breast tumors produce BSP, a major non-collagenous protein normally found only in mineralizing tissues. More recent studies indicate that there is a positive correlation between the expression level of BSP and metastasis of tumor cells to bone. The purpose of this investigation was to determine the effect of BSP over-expression in promoting bone metastasis of breast cancer cells. We used a human breast cancer cell line (MDA-231), a DNA transfection technique and an in vivo mouse model in this study. We first established the cultures of MDA-231 cells by stable transfection of DNA constructs of pIRES2-EGFP (green fluorescent protein) expressing human BSP (hBSP) cDNA (231S) under a CMV promoter, or antisense sequence of hBSP cDNA (231AS), or an empty vector as a control (231CTL), respectively. The cells were selected by G418 for Neor expression and flow cytometry for GFP expression. The resultant cultured cells expressed different level of hBSP as detected by RT-PCR and Western blot. Among the three, 231S expressed the highest level of hBSP while 231AS expressed the lowest. The capacity of tumor cells causing bone metastasis was determined in nude mice (5 in each group) by intracardiac injection of the cells from three different groups. Four weeks after inoculation a radiological examination revealed that all the five mice in 231S cell group showed osteolytic bone metastases. In 231AS group only one mouse demonstrated metastatic bone lesion while three out five mice in control group developed metastatic lesions in bones. We concluded that these results strongly suggest that BSP over-expression in human tumor cells enhance bone metastasis and an antisense of BSP cDNA inhibits this effect in this mouse model. (Supported by an NIH/NIDCR grant DE11088-08)

P142 W

A NOVEL BONE-TARGETED SRC TYROSINE KINASE INHIBITOR, AP23451, PREVENTS BONE LOSS IN VITRO AND IN VIVO

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Src is a non-receptor tyrosine kinase whose expression is required in osteoclasts for osteoclast activation and bone resorption. Thus, it is a well-validated therapeutic target for the prevention of postmenopausal and other forms of bone loss.

To this end, we have developed a series of novel, potent (low nM IC50s) non-peptide Src tyrosine kinase inhibitors (MW <500) using structure-based drug design. We incorporated bone-targeting chemical moieties into these Src kinase inhibitors that confer them with high affinity for hydroxyapatite along with cellular selectivity for osteoclasts. In addition, they have significant selectivity (>100-1000 fold) to inhibit Src relative to a panel of more than 30 protein kinases.

A lead compound (AP23451): 1) dose-dependently inhibited osteoclastic resorption in vitro (pit area and number reduced by 55-97% at 1 micro M and by 98-100% at 10 micro M), effects comparable to alendronate at 1 and 10 micro M; 2) prevented PTH-induced hypercalcemia (100% inhibition) and bone resorption (osteoclast number/mm calvarial bone surface (AP23451+ PTH: 1.7 ±1.7 vs. 14.4 ±3.3, PTH alone) when given twice daily sc. (10 mg/kg) to mice for 5d; 3) prevented Ovx-induced vertebral (DEXA: 0.069 ±0.002 vs. 0.057 ±0.002 g/cm^2) and femoral metaphyseal (pQCT: 660 ±14 vs. 455 ±17 g/cm^3) bone loss (10mg/kg sc. once daily for 35d); 4) prevented osteolytic metastases (10mg/kg ip. once daily for up to 28d) following intracardiac inoculation of MDA-231 breast cancer cells into nude mice by a cellular mechanism different from that of zoledronate; and 5) dose-dependently (50-100 micro M) inhibited invasion of MDA-231 cells through Matrigel basement matrix gel in vitro.

Our findings indicate that this specifically-designed, novel bone-targeted Src tyrosine kinase inhibitor has potent in vitro and in vitro anti-resporptive properties for use in treating malignant hypercalcemia, bone metastases and postmenopausal osteoporosis.

P143 F

MYELOMA CELLS SUPPRESS OSTEOBLAST DIFFERENTIATION: IMPAIRED BONE FORMATION IN MULTIPLE MYELOMA

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Multiple myeloma (MM) develops and expands in the bone marrow, and generates a devastating bone destruction. Besides enhanced osteoclastic bone resorption caused by complex cellular interactions between MM and bone cells and MM-derived soluble factors such as MIP-1alpha and IL-6, clinical evidence has also suggested suppression of bone formation as a contributing factor to the bone loss in MM. Consistently, we have demonstrated that serum levels of a bone formation marker osteocalcin were relatively suppressed compared to apparently increased levels of a bone resorption marker urinary deoxypyridinoline excretion in MM patients. In the present study, we therefore tested a hypothesis that MM cells produce a soluble inhibitor of osteoblast differentiation and function. When murine osteoblastic MC-3T3-E1 cells were cultured in osteogenic induction medium containing beta-glycerophosphate and ascorbic acid, alkaline phosphatase (ALP) activity in the cells was increased 5-fold at day 10 comparing to control cultures, and further increased up to 25-fold in the presence of BMP-2 at 50 ng/ml. Consistent with our hypothesis, we found that the BMP-2-induced ALP activities were reduced to 90, 70, and 60% by addition of 10% conditioned media from MM cell lines, ARH77, U266, and RPMI8226, respectively. Interestingly, MM cell conditioned medium showed minimal effects on osteogenic medium-induced ALP in the absence of BMP-2. ARH77 conditioned media also delayed osteocalcin mRNA induction, and almost completely suppressed mineralized nodule formation in primary osteoblasts as well as MC-3T3-E1 cells stimulated by BMP-2. Neither MM-derived bone resorptive factors such as MIP-1 nor IL-6 were able to mimic such inhibitory effects. TNF-alpha was not detectable in MM conditioned medium in these experiments, either. These results suggest that MM cell-derived unknown soluble factor(s) suppress osteoblast differentiation, in which blockage of BMP-2 signaling pathways may be involved. In conclusion, cellular interplays between MM cells and bone cells result in defective bone formation as well as efficient activation of osteoclastic bone resorption, leading to an uncoupling state of bone turnover which ultimately causes rapid loss and destruction of bone.

P144 S

INCREASED CYCLOOXYGENASE-2 EXPRESSION IN BREAST CANCER CELLS METASTASIZED IN BONE

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Breast cancers preferentially disseminate to bone. Evidence is accumulating that breast cancer products play important roles in the pathophysiology of bone metastasis. Early clinical studies suggested a positive correlation between prostaglandin (PG) production and breast cancer metastasis to bone. In the present study, we examined the relationship of the expression of cyclooxygenase-2 (COX-2), a rate-limiting enzyme in prostaglandin (PG) synthesis, and resulting PG synthesis with bone metastasis in breast cancer. First, we examined COX-2 expression in vivo by inoculating MDA-MB-231 (MDA-231) human breast cancer cells into orthotopic mammary fat pad or the heart in female nude mice. Immunohistochemical examination showed that COX-2 expression was undetectable in MDA-231 cells in the orthotopic site. On the other hand, MDA-231 cells in bone metastases demonstrated substantial COX-2 expression, especially cells in the close vicinity of residual bone. Of interest, the bisphosphonate ibandronate decreased COX-2 expression and suppressed bone metastases with reduced number of osteoclasts in MDA-231 cells in bone. From these results, we hypothesized that inhibition of bone resorption by ibandronate decreased COX-2 expression and PG production in MDA-231 cells through limiting a supply of bone-stored growth factors. In support of this notion, TGF-beta (TGFb) increased COX-2 expression in MDA-231 cells, whereas TGFb did not affect COX-1 expression. IGF, FGF and PDGF showed no effects on COX-2 expression. Conditioned medium obtained from TGFb-treated MDA-231 cells significantly stimulated osteoclast-like cell formation in mouse bone marrow cultures and this stimulation was not observed in the presence of a selective COX-2 inhibitor NS-398. ELISA demonstrated that TGFb significantly increased PGE2 production in MDA-231 cells, which was dose-dependently decreased by NS-398. RT-PCR analysis showed that PGE2 increased RANKL and decreased osteoprotegerin mRNA expression in primary bone marrow stromal cells. In conclusion, our results suggest that bone-derived TGFb increases PG production by breast cancer cells through up-regulation of COX-2 expression, which in turn stimulates osteoclastic bone destruction, leading to the progression of bone metastases. Our results also suggest that COX-2 may be a potential therapeutic target in the treatment of bone metastases in breast cancer.

P145 W

METHOD FOR ASSESSMENT OF SPATIALLY REFERENCED BONE STRUCTURE LOSS FROM BONE METASTASIS IN THE MOUSE BY *IN-VIVO* AND *EX VIVO* MICRO-CT

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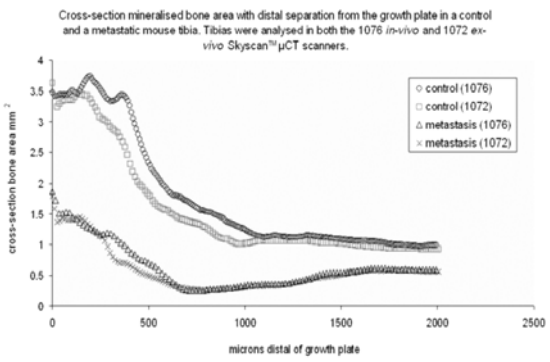
Bone metastasis is an important field of preclinical research with rodent models. Micro-CT has potential to play a significant role in assessment of metastasis in bone. Metastasis can be studied by micro-CT in two ways. Firstly, if a single metastasis is mostly bordered by intact bone, then 3D polygonal region of interest selection software can delineate the metastasis and calculate its volume. However when metastases are multiple and advanced, such delineation is impossible since a high proportion of the bone structure is lost. In this situation metastasis can be studied as a special case of osteoporosis. Proximal tibias from a (nude) mouse with (MDA-MB-231 breast cancer cell induced) metastases and a control mouse were studied using the growth plate (GP) as a spatial reference. Micro-CT analysis of the tibial section extending 2mm distal of the GP provided histomorphometric parameters and the distribution of structure thicknesses, integrated over the whole structure. In addition, a slice-by-slice analysis of structural parameters in transverse section moving distally from the GP allowed comparison of spatially referenced profiles of histomorphometric parameters between normal and metastasised tibiae. Results showed substantial bone volume loss, and an increase in TBPf and decrease in Euler connectivity reflecting structural dissociation due to metastasis. Bone structure loss and dissociation were greatest close to the GP at the primary and early secondary spongiosa (figure 1). The thickness distributions of control and metastasised bone were similar reflecting loss of cortical and trabecular structures with little preference for structure thickness. All the measurements described were performed on the mouse tibiae using both the Skyscan 1072 *ex-vivo* and 1076 *in-vivo* scanners for comparison. Image pixel sizes of 6.5 and 8.9 microns and resolutions of 8 and 17 microns respectively were attained. Histomorphometric parameters measured showed consistency between the two scanners.



Cross-section mineralised bone area with distal separation from the growth plate in a control and a metastatic mouse tibia. Tibias were analysed in both the 1076 *in-vivo* and 1072 *ex-vivo* Skyscan™ µCT scanners.

P146 F

BISPHOSPHONATES INDUCE APOPTOSIS IN STROMAL TUMOR CELLS OF GIANT CELL TUMOUR

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Giant cell tumor of bone (GCT) is an aggressive primary neoplasm of bone that produces osteolytic lesions. The stromal cells are the main neoplastic components of this tumor and regulate the formation of osteoclastic-like giant cells ultimately responsible for bone destruction. Bisphosphonates prevent bone resorption by inhibiting osteoclast activity, promoting osteoclast apoptosis, and they can also induce apoptosis of primary neoplastic cells such as breast and prostate cancer. We hypothesized that bisphosphonates may induce apoptosis not only in giant cells but also neoplastic stromal cells of GCT, and such systemic treatment may mitigate osteolysis in GCT patients. Eight GCT primary cell cultures were treated with zolendronate, pamidronate or alendronate for 48 hours with different dosage (3, 30, 150 uM) and subjected to apoptosis assay by flow cytometry following fluorescent Annexin-V labeling. Surgical specimens were collected from 15 patients with GCT before and after pamidronate administration at dosage of 90 mg given weekly for 4-8 weeks. TUNEL assay was used to study the morphological changes associated with apoptosis and an image analysis program was used to calculate the percentage of apoptotic cells in representative sections. All three bisphosphonates significantly induced stromal tumor cell apoptosis in the cultures. Zolendronate being the most potent inducing 20% apoptosis at 30uM, followed by alendronate 13% and pamidronate 11%. Significant apoptosis was observed in both stromal tumor cells and multinucleated giant cells in GCT patients followed pamidronate treatment. Our observations suggest that bisphosphonates induce apoptosis in both stromal tumor cells and multinucleated giant cells and these drugs may be useful adjuvant in the treatment of GCT of bone.

P147 S

EFFECT OF INCADRONATE ON PROLIFERATION OF MESENCHYMAL TUMOR CELLS WITH OR WITHOUT ACTIVATED RAS MUTATION

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Nitrogen-containing bisphosphonates (e.g. incadronate etc) induce apoptosis in osteoclasts and myeloma cells through the inhibition of mevalonate pathway and decrease tumor burden of metastatic breast cancer in bone. Mesenchymal tumors sometimes arise within bone and have tendency to metastasize to bone, however, the effect of bisphosphonates on these tumors were not well understood. We investigated in this study the effect of bisphosphonates on the mesenchymal tumors and the mechanisms fo inhibition in these tumor cell proliferation. HT-1080 (fibrosarcoma cell line) and NMS-2 (neurofibromatosis type 1 cell line) were significantly inhibited cell- proliferation, however the other cell lines showed no influence. HT-1080 and NMS-2 have increased-Ras activity because of the mutation of Ras gene or NF1 gene. We confirmed the other cell lines tested in this study have no mutation in any of the N-, K-, or H-ras alleles by direct sequencing method. Luckman et al. have shown previously that nitrogen-containing BPs- induced apoptosis could be suppressed by the addition of FPP or GGPP in macrophage cells. In order to determine whether the mechanism of incadronate-induced inhibition on proliferation was similar to the apoptosis in macrophage cells, we implemented an add-back approach to identify which products of the mevalonate pathway were critical to incadronate-induced inhibition on proliferation. FPP partially prevented incadronate-induced anti-proliferation of HT-1080 and NMS-2 cells. GGPP directly bypassed the site of incadronate inhibition and completely restored inhibition of proliferation. Non nitrogen-containing bisphosphonate (etidronate) showed no influence on these tumor cells proliferation. These indicate that incadronate inhibits the mevalonate pathway and prevent post-translational prenylation of Ras. As a result, oncogenic Ras signaling was blocked, and HT-1080 and NMS-2 tumor cell proliferation was reduced. To confirm the speculation, oncogenic Ras transfected BALB/3T3 cell line (Bhas 42) was also compared the influence of incadronate with parental BALB/3T3 cell line. Although parental BALB/3T3 cells showed only a little inhibition by treatment of incadronte, proliferation of Bhas 42 cells were reduced significantly. These results suggests that incadronate suppress oncogenic Ras activated mesenchymal tumors in through the inhibition of Ras signaling pathways.

P148 W

GDF5 EXPRESSION PATTERN OF LIGAMENT/TENDON CELL IN VIVO AND IN VITRO

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Growth and differentiation factor (GDF)-5 is a member of the bone morphogenetic protein (BMP) family. Like other members of the BMP family, importance of GDF-5 in bone development is well established. It is also demonstrated that GDF-5 induces ectopic formation of tendon- or ligament-like tissue and promotes tendon repair. In addition, GDF-5-deficient mice have a little weakness in Achilles tendons. These observations strongly suggest that GDF-5 plays a role in development and maturation of tendons and ligaments. Little is known, however, about development and organogenesis of these tissues due to lack of cell lines and specific markers. To examine possible roles of GDF-5 in tendons and ligaments, we used recently established cell lines derived from the periodontal ligament (PDL), together with primary culture of Achilles tendon and osteoblastic MC3T3-E1. RT-PCR analysis demonstrated that GDF-5 expression is more abundant in the PDL cells and Achilles tendon cells than in MC3T3-E1 cells, and is inversely proportional to matrix mineralization potential of the cell. When MC3T3-E1 was cultured in differentiation medium, GDF-5 expression gradually decreased as mineralization progressed. In contrast, GDF-5 expression in PDL-L2, was maintained throughout the culture period and no mineralized nodule was produced. PDL-L2 is indistinguishable from the periodontal ligament (PDL) fibroblasts in vivo in terms of its gene expression pattern and inability of inducing matrix mineralization despite expressing a substantial amount of Runx2/Cbfa1/Osf2, and thus is appeared to be a typical PDL fibroblast. Since the PDL is constantly exposed to widely varying mechanical stress, we next

examined the effect of mechanical stress on GDF-5 expression in PDL-L2 using Flexorcell (8 kPA, 6 cycles/min). Interestingly, the expression level markedly increased. This is intriguing in view of the fact that the PDL never calcifies in vivo. Furthermore, in situ hybridization study demonstrated that GDF-5, in vivo, is abundantly expressed in cells of Achilles tendon and PDL but neither in osteoblasts nor in cementoblasts. These observations suggest an importance of GDF-5 in regulating functions of tendons and ligaments.

P149 F

ROLE OF BETA1 INTEGRIN-MEDIATED SIGNALING IN OSTEOBLAST FUNCTION

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Although osteoblasts play a central role in the bone metabolism, relevance of interaction between the cells and the surrounding bone matrices including fibronectin and collagen to the intracellular signaling and functions remains unclear. We, therefore, assessed characteristics of human osteoblasts, shedding light on signaling mediated by beta1, major receptors for these matrix glycoproteins. 1) Integrins beta 1, alpha 2, alpha 4, alpha 5, alpha 6 and alpha v were highly expressed on primary osteoblastic cells. 2) Crosslink of beta1 on osteoblasts by antibody or ligand matrices, such as fibronectin or collagen, augmented production of intracellular osteocalcin and type I collagen as well as expression of ICAM-1 and RANKL on the surface. 3) Engagement of beta1 induced TRAP+ MNC formation in the coculture system of osteoblasts and peripheral monocytes. 4) Up-regulation of ICAM-1 and RANKL on osteoblasts by beta1-stimulation was completely abrogated by pretreatment with herbimycin A and genistein, tyrosine kinase inhibitors, or transfection of dominant negative truncations of focal adhesion kinase (FAK). Our results indicate that beta1 integrin-dependent adhesion of osteoblasts to bone matrices induces bone matrix synthesis as well as ICAM-1 and RANKL expression and osteoclast formation via tyrosine kinase, especially FAK. We here propose that beta1/FAK-mediated signaling on osteoblasts could be involved in high turnover on bone metabolism through two paradoxical features of bone formation and bone resorption.

P150 S

THE EFFECT OF THE 5' END OF THE OPEN READING FRAME OF CEF-10/CYR61 MRNA AS A *CIS* ELEMENT OF GENE EXPRESSION

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[OBJECTIVE] CEF-10/Cyr61 is a cystein-rich growth factor which belongs to the CCN family, and has been suggested to play important roles in chondrogenesis and osteogenesis. The regulatory system on CEF-10/Cyr61 gene expression, however, still remains unclear. In this context, we were interested in a GC rich region that exists in the 5' region of open reading frame (ORF) of chicken *cef-10/cyr61* mRNA. In the present study, we investigated the effects of the region on the post-transcriptional regulation of gene expression.

[METHODS] The 5' region of ORF of *cef-10* was amplified by RT-PCR from chicken embryo fibroblasts (CEF cells), and the amplicon was subcloned into pGEM T-Easy (Promega). The labeled RNA was prepared by *in vitro* transcription in the presence of alpha-[^{32}P] CTP, and RNA *in vitro* folding assay and RNA gel shift assay were carried out. Thereafter, chimeric gene containing the 5' cDNA fragment at the upstream or downstream of firefly luciferase gene was constructed, respectively. These molecular constructs were transfected into CEF cells, and dual luciferase assay was carried out, in order to investigate the effects of the fragment on expression of reporter gene. RNase protection assay of total RNA or ribosmal RNA of transfected cells was carried out, in order to investigate the effect of the fragment on ribosome entry of the reporter gene.

[RESULTS & DISCUSSION] The length of the RT-PCR amplicon was 200 bp shorter than expected length that was designed to overcome GC-rich structures. However, in the presence of PCRx Enhancer (Invitrogen), the length of the amplicon was the same as expected. Computer analysis of the deleted region revealed that the region was GC-rich, and was predicted to form a stabile secondary structure. RNA *in vitro* folding assay revealed that there existed a folded structure of 100 bases in the region, and RNA gel shift assay revelead that the region bound to a cytosolic protein. Furthermore, dual-luciferase assay revealed that the corresponding cDNA fragment enhanced the gene expression, and RNase protection assay revealed that it enhanced the mRNA entry into ribosome, when it was connected immediately upstream of the reporter gene. An RNA *cis* element with a stabile secondary structure in the ORF of chiken *cef-10* was suggested enhancing effect on gene expression.

P151 W

IDENTIFICATION OF A PARATHYROID HORMONE IN THE FISH, FUGU RUBRIPES

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A parathyroid hormone (PTH) gene has been isolated from Fugu rubripes and the eighty amino acids of the protein coding region determined. The N-terminal 34 residues of fugu PTH (fPTH) share 15 residues with other known PTH sequences and is most closely related to chicken PTH(1-88) (cPTH). In the amino acid sequence of fPTH after the first 34 amino acids there is no significant homology to either human PTH (hPTH) or cPTH, indicating weak evolutionary pressure to conserve the C-terminus of the PTH molecule. The potency of fPTH(1-34) in promoting cyclic adenosine monophosphate (cAMP) formation in UMR106.01 cells is consistently less than hPTH(1-34), human parathyroid hormone-related protein(1-34) (hPTHrP) and fugu parathyroid hormone-related protein (1-34) (fPTHrP) but the maximum amplitude of response was significantly greater than that achieved with the highest concentrations of hPTH or human or fugu PTHrP. The cAMP studies indicate that fPTH(1-34) acts through the PTH/PTHrP receptor, (PTH1R). But fPTH(1-34) is not recognized immunologically by a number of antisera raised to the N-terminus of hPTHrP or hPTH. Therefore fPTH is a member of the PTH family with only N-terminal homology to other family members, but with the least homology with any PTH so far sequenced.

P152 F

INVOLVEMENT OF A FARNESYLATED PROTEIN IN RAPID DOWN REGULATION OF RENAL TYPE IIA TRANSPORTER IN RESPONSE TO PARATHYROID HORMONE

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Renal handling of inorganic phosphate (Pi) is primary importance to control the extracellular concentration of Pi. The type IIa sodium dependent phosphate co-transporter (Npt2a) is a key player in overall phosphate homeostasis and bone remodeling. Npt2a is internalized from apical membrane and degraded in the lysosome by parathyroid hormone (PTH) treatment or intake of high Pi diet. For its PTH-induced retrieval, it is known that a dibasic amino acid motif (KR) in the 3rd-intracellular loop of Npt2a.

Here we described the identification of the interacting protein for the endocytic motif of the Npt2a using yeast two-hybrid method. We identified a strong interaction of Npt2a with a small protein first cloned and termed NaPi-3LAP, described as the human peroxisomal farnesylated protein PxF/PEX19/Pex19p. NaPi-3LAP can bind the KR motif, but not NI mutant in the regions. NaPi-3LAP is highly expressed in mouse and rat kidney. Western blot analysis indicates that NaPi-3LAP is located in cytosol and brush border membrane fractions (microvilli and subapical component). Immunohistochemical study indicates that NaPi-3LAP rapidly induced by PTH administration or high Pi diet. Farnesylation of NaPi-3LAP was an important determinant in the affinity of peroxisomal protein and the interaction of Npt2a. In addition, NaPi-3LAP also interacts with Na/H exchanger regulatory factor 1 (NHERF1). In conclusion, the present study indicates that NaPi-3LAP may be actively involved in controlling the internalization and trafficking of Npt2a.

P153 S

ESTROGEN ACTIVATES CYCLIN-DEPENDENT KINASES 4 AND 6 THROUGH INDUCTION OF CYCLIN D IN RAT PRIMARY OSTEOBLASTS

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Estrogen plays important roles in maintaining bone density and protecting against osteoporosis, but the underlying mechanisms of estrogen action via estrogen receptors (ERs) in bone remain to be clarified. In the present study, we isolated primary osteoblasts derived from transgenic rats harboring a dominant negative ER mutant, namely dnER, and from their wild-type littermates. We observed that the rate of cell growth of osteoblasts from the dnER transgenic rats was reduced compared to that of wild-type osteoblasts. To identify which genes are differentially expressed in osteoblasts from the dnER transgenic rats compared to those from wild-type rats, cDNA microarray analysis was performed with mRNAs obtained from the two sources of osteoblasts. Among differentially expressed genes that are related to cell cycle regulations, cyclin D2, a member of D-type cyclins, was one of the downregulated genes in osteoblasts from the dnER transgenic rats. We investigated whether 17beta- estradiol (E2) modulated expression of cyclin D2 in wild-type rat primary osteoblasts. The protein levels of D-type cyclins including cyclin D2 and

cyclin D3 but not cyclin D1 were elevated in wild-type osteoblasts with E2 (10^{-8} M) treatment, resulting in the activation of cyclin-dependent kinases 4 and 6 (Cdk4/6) activities and the promotion of cell growth. An anti-estrogen ICI 182,780 (10^{-7} M) abolished the induction of the expression of D-type cyclins by E2 suggesting the induction of cyclin D2 and cyclin D3 is mediated through the ERs. D-type cyclins promote G1/S-phase transition by binding to Cdk4/6. E2 treatment increased the amounts of cyclin D2-Cdk4/6 and cyclin D3-Cdk4/6 complexes in primary osteoblasts. Activation of cyclin D-Cdk4/6 complexes is an early-to-mid G1-phase event that elicits hyperphosphorylation and inactivation of retinoblastoma protein (Rb). We analyzed the Cdk4/6 kinase activities in primary osteoblasts using GST-Rb as a substrate. E2 treatment markedly enhanced cyclin D2-Cdk4/6 and cyclin D3-Cdk4/6 kinase activity. These findings suggest that the regulation of the expression of cyclin D2 and cyclin D3 has a role in the G1 progression of primary osteoblasts by E2 through ERs.

P154 W

GLUCOCORTICOID INDUCES LOW TURNOVER OSTEOPOROSIS IN GROWING MINIPIGS

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The purpose of the present experiment was to evaluate osteoporotic changes induced by glucocorticoids in young Gottingen minipigs, an otherwise often used animal model for human disease.

Fifteen Gottingen minipigs were respectively assigned to 3 experimental groups at the age of 8 months: Baseline control group (BC, n=5), Control group (C, n=5), Glucocorticoid group (GC, n=5). The minipigs in Group GC were administered with predonisolone at dose of 0.5 mg/kg body weight 5 times a week for 6 months. Body weight, serum Ca, urine Ca, and bone metabolic markers, such as serum bone alkaline phosphatase (BAP), serum osteocalcin (OC), and urine NTX, were measured at 0, 4, 12, and 24 weeks after the start of the experiment. The minipigs were sacrificed in Group BC at day 0 and in Groups C and GC at 24 weeks. BMC and BMD were measured by DXA and pQCT. Three-dimensional microarchitecture was evaluated by micro-CT. Ultimate compressive load was obtained by the materials-testing machine. Specimens of lumber body were assessed with histomorphometric analysis.

The values of serum BAP, serum OC and urine NTX in Group GC significantly decreased compared with those in Group C. Histomorphometric analysis revealed that bone formation rate (BFR/BS) decreased in Group GC. DXA and pQCT showed that BMD value of lumber vertebra significantly decreased in Group GC. In the results of micro CT, BV/TV and Tb.Th decreased, and BS/BV,SMI and TBPf increased in Group GC compared with those in Group C.

In conclusion, glucocorticoid induces low turnover osteoporosis, leading to deterioration of cancellous bone and reduction of bone strength, in growing Gottingen minipigs.

P155 F

ISOLATION OF UPSTREAM REGION OF THE HUMAN TISSUE-NONSPECIFIC ALKALINE PHOSPHATASE GENE AND ITS REGULATION BY ALL-TRANS RETINOIC ACID

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Although human tissue-nonspecific alkaline phosphatase (TNSALP) is a well-known marker of bone formation, its precise function in bone mineralization and its regulation are still unclear. In addition, effect of fat-soluble vitamins on expression of TNSALP has not been sufficiently analyzed. The human TNSALP gene consists of 12 exons, in which exons 2 to 12 are coding exons. Of two leader exons, 1B for bone type and 1L for liver type, 1B is transcribed in osteoblasts. To study the effect of retinoic acid on expression of TNSALP in osteoblastic cell lines, we isolated and characterized the 5 prime-upstream region of exon 1B.

Approximately 4.5 kb of the 5 prime-upstream region from the transcription start site was cloned using GenomeWalker kit (Clontech) and the nucleotide sequences were determined. The upstream region was subcloned into the luciferase reporter vectors, pGL3PV and pGL3BV (Promega), and then a series of deletion mutants were created. Osteoblastic cell lines SaOS-2 were incubated with 10^{-6} M all-trans retionoic acid. Enzymatic activity of ALP and mRNA of bone type ALP were estimated at 6, 24, 48 and 72 h after the addition of retinoic acid. For luciferase assay, the luciferase reporter vectors and Renilla luciferase vector, pRL-Tk, were transfected with LipofectAMINE 2000 (Invitrogen) into SaOS-2 cells, and then the cells were incubated with retinoic acid. The cells were harvested at 72 h after the addition of retinoic acid and the cell lysate was analyzed by dual luciferase assay.

The nucleotide sequences of the upstream region showed 55% homology with the promoter region of the mouse TNSALP gene. ALP activity was increased in 48 to 72 h after the incubation with retinoic acid in SaOS-2 cells. The concentration of bone type mRNA was also increased in 24 to 48 h after retinoic acid treatment. Luciferase assay revealed that the upstream sequence encompassing from minus 1313 to minus 724 containing a retinoic acid responsive element-like motif participate in this enhancing reaction.

Osteoblasts

P156 S

THE PROTECTIVE EFFECT OF PYRUVATE ON CELL DEATH IN CULTURED OSTEOBLASTS

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We have previously reported that pyruvate deficiency induced cell death in proliferative cultured calvarial osteoblasts (Hinoi et al., 2002, Biochem Biophys Res Commun.). In this study, to clarify protective mechanism of pyruvate against cell death, we have investigated the transport system of pyruvate via particular monocarboxylate transporters (MCTs) in osteoblasts. Osteoblasts were prepared from calvaria of 1 or 2 days old Wistar rats by sequential enzyme digestion and cultured in alpha-MEM containing 10% FBS. After replacement of standard culture medium (alpha-MEM) with DMEM, cell survival was determined using cell counting kits. The uptake of pyruvate was performed by measurement of transport activity of [^{14}C] pyruvate in cultured osteoblasts for 3 and 7 DIV. Expression of MCTs was confirmed by RT-PCR, immunoblotting and immunocytochemistry. Cell survival was significantly decreased in osteoblasts when medium change was made at Day 0, 1 and 3, but no marked alternation was found at Day 5, 7 and 21. On the other hand, pyruvate and cysteine, the components contained in alpha-MEM but not in DMEM, prevented the cell death induced by medium replacement at Day 1 and 3. Of MCTs tested, expression of high affinity MCT2 was seen in osteoblasts cultured for 3 and 7 DIV, whereas the uptake of pyruvate was detected in osteoblast cultured for 7 DIV but not for 3 DIV. These results suggested that functional expression pattern of MCT2 may be associated with the protective effect of pyruvate on cell death induced by medium replacement.

P157 W

TRAIL IS EXPRESSED IN QUANTITIES SIMILAR TO RANKL IN HUMAN PERIPROSTHETIC PSEUDOMEMBRANE, OSTEOARTHRITIC STROMAL CELLS AND OSTEOBLASTS

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Apoptosis is critical in bone development and in turnover of mature bone. Members of the TNF cytokine family play important roles in bone cell survival and apoptosis. One of the TNF family members, the Apo2 ligand, TRAIL (TNF-related apoptosis inducing ligand), mediates apoptosis in a wide variety of cells. As well as binding to its apoptosis-inducing receptor, TRAIL binds to soluble 'decoy' receptors that regulate the activity of TNF-family proteins. One of these decoy receptors, osteoprotegerin (OPG) is an important regulator of bone turnover via interactions with the ligand for the receptor-activator of nuclear factor kB (RANK). Because of the importance of TRAIL in regulating apoptosis, and because of the interaction of TRAIL and OPG, we hypothesized that TRAIL is expressed in bone and co-regulated, along with RANKL, by OPG. The initial phase of investigation of this hypothesis has been a survey of TRAIL occurrence in human osteoblasts and bone-associated stromal cells in normal and pathological conditions. Human osteoblasts and stromal cells were obtained by enzymatic digestion of excess bone and joint tissue and grown in vitro. MG-63 osteosarcoma cells were also studied. Semi-quantitative RT-PCR amplification of 300 bp segments of TRAIL or RANKL from uniform 5 micro gram isolates of RNA and Western analysis on aliquots of proteins from 2 x 10^6 cells, were performed. We found that TRAIL mRNA is expressed in MG63 cells and stromal cells from normal bone, arthritic bone and synovial fibroblasts. A liposarcoma from bone, did not express measurable quantities of TRAIL. Analysis by immunoprecipitation, using rabbit anti TRAIL or semi-quantitative PCR, suggested that TRAIL production and expression is often similar to RANKL. Western blot results showed 20 kD mature TRAIL in all bone cell culture extracts. Recombinant human TRAIL (19.6 kD) was used as a positive control. Controls for antibody specificity included reaction with

RANKL which were non-reactive with the antibody. Thus, our data indicate that the TRAIL system is highly likely to be important in bone apoptosis and expression of OPG is certainly important not only in regulation of osteoclasts but in regulation of apoptosis via the Apo2-TRAIL system.

P158 F

ADDITIONAL BONE FORMATION IS ESSENTIAL FOR OSTEOCLASTIC BONE RESORPTION IN RAT FEMORAL OSTEONECROSIS

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Additional bone formation is believed to play an important role in bone regeneration after osteonecrosis. To understand the mechanism of bone regeneration is useful to develop the treatment for osteonecrosis of femoral head. We analyzed histological and molecular features of additional bone formation in rat experimental femoral osteonecrosis. Proximal femora were harvested on day 2, 7, 21, 42 and 84 postoperatively. Osteonecrosis was defined by empty lacunae and/or pyknotic nuclei of osteocytes. These osteonecrotic features were initially detected on day 2 and the number of empty lacunae increased with time. Almost all the osteocytic lacunae in the femoral heads and necks became empty by 3rd week postoperatively. Pyknotic nuclei showed positive signal for TUNEL, indicating apoptosis. Additional bone formation were initially detected on day 21 at the interface between viable and necrotic bone. The amount of newly formed bone increased gradually. By 6th week, undifferentiated mesenchymal cells appeared in necrotic area. These mesenchymal cells were from the revascularized bone marrow, as well as the hypercellular fibrous tissues in the thickened capsule. Cuboidal osteoblasts, lining the newly formed trabecula, expressed type I collagen, osteopontin, and osteocalcin mRNAs detected by in situ hybridization. These bone forming cells were positive for immunohistochemistry of alkaline phosphatase. TRAP positive multinuclear cells, possibly osteoclasts, were detected only on the surface of newly formed bone in the necrotic area, whereas osteoclasts never lined the necrotic bone surface. These data suggested that osteoblasts on the newly formed bone by additional bone formation were essential for osteoclasts to replace the bone matrix.

P159 S

SERUM OSTEOPROTEGERIN LEVELS IN FEMALE PATIENTS WITH ANOREXIA NERVOSA

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Introduction: Female patients with anorexia nervosa (AN) show decreased bone mineral density due to hypogonadism, malnutrition and glucocorticoid excess. Recently, osteoprotegerin (OPG) has been identified as a cytokine that inhibits osteoclast differentiation and activity. The aim of our study was to clarify the correlation between OPG and other markers of bone metabolism as well as hormone levels in female patients with AN.

Patients and Methods: In order to assess the relationship between serum OPG, biochemical markers of bone formation and biochemical marker of bone resorption the following parameters were determined: osteocalcin (OC), type I procollagen carboxyterminal propeptide (PICP), bone specific alkaline phosphatase (BAP), and serum carboxyterminal pyridinoline cross linked telopeptide (ICTP). All these parameters were investigated in 33 female patients with anorexia nervosa (AN; age: 23.5 ± 6 yrs) and 18 healthy age matched control subjects (CO; age: 26.3 ±2.5 yrs). Additionally, we have assessed the correlation of OPG with serum levels of estrogen, progesteron, follicle stimulating hormone and luteinising hormone.

Results: Serum OPG levels were significantly lower in the anorexia nervosa group as compared to control subjects (0,07 ± 0,003 vs. 64,2 ± 11,3 ng/ml; p <0,0001). There were no significant differences between serum OC, PICP and BAP levels between the two groups. Serum ICTP levels tend to be increased in AN, however not statistically significant in comparison with healthy controls (8,8 ± 1,6 vs. 4,2 ± 0,3; p=0.1). Serum OPG levels correlated only with serum estrogen levels in the AN group (r=0,38; p<0,05).

Summary: The pathophysiology of decreased bone mineral density is still quite controversial. The main mechanisms are hypogonadism, malnutrition and hypercortisolism. Many authors show a shift between bone resorption and bone formation, either towards increased bone resorption or decreased bone formation. OPG inhibits osteoclast differentiation and activity. We found significantly decreased serum levels in patients with AN without increase of bone resorption markers.

Conclusion: We conclude that decreased serum levels of OPG in AN patients are an expression of deficient estrogen regulation due to hypogonadism in anorexia nervosa without an activating effect on bone resorption.

P160 W

ACTIVATION OF THE PERIPHERAL SYMPATHETIC NERVOUS SYSTEM MODULATES OSTEOBLASTIC ACTIVITY IN MOUSE CALVARIA

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It has been demonstrated that human osteoblastic as well as osteoclastic cells are equipped with adrenergic receptors and neuropeptide receptors and that they constitutively express diffusible axon guidance molecules that are known to function as a chemoattractant and/or chemorepellent for growing nerve fibers. Pharmacological stimulation of beta-adrenoceptor was demonstrated to increase the synthesis of interleukin (IL)-6 and prostaglandin (PG) E2, well known to modulate bone metabolism by regulating the development and function of osteoclasts and osteoblasts, in cultured osteoblastic cells. These *in vitro* findings suggest that the expression of axons of sympathetic and peripheral sensory neurons to osteoblastic and osteoclastic cells is required for the dynamic neural regulation of local bone metabolism. Recently, intracerebroventricular (i.c.v.) injection of lipopolysaccharide (LPS), which caused the inflammatory stimuli in the brain, was demonstrated to increase the outflow of the peripheral sympathetic nervous system. In this study, to prove the physiological role of sympathetic nerves in bone metabolism *in vivo*, we examined by RT-PCR analysis the effects of a restraint stress (30 min) and i.c.v. injection of LPS (50 ng/mouse) on COX-2 and IL-6 mRNAs expression in mouse calvaria. The expression of COX-2 and IL-6 mRNAs were increased by i.c.v. injection of LPS in mouse calvaria. Both increases were inhibited by the treatment with the neurotoxin 6-hydroxydopamine (6-OHDA, 100 mg/kg/day, i.p., for 3 days) or beta-blocker, propranolol (25 mg/kg, i.p.). Similarly, a restraint stress induced the expression of IL-6 mRNA in mouse calvaria. The induction was not influenced by 6-OHDA, but inhibited by propranolol. In addition, the treatment of calvaria with isoprenaline (100 microM) or noradrenaline (100 microM) increased PGE2 and IL-6 synthesis in the organ culture system. These findings show that gene expressions increased by a restraint stress and i.c.v. injection of LPS was mediated by the activation of sympathetic nerve fibers and beta-adrenoceptor in mouse calvaria and suggest that *in vivo* activation of the sympathetic nervous system modulates bone metabolism.

P161 F

HOMEOBOX PROTEIN MSX2 IS A KEY REGULATOR INHIBITING MATRIX MINERALIZATION IN LIGAMENT

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Little is known about the precise character of ligament fibroblasts, due to the absence of specific markers and cell lines. We have focused on the periodontal ligament (PDL). The PDL is located between the cementum of teeth and alveolar bone of mandibula, and is believed to play an integrating role in the maintenance and regeneration of the periodontal tissue.

To clarify the biological functions of PDL cells, we established several cell lines of the PDL. The established cell lines have heterogeneity with respect to pattern of mineralization and expression of osteoblast differentiation markers. One of these cell lines, PDL-L2, shares with osteoblasts expression of genes for type I collagen, Runx2/Cbfa1/Osf-2, periostin, but not for Osterix, bone sialoprotein and osteocalcin. This profile of gene expression in PDL-L2 exactly matches those of majority of the PDL fibroblastic cells in vivo. Unlike osteoblastic MC3T3-E1 cells, PDL-L2 failed to form mineralized nodules in the absence of rhBMP-2, even though alkaline phosphatase and Runx2 genes were expressed. Thus, there may be a regulatory mechanism preventing differentiation of PDL cells toward osteoblasts. cDNA microarray analysis revealed high expression of Msx2 in PDL-L2 cells compared with MC3T3-E1 cells. Msx2 expression is maintained in PDL-L2 cells throughout the culture period in the differentiation medium, but is gradually downregulated in MC3T3-E1 cells as mineralized nodules are produced. Msx2 is a transcription factor that stimulates cell proliferation and represses osteocalcin expression in the early stage of osteoblast differentiation. In addition, we found that Msx2 interacts and colocalizes with Runx2/Osf2, and represses Runx2 transcriptional activity. Moreover, stable expression of Msx2 antisense RNA in PDL-L2 cells induces matrix mineralization even in the absence of rhBMP-2. We also found that Msx2 is prominently expressed in tendons and the expression of Msx2 is downregulated at the calcified region in patients with ossification of the posterior longitudinal ligament (OPLL). In addition to rhBMP-2, vitamin D represses expression of Msx2 mRNA and induces matrix mineralization in PDL-L2. Taken together, these findings indicate that Msx2 plays a central role in preventing osteoblastic differentiation of ligament fibroblasts by repressing Runx2/Osf2 transcriptional activity.

P162 S

CHARACTERIZATION OF DLX5 RESPONSIVE ELEMENTS IN RUNX2 PROMOTERS

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Intramuscular injection of BMP-2 induces ectopic bone formation in vivo. Similarly, BMP-2 treatment blocks myogenic differentiation and induces osteoblastic transdifferentiation of premyoblastic C2C12 cells. Our previous results indicated that homeodomain transcription factor Dlx5 is a key mediator of BMP-2-induced osteoblast differentiation that triggers osteogenic master gene, Runx2, and ultimately results in the bone marker gene expression in BMP-induced osteogenic transdifferentiation. However, little is known about the molecular mechanisms by which Dlx5 regulates Runx2 expression in response to BMP-2 signaling. Two major isoforms of Runx2 have been reported, and are caused by different promoter usage. One of these isoforms, denoted as Runx2-typeI/p56 (starting with the sequence MRIPV), use the proximal promoter and the other, denoted as Runx2-type II/p57 (starting with the sequence MASNS), use the distal promoter. In this study, we found that Dlx5 overexpression strongly stimulated Runx2 expression and the bone specific Runx2-typeII expression was specifically stimulated either by BMP treatment or by Dlx5 overexpression. As Runx2 isoforms are distinctly regulated by two different promoters, we first tested Dlx5 responsiveness of each promoter-reporter constructs. The promoter activity of Runx2-typeII was strongly stimulated by Dlx5 overexpression while that of Runx2-type I by Dlx5 was marginally stimulated. The different responsiveness of the two promoters was highly correlated with the higher expression of Runx2-II by BMP-2 signalling. To identify Dlx5 response elements in Runx2-II promoter, serial promoter deletion constructs were generated. Cotransfection of the Dlx5 expression vector and the promoter deletion constructs indicated that Dlx5 response element is between -1kb and -0.45kb of Runx2-II transcription start site. There were 3 putative homeodomain binding consensus sequences between -1kb and -0.45kb in the Runx2-II promoter. We found that all 3 putative binding sites effectively interacts with Dlx5 by gel mobility shift assay. Thus, we identified Dlx5 responsive elements in Runx2-II promoter and this is also the direct evidence of the role of Dlx5 which binds and stimulates bone specific Runx2-II promoter to stimulate osteoblast differentiaiton.

P163 W

1,25-DIHYDROXYVITAMIN D3 SUPPRESSES RUNX2/CBFA1 GENE PROMOTOR ACTIVITY, BUT NOT VIA VDRE

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Runx2 is an essential regulator of bone specific cell differentiation. We observed the higher expression of Runx2 in vitamin D receptor knockout (VDR KO) mice than in wild type.

To investigate the effect of VD3 for Runx2 gene promotor activity, we examined tansient reporter gene assay of mouse Runx2 gene promotor (-1817 to -8: pGL3 1.8kb) in MC3T3 E1 cell. The activity of pGL3 1.8kb was suppressed in the presence of VD3 dose dependently with co-transfecting Vitamin D receptor (VDR). In the presence of 10^{-8} M of VD3, transfected MC3T3E1 cells exhibited 60% reduction of promotor activity. Mimicking hyper VD3 status like VDR KO mice up to 10^{-6}M of VD3, Runx2 promotor activity was markedly reduced to 40%. No suppression effect was observed in COS-1 cell which does not express Runx2. We hypothesized that liganded VDR is acting as a repressor for up-regulated Runx2 gene promotor activity by somewhat unknown mechanism, whereas VDR KO mice highly expresses Runx2 since repressor free. To address the functional region for VD3 induced suppression of Runx2 gene promotor activity, truncated mutants, -444 to -8, -345 to -8, -246 to -8 and -165 to -8 of pGL3 1.8kb were examined respectively. Although the activities of all mutants but -165 to -8 were suppressed by VD3, the effects of VD3 were blunted by truncating around -444 to -345. We speculated this approximately a hundred bp region could be critical for up-regulating Runx2 gene promotor activity and the suppressive effect of VD3.

Recently, sequences (-89 to -74) contain a functional DR3 typeVD3 responsive element (VDRE) that binds a VDR/retinoid X receptor heterodimer have been reported. However, the promotor activities of the site-directed mutants of this sequence eliminating the binding affinity to VDR/RXR heterodimer were also suppressed as well as that of the wild type in the presence of VD3. Our data suggest that this VDRE does not act as a key trans element for suppressing RUNX2 gene promotor activity.

In summary, VD3 down regulates Runx2 promotor activity in osteochondrogenic lineage in which Runx2 specifically express. That effect is perhaps related to -444 to -345 small region and independent from VDRE (-89 to -74).

P164 F

IDENTIFICATION OF POTENTIAL OSTEOBLAST SPECIFIC ETS1 AND ETS2 TARGETS BY CDNA MICROARRAY ANALYSIS

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Maturation of MC3T3-E1 cells can be divided into three major stages, namely, proliferation, differentiation, and mineralization. The differential expression of Ets1 and Ets2 transcription factors in these cells suggests that they regulate unique bone specific genes during the development of MC3T3-E1 cells and in this way regulate the process of osteogenesis.

Our microarray analysis of 9000 cDNAs using RNA prepared from proliferating and differentiated osteoblasts identified approximately 250 genes with expression ratio of greater than 2 associated with each of these stages. Specifically, we found RNF11/sid1669 to be abundantly expressed during the proliferation stage of MC3T3-E1 development and weakly expressed during differentiation phase. RNF11 and Ets1 have similar patterns of expression during osteoblast maturation in the MC3T3-E1 in vitro model. Bioinformatic analysis of the 5' flanking sequence of the human and mouse RNF11 revealed three Ets transcription factor binding sites (EBS). We investigated the binding of the human EBSs with Ets1, Ets2, Elk1, and EWS-Fli1 factors and found that only Ets1 binds to a GGAT core containing site. This EBS core is known to bind either Ets1 or the related Ets factor PDEF, which we have shown to be not expressed in osteoblasts. Thus, our data suggests that RNF11 is a target of Ets1 factor. Our earlier work showed that Ets1 mRNA is expressed in developing bone, and here we demonstrate that RNF11 is also expressed at the time of bone formation. Immunohistochemistry using a RNF11 specific antibody revealed that it is expressed in human osteoblasts in vivo during embryonic jawbone formation. Similarly, osteoblasts of the mouse mandible and long bones of the day 16 mouse embryo are strongly positive for RNF11 protein, whereas the cartilage and undifferentiated mesenchyme are negative. This antibody also shows that RNF11 is expressed in the cytoplasm and nucleus of SAOS-2 human osteosarcoma cells suggesting that one of its interactions may be with proteins of transcriptional machinery. This may be possible as the RNF11 protein contains a consensus Ring-H2 finger domain in the carboxyl terminal region and a consensus PY motif in the amino terminal region indicative of roles in protein-protein and/or DNA-protein interactions.

P165 S

THE EXPRESSION OF CLOCK GENES IN BONE CELLS

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In mammals, the central circadian rhythm that exists in suprachiasmatic nucleus (SCN) is generated by cell-autonomous negative transcription-translation feedback loop. The main oscillators are three homologs of the *Drosophila* genes period (*mPer1, mPer2, mPer3*), two cryptochrome genes (*mCry1, mCry2*), and the transcriptional activator genes (*Clock* and *Bmal1*). CLOCK:BMAL1 heterodimers bind to the E-box (CACGTG), drive the rhythmic transcription of clock genes. As the per and cry proteins are translated, they form PER:CRY complexes that are translocated to the nucleus. In the nucleus, the complexes act as negative regulators by directly interacting with CLOCK and/or BMAL1 to inhibit transcription, forming a negative feedback loop. Recently, it have emerged that clock genes exist not only in the SCN but also in a variety of peripheral tissues including liver, muscle, kidney and lung. In this study, we have evaluated possible expression of clock genes in bone cells. Expression of each clock genes was ascertained by RT-PCR in primary osteoblasts, chondrocytes, MC3T3-E1 and ATDC5, at both 7 and 21 days in vitro (DIV). MC3T3-E1 cultured for 7 DIV was treated with rat parathyroid hormone (rPTH) at 1-100 nanoM, insulin growth factor I (IGF I) at 1-100 nanog/miliL, or basic fibroblast growth factor (bFGF) at 1-100 nanog/miliL for 1 hr, and subsequent extraction of total RNA at indicated time, followed by analysis of mRNA expression of clock genes by Northern blotting. Expression pattern of clock genes differed between primary and cell lines, osteoblasts and chondrocytes. Transient stimulation of rPTH significantly induced rapid surge of expression of *mPer1* at concentration dependent manner in MC3T3-E1. Induction of *mPer1* was also seen when stimulated with IGF I and bFGF. These results suggest that clock genes may play an unidentified role in mechanisms associated with osteoblastgenesis.

P166 W

FUNCTIONAL DOMAINS OF PAIRED-LIKE HOMEOPROTEIN CART1 AND THE RELATIONSHIP BETWEEN DIMERIZATION AND TRANSCRIPTION ACTIVITY

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Cart1 encodes the paired-like homeodomain in the central portion of the gene, and plays a crucial role in the developmental lineage of bone and cartilage, especially in head formation. However, its transactivation mechanism is still poorly understood, including the target gene. Here, we report biochemical dissections of Cart1 functional domains and a relationship between dimerization and transcription activity.

Deletion studies of GAL4-fused Cart1 indicated that the transactivation domain is located in the middle portion of the C-terminal domain, but the N-terminal is also required for full activation of the consensus palindromic binding site (TAATNNNATTA). Analysis of the basic amino acid residues at both ends of the homeodomain revealed that both sides act as nuclear localization signals, and are necessary for the cooperative binding to the palindromic sequence. In this study, two additional Cart1 isoforms that behave as dominant negatives were identified from rat chondrosarcoma cells. These isoforms suppressed the transcription activity of the wild-type despite loss of DNA binding ability, and could interact with the wild-type in yeast. These isoforms could not be detected from RT-PCR analysis of mice embryos as well as an EST database search. However, we confirmed the presence of these isoforms in several species prepared from human chondrosarcoma but not from benign tumors from connective tissues, suggesting the possible involvement of these isoforms in the tumorgenesis of chondrosarcoma. Finally, we demonstrated that wild-type Cart1 forms DNA-independent homodimer in *in vivo* conditions, and that the transactivation of wild-type Cart1 was suppressed by the N- or C-terminal domain which was expressed in the nucleus.

These results thus revealed that homodimerization through direct interaction is necessary for potent transcription activity of Cart1.

P167 F

PROSTAGLANDIN E2 ACTIVATES THE CORE BINDING FACTOR A1 IN OSTEOBLAST CELL LINE MG-63: THE ROLE OF CORE BINDING FACTOR A1 IN POST-TRAUMATIC HETEROTOPIC OSSIFICATION

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Introduction: Post-traumatic Heterotopic Ossification (HO) is one of the most unsolved problems in trauma and orthopedic surgery. Nonsteroidal anti-inflammatory drugs (NSAIDs) are frequently used to prevent HO formation. Prostaglandin (PGE), especially PGE_2, the important metabolite of NSAIDs target enzymes Cyclo-oxygenases, has multiple effects on bone, including stimulation of both resorption and formation. PGE_2 has also been suggested as a possible diagnostic parameter for HO. Core Binding Factor a1 (Cbfa1), a transcription factor for osteoblast differentiation, controls osteogenesis at multiple stages. Recently we and others could demonstrate that human osteoblast derived from HO behaves differently compared to osteoblasts derived from iliac crest. The aim of this study was to investigate the PGE_2 influenced Cbfa1 expression in osteoblasts.

Methods: We examined the expression of PGE_2 receptors $EP_{1\text{-}4}$, and Cbfa1 mRNA in PGE_2 ($2.5\text{-}5X10^{-7}$mol/l) treated osteoblast cell line MG-63, and in human osteobalsts derived from iliac crest and HO by RT-PCR/Real time PCR. Cbfa1 Protein in PGE_2 stimulated MG-63 was detected using western blotting. Additionally, Cbfa1 binding activity was investigated in both PGE_2 stimulated MG-63, and in human osteoblasts.

Results: In MG-63, PGE_2 stimulated the expression of Cbfa1 markedly accompanying a weakly improved regulation of EP_2 and EP_4; Cbfa1 protein was also weakly improved regulated by PGE_2. PGE_2 activates the binding activity of Cbfa1 markedly. This stimulatory effects of PGE_2 displayed a time depended manner. The expression of Cbfa1 in osteoblasts derived from HO was weakly higher than those derived from iliac crest, however the Cbfa1 binding activity produced no differences between these human osteoblasts.

Conclusion: These results indicate that PGE_2 activates the binding activity of Cbfa1 via EP_2 and EP_4, thereby influences bone formation. PGE_2 is involved in hetrotopic ossification formation, and suggests that Cbfa1 plays an important regulation role in this pathological process.

P168 S

TOB FAMILY PROTEINS SUPPRESS STEROID HORMONE RECEPTOR-DEPENDENT TRANSCRIPTIONAL ACTIVATION

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Although androgens have significant effects on bone metabolism, the molecular mechanism of these actions is still unclear. It was recently shown that Tob protein, a member of an antiprolifelative protein family, negatively regulated osteoblast proliferation and differentiation. Since Tob proteins carry a LXXLL motif that mediates the binding of coactivators to steroid hormone receptors, we examined the functional relationship between androgen receptors (AR) and Tob family proteins. Luciferase assay using MMTV promoter carrying androgen-responsive element revealed that both Tob1 and Tob2 proteins suppressed AR-dependent transcriptional activation in MC3T3-E1 osteoblastic cells. However, mutant Tob proteins carrying amino acid substitution at LXXLL motif showed the same level of inhibition of the transcriptional activation as wild type. Expression of Tob protein can also inhibit the transcriptional activation by estrogen receptor (ER) and glucocorticoid receptor (GR). Using AR tagged with green fluorescent protein (GFP) and conforcal laser scanning microscope, we previously reported that after the treatment of dihydrotestosterone AR was translocated into nucleus and produced intranuclear foci. This foci formation was closely associated with AR transcriptional activation. Interestingly, Tob1 expression inhibited the formation of nuclear foci of AR. These results suggest that Tob family proteins may negatively regulate proliferative action of androgen and estrogen on bone and other non-genotropic cells.

P169 W

OSTEOBLAST-SPECIFIC DISRUPTION OF VDR GENE IN MICE

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1alpha,25-Dihydroxyvitamin D_3[1alpha,25$(OH)_2D_3$], an active form of vitamin D, has roles in many biological phenomena such as calcium homeostasis and bone formation, which are thought to be mediated by the 1alpha,25$(OH)_2D_3$ receptor (VDR), a member of the nuclear hormone receptor superfamily. To investigate the physiological roles of VDR, we had generated VDR deficient mice by a conventional gene targeting, and found that $VDR^{-/-}$ knockout (VDRKO) mice showed features typical of vitamin D-dependent type II rickets after weaning, including growth retardation, impaired bone formation, hypocalcemia, hypophosphatemia and alopecia (Yoshizawa *et al. Nature genet.* 1997). However, it is impossible to account for all these phenotypes only by direct VDR functions, because, as the observed impaired bone formation with hypocalcemia and hypophosphatemia in the VDRKO mice might be caused by secondary hyperparathyroidism. Moreover, the direct function of VDR in bone cells still remains controversy.

To define VDR function in bone formation, a conditional gene inactivation scheme was undertaken using the bacteriophage P1 recombinase Cre/loxP system. Mice with loxP sites in both sides of the VDR gene exon 2 ($VDR^{L2/+}$) were generated, and crossed with transgenic mice with the Cre recombinase under the alpha1(I)-collagen promoter (Dacquin *et al. Dev. Dyn.* 2002), which is functional only in osteoblasts. The total $VDR^{L-/L-}$ KO mice exhibited the same phenotypes that were seen in $VDR^{-/-}$ mice. Osteoblast specific $VDR^{L-/L-}$ mice are under analysis and the bone phenotype will be presented.

P170 F

THE EXPRESSION OF GABA TRANSPORTER SYSTEM IN OSTEOBLASTS

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In the central nervous system (CNS), Glutamate (Glu) and gamma-aminobutyric acid (GABA) are major excitatory and inhibitory neurotransmitters, respectively. Recent studies show that osteoblasts and osteoclasts express diverse Glu receptors and transporters that are pharmacologically similar to those expressed in the CNS. These previous findings led to examine the possible expression of inhibitory GABA signal system in bone cells as well as that of Glu. We previously reported that metabotropic $GABA_B$ receptor expressed in cultured calvarial osteoblast and demonstrated function associated with the modulation of intracellular cAMP formation (Fujimori, et al., 2002, Biochem Biophys Res Commun.). In this study, we investigated the functional expression of GABA transports (GATs) system in cultured rat calvarial osteoblasts. Primary osteoblasts isolated from calvaria of 1- or 2- day- old Wistar rats using by the sequential digestion method were cultured in alpha-MEM containing 10 percent FBS for different day in vitro (DIV). The expression of GATs was confirmed in osteoblasts using RT-PCR and immunoblotting techniques, while analysis of GABA uptake was conducted using [3H] GABA. The expression of GAT3 was detected at mRNA and

protein level in osteoblasts cultured for 7 and 21 DIV. In the transport assay, uptake of [3H] GABA linearly increased depend on incubation time at 37 °C up to 50 min, whereas no marked uptake of [3H] GABA was found at 2 °C in osteoblasts cultured for 7 DIV. Moreover, [3H] GABA uptake was significantly inhibited by nipecotic acid and beta-alanine at concentration dependent manner as well as substitution of sodium with choline or chloride with gluconate in uptake buffer. These results suggested that GATs system expressed in osteoblasts in addition to metabotropic $GABA_B$ receptor may play an unidentified role in mechanisms associated with cellular proliferation, differentiation and/or development.

P171 S

ANALYSIS OF GENE EXPRESSION PROFILES AND DIFFERENTIATION PATTERNS OF HUMAN MESENCHYMAL STEM CELL (HMSC) CLONES

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Purposes: Bone marrow stroma-derived mesenchymal stem cells (MSCs) are pluripotent somatic stem cells and thought to be suitable for regeneration of bone and cartilage of one's own. The present problems of MSCs in application to regenerative research are its heterogeneity and low ability of proliferation. Recently, Kosaka and Kobayashi established the immortalized MSC clones by introducing hTERT gene into primary MSCs. These cells continuously proliferate and can be differentiated into osteoblasts and chondrocytes. In this study, we analyzed the expression of surface markers of these cells by using flowcytometer and the gene expression profiles by using DNA microarray to understand the heterogeneity and differentiation patterns of these cells.

Methods: MSCs were transfected with hTERT gene and the stable transformants were cloned. Cell proliferation was measured by using Cell Proliferation Assay (CPA). Cell surface markers were analyzed by using flowcytometer (EPICS Elite). Gene expression was analyzed by using DNA Glassarray (Clontech) and the expression profiling was performed by using Genepix analyzer (Axon).

Results: MSC clones showed various patterns of proliferation. Analysis with flowcytometer also showed variations of marker expression on MSCs. For example, the expression of CD44 was almost positive in every clone, but highly proliferative clone no.4 showed the expression of CD34 and SSEA, and less proliferative clone no.12 showed high expression of CD44. The profile of gene expression was also different between these clones. Especially, several genes were highly expressed in clone 12 with low proliferation ability and high potential of differentiation. These data show heterogeneity of MSC clones in their molecular and cellular aspects.

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P172 W

SOST GENE EXPRESSION CLOSELY ASSOCIATES WITH OSTERIX GENE EXPRESSION DURING EMBRYONIC SKELETOGENESIS

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Bone morphogenetic proteins (BMP) play a pivotal role in growth and differentiation of skeletal cells. Several BMP inhibitors are expressed in skeletogenesis and are known to modulate intrinsic BMP signals. Among the BMP inhibitors, sclerostin (SOST) is an essential molecule of skeletogenesis since mutation of SOST gene causes bone dysplasia in human. To understand function of SOST in developmental skeletal tissue formation, we examined SOST expression in in vitro and in vivo embryonic osteogenesis. During osteoblastic differentiation in primary murine calvarial cells, the levels of SOST expression were increased along with those of alkaline phosphatase activity and nodule formation. In situ hybridization revealed that SOST mRNA expression was observed in osteogenic front in E16.5 calvariae and persisted in later developmental stage (E18.5). These temporal and spacial expression patterns were in parallel to those of Osterix, which is a critical transcriptional factor for bone formation. Expression of both SOST and Osterix was enhanced by BMP treatment in primary calvarial cells. Furthermore, in the organ cultures of embryonic calvariae, we observed upregulation in both of the SOST and Osterix mRNA expression. Thus, expression of SOST and its regulation were closely associated with those of Osterix in vivo and in vitro. These data suggest that coordinated regulation of SOST and Osterix are required for proper embryonic bone formation.

P173 F

MEPE PROTEIN IS HIGHLY EXPRESSED IN OSTEOCYTES IN HUMAN BONE

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Matrix extracellular phosphoglycoprotein (MEPE) gene is highly expressed in tumors which cause oncogenic hypophosphatemic osteomalacia (OHO). MEPE is also known to be one of the bone-tooth matrix proteins, which might be one of the inhibitory factors of bone mineralization. We have performed cloning of human MEPE gene from cDNA library of human nasal tumor causing OHO. Then we have obtained recombinant human MEPE protein (rhMEPE) and developed a rabbit polyclonal antibody against rhMEPE. We have confirmed that anti-rhMEPE antibody, thus obtained, has been available for detection of the rhMEPE expressed in *E.coli*, CHO and insect cells. Using this polyclonal antibody, we analyzed the distribution of MEPE protein in human bone by immunohistochemistry. The data from four normal control people revealed that MEPE protein was predominantly expressed in osteocytes, including their dendritic processes and pericellular bone matrix but not by osteoblasts or bone-lining cells. However the bone specimen from OHO patient revealed that MEPE was focally expressed in deeply-located osteocytes. Then we have compared the MEPE positivity of osteocyte between mineralized area and non-minerarized osteoid area using serial sections from undecalcified bone, which were embedded in methylmethacrylate, obtained from four osteomalacia and four osteoporosis patients. Osteomalacia patients consisted of two OHO, one Fanconi's syndrome, and one vitamin D-deficient rickets. For osteomalacia MEPE positivity of osteocyte in mineralized bone is 87.5 ±8.6 % and that in osteoid is 7.8 ±6.4 %, meanwhile for osteoporosis MEPE positivity of osteocyte in mineralized bone is 95.3 ±0.5 % and that in osteoid is 4.9 ±5.7 %. In specimens from osteomalacia patients, regardless of the cause of osteomalacia, MEPE protein was mainly localized in osteocytes embedded in the matrix of mineralized bone. Similar results were obtained with samples from osteoporosis patients. Our data provide the first evidence that MEPE protein is expressed by osteocytes in human bone tissue and MEPE positive osteocytes are predominantly localized to mineralized bone.

P174 S

STIMULATORY EFFECT OF PROSTAGLANDIN F2ALPHA ON NA-DEPENDENT PHOSPHATE TRANSPORT IN OSTEOBLAST-LIKE CELLS

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Prostaglandins are important regulators of bone formation and resorption. Among them, prostaglandin F2alpha (PGF2alpha) has been reported to activate protein kinase C (PKC) through both phospholipase C and D, resulting in the proliferation of osteoblast-like cells. In addition, it has also been reported that Erk mitogen-activated protein kinase is also involved in the mechanism of PGF2alpha-induced proliferation of these cells. Recently, we have reported that several growth factors stimulate Na-dependent phosphate transport (Pi transport) activity of osteoblast-like cells, which has been recognized to play an important role in their mineralization. In the present study, we investigated the effect of PGF2alpha on Pi transport in MC3T3-E1 osteoblast-like cells. PGF2alpha stimulated Na-dependent Pi transport dose-dependently in the range between 1 nM and 10 microM in MC3T3-E1 cells. The effect was time-dependent up to 24 h. Kinetic analysis revealed that PGF2alpha induces newly synthesized Pi transporter. Pretreatment with actinomycin D and cycloheximide suppressed PGF2alpha-induced enhancement of Pi transport. Combined effect of PGF2alpha and PMA, a PKC-activator, was not additive in Pi transport. Calphostin C, a PKC inhibitor, dose-dependently suppressed Pi transport induced by PGF2alpha. On the contrary, U0126, which inhibits an upstream kinase of Erk, did not affect PGF2alpha-induced enhancement of Pi transport.

In conclusion, PGF2alpha stimulates Pi transport through activation of PKC in osteoblast-like cells.

P175 W

COUPLING OF RUNX2 AND INSULIN/IGF SIGNALING IN SKELETAL CELL DIFFERENTIATION

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Runx2 is an essential transcription factor for osteoblast differentiation and plays an important role in chondrocyte maturation. Mice deficient in insulin-like growth factor (IGF) -I receptor are small and show a delay in bone development, indicating that IGF

signaling also plays an important role in skeletal cell differentiation. However, it is unknown whether IGF signaling is involved in Runx2-dependent skeletal cell differentiation. To resolve this issue, we examined the interaction between Runx2 and insulin/IGF signaling in osteoblast and chondrocyte differentiation. Overexpression of Runx2 in C3H10T1/2, MC3T3-E1, and ATDC5 cells induced alkaline phosphatase activity and mineralization, and the induction was inhibited by the introduction of dominant negative (dn)-Akt or LY294002 treatment. Further, Runx2 increased the size of the cells and enhanced their chemotaxis. Runx2 induced the expression of PI3K and Akt in these cells. In growth plates of wild-type mice, the expression of all of Runx2, PI3K, and Akt proteins were upregulated at prehypertrophic chondrocytes, whereas the upregulation of PI3K and Akt was not observed in dn-Runx2 transgenic mice under the control of type II collagen promoter. In addition, the introduction of dnAkt or LY294002 treatment inhibited both DNA binding of Runx2 and Runx2-dependent transcriptional activation, suggesting that PI3K-Akt signaling regulates Runx2 function. These findings indicate that Runx2 regulates osteoblast and chondrocyte differentiation through the interaction with insulin/IGF signaling.

P176 F

GLUCOCORTICOIDS INHIBIT OSTEOBLAST PROLIFERATION VIA TRANSCRIPTIONAL UPREGULATION OF MKP-1 : A PRIME TARGET FOR THE DEVELOPMENT OF NOVEL OSTEO-ANABOLIC AGENTS

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Glucocorticoid (GC)-induced osteoporosis (OP) is characterised by decreased osteoblast proliferation. We have found that the GC, dexamethasone (DEX), markedly inhibits the proliferation of immature mouse (MBA-15.4) and human (MG63) osteoblast cell lines. This correlates with a 40% decrease in mitogen stimulated MAP-Kinase (ERK) activity. DEX rapidly (<2h) inhibits ERK activation, primarily affecting the sustained phase of ERK activation required for nuclear shift and mitogenesis. This inhibition is reversed by co-treatment with the protein synthesis inhibitor cycloheximide, the GC-receptor antagonist RU486, as well as the protein tyrosine phosphatase inhibitor orthovanadate.

In vivo administration of vanadate also prevented the densitometric (DEXA-confirmed osteopenia), histologic (decreased bone formation assessed with quantitative bone histology following time-spaced tetracycline labelling) and physical abnormalities (tibial/vertebral breaking strengths) induced by prednisolone (3.5mg/kg/day s.c. for 9 weeks) in Sprague-Dawley rats.

Taken together, these data suggested that GC-induced OP is caused, at least in part, by up-regulation of a tyrosine phosphatase(s). Employing quantitative, real-time PCR, expression of both human and mouse MAP-Kinase Phosphatase 1 (MKP-1) was shown to be 10-fold upregulated within 30 min of DEX treatment. MKP-1 protein was also markedly upregulated following 2-8h of DEX treatment, and this correlated precisely with dephosphorylation of ERK. We therefore propose MKP-1 upregulation as a novel mechanism accounting for the direct negative effects of GC on osteoblasts, and suggest that ERK regulators like MKP-1 should be seen as prime targets in the development of new bone anabolics.

P177 S

CAVEOLIN IN THE MATRIX VESICLES RELEASED FROM OSTEOBLAST

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Matrix vesicles (MVs) are released from osteoblast and chondrocyte for mineralization. MVs can increase intravesicular calcium and inorganic phosphate concentration to promote generation of initial hydroxyapatite mineral crystals. This would be the first step of biological mineralization. MVs can be generated by budding from surfaces of osteoblast and pinched off and then released from the membrane. However, it has not been clarified how MVs are generated. Furthermore, MVs have several specific functional proteins such as annexin II, annexin V, alkaline phosphatase, phosphate transporters, and so on. Until now, nobody knows how these proteins can be concentrated in MVs. To address those problems, we hypothesized that caveolae would play an important role in the generation and maturation of MVs, and investigate the existence of caveolin that is a marker protein of caveolae in MVs and the lipid compositions of plasma membrane, caveolae membrane and MVs in the MC3T3-E1 osteoblastic cell line during calcification. First, MVs were prepared from the MC3T3-E1 cells that treated ascorbic acid and sodium phosphate for 7 days. The caveolin was expressed in the MC3T3-E1 cells and was localized in the MVs as much as caveolae membrane of the cells by immunoblot analysis. Annexin II and V was also enriched in the both MVs and caveolae, however, clathrin was not detected in MVs. Furthermore, we observed caveolin immunoreactivity in the MVs by means of immunoelectron microscopy. Second, MVs were subjected to lipid composition analysis, cholesterol was enriched in the MVs as much as caveolae (The cholesterol:phospholipid ratio of CM and MVs was twice of plasma membrane.). The sphingomyelin was also enriched in the MVs. Cholesterol and sphingomyelin are enriched in caveolae, and previous works reported that annexin II, annexin V, alkaline phosphatase and Ca2+-ATPase are localized in caveolae. Therefore this study indicates that caveolae may play an important role in the enrichment of annexin II and V into MVs and/or MVs may be derived from caveolae. This is also the first report that caveolin can be released from the cells as membrane vesicles but not lipoprotein particles as reported by Liu et al. (Nat Cell Biol. 1, 328-338).

P178 W

OSTEOPONTIN EXPRESSION IN OSTEOBLASTS AND OSTEOCYTES DURING BONE FORMATION UNDER MECHANICAL STRESS IN THE CALVARIAL SUTURE IN VIVO

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Bone formation is known to be stimulated by mechanical stress, however, molecules involved in stress-dependent regulation of bone formation have not yet been fully characterized. Extracelluar matrix proteins such as osteopontin (OPN) could play a role in mediation of the mechanical stress signal to osteoblasts. However, the function of OPN in bone formation under mechanical force is not known. Therefore, we examined the expression and the role of OPN in bone formation in vivo under tensile mechanical stress. Sagittal sutures of mice were subjected to expansion mechanical stress by setting orthodontic spring wires and OPN expression during bone formation within the suture gap was examined. Expansion of the sutures resulted in bone formation at the edges of the parietal bones within the sagittal suture. Immunohistochemical analysis revealed abundant accumulation of OPN protein in the matrix of newly formed bone on the inner edge of the parietal bone within the mechanically expanded sutures. Osteoblasts forming bone within the suture subjected to tensile stress also exhibited high levels of OPN protein expression. RT-PCR analysis indicated that OPN mRNA expression was enhanced in wild type calvariae subjected to expansion force compared to the control calvariae where dead spring wires were set without expansion stress. In addition, type I collagen mRNA was also expressed in the calvariae under the mechanical stimuli. To understand the function of OPN, sagittal sutures in OPN-deficient mice were subjected the expansion stress and bone formation within the suture to fill the expanded gap was compared to that observed in wild type mice. OPN-deficiency reduced bone formation at the edge of the parietal bone in contact with the expanded suture gap. These observations revealed that OPN plays a pivotal role in bone formation under the tensile mechanical stress.

P179 F

ULTRASOUND TREATMENT OF OSTEOBLASTS: THE EFFECTS OF INCREASING TREATMENT FREQUENCY

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Low intensity ultrasound (LIUS) has been shown to accelerate fracture healing in animal and clinical studies. These studies have standardized treatments to 20 minutes once a day but there is insufficient data to support this choice of treatment duration. It is hypothesized that increasing treatment frequency will increase osteoblast activity.

Bovine fetal osteoblasts were seeded at a density of 1.5 million cells per well and treated with LIUS for 28 days. The ultrasound unit (Smith-Nephew) generated a pulsed (1.5 MHz) ultrasound wave (30-mW/cm^2). Alkaline phosphatase activity (ALP) was measured using a colorimetric assay (Sigma) and calcium content was determined by ash weight.

Initially, cells were treated for 20 minutes a day with LIUS; controls were not treated. ALP activity was measured every three days. Ash weight was measured at days 21 and 28. Following verification of the model, a second group of cells were treated with zero (control), one (1x), two (2x), or three (3x) LIUS treatments per day. ALP activity was measured approximately every other day.

Mineralization was visible in both groups by day 14. LIUS treated cells demonstrated earlier increases in ALP activity. ALP activity peaked in the LIUS treated cells at day 15 compared to day 21 in the controls ($p=0.002$). Calcium content was significantly increased in the LIUS group after 21 and 28 days of treatment ($p=0.029$). These findings were replicated in the second experiment. Furthermore, ALP levels in the LIUS 2x and 3x peaked significantly earlier (day 10 versus day 13) compared to the LIUS 1x group ($p=0.002$) and reached higher peak values ($p=0.004$). There was a non-significant trend towards an earlier peak in ALP expression in the LIUS 3x group compared to LIUS 2x.

In conclusion, LIUS positively affected mineralization in osteoblasts as demonstrated by an earlier peak in alkaline phosphatase activity and an increase in calcium content in the stimulated cells. Alkaline phosphatase activity increased with more frequent treatments. These results indicate that increasing LIUS treatments to two or three times per day may increase bone formation compared to one daily 20-minute treatment.

P180 S

CONTINUOUS COMPRESSIVE FORCE INDUCES APOPTOSIS IN HUMAN OSTEOBLAST-LIKE CELLS

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In orthodontic tooth movement, various mechanical stresses, such as compressive and tension forces, are loaded on the periodontium, and periodontal remodeling takes place in response to these stresses. The application of a light orthodontic force results in direct alveolar bone resorption on the pressure side, while the application of too much orthodontic force results in excess compressive force, which induces a local lack of blood supply, tissue hyalinization, and cell death in the periodontal ligament. There are two modes of cell death: necrosis and apoptosis. However, the nature of compressive stress-induced cell death is not clear. This study examined whether the *in vitro* application of a continuous compressive stress induces apoptosis in MG-63 human osteoblast-like cells.

MG-63 cells were subjected to 1 to 4 g/mm^2 of continuous compressive force directly, and then cell viability and apoptosis induction were examined. After 24 hours, the morphology of cells subjected to compression had changed; cells became aligned irregularly and the spaces between them increased. Cell viability decreased in a time- and force-dependent manner, suggesting that cell death was induced by the continuous compressive stress.

In order to examine whether the compressive force-mediated cell death was apoptosis, we performed terminal deoxynucleotidyl transference-mediated nick end labeling (TUNEL) analysis. The number of apoptotic cells was significantly increased in a time- and force-dependent manner. Furthermore, the compressive force significantly increased caspase-3 activity.

We conclude that an *in vitro* compressive force induces apoptosis with caspase-3 activation in MG-63 cells. Apoptosis might act in periodontal remodeling during orthodontic tooth movement.

P181 W

THE RELATION BETWEEN BONE REMODELING AND NITRIC OXIDE BY CYCLIC TENSILE STRAIN WHICH INDUCED P38 MAPK ACTIVATION AND OSTEOPROTEGERIN SYNTHESIS IN HUMAN OSTEOBLASTS

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The bone remodeling is regulated by osteoblasts and osteoclasts. It is thought that mechanical stress play an important role in bone metabolism, as well as a number of biochemical factors. Many investigators have reported that nitric oxide (NO), as one of biological factors, is induced in bone by adding mechanical stress. Although NO is thought to be a parameter of inflammation process and an intracellular messenger in tissues, it has been poorly understood on the relation between NO and bone remodeling. In this context, using a model of cyclic tensile strain (CTS) as mechanical stress on normal human osteoblasts, we investigated NO production and the expression and/or activation of NO synthase (NOS), cyclooxygenase 2 (Cox-2), and the family of mitogen-activated protein kinases (MAPKs). It was found that 7 % and 0.25 Hz CTS was significantly increased NO production. Adding CTS against osteoblasts for 4 hrs or 12 hrs, NO production and the expression for iNOS mRNA were induced. Also, CTS was shown to increase the expression for Cox-2 mRNA in osteoblasts, attempting the quantitative RT-PCR. On the intracellular signal transduction, osteoblasts adding CTS through 3 days for 4 hrs a day, p38 MAPK activation was found to be kept for 3 days, however, the extracellular signal-regulated kinase (ERK1/2) activation down-regulated day by day, simultaneously. Concerning on bone remodeling, recent studies discussed about important roles for receptor activator of NF-kappaB ligand (RANKL) and osteoprotegerin (OPG) from osteoblasts. Particularly, it was known that soluble RANKL (sRANKL) separating from RANKL activated osteoclasts though RANK receptor. While, OPG reduced the function of osteoclasts. In this study, adding CTS against osteoblasts three times every day for 4 hrs a day, sRANKL release was decreased but OPG synthesis was increased from osteoblasts, simultaneously. Activation of osteoblasts by CTS was revealed consequently to induce NO production, the increase of the expression for Cox-2 mRNA and the enhancement of p38 MAPK activation. Furthermore, CTS was shown to inhibit sRANKL release and to increase OPG synthesis, simultaneously. From these results, it was suggested that CTS might enhance the function of osteoblasts to form new bone.

P182 F

THE CANCELLOUS BONE FORMATION BY ACTIVATION OF EP4

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Recently, it has been reported that an activation of EP4, one of prostaglandin E receptor subtype, induced the cancellous bone formation (PNAS 99:4580-4585, 2002). However, the mechanism of the bone formation by activation of EP 4 remains unclear. The aim of this study was to elucidate the mechanism of the cancellous bone formation by activation of EP4.

A total 60 male Wistar rats, 6-weeks old were used in this study,

An osmotic pump was implanted to the rats on the back subcutaneous and EP4 agonist was administered to the rat at dose of 100 ng/kg/min for up to 28 days. Thirty sham-operated rats administrating saline were used for the control of each time period. Histological and histomorphometrical changes of the femur were observed on Day 0, 1, 3, 5, 7, 14 and Day 28.

In the EP4 agonist-treated rats, proliferation of fibroblast-like cells adjacent to the endosteal bone surface in the diaphysis was initially detected on Day 5. The cancellous bone formation in the diaphysis was observed from on Day 7 and peaked on Day 28. The number of osteoblasts and osteoclasts on the cancellous bone showed an increased from on Day 3 and reached plateau on day 14. Numerous cement lines on bone- formation sits demonstrated high remodeling activity in newly formed cancellous bone. In addition, the number of adipocytes in the bone marrow was markedly reduced in the EP4 agonist-treated rats as compared with that of the controls.

These results demonstrate that EP4 agonist administration promotes both bone formation and resorption in cancellous bone and suggest that EP4 agonist may inhibit adipocyte differentiation and promote early osteoblast differentiation in bone marrow stromal cells and then it will work in mature osteoblasts for induction of osteoclasts on the newly formed cancellous bone.

P183 S

CIRCADIAN RHYTHMS IN THE COLLAGEN-SYNTHETIC AND SECRETORY ACTIVITIES OF OSTEOBLASTS AND CARTILAGE CELLS IN RATS

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Blood concentrations of calciotropic hormones as well as biochemical parameters of bone formation and resorption display characteristic circadian rhythmicity. However, the mechanisms underlying these rhythms have not yet been fully understood. In the present study, we investigated whether there is a circadian rhythm in the collagen-synthetic and secretory activities of rat osteoblasts and cartilage cells by radioautography with ^{3}H-proline as a tracer. We also investigated the rhythmic expression of type-I collagen mRNA in bone cells by northern blot analysis. Six different time groups of Wistar rats, 6-week-old, were injected with ^{3}H-proline at 00:00, 04:00, 08:00, 12:00, 16:00, or 20:00 after the animals had become acclimated to a 12/12-hour light-dark illumination cycle for 2 weeks. One hour after the injection, the animals were killed. Femora, mandibulae and calvariae were removed and processed for radioautographic study. In another experiment, six groups of rats were killed at 4-hour interval over 24 hours. Calvariae were removed and processed for northern blot analysis for type-I collagen mRNA. In all bone tissues examined, the radioautographic silver grains of ^{3}H-proline were most intense over osteoblasts and bone matrix, and over cartilage cells and surrounding matrix during the environmental light period (at 13:00), while the nadir occurred during the dark period (at 01:00). The peak values were approximately 1.4 to 2.5-fold higher than the minimum values. The result of northern blot analysis showed that expression of type-I collagen mRNA also followed a circadian rhythm with a peak during the light period and with a nadir during the dark period. The above results clearly demonstrated that both osteoblasts and cartilage cells synthesize and secrete collagen according to a circadian rhythm with a peak during the resting period of animals and with a nadir during the active period of animals.

P184 W

BASIC FGF PROTEIN PROMOTES BONE FORMATION BY GENE THERAPY USING BMP -2 GENE

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In this study, the bone defects created on animal cranium which treated by BMP gene (cDNA plasmid) combined with or without bFGF introduced with porous HAP and the bone formation was analyzed histpathologically. The bone defect of 1.2cm in the diameter was made on rabbit's cranium and HAP pellets of exactly 1.0 cm in diameter were implanted after completion of hemostasis. In the HAP which containing BMP-2 gene alone group, although it revealed that new bone formation was evident surrounding the HAP pellets at three weeks after the operation, the inducted bone tissue did not fill to the entire pores of the HAP pellets even at 9 weeks after the operation. The effect of basic fibroblast growth factor protein (bFGF:1 mcg) combined with BMP-2 cDNA plasmid(10 mcg) was also examined in this research. As a result, in the bFGF-BMP2 cDNA combined group, new bone was not filled the large part of the pores of the HAP pellet in addition to the bone formation around HAP pellet. As a result of this study, it revealed that is the combination of BMP-2 cDNA plasmid with bFGF protein is much more effective. Mechanism of this effect

manifestation is not clear, but we regard that the effective gene induction to the osteogenetic cells in addition to a cell growth and a neovascularisation effects by bFGF administration. It is regarded that the clinical significance of gene therapy for the purpose of bone formation which uses the BMP-2 gene was confirmed by animal cranial defect model. In addition to that, through the results of this research, the bone formation by the gene transfer which uses BMP-2 cDNA plasmid combined with the bFGF protein together is thought to be a technique that is effective because it is assumed to be more efficient strategy clinically.

P185 F

ACTIVATION OF PROSTAGLANDIN E RECEPTOR EP2 DURING THE OSTEOPROGENITOR PROLIFERATION-DIFFERENTIATION TRANSITION IS THE MAJOR PATHWAY MEDIATING THE ANABOLIC EFFECTS OF PGE2 ON OSTEOBLAST DEVELOPMENT IN RAT CALVARIA CELL CULTURES

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Of four prostaglandin E (PGE) receptor subtypes (EP1-EP4), EP4 has been proposed, based on analysis of the four different EP knockout mice treated with PGE2, to be the sole receptor mediating the anabolic effects of PGE2 on bone formation. However, local injection of EP2 selective agonist into the rat tibia is also known to increase bone formation. Thus, there remain questions about which EP pathways contribute to the effects of PGE2 on bone formation. To dissect the role of the EP2 versus EP4 pathways in osteoblast development, we assessed the effects of EP2 (EP2A, ONO-AE1-259-01) and EP4 selective agonist (EP4A, ONO-AE1-437) in rat calvaria (RC) cell cultures. Both EPAs enhanced osteoblast development and matrix mineralization as did PGE2, the latter at doses consistent with previous results. Treatment with PGE2 or either of the EPAs increased the number of alkaline phosphatase-positive nodules but decreased their size. This change in size appeared to be due to inhibition of proliferation of osteoprogenitor cells as well as acceleration of proliferation of more committed cells in nascent/immature nodules and/or their more rapid differentiation. The increase in osteoblast development was seen not only with chronic treatments but also in cells pulse-treated during the osteoprogenitor proliferation-differentiation transition but not during late nodule formation or later phases of cultures. PGE2 and EPA effects were diminished (with PGE2 or EP2A) or eliminated (with EP4A) when cells were cultured with dexamethasone, a potent stimulator of osteoblast development in the RC model. The efficacy of EP2A was comparable to that of PGE2 but much higher than EP4A in all parameters examined. Interestingly, EP2A markedly, while EP4A only slightly, increased EP1, EP2 and EP4 mRNA levels during the osteoprogenitor proliferation-differentiation transition. Concomitantly, the cAMP response to EP2A during this developmental time period was much higher than that to EP4A. When discrete RC osteoblast colonies were analyzed, EP2 mRNA was more abundant in osteoprogenitor colonies while EP4 mRNA was more abundant in mature osteoblast colonies. In conclusion, PGE2 acts, in a biphasic manner, to stimulate osteogenesis in the osteoprogenitor proliferation-differentiation transition with the EP2 pathway predominating in the RC cell model.

P186 S

IDENTIFICATION OF DRUGABLE TARGETS IN RHEUMATOID ARTHRITIS AND OSTEOPOROSIS BY FUNCTIONAL GENOMICS

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Several bone diseases with high prevalence such as rheumatoid arthritis (RA), osteo arthritis (OA) and osteoporosis are still unsatisfactorily treated in the clinic. Current treatments are symptomatic but offer no relieve towards e.g. bone and cartilage degradation let alone regeneration. The identification of compounds with bone or cartilage forming potential is therefore of commercial importance, considering the huge market potential.

New approaches are needed to develop novel drugs in this field, and our unique functional genomics platform and assay development capabilities will give Galapagos Genomics an advantage to mine the bone disease areas for new drug targets.

With its current research project in RA and osteoporosis, Galapagos has already built know-how in this area.

On the one hand, primary human mesenchymal stem cells are used to develop high-throughput cellular assays allowing the identification of anabolic bone factors. The upregulation of the endogenous early osteoblast marker bone alkaline phosphatase (BAP) was used as a reporter. BAP is cell-membrane bound and easy to detect with high sensitivity. The assay was successfully used to screen 112.000 viruses from Galapagos' proprietary PhenoSelect arrayed adenoviral cDNA libraries. This has led us to identify novel osteogenic pathways. The identified factors are then validated for their osteogenic potential (e.g. bone mineralization) and for their contribution to the disturbance or maintenance of the fine-tuned balance between osteoclasts and osteoblasts needed for proper bone homeostasis.

On the other hand, primary human synovial fibroblasts are used to develop assays allowing the identification of drugable targets that i) regulate synoviocyte proliferation or ii) that induce bone or cartilage extracellular matrix degradation. Identified targets are further validated to obtain insight into their mechanism of action and for their upregulation of inflammatory agents such as TNFa or IL-1b.

P187 W

ELECTROPORATION TO BONE MARROW AS A TOOL FOR GENE THERAPY OF BONE DISEASES

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Electroporation is one of the altertative tools of gene transfer for clinical use. Whereas this procedure is easy, costless and rarely induces immuno-response, the efficacy of gene transfer is lower than those with viral vectors. The pCAGGS vector, recently we developed, improved this problem. This vector demonstrated acceptable efficacy when it was injected into muscle by electroporation. Bone marrow is rich with progenitor cells for tissue regeneration and will be reasonable target for gene therapy. The aim of this study was to establish a gene transfer into bone marrow by in vivo electroporation. To determine the optimum condition for gene transfer, we transferred the pCAGGS vector, harboring luciferase gene, into bone marrow in the proximal femora of the Wistar rats. The DNA solution was injected into the intertrochanteric lesion of the proximal femur. The electrodes were placed on the femoral head and intramedullary space. Square pulses were produced between the electrodes by using of a pulse generator (CUY21EDIT, Neppagene, Japan). The efficacy of gene transfer depended on the volt and current applied to the DNA injected site. The optimal gene transfer could be achieved when 12 square pulses of 100V each were delivered to the injection site. The pulse rate was 1 Hz and each pulse lasted for 50 ms. The luciferase protein was detectable for 6 weeks after gene transfer with its peak on day 2. Histological analysis of the femur never showed irreversible damages around the electroporation site. This is the first report to transfer a foreign gene into bone marrow by in vivo electroporation, which will be clinically useful to treat bone diseases.

P188 F

EFFECT OF BMP-2 OR TROGLITAZONE, AS AN INDUCER FOR OSTEOGENIC CELLS OR ADIPOCYTES, ON DIFFERENTIATION OF TBR31-2 CELLS

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TBR31-2 cells belong to one of the stromal cell lines established from the marrow of transgenic mice harboring the temperature-sensitive SV 40 T-antigen gene. These cells show the characteristics of undifferentiated cells and possess the capacity to differentiate toward adipocytic and osteoblastic cells during long-term culture. In the present study, we investigated the effect of BMP-2 or troglitazone, which is an inducer of osteoblasts or adipocytes, on the differentiation of TBR31-2 cells.

Differentiation toward osteogenic cells and calcification was observed when TBR31-2 cells were cultured for 4 weeks at 37 ºC with alpha-MEM supplemented with 10% FBS, 0.2 mM ascorbic acid and 5 mM beta-glycerophosphate. One of the osteogenic differentiation markers, alkaline phosphatase (ALP) activity, increased during 2 weeks of culture at 37 ºC and reached maximum at 4 weeks. In the osteogenic differentiation, BMP-2 in the range of 50-400 ng/ml stimulated ALP activity within 1 day in a dose- and time-dependent manner.

Troglitazone is one of the thiazolidinediones that activate the PPARgamma. After the TBR31-2 cells had reached confluency, they were cultured at 37 ºC with alpha-MEM supplemented with 10% FBS and 10 microM troglitazone for 12 days. Oil droplet accumulation, an adipocyte differentiation marker, was found to be time-dependent when examined using Nile red staining. Furthermore, the increase of ALP activity was completely suppressed by the addition of troglitazone.

RT-PCR analysis was performed to determine the expression of the specific gene for the osteogenic cell differentiation marker, such as Cbfa1, ALP, type I collagen, BGP and osteopontin, and for the adipocyte differentiation marker, such as PPARgamma adipsin and lipoprotein lipase. Confluent cultures of TBR31-2 cells were kept at 37 ºC in differentiation medium with 400 ng/ml BMP-2 or 10 microM troglitazone for 3, 6 and 12 days. In the presence of BMP-2, an increase of mRNA expression in the osteogenic cell differentiation markers and a decrease of mRNA expression in the adipocyte differentiation markers were observed. On the other hand, troglitazone treatment produced the opposite tendency.

These findings indicate that TBR31-2 cells can be a bipotent progenitor for osteoblasts and adipocytes under the effect of a differentiation inducer during short-term culture.

P189 S

THE ADMINISTRATION OF EP4 SELECTIVE AGONIST ACCELERATES CORTICAL BONE HEALING AFTER DRILL-HOLE INJURY IN RATS

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Prostaglandin E2 (PGE2) stimulates bone formation and increases bone mass when dosed systemically or locally. Of the four PGE2 receptor subtypes (EP1-EP4), EP4 is considered to be the major receptor that mediates an anabolic effect on bone. This investigation was performed to clarify the hypothesis that EP4 selective agonist accelerates cortical bone healing in drill-hole injuring rats. A total of 128 male Wistar 12-week-old rats were studied. In this model, a hole measuring approximately 2.0 mm in diameter penetrating the bone marrow was drilled in the anterior portion of the diaphysis of bilateral femurs. Rats were injected subcutaneously with vehicle or two doses (10ug/kg, and 30ug/kg body weight) of EP4 selective agonist (ONO-4819 CD) twice a day. We started the administration on the next day of the surgery and continued it until a day before killing. At days 0, 5, 7, 14, 21, and 28 after surgery, the injured rats (n=8 in each group) were sacrificed. The injured sites of the femurs were analyzed using peripheral quantitative computed tomography (pQCT), bone histomorphometry, and biomechanical testing in three point bending. The results of pQCT showed that in the EP4 agonist-administered rats, cortical bone mineral content and cortical bone mineral area increased significantly and dose-dependently at day 21 compared with those in the vehicle-administered control rats. Histomorphometric analysis showed that in the EP4 agonist-administered rats, the cortical bone volume (BV/TV) increased significantly and dose-dependently at days 14 and 21 compared with that in the control rats. The values of ultimate load in the EP4 agonist-administered rats increased dose-dependently. The values of bone metabolic markers, urinary deoxypyridinoline and serum osteocalcin, in the EP4 agonist-administered rats did not differ significantly from those in the control rats. Bone mineral density (BMC) of the fifth lumbar body in the EP4 agonist-administered rats did not differ from that in the control rats. We concluded that the administration of EP4 selective agonist accelerates cortical bone healing after drill hole injury in rats. The EP4 agonist at the doses used in this experiment did not affect the systemic bone turnover, but the injured sites selectively.

P190 W

CYCLOOXYGENASE-2 INHIBITOR DELAYS THE FRACTURE HEALING ESPECIALLY IN EARLY STAGE

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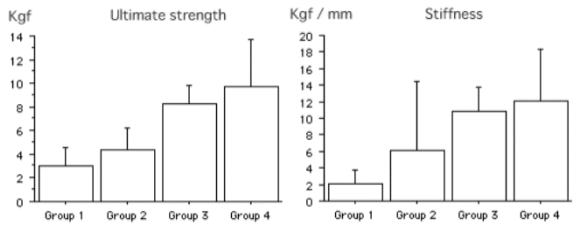
Introduction: Recently, Cyclooxygenase-2 (COX-2) inhibitor has been reported to delay the fracture healings. Inflammation at the fracture site is usually self-limiting in its nature. So only the short period administration of the COX-2 inhibitor may have the risk of delaying the fracture healing. The purpose of this study was to elucidate this point.

Methods: Twenty-two female Wister rats were used in this study. After non-displaced closed femoral shaft fracture were operated on, the rats were randomly assigned to four groups by the differences of the drugs administrated period; seven rats received intraperitoneally administered etodolac (a cox-2 specific inhibitor) at a dose of 20 mg/kg body weight per day for three weeks (group 1). Five rats had same dose from operation to 7th day after operation, that was early administrated group (group 2). Five rats had same dose from 14th to 21st day, that was late administrated group (group 3). While five rats were in the vehicle control group (group 4). Immediately after surgery, and at one-, two- and three- weeks following surgery radiographs were taken to evaluate the callus formation and bone healing. A radiographic scoring system for fracture healing was used for the evaluations. Three weeks after surgery femurs were harvested from all rats, and all soft tissue and intramedurally inserted K-wires were removed. A three point bending test was used for the mechanical evaluation.



Results: At the three weeks, bone union was observed in the groups 3 and 4, while in the groups 1 and 2 bone union had been poor. Moreover the mechanical testing also showed delayed maturation in the groups 1 and 2, these were etodolac administrated groups. The results of this study indicated that bone union and callus formation were delayed by the administration of etodolac especially in the early stage of the fracture healing.

P191 F

PARATHYROID HORMONE(PTH) INFLUENCES IMMATURE OSTEOBLASTIC CELLS AND EXERT ANABOLIC EFFECTS ON MINERALIZED TISSUE FORMATION

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PTH is a major regulator in calcium homeostasis and it is well known that intermittent administration of PTH increases bone mass, stimulating remodeling activity not only in post-menopausal animal model but also in intact one. Large number of human studies also confirmed PTH can recover bone mass in osteoporotic patients. The anabolic effects of PTH on bone formation have not been clearly reproducible in vitro and detail of the PTH anabolic effects is not well understood. We have previously established primary culture system of rat bone marrow cells and examined PTH effects on mineralized tissue formation. In this culture system, PTH/PTHrP receptor was expressed at early culture stage, in which osteopontin(OPN), osteocalcin(OC) or bone sialoprotein(BSP) mRNAs expression was not detected by Northern blotting analysis, and the anabolic action of PTH on mineralized nodule formation was observed after pulse treatment with PTH (10 or 100 nM) at early culture stage (day 4). In contrast, treatment with PTH at later stage did not affect or inhibited it. Interestingly, this early stage pulse treatment up-regulated OPN, OC and BSP mRNA expression and ALP activity while DNA contents and [^{3}H]thymisin incorporation were comparable between PTH treatment group and control group. On the contrary, PTH treatment at later stage down-regulated OPN, OC and BSP expression. Furthermore, PTHrP also exerted similar effects to PTH in this culture system. These results suggest that target cells for PTH, exerting anabolic effect, could be immature cells of osteoblastic lineage that are expressing PTH/PTHrP receptors but not another osteoblastic markers such as OC and BSP. Moreover, PTH may stimulate osteoblast differentiation without cellular proliferation. Therefore, we concluded that PTH could influence immature osteoblastic cells and exert anabolic effects on mineralized tissue formation.

P192 S

STATIN INDUCED MORPHOLOGICAL CHANGES IN HUMAN OSTEOBLASTS THROUGH INDUCTION OF BMP EXPRESSION

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Objective; Statin reduces the synthesis of cholesterol through inhibiting HMG-CoA reductase in mevalonate pathway of the liver, whereas statin increased BMP-2 and BMP-4 expressions in osteoblasts and enhance bone formation. Isoprenoid intermediates in mevalonate pathway (FPP and GGPP) are known to play an important role in post translational prenylation, translocation, and activation of small GTP-binding proteins, such as Rho A. Rho is thought to play an important role of cytoskeleton via target proteins. Recently we found that statin induced morphological changes in normal human osteoblasts. In this study we examined the mechanism of statin action in human periosteal osteoblasts in terms of Rho-associated kinase. *Materials and methods*; Human osteoblasts (SaM-1) from ulnal periosteum have been established in culture (Koshihara et al., 1989). SaM-1 cells were identified as normal osteoblasts, accordingly to the characteristic features displayed, and with mortality. The confluent cells at 19 population doubling levels (PDL) were incubated with cerivastatin, lovastatin, sinvastatin or 1,25$(OH)_2$D3 for 24 hrs. Total RNA was extracted by AGPC method, and subsequently used to investigate BMP-2 and BMP-4 mRNA expressions by RT-PCR. The level of mRNA expression was quantfied by densitometry in comparison with the level of GAPDH mRNA. *Results* ; All kinds of statin increased BMP-2 expressions and slightly increased BMP-4 expressions in dose dependent manner. Of all three kinds of statin, cerivastatinwas 100 times more potent than the others. However, 1,25$(OH)_2$D3 did not affect the expression. Cerivastatin-induced BMP-2 expression was inhibited by adding farnesyl PP (FPP) or geranylgeranyl PP (GGPP) into the medium. Cerivastatin (10^{-7}M) inhibited the translocation of Rho A protein from soluble fraction to particulate fractions. This inhibition was rescued by adding FPP and GGPP. When cerivastatin was added to human osteoblasts, it caused morphological changes from a fibroblastic shape to a rounded shape. By rhodamine phalloidin staining, cerivastatin- induced a breakdown of actin filament in a cell was seen. These morphological changes were recovered with adding FPP and GGPP into medium. *Conclusions* ; Statin stimulated BMP-2 and BMP-4 expressions by inhibiting Rho-associated kinase, and induced morphological changes by a breakdown of actin filaments.

P193 W

ALUMINIUM STIMULATES OSTEOBLAST FUNCTIONS DUE TO THE INHIBITION OF ADENYLATE CYCLASE AND ACTIVATION OF PHOSPHOLIPASE C

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The accumulation of aluminium (Al^{3+}) in bone has been associated with the development of osteomalacia due to a defect in the mineralization of bone with an accumulation of osteoid. However, the mechanisms underlying these changes are still not known. The effects of prostaglandin E_2 (PGE_2) are mediated by four characterized receptors, EP_1-EP_4. EP_1 is coupled with intracellular Ca^{2+} transport, whereas EP_2 and EP_4 are coupled with cAMP production through the activation of adenylate cyclase. We have shown previously that an EP_1 agonist stimulates the differentiation of osteoblasts and an EP_2/EP_4 agonist has an opposite effect on osteoblasts. The purpose of this study is to examine the effects of Al^{3+} on the differentiation of osteoblasts through PGE_2 receptors. Osteoblasts were enzymatically isolated from calvariae of 4- and 25-month-old female rats. In both cells, EP_1, EP_2 and EP_4 were expressed. The expression level of EP_1 was decreased with aging, whereas EP_2 and EP_4 were constantly expressed. An exposure to Al^{3+} resulted in a dose-dependent increase in bone formation regardless of cell donor ages. Al^{3+} decreased EP_2/EP_4 agonist-induced suppression of bone formation due to the decrease in cAMP production. Al^{3+} stimulated the production of inositol-1,4,5-triphosphate. Al^{3+}-induced effects were inhibited by the pretreatment with pertussis toxin as an uncoupler of G_i protein. In conclusion, Al^{3+} acts as a promoter for bone formation due to a inhibition of adenylate cyclase and activation of phospholipase C through G_i protein.

P194 F

TRANSFORMATION OF OSTEOBLASTS TO OSTEOCYTES IS ACCOMPANIED BY DRAMATIC CHANGES IN THE DISTRIBUTION OF ACTIN-BINDING PROTEINS

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Osteocytes are derived from a select group of osteoblasts that have undergone a final differentiation. We previously reported that osteocyte shape is dependent on actin filaments. To analyze the transformation from osteoblasts to osteocytes, we have investigated the actin-binding proteins, which are the control elements in the dynamic organization of the actin cytoskeleton. We used primary chick osteocytes and osteoblasts, the phenotypes of which were confirmed by use of OB 7.3, a chick osteocyte-specific monoclonal antibody and alkaline phosphatase activity, respectively. Immunofluorescence staining showed the presence of fimbrin and alpha-actinin in the processes of osteocyte, and especially strong signals indicating fimbrin were observed at the site of divarication of the processes. Anti-villin was reactive with the osteocyte cytoplasm but not with the processes. Interestingly, anti-villin immunoreactivity in osteocytes was much stronger than that in osteoblasts. Filamin was localized in entire stress fibers of osteoblasts, but was seen only in those of the proximal base of osteocyte processes. Myosin and tropomyosin were identified as having a similar pattern in both stress fibers of osteoblasts and osteocyte processes. The change in the distribution of anti-spectrin staining was highly dramatic. Immunostaining of osteoblasts with anti-spectrin showed punctate signals on their cytoplasmic membranes, whereas anti-spectrin in osteocytes detacted a filamentous organization, and the spectrin was totally colocalized with actin from the distal portion of the cytoplasmic processes to the cell center. In conclusion, we revealed that transformation of osteoblasts to osteocytes was accompanied by highly dramatical changes in the distribution of actin-binding proteins.

P195 S

EFFECTS OF INSULIN-LIKE GROWTH FACTOR-I, PTH, AND PTHRP ON PROLIFERATION AND DIFFERENTIATION OF STROMAL BONE MARROW CELLS IN ENDOTHELIAL NITRIC OXIDE SYNTHASES KNOCKOUT MICE

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Nitric oxide (NO) is a free radical and a regulator of bone remodelling. It is synthesised from L-Arginine by nitric oxide synthases (NOS) in bone cells. Endothelial NOS (eNOS) is the most abundant NOS in bone. Previous studies on eNOS knock-out (eNOSKO) mice have demonstrated decreased bone volume and marked abnormalities in bone formation.

In this study we investigated the effects of IGF-I (1ng/ml), PTH_{1-34} (10^{-9}M) and $PTHrP_{1-34}$ (10^{-9}M) on proliferation and differentiation of marrow stromal cells in adult eNOSKO mice compared to wild type (WT) counterparts. The cells were cultivated with osteogenic supplements.

The WT marrow stromal cells had positive staining for alkaline phosphatase activity and they produced osteocalcin indicating an osteoblast-like phenotype. In the eNOSKO cells the ALP staining was positive but weaker than in the WT cells. Osteocalcin was stimulated with PTHrP together with vitamin K 10^{-5} M ($p<0.01$) in WT cells while in the eNOSKO cells it was below detection level.

In WT cells, IGF-I increased cell proliferation with 42 % ($p<0.01$) while cell proliferation in eNOSKO was unaffected by IGF-I. PTH and PTHrP increased cell proliferation significantly by 2-3 fold in cell-cultures, 14-18 days old in both WT and eNOSKO. In cells cultivated for 21-22 days the proliferation was significantly inhibited in both WT and eNOSKO.

In conclusion: The anabolic response of IGF-I on cell proliferation was decreased in adult eNOSKO mice compared to wild type animals. This demonstrates that an impaired osteoblast function is present also in adult eNOS-deficient animals. The impaired IGF-I response may contribute to the defect bone formation seen in theses animals. PTH and PTHrP had similar effects on cell proliferation in both WT and eNOSKO mice suggesting a mechanism independent of a functional eNOS.

P196 W

ACCELERATION OF OSTEOBLASTIC CELL DIFFERENTIATION AND MINERALIZATION BY FIBROBLAST GROWTH FACTOR RECEPTOR (FGFR) 2 WITH APERT MUTATION

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Apert syndrome is an autosomal dominant disease, which is characterized by craniosynostosis and bony syndactyly. This syndrome is associated with point mutations (S252W, P253R) in the fibroblast growth factor receptor (FGFR) 2 which may affect ligand-dependent activation. This suggests that abnormal FGF/FGFR2 signaling in osteogenic cells by Apert mutations might contribute to the pathogenesis of the skeletal development in the patients. Therefore, we investigated the role of Apert mutation (S252W) in FGFR2 on osteoblastic cells in this study. We isolated osteoblastic cells (ApOB1, 2) derived from two independent Apert patients (S252W) and confirmed the expression of FGFR2 mRNA with S252W mutation, higher alkaline phosphatase (ALP) activity, osteocalcin and osteopontin mRNA expression and mineralization capacity in comparison with control osteoblastic cells (HOB1, 2) derived from two independent non-syndromic polydactyly patients. For better understanding of the role of FGFR2IIIIc with S252W (FGFR2IIIcS252W) on osteoblastic cells, we established three stable cell lines (MG63-IIIc#1~3) overexpressing wild-type FGFR2IIIc and five stable cell lines (MG63-Ap#1~5) overexpressing FGFR2IIIcS252W from MG63 human osteosarcoma cell line. MG63-IIIc and MG63-Ap cells similarly showed a significant reduction of BrdU incorporation and an evident increase in apoptotic cell numbers in the differentiation medium as compared with parental or mock-transfected MG63 cells. On the contrary, MG63-Ap cells showed higher RUNX2, osteocalcin and osteopontin mRNA expression than MG63-IIIc cells. Furthermore, the mineralization activity was dramatically accelerated in MG63-Ap cells as compared with MG63-IIIc cells and was completely inhibited by the administration of soluble FGFR2IIIcS252W lacking transmembrane and cytoplasmic domains. These results indicate that FGFR2IIIc causes less proliferation and more apoptosis induction in osteoblastic cells regardless of Apert mutation. However, FGFR2IIIc with Apert mutation (S252W), but not FGFR2IIIcc, could enhance osteoblastic marker gene expression and in vitro mineralization in MG63 cells. Therefore, it is strongly suggested that Apert mutation in FGFR2 may play a key role in the enhancement of osteoblastic differentiation and mineralization.

P197 F

ALTERNATIVE TREATMENTS, NON-PHARMACOLOGICAL, IN THE STIMULATION OF OSTEOGENESIS

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Mechanical loads cause bone deformation leading to an increase in local bone formation. Physical activity promotes cellular proliferation, differentiation, morphogenesis and gene expression. Despite the fact that genetics is responsible for 80% of the individual bone mass, physical activity associated with a healthy diet and life style can provide a high bone amount. Electrical stimulation and ultrasound can be used as stimulus for bone formation, because both cause bone deformation, which do increase calcium deposition in bone. Moreover, laser action can stimulate the osteogenesis, consequently accelerating bone repair, due to increase speed of cellular

mitosis through biochemical, bioelectrical and bioenergetic effects. Application of such techniques can be shown in disuse osteoporosis pathologies (i.e., spinal cord injuries).

P198 S

BONE REGENERATION OF THE SEGMENTAL TIBIAL DEFECTS BY DIRECT GENE TRANSFER TECHNIQUE

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Bone morphogenetic proteins (BMPs), which have an ability to induce ectopic bone, are expected to be applicable to bone regeneration. However, a large amount of the recombinant BMP is required to induce ectopic bone in human. Gene transfer technique with naked plasmid DNA containing a sequence of a bone inducing protein has been recently applied to bone regeneration. Although application of gene transfer to bone regeneration was effective, the efficiency of this gene transfer was extensively low in previous studies. To solve this problem, the following experiment was conducted.

A segmental bone defect, five millimeters length, which is critical size of a bone defect, was created in the right tibia of adult male Wistar rats (10 weeks old). Fixation in anatomical orientation was achieved with four stainless screws, wire and resin. All animals were divided into four groups (group A to D). In group A, the defect was implanted with a material, which consist of plasmid vector containing 25 microg of BMP2 cDNA (BMP-plasmid), calcium phosphate (CaP) and bovine type I atelo-collagen (collagen). In group B, a defect was implanted with a material, which consists of BMP-plasmid and collagen. In group C, a defect was implanted with a material consist of collagen alone. In group D, the defect was not filled. Animals were sacrificed at four and six weeks after the implant operation. The regeneration and healing of bone defects were evaluated radiographically.

Radiographic analysis demonstrated the complete bone fusion in group A. Only modest and partial bone regeneration was observed in group B and C defects. The defects of group D showed little or no radiographic evidence of bone regeneration.

These results confirm that direct gene transfer technique is effective for regeneration of segmental bony defects. Furthermore, it has been demonstrated that combination of BMP-plasmid with CaP and collagen enhances the efficiency of gene transfer compared to the combination of BMP-plasmid with collagen, which was previously reported as 'gene acitivated matrix'. This new gene transfer technique (plasmid + CaP + collagen) would be useful in bone regeneration.

P199 W

THREE-DIMENSIONAL QUANTITATIVE EVALUATION OF OSTEOCYTES IN BONE

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It is thought that cell morphology is strongly related to cell function. Thus, dynamic morphological changes from cuboidal shape of the osteoblasts to stellate shape of the osteocytes would contribute to biological function of osteoblasts and osteocytes. However, osteocytes are surrounded by hard bone tissue, so it has been impossible to image whole figure of the osteocytes. In our previous study, we have reported the possibility to observe fluorescently labeled osteocytes in bone by using confocal laser scanning microscopy. In this study we conducted an extensive analysis of the three-dimensional structure of osteocyte visualized as shadow projections from confocal images by use of IMARIS software. Furthermore, the length of processes, surface area, and volume of osteocytes were analyzed with morphological analysis software. For this study, 16-day-old embryonic chick calvariae were fixed, decalcified, and permealized. The samples were stained with Texas Red-X-conjugated phalloidin and were observed by using an LSM510 confocal laser scanning microscope from the surface of the bone matrix to a depth of 4.5 microm. Thus, the visualized osteocytes were regarded as osteoid-osteocytes. From the images of IMARIS, we calculated that one osteocyte occupied 4180 ±673 microm^3 of bone matrix. The distance between adjacent osteocytes was 24.1 ±2.78 microm. The length of processes, surface area, and volume of osteocyte were analyzed by using NEURON TRACER and SURPASS software. NEURON TRACER is a software specifically designed to automatically map and analyze the three-dimensional structure of dendritic models. The length of osteocyte processes was 0.256 ±0.025 microm per 1 microm^3 bone matrix. SURPASS is the software that reconstructs a binary model of a cell from serial confocal images and can analyze its surface area and volume. The surface area of osteocytes was 0.526 ±0.064 microm^2 per 1 microm^3 bone matrix. The volume ratio of osteocyte to bone matrix was 18.36 ±2.24 % . As a result, the total length of processes, the surface area, and the volume of one osteocyte were 1070 microm, 2198 microm^2, 767 microm^3, respectively. We conclude that it is possible to reconstruct whole figures of osteocytes in bone and obtain morphometrical data from them.

P200 F

PROMOTED OSTEOBLASTIC ACTIVITY AND BONE REMODELING IN OSTEOPROTEGERIN DEFICIENT MICE

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Osteoprotegerin (OPG) deficient mice have been reported to show enhanced osteoclastogenesis and subsequent stimulated bone resorption, resulting in severe osteoporosis. However, serum alkaline phosphatase (ALP), a hallmark of bone forming markers, was elevated in the OPG deficient mice. In the present study, therefore, we have attempted to investigate histological alterations on osteoblastic activity and bone remodeling in this mutant mouse. As expected, tartrate-resistant acid phosphatase (TRAP) positive osteoclasts were abundantly localized on trabeculae of OPG deficient mice, consistent with markedly reduced numbers of trabeculae. On the contrary, thick layers of strong ALP positive osteoblasts overlying bone surfaces and numerous calcein depositions on the trabeculae were found in the OPG deleted mice, indicating accelerated osteoblastic activity. Electron microscopy, coincidently, demonstrated abundant osteoblasts and preosteoblasts in the intertrabecular region. Quantitative analysis employing real-time PCR verified upregulation of ALP and Cbfa-1/Runx2 genes in OPG deficient mice, relative to their wild-type counterparts. It seems likely that osteblastic proliferation and subsequent differentiation are promoted in this mutant mouse. A meshwork of numerous cement lines, which were envisioned by TRAP and osteopontin as boundary lines between newly-formed and the pre-existing bones, ensured highly accelerated bone remodeling in the OPG deficient mice. Under electron microscopy, a cement line of the wild type bone was observed as a narrow osmiophilic linear structure, whereas the OPG deficient mouse revealed this line as a thick translucent fissure, indicating less attachment of bones. In addition, collagen fibers of the OPG depleted bone matrix were dispersed in irregular directions, indicative of fragility. Thus, we concluded, in OPG deficient mice: 1) osteoblastic activity and resultant bone remodeling are stimulated in co-operation with promoted osteoclastic bone resorption, and 2) high turnover ensures less quality of bone as evidenced by poor attachment of fragile bones at the cement lines.

P201 S

OPTIMISATION OF CASE FINDING WITH BONE MINERAL DENSITY

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The aim of this study was to develop a methodology to optimise the role of bone mineral density (BMD) measurements in a case finding strategy. We studied 2113 women aged 75 years or more randomly selected from Sheffield UK. Baseline assessment included hip bone mineral density (BMD) and clinical risk factors. Outcomes included death and fracture in women followed for 6723 person years. Poisson models were used to identify significant risk factors for all fractures and for death with and without BMD. The hazard functions were used to compute fracture probabilities. Women were categorised by fracture probability with and without a BMD assessment. An arbitrary 10 year fracture probability threshold of 35% was taken as an intervention threshold and a threshold of 37% and 27% in sensitivity analyses. Age, prior fracture, use of corticosteroids and low body mass index (BMI) were identified as significant clinical risk factors. 16.8% of women were classified as high risk on the basis of these clinical risk factors. The average BMD in these patients was approximately 1 SD lower than in low risk women. 21.5% of women were designated to be at high risk with the addition of BMD. 15% of all women were re-classified after adding BMD to clinical risk factors, most of whom lay near the intervention threshold. When a high probability of re-classification was accepted (without a BMD test) for high risk to low risk ($P1 < 0.8$) and a low probability accepted for low to high risk ($P2 < 0.2$), BMD tests would be required in only 21% of the population and the proportion of misclassified women would be reduced from 15 to 8%. The proportion of high risk individuals not detected would decrease from 46 to 13%. We conclude that the use of clinical risk factors can identify elderly women at high fracture risk and such patients have a low average BMD. BMD testing is required, however, in a minority of women, a fraction that depends upon the probabilities accepted for misclassification and the thresholds of risk chosen.

P202 W

INHIBITION OF OSTEOBLASTIC DIFFERENTIATION BY ARACHIDONIC ACID TREATMENT THROUGH INCREASE OF PROSTAGLANDIN E_2 IN MC3T3-E1 CELLS

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Arachidonic acid, a precursor of prostaglandins (PG_s), is released by phospholipase A_2 (PLA_2) and plays an important roles in biological reactions. To determine the action of arachidonic acid on osteoblastic differentiation, we investigated the effect of exogenous arachidonic acid on osteoblastic clone MC3T3-E1 cells.

Arachidonic acid dose-dependently decreased ALP activity and increased PGE_2 production after 7 days in culture. The cell shape of MC3T3-E1 changed from polygonal to fibroblastic by the treatment of arachidonic acid. This effects were recovered by NS-398; a specific inhibitor for cyclooxygenase-2 (COX-2), and indomethacin; an inhibitor for both COX-1 and COX-2. Arachidonic acid at 100 microM increased COX-2 mRNA over 60-fold of the control level at 2-3h. PGE_2 production was increased in parallel with the COX-2 mRNA expression. This increase was biphasic; the first small peak was detected within 30 min and the second marked peak appeared later on 3 h. These results suggest that arachidonic acid was converted to PGE_2 by COX-2 in early times and then the secreted PGE_2 inhibited differentiation of MC3T3-E1 cells with a paracrine manner.

Exogenous arachidonic acid induced the release of cellular arachidonic acid, however, NS-398 and indomethacin did not completely block it at 5 min. MAFP (an inhibitor for $cPLA_2$) suppressed this arachidonic acid release at 5 and 30 min, while NS-398 and indomethacin suppressed at 30 min. MAFP also suppressed the expression of COX-2 mRNA expression augmented by arachidonic acid. These results indicate that exogenous arachidonic acid enhanced PGE_2 production by coupling with the increase of $cPLA_2$ and COX-2. Present findings have shown the significance of arachidonic acid as an amplifier of PGE_2 and as an inhibitor of osteoblastic differentiation.

P203 F

OSTEOGENIC GENE REPROGRAMMING BY HYPOXIA

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New bone formation following injury can occur through two distinct processes. If bone segments are stabilized (e.g. distraction), mesenchymal cells differentiate directly into osteoblasts. In a biomechanically unstable fracture (e.g. long bone fracture), bone formation occurs via a cartilage intermediate in which low oxygen tension is required. We have shown that bone osteoblasts and osteocytes sense and respond to hypoxia by elevating the level of the hypoxia-inducible factors (HIFs), evolutionarily conserved master regulators of the hypoxic response, which transcriptionally activate oxygen-sensitive genes. We therefore hypothesized that oxygen tension dictates the developmental fate of stromal mesenchymal cells. To test this hypothesis, mouse ST2 bone stromal cells were maintained in either 1% or 21% oxygen and RNA was isolated over a 48 h period. Microarray analysis was performed with the Incyte mouse gene chip (GEM1) containing 8,734 clones. Of 729 genes with significant ($p<0.01$) alterations in expression, 10 genes known to be oxygen sensitive were verified using real-time PCR. Subsets of genes that exhibited distinct expression patterns over time were annotated. ESTs were identified by comparing them with mouse and other genomes using the NCBI BLAST program. Eight genes known to be involved in either osteoblast or chondrocyte differentiation were identified. Genes known to be expressed in cells in the chondrocyte lineage included: connective tissue growth factor (+6 fold), lysyl oxidase (+3 fold), annexin 2 and 5 (+6 fold), chondroitin 4-sulfonotransferase 2 (+6 fold), and lectin galactose binding 3 (+6 fold). Conversely, genes associated with repression of the osteoblast phenotype included: retinoic acid receptor-related orphan receptor alpha (+3 fold) and calpain (-5 fold). Analysis of the promoter regions of connective tissue growth factor, annexin 5 and lectin galactose binding 3 revealed consensus HIF binding sites. These data suggested that hypoxia might promote a chondrocytic differentiation pathway. Consistent with this concept, forced expression of a stable HIF-1 construct in the pluripotential C3H10T1/2 cells resulted in an increase in chondrocyte-specific gene expression and a decrease in osteoblast-specific gene expression. Current studies are underway to examine the importance of HIF in vivo using Cre-loxP technology to inactivate HIF-1 in mouse bone cells.

P204 S

THE ESTROGEN RECEPTOR ALPHA PATHWAY IS THE MAJOR ROUTE FOR GENISTEIN'S BONE PROMOTING EFFECTS

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Estrogen deficiency in post-menopausal women is associated with an increased risk of osteoporosis. Hormone replacement therapy has been shown to reduce the incidence of osteoporosis in women but increase the risk of breast cancer. This problem may be overcome by selective estrogen receptor modulators (SERMs) that display tissue specific actions. The phytoestrogen genistein has no estrogenic effect on uterine tissue and prevents bone loss in ovariectomized mice, rats and in early postmenopausal women. We established cultures of human osteoblastic cells (HOB) in order to determine whether the bone protective effect of genistein is mediated by the same or by different estrogen receptors (ERs). The HOB cells expressed ERalpha and three ERbeta splice variants: ERbeta5 and, at much lower levels, ERbeta1 and ERbeta2. Targeted inactivation of ERalpha by short interfering RNA (siRNA) was used to generate HOB cells that expressed the ERbeta splice variants but not ERalpha. In contrast to untreated cells, ERalpha-deficient cells failed to upregulate osteoprotegerin in response to genistein treatment. We conclude that genistein shares at least one important mechanism of bone protection with estrogen, and this effect is dependent on the ERalpha pathway

P205 W

$P57^{KIP2}$ IS A VITAMIN D_3 SIGNALING MEDIATOR IN OSTEOBLAST

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The cyclin-dependent kinase(CDK) inhibitor, $p57^{kip2}$ is a negative regulator of the cell cycle. Mice with a disruped p57gene show cleft palate, endochondral bone ossification defects with incomplete differentiation of hypertrophic chondrocytes. We have also reported that the amount of $p57^{kip2}$ protein increased in rat osteoblasts treated with $1,25(OH)_2$ vitamin D_3. However, the role of $p57^{kip2}$ in bone metabolism is still unknown. In the present study osteoblasts derived from p57null(-/-) mutant calvaria showed increased the proliferation rate and higher alkaline phosphatase activity than that of wild type(WT). However p57(-/-) cells preserved the similar ability in contact inhibition to WT. The activity of cyclin dependent kinase(CDK) 4 is expressed at very high level in p57(-/-) osteoblasts. CDK2 and CDK6 activity was also increased. Immunohistochemistry shows that p57 protein was expressed in WT osteoblast and hypertrophic chondrocyte. In nodule formation assay, mineralization was siginificantly delayed in osteoblasts derived from -/- mice in the absence or presence of $1,25(OH)_2$ vitamin D_3. Surprisingly, osteopontin mRNA expression was supressed by the addition of $1,25(OH)_2$ vitamin D_3 by using semiquantitative reverse transcriptase-PCR. However -/-osteoblasts expressed the comparable amount of Vitamin D receptor mRNA to that of wild type. Moreover we have investigated the role of $p57^{kip2}$ on osteoclast formation. Osteoclast formation was also reduced in a co-culture system of -/-osteoblasts and wild type bone marrow cells in the presence of $1,25(OH)_2$ vitamin D_3. Taken together, these results may suggest a possible involvement of $p57^{kip2}$ related to $1,25(OH)_2$ vitamin D_3 signaling in the control of both osteoblast proliferation and osteoblast/osteoclast differentiation.

P206 F

INCREMENT OF DDR(DISCOIDIN DOMAIN RECEPTOR) MRNA AND PROTEIN LEVEL IN OSTEOBLASTS BY BMP THROUGH RUNX2/CBFA1 AND MAPK PATHWAY

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Osteoblastic differentiation and function are regulated by bone related cytokines and growth factors. Recent studies indicated that collagens, main components of bone matrix, also effect on the proliferation, differentiation and apoptosis of mesenchymal cells including osteoblasts. DDRs, discoidin domain receptors, are recently identified non-integrin type receptor tyrosine kinase family molecules those bind to collagen fibrils. Recent studies reported that collagen binding to DDR2 results in the upregulation of MMP1(Matrix metalloprotease1), suggested that DDRs function something as a sensor molecules to recognize and reconstruct micro-environment surrounding mesenchymal cells. In this study, to elucidate the regulation and function of DDRs in the proliferation, differentiation and apoptosis of osteoblasts, we investigated the expression and regulation of DDRs in osteoblasts. We found that two isoforms of DDRs, DDR1 and DDR2, are constitutively expressed in osteoblast-like cell line, MC3T3E1 cells and 200 ng/ml of BMP2 transiently enhances the amounts of DDR mRNA and protein within 2 days. DDR expression level was relatively low in osteoblast-specific transcription factor Runx2/Cbfa1 deficient primary calvarial cells compared to wild type, and ectopic expression of Runx2/Cbfa1 in MC3T3E1 cells enhances the amounts of DDR1 mRNA. These indicated that enhancement of DDR mRNA expression by BMP is mediated by Runx2/Cbfa1. Increment of the amount of DDR1 mRNA by BMP2 was also observed in the presence of 25mg/ml of DRB, a transcription inhibitor, suggesting that BMP enhances the stability of DDR1 mRNA. Cycloheximide, a translation inhibitor, also increased the amount of DDR1 mRNA even in the absence of BMP2, indicating that de novo protein synthesis is needed for the degradation of DDR1mRNA. These results indicated that BMP2 inhibit the expression of undefined proteins those instabilize DDR1 mRNA through Runx2/Cbfa1 pathway in osteoblasts. Recent study indicates that AU-rich element binding factor, which binds to AUUUA motif in UTR region of target mRNA and enhance mRNA degradation, is inhibited by the activation of MAP kinase. But the increment of DDR1 mRNA by BMP2 is cooperatively enhanced by U0126, a comprehensive MEKK inhibitor. This indicated that undefined DDR1 instabilizing factor is a novel type of modulator of mRNA expressed in MC3T3E1 cells.

P207 S

FIBROBLAST GROWTH FACTOR (FGF) SIGNALING IN IMMATURE AND MATURE OSTEOBLASTS

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Many human skeletal genetic disorders such as dwarfism and craniosynostosis are due to unregulated fibroblast growth factor (FGF) signaling. FGF signaling in osteoblasts leads to the activation of the classical MAP kinase (ERK) as well as the p38 MAP kinase pathways. The ERK pathway is generally associated with growth promoting signals, while the p38 pathway was originally identified as a stress induced signal involved in apoptosis. In addition, the PI-3-kinase/ AKT anti-apoptotic pathway is also activated by FGF signaling. We have previously shown that immature osteoblasts respond to FGF with growth stimulation, while mature osteoblasts lose this proliferative response and instead undergo increased apoptosis in response to continuous FGF signaling.

To determine how FGF signaling leads to these divergent cellular responses depending on the maturation stage of the osteoblasts, we have studied FGF signaling pathways in osteoblastic cell lines that can be induced to differentiate in two weeks. Activation of ERK, p38 and AKT was compared in immature cells, and cells that had been differentiated for two weeks. We find that ERK and p38 are activated at both stages, but AKT activation is induced in immature cells but is constitutively on in mature cells.

To assess the contribution of these pathways to the FGF response, we have used a combination of specific chemical inhibitors and dominant negative constructs for each pathway.

In this report we show that the interaction between these pathways differs in immature and mature osteoblasts as well as the activation of downstream targets such as CREB. P38 signaling contributes to activation of the AKT pathway in differentiated osteoblasts but not in immature osteoblasts.

We also show that ERK signaling is required for the FGF-induced morphological transformation and growth stimulation, while p38 acts as a brake during osteoblast proliferation and also plays a role in osteoblast differentiation. The implications of our findings will be discussed.

P208 W

ANTI-OSTEOPENIC EFFECT OF TAURINE: POSSIBLE INVOLVEMENT OF ACTIVATED MEK-ERK-CBFA1 SIGNALING

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Previously we first noted that taurine (TR) has anti-osteopenic effect on low Ca diet-induced osteopenia in rats (1). Employing osteoblastic MC3T3-E1 cells, the mechanism of the anti-osteopenic effect was explored in vitro. TR (1mM) was found to promote mineralization of extracellular matrices and upregulation of alkaline phosphatase activity. Gel shift assay using 32P-labeled OSE2 (osteoblast-specific cis-element 2: the consensus sequence for Cbfa1, refer to 2) indicated that TR (1mM) increased the nuclear localization of Cbfa1, just as PTH (1-34) (3,4) and bisphosphonates would do (5). In addition, TR was found to stimulate ERK phosphorylation. PD98059, a MEK inhibitor, suppressed effects of TR on both Cbfa1 transactivation and ERK activation. The results strongly suggest that TR first activates intracellular MEK-ERK-Cbfa1 signaling system thereby promoting mineralization and finally exerting to its bone anabolic action.

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P209 F

FGF SIGNALING INITIATES MULTIPLE PATHWAYS TO INDUCE GROWTH ARREST AND PROMOTE HYPERTROPHIC DIFFERENTIATION OF CHONDROCYTES

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The extent of longitudinal bone growth depends on the rates of chondrocyte proliferation and maturation. A role for FGF signaling in these processes was indicated by the observation that activating mutations within FGF receptor 3 underlie the human dwarfism disorders achondroplasia, hypochondroplasia, and thanophoric dysplasia. Subsequent mouse models have suggested that FGF signaling restrains chondrocyte proliferation and differentiation.

Although STAT proteins and p21 have been implicated as mediators of FGF signaling in chondrocytes, neither STAT1- nor p21- deficient mice exhibit overt skeletal abnormalities. In contrast, as we previously showed, the Rb-related proteins p107 and p130 are required for FGF-mediated growth arrest of chondrocyte cultures and the proper growth arrest and differentiation of growth plate chondrocytes in vivo.

To gain further insight into the mechanisms of FGF signaling in chondrocytes, we have used microarray analysis to elucidate the cascades of gene expression following FGF treatment of cultured RCS cells. Among the data to be discussed, and consistent with the role of Rb family proteins in FGF-mediated chondrocyte growth arrest, we find a subset of genes previously identified as targets of the E2F proteins to be rapidly downregulated prior to growth arrest. Repression of these genes is most likely mediated by p107 since we find p107 to be rapidly dephosphorylated, independently of cyclinE-cdk2 kinase inhibition, following fgf treatment. In contrast, hypophosphorylated forms of pRb and p130 appear with later kinetics that correlate with inhibition of the CyclinE-cdk2 complex. Together our results are consistent with a model in which FGF inhibition of the chondrocyte cell cycle proceeds by a 'two step' mechanism: 1) direct signaling to negatively regulate the activities of key cell cycle components ('initiation of growth arrest') and 2) the transcriptional downregulation of critical cell cycle protein genes ('maintenance of growth arrest').

In contradiction to current models, we also noted a pattern of gene expression changes that is consistent with the notion that fgf promotes aspects of hypertrophic differentiation. These data have been confirmed using in situ analyses of growth plates from mice harboring an activated FGFR3. Together these results provide new insights regarding the role of FGF in bone development.

P210 S

BMP-2 DOWNREGULATES ESTROGEN RECEPTOR GENE EXPRESSION POSSIBLY THROUGH PROTEIN KINASE C PATHWAY

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Estrogens play important roles in a variety of tissues including bone. The hormones exert their effects mainly via two nucleus-localized receptors, estrogen receptors (ERs) alpha and beta. The two receptors have been reported to have distinct functions; therefore regulation of the expression level of each ER is crucial for cellular responses to estrogens. In this study, to explore factors that affect the gene expression of ER alpha in osteoblasts, we have established the screening system of MC3T3-E1 osteoblastic cells stably transfected with a mouse ER alpha gene promoter-luciferase construct. With this system we have found that recombinant human bone morphogenetic protein (rhBMP)-2 and phorbol 12-myristate 13-acetate (PMA) significantly inhibit the activity of the ER alpha gene promoter. RT-PCR-southern analysis revealed that both rhBMP-2 and PMA decreased the mRNA level of ER alpha in MC3T3-E1 cells with a maximum effect at 6-12 hr after the initiation of treatment. Interestingly, these molecules also inhibited ER beta gene expression in MC3T3-E1 cells as well. Consistent with the results, the estrogen-mediated activation of an estrogen responsive element (ERE)-containing promoter was suppressed by the pretreatment of the cells with rhBMP-2, indicating that the protein levels of functional ERs also diminished. The BMP-2-dependent downregulation of ER gene expression was inhibited by cycloheximide and calphostin C, respectively, indicating that the effect is dependent upon *de novo* protein synthesis and a protein kinase C (PKC)-mediated pathway. Since PMA also downregulates ER gene expression via *de novo* protein synthesis, we propose that BMP-2 exerts the above effect by directly stimulating a protein kinase C pathway, which in turn leads to the production of secondary factor(s) that downregulates the ER gene expression, although the possibility that it is mediated by the Smad pathway can not be excluded. We are currently trying to identify such BMP-2-inducible protein(s) that affects ER gene expression, which is expected to leads to the development of new therapeutic agents that modify osteoblast functions.

P211 W

THE TYROSINE KINASE RECEPTOR RON AND ITS LIGAND MACROPHAGE STIMULATING PROTEIN (MSP) ACTIVATE INTRACELLULAR PATHWAYS POSSIBLY RELATED TO OSTEOCYTIC DIFFERENTIATION OF OSTEOBLASTS

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Macrophage Stimulating Protein, MSP, is a member of the 'Plasminogen related Growth Factor' family, whose tyrosine kinase receptor Ron is expressed in mouse and human embryonic bone tissues. We show here that Ron is expressed in adult human OBs and that MSP treatment induces receptor tyrosine autophosphorylation and activation of ERK2 and AKT pathways. These events are accompanied by intracellular Calcium increase.

MSP signaling in OBs requires lipid-raft integrity, because after exposure to methyl-α-cyclodextrin, known to disaggregate lipid rafts, MSP failed to increase intracellular Ca2+ and to activate ERK2 and AKT. Moreover, Ron was tyrosine phosphorylated in the absence of the ligand upon cell adhesion to the extracellular matrix, but this phosphorylation was ineffective on intracellular pathways activation.

MSP stimulation induced a significant decrease in Alkaline Phosphatase activity and in Osteocalcin production, 'hallmark' proteins characterizing osteoblast differentiation. These two parameters are known to be differently modulated during OBs differentiation, with high levels of ALP and Osteocalcin in early and mature OBs respectively and lower levels thereafter.

The expression of Ron is markedly modulated during OBs lifespan, being the expression of the receptor higher during the first 2 weeks of culture in differentiating conditions and decreasing in later stages. Continuous stimulation of OBs with MSP for 5 days led to a strong increase in RANKL production, compared with the untreated control. Moreover, OBs exposed to MSP underwent a dramatic shape change, characterized by the formation of branched cytoplasmic protrusions, resulting in an osteocyte-like shape.

This study establishes OBs as novel target cells for MSP and unravels a new pivotal role for Ron in Osteoblast differentiation.

P212 F

BONE MORPHOGENETIC PROTEIN (BMP) RECEPTOR IB SIGNALING MEDIATES APOPTOSIS INDUCED BY BMP-2 IN HUMAN OSTEOBLASTIC CELLS

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Bone morphogenetic protein-2 (BMP-2) is an important regulator of osteoblast differentiation by activating distinct signaling pathways mediated by BMP receptors (BMP-R) I and II. We have recently shown that BMP-2 activates apoptosis by activating protein kinase C (PKC) in human osteoblasts. However, the signaling mechanism involved in this effect remained unknown. We have now identified the BMP receptor and the signaling pathway involved in BMP-2-induced apoptosis in human osteoblastic cells. RT-PCR and western blot analyses showed that OHS4 and SaOS2 osteoblastic cells express BMP-RI and BMP-RII as well as Smad-1, -4, -5 and -8 that are downstream effectors required for BMP signaling. In contrast to BMP-RIA, BMP-RIB mRNA and protein were undetectable in OHS4 cells. BMP-2 increased alkaline phosphatase, N-cadherin and Cbfa1/Runx2 expression in both OHS4 and SaOS2 cell lines, suggesting that promotion of these osteoblast differentiation genes by BMP-2 does not require BMP-RIB expression in these cells. In contrast, BMP-2 increased caspase-9 and caspase-3, -6, -7 activity and apoptosis in SaOS2 but not in OHS4 cells, suggesting a role for BMP-RIB in BMP-2-induced apoptosis. Consistently, overexpression of BMP-2 in OHS4 cells increased Cbfa1/Runx2 mRNA expression but not caspase activity. BMP-2 increased protein kinase C (PKC) activity and apoptosis in SaOS2 cells, which was blocked by calphostin C, but had no effect in OHS4 cells, indicating that BMP-RIB signals through PKC. Transient transfection of SaOS2 cells with wild-type BMP-RIB promoted BMP-2-induced apoptosis. In contrast, transfection with a dominant-negative BMP-RIB effectively blocked the BMP-2-induced increase in caspase-3 and -9 in SaOS2 cells. Transfection of OHS4 cells with BMP-RIB rescued the pro-apoptotic effect of BMP-2 in these cells, confirming that the BMP-RIB signaling pathway is involved in BMP-2-induced apoptosis. Furthermore, a constitutively active form of BMP-RIB activated caspase activity in BMP-RIB-deficient OHS4 cells. Altogether, these data provide the first evidence that BMP-2-induced activation of apoptosis is mediated by BMP-RIB in human osteoblasts.

P213 S

CYCLIC TENSILE STRETCH LOADED ON OSTEOBLAST CELLS ENHANCED REACTIVE OXYGEN SPECIES INDUCTION

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Introduction: The macro- and microscopic morphology of bone changes depends on physical circumstances such as mechanical stress. Though it is clear that bone remodels according to the load placed on it, knowledge of the mechanism is relatively slight. Reactive oxygen species (ROS) are molecules with electrons in their outer orbit. Although ROS production by the osteoblast with ionizing radiation or reoxygenation was reported, ROS production with mechanical stress has not been reported. The purpose of this study was to examine the ROS induction from osteoblast-like cells with mechanical stress.

Materials and Methods: We used HT-3 cells, which possess an osteoblastic phenotype. Cyclic tensile stretch was loaded using a pressure-operated instrument (Flexercell Strain Instrument). The regimen was set at 10 cycles per hour, i.e., every 6 minutes osteoblasts were stretched for 3 seconds with 10 kPa of stress. ROS activity was measured using chemiluminescent probe (L-012). The SOD activity was measured using a water-soluble tetrazolium.

Result and Discussion: Osteoblast-like cells grown in flexible-bottom culture plates were subjected to cyclic tensile stretch. To prevent ROS being toxic, cells possess a co-ordinated antioxidant enzyme system. Superoxide dismutase (SOD) being the first enzyme in this radical-scavenging system. To determine the induction of ROS with mechanical stress, we first measured SOD activity and found that activity of SOD was enhanced with cyclic tensile stretch. We also examined whether the cyclic tensile stretch enhances the ROS synthesis in osteoblast-like cells. Cells exposed to cyclic tensile stretch for 12 hours were washed and intracellular ROS levels were measured. Cyclic tensile stretch clearly enhanced ROS synthesis. These data suggest that ROS play an important role in the stress-mediated remodeling of the bone.

P214 W

$P2X_7$ NUCLEOTIDE RECEPTORS MEDIATE PORE FORMATION AND MEMBRANE BLEBBING IN MURINE OSTEOBLASTS

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Osteoblast activity is tightly controlled by local and systemic factors. In response to inflammatory and mechanical stimuli, nucleotides are released into the extracellular fluid where they can signal through P2 nucleotide receptors on the surface of many cell types, including osteoblasts. There are two classes of P2 nucleotide receptors, P2X (ligand-gated ion channels) and P2Y (G protein-coupled receptors). Mice genetically modified to disrupt the $P2X_7$ receptor exhibit decreased periosteal bone formation, but it is not clear whether this defect is due to the absence of $P2X_7$ receptors on osteoblasts or another cell type that regulates their activity. There are conflicting reports concerning expression of $P2X_7$ receptors in cells of the osteoblast lineage. The purpose of this study was to determine whether $P2X_7$ receptors are present on osteoblasts. Osteoblast-enriched cultures were obtained by sequential collagenase digestion of calvariae from neonatal wild-type (WT) and $P2X_7$ receptor knockout (KO) mice. Reverse transcription-PCR revealed the presence of $P2X_7$ mRNA in WT, but not KO osteoblasts. $P2X_7$ receptors are bifunctional, behaving as ligand-gated ion channels and mediating the formation of large membrane pores. To examine pore formation, we monitored uptake of ethidium bromide using fluorescence microscopy. When incubated for 12 min in buffer containing a low concentration of Ca^{2+} and Mg^{2+}, benzoylbenzoyl-ATP (BzATP, a relatively potent $P2X_7$ agonist) induced staining of approximately 30% of cells in WT cultures. In contrast, there was no significant effect of BzATP on ethidium bromide uptake by osteoblasts from KO animals. In many cell types, activation of $P2X_7$ receptors leads to cytoskeletal rearrangements. Time-lapse video microscopy revealed that BzATP induces dynamic membrane blebbing in a subpopulation of cells from WT, but not KO mice. These effects were reversible and not mimicked by UTP, which activates P2Y but not $P2X_7$ receptors on osteoblasts. In conclusion, use of the KO mouse model has allowed us to unequivocally demonstrate the presence of functional $P2X_7$ receptors in a subpopulation of calvarial bone cells. Loss of this receptor from osteoblasts may contribute to the defective osteogenesis observed in KO mice. These studies were supported by the Canadian Institutes of Health Research (CIHR).

P215 F

OXYGEN TENSION REGULATES THE BALANCE BETWEEN STEMNESS AND DIFFERENTIATION OF HUMAN MARROW STROMAL STEM CELLS

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Oxygen is involved in maintaining skeletal homeostasis, from development to angiogenesis to wound repair. The physiological microenvironment of human marrow stromal stem cells (hMSCs) in vivo is hypoxic, ranging between an oxygen tension of 1 and 10%. We investigated the role of oxygen as a regulator of the balance between stemness (self-renewal) and osteogenic differentiation hMSCs. Human MSCs were isolated from cadaveric vertebral bodies (T1-L5) and grown under hypoxic conditions (3-10% oxygen) or air (20% oxygen). Our results show that when multicolony derived hMSCs from a 16-year-old male were exposed to hypoxia for 7 days the total number of cells increased 1.5-fold compared to cells cultured in air. Moreover, the inclusion of osteogenic promoters such as dexamethasone (Dex) and parathyroid hormone (PTH) during in vitro culture of the hMSCs enhanced the hypoxic effect up to 3-fold compared to untreated cells in air. Similar results were obtained with hMSCs tested from other donors (3-59-years-old, male and female). The number of cells obtained under 3% oxygen tension was consistently higher (3- to 6-fold) independent of age or gender when initially plated at low density after 7-15 days in culture. On the contrary, alkaline phosphatase activity was significantly lower in cells exposed to hypoxia compared to cells grown in the presence of air in untreated cells or in Dex- and/or PTH-treated cells after 7 to 23 days in culture. Furthermore, hypoxia appeared to abolish biomineralization in long-term cultures even using osteogenic conditions. Finally, we examined the effect of oxygen tension on the mRNA expression of HIF-1alpha, a transcription factor mediating hypoxic effects. HIF-1alpha was upregulated after a 2-h exposure to 3% oxygen, suggesting that hypoxia-induced HIF-1alpha activation may be involved in the activation of genes necessary for optimal growth and self-renewal of hMSCs at low oxygen tension. These results support the idea that maintaining the cells at low oxygen in vitro favors stemness over differentiation. Our data is consistent with normal in vivo physiological conditions where hMSCs in their natural marrow microenvironment are at low oxygen tension, while osteoblasts are closer to blood vessels and hence exposed to higher oxygen tensions.

P216 S

IN VITRO GROWTH AND OSTEOBLASTIC DIFFERENTIATION OF HUMAN BONE MARROW STROMAL CELLS SUPPORTED BY AUTOLOGOUS PLASMA

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Autologous bone marrow stromal cells have been proposed as an adjuvant in the treatment of bone nonunion. This cell therapy requires the establishment of culture conditions that permit the rapid expansion of these cells ex vivo while retaining their potential for further differentiation. Several culture models have already been proposed, all of them using fetal calf serum (FCS) as a source of growth factors. For subsequent autologous implantation the use of FCS is problematic because of the possible disease transmission. Our aim, therefore, was to set up a similar cell culture model replacing the standard FCS by autologous plasma recovered from bone marrow (APM).

We first tested the ability of APM versus FCS to recruit osteoprogenitor cells in short term cultures of human bone marrow stromal (HBMS) cells in mineralizing conditions (DMEM with 10^{-8}M dexamethasone, 50 microg/ml ascorbic acid and 3 mM phosphate) by counting the number of alkaline phosphatase-positive colonies per 10^6 nucleated marrow cells, 9 days after plating. Cultures grown with APM exhibited a significantly higher number of alkaline phosphatase-positive colonies than those grown with FCS (matched pairs t-test, $p<0.05$).

We also established growth curves of primary cultures of HBMS cells in DMEM supplemented with 10% either FCS or APM, by direct counting of the percentage of dish area occupied by cells at different times ranging from day zero after plating until confluence. For both conditions we obtained typical growth curves with a linear stage followed by a plateau. The time to reach confluence could differ whether the culture was run with FCS or APM, but the cell number obtained at confluence was similar in both cases.

First passage cells were then subcultured in mineralizing conditions with either 10% FCS or 10% APM during 28 days. An osteoblastic differentiation was observed in both cases as the cell layers formed nodular structures and expressed alkaline phosphatase.

Our study shows that proliferative capacity and osteoblastic differentiation potential of HBMS cells are maintained when cultured with APM. Thus, this differentiation model could provide a new and safer tool to elaborate an autologous cell therapy designed to enhance osteogenesis.

P217 W

STUDY ON OSTEOGENIC POTENTIAL OF CULTURED MESENCHYMAL STEM CELLS TRANSFECTED WITH AD-BMP-2

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Objective: To study the influence of Ad-BMP-2 on osteogenic potential of cultured MSCs.

Methods: MSCs were obtained from dual femoral greater trochanter of twenty Japan Big Ear rabbits. The experimental group cells were from the left trochanters, the control group cells were from the right ones. MSCs were transfected with Ad-bmp-2 and through the methods of RT-PCR and immunohistochemistry stain to test the expression of BMP-2 protein. After the MSCs were treated with the protein, Gomori stain and Von Kossa stain were used tested ALP and mineralized matrixes forming. Besides that, ALP testing kit were used to measure ALP activity; BGP immumoradiatory testing kit were used to measure OCN. In addition, the collagen type one were tested with the methods of RT-PCR and immunohistochemistry stain.

Result: The MSCs transfected with Ad-BMP-2 can express BMP-2 protein effectively. After treated with the gene transfection method, the MSCs gained the morphological characters which were similar to those of osteoblasts. The ALP activity was in the Ad-BMP-2 transfected group as compared with in the control group. PI value of collagen type one expression increased remarkably after transfection (in the experimental group versus in the control group). The OCN was in the experimental group and in the control group.

Conclusion: The MSCs transfected with Ad-BMP-2 can express BMP-2 protein effectively. And through the method of gene transfection, the osteogenic potential of MSCs can be promoted greatly.

P218 F

EXPRESSION AND LOCALIZATION OF BMP RECEPTORS IN MUSCLE OF MICE DURING BMP-2 INDUCED ECTOPIC BONE FORMATION

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[Purpose] Ectopic bone formation is elicited by implanting BMP-retaining collagen pellets into the back muscles of adult mice. Three weeks after implantation, the pellets has been replaced by the newly formed bone mass. However, the modulation of BMPRs expression and localization during the process of BMP induced bone formation is not yet known or completely understood. Changes in the temporal and spatial expression profiles of BMP receptors (BMPRs) were examined by some molecular methods.

[Methods] Northern blot, Immunohistochemistry were performed.

[Results] Expression of BMPRs was enhanced in the early phase of BMP-induced bone formation, and was also enhanced in undifferentiated mesenchymal cells located close to the BMP-retaining implants.

[Conclusion] These results indicate that up-regulated expression of BMPRs and the coordinate expression of these molecules points to a potential regulatory mechanism involving those positive regulators for BMP signaling.

P219 S

ALKALINE PHOSPHATASE EXPRESSION DURING OSTEOGENIC DIFFERENTIATION OF RAT BONE MARROW STROMAL CELLS

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Introduction: Bone marrow contains population of stem cells that capable to differentiating to osteoblasts and to form the bone nodule by using dexamethasone.

Aims of Investigation: Differentiation of osteoblasts from bone marrow stromal cells and expression of Alkaline Phosphatase.

Materials and Methods: The stromal cells of bone marrow obtained from 4 to 6 weeks old Spruge-Dawely male rats were grown in primary culture for 7 days and sub cultured for 18 days. The cells were cultured in DMEM medium containing 15% fetal calf serum and antibiotics. All mediums and controls supplemented by osteogenic supplements (OS) include: 10miliM Na-B glycerophosphate (Na-BGp), 10 nM Dexametasone (Dex.) and 50 g/ml ascorbic acid (AsA) as examined for mineralization and Alkaline Phosphatase (Apase) expression.

Results: Mesenchymal stem cells (MSCs) in examined cultures underwent a dramatic change in cellular morphology and significant increase in Apase activity by day 12. The deposition of a calcified matrix on the surface of the culture flasks became evident between days 12 and 18.

Discussion: Results of this investigation confirmed by Rickard et al. They believed that, treatment of rat marrow stromal-drived cells with Dex. increase population of undifferentiated cells which retain the capacity to change osteoblastic cells and bone nodule formation.

Conclusion: The addition of osteogenic supplements (OS) to MSCs cultures induced Apase expression that contributes to cellular differentiation and mineralization of extra cellular matrix.

P220 W

EFFECTS OF SIMVASTATIN ON THE PROLIFERATION AND DIFFERENTIATION OF HUMAN BONE MARROW-DERIVED STROMAL CELL

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Statins have been postulated to affect bone metabolism. Recent experimental and epidemiologic studies have suggested that statins may have bone protective effects. We assessed the effects of simvastatin on the proliferation and differentiation of human mesenchymal stem cells (MSCs) in ex vivo culture. The present study is the first one to describe the influence of statin on primary human osteoblastic cells.

Bone marrow was obtained from healthy donors. Mononuclear cells including mesenchymal stem cells were then isolated and cultured to osteoblastic lineage. Also, different doses of simvastatin (control, 10-8 M, 10-6 M) were added to culture media. At the 15th day of primary culture, simvastatin diminished the average size of CFU-Fs (colony forming unit-fibroblastic) but increased the number of alkaline phosphatatse positive colony in a dose dependent fashion. Measurement of matrix calcification

showed that simvastatin enhanced matrix calcification, a late marker of osteoblastic maturation. At near confluence, bone marrow stromal cells were sub-cultured. Thereafter, alkaline phosphatase activities of each group were measured by the time course of secondary culture. Simvastatin dose dependently enhanced alkaline phosphatase activity and this stimulatory effect was more evident during the early period of culture (day 5; 269.5 ±34, 290.8 ±44, 378.4 ±52; control, 10-8 M, 10-6 M respectively; mean Alp/protein (U/mg)). To clarify the effect of simvastatin on the proliferation of MSCs, MTT assay were performed during secondary culture. As compare to control group, simvastatin significantly lessened total cell amount of each culture well. Total RNA was extracted at the 12th day of secondary culture and expression of osteocalcin mRNA was examined by RT-PCR. Simvastatin significantly increased osteocalcin mRNA expression (75.7 ±5.4, 76.6 ±4.5, 97.6 ±5.0; control, 10-8 M, 10-6 M respectively, p<0.01).

Our study shows that simvastatin has stimulatory effect on bone formation through osteoblastic differentiation and has inhibitory effect on proliferative potential in human mesenchymal stem cell culture. This beneficial influence of simvastatin on bone metabolism could allow statins to become effective anabolic agent for the treatment of common metabolic diseases such as osteoporosis.

P221 F

ANDROGENS EXERT ESTROGEN-LIKE EFFECTS ON THE DIFFERENTIATION OF BIPOTENTIAL BONE MARROW STROMAL CELLS

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Bone marrow stromal cells (BMSCs) are common progenitors for bone forming osteoblasts and adipocytes. Because age-related bone loss is often associated with both impaired bone formation and increased bone marrow adipose, a reciprocal regulation of BMSC differentiation into osteoblasts/adipocytes has been proposed. Consistently, we and others reported that 17beta-estradiol (E2) promotes osteoblastic whereas inhibits adipocytic differentiation of BMSCs. Similar to estrogen deficiency, reduced androgen action is also known to cause osteoporosis. However, clinical and experimental evidence has suggested that a large part of androgen action is mediated by estrogen, and contribution of direct androgen action is currently unclear. In the present study, we therefore examined androgen effects on the differentiation of BMSCs using 5alpha-dihydrotestosterone (DHT), a non-aromatizable androgen, and dehydroepiandrosterone (DHEA), an aromatizable adrenal androgen. Cell models used were, mouse cell lines MC3T3/PA6 (PA6), wild-type ST-2, ST-2 stably transfected with an human estrogen receptor (ER) alpha or ERbeta expression vector (ST2ERalpha or ST2ERbeta, respectively). In all the cell populations above, bone morphogenetic protein-2 (BMP-2) increased the number of alkaline phosphatase (ALP) positive osteoblasts as well as oil-red O staining positive adipocytes. Both DHT (100 nM) and DHEA (100 nM) enhanced ALP induction by BMP-2 in ST2ERalpha and ST2ERbeta, but not in wild-type ST-2 or PA6 cells, despite comparable levels of androgen receptor mRNA expression. DHT and DHEA dose-dependently decreased the number of adipocytes in association with a decrease in PPARgamma2 mRNA expression in ST2ERalpha and ST2ERbeta, but not in the other cells. DHT and DHEA effects at 1 microM were comparable to 1 nM E2 effects, and were completely abolished with an ER antagonist ICI182780, but not with an AR antagonist hydroxy-flutamide or aromatase inhibitors. We conclude that DHT and DHEA promote osteoblastogenesis while inhibiting adipogenesis of BMSCs in a manner similar to but independent of estrogen. In light of recent report that not only AR but also ERs can confer anti-apoptotic effect of androgen in osteoblastic cells, our results suggest that these androgen actions on stromal cell differentiation may be in part mediated by ER, or influenced by the abundance of ER.

P222 S

OSTEOINDUCTIVITY OF DIFFERENT SPECIES BMPS DURING OSTEOGENIC DIFFERENTIATION OF RAT BONE MARROW STROMAL CELLS

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Osteoinductivity of different species bone morphogenetic proteins(BMPs) were tested during osteogenic differentiation of rat bone marrow stromal cells(MSCs)culture.

Materials and methods: Bovine, human, ostrich and emu BMPs extraction were performanced by Urist method,12 week-old SD rats femur MSCs were collected by flushing with a culture medium and cultured untill day 7,subcultures into 24-well plate at 5 x 104 cells/well. Another group added 10nMol dexamethasone to culture medium. After 12hrs,100ug of each species BMPs were feed into each well for 14 days. Samples were assayed following Sigma protocol 104 for alkaline phosphatase(ALP)assay. Enzyme activity was measured by spectrophotometry at 405 nm. Standard curves were generated using p-nitrophenol standard solution in 0.08N NaOH, the 2-N Sigma enzyme controls were also used as a positive control.

Results: ALP activity in MSCs cultures was elevated by BMPs in 2-10 folds (P<0.05-0.001) by day 3.Bovine BMPs elevated ALP by day 1 and continued to climb until peaking at day 5, human BMPs elevated ALP and peaking by day 3,ostrich and emu BMPs elevated ALP by day 3 and the peaking at day 7. Bovine BMP is more than human BMPs in elevating ALP(P<0.01) during day 3 to 7. Human BMPs intensified to elevate ALP more than bovine BMPs at day 14(P<0.01). Ostrich and emu BMP keeping the same level of ALP after day 1. Dexamethasone effected ALP as same as normal medium at culture of 24hrs,and declined to lower level at day 3(P<0.05-0.01), but after 3 days, bovine and human BMPs were elevated up to 35%-125%, ostrich and emu BMPs increased by day 14, day 5 and 7(P<0.05).

Conclusion: ALP activity in MSCs cultures was elevated by BMPs culture 3 day later. Bovine BMPs elevated more ALP than others,however,at day 14,the others were more than bovine BMPs.Dexamethasone enchances the abilities of BMPs to stimulate the differentiation of osteoblast-like cells and increases activity of ALP after day 3 culture,and keeping in a higher level.

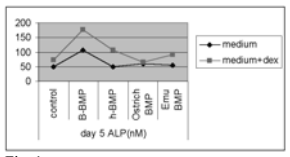

Fig. 1

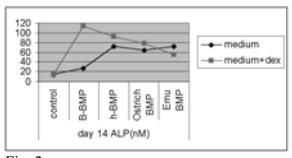

Fig. 2

P223 W

EXPRESSION OF PRE-OSTEOBLAST MARKERS AND CBFA-1 AND OSTERIX GENE TRANSCRIPTS IN STROMAL TUMOR CELLS OF GIANT CELL TUMOR OF BONE

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In giant cell tumor of bone (GCT), the mononuclear stromal cells which represent neoplastic component of this tumor, regulate the formation of multinucleated osteoclast-like giant cells. However, the origin of stromal tumor cells has not yet been clearly defined. In this study, we have initially evaluated several osteoblast markers, collagen type I, BSP, osteonectin and osteocalcin in GCT stromal tumor cells (GCTSC) by immunohistochemistry. We have further examined the gene expression of Cbfa-1, Osterix, osteocalcin and ALP in GCTSC and subsequently determined the regulation of these genes expression by BMP-2 using real-time quantitative RT-PCR analysis. Totally 13 cases of GCT specimens and 7 GCT stromal cell cultures were included. Despite a high individual variability in protein or gene expression levels, we identified the following significant patterns: i) the majority of stromal tumor cells were intensively positive for BSP and osteonectin, less intensively positive for collagen type I, but negative for osteocalcin; ii) Cbfa-1 gene transcripts, a low extent Osterix mRNA and osteocalcin mRNA were identified in GCTSC, but ALP was undetectable; iii) BMP-2 was able to up-regulate the expression of Osterix gene transcripts in GCTSC cultures; up-regulation of Cbfa-1 and osteocalcin mRNA by BMP-2 were less intensively observed. In conclusion, our data suggest that the stromal tumor cells of GCT are of the pre-osteoblast characters. Further studies are necessary to elucidate whether these stromal tumor cells may exhibit osteoblastic differentiation exactly under appropriate osteoinductive conditions.

Table 1. The intensity and extent of immuno-staining

		stromal tumor cells				**multi-nucleated giant cells**			
		-	+	++	+++	-	+	++	+++
collagen type I	0	4	7	2	9	3	1	0	
BSP	0	2	4	7	2	4	4	3	
osteonectin	0	3	4	6	9	3	1	0	
osteocalcin	10	1	2	0	13	0	0	0	

Osteoclasts

P224 F

LOCALIZATION OF MATRIX METALLOPROTEINASE (MMP)-13 ON THE BONE SURFACES UNDER RUFFLED BORDERS OF OSTEOCLASTS IN RAT TIBIA

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Matrix metalloproteinase (MMP)-13, also called interstitial collagenase or collagenase-3, is involved in the degradation of extracellular matrices including type I, II, III collagens and aggrecan. We investigated the localization of MMP-13 in rat tibia to clarify the role of MMP-13 in bone resorption by immunohistochemistry and Western blotting.

MMP-13 reactivity was mainly seen on bone surfaces under osteoclasts, some osteocytes and their lacunae adjacent to osteoclasts. In bone matrices, MMP-13 was also localized on cement lines in epiphysis. In growth plate erosion zone, perivascular cells also showed MMP-13 positive reactivity. However, immunoreactivity was not seen in chondrocytes or osteoclasts. Immunoelectron microscopy revealed that MMP-13 was localized on the bone surfaces under the ruffled borders and some clear zones of osteoclasts. Gold particles were also detected in Golgi apparatus of osteocytes adjacent to osteoclasts. Western blotting demonstrated that anti-MMP13 antibody reacted with a 62 kD band in the EDTA-extract from rat tibia, suggesting that pro-MMP-13 was mainly associated with the mineralized bone matrices. These findings indicate that MMP-13 synthesized by osteocytes may be transported through lacunae-canaliculi channel and localized under the ruffled borders of osteoclasts. MMP-13 may play an important role in the degradation of organic components in bone matrices in concert with cathepsin K and MMP-9 produced by osteoclasts. On the other hand, MMP-13 in perivascular cells may be involved in the removal of cartilage matrices, such as type II collagen and aggrecan.

P225 S

PHOSPHATIDYLINOSITOL-3 KINASE PLAYS A ROLE IN VESICLE TRANSPORT IN THE SECRETORY PATHWAY OF CATHEPSIN K AND CYSTATIN C IN OSTEOCLASTS

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Bone resorption involves the degradation of bone organic matrix and the decalcification of inorganic bone components. The organic components are degraded by proteinases released from the ruffled border beneath osteoclasts. A cysteine proteinase, cathepsin K (CK), is the key proteolytic enzyme in bone resorption. Recently, we analyzed the localization of an endogenous cysteine proteinase inhibitor, cystatin C (CC), in osteoclasts (Bone, 2001). However, the kinetics of the secretory pathways of CK and CC are unclear. Therefore, this study examined the secretion of the proteinase and its inhibitor in osteoclasts. Furthermore, we also investigated the function of phosphatidylinositol-3 kinase (PI3-kinase) in the CK and CC secretory pathways in osteoclasts in vivo and in vitro. Using in vivo immunoelectron microscopy, CK was found both intracellularly in vesicles and vacuoles and extracellularly under the ruffled border of osteoclasts in the absence of wortmannin (WT). Ten minutes after injecting mice with the PI3-kinase inhibitor wortmannin, CK was observed in the vesicles, but not under osteoclasts with an undeveloped ruffled border. Thirty minutes after the injection, many CK-positive vacuoles were present in osteoclasts. Osteoclast-like cells from mouse bone marrow were cultured in medium containing 1a,25 (OH) vitamin D3 for 6-7 days. Immunofluorescence microscopy showed that CK and CC were co-localized in the vesicles of control osteoclasts. Ten minutes after WT treatment, small CK- or CC-positive vesicles were dissociated in osteoclast-like cells; the co-localization of CK and CC was reduced. Thirty minutes after WT treatment, CK and CC were observed in large vesicles, although they were no longer co-localized. By contrast, in osteoclasts treated with a vacuole-type proton pump inhibitor, bafilomycin A, for 30 min, the distribution of CK- and CC-positive structures was similar to that in cells treated with WT. These results indicate that CC accumulates in the acidic compartment containing CK in the secretory pathway in osteoclasts. It also suggests that PI3-kinase controls vesicle transport to the ruffled border in osteoclasts via the acidification process.

P226 W

EFFECT OF RGD-POLYPEPTIDE(224) ON ACTIVITIES OF BONE RESORPTION AND GENE EXPRESSION OF CARBONIC ANHYDRASE II IN HUMAN OSTEOCLAST-LIKE CELLS

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Objective: In this study, We explored the mechanism of anti-bone resorption of RGD-polypeptide(224) (RGD) using osteoclast-like cells (OLCs) from giant cell tumor of bone as an in vitro model.

Methods: The function of bone resorption was observed and the staining for tartrate-resistant acid phosphatase(TRAPase) was idenitified. The carbonic anhydrase II (CAII) gene expression, apoptosis and the adhesion of OLCs were determined by in situ hybridization, TUNEL staining and adhesion test, after treatment of RGD and echistatin respectively.

Results: OLCs were TRAP positive and had bone resorptive function. The numbers of TRAP-positive multinucleated cells decreased in a dose-dependent manner after the treatment of 10^{-5}, 10^{-6}, 10^{-7}mol/L echistatin respectively and RGD. The expression of CAII mRNA decreased. the number of OLCs apoptosis increased in a dose-dependent manner ($P<0.001$). After pretreated with 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} mol/L RGD , the numbers of adhered OLCs decreased. OLC adhesion was inhibited obviously with increasing concentrations of RGD and echistatin($P<0.05$).

Conclusions: Both RGD-polypeptide(224) and echistatin inhibited the expression of CAII mRNA and induced the OLC apoptosis in dose-dependent manner.

P227 F

A NOVEL ANTAGONIST OF ALPHA$_v$BETA$_3$ INTEGRIN IS A POTENT INHIBITOR OF BONE RESORPTION IN VIVO

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The alpha$_v$beta$_3$ integrin was shown to play a major role in bone resorption *in vitro* and *in vivo*. alpha$_v$beta$_3$ is highly expressed in osteoclasts and binds to the RGD sequence in extracellular matrix proteins such as osteopontin and bone sialoprotein. The pharmacological properties of a low molecular weight RGD mimetic (compound A) and its *in vivo* activity in several models of bone resorption is described below. 3H-Compound A binds with high affinity (Kd 0.33 nM) to purified human alpha$_v$beta$_3$.

In cell assays *in vitro* Compound A: (i) is a potent inhibitor of osteoclast formation and bone resorption with IC50s of ~ 10 nM; (ii) inhibits attachment to vitronectin of HEK 293 cells, transfected with either alpha$_v$beta$_3$ or alpha$_v$beta$_5$ integrin, with IC50s of 0.6 and 25 nM, respectively; and (iii) inhibits alpha$_{IIb}$beta$_3$-dependent human platelet aggregation with an IC50 of 35 uM.

In pharmacological assays of *in vivo* bone resorption compound A: (i) increases bone mineral density (BMD) significantly in growing male rats, when administered at 10 and 30 mg/kg P.O., b.i.d. for 10 days; (ii) increases BMD dose dependently in growing male rats by 4.7 to 32%, at increasing steady state concentrations between ~70 and 1550 nM, respectively, obtained by administering the drug S.C. with minipumps; (iii) in 6 month old ovariectomized (ovx) rats (a) increases BMD of distal femur; (b) increases bone volume of proximal tibial cancellous bone determined by histomorphometry; and (c) reduces bone turnover rate, when administered orally at 10 and 30 mg/kg, b.i.d. for 4 weeks, all relative to ovx rats receiving vehicle; and (iv) suppresses urinary N-telopeptide (uNTx) in ovx rhesus monkeys, when administered orally at 5, 15 and 40 mg/kg/day.

In conclusion, Compound A, a potent and selective alpha$_v$beta$_3$ integrin ligand, is a powerful inhibitor of bone resorption; it prevents bone loss in several animal models when given orally, without eliciting detectable changes in other tissues examined.

P228 S

THREE-DIMENSIONAL (3D) ARCHITECTURE OF THE INNER SURFACE OF VENTRAL MEMBRANES IN CULTURED OSTEOCLASTS

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Osteoclasts can adhere to the substrate via F-actin containing close contact structures termed podosomes. Taking advantage of an in vitro system of osteoclastic adhesion together with cell-sheaaring, quick-freeze, freeze-drying, and rotary replication, we investigated the 3D-architecture in the cytoskeleton of osteoclasts cultured on the glass or synthetic apatite plates. After exposure of the cytoplasmic surface of the ventral membrane by cell-shearing, the organizational structure of the

podosomes and clathrin sheets were left behind and were clearly distinguishable in 3D by viewing through eye glasses with blue-red filters. The core of single podosomes contained packed microfilaments with detergent-resistant materials from which actin filaments appeared to radiate out in rosette fashion. Most of microfilaments seemed to terminate at membrane-associated particles. Although the clathrin sheets showing various shapes were observed in the vicinity of the podosomes, few endocytic coated pits or vesicles were seen on the ventral membranes. In this study we have found that in vitro osteoclasts adhere to the substratum via two types of contacts: one is a close contact podosomes associated with actin microfilaments, and the other a cytoskeleton independent clathrin sheets.

P229 W

DIFFERENTIAL REGULATION BY CELL ADHESION ON CT-INDUCED ERK AND P38 MAPK SIGNALING IN MATURE MOUSE OSTEOCLASTS

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Calcitonin (CT) inhibits osteoclastic bone resorption through alteration of cytoskeletal organization and attachment to matrix. The consequences of CT treatment would elongate osteoclast (OC) survival by the reduction of apoptosis. It has been shown recently that Ras/ERK pathway is crucial for cell survival. However current understanding on the molecular mechanism by which survival factors prevent osteoclast apoptosis is somewhat limited. In previous studies, we have shown that activation of p38 MAPK is indispensable for maturation of OCs from the progenitors, and OCs previously exposed to CT regained bone resorbing capability in a certain period of time (CT escape). In this study, we examined possible roles of ERK and p38 MAPK in mature OCs, either adherent or nonadherent cells. When OCs adherent to the dishes were treated by CT (10-9 M), basal phosphorylation of Erk was diminished and dephosphorylation was evident 60 min after the treatment. Detachment of OCs from the dishes reduced basal phosphorylation of ERK, while treatment with CT markedly phosphorylated ERK of floating OCs in media. These CT effects were mimicked by forskolin, a direct activator of adenylate cyclase. Interestingly, treatment with CT also induced phosphorylation of p38 MAPK, which was observed either adherent or non-adherent OCs. In contrast, phosphorylation of p38 MAPK was neither mimicked by the treatment of forskolin nor phorbol esters. To explore the differential effects of CT on cell adhesion, we investigated the roles of FAK and PYK2 in the processes. Treatment with CT disrupted actin ring formation. FAK and PYK2 distributed in the contracted central region of OCs, and CT induced tyrosine phosphorylation of FAK. We are currently investigating the effects of PD98059, MAPK/ERK inhibitor and SB203580, P38 MAPK inhibitor, to clarify the down-stream signaling effects of CT, along with possible interaction of cell adhesion molecules in OCs. Cross-talk between outside-in and inside-out signaling by peptide hormone and adhesion molecules may result in various modifications of OC function in vivo.

P230 F

ENHANCED GENE EXPRESSION OF OSTEOCLASTOGENIC FACTORS IN HUMAN OSTEOPOROTIC TRABECULAR BONE

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The eitiology of osteoporosis (OP) is multifactorial but the potential role of local regulatory factors including cytokines and growth factors requires further investigation. Recently identified factors that regulate the differentiation and activity of osteoclasts include; Receptor Activator of Nuclear Factor Kappabeta Ligand (RANKL), RANKL receptor (RANK) and osteoprotegerin (OPG). The aim of this project was to investigate these key regulatory molecules of osteocalstogenesis in the local human bone micorenvironment.

Cancellous bone cores from the human proximal femur were obtained from 22 autopsy cases (11 females, 11 males; median age 71.5 years) with no evidence of skeletal abnormality, and 14 surgical cases (9 females, 5 males; median age 81.5 years) from patients undergoing surgery due to an OP hip fracture. Total RNA was extracted from the bone core, and RT-PCR was performed using a panel of oligonucleotides designed to amplify mRNA encoding; RANKL, RANK and OPG. PCR results were normalised according to the expression of the housekeeping gene, GAPDH and analysed semi-quantitatively. Non-parametric statistics were used and the data are reported as the median [interquartile range].

Gene expression patterns of the key factors involved in osteoclastogenesis have emerged. The RANKL/OPG mRNA ratio was significantly higher in OP bone (5.1 [4.0] > 2.4 [2.2]; $p<0.003$). OP bone showed significantly elevated RANKL (1.6 [2.1] > 0.9 [0.8]; $p<0.02$) and RANK (2.12 [0.8] > 0.8 [1.5]; $p<0.007$) mRNA expression. The RANK/OPG mRNA ratio also showed increased expression in OP (8.2 [5] > 1.4 [2.7]; $p<0.003$). There were no significant differences in the expression of OPG mRNA or the RANKL/RANK mRNA ratio between OP and control groups.

The RANKL/OPG ratio as well as the RANK/OPG ratio is consistent with increased osteoclast promotion and activity in OP bone. The fact that OPG was no different in control and OP groups; suggests that the regulation of OPG is not altering osteoclastogenesis in osteoporosis. The balance in the RANKL/RANK ratio suggests the coordinated regulation of these two factors in the bone microenvironment. The difference in gene expression between the OP and control groups is consistent with the hypothesis that altered levels of pro-osteoclastogenic factors contribute to the deterioration of bone in OP.

P231 S

RANKL EXPRESSION AND OSTEOCLAST LIKE FORMATION OF PERIPHERAL MONONUCLEARED CELLS FROM PATIENTS WITH LANGERHANS CELL HISTICYTOSIS

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Introduction: Receptor activator of NF-kB(RANK) is a recently identified member of the tumor necrosis factor receptor superfamily and is expressed on activated T cells and dendritic cells. Its cognate ligand (RANKL) plays significant roles in the activation of dendritic cell function and osteoclast differentiation. However, little is known about RANKL plays roles in Langerhans cell histiocytosis. We examined RANKL expressions and osteoclast formation of peripheral mononucleared cells from LCH.

Materials and Methods: PBMCs from LCH patients with multi-osteolytic lesions and healthy controls were obtained. We examined RANKL expressions on PBMCs by flow cytometer. Then, PBMCs were cultured for 9 days in the presence of human M-CSF and RANKL. Number of tartrate-resistant acid phosphatase (TRAP)-positive cells containing more 3 nuclei was counted as osteoclast like cell formation.

Results: Peripheral activation T lymphocytes expressed RANKL higher in LCH patients than healthy controls four times. RANKL expressions were decreased in lCHwithout progressive osteolytic lesions. In vitro osteoclast like cells formation in the presence of human M-CSF and RANKL were increased in LCH patients than healthy controls. Bisphosphonate and Cyclooxgenase-2 inhibitors inhibited osteoclast like cell formation in PBMC from LCH patients.

Conclusion: Peripheral activation T cells in LCH patients express RANKL. In vitro LCH peripheral monocyte differentiates earlier in activated osteoclast in the presence of RANKL. This fact opens the possibility that RANKL may play important roles osteoclastgenesis LCH patients.

P232 W

MIP-1ALPHA/CCR5 PATHWAY INDUCES OSTEOCLAST MATURATION AND ITS ANTAGONISM IS A POTENT TARGET FOR BONE RESORPTION IN RHEUMATOID ARTHRITIS

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We have reported that macrophage inflammatory protein (MIP)-1alpha, a major ligand of CC-chemokine receptor 5 (CCR5), induces integrin-mediated adhesion and migration of T cells and, thereby, plays a pivotal role in the inflammatory processes of rheumatoid arthritis (RA). We here document the functional role of MIP-1alpha in osteoclastogenesis. 1) T cells infiltrated into RA synovium produced high amounts of MIP-1alpha in vivo and in vitro. 2) CCR5 was upregulated on peripheral blood mononuclear cells (PBMC) of RA patients, compared to those of healthy control. 3) PBMC of RA patients and controls were differentiated into TRAP-positive multinucleated cells (MNC) when cultured with RANKL and M-CSF, and MIP-1alpha further induced TRAP-positive MNC formation. 4) MIP-1alpha also augmented Ca-release from mouse calvaria by Raisz method. 5) CCR5 antagonists inhibited MIP-1alpha-induced osteoclast formation from PBMC and subsequent osteoclastic bone resorption, in which 1000-fold higher concentration of CCR5 antagonist was required when PBMC of RA patients were used for the induction, compared to those of OA. These results imply that MIP-1alpha plays an pivotal role in the maturation of a monocyte-osteoclast lineage and subsequent bone resorption and we here propose that the chemokine antagonism such as a CCR5 antagonist would be a novel therapeutic agent for inflammatory processes and bone resorption and/or joint destruction in RA.

P233 F

THE SYSTEM OF EASILY EVALUATING OSTEOCLAST FORMATION AND THE DIFFERENT OSTEOCLAST FORMATION FROM CIRCULATING PRECURSORS OF PRE-/POSTMENOPAUSAL JAPANESE WOMEN

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As the arrival of the aging society, there is a dramatical increase in the number of the patients suffering from osteoporosis. So it is noticed that how to reduce the risk of fractures and the decline of the activity of daily life caused by the osteoporosis. Osteoporosis is more common in elderly women. A dramatical reduction of bone mineral density is done after acquiring menopause. One of the causes of reducing it in postmenopausal women is whether HPBMC changes the character after acquiring menopause. Recently, there were many reports about methods to induce and evaluate the osteoclast formation from human peripheral blood mononucleated cells (HPBMC) in vitro. At present, human osteoclast formation in vitro needs to culture for more than 2 weeks, and the efficiency is extremely lower than the mouse system.

In this study, we developed the culture system combined recombinant human M-CSF and RANKL with a mouse stromal cell line. When CD14-positive HPBMC enriched by the magnetic beads were used as a source of osteoclast precursors, osteoclastogenesis was significantly increased on 14th day of cultures.Moreover, even on 7 th day, tartrate resistant acid phosphatase (TRAP)-positive multinucleated cells (MNC) were efficiently detected. There is a report that osteoclast formation induced from HPBMC is not different to between pre-/postmenopausal women. However, using this culture system, we find the interesting differences of osteoclast formation induced from CD14-positive peripheral blood mononucleated cells of the patients of rheumatoid arthritis, pre-/postmenopausal Japanese women and normal persons.

Now, we assess the correlation between the number of osteoclasts in our culture system, the percentage area of lacunar resorption on dentine slice formed by multinucleated osteoclasts, and the amount of TRAP activity measured by the absorption spectrophotometer. Here, we show an easier and faster method of evaluating osteoclast formation and activity induced from CD14-positive HPBMC in vitro.

P234 S

FGF-2 INDUCES RANKL EXPRESSION AND OSTEOCLAST MATURATION BY BINDING TO HEPARAN SULFATE PROTEOGLYCAN ON RHEUMATOID SYNOVIAL FIBROBLASTS

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Rheumatoid arthritis (RA) is characterized by progressive joint destruction resulting from inflammation in synovial joints. Although RA synovial fibroblasts (RASF) play a central role in RA synovitis, mechanisms of RASF in bone resorption have not been clarified. We here document the role of fibroblast growth factor (FGF)-2 produced by RASF in osteoclastogenesis. 1) RASF highly produced FGF-2 and FGF-2 augmented RASF proliferation. 2) RANKL and ICAM-1 were expressed on RASF more than on synovial fibroblasts of osteoarthritis (OASF). 3) FGF-2 induced RANKL and ICAM-1 expression on RASF, which was inhibited by an antibody against human FGF-2. 4) Although FGF receptor (FGFR)-1 was equally expressed on RASF and OASF, heparan sulfate proteoglycan (HSPG), a co-receptor for FGF-2, was highly expressed on RASF, but was absent on OASF. 5) FGF-2-mediated RANKL induction on RASF was reduced by removal of HS or HSPG with heparinase or heparitinase respectively. 6) TRAP-positive MNC formation in cocultures of RASF and PBMC was strongly reduced in the presence of heparitinase. 7) RANKL up-regulation on RASF and MNC formation by FGF-2 were also inhibited by PD-98059, an inhibitor of ERK. Taken together, FGF-2 is transferred to FGFR-1 thorough binding to HSPG characteristically expressed on RASF, resulting in RANKL and ICAM-1-mediated maturation of osteoclasts via ERK activation. Thus, FGF-2 not only augments RASF proliferation but also is involved in osteoclast maturation which leads to bone destruction and osteoporosis of RA and we propose that FGF-2 and HSPG-mediated signaling could be a potent target for the treatment of RA.

P235 W

POTENTIAL ROLE OF LEPTIN IN MOUSE OSTEOPOROSIS INDUCED BY OVARIECTOMY

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Osteoporosis is a metabolic bone disease characterized by low bone mass, microarchitectural deterioration of bone tissue and increase of intramedullary adipose tissue, leading to enhanced bone fragility and a consequent increase in fracture risk. In the primary osteoporosis, not only bone resorption but also bone formation is known to be enhanced. However, the mechanism of this enhanced bone formation is still unclear. Leptin, a 16-kD circulating hormone secreted by white adipose tissue, is a product of the obese (*ob*) gene. It influences body weight homeostasis through its effects on food intake and energy expenditure by negative feedback at the hypothalamic nuclei. Recent study showed that leptin regulated the osteogenic and adipogenic differentiation of bone marrow stromal cells. From these facts, leptin may play an pathophysiological role in occurrence and/or progress of osteoporosis. To elucidate a possible role in osteoporosis, we immunohistochemically investigated the expression of leptin in tibiae of normal and ovariectomized mice (8 weeks old, ICR strain). In addition, the effects of leptin on the proliferation and differentiation of MC3T3-E1, a mouse osteoblast cell line, and MMSC-8, a bone marrow stromal cell line were also investigated *in vitro* by crystal violet staining method and reverse transcription-polymerase chain reaction, respectively.

As a result, leptin was observed to be expressed in osteoblasts in the primary spongiosa beneath the growth plate, hypertrophic chondrocytes and bone marrow cells in normal mice. Interestingly, the expression level of leptin in osteoblasts in primary spongiosa strongly elevated in ovariectomized mice. *In vitro* study showed that leptin had no effects on the proliferation or differentiation of MC3T3-E1. In contrast, the osteoblastic differentiation of MMSC-8 was significantly enhanced by leptin.

These results suggested that ovariectomy-induced elevation of leptin expression in bone tissue may be involved in the mechanism by which the bone formation is enhanced in osteoporosis as well as bone resorption.

P236 F

BONE LOSS IS INDUCED IN REGUCALCIN TRANSGENIC RATS

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The rat regucalcin gene is localized on chromosome Xq11.1-12 proximal end, and the gene has been demonstrated in human, mouse, bovine, monkey, dog, rabbit, and chicken but not yeast. Comparison of the nucleotide sequences of regucalcin from vertebrate species was highly conserved throughout evolution (Int J Mol Med 6:191-196,2000; Life Sci 66:1769-1780,2000; BBRC 276:1-6,2000).

The role of endogenous regucalcin in the regulation of bone metabolism was investigated in regucalcin transgenic rats (RC Tg) (J Cell Biochem 86:520-529,2002). This animals were supplied from Japan SLC (Hamamatsu, Japan).

Regucalcin (RC) levels were significantly increased in the femoral-diaphyseal and -metaphyseal tissues of RC Tg female rats. Morphologic change was great in the femoral tissues of female rats as compared with that of male rats (5 weeks old). Mineral content, mineral density and polar strength strain index in the femoral-diaphyseal and -metaphyseal tissues were markedly reduced in RC Tg female rats. A significant decrease in cortical thickness was seen in the femoral diaphysis of RC Tg female rats. Calcium content in the femoral-diaphyseal and -metaphyseal tissues was significantly decreased in RC Tg male and female rats; a remarkable decrease was seen in female rats. Femoral-metaphyseal alkaline phosphatase activity was significantly lowered in RC Tg female rats. A significant decrease in DNA content was seen in the metaphyseal tissue of RC Tg male rats and in the diaphyseal and metaphyseal tissues of the TG female rats.

This study demonstrates that bone loss is induced in the femoral tissue of regucalcin transgenic RC Tg) rats, and that a remarkable decrease in bone morphogenic index and biochemical component was ssen in the female young rats. Regucalcin may be involved in the regulation of bone metabolism. Regucalcin has also been found to be expressed in bone marrow cells. Presumably, regucalcin is involved in the regulation of bone resorption.

P237 S

IDENTIFICATION OF MELANOCORTIN-4 RECEPTOR ON OSTEOCLASTS

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The hypothalamic melanocortin system appears to play a significant role in the central regulation of food intake and metabolism. Genetic deficiencies in the receptors for the melanocortin peptides, namely the melanocortin-3 receptor (MC3-R) and the melanocortin-4 receptor (MC4-R), result in obesity in humans and rodents. The presence of these receptors in the hypothalamus has long been established but because

obesity is associated with high bone mineral density we sought to determine if these receptors are also present peripherally in bone. For the first time we demonstrate the presence of MC4-R on the osteoclast population in the bone marrow. Immunohistochemistry performed on rat tibial sections has shown the presence of this receptor on large multinucleated cells in areas of active resorption, presumably osteoclasts. An adjacent section stained for tartrate-resistant-acid-phosphatase (TRAP) indicates that the MC4-R positive multinucleated cells are also TRAP positive. Both immunocytochemistry and northern analysis of in vitro osteoclast preparations confirmed these data. Non-adherent bone marrow cells from 4-week old female Sprague-Dawley rats were grown in alpha-MEM medium supplemented with 10% FBS, 50ug/ml ascorbic acid, 50ng/ml m-CSF and 50ng/ml OPGL to induce osteoclast formation. At the peak of formation, day 5, osteoclast cells were processed for immunocytochemistry or extracted for RNA. Immunocytochemical preparations were also stained with TRAP to identify the osteoclast phenotype. TRAP positive osteoclasts were immunochemically positive for MC4-R, confirming the northern analysis of total RNA preparations, which were also positive for this receptor. The identification of MC4-R in the osteoclast population suggests its involvement in the regulation of bone re-modeling and opens a whole new area of bone mineral homeostasis research.

P238 W

THE EFFECTS OF QUERCETIN ON PROLIFERATION AND APOPTOSIS OF SD RAT OSTEO- CLASTS IN VITRO

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OBJECTIVE: To study the effects of quercetin on proliferation and apoptosis of SD rat osteoclasts in vitro.

METHODS: Osteoclasts and their precursors of SD rats were mechanically isolated from long bones. Cells were cultured in four classes of Minimum Essential Medium(MEM) of which quercetin concentration was 0, 2.5uM, 10uM, or 40uM, respectively. Dynamic changes in morphology, numbers and apoptosis of the osteoclast were detected with Wright's or TUNEL staining at 72, 96, 120 and 144hrs from the beginning of culture.

RESULTS: The number of osteoclast was decreased but the number of apoptic osteoclast had a tendency of augmentation with increasing concentration of quercetin.

CONCLUSIONS: Quercetin could inhibit the proliferation and induce apoptosis of osteoclasts cultured in vitro.

P239 F

GAMMA-GLUTAMYL TRANSPEPTIDASE AS A NOVEL BONE-RESORBING FACTOR

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gamma-Glutamyl transpeptidase (GGT) is an ectoenzyme expressed in kidney, liver and pancreas, and its serum concentrations have been used mainly for the diagnosis of liver diseases. By screening bone-resorbing activity produced by murine T-lymphoma cells, using *Xenopus* oocyte expression cloning system, we identified GGT as a novel bone-resorbing factor. Addition of purified GGT (5-625ng/ml) to murine bone marrow cell cultures dose-dependently induced the formation of TRAP-positive multinucleated cell, with calcitonin receptor and the capacity to form resorption pits on dentin slices. Moreover, anti-GGT antibody dose-dependently supressed osteoclast formation induced by GGT, but not the enzymatic activity. Taken together with the findings that inactivated form of GGT, whose enzymatic activity was blocked through chemical modification by acivicin, supported osteoclast formation, it was indicated that the osteoclastogenetic activity of GGT does not require its enzymatic activity and may involve a putative receptor molecule. Not only native GGT but also inactive GGT induced the expression of RANKL mRNA and protein in bone marrow stromal cells, and osteoprotegerin (OPG), a decoy receptor for RANKL, completely supressed GGT-induced osteoclast formation, suggesting that GGT stimulates osteoclastogenesis through RANKL expression in stromal cells.

In collagen-induced arthritis, mouse model of rheumatoid arthritis, GGT expression was markedly increased in synovial tissue. Culture of isolated cells from arthritic paws spontaneously induced the formation of osteoclasts, which was supressed by anti-GGT antibody. Increased levels of GGT are found in hepatic diseases and chronic alcoholism, conditions frequently associated with osteopenia. Thus, the involvement of GGT in secondary osteoporosis associated with these hepatic diseases should warrant further investigatation.

P240 S

INVESTIGATION OF THE LAW AND PHENOMENA ABOUT APOPTOSIS OF OSTEOCLASTS OF SD RAT CULTURED IN VITRO

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Objective: To study the phenomena and the law of apoptosis about osteoclasts of SD rat cultured in vitro.

Methods: The phenomena about apoptosis of osteoclasts of SD rat cultured in vitro were investigated through morphology and DNA 3-OH terminal deoxynucleotidyl transferase-mediated deoxyuride tiphosphate nick-end labeling by microscope, and the law of apoptosis was suspected.

Result: The phenomena of apoptosis was observed, the number and the ratio of osteoclasts in apoptosis were increasing by the time of culture. The ratio of apoptosis of osteoclasts were 10, 14, 34 and 50 percent at the date of 3, 4, 5 and 6 respectively.

Conclusion: Apoptosis was existed in osteoclasts of SD rat cultured in vitro, the ratio of apoptosis of osteoclasts was increasing by the time of culture.

P241 W

$1,25\text{-}(OH)_2D_3$ DIRECTLY INDUCE OSTEOCLAST (OCL) FORMATION BY OSTEOCLAST PRECURSORS FROM PAGET'S DISEASE (PD) PATIENTS IN THE ABSENCE OF RANK LIGAND (RL)

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1,25 dihydroxyvitamin D_3 ($1,25\text{-}(OH)_2D_3$) is believed to induce OCL formation indirectly through induction of RL on marrow stromal cells and osteoblasts. Consistent with this observation, RL or RANK receptor knockout mice are profoundly osteopetrotic. Although VDR is expressed by normal OCL precursors, they only form OCL in response to supraphysiologic levels of $1,25\text{-}(OH)_2D_3$ (10^{-9} to 10^{-8} M). In contrast, OCL from PD patients are hyperresponsive to $1,25\text{-}(OH)_2D_3$. These data suggested that $1,25\text{-}(OH)_2D_3$ also could act directly on OCL precursors, and that in contrast to normals, OCL precursors from PD patients may form OCL at physiologic concentrations of $1,25\text{-}(OH)_2D_3$ (10^{-10} M) in the absence of RANKL. Therefore, marrow cultures from PD patients or normals were treated with 10^{-11} to 10^{-7} M $1,25\text{-}(OH)_2D_3$ or 50nanog/ml RANKL ±50 nanog/ml osteoprotegerin (OPG), which completely inhibits OCL formation induced by 50 nanog/ml of RANKL. OPG inhibited OCL formation induced by $1,25\text{-}(OH)_2D_3$ approximately 40 percent in normal or PD cultures. Normal marrow cultures only formed OCL in the presence of high levels of (10^{-9} to 10^{-8} M) $1,25\text{-}(OH)_2D_3$. In contrast, OCL formed in PD marrow cultures with 10^{-11} M to 10^{-10}M $1,25\text{-}(OH)_2D_3$. The OCL that formed in the absence of RANKL resorbed dentine. OPG (50 nanog/ml) totally blocked OCL formation induced by RL. In marrow cultures treated with $1,25\text{-}(OH)_2D_3$ (10^{-8}M) or media, RL levels were 732 ±47 picog/ml or 177 ±5 picog/ml, respectively. Importantly, OPG did not inhibit OCL formation in cultures of highly purified early human osteoclast precursors treated with 10^{-11} to 10^{-7} M $1,25\text{-}(OH)_2D_3$, but totally blocked the effects of RL. RL was undetectable in cultures of highly purified OCL precursors treated with 10^{-8}M $1,25\text{-}(OH)_2D_3$ consistent with the absence of stromal cells in these cultures. These data demonstrate that $1,25\text{-}(OH)_2D_3$ can induce OCL formation by normal OCL precursors at supraphysiologic concentrations of $1,25\text{-}(OH)_2D_3$ (10^{-8}M). In contrast, PD OCL precursors can form OCL at physiologic levels of $1,25\text{-}(OH)_2D_3$, (10^{-10} to 10^{-11} M) in the absence of RANKL. These data suggest that $1,25\text{-}(OH)_2D_3$ may act both directly and indirectly to induce OCL formation in PD patients.

P242 F

BMP-2 AND ASCORBIC ACID STIMULATE OSTEOCLASTOGENESIS BY OSTEOBLAST-LIKE CELLS ESTABLISHED FROM RUNX-2-DEFICIENT MOUSE IN THE PRESENCE OF VITAMIN D

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The interaction of osteoclast precursors with osteoblasts or stromal cells is essential for the formation of mature osteoclasts and the resorption of bone. We previously found that the formation of osteoclasts required ascorbic acid and ascorbic acid increased the expression level of mRNA for the receptor activator of nuclear factor-kappa B ligand (RANKL) (Endocrinology, 141:3006-3011, 2000). Furthermore, we reported that treatment of myoblastic C2C12 cells with BMP-2 stimulated the vitamin D-induced formation of osteoclasts by stimulating of expression level of RANKL. It seems possible that the formation of osteoclasts stimulated by ascorbic acid and BMP-2 might be mediated by signal transduction pathways that involve runx-2. It is also reported that runx-2 contributed to the expression of osteoprotegerin. Therefore, in the present study, we examined the effects of ascorbic acid or BMP-2 on osteoclastogenesis using osteoblast-like cells established from *runx-2*-deficient mouse (C6 cells).

We used coculture system of mouse spleen cells with runx-2-deficient osteoblast-like cells (C6 cells) in this study. At first, we examined dose-dependency of 1alpha,25-dihydroxyvitamin D_3 [1alpha,25$(OH)_2D_3$] for the formation of osteoclasts in our coculture system. Exposure of C6 cells to 1alpha,25$(OH)_2D_3$ dose-dependently induced the differentiation of mouse spleen cells into tartrate-resistant acid phosphatase-positive (TRAP-positive) multinucleated cells. We confirmed that, at 10^{-7} M of 1alpha,25$(OH)_2D_3$, expression levels of mRNAs for RANKL and osteoprotegerin (OPG) increased and decreased, respectively, in C6 cells. Furthermore, ascorbic acid and BMP-2 dose-dependently stimulated the formation of osteoclasts by C6 cells. Our findings suggest that runx-2 does not regulate the expression of RANKL and OPG by 1alpha,25$(OH)_2D_3$, and ascorbic acid and BMP-2 might stimulate osteoclastogenesis independent on runx-2.

P243 S

TRIBUTYLTIN INHIBITS THE FORMATION OF OSTEOCLAST: ELUCIDATION OF INHIBITORY MECHANISM

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Environmental endocrine disrupting chemicals are reported to be involved in carcinogenesis, immunodeficiency, and fetotoxic manifestations. However, information on action and signal pathways of these compounds in the modeling and remodeling of mammalian bone is not fully available. Recently, we reported that 3-methylcholanthrene, a ligand of dioxin receptor, inhibited the proliferation and differentiation of cultured osteoblastic cells and caused delay of ossification *in vivo* (Endocrinology, 143:3575-3581, 2002). In the present study, we attempted to investigate the effects of tributyltin (TBT), one of environmental contaminants, on osteoclastogenesis.

At first, we investigated effects of TBT on the formation of osteoclasts. TBT decreased dose-dependently the formation of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells (osteoclasts) from coculture system of mouse spleen cells with mouse stromal cell ST2 and TBT even at 10^{-9} M inhibited the formation. By contrast, monobutyltin did not affect the formation of osteoclasts, dibutyltin moderate, and triphenyltin did it more potent than TBT. When we administrated TBT into culture system for RAW 264.7 cells or spleen cells stimulated with soluble receptor activator of NF-kappaB ligand (sRANKL), TBT inhibited the formation of osteoclastic cells. These results suggested that TBT affected the monocyte/macrophage directly. Next, we examined how TBT inhibited the formation of osteoclasts. TBT has been known to have androgen-like actions and inhibit an activity of aromatase, but testosterone and aromatase inhibitor aminoglutethimide did not reproduce the inhibitory effect of TBT on the formation of osteoclasts. By contrast, we found that the proliferation of RAW 264.7 cells was accelerated by the exposure of TBT. Moreover, we clarified the relationship with TBT between the stimulation of proliferation of Raw 264.7 cells and inhibition of the formation of osteoclastic cells. Our findings indicate that TBT might have critical effects on osteoclastogenesis and environmental endocrine disrupting chemicals.

P244 W

ESTIMATION OF GLUCOCORTICOID EFFECTS ON BONE METABOLISM MARKER AND BONE MINERAL DENSITY IN RATS

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Introduction: Glucocorticoid induced osteoporosis was characterized by decreased of osteoblast numbers and a marked impairment of new bone formation. The present study evaluated the effect of methylprednisolone Acetate on metabolism and bone mineral density of rats.

Methods and Material: Total duration of the experiment was four weeks. Eighteen male spargue Dawly rats (8 week old and 180 gr weight) were randomly divided into three groups: Group A (n=6), was a base line control or normal animal. Group B (n=6), get only normal saline (0.9%) and group C (n=6), get methylprednisolne acetate (0.2 mg/kg) subcutaneously 3 times for 4 weeks. For evaluation on Biochemical agents changed in the serum calcium, Acid phosphatas and osteocalcine were measured before and after treatment. Also, bone mineral density (BMD) of lumber vertebrae was measured by dual energy x-ray absorptiometry (DEXA).

Results: The results showed that, the serum calcium level unaffected ($p<0.05$) by methylprednisolone acetate, but the serum Acid phosphatase level was significantly ($p<0.05$) increased after 4 weeks treatment. Also, the serum osteocalcine level and bone mineral density of lumbar vertebrae were significantly ($p<0.05$) decreased by methylprednisolone acetate treatment compared with the other groups.

Conclusions: The findings indicate that by administration of methylprednisolne actate bone formation lumber vertebrae was decreased and bone resorption in lumber vertebrae was increased.

P245 F

CIRCADIAN RHYTHM OF OSTEOCLASTOGENESIS ACTIVITY IN RAT SERUM

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Calciotropic hormones as well as biochemical parameters of bone formation and resorption show circadian rhythms. Previous studies have described circadian rhythms in bone-resorbing activity both in rat and human serum. To understand this mechanism, we investigated whether there was a circadian rhythm in the activity of osteoclast formation in rat serum. Male Wistar rats, 6-week-old, were adapted for 2 weeks to an environmental room equipped with an automatically timed 12:12h light-dark illumination program. On day 15, they were killed at 4-hour interval over 24 hours (at 0100, 0500, 0900, 1300, 1700 and 2100). At the time of killing, serum was collected from carotid artery under light ether anesthesia. To access the osteoclast-forming activity of the collected serum, osteoclastic progenitors were obtained by treating mouse (dddy) bone marrow cells with Sephadex G10 column. They were cultured for 8 days in alpha-MEM supplemented with CSF-1 (20nanog/ml), sRANKL (60nanog/ml) and 15% rat serum collected as described above. The number of TRAP positive multinucleated cells (MNC) was counted after the culture. The expressions of mRNA for TRAP, calcitonin receptor, RANK and c-fms were examined by RT-PCR using the cells cultured for 2 or 5 days in the medium supplemented with serum collected at 0900 or 2100. The following results were obtained. (1) Counting of TRAP positive MNC revealed that osteoclast-forming activity of rat serum followed a marked circadian rhythm with a peak at around 2100-0100 and a nadir at around 0900-1300. (2) RT-PCR analysis showed that in 5-day-culture (but not in 2-day-culture), all mRNA expressions were higher in the cells cultured in the medium supplemented with the serum collected at 2100 than that collected at 0900. The above results suggest that some humoral factor(s) would regulate circadian rhythm in bone resorption via osteoclast formation.

P246 S

ROLES OF CXC-CHEMOKINE IN LPS-INDUCED PERIODONTAL TISSUE DESTRUCTION

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Lipopolysaccharide (LPS) elicits various periodontal tissue responses, including recruitment of neutrophils and stimulation of osteoclastic bone resorption via release of proinflammatory cytokines from host cells. CXC-chemokine has been known to be an important cytokine, which mediates selective neutrophil recruitment and activation. The purpose of this study was to examine the role of CXC-chemokine in periodontal tissue destruction caused by LPS-challenge.1) Topically applied 5mg/ml *E. Coli* LPS from rat gingival sulcus rapidly provoked inflammatory changes in junctional epithelium (JE) and sub-JE area. In addition, stimulation of osteoclastic bone resorption along the alveolar bone surface showing a biphasic response peaking at 3 hours and 3 days was observed. 2) A transient immuno-expression of macrophage inflammatory protein-2 (MIP-2), which is an important murain CXC-chemokine, in JE cells peaking at 1 day was observed. Corresponding to excessive MIP-2 expression, LPS application induced a significant increase in number of neutrophils in JE and sub-JE area. 3) Various concentrations of recombinant rat MIP-2 (0.05, 0.5, 5, 50microg/ml) applied from gingival sulcus caused not only neutrophil recruitment into JE and sub-JE area but also osteoclasts amassment along the alveolar bone surface in a dose dependent manner. 4) Injection of the anti-MIP-2 antibody remarkably reduced the recruitment of neutrophils into JE and sub-JE area and significantly decreased the appearance of osteoclasts at 3 days after LPS application. On the other hand, the first increase of osteoclasts at 3 hours, which is seemed to be a direct effect of LPS, was not inhibited by the anti-MIP-2 antibody treatment. Theses findings indicated that MIP-2 may be one of powerful CXC-chemokines for neutrophil recruitment into the periodontal disease site and may play an important role in the initiation of inflammation. Moreover, the over secretion of CXC-chemokine in the periodontal disease site may induce production of various osteoclast stimulating factors resulting in the subsequent alveolar bone destruction. It is suggested a possibility of establishment new periodontal therapy targeting CXC-chemokine.

P247 W

EFFECT OF HIGH PHOSPHATE CONCENTRATION ON OSTEOCLAST DIFFERENTIATION AS WELL AS BONE-RESORBING ACTIVITY

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Previous study has shown that high inorganic phosphate concentration ([Pi]) in culture media inhibits generation of new osteoclasts and also inhibits bone resorption by mature osteoclasts. However, the precise mechanism by which high [Pi] inhibits

osteoclast differentiation and osteoclastic bone resorption has not been elucidated. The present study was performed to investigate these issues. We employed mouse preexistent osteoclast-free bone cells cultures and osteoclast precursor cells cultures derived from mouse spleen cells to evaluate osteoclast differentiation in the presence and absence of stromal cells, respectively, and the pit assay by isolated rabbit osteoclasts to evaluate bone-resorbing activity by mature osteoclasts in the absence of stromal cells. Northern blot analysis and RT-PCR analysis were performed to evaluate the expressions of osteoprotegerin (OPG) and receptor activator of NF-kappa B ligand (RANKL) mRNA. Apoptosis was evaluated by TUNEL assay. Increase in extracellular [Pi] ([Pi]e) (2.5-4 mM) concentration-dependently inhibited $1,25(OH)_2D_3$- or PTH-(1-34)-induced osteoclast formation from unfractionated bone cells in the presence of stromal cells. Increase in [Pi]e (2.5-4 mM) concentration dependently inhibited $1,25(OH)_2D_3$-, PTH-(1-34)- or RANKL and M-CSF-induced osteoclast-like cell formation from hemopoietic blast cells in the absence of stromal cells. Increase in [Pi]e (2.5-4 mM) dose-dependently stimulated the expression of OPG mRNA and increased the expression of OPG mRNA suppressed by PTH-(1-34) or $1,25(OH)_2D_3$ in unfractionated bone cells, while it did not affect RANKL mRNA. Increase in [Pi]e (2.5-4 mM) concentration-dependently inhibited the bone-resorbing activity of isolated rabbit osteoclasts. Increase in [Pi]e (4 mM) induced the apoptosis of isolated rabbit osteoclasts. High [Pi]e (4 mM) did not affect apoptosis of osteoclast progenitors and C7 cells. These results indicate that increase in [Pi]e inhibits osteoclast differentiation both by up-regulating OPG expression and by direct action on osteoclast precursor cells. It is also indicated that increase in [Pi]e inhibits osteoclastic activity at least in part by the direct induction of apotosis of osteoclasts.

P248 F

EFFECT OF ARTEMISIA ASIATICA ON EXPRESSION OF OSTEOCLAST DIFFERENTIATION FACTOR INDUCED BY ORAL SPIROCHETES

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Artemisia asiatica (A.asiatica) has been used in traditional oriental medicine as antiinflammatory agents, but its effect on bone tissue is not documented. Osteoclastogenesis is induced by osteoclast differentiation factor (ODF) and inflammatory cytokines including IL-1beta and TNF-alpha stimulate ODF expression. Periodontitis is an inflammatory disease and alveolar bone destruction is a significant characteristic. In our previous study, we observed that *A.asiatica* has inhibitory effect on osteoclast formation induced by *Treponema socranskii* and *Treponema denticola* which are involved in periodontitis. To clarify the inhibitory mechanism of *A.asiatica* on osteoclast formation induced by *T.socranskii* and *T.denticola*, the effect of *A.asiatica* on expression of ODF, IL-1beta and TNF-alpha mRNA was observed in mouse calvaria-derived osteoblastic cells by RT-PCR. The osteoclast formation was determined in coculture system using mouse calvaria-derived osteoblastic cells and bone marrow cells. Both *T.socranskii* and *T.denticola* sonicates increased the expression of ODF, IL-1beta and TNF-alpha mRNA. IL-1beta receptor antagonist and TNF-alpha receptor R1 Ab decreased the number of osteoclast induced by sonicates of *T.socranskii* and *T.denticola*. In addition, *A.asiatica* decreased the level of ODF, TNF-alpha and IL-1beta induced by *T.socranskii* and *T.denticola* in osteoblastic cells. These results suggest that *A.asiatica* inhibits osteoclast differentiation induced by *T.socranskii* and *T.denticola* through inhibition of TNF-alpha and IL-1beta, which are involved in ODF expression, in osteoblastic cells.

P249 S

ESSENTIAL ROLE OF P38 MAP KINASE SIGNALING PATHWAY FOR RECEPTOR ACTIVATOR OF NF-KB LIGAND (RANKL)-INDUCED EXPRESSION OF MARKER GENES IN OSTEOCLASTOGENESIS

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The receptor activator of NF-kB ligand (RANKL) induces osteoclastogenesis in which osteoclasts express several markers including tartrate-resistant acid phosphatase (TRAP) and Cathepsin K. Our previous studies showed that treatment of bone marrow cells with p38 mitogen-acitvated protein kinase (MAPK) specific inhibitor, SB203580, inhibits osteoclast differentiation via inhibition of the RANKL-mediated signaling pathway, and that p38 MAPK pathway is essential for osteoclastogenesis. However, much remains to be learned about its mode of action. The aim of this study is to elucidate the role of p38 MAPK pathway in the context of RANKL-induced osteoclastogenesis at the molecular level. We employed RAW264 cells which could differentiate into osteoclast-like cells following treatment with RANKL alone. Treatment with the p38 MAPK specific inhibitor suppressed RANKL-induced expressions for *TRAP* and *Cathepsin K* genes. We identified novel PU.1-binding motifs in the Cathepsin K gene promoter and observed specific binding of PU.1 to the sites by gel shift assay. Analysis of transient reporter gene indicated that PU.1 activated *Cathepsin K* gene promoter in concert with microphthalmia transcription factor (MITF), which is a master regulator of RANKL-induced gene expression for *TRAP* and *Cathepsin K*. Interestingly, we found that p38 MAPK activated these promoters synergistically with MITF and PU.1. Mutation analysis of MITF determined that the amino acid residue of Ser(397) was important for the synergism between p38 MAPK and the transcription factors. p38 MAPK did not phosphorylate directly neither MITF nor PU.1 in *in vitro* kinase assay, suggesting the possible involvement of another candidate(s) in the synergism. Analysis to identify the downstream molecule(s) of p38 MAPK is ongoing. We also observed that c-Jun had functional interaction with MITF and PU.1 as a negative regulator for transactivations of these genes, and that p38 MAPK was phosphorylated in matured osteoclasts stimulated with calcitonin. These results suggest that osteoclast differentiation is regulated by p38 MAPK-mediated cross-talk signaling at the transcriptional level. Taken together, activation of p38 MAPK pathway could play a critical role not only in RANKL-induced osteoclastogenesis but also in the control of bone resorption by peptide hormone such as calcitonin.

P250 W

TRANCE-INDUCED OSTEOCLAST DIFFERENTIATION REQUIRES PRODUCTION OF REACTIVE OXYGEN SPECIES

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Bone resorption and remodeling is a controlled, physiological process that requires the function of osteoclasts. The processes governing both the differentiation and activation of osteoclasts involve signals induced by TRANCE, a TNF family member, and its cognate receptor TRANCE-R. The molecular mechanisms by which TRANCE activates various downstream kinases, leading to osteoclastogenesis remain to be elucidated. Here, we demonstrate that TRANCE stimulation of both bone marrow monocytes and RAW 264.7 cells, a monocytic cell line, transiently increased the intracellular concentration of reactive oxygen species (ROS) through a signaling cascade involving TRAF6 and PI3-kinase. Treatment of PI3-kinase inhibitors specifically inhibited TRANCE-induced p38 MAP kinase activation but not ERK and JNK activation. TRANCE-induced osteoclast differentiation of bone marrow cells was inhibited when ROS production was prevented by PI3-kinase and p38 MAP kinase inhibitors. These data suggest that TRANCE generates ROS through TRAF6-PI3-kinase-dependent pathway. The ROS may, in turn, activate p38 MAP kinase, leading to osteoclast differentiation.

P251 F

FIBRONECTIN IS ESSENTIAL FOR STROMAL CELL-DEPENDENT OSTEOCLASTOGENESIS AT THE EARLY STAGE OF DIFFERENTIATION

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RANKL is expressed on various tissues and hematopoetic cells, however, osteoclastogenesis is regulated mainly on bone marrow stromal cells. We tried to isolate surface molecules regulating osteoclastogenesis on stromal cells. The mouse stromal cell line, TSB13, which can support osteoclastogenesis, was immunized into a rat, and a monoclonal antibody, A15-1, was chosen. A15-1 inhibited osteoclastogenesis dose-dependently at the early stages of osteoclastogensisis from osteoclast progenitors to mononuclear osteoclasts. However, inhibition was not observed in the stroma-free, sRANKL-induced osteoclastogenesis. We identified that the A15-1 antigen is fibronectin, a heterodimeric ECM glycoprotein. Antisense oligonucleotides against the mouse fibronectin mRNA were transfected into TSB13, resulting remarkable decrease of the ability of osteoclastogenesis. The pro-B and pre-B cell lines which highly express RANKL on the surface were not able to support osteoclast differentiation. Because the expression of the fibronectin is limited to mesenchymal lineage cells, the lack of fibronectin in other lineage cells implies the defect in osteoclastogenesis. In the in-vivo analyses, treatment with A15-1 significantry decreased both the number of mature osteoclasts and the area of erosion surface in PTH-induced hypercalcemia model mice. In this experiment, we firstly demonstrated the novel function of fibronectin on stromal cells in osteoclast differentiation. In addinng to RANKL and M-CSF, the third molecule, fibronectin, is necessary as the differentiation factor in osteoclastogenesis.

P252 S

ROLES OF TOLL-LIKE RECEPTOR (TLR) 2 AND TLR4 SIGNALS IN PGE PRODUCTION BY OSTEOBLASTS AND PERIODONTAL BONE LOSS

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Toll-like receptors (TLR) are involved in the innate immunity and play important roles in the host defense against pathogens. TLR2 and TLR4 have shown to mediate the signaling for bacterial infection, but roles of TLR2 and TLR4 in inflammatory bone loss of periodontal disease are not fully understood. In this study, using TLR2-specific ligand (TLR2-L: Pam3-Cys-Ala-Gly) and TLR4-specific ligand (TLR4-L: synthetic Lipid A), we examined the effects of TLR2-L and TLR4-L on PGE2 production by osteoblasts, osteoclast formation and inflammatory bone loss of alveolar bone in mouse mandible.

TLR2-L and TLR4-L individually stimulated PGE2 production by osteoblasts isolated from newborn mouse calvaria. In coculture of mouse bone marrow cells and osteoblasts, TLR2-L and TLR4-L induced osteoclast formation and PGE2 production, and adding indomethacin or NS-398, an inhibitor of COX-2, completely suppressed the TLR-induced osteoclast formation. To examine the role of TLR signal in inflammatory bone loss associated with periodontal disease, we isolated alveolar bone from mouse mandible and cultured in vitro, and found that the TLR signal stimulated bone-resorbing activity measured by medium calcium and PGE2 secretion from alveolar bone. In vivo, the injection of TLR2-L or TLR4-L into the left side of alveolar gingiva reduced bone mineral density of the left alveolar bone in mice compared with that of the right side of alveolar bone injected with PBS. Next, we examined the effects of simultaneous treatment with TLR2-L and TLR4-L in vitro and in vivo. Simultaneous addition of low doses of TLR2-L and TLR4-L, which showed no individual effect in the dose, clearly induced osteoclast formation and PGE production in vitro and elicited marked loss of alveolar bone of mandible in vivo. Osteoblasts express both TLR2 and TLR4 on the surface, and the expression level of TLR4 was higher than that of TLR2. Treatment with TLR4-L and TLR2-L enhanced the expression of TLR2 in osteoblasts, suggesting the amplification pathway of TLR signal in osteoblasts. These results suggest that both TLR2 and TLR4 signals synergistically induce PGE-mediated bone resorption associated with inflammation, which may be involved in the pathogenesis of periodontal disease.

P253 W

ROLE OF PGE_2-RECEPTOR-MEDIATED SIGNALS IN OSTEOCLAST DIFFERENTIATION AND FUNCTION

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It has been believed that PGE_2 induces osteoclast formation and bone resorption mainly through the expression of RANKL in osteoblasts mediated by its receptors (EP2/EP4). Recently, it was shown that PGE_2 stimulates bone resorption through a direct action on osteoclast precursors, with a synergistic effect on the ability of RANKL to induce osteoclastic differentiation. We studied the precise mechanism of PGE_2 action on osteoclastic bone resorption. Using an RT-PCR technique, we first investigated the expression of PGE_2 receptor subtypes (EP1, EP2, EP3, EP4) in mature osteoclasts and osteoclast precursors such as bone marrow macrophages (BMMphi) and RAW 264.7 cells. Both BMMphi and RAW 264.7 cells expressed EP1, EP2 and EP4 mRNAs, while mature osteoclasts expressed only EP1 mRNA. PGE_2 at 10^{-6}M significantly enhanced TRAP(+) osteoclast formation induced by RANKL in cultures of RAW 264.7 cells. The synergistic action was mimicked by db-cAMP (100 microM) and was completely inhibited by H-89 (20 microM), a specific PKA inhibitor. These results suggest that the synergistic effect of PGE_2 is mediated by the cAMP-PKA system in osteoclast precursors. RANKL (50 ng/ml) induced both degradation of IkappaB and phosphorylation of p38MAPK, Erk and JNK in RAW 264.7 cells. PGE_2 at 10^{-6}M markedly enhanced RANKL-induced degradation of I kappa B and phosphorylation of p38MAPK in RAW 264.7 cells. The phosphorylation of Erk and JNK was less affected by PGE_2. These results suggest that PKA-mediated signals cross-talk with NF-kappa B- and p38 MAPK-mediated ones in osteoclast precursors. We finally studied whether functional PGE_2 receptors are present on osteoclasts. Calcitonin (eel, 10^{-9}M) significantly increased cAMP production, but PGE_2 failed to increase it in mature osteoclasts. Calcitonin (10^{-9}M) as well as RANKL (100 ng/ml) significantly promoted the survival of osteoclasts. $PGE(_2 10^{-6}$M), EP1 agonist (ONO-DI-004, 10^{-6}M) and EP4 agonist (ONO-AE1-329, 10^{-6}M) failed to support the survival of osteoclasts. Thus, PGE_2 did not mimic the effect of calcitonin on mature osteoclasts. These results confirmed that mouse mature osteoclasts have no functional EP2/EP4 receptors. It is also suggested that although osteoclasts express EP1 mRNA, EP1-mediated signals do not bear the key role on osteoclast survival.

P254 F

CONTRIBUTION OF PMA-ACTIVATED VOLTAGE-GATED H^+CHANNELS TO REGULATION OF INTRACELLULAR PH AND THE MEMBRANE POTENTIAL IN MURINE OSTEOCLASTS

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Diverse pH regulating mechanisms are involved in regulation of osteoclast functions. The voltage-gated proton (H^+) channel is a H^+ conductive pathway and characterized by extremely-high H^+ extrusion rate, ~100 times as strong as H^+ pumps and Na^+/H^+ exchangers. The H^+ channel is thought to be activated when cells are acidified and depolarized, but there is little knowledge on how and when the channel is activated in osteoclasts. As phorbol ester activates H^+ channels in various cell-types, we investigated the channel activity in osteoclasts stimulated with phorbol 12-myristate 13-acetate (PMA). The H^+ currents were recorded from murine osteoclasts generated from mononuclear precursors in the presence of M-CSF and sRANKL, using the permeabilized patch clamp technique. PMA (10-1000 nM) increased the H^+ current amplitude and the activation rate on depolarization in dose-dependent manner. PMA also shifted the voltage dependency for activation to more negative voltages. At -60 mV, PMA often induced inward currents that were reverted by diphenylene iodinium (DPI), an inhibitor for NADPH oxidases, suggesting that PMA activated the oxidase in osteoclasts. However, the PMA-induced potentiation of the H^+ current remained in the presence of DPI, implying that NADPH oxidases were not essential for the channel activation. In clamped cells, PMA shifted the reversal potential for the H^+ channel to more negative potentials, suggesting that intracellular acidification occurred. PMA-induced cell acidosis was confirmed in intact cells using BCECF. DPI had no effects on the cell acidosis, so that PMA generated cell acidosis without actions of NADPH oxidases. Although cell acidosis itself could enhance the H^+ currents, the PMA-induced potentiation was observed even at potentials of the same driving force for H^+. Therefore multiple factors, one of which may be cell acidosis, may be involved in activation of the H^+ channel by PMA. In the current-clamp mode, the membrane potential was hyperpolarized together with negative shifts of the reversal potential. These data suggest that the H^+ channel may contribute to regulation of both pH and the membrane potential in osteoclasts when protein kinase C is activated.

P255 S

ASCORBIC ACID DIRECTLY INFLUENCES OSTEOCLASTOGENESIS FROM ES CELLS

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Ascorbic acid is known to influence the osteoblast development, however, few studies are reported about the effect on the osteoclast lineage. To investigate the effect of ascorbic acid on whole process of osteoblastogenesis and osteoclastogenesis, we developed a new culture system. In this culture, only undifferentiated embryonic stem (ES) cells are sowed on cell culture dishes, and cultured in ascorbic acid-free modified alpha-MEM with or without ascorbic acid. On 8 days of culturing, $1,25(OH)_2D_3$ and dexamethasone are added, and cultured for further 6 days. Osteoclast lineage cells are detected as TRAP-positive cells.

In the presence of ascorbic acid during initial 8 days, the number of TRAP-positive cells is increased compared without ascorbic acid. To assess whether ascorbic acid affects the gene expression of the osteoblast lineage, a semi-quantitative RT-PCR for genes of Cbfa1 and Collagen type-I, is performed. However, no significant difference of their gene expression on day 8 is observed between presence and absence of ascorbic acid in cultures.

To examine the effect of ascorbic acid directed to the osteoclast lineage, M-CSF and RANKL are added to last 6 days, instead of $1,25(OH)_2D_3$ and dexamethasone. The number of TRAP-positive cells is increased by addition of ascorbic acid. Increase of osteoclast precursors is also observed by using a limiting dilution assay. Moreover, results from the colony-formation elicited by hematopoietic growth factors showed that the colony-forming cells harvested on day 8 of cultures are also increased in addition of ascorbic acid. Although one of functions of ascorbic acid is known to be a reducing activity, these effects can not be replaced by other reducing agents. These results suggest that ascorbic acid directly influences the development of osteoclast lineages.

P256 W

INTERLEUKIN-12 INHIBITS RANKL-INDUCED OSTEOCLAST FORMATION IN MOUSE BONE MARROW CELLS BY T CELL AND IFN-GAMMA-INDEPENDENT MECHANISM

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IL-12 was shown to have the potential to inhibit osteoclast formation in mouse bone marrow cells treated with macrophage colony-stimulating factor (M-CSF) and receptor activator of NF-kappaB ligand (RANKL). When bone marrow macrophages (BMM) were used as osteoclast precursors, IL-12 failed to inhibit M-CSF and RANKL-induced osteoclast formation from BMM. Supernatants of IL-12-stimulated whole bone marrow cells inhibited osteclast formation from BMM. These results strongly suggested that IL-12 indirectly affected M-CSF and RANKL-induced osteoclastogenesis by a humoral factor(s) from IL-12-stimulated whole bone marrow cells. The inhibitory effect of IL-12 on osteoclast formation was also seen in the T cell-depleted bone marrow cells of normal mice and the whole bone marrow cells of athymic nude mice. In coculture experiments using transwells, IL-12 failed to inhibit osteoclast formation from BMM cocultured with T cells, whereas IL-12 did inhibit osteoclast formation from BMM cocultured with whole bone marrow cells. These results strongly suggested that T cells might not be involved in the inhibitory effect of IL-12 on osteoclast formation from bone marrow cells. Experiments with anti-interferon (IFN)-gamma antibody and bone marrow cells from IFN-gamma receptor knockout mice revealed that IFN-gamma might not be the putative humoral factor causing inhibition of osteoclast formation in this system. The inhibitory effect of IL-12 on M-CSF and RANKL-induced osteoclastogenesis might not be caused by death of the adherent cells of bone marrow cells because of no viability loss of bone marrow cells treated with M-CSF, RANKL and IL-12 and no expression of Fas on the adherent cells, in contrast with our previous finding that the inhibitory effect of IL-12 on M-CSF and TNF-alpha-induced osteoclastogenesis is attributable to Fas and FasL-mediated apoptosis. TNF-alpha and IL-12 are produced in response to infection by microorganisms. IL-12 may regulate pathological TNF-alpha-induced osteoclastogenesis in an irreversible way, apoptosis of the adherent cells, while IL-12 may regulate physiological RANKL-induced osteoclastogenesis in a reversible way without cell death.

P257 F

EXPRESSION AND CHARACTERIZATION OF A NOVEL V-ATPASE ACCESSORY SUBUNIT ATP6S1 IN OSTEOCLASTS

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Osteoclasts are multinucleated bone resorbing cells, that derived from haematopoietic mononuclear cells. Osteoclasts resorb bone by secreting protons into an extracellular resorption zone through vacuolar-type proton pumps (V-ATPase) located in the ruffled border. Using PCR-selective substraction hybridization of cDNAs from osteoclasts and their precursor cells, we have identified a cDNA fragment encoding the mouse vacuolar H+-ATPase accessory subunit, ATP6S1, from osteoclasts. Moreover, RT-PCR analysis showed that ATP6S1 transcripts were expressed highly in mouse osteoclast and brain, and at various level in other tissues including heart, kidney, muscle, spleen, liver and lung. The expression of ATP6S1 was slightly up-regulated in osteoclast compared with in osteoclast precursor cells. Both ATP6S1-EYFP and ATP6S1 mutant EYFP were generated and expression in COS-7 cells. Confocal microscopy showed that both proteins were similarly localized to perinuclear region and vacuolar structure in the cytoplasm. And ATP6S1-EYFP or ATP6S1 mutant EYFP colocalize with V-ATPase subunit c-Flag fusion protein. Using immunohistochimestry with anti-ATP6S1 antibody, ATP6S1 was partially colocalized with pH- dependent lysotracker and recycling endosomal marker tranferin in mouse osteoclasts. In addition, expression of antisense ATP6S1 affected the distribution of lysotracker and bone resorption. Furthermore, over-expression ATP6S1-EYFP increased the uptake of transferrin in RAW264.7 cells. In all, these results indicate that ATP6Si is associated with endosomal/lysosomal pathways and implicate a role for ATP6S1 in osteoclast acidifaction and endocytosis.

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P258 S

SUBCELLULAR LOCALIZATION OF THE RECEPTOR ACTIVATOR OF NF-KAPPAB AND SRC KINASE IN DETERGENT INSOLUBLE FRACTIONS OF OSTEOCLASTS

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To investigate the molecular events involved in osteoclastogenesis induced by macrophage-colony stimulating factor (M-CSF) and the receptor activator of NF-kappa B ligand (RANKL), we analyzed gene expression using cDNA microarray hybridization. Significant inductions of 41 clones including caveolin were observed. Caveolin, primarily identified as a multifunctional lipid raft associated protein, is a component of the caveolae architecture and substrate of Src kinase. Previous studies show that many signaling molecules localize in microdomains of the plasma membrane, lipid rafts, similar to membranes derived from caveolae. A number of subsequent studies indicate that glycosphingolipid and cholesterol enriched membrane fraction present in the plasma membrane are clustered with receptors and signal transducing molecules to form lipid rafts and can be recovered as low density, detergent insoluble membrane (DIM) fractions when cells are homogenized in 1% Triton X-100 followed by sucrose density gradient centrifugation. Our recent study provides evidence that D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (D-PDMP), a glucosylceramide synthase inhibitor, inhibits the osteoclast formation induced by M-CSF and RANKL in a dose-dependent manner. This data suggest that glycosphingolipid is necessary for osteoclastogenesis and may suggest that DIM fractions are present in the plasma membrane, and these fractions may control the segregation of receptors. In this study, we confirmed the expression of caveolin in osteoclasts using RT-PCR. Moreover, DIM fractions from osteoclasts were prepared by sucrose density gradient centrifugation. The caveolin-1 was enriched in low-density DIM fraction. We further investigated subcellular localization of receptor activator of NF-kappa B (RANK), c-fms (receptor for M-CSF), alpha-v integrin and Src kinase. Neither c-fms or alpha-v integrin were localized in DIM fractions, however RANK and Src kinase localize in DIM fractions, suggesting that structural and functional microdomains exist in plasma membrane and regulate the localization of RANK related signaling molecules and control the signal trasduction for osteoclastogenesis. Although the function of caveolin is still unclear, it may participate intracellular transport and assembly of signaling complexes and signal transductions in osteoclasts.

P259 W

EXPRESSION OF RECEPTOR ACTIVATOR OF NF-KAPPAB LIGAND, OSTEOPROTEGERIN AND INTERLEUKIN-1 BETA IN MECHANICAL FORCE-INDUCED BONE RESORPTION

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INTRODUCTION: The balance between receptor activator of NF-kappaB ligand (RANKL) and osteoprotegerin (OPG) is an important factor to osteoclastogenesis in inflammatory condition. RANKL accelerates osteoclast formation and OPG inhibits it and the production of RANKL and OPG is regulated by proinflammatory cytokines. However, the functions of RANKL, OPG and proinflammatory cytokines are not clear in mechanical force-induced osteoclastogenesis. The purpose of this study was to investigate the involvement of RANKL, OPG and interleukin (IL)-1 beta in occlusal traumatic bone resorption.

METHODS: Setting metal inlay on rat molar induced excessive occlusal force. Histopathological changes were observed in interradicular septa of the molar on 12 hours, 1, 2, 5 and 7days after setting metal inlay. Tartrate-resistant acid phosphatase (TRAP) positive multinucleated cells were counted in the unit area of bone surface. The expression of RANKL, OPG, IL-1 beta and hypoxia inducible factor (HIF)-1 alpha were immunohistologically determined. The numbers of RANKL positive cells and OPG positive cells were calculated in vascular endothelial cells (VECs), fibroblastic cells and osteoblasts.

RESULTS: No inflammation was observed through the experimental period. The number of osteoclasts significantly increased from the day 2 to the day 7. The ratio of RANKL positive cells to OPG positive cells (RANKL/OPG ratio) significantly elevated in VECs from the 12 hour to the day 5 and in fibroblastic cells on the day 5 and day 7. On the other hands, RANKL/OPG ratio in osteoblasts did not significantly change during this experiment. The number of IL-1beta positive cells increased from the 12 hour to the day 7. Moreover, positive reaction of HIF-1 alpha was detected in interradicular septa on the 12 hour.

DISCUSSION: These results suggested that the balance between RANKL and OPG produced in VECs and fibroblastic cells would have an important role of osteoclastogenesis on mechanical force-induced bone resorption. Considering that IL-1 beta positive cells increased when high RANKL/OPG ratio was observed, it is likely that IL-1 beta involves in the regulation of RANKL and OPG. The expression of HIF-1 alpha on the 12 hours suggested that IL-1 beta production was induced by hypoxia.

P260 F

LOWER OSTEOCLAST DIFFERENTIATION AT HIGHER RATIO OF CELL NUMBER TO CULTURE MEDIA VOLUME INDICATES THAT OSTEOCLAST PRECURSOR CELL SECRETE AUTOCRINE FACTORS INHIBITING ITS DIFFERENTIATION

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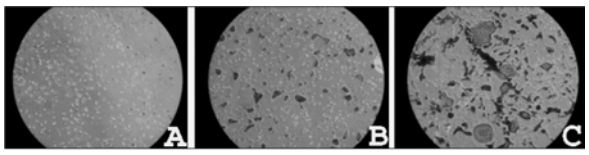
While osteoclast differentiate from hematopoietic bone marrow cells under influence of NF-kappaB ligand (RANKL) along with macrophage colony stimulating factor (M-CSF), other cytokines are also known to affect osteoclast differentiation. In the present study, we examined the effects of ratio of cell number to culture media volume on differentiation of osteoclasts to know if there is(are) autocrine factor(s) secreted from osteoclast precursor cells influencing osteoclast differentiation. 5 x 104 mouse bone marrow cells were cultured in 96 wells for 1 day in 200 microliter alpha-MEM containing 5 ng/ml M-CSF and unattached cells were cultured for additional 3 days in 100, 200, 300 microliter alpha-MEM containing 30 ng/ml M-CSF respectively. Then attached cells were more cultured for 3 days in 100 (Fig. A), 200 (Fig. B), 300 (Fig. C) microliter alpha-MEM containing 30 ng/ml M-CSF and 70 ng/ml RANKL. In other experiment, 5 x 10^3, 5 x 10^4, 5 x 10^5 cells were cultured in 200 microliter alpha-MEM in a same way described above. To examine the effect of autocrine factor(s) from pure osteoclast precursor cells, 5 x 10^4 cells that were induced to attach to the surface for 3 days in the same volume of culture media (200 microliter) containing 30 ng/ml M-CSF were further cultured in 100, 200, 300 microlter of alpha-MEM containing 30 ng/ml M-CSF and 70 ng/ml RANKL for 3 days. Higher ratio cell number to volume of culture media was, lower osteoclasts differentiated. Differentiation of osteoclast was totally inhibited when highest ratio of cell number to media volume, while number of cells decreased by apoptosis. These results strongly suggest that osteoclast precursors secrete factor(s) to inhibit differentiation of osteoclast. In addition, these results indicate that standardization of ratio of cell number to media volume is crucial in studying of osteoclast differentiation.



P261 S

AN IMMUNOHISTOLOGICAL STUDY ON EXPRESSION OF RECEPTOR ACTIVATOR OF NF-KAPPA B LIGAND IN LIPOPOLYSACCHARIDE-INDUCED BONE RESORPTION

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INTRODUCTION: The receptor activator of NF-kappa B ligand (RANKL) is the essential signal required for osteoclast development, activation, and survival. Moreover, RANKL is known to play a key role in bacterial lipopolysaccharide (LPS)-induced bone resorption. However, it is unclear whether RANKL expression changes in conjunction with inflammatory condition. In the present study, we observed the immuno-histological changes of the type of RANKL-expressing cells and their number with appearance of osteoclasts in inflammatory bone resorption.

METHODS: LPS were repeatedly injected with 8 times every 48 hours into mice gingiva (the base-line group). After inducing bone resorption, LPS or PBS was additionally injected (the LPS- or PBS-injected group). The serial sections from each group were stained with haematoxylin and eosin. Tartrate-resistant acid phosphatase (TRAP) staining was employed to observe osteoclasts and immunohistological staining was performed to detect RANKL expressing cells. The numbers of TRAP positive multinucleated cells and RANKL expressing cells were counted in the unit area.

RESULTS: Some TRAP positive multinucleated cells were observed within the resorption lacunae on the surface of the alveolar bone. RANKL expression was detected in vascular endothelial cells, fibroblastic cells and osteoblasts in the base-line group. The total number of TRAP positive multinucleated cells and RANKL positive cells increased significantly more in the LPS-injected group than in the base-line group. RANKL expression was mainly detected in vascular endothelial cells and fibroblastic cells, but not in osteoblasts. On the other hand, few TRAP positive multinucleated cells existed in the PBS-injected group. RANKL expression was mainly detected in osteoblasts within the resorption lacunae, although the total number of RANKL positive cells was similar to that in the base-line group.

DISCUSSION: In the LPS-injected group, the numbers of RANKL expressing vascular endothelial cells and fibroblastic cells were elevated with the increasing number of osteoclasts. On the other hand, RANKL expressing osteoblasts increased with the disappearance of osteoclasts. These results suggested that the type of RANKL-expressing cells related to the appearance of osteoclasts in the LPS-induced bone resorption.

P262 W

PD-1 DEFICIENT MICE SHOWED THE MILD OSTEOPETROTIC PHENOTYPE BUT DECREASED THE BONE VOLUME BY ESTROGEN DEFICIENCY

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The decreased bone volume and the increased number of osteoclasts in tibia and femur of the cytotoxic T lymphocyte associated antigen 4 (CTLA-4) deficient mice were shown (Nature 402: 304-309, 2000). CTLA-4 is a negative regulator of T cell activation, and actually the defect of this molecule induces T cell activation. On the other hand, Programmed Death-1 (PD-1) molecule is structurally similar to CTLA-4 working as a negative regulator of T cell activation similarly. We have already reported the mild osteopetrotic phenotype in PD-1 deficient mice, which is quite different from the CTLA-4 deficient mice (JSBMR,2002). Histomorphometric analysis revealed the decreased osteoclast number and no changes in bone formation parameters. These results raised the question whether the osteoclast recruitment in PD-1 deficient mice is also retarded under the condition that bone resorption is promoted. To address this question, we performed the ovariectomy to promote the osteoclastic activity. 9 week-old PD-1 deficient mice and the age-matched controls were ovariectomized (OVX) or sham-operated, and were sacrified 5 weeks after the operation. Then the tibiae were dissected and bone mineral density (BMD) of tibiae were measured by Dual X-ray absorptiometry (DXA). The BMD (mg/cm^2) in OVX group of the control mice was decreased compared to the sham group of the controls (24.08 ±1.26 vs 17.90 ±1.00; -25.7%). The BMD in the OVX group of the PD-1 deficient mice was decreased compared to the sham group of the knockout mice (26.60 ±1.15 vs 19.70 ±2.05; -25.9%). The percentage of the decreased BMD in PD-1 deficient mice was almost the same as in the controls. These results indicate that PD-1 molecule may not be involved in the promoted function of bone resorption induced by estrogen deficiency. PD-1 deficient mice showed the mild osteopetrotic phenotype with decreasing the number of osteoclasts but decreased the bone volume by estrogen deficiency in the same extent as in controls.

P263 F

CORRELATION BETWEEN BETA C-TERMINAL TELOPEPTIDES RELEASED FROM DENTINE WAFERS AND AREA OF RESORPTION BY SOLUBLE RANKL-GENERATED HUMAN OSTEOCLASTS

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Point counting by reflected light microscopy has been used for many years to determine the area of resorption lacunae excavated by osteoclasts (OCs) cultured on calcified substrates *in vitro*. However, this method to identify and quantitate the activity of OCs is time consuming and can be inaccurate. The aim of this study was to evaluate a simple technique to monitor the activity of OCs on dentine wafers generated by human recombinant receptor activator for nuclear factor-$_K$B ligand (RANKL). Precursors of OCs (preOCs), present in the monocyte fraction of whole blood, were harvested from peripheral blood collected from subjects with Paget's disease of bone (n=4). PreOCs were cultured in medium containing either 10% FCS or 1% or 10% human serum derived from the same individual in the presence of 30 nano-grams per millilitre RANKL and 25 nano-grams per millilitre macrophage-colony-stimulating factor (M-CSF). Cell culture medium was harvested at specific time points (7, 14, and 21 days) during a 3-week incubation period for measurement of beta c-terminal telopeptides (beta-CTx) by electrochemiluminescence immunoassay (ROCHE, Lewes UK). End-point area of resorption was evaluated by point counting using reflected light microscopy. Incubation of Pagetic preOCs with 10% Pagetic serum or 10% FCS resulted in a significantly higher release of beta-CTx and a greater area of resorption than from cells cultured in 1% Pagetic serum, and beta-CTx was significantly correlated with the area of resorption (r=0.92, n=62). It is suggested that determination of beta-CTx release from dentine wafers is a more convenient method than point counting by reflected light microscopy for monitoring the resorptive activity of mature OCs.

P264 S

INTERLEUKIN-18 INDUCES APOPTOSIS OF BONE MARROW CELLS IN TUMOR NECROSIS FACTOR-ALPHA MEDIATED OSTEOCLASTOGENESIS

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In recent years, much importance has been attached to the relationship between cytokines and bone metabolism. Although RANKL is an essential cytokine for inducing the differentiation of osteoclasts, it has recently been found that the differentiation of osteoclasts is also induced by TNF-alpha. We previously reported that IL-12 induced apoptosis in bone marrow cells treated with TNF-alpha resulting in inhibition of osteoclast formation. Interleukin-18 is also an important cytokine such as IL-12 in cellular immunity. However, few studies have clarified the relationship between bone metabolism and IL-18. In this study, we investigated the effects of IL-18 on TNF-alpha-mediated osteoclastgenesis. Whole bone marrow cells collected from ddY mice were cultured in the medium containing M-CSF and TNF-alpha with or without IL-18. When bone marrow cells were cultured in the medium without IL-18, a number of osteoclast-like cells were formed. On the other hand, when they were cultured with IL-18, the number of adherent cells decreased in a dose-dependent manner. We found morphological changes such as cell atrophy, and nuclear and cellular fragmentation, and biological changes such as DNA fragmentation in the adherent cells treated with IL-18, indicating apoptosis of these cells. Direct effects of IL-18 on TNF-alpha-inducing osteoclastgenesis were determined using M-CSF-dependent bone marrow macrophage as an osteoclasts precursor (preOC). Apoptotic effects such as cell atrophy were not observed in M-CSF-dependent bone marrow macrophage, suggesting that apoptosis would not be induced by a direct effect of IL-18 on preOC, but would be induced by a factor formed by IL-18-treated non-preOC cells in whole bone marrow cells. In this study, IL-18 was found to induce apoptosis of the adherent cells in TNF-alpha-mediated osteoclast formation of mouse bone marrow cells, suggesting that IL-18 can inhibit osteoclast formation that is related to pathological bone resorption induced by TNF-alpha.

P265 W

17-ALLYLAMINOGELDANAMYCIN (17AAG) STIMULATES OSTEOCLAST FORMATION IN VITRO AND INCREASES BONE METASTASIS IN A NUDE MOUSE INTRACARDIAC TUMOUR CELL INJECTION MODEL

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17AAG is a geldanamycin derived Hsp90 blocker that reduces tumour growth and soft tissue metastasis, and is currently undergoing phase I clinical trials. However, 17AAG effects on bone metastasis have not been investigated. We studied this with a nude mouse model employing cardiac injection of MDA-MB-231 Tx-P cells (derived from MDA-MB-231 cells by serial passage through nude mice) that results in bone metastases. In mice treated with 17AAG (70mg/kg/day) the size and incidence of osteolytic bone metastases observed by Faxitron x-ray analysis were greatly increased. Similar 17AAG treatment of mice with no tumour challenge did not detectably affect their femoral osteoclast/osteoblast numbers or microarchitecture.

To investigate whether 17AAG increases tumour invasion and destruction of bone by stimulating osteoclast formation we studied its effects on in vitro osteoclast differentiation. In co-cultures of calvarial osteoblasts and bone marrow cells (stimulated by dihydroxyvitamin D3 and prostaglandin E2) osteoclast formation was increased approximately 2.4-fold further by 30nM 17AAG. Similarly, osteoclast formation in bone marrow cells and bone marrow derived macrophages stimulated by receptor activator of NF kappaB ligand (RANKL; 100ng/ml) and macrophage colony stimulating factor (20ng/ml) were also dose dependently increased by 17AAG beyond the maximal RANKL stimulation. Osteoclast formation in RAW264.7 cells stimulated by RANKL (50ng/ml) was also dose-dependently increased by 17AAG treatment, with 300nM 17AAG causing a greater than 4-fold increase in osteoclast numbers. The action of geldanamycin in these assays was unclear due to its high cytotoxicity above 10nM.

Our data suggest that 17AAG powerfully stimulates osteoclast formation by direct action on osteoclast progenitors, and this may underlie the increased bone destruction and metastasis in our in vivo model. However, 17AAG may not affect normal bone turnover, which is tightly regulated. These results emphasise differences between tumour growth in soft tissues and bone. In soft tissues, 17AAG reduces tumour growth by its action on tumour cells. In bone, however, tumour growth depends on osteolysis caused by osteoclasts recruited by the tumour, a process stimulated by 17AAG that may thereby enable greater tumour invasion.

P266 F

INTERFERON-GAMMA (IFN-G) PRODUCING HUMAN T CELLS DIRECTLY INDUCE OSTEOCLASTOGENESIS FROM HUMAN MONOCYTES VIA THE EXPRESSION OF RANKL: POSSIBLE ROLE OF TH1 CELLS IN BONE RESORPTION

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We previously demonstrated that activated human T cells expressing the receptor activator of NF-kB (RANKL) induce osteoclastogenesis from human monocytes. This finding suggested that excess production of RANKL by activated T cells increases the level of soluble RANKL (sRANKL) in synovial fluid and may contribute to osteoclastic bone resorption in patients with rheumatoid arthritis (RA). It was, however, reported that interferon-g (IFN-g) inhibits osteoclastogenesis by degrading the RANK adapter protein, tumor necrosis factor receptor-associated factor 6 (TRAF6). This study explored our hypothesis that IFN-g producing T cells inhibit osteoclast formation.

Activated T cells derived from human peripheral blood were divided into IFN-g producing T cells [IFN-g(+) T cells] and IFN-g non-producing T cells [IFN-g(-) T cells] by a magnetic cell sorting method. These T cells were cultured with human peripheral blood monocytes in the presence of M-CSF alone or M-CSF and sRANKL. Surprisingly, IFN-g(+) T cells, but not IFN-g (-) T cells, induced osteoclastogenesis in the presence of M-CSF alone. Additionally, IFN-g(-) T cells, but not IFN-g(+) T cells, inhibited the osteoclastogenesis induced by M-CSF and sRANKL. The expression of mRNA RANKL and the levels of both soluble and membrane type RANKL were elevated in IFN-g(+) T cells. IFN-g(+) T cells and IFN-g(-) T cells included T cells identified as TH1 cells and TH2 cells, respectively, by flow cytometry using antibodies against intracellular IFN-g and IL-4. IFN-g (1 ng/ml) completely blocked the osteoclastogenesis from monocytes by sRANKL. In addition, IL-4 also dose-dependently inhibited the osteoclastogenesis by sRANKL.

Contrary to our hypothesis, these findings demonstrated that IFN-g(+) T cells induce osteoclastogenesis through the expression of RANKL and that IFN-g(-) T cells inhibit osteoclastogenesis, at least in part, through the expression of IL-4. In addition, the present findings suggest that TH1 cells play a direct role in bone resorption in TH1 dominant diseases such as RA.

P267 S

INHIBITION OF RANKL INDUCTION AS A STRATEGY FOR PALLIATIVE TREATMENT OF OSTEOLYTIC COMPLICATIONS OF CANCER, INFLAMMATORY AND OTHER DISEASES

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The strength and integrity of bones depend on maintaining a delicate balance between bone resorption by osteoclasts and bone formation by osteoblasts. Pre-osteocalsts arise from stem cells of the monocyte/macrophage lineage, requiring macrophage colony stimulating factor for their proliferation. Further maturation, survival and acquisition of the ability to resorb bone by pre-osteoclast depends on the expression of the specific receptor RANK and its engagement by its ligand RANKL. Inhibition of RANK-RANKL mediated pathways, should have important clinical application, in malignant diseases that involve bone destruction (multiple myeloma, osteosarcoma, bone metastases of cancer) and in benign bone diseases accompanied by osteolysis (rheumatoid arthritis, periodontal disease) and osteopenia (osteoporosis).

We propose two approaches for independent identification of small molecules that might down regulate RANKL expression and activity.

The first approach is aimed to the discovery of specific target genes for inhibition of RANKL expression using QBI's proprietary BiFAR technological platform. For this, we have successfully established a functional selection system based on direct measurement of membrane RANKL expression on mouse pre-B cells (alternative selection systems based on T-cells or stroma cells also exist). Introduction of Genetic Inhibitory Elements (GIE) library prepared from the same cell source will result in random functional inhibition of one gene per cell. In a subset of the cells the GIEs activity is expected to interfere with induction of membrane RANKL expression in response to certain stimulatory treatments by virtue of inhibition of the corresponding endogenous genes important in the pathway. Such cells with reduced membrane RANKL expression may be easily isolated through the developed technique leading to further rescue of active GIEs and identification of important functional genes.

The second approach is based on the direct screening of small molecules in a cell-based bioassay. Monitoring the changes of RANKL expression on the plasma membrane following specific cell treatments is used for the identification of small molecules that inhibit the RANKL pathway.

P268 W

LIPID RAFT MICRODOMAINS PLAY A CRITICAL ROLE IN RANK SIGNALING

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Receptor activator of nuclear factor kappa B (RANK) is an important integral membrane receptor molecule that governs multiple aspects of osteoclast regulation, which include differentiation, fusion, resorption function, and survival. Lipid rafts play a key role in immune cell activation by recruiting and excluding specific signaling components of immune cell surface receptors upon the receptor engagement. Despite this, the role of these microdomains in the regulation of osteoclasts by receptor activator of nuclear factor kappa B (RANK) has not established. In this study, we demonstrate that the raft microdomain expression plays an essential role in RANK signaling in osteoclasts. Expression of raft component flotillin greatly increased during osteoclast differentiation. Engagement of RANK induced the translocation of TNF receptor-associated factor (TRAF) 6 to rafts where Src was constitutively resident. Disruption of rafts blocked the raft-association of TRAF6 induced by RANKL stimulation. Furthermore, the Akt activation by RANK ligand was suppressed by treatment with raft-disrupting agents. However, RANKL-induced activation of NF-kappaB and MAPKs was not inhibited. Our observations demonstrate for the first time that the expression and integrity of lipid raft microdomains are important for RANK-mediated signaling in osteoclasts.

P269 F

DISTINCT OSTEOCLAST PRECURSORS IN BONE MARROW AND EXTRAMEDULLARY ORGANS

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Osteoclasts are always observed to be tightly attached to bone matrix, whereas, the cells that have the potential to differentiate into osteoclasts in cultures are widely distributed through whole bodies. Specific microenvironments only in the bone tissues may support osteoclast development. However, these bone specific molecules are not obvious, because M-CSF and RANKL are the essential molecules for osteoclastogenesis produced by osteoblasts, but they are also produced out of bone. In contrast, few studies were reported the difference of osteoclast precursors in each tissue.

Indicating effects of toll-like receptor (TLR) ligands and TNF-alpha on osteoclastogenesis, we assessed osteoclast precursors. In the presence of recombinant human M-CSF and soluble RANKL, unmethylated CpG oligonucleotides (CpG: TLR9 ligand) inhibited the osteoclastogenesis from the precursors in all of tissues tested. Lipopolysaccharide (LPS: TLR4 ligand) and peptidoglycan (PGN: TLR2 ligand) inhibited osteoclast development from adult spleen and peritoneal cells, but not from adult bone marrow, fetal liver, or newborn spleen cells.

In the presence of M-CSF, TNF-alpha induced osteoclasts from adult bone marrow and spleen cells as RANKL. In contrast, TNF-alpha functioned as an inhibitor for peritoneal cells. Bone marrow cells pre-cultured with M-CSF became sensitive to LPS, and were inhibited osteoclastogenesis by LPS. Their response to TNF-alpha also decreased. Osteoclastogenesis of cloned C7-TY cells was sensitive to TLR-ligands, and did not require M-CSF.

Based on these results, we classify osteoclast precursors. (1) Precursors in freshly prepared bone marrow cells are sensitive to CpG, but resistant to LPS and PGN. (2) Those in adult spleen are LPS-sensitive and are induced osteoclastogenesis by TNF-alpha as well as RANKL. (3) Bone marrow cells precultured with M-CSF become LPS-sensitive, and respond to TNF-alpha less efficiently than RANKL. (4) TNF-alpha functions to peritoneal cells as an inhibitor for M-CSF and RANKL-induced osteoclast development. (5) The osteoclastogenesis is induced only RANKL without M-CSF as C7-TY cells. Therefore, osteoclast precursors maintained in the bone marrow and extramedullary tissues may be different in responsiveness to TLR ligands and TNF-alpha.

This is a collaborative work with Drs. Sachiko Akashi, Kensuke Miyake (Tokyo Univ.), Leonard Shultz (Jackson Lab.), and Masayuki Takahashi (Otsuka Pharm.).

P270 S

ISOLATION AND CHARACTERIZATION OF OSTEOPROTEGERIN GENES IN THE TELEOST MEDAKA (ORYZIAS LATIPES)

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In mammals, osteoprotegerin (OPG) encodes a secreted protein of 401 amino acids belonging to the tumor-necrosis-factor receptor (TNFr) superfamily and is involved in the regulation of osteoclast formation. It binds to the receptor-activator-of-NFkappaB ligand (RANKL) present on osteoblast/stromal cells and thereby prevents RANKL interaction with its receptor RANK on osteoclasts and their precursor cells. By this mechanism, OPG inhibits the maturation and activation of multinucleated osteoclasts as well as their survival and therefore antagonizes bone resorption. We want to use the advantages of the small teleost fish medaka as a genetic model to analyze osteoblast and osteoclast formation at the molecular level. We identified two related, but significantly different opg cDNA sequences in the medaka. Our phylogenetic analysis showed that both genes are true opg orthologs and are therefore named opg1 and opg2. Both sequences contain open reading frames for putative proteins that are significantly shorter than the human OPG (OPG1: 197 amino acids, OPG2: 292). In comparison to human OPG, medaka OPG1 shows 42% amino acid identity (58% similarity). For OPG2, the numbers are even higher (45% identity; 62% similarity). When the human and medaka OPG sequences were aligned, we found a high degree of sequence conservation in the N-terminus of the protein (the so-called cystein-rich domain), which is responsible for the osteoclast-inhibiting activity in mammals. Interestingly, the homology was low (OPG2) or absent (OPG1) in the C-terminal part that contains the death-domain in mammals. RT-PCR analysis showed that both medaka opg genes are expressed during embryogenesis, in adult tissues and in primary osteoblast-like cell culture, albeit with different temporal and spatial patterns. While opg1 appears to be expressed ubiquitously during all stages analyzed, opg2 transcripts are detected at significant levels only starting from day 10 of development (hatching stage). Interestingly, this coincides with the onset of bone mineralization in medaka. We also isolated the promoter region of opg2 in order to produce transgenic GFP fish and identified two consensus binding sites for CBFA1, a key regulator of osteoblast formation. Taken together, this suggests that OPG activity and its transcriptional regulation might be conserved between mammals and teleost fish.

P271 W

PRELIMINARY CHARACTERIZATION OF OSTEOCLAST PRECURSORS IN HUMAN BONE MARROW CULTURES

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Studies employing murine bone marrow (BM) cultures have helped to characterize the phenotype of osteoclast precursors. Our studies were undertaken to define the optimal conditions for culturing osteoclasts from human BM and to characterize the phenotypic features of osteoclast precursors. Human BM was obtained from patients undergoing total hip replacement for end-stage osteoarthritis. The BM cells were diluted in alpha-MEM and mononuclear cells were separated by passage over Histopaque (Sigma). Hematopoietic precursors were isolated by adherence depletion followed by culture in semi-solid or liquid culture conditions in the presence of GM-CSF for 0, 1, 4, or 7 days. Cells were then cultured in the presence of soluble RANKL (sRANKL)(Research Diagnostics) and M-CSF (Research Diagnostics) with or without anti-osteoprotegerin (OPG) antibody (R & D Systems). TRAP-positive calcitonin receptor positive multinucleated osteoclast-like cell (OCL) formation peaked 6 or 7 days after addition of sRANKL and M-CSF. Bone resorbing activity (assessed by culture on dentine slices) was detected 10-14 days after treatment and was dependent on the length of GM-CSF exposure during the preincubation period. In 50% of the BM cultures, addition of anti-human OPG antibody increased the number of TRAP positive OCL, suggesting that BM-derived osteoclast differentiation inhibitory factors may be present in some cultures. To isolate osteoclast precursors, BM cells cultured in the presence of GM-CSF for 1 week were stained with fluorescence-conjugated antibodies (c-kit, c-fms, or CD11b) and sorted using a Fluorescent Activated Cell Sorter. The eight sorted fractions were cultured independently in the presence of M-CSF and sRANKL for 7-14 days. Only the cells in the fraction containing c-kit+, c-fms-, CD11b- cells differentiated into multinucleated OCL. The efficiency of TRAP-positive multinucleated cell formation was increased by culture at high cell density.

These results demonstrate the utility of human BM culture systems to study osteoclast differentiation and provide insight into the identity of the phenotype of human OCL precursors.

P272 F

MEMBRANE RAFTS ARE CRUCIAL FOR OSTEOCLAST FUNCTION

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Rafts are specialized membrane microdomains enriched in glycosphingolipids, cholesterol, and glycosylphosphatidylinositol-anchored proteins. The involvement of rafts has been implicated in many important cellular processes, which include generation and maintenance of cellular polarity, chemotactic migration, and cell surface receptor signaling. For T cell and B cell antigen receptors, raft domains function as signaling platforms where selective signaling molecules are recruited or segregated away. Despite this, the role of these microdomains in the regulation of osteoclasts by receptor activator of nuclear factor kappa B (RANK) has not established. In this study, we demonstrate that the raft microdomain expression plays an essential role in osteoclast function and differentiation. During osteoclast differentiation the expression of raft component flotillin greatly increased. More

importantly, the actin ring formation and bone resorption by osteoclasts were found to require the integrity of rafts. Also the survival of mature osteoclasts was suppressed by treatment with raft-disrupting agents, which was accompanied with increased apoptosis. Our observations demonstrate for the first time that osteoclast function is critically dependent on the expression and integrity of raft membrane microdomains.

P273 S

OSTEOCLAST MEDIATED BONE DESTRUCTION IN COLLAGEN-INDUCED ARTHRITIS IS REVEALED BY TARGETING THE RANKL-RANK INTERACTION WITH OSTEOPROTEGERIN

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Bone destruction is a characteristic feature of rheumatoid arthritis (RA) and constitutes a major cause of progressive disability. While matrix metalloproteinases are implicated in the cartilage erosion, bone loss involves osteoclasts, which are abundant at sites of joint destruction in RA and animal models such as collagen-induced arthritis (CIA). Several studies have shown that receptor activator of NFkappaB ligand (RANKL) and its cognate receptor; RANK is expressed on cells of the synovial membrane in RA. Osteoprotegerin (OPG), a decoy receptor for RANKL, impinges on this system and regulates osteoclast numbers and activity. We established that the infiltrating T-cells in RA synovium expressed RANKL mRNA and that activated peripheral blood T cells supported osteoclast formation in vitro. RANKL was expressed in CIA, where collections of TRAP+/calcitonin receptor (CTR)+ osteoclasts are located at sites of bone destruction. To elucidate the role of RANK signaling events in the effector phase of CIA, we investigated effects of Fc-OPG fusion protein (Fc-OPG) in CIA. After the induction of CIA in rats, test animals were treated with or without Fc-OPG (3mg/kg/day) S.C. for 5 days. Paraffin-embedded joints were analysed histologically and adjacent bone was assessed by histomorphometry. Osteoclasts were identified using TRAP, and expression of mRNA for OPG and RANKL was verified by in situ hybridization. Our results indicated that Fc-OPG effectively prevented joint destruction, even though it had no impact on synovitis. Fc-OPG depleted osteoclast numbers by at least 75% and diminished bone erosion scores by 60%. Although cartilage loss was reduced, the effects of OPG on cartilage were less striking than those on bone. In arthritic joints, OPG mRNA was expressed and co-localised with RANKL. Treatment with Fc-OPG did not affect the expression of endogenous RANKL or OPG mRNA. Others and we have now demonstrated that OPG has powerful anti-erosive effects in arthritis that are dissociated from effects on synovitis. These findings indicate that targeting osteoclasts or disrupting the RANK-signaling cascade, independently of inflammation, may prevent crippling joint destruction in RA.

P274 W

BIOMECHANICAL AND PHYSICAL PROPERTIES OF FEMUR FROM OVARIECTOMIZED RATS TREATED WITH FLUORIDE

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Ovariectomized rat had been used as an animal model of experimental osteoporosis testing many therapeutic agents to prevent bone mass loss. The aim of this study was to investigate the effect of fluoride treatment on the osteopenia induced by castration in sexually mature female rats. Adult female Wistar virgin rats with 4 months of age were randomly divided in the following groups: basal group; intact control, ovariectomized control and ovariectomized treated with fluorine. The animals of basal group were sacrified at the beginning of the experiment and the others groups after 2 and 4 months of treatment. The treatment consisted of tap water or 40 ppm of NaF in the drinking water.

The osteopenia induced by ovariectomy is indicated by physical parameters promoted lower biomechanical resistance in the 3 points bending test. The animals treated with NaF during 2 and 4 months presented better physical and biomechanical properties showing that fluorine inhibited the bone mass loss improving the bone quality .

Groups	**Max Force**	**Failure Force**	**Stiffness**	**Bone Density**	**Bone Mineral Density**	**Bone Fluorine Content**
	(N)	(N)	(10^3N/m)	(g/cm^3)	(g/cm^3)	(ppm)
basal	75.69(5.03)	81.35(5.76)	140.97(14.62)	1.72(0.04)	0.69(0.05)	540.13(38.52)
intact 2m	105.01(8.06)	89.03(8.37)	150.29(19.32)	1.79(0.05)	0.72(0.07)	681.34(55.16)
intact 4m	96.69(7.62)	93.39(9.16)	165.96(18.38)	1.77(0.07)	0.76(0.07)	703.20(82.18)
ovx 2m	76.68(5.74)	75.67(4.39)	141.66(12.73)	1.70(0.09)	0.62(0.08)	661.58(75.95)
ovx 4m	79.42(4.11)	75.58(5.22)	134.04(26.66)	1.71(0.07)	0.65(0.07)	711.72(97.68)
ovx+NaF 2m	118.28(6.54)	90.90(9.37)	204.19(29.81)	1.80(0.11)	0.78(0.11)	2082.76(272.23)
ovx+NaF 4m	111.68(10.90)	93.43(6.84)	230.83(24.14)	1.81(0.15)	0.82(0.12)	2191.02(364.93)
Mean(SD)- n= 9-10 Finantial support:FAPESP 00/01627-7						

P275 F

AMG 162, A FULLY HUMAN MONOCLONAL ANTIBODY AGAINST RECEPTOR ACTIVATOR OF NF-KAPPA B LIGAND (RANKL), RAPIDLY AND PROFOUNDLY SUPPRESSES BONE RESORPTION IN CYNOMOLGUS MONKEY

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AMG 162 is a fully human monoclonal antibody that binds with high affinity and specificity to RANKL. Single dose pharmacodynamics (PD) and pharmacokinetics (PK) of AMG 162 following intravenous (IV) administration of 0.1 to 10 millig/kilog (n=2/group) and subcutaneous (SC) administration of 1.0 millig/kilog (n=6) have been investigated in cynomolgus monkeys. AMG 162 was a highly effective bone antiresorptive agent, producing rapid suppression of urinary N-telopeptide/creatinine (NTx) one day after dosing and reaching nadirs between one and 19 days post dose. Average maximum suppression (SD) increased from 81% (12) to 94% (1.6) as IV doses increased, and was 93% (2.7) after SC administration. Duration of NTx suppression increased with dose to a maximum of 91 days in the 10 millig/kilog IV dose group. Both extent and duration of effect were similar following 1 millig/kilog IV or SC. In placebo treated animals the maximum decrease in NTx was 18% (n=2). Suppression of serum alkaline phosphatase (ALP) levels was delayed relative to NTx suppression, consistent with a reduction in activation frequency of basic multicellular units (BMUs).

AMG 162 demonstrated non-linear PK with clearance decreasing 3-fold from 0.401 to 0.120 milliL/hour/kilog as doses increased from 0.1 to 10 mg/kg. In general, as serum levels of AMG 162 decreased below 2000 nanog/milliL NTx levels started to return towards baseline. Estimates of volume of distribution indicated limited distribution of AMG 162 outside the systemic circulation. Serum profiles following IV administration were generally tri-phasic, exhibiting a rapid distribution phase (~6 hours), a slower dose-dependent secondary phase (87 to 444 hours), and a rapid terminal phase (~32 hours). Following a 10 millig/kilog IV dose, detectable concentrations of AMG 162 were observed at the end of study (18 weeks). Following a 1 millig/kilog SC dose, AMG 162 peak concentrations were observed 3 to 5 days post-dose and bioavailability was approximately 89%. In conclusion, AMG 162 has demonstrated favorable PD and PK profiles following single dose administration to cynomolgus monkeys. These results suggest that AMG 162 may allow for infrequent SC dosing in the treatment of osteolytic bone disorders, such as osteoporosis and cancer related bone diseases.

P276 S

ACUTE EFFECTS OF ENDOGENOUS CALCITONIN ON OSTEOCLASTIC BONE RESORPTION IN HUMANS: RESULTS IN THYROIDECTOMIZED PATIENTS COMPARED WITH NORMAL SUBJECTS

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The exact physiological role of calcitonin (CT) in calcium and skeletal homeostasis has not been established in humans. The aim of the present study was to test a hypothesis that the decrease in the bone resorption in response to the calcium challenge is effectively influenced by the endogenous secretion of CT in humans. The acute effects of an intravenous calcium infusion on the secretion of PTH, and CT, and concentrations of the plasma C-terminal telopeptide of collagen type 1 (beta-CTX) were evaluated in 9 patients after total thyroidectomy (aged 29.2 ± 8 years) compared with 9 healthy subjects. The two groups were matched for age, gender and the body mass index. The patients did not have any clinical or biochemical features of hypoparathyroidism, and did not require a supplementation with calcium and/or vitamin D. The patients were substituted with a stable dose of L-thyroxine for at least 1 year. The baseline concentrations of serum ionized calcium (S-iCa), plasma CT, PTH and beta-CTX did not differ significantly between the healthy and thyroidectomized subjects. After overnight fasting, intravenous infusions of 1,7 mg of elemental calcium / kg body weight over a 10 min period were given. Blood samples for measurements of S-iCa, plasma intact CT and PTH and plasma beta-CTX were obtained 3 min before and at 13, 30, 60, 90 and 150 min after the start of the infusion. A similar increase in S-iCa and decrease in plasma PTH levels were observed in both groups. However, only in the group consisting of healthy individuals, the plasma CT increased significantly by 13 min ($p<0.05$, ANOVA) and beta-CTX significantly decreased as early as at 30 min and 60 min as compared with the baseline ($p < 0.05$, ANOVA). In the thyroidectomized group, the plasma beta-CTX response to calcium load was significantly diminished throughout the study period as compared with the healthy subjects ($P < 0.01$, AUC). In conclusion, the immediate secretory response of endogenous CT to intravenous calcium stimulus is responsible for rapid initial decrease in the osteoclastic bone resorption following an acute intravenous calcium load in healthy subjects. The data document the importance of endogenous CT in emergency metabolic situations (i.e., combat hypercalcemia) in humans.

P277 W

MATRIX METALLOPROTEINASE-9 ANTISENSE OLIGODEOXYNUCLEOTIDE INHIBITS OSTEOCLASTIC BONE RESORPTION ON MATRIGEL-COATED DENTINE SLICES

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The degradation of bone collagen by osteoclasts is mainly carried out by two types of proteases, matrix metalloproteinases (MMPs) and lysosomal cysteine proteases. Of these proteases, particularly MMP-9 (gelatinase B) and cathepsin K have been shown to be abundantly expressed in human and rabbit osteoclasts and multinucleate giant cells of human osteoclastoma. We have previously demonstrated that the non-specific inhibitor of MMPs, BB-94, inhibited the osteoclastic pit formation on dentine slices coated with reconstituted basement membrane, MATRIGEL[1]. The inhibitor had no effect on osteoclastic pit formation on naked dentine slices, indicating that MMPs are necessary for the migration of preosteoclasts and/or immature osteoclasts to bone surfaces through basement membranes, but not for direct bone resorption. However, it still remains to be demonstrated that which MMP plays the most crucial role in the degradation of basement membranes. In the present study, we have investigated the effect of MMP-9 antisense phosphothiorate oligodeoxynucleotide (S-ODN) on the osteoclast-mediated degradation of MATRIGEL-coated dentine slices, since MMP-9 is a most abundant MMP in osteoclasts. Rabbit osteoclasts were cultured on the dentine slices for 24 h in the presence or absence of the antisense S-ODN in a medium containing 100 nM Tfx^{TM}-50, polycationic liposome, as a carrier of the S-ODN. The antisense S-ODN-treated osteoclasts showed a marked decrease in the amount of MMP-9 protein. The antisense S-ODN inhibited the osteoclastic pit formation in a concentration-dependent fashion. At 10 μM the antisense S-ODN reduced the total pit volume by approximately 40%. On the other hand, the sense S-ODN of MMP-9, which was used as a negative control, had no effect on the pit formation. The inhibitory effect of the antisense S-ODN similar to that of BB-94 demonstrates that MMP-9 is a MMP playing a major role in the migration of preosteoclasts and/or immature osteoclasts through basement membrane.

[1]Inui et al. Biochem. Biophys. Res. Commun. 258, 173-178, 1999

P278 F

INHIBITORY EFFECTS OF MINODRONIC ACID (YM529) ON TUMOR-INDUCED OSTEOLYSIS IN BONE METASTASES MODEL OF LUNG CANCER CELLS

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The effects of minodronic acid (YM529) on osteolysis due to bone metastases of lung cancer were investigated and compared with those of other bisphosphonates. Metastases were induced by intracardiac injection of the human lung cancer cell line SBC-5 into C.B-17/Icr-scid mice. Test compounds were intravenously given to mice after osteolytic metastases were radiologically defined. Histological examination confirmed that numerous TRAPase-positive (osteoclastic) cells appeared on the bone surface near the tumor in the bone metastases model. In this model, intravenous administration of minodronic acid reduced the number of osteoclasts and the ratio of osteoclast surface to bone surface at each tumor site in a dose-dependent manner. The effects of minodronic acid were statistically significant at doses of 0.01 and 0.1 mg/kg i.v. Zoledronic acid also induced significant reduction of these parameters at the same doses as minodronic acid. In contrast, a dose of 10 mg/kg i.v. was necessary for pamidronate to cause a significant decrease in the number of osteoclasts and the ratio of osteoclast surface to bone surface. In conclusion, minodronic acid inhibited osteolysis caused by bone metastases of SBC-5 cells. Consequently, minodronic acid holds promise as an intravenous treatment for bone lesions arising from metastases of lung cancer to bone. In addition, minodronic acid may have the same potency as zoledronic acid and be more potent than pamidronate.

P279 S

MICROARRAY ANALYSIS OF BONE CELL GENE EXPRESSION EARLY AFTER CADMIUM GAVAGE IN MICE

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We developed an in vivo model for Cd-induced bone loss in which mice excrete bone mineral in feces starting 8 hours after Cd gavage. In both mice and dogs, this bone response starts at 2-5 microg Cd/l blood, below current USOSHA standards for industry. Female mice of 3 strains [CF1; metallothionein-wildtype (MTN); MT1,2-deficient (MT1,2KO)] were placed on a low Ca diet for 2 weeks. Each mouse was gavaged with 200 microg Cd or vehicle only. Fecal Ca was monitored for 8 days to document the bone response. For CF1 mice, bones were taken from 4 groups: plus/minus Cd, 2 hours after Cd; plus/minus Cd, 4 hours after Cd. The MTN and MT1,2KO strains had two groups each: plus/minus Cd, 4 hours after Cd. PolyA+ RNA was isolated from marrow-free shafts of femura and tibiae, and each plus/minus Cd pair was submitted to Incyte Genomics for microarray analysis. To validate microarray results, the same CF1 4-hour RNA preparations were subjected to Northern analysis; probes were prepared from 18 clones of genes shown by microarray to be key to the bone cell response to Cd, standard housekeeping genes, and other genes with no Cd response via microarray. Fecal Ca results showed that bone calcium excreted after Cd differed for the three mouse strains: CF1 0.24 ±0.08 mg; MTN 0.92 ±0.22 mg; MT1,2KO 1.7 ±0.4 mg. Gene microarray results showed that, among the approximately 8500 arrayed genes, three categories were significantly induced by Cd: cell protector genes (e.g., MT1, MT2, transferrin receptor); cell signaling genes (e.g., p38 MAP kinase); and genes involved in osteoclast-mediated bone resorption (e.g., vacuolar proton pump, integrin alpha V, src-like adaptor protein). Cd also induced two genes encoding unknown proteins, one by 18-fold. No genes were clearly down-regulated by cadmium. In particular, genes for bone formation, stress response, growth factors, and signaling molecules other than p38MAPK did not change in expression in bone cells early after cadmium gavage. Results support the hypothesis that Cd increases bone demineralization via a p38 MAPK pathway that results in stimulation of osteoclast-mediated bone resorption.

P280 W

ACTIN-RING DERANGEMENTS IN HUMAN OSTEOCLASTS BY CATHEPSIN K ANTISENSE OLIGONUCLEOTIDE TREATMENT

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Human cathepsin K, a cysteine protease that is highly and selectively expressed in osteoclasts, displays a powerful proteolytic activity against type I collagen. Knockout of this enzyme in mice, as well as lack of functional enzyme in the human condition pycnodysostosis, results in osteopetrosis. On this basis, cathepsin K targeting may offer as a promising tool for protection from bone loss in conditions of elevated resorption activity, such as osteolytic bone metastasis. Despite the well-proven crucial involvement of cathepsin K in bone resorption, different sites of activity have been suggested besides simple collagen proteolysis. In fact, a reduction of cathepsin K function cannot completely inhibit pit formation, but remarkably influences the depth of resorption lacunae. To investigate the contribution of cathepsin K to cytoskeletal organization, osteoclast polarization, and, in turn, osteoclastic bone resorption through continuous cavitation, we studied the effects of cathepsin K completely phosphorothioate antisense oligodeoxynucleotides (AS-ODNs) on human osteoclasts obtained from peripheral blood monocytes. An effective uptake of AS-ODNs by osteoclasts was demonstrated by using fluorescein-labeled AS-ODNs at different times of exposure (2,4 and 7 days) and dosage (1, 10, 20 microM). Treatment with antisense significantly decreased the amount of cathepsin K in osteoclasts, as verified by immunofluorescence and Western-blot. Under the same conditions, osteoclast viability was unaffected, as shown by the number of TRAP-positive cells. At a 10 microM dose, AS-ODN treatment inihibited pit formation, and, interestingly, also impaired the organization of pseudopods that failed to form regular or complete actin-rings. Sense ODNs that were used as negative controls had no effect on cathepsin K protein level, on actin-ring formation, or on resorption activity of osteoclasts. These data show that inhibition of cathepsin K in human osteoclasts results in a reduction of the resorption activity that is associated with a derangement in the cytoskeletal organization of the actin-ring.

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P281 F

DOCETAXEL, AN ANTI-TUMOR COMPOUND, STRONGLY INHIBITS OSTEOCLAST DIFFERENTIATION BUT NOT OSTEOCLAST FUNCTION

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Pacritaxel (taxol) is an anti-tumor agent used for the treatment of various human cancers. Taxol has a unique mechanism of action to suppress growth of tumor cells: it promotes and stabilizes microtubule formation, which results in the inhibition of tumor cell proliferation. As tumor-induced hypercalcemia is usually caused by enhanced osteoclastic bone resorption, the inhibition of bone resorption by anti-bone resorbing agents such as bisphosphonates is applied for the hypercalcemia. Recently, the discovery of RANKL elucidates the precise mechanism of osteoclast differentiation and function regulated by osteoblasts. In this study, the effects of taxol on osteoclast differentiation was examined in co-cultures of mouse osteoblasts and bone marrow cells treated with $1,25(OH)_2D_3$ and PGE_2. Effects of taxol on osteoclast function were also examined in the pit-formation assay using dentine slices and osteoclasts formed in the co-culture. When increasing concentrations of taxol were added to the co-culture for the entire culture period of 7 days, osteoclast formation

induced by $1,25(OH)_2D_3$ and PGE_2 was inhibited by taxol in a dose-dependent manner. Taxol at 10^{-8} M almost completely inhibited TRAP-positive cell formation in the co-culture. In contrast, when taxol at 10^{-8} M was added on day 3 to the co-culture treated with $1,25(OH)_2D_3$ and PGE_2, many TRAP-positive cells were observed on day 7. Thus, the inhibitory effect of taxol on osteoclast formation was observed, only when the co-culture was treated with taxol from the beginning of the culture. When osteoclasts were cultured on dentine slices in the presence of osteoblasts for 24 h, many resorption pits were observed on dentine slices. The pit forming activity of osteoclasts was not affected by 10^{-8} M of taxol added to the pit formation assay. Taxol at 10^{-8} M significantly inhibited proliferation of bone marrow macrophages of osteoclast precursors and cancer cells such as a squamous cell carcinoma and an adenoid cystic carcinoma but not that of osteoblasts. These results suggest that taxol at the effective dose for cancer cells suppress bone resorption through the inhibition of osteoclast formation, probably due to inhibiting proliferation of osteoclast progenitors. Thus, taxol is an anti-tumor agent with a suppressive effect on hypercalcemia.

P282 S

RALOXIFENE PREVENTS BONE LOSS INDUCED BY GNRHA AGONIST IN MICE

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Estrogen deficiency causes marked bone loss that can be prevented by estrogen replacement therapy. Raloxifene, a selective estrogen receptor modulator, has been reported to reduce bone loss without estrogenic effects on the uterus. GnRH agonist (GnRHa) decreases the rhythmical secretion of gonadotropin and is therefore useful for the treatment estrogen-dependent disease. However, long-term treatment with GnRHa can lead to bone loss caused by increased bone resorption. In the present study, we examined secondary bone loss associated with the use of GnRHa in mice and studied the effects of raloxifene on such bone loss.

Female ddy mice (8 weeks old) were given injections of GnRHa (5 mg/kg) every 4 weeks for 4 to 12 weeks. Some of the GnRHa-treated mice concurrently received raloxifene (1 mg/kg/day) or 17beta-estradiol (1 microg/kg/day), given subcutaneously via an Alzet pump. Another mice underwent ovariectomy (OVX) and received raloxifene or 17beta-estradiol.

OVX mice showed a marked decrease in uterine weight associated with uterine atrophy. In GnRHa-treated mice, uterine weight decreased only slightly. However, the serum FSH level markedly was higher than the control value, suggesting that uterine atrophy was caused by GnRHa. The femoral bone mineral density (BMD) in GnRHa mice was significantly reduced and was similar to the BMD in OVX mice. In OVX mice, 17beta-estradiol prevented both uterine weight loss and bone loss, whereas raloxifene prevented only bone loss. Similarly, in GnRHa-treated mice, raloxifene prevented bone loss, but had no effect on the uterus. The serum OPG level was higher in GnRHa-treated mice than in control. Raloxifene decreased the serum OPG level to lower than that in control.

In conclusion, our results indicate that treatment with GnRHa causes bone loss in female mice. Raloxifene can prevent such bone loss without estrogenic effects on the uterus. Raloxifene may therefore be useful for add-back therapy in women receiving GnRHa.

P283 W

VITAMIN K2 DID NOT AFFECT OSTEOCLAST APOPTOSIS INDUCED BY N-CONTAINING BISPHOSPHONATE

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Nitrogen-containing bisphosphonates (N-BP) are important class of anti-resorptive drugs used in the treatment of metabolic bone diseases. These drugs inhibit bone resorption by inhibiting enzymes in the biosynthetic pathway leading from mevalonate to cholesterol. It is widely recognized that like statins, N-BP caused to the loss of geranylgeranylated proteins and induce osteoclast apoptosis. Recent studies have shown that geranylgeraniol (GGO) abrogated the effect of N-BP on bone resorption. Vitamin K2 (menatetrenon, MK4), an anti-osteoporosis drug has a GGO in the molecule, suggesting that MK4 as well as GGO prevents anti-resorptive effect of N-BP. Therefore, we examine whether MK4 affects to prevent the osteoclast apoptosis induced by N-BP. TRAP-positive multinucleated cells was obtained by co-culture of spleen cells with cloned stromal cells isolated mouse bone marrow with 1,25(OH)2D3 and identified osteoclast by calcitonin receptors, beta-3 integrin and pit formation. After 6 days culture, we treated with a combination of MK4, GGO and N-BP, risedronate, alendronate for 24hr. Osteoclast apoptosis was assessed by actin-ring degradation. As expected, risedronate and alendronate induced osteoclast apoptosis and simultaneous addition of GGO abrogated it. In contrast, MK4 did not affect the osteoclast apoptosis induced by risedronate and alendronate. In the previous observation, we have already reported that MK4 suppressed osteoclastogenesis and the mechanism depended on GGO. Therefore, we next explore the mechanism of MK4 on osteoclastogenesis again. As a result, we were able to isolate some clones of stromal cells which have a distinct inhibitory effect of MK4 and GGO on osteoclast formation. Using these clones, it was demonstrated that MK4 suppressed a secretion of prostaglandin E2 (PGE2) through a decrease in the induction of cyclooxygenase-2 (COX-2) and that GGO inhibited the expression of RANKL. These results indicate that the mechanism for anti-resorptive effect of MK4 is independent to that of GGO and that the combination therapy of BP and MK4 does not affect therapeutical effects each other.

P284 F

COOPERATED INTERACTION BETWEEN MICROTUBULES AND ACTIN FILAMENTS IN FUNCTIONING OSTEOCLASTS

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Cytoskeletons are composed of three independent elements: microtubules, actin filaments and intermediate filaments. Although these three elements play their own roles, they are believed to relate to each other in many aspects of cell function. Bone-resorbing osteoclasts form unique actin structures called actin rings which are observed as a ring like alignment of F-actin dots (podosomes). The actin dynamics in osteoclasts has been thoroughly investigated, because the actin ring is a prerequisite structure for bone resorption. Here, we studied interaction between microtubules and actin filaments in osteoclasts placed on glass cover slips and on dentine slices. Osteoclasts were prepared from the co-culture of mouse osteoblasts and bone marrow cells. Nocodazole and cytochalasin D were used as drugs to disrupt microtubules and actin filaments, respectively. Calcitonin (eel calcitonin) was used as a physiological modifier of the actin configuration in osteoclasts. Beta-tubulin and F-actin were illustrated with FITC-conjugated anti-beta-tubulin antibody and rhodamine-phalloidin, respectively, and their distribution was observed using a fluorescence microscope and a confocal laser scanning microscope. Pit-forming activity of osteoclasts on dentine slices was also determined in the presence or absence of nocodazole, cytochalasin D and calcitonin. Confocal microscopic observation revealed that the upper portion of podosomes was co-localized with the end of microtubules at the periphery of osteoclasts placed on glass. Nocodazole dose-dependently and reversibly disrupted microtubule networks in osteoclasts. At the same time, the nocodazole treatment induced the dispersion of podosomes and plasma membrane perturbation of osteoclasts. Nocodazole also inhibited pit-forming activity of osteoclasts with a similar dose-response effect to that on the disruption of microtubules. Cytochalasin D induced the dispersion of podosomes with the disruption of microtubule networks in osteoclasts on glass. Pit-forming activity of osteoclasts on dentine was also inhibited by cytochalasin D. Like cytochalasin D, calcitonin induced the dispersion of podosomes and inhibited pit-forming activity of osteoclasts. However, the microtubule network in osteoclasts was less affected by calcitonin. These results suggested that microtubule and actin configurations are mutually linked in osteoclasts, and that the calcitonin-induced disruption of podosomes is essentially different from that induced by cytochalasin D in osteoclasts.

P285 S

GERANYLGERANYLACETONE, AN ANTIULCER DRUG, POTENTLY INHIBITS FORMATION AND FUNCTION OF HUMAN OSTEOCLASTS IN VITRO AND BONE LOSS IN OVARIECTOMIZED RATS

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Vitamin K is widely used for protecting osteoporosis. Recently, it has been reported that the inhibitory effect of vitamin K2 (menatetrenone) on bone resorption may be related to its side chain. Geranylgeranylacetone (GGA), known as teprenone, an antiulcer drug, has almost the same chemical structure as that of the side chain of menatetrenone. We investigated whether GGA also has an inhibitory effect in osteoclastogenesis both in vitro and in vivo. GGA inhibited directly osteoclastogenesis from human monocytes induced by soluble receptor activator of nuclear factor kB ligand (sRANKL). In addition, GGA induced degradation of actin ring in mature osteoclast. Moreover, GGA increased bone mineral density of total femur, proximal epiphysis and diaphysis of femur in ovariectamized rats. These results indicate that GGA increases bone mass by maintaining a positive balance of bone turnover by suppressing both the formation and the activity of osteoclasts. Thus, GGA could be used to protect and improve osteoporosis.

P286 W

EVIDENCE FOR THE INHIBITORY EFFECTS OF BISPHOSPHONATES ON OSTEOCLAST DIFFERENTIATION AND ACTIVATION OBTAINED USING LASER SCANNING CONFOCAL MICROSCOPY

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Several mechanisms have been proposed to describe the anti-resorptive action of bisphosphonates (BPs). These include inhibition of osteoclast (OC) formation or differentiation, prevention of OC attachment to bone, and disruption of the OC cytoskeleton necessary for bone resorption. To evaluate the effects of BPs on OCs in a living bone, we have used confocal microscopy combined with immunohistochemistry to examine osteoclastic resorption in mouse calvaria. Neonatal calvaria were cultured for 48hours with or without BPs; clodronate at 1, 5 or 25 microM, or risedronate at 0.1, 0.5 or 2.5 microM. We also used either 10^{-8}M PTH or 10 microg/ml LPS to stimulate bone resorption. The following results were obtained: 1) TRAP staining showed that long pseudopodia observed in relatively small non-resorbing, multinucleated OCs were completely retracted in some OCs in the presence of BPs, indicating the inhibition of fusion. BPs also caused vacuolization of the cytoplasm in the OCs. 2) Co-localized expression of F-actin and beta 3 integrin/osteopontin along the attachment border of OC on the bone surface, which is one of the characteristic features of bone-resorbing OCs, was dramatically reduced in the presence of BPs. Both the area and the depth of resorption lacunae under the OCs were reduced in a dose-dependent manner. 3) Strong staining for osteopontin, observed at the bottom of the resorption lacunae, was greatly diminished by the addition of BPs. 4) X-Z scanning (depth analyses) confocal images of calvaria stained with Alexa Fluor 488-labeled phalloidin and Alizarin Red S showed that resorption lacunae induced by PTH or LPS were filled with populations of newly-proliferated alkaline phosphatase- positive osteoblastic cells that secreted matrix. Interestingly, this anabolic phenomenon was not observed in calvaria cultured without PTH or LPS stimulation. Moreover, the effect was not altered by BPs treatment. In summary, BPs inhibited both the differentiation and the activation of OCs dose-dependently, but did not interfere with bone formation that is coupled with bone resorption.

P287 F

EFFECTS OF D-003, A NEW HYPOCHOLESTEROLEMIC DRUG, ON BONES OF OVARIECTOMIZED RATS

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Osteoporosis is characterized by reduced bone mass and abnormal bone architecture, which increases the risk for fractures, mainly in elderly individuals. Mevalonate is a precursor of lipoids participating in osteoclast activity, so that changes of its biosynthesis can affect bone metabolism. Nutritional and pharmacological strategies are considered for osteoporosis management, recent evidences showing that cholesterol-lowering drugs inhibiting HMGCoA reductase activity might increase new bone formation in rodents and human cells in vitro through the inhibition of mevalonate production. D-003 is a mixture of high molecular weight aliphatic acids purified from sugar cane wax, with cholesterol-lowering effects associated to inhibition of cholesterol biosynthesis via an indirect regulation of HMGCoA reductase. The purpose of this study was to determine whether D-003 could prevent bone loss induced by ovariectomy in Sprague Dawley rats. Female rats were bilaterally ovariectomized (ovx) or sham operated, being randomly distributed in 4 groups: a control ovx group treated with Tween/H20 vehicle, a group treated with alendronate (3 mg/kg) and two groups treated with D-003 (50 and 200 mg/kg). All treatments were administered for 3 months. At sacrifice, bones (femoral neck, distal femur and lumbar vertebrae) were removed and taken for histomorphometry. Measurements related to trabecular bone volume and structure were performed and determinations related to bone resorption were also achieved. Results showed that ovariectomy reduced trabecules number and thickness, while increased trabecular separation. Alendronate prevented ovx-induced bone loss and decreased bone resorption. D-003 significantly prevented the ovx-induced decrease on the number of trabecules and trabecular thickness, as well as the increase on trabecular separation induced by ovariectomy. The effects of D-003 on distal femur and femoral neck were larger than those induced with alendronate. The increase of osteoclast number and perimeter induced in ovx-rats were significantly decreased in alendronate and D-003 groups compared with ovx controls. Alendronate, but not D-003, significantly reduced osteoblast surface compared with ovx-controls. It is concluded that D-003 was effective to prevent bone loss and decreasing bone resorption in ovx rats. These data suggest that D-003 should be potentially useful in the prevention of bone loss associated to osteoporosis.

P288 S

RESPONSE OF BIOCHEMICAL MARKER OF BONE TURN-OVER TO CLACITONIN SHORT TERM INJECTION IN POSTMENOPAUSAL OSTEOPOROSIS PATIENTS

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Objective :

To evaluate the influence of calcitonin short term injection on bone resorption we investigated a change of urinary deoxypyrydinoline excretion and serum osteocalcin.

Method :

Beginning November 2001, the study was carried out in 20 post-menopausal inpatient women who have lumbar or thoracic spine compression fracture with osteoporosis.

When the patient admitted, we estimated serum osteocalcin and urine deoxypyrydinoline.

The patient was treated with elcitonin 20IU IM per day for 21 consecutive days. After elcitonin injection treatment was finished, we estimated serum osteocalcin and urine deoxypyrydinoline again. Urine was collected on first voiding in the morning and serum osteocalcin was collected on an empty stomach in the morning because of the diurnal variation.

Results :

On the day of treatment, serum osteocalcin estimated 3.05 ng/ml±1.21 and urine deoxypyridinoline excreated 37.61nmol/gm/dl±20.34. After 21 consecutive days elcitonin injection treatment was finished, serum osteocalcin estimated 2.68ng/ml±1.41($p=0.139$) and urine deoxypyridinoline excreated 31.39nmol/gm/dl±15.29($p<0.05$). The data were analyzed by paired T-test. Only urine deoxypyridinoline was significantly decreased after calcitonin daily injection therapy.

Conclusion :

These results suggest that shorterm injection of calcitonin in postmenopausal osteoporosis patient has meaningful selective inhibitory action on deoxypyridinoline urinary excretion.

P289 W

EFFECTS OF ANGIOGENESIS INHIBITOR TNP-470 ON OSTEOCLASTIC BONE RESORPTION

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Antiangiogenic therapy has been the focus of recent attention as one of the most promising strategy for tumor growth and metastasis, because angiogenesis is an essential step in the progression of tumor growth, invasion and metastasis. Angiogenesis is also critical involved in bone development and remodeling, and influence osteoclast recruitment, formation, and activity. Therefore, it is suggested that angiogenesis inhibitors might be useful for osteoclastic bone resorption. We previously demonstrated that the potent angiogenesis inhibitor, a semisynthetic analogue of fumagillin, TNP-470, inhibited not only local osteolytic bone destruction in bone metastasis but also hypercalcemia, which is systemic osteolysis induced by tumor-produced parathyroid hormone-related protein (PTHrP), in experimental animal models. It is suggested that TNP-470 should be a beneficial drug to treat cancer-induced bone diseases, because TNP-470 has both antitumor activity through antiangiogenesis and the direct osteoclast-inhibitory activity. However, the mechanism of inhibitory effects of TNP-470 on osteoclastic bone resorption is completely uncertain. In the present study, we examined the molecular regulation of osteoclastic bone resorption by TNP-470. In vitro, TNP-470 dose-dependently inhibited the osteoclast formation and bone resorbing activity stimulated by 1,25(OH)2D3 or PTHrP. Receptor activator of NF-kappa B ligand (RANKL), osteoprotegerin (OPG) and macrophage colony-stimulating factor (M-CSF) are regulating factors for osteoclast formation and activity. We examined whether TNP-470 regulated these factors in osteoclastgenesis. TNP-470 did not regulate the expression of mRNA of RANKL, OPG, and M-CSF in bone marrow stromal cells. TNP-470 also did not regulate the expression of mRNA of c-fms and RANK, the receptor for M-CSF and RANKL respectively, in osteoclast precursors. Moreover, TNP-470 did not show the cytotoxity and inhibitory activity against the proliferation of various cell types, which are related to osteoclastogenesis, at concentrations of the inhibition of osteoclast formation. On the other hand, TNP-470 inhibited the osteoclast formation from spleen cells induced by soluble RANKL and M-CSF in the absence of stromal cells. These findings indicate that TNP-470 might directly affect osteoclast precursors and influence RANK-mediated signaling system for osteoclastgenesis.

P290 F

EFFECTS OF POLICOSANOL ON BONES OF OVARIECTOMIZED SPRAGUE DAWLEY RATS

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Osteoporosis is frequent disease, mainly in elderly people, characterized by reduced bone mass, abnormal bone architecture and a high fracture risk. Mevalonate is a precursor necessary for the production of lipoids important in osteoclast activity, so that inhibition of mevalonate synthesis has been linked to bone metabolism. Nutritional and pharmacological strategies are considered for osteoporosis management. Recently, evidence has shown that cholesterol-lowering drugs acting by inhibiting HMGCoA reductase activity might increase new bone formation in rodents and human cells in vitro through the impairment of mevalonate production. Policosanol is a cholesterol-lowering drug isolated from sugar cane wax acting by inhibiting cholesterol biosynthesis in a step located between acetate consumption and mevalonate production. Policosanol also shows pleiotropic effects, such as inhibition of LDL lipid peroxidation and platelet aggregation, proven in experimental and clinical studies. Hence, the purpose of this study was to determine whether policosanol could prevent bone loss in bones of ovariectomized (ovx) rats. Sprague Dawley female rats were bilaterally ovx or sham operated at 3 months of age. The ovx rats were treated with Tween/H20 vehicle, 17 b estradiol at 30 µg/kg/day or policosanol at 50 and 200 mg/kg day for 3 months. At sacrifice, bones (femoral neck, distal femur and lumbar verteberae) were removed and used for histomorphometry. Measurements related to trabecular bone volume and structure were performed and determinations related to bone resorption were also achieved. Results indicated that ovariectomy reduced trabecular number and thickness, while increased trabecular separation. Estradiol and policosanol prevented the decrease on the number of trabecules and trabecular thickness and the increase on trabecular separation induced by ovariectomy, so that both treatments prevented ovx-induced bone loss and decreased bone resorption. Both treatments also prevent the increase on osteoclast number and perimeter in ovx rats compared with both sham and ovx controls. It is concluded that policosanol at 50 and 200 mg/kg prevents bone loss and decreased bone resorption in ovx rats similarly as estrogen does. These data suggest that it should be potentially useful in the prevention of bone loss in postmenopausal women.

Osteoporosis: Diagnosis

P291 S

PERIMENOPAUSAL AND POSTMENOPAUSAL CHANGES IN BONE METABOLIC MARKERS

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[Background]. We measured the bone metabolic markers NTx and BAP in perimenopausal and postmenopausal women to examine whether the dynamics of bone metabolism could be explained on the basis of the balance between bone resorption and formation. [Subjects]. A total of 265 women (101 premenopausal, 164 postmenopausal) who presented at our climacteric outpatient clinic were studied. The premenopausal women were divided into 50 with regular menses (REG) and 51 with irregular menses (IRREG). The postmenopausal women were divided into five groups according to the number of years since menopause (YSM, 0-2, 3-5, 6-10, 11-15, and 16-). NTx and BAP were measured in each group and were expressed as the percent change (%) from the mean values in the REG group. NTx/BAP ratios were calculated to study the balance between bone resorption and formation. [Results]. In the REG and IRREG groups, both NTx and BAP were significantly increased ($P<0.01$). These increased levels persisted after menopause. Peak levels were attained in YSM 0-2 for NTx and YSM 3-5 for BAP; levels of both markers were about 90% higher than those in the REG group and then gradually declined. In the IRREG group, bone resorption exceeded bone formation in YSM 0-2, whereas bone formation exceeded resorption in YSM 3-15. The NTx/BAP ratio increased from YSM 0-2 as compared with the REG group, but then rapidly decreased from YSM 3-5 and fell below the value in the REG group, indicating an improvement in the resorption-predominant balance. [Conclusions]. Bone mineral density starts to decrease from about 45 years of age and is most distinct during perimenopause. Subsequently, bone mineral density declines gradually. Our results showed that NTx and BAP were excellent markers that closely reflected the balance between bone resorption and formation. A number of years after menopause the decrease in mineral density levels off, despite persistently increased levels of bone resorption. This apparently paradoxical mechanism can be explained by the fact that increased bone resorption during this period is compensated for by bone formation.

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RESEARCH OF NORMAL POPULATION PHALANGEAL BONE DENSITY IN THE SUBURBS OF BEIJING BY RADIOGRAPHIC ABSORPTIONETRY

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Preface:MetriScan Bone Densitometer is a popular portable osteoporosis diagnostic instrument to estimate phalangeal BMD. We conducted this research to develop a normative database for the Chinese people.

Method: Metriscan is an independent desktop bone density measuring device which uses radiographic absorptiometry (RA) to estimate the corresponding BMD value and T-score. As

a result,osteoporosis can be diagnosed and fracture risk can be predicted. Grouping:we selected 8 groups of ordinary people aging from 10 to 89. Each group has 50 people with men and women counted separately. To obtain more accurate estimate of peak bone mass,age groups from 20 to 29 and from 30 to 39 include 125 people each. People each.People who have taken osteoposis drugs or who have suffered from diabetes, ovariotomy, etc.are excluded from the selected groups.

Result: For women, peak bone mass appears in the age group from 30 to 39 and from 29 to 35.As age increases, bone mass loss is evident with considerable regularity. For men ,peak bone mass appears in the age group from 30 to 39 and 25 to 32. As age increases,loss of bone mass slows down.

Discussion: A.The brand new MetriScan integrating Radiographic Absorptiometry and Digital Graph Analysis is suitable for osteoporosis diagnosis for the Chinese people. Before SPA came into being, X-ray was used to diagnose osteoporosis. Affected by many factors, osteoporosis was diagnosed only when bone mass loss reached 30 to 50 percent. Digitalization and RA technology brought a new member to the early-stage osteoporosis diagnostic instrument family. The result obtained by using MetriScan to measure the normal group from Northern China is similar to that obtained by a task team headed by Prof.Liu Zhong-hou using SPA to measure bone density of the forearm (radius and ulna) 1/3 of 40,000 people.

B.MetriScan is an independent bone density diagnostic instrument with a low price. As it is easy to operate, it is suitable for small hospitals. In big hospitals, Metriscan is supplementary to DEXA.

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CROSS-CALIBRATION OF THREE TYPES OF DXA HOLOGIC DEVICES USING THE ESP PHANTOMS IN THE LONGITUDINAL MULTICENTRE STRONTIUM RANELATE TRIAL

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In order to pool in vivo data acquired on different DXA devices in a multicentre clinical trial, cross-calibration (X-cal) is needed. The Strontium Ranelate phase 3 study's quality control program, involves 6740 postmenopausal women. Multiple cross-calibration were completed on three types of Hologic DXA scanners using the European Spine Phantom (ESP).

DXA measurements were performed on 77 Hologic DXA scanners: 24 QDR1000, 19 QDR2000 and 34 QDR4500. Those were cross calibrated using three ESP phantoms (138, 140 and 142) measured 20 times each. Differences between phantoms and between apparatus were measured, and the consistency of results found between the three types of scanner over several X-cal was determined.

The average ESP precision was 0.47%, 0.56% and 0.42% for QDR1000, 2000 and 4500 respectively. Since no significant difference was found and because the maximum difference did not even reach the precision of the ESP, results were pooled.

Percentage of differences between two types of devices (DXAdiff) were significantly different from zero between pairs of devices (see table). In addition, these inter-types differences (QDR1000 vs 2000, etc) were larger than the observed intra-types variability. Finally, no significant difference was found between the successive cross-calibrations for the diverse DXA.

DXAdiff	**QDR1000/2000**	**QDR1000/4500**	**QDR2000/4500**
ESP 138	-8.2	-5.3	2.7
ESP 140	-7.7	-6.1	1.5
ESP 142	-10.2	-6.2	3.6
Mean diff.	-8.7*	-5.9*	2.6*
DXAdiff = [(meanBMD DXA1-meanBMD DXA2)/ meanBMD DXA1]*100 * $p< 0.05$			

Our results confirm the importance of a stringent cross-calibration procedure in multicentre clinical trials involving different types of devices. Besides, the observed stability between the three cross-calibrations suggests that their number could be reduced in the future except in case of hardware modifications assuming a strict daily quality control check.

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COMPUTER MORPHOMETRIC DENSITOMETRY CAN DETERMINE LOW BONE DENSITY IN PATIENTS WITH DIABETES TYPE 1

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Background: The aim of this study was to evaluate how Computer Morphometric Densitometry (CMD) can be a more potent method of evaluation of low bone mass density (BMD) in comparison with other methods - x-ray, dual x-ray absortiometry (DEXA).

Materials and Methods: 12 patients (5 female, 6 male) with type 1 diabetes mellitus (age 39 ±2.1 years, duration of diabetes 14 years ±3.2). All patients did not receive any antiresorbtive or bone formation drug. X-ray was performed in L2-L4 region and in the foot. BMD was measured by DEXA in lumbar spine. Using the CMD method we investigated L2,L3,L4 of the spine and foot region.

Results: After X-ray investigation we received such results - in the L2-L4 region 3 female and 5 male observed normal bone status, 1 female and 1 male observed osteopenia and 1 female had osteoporosis. X-ray of the foot: 2 female and 2 male had normal condition of the foot, 3 female and 4 male observed osteoarthropathy (70%). DEXA of the spine showed normal BMD in 3 female and 3 male, 1 female and 3 male observed osteopenia, 1 female observed osteoporosis. The CMD method showed in L2, L3, L4 region low bone density of different amplitude in all 12 patients (100%). The same result with a higher amplitude was observed in the foot region in 12 patients (100%).

Conclusions: The CMD method in our study showed a high level of diagnostic measurement. Probably this method could be extremely useful for the early investigation of foot complication in patients with diabetes. More data is needed to provide a result of high accuracy.

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QUALITY ASSESSMENT OF DXA SCANS IN A MULTICENTER STUDY: THE PHASE 3 STRONTIUM RANELATE PROGRAM

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DXA scan's quality assessment is of great importance in multicenter clinical trials assessing bone mineral density, and includes appropriate patient positioning, training of technologists, daily quality control of DXA equipment, cross-calibration of the devices and quality assessment of the performed scans. This latter condition has been studied in the Strontium Ranelate Phase III studies (SOTI: Spinal Osteoporosis Therapeutic Intervention) and TROPOS (Treatment of Peripheral Osteoporosis), over more than three years, involving 6740 osteoporotic women (mean age 75.0 ± 6.4 years) and 75 investigation centers.

Lumbar spine (L1-L4) and hip were measured every 6 months since baseline visits in both placebo and treated groups, using DXA densitometers (Hologic Inc. Waltham USA). Beside central bone scan analysis, systematic quality assessment was also performed by GEQAP. It assessed technological, morphological or structural criteria for each vertebrae (L1 to L4) as well as for the total spine (L1-4). Similar approach was used for the hip. When concomitant problems occurred at one scan, it was decided to take into account the most impacting factor on bone density for the final grading of this scan. Results of qualitative criteria are summarized in the following tables.

Among 32026 visits, we counted 30686 spine and 30863 hip scans (177 bilateral hip scans).

Because the presence of criteria does not necessarily lead to the exclusion of the whole spine scan (the exclusion was driven by the severity of our global grading), only 628 (2%) individual or grouped vertebrae were excluded. The main reasons of exclusion were incorrect field of view (48.6%), overlying (33.9%) or motion artifacts (13.5%). Similarly 0.3% of hip scans were not assessable.

Careful and global quality program is necessary throughout a longitudinal study, especially when it involves patients potentially affected by degenerative disorders.

Scan evaluation criteria	**Spine(n=30686)**	**Hip(n=30863)**
Positioning difficulties	13.6%	17.1%
Motion artifact	0.8%	0.4%
Incorrect field of view (FOV)	0.9%	0.2%
Soft tissues artifact	6.9%	0.8%
Localized bone hyperdensity	66.2%	0.7%
Bone overlying artifact	1.1%	0.6%
No specific remark	10.6%	80.2%

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LONGITUDINAL QUALITY CONTROL PROCEDURE USING THE HOLOGIC SPINE PHANTOM DURING A MULTI-CENTER STUDY: THE PHASE 3 STRONTIUM RANELATE PROGRAM

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During the phase 3 program of Strontium Ranelate, a new compound for the treatment of osteoporosis, involving 9196 post-menopausal women, DXA measurements were performed in 77 Hologic DXA scanners.

In our study's quality control program, we investigated the long term stability over six years of three types of Hologic DXA scanners (QDR 1000, 2000 and 4500) using the local Hologic spine phantoms (LHSP).

Baseline measurements of each LHSP were performed by trained technician from the Quality assurance centre (GEQAP) prior the recruitment of the 1st patient. Then, while daily acquisitions of this phantom were performed by the technician in each investigation centre, GEQAP carried out the central analysis of the scans as well as the statistical evaluation using Shewhart rules and Cusum analysis. Each malfunction were also recorded by the QA centre. Summary results are given in the following table.

Overall the stability of the involved devices are very satisfactory. QDR 2000 appears to have a number of alarms and malfunctions slightly higher than the QDR 1000 and 4500 series, and the mean CV is above 0.5% which is usually the accepted limit. The QDR 4500 seems to be the most stable device and the QDR 1000 the most robust. Careful long term QC remains to be performed and stringent procedures must be applied to ensure optimal quality in long-term multicentrique clinical trials.

	QDR 1000 n=24	**QDR 2000 n=19**	**QDR 4500 n=34**
Local spine phantom scan number	28951	28120	51358
Mean spine phantom BMD (g/cm2)	1.022	1.039	1.000
SD	0.020	0.013	0.017
Mean CV %	0.46	0.52	0.44
Mean Shewhart alarms per devices	24	27	14
Mean Cusum alarms per devices	35	44	28
Number of malfunction report per devices	4	7	5

P297 S

THE EDUCATION OF PATIENTS AND SUBSEQUENT TREATMENT FOLLOWING BONE DENSITOMETRY

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Introduction: Appropriate treatment for osteoporosis is often not started or continued long enough to be of benefit. The reasons may include poor communication and lack of patient education by physicians. This study investigates how patients are educated and treated after their first bone densitometry.

Methods: 1434 patients who had bone densitometry (DEXA, Lunar DPX-IQ) were asked to complete a questionnaire. They were asked if they had been informed of their results and what treatment had been prescribed. Patients' T-scores and the specialty of the clinician ordering the test and received the interpretation was also recorded.

Results: 1014 responses were received. 54 individual physicians, physician assistants and nurse practitioners ordered studies. Internists, family practitioners, nurse practioners and physician assistants ordered 97% of the studies. Results were normal in 341 participants, 309 had osteopenia and 364 had osteoporosis. Eighty percent of study participants responded that they had been informed of the results. Of those who had had normal results, 64% knew the correct results, 31% of those with osteopenia and 50 % of those with osteoporosis. The patients' reporting of correct results did not depend on the specialty of the health care provider and older patents were not more likely to report inaccurate results. For those who had not been taking calcium prior to the DEXA, calcium supplements were recommended to 65%. Internists were more likely to recommend calcium (p=.0003). Following the initial DEXA, 339 patients were started on medications(33%). Of those, 86% were still taking some form of prescribed therapy, but 140 (41%) were not taking the original medication. Reasons for discontinuation included sideffects (48%) and cost (26 %). The selection of medications was similar amongst medical specialties. Patients with osteopenia or osteoporosis, who correctly reported their results, were more likely to be prescribed a medication and continue to take it (p<.0001).

Conclusion: Most patients are informed of the results of their bone densitometries, but the information they retain may not be accurate. Patients who correctly reported the results of their DEXAs were more likely to be treated and to remain on treatment.

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P298 W

PREDICTORS FOR THE PROGRESSION TO OSTEOPOROSIS IN OSTEOPENIC WOMEN: JPOS COHORT STUDY

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Aims: To predict which subjects with osteopenia at present will develop osteoporosis in near future.

Methods: Bone mineral density (BMD) was measured at baseline and 3 years after it by DXA at the spine (LS), total hip (TH) and distal 1/3 site of the radius (DR) in 1,285 women aged 15 to 79 years at baseline. The JSBMR criterion was applied to form the diagnosis of osteopenia (BMD ranging 70%-80% of the young adult mean (YAM)) and osteoporosis (BMD<70% of YAM). Biochemical markers of bone turnover were determined at baseline including serum osteocalcin (OC) and bone alkaline phosphatase, and type I collagen C-terminal telopeptide and free and total deoxypyridinoline (tDPD) in fasting urine. 1,153 women without any disease or medication affecting bone metabolism were analyzed.

Results: Among the subjects, 163, 177 or 139 subjects were judged to have osteopenia at LS, TH or DR, respectively, at baseline. 21.5%, 13.0% or 13.0% of them, respectively, had developed osteoporosis during the subsequent 3 years. Most of these osteoporotic patients originated from the subjects whose BMD at baseline were lower than 75% of YAM. We compared, therefore, baseline characteristics between in the subjects with and without progression to osteoporosis among those with BMD ranging from 70% to 75% at baseline. We observed that 32 subjects developed osteoporosis and 58 remained to be osteopenic at LS. No difference was found between these two groups in age, height or weight but the former subjects had significantly higher level of OC than the latter. 23 developed osteoporosis and 48 remained osteopenic at TH. The former were significantly older and shorter than the latter. No other difference was found. We found 14 and 58 subjects with and without the progression to osteoporosis at DR, respectively. The subjects with progression showed greater physique and higher values of OC and tDPD.

Conclusion: Several significant differences were found in the baseline characteristics between the subjects with and without progression from osteopenia to osteoporosis. Although they were not consistent over the skeletal sites, an elevated level of OC may predict the progression to osteoporosis in osteopenic subjects.

P299 F

THE EFFECT OF BONE QUALITY ON THE INTRAMEDULLARY FIXATION OF PROXIMAL HUMERAL FRACTURES

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Introduction: We report an evaluation of an implanted retro grade humeral nail and the effect of Bone Mineral Density (BMD) on its intramedullary fixation in proximal humeral fractures.

Methods: Ten fresh frozen whole adult humeri were obtained at post-mortem. All specimens underwent standard morphometrical measurements. Each specimen was assessed for proximal and distal bone quality using a Lunar- Expert DXA scanner. Each specimen was instrumented with the correct size retrograde nail and an osteotomy recreated a proximal humeral fracture.

Each specimen was tested for failure through the axis of the nail, within the bone. The testing protocol allowed for assessments of torque hold at 10, 20 and 30* rotation. Failure of torque hold was defined as a 10% drop in measured pure torque.

Results: Two specimens proved inadequate, eight were tested to full failure at the proximal end. The mean failure of torque hold for the nail was 1.25Nm * 0.14, median 1.2Nm. The mean rotation observed at failure was 29.2** 17.43, median 34.3*. In sub-failure testing to 10 * rotation a mean recoil of only 54% was observed, with significant deformation of the trio wire, similarly at 20*, 50.5% and at 30*, 38%.

There was no significant correlation at the 5% ($p<0.05$) level between the observed bone mineral density of either the humeral head, neck, tuberosity or total proximal bone mineral density and the observed torque hold at failure. At 10% ($p<0.1$) a significant relationship between the bone mineral density of the head and the torque at failure was observed, $r=0.63$ $p<0.1$.

Distally, no failures of the fixation were observed. Reproducibility of the tests was proven with strong correlation observed between failure torque at 10, 20 and 30*s.

Discussion: It would appear that proximal humeral bone quality is not important in determining the suitability or not for a retrograde nail with a trio wire. No significant relationship between BMD and nail function was identified. The retrograde humeral nail used in this study (Halder Nail) exhibits poor rotational stability in varied quality bone.

P300 S

PRECISION AND CONCORDANCE OF A TEN SECOND DXA SCANNING MODE

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Hologic's newest densitometers, the Discovery QDR Series A/SL/W and C, come with a 10s 'Express' scanning mode for the AP Spine and hip. The *in vivo* precision of this new 10s DXA scanning mode was measured along with its concordance to the default Delphi (30s) DXA scanning mode.

To obtain a large BMD range, sixty women were recruited in two groups of thirty at Puget Sound Osteoporosis Center in Seattle, WA. Group One had an age range of 21-40 years, while Group Two had an age range of 50-70 years. The precision of the 10s scanning mode was measured at the spine and hip on each subject using triplicate measurements, with the subject getting off the table after each measurement. One subject from Group Two did not have a complete set of measurements and was excluded from the precision analysis. The precision of the two groups was not statistically different, so the results were pooled.

In addition to the 10s scans, each women had one 30s scan done of her spine and left hip to measure the concordance between the 10s and 30s scan mode.

The *in vivo* precision of the 10s DXA scanning mode was measured to be 0.9% for the AP Spine and 1.0% for the Total Hip. The correlation of the linear regression between the 10s and 30s modes for the AP Spine, Total Hip, and Femoral Neck was $r = 0.99$, $r = 0.98$, and $r = 0.98$, respectively. None of the offsets of the linear regressions were significantly different from zero; therefore the slopes were fit with the intercept restricted to zero. The slopes were not statistically different from unity.

The 10s DXA scanning mode of the Discovery is precise and agrees strongly with the default scanning mode of the Delphi.

P301 W

RATIONAL SCREENING FOR OSTEOPOROSIS AMONG GENERAL POPULATION OF POSTMENOPAUSAL WOMEN

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Early identification of postmenopausal women with osteoporosis by means of bone mineral density (BMD) measurement is a prerequisite for reducing the incidence of osteoporotic fractures. Objective of our study is to assess simple decision rules that could enhance identifying patients with high risk of fracture in the general population of postmenopausal women.

In the local women health magazine, we invited to the DEXA measurement of lumbar spine (L1-L4) and hip all women who were postmenopausal for at least 5 years, had body mass index (BMI) less than 26 kg/m2, and have never been diagnosed with osteoporosis. Between February and May 2002, 201 women were enrolled in the study; 133 women (66%) were identified as osteoporotic and 46 (23%) as osteopenic. Approximately 29% of all women (58 women) suffered from previous low-trauma fracture; within this fracture-group, 74% of patients were diagnosed with osteoporosis and 24% had osteopenia. The fracture status of 58 women was as follows: 53 of women suffered the wrist fracture, 3 the hip fracture, and 6 vertebral fracture; 6 women had multiple fractures. None of the women enrolled in the study were treated for osteoporosis; about half of the patients were given NSAIDs, while the other half received no treatment.

Results of our study suggest that three simple decision rules provide efficient guidance for BMD measurement referrals even among general population of postmenopausal women that would otherwise rarely see their GP. This is of importance since this study corroborates and expands our earlier study in which these decision rules proved to work well in the setting of primary health care. In spite of the fact that women enrolled in our study were at relatively high risk of fracture (90% had either osteopenia or osteoporosis), they were left undiagnosed and untreated. These rules should be thus recognized as an effective tool for identifying patients with osteoporosis in the general population that are still active and unaware of their disease.

P302 F

HIGHER CUT OFF VALUES OF PERIPHERAL BONE MASS MEASURED AT VARIOUS SITES FOR DETECTING THE PREVALENT VERTEBRAL FRACTURES IN WOMEN ON LONG-TERM GLUCOCORTICOID USE

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Since long-term glucocorticoid (GC) users are known to be at high risk of fragility fracture, clinical guidelines recommend pharmaceutical interventions to be initiated if bone mineral density determined by DXA is below the levels of osteopenia. However, little is known whether this is also true in peripheral bone mass determined by other devices. In a hospital setting we simultaneously measured bone mass at various sites as follows; Lumbar spine (DXA), heel (QUS), metacarpal (RA), and distal radius

(pQCT). Enrolled in the present study were 65 ambulatory women on long term GC use (GC group) and 355 postmenopausal women (PM group). Vertebral fractures (Vfx) are defined by level specific criteria proposed by McCloskey. ROC analysis was employed to determine cut off values to effectively discriminate the subjects with and without one vertebral fractures or more in the two groups. Although the prevalence of Vfx in GC group was similar to that in PM group (29.2% vs. 27.6%), frequency of Vfx distributed differently at vertebral levels in two groups. Cut off values expressed by percentage of values of young adult mean (YAM) at sites measured in GC group were higher than those in PM group as shown in Table. There results imply that pharmaceutical intervention should be initiated at higher cut off values of peripheral bone mass in long term GC users irrespective of sites measured.

	Lumbar spine	**metacarpal**	**heel**	**distal radius**
	DXA	**RA**	**QUS**	**pQCT**
PM Group	66	73	78	64
GC Group	72	86	83	74
Cut off values expressed as %YAM YAM: Young adult mean (20-44 years)				

P303 S

VALIDATION OF A NEW SINGLE-PHOTON SYSTEM OF RADIOABSORPTIOMETRY OF THE PHALANX FOR MEASUREMENT OF BONE MINERAL DENSITY

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Background: A new method for the measurement of bone density of the phalanx using digitised conventional X-ray films of the hand has been developed in our centre.

Objectives: To test the reliability and internal validity (test-retest) of this method, especially regarding the digitalisation process and the software developed for segmentation and optical density analyses.

Methods: The non-dominant hand is radiographed together with an aluminium wedge used for calibration and correction of the variability associated with the XR technique and digitalisation process. A single exposure at 46 kV is performed and the resulting film is read with an AgfaScan T1200 device with predefined settings.

Results: To assess the reproducibility of our technique, XR hand films of 17 patients were scanned 5 times in different days. CV was found to be 0,913 (95% CI 0,650-1,825) for the middle phalanx; 0,632 (95% CI 0,470-1,265) for the proximal phalanx; and 1,194 (95% CI 0,918-2,387) for the central area of the third metacarpal. A test-retest experiment with 2 films in 50 patients gave an almost perfect internal correlation (Pearson's R of 0,985; 0,995 and 0,986) for these same sites. We also validated our technique in a sample of 171 women referred by general practitioners with a clinical diagnosis of osteoporosis. Construct validation was performed against conventional lumbar spine and hip DXA (Lunar DPX). Also,78 of these women had a phalanx DXA measurement with a commercial device (AccuDXA). Correlations between BMD in several sites are shown in the attached table.

Conclusion: The reproducibility and internal correlation of measurements made with our newly developed technique is very high for the three areas of the hand studied. Correlation of BMD of the hand with BMD in the lumbar spine and hip is moderate and similar to that found among these places themselves.

Correlation among different BMD mesaurement procedures					
	Prox Phal	**MCP**	**Spine**	**Hip**	**AccuDXA**
MiddIe Phal	0,851*	0,755*	0,562*	0,565*	0,674*
Prox Phal		0,845*	0,551*	0,580*	0,742*
MCP			0,548*	0,603*	0,713*
Spine				0,708*	0,510*
Hip					0,623*
(*) p<0.005					

P304 W

DUAL-ENERGY X-RAY ABSORPTIOMETRY (DXA) EVALUATION OF PERIPROSTHETIC BONE MINERAL DENSITY OF THE ACETABULUM DISCRIMINATES BETWEEN OPERATIVE AND NON-OPERATIVE HIP IN PATIENTS FOLLOWING TOTAL HIP ARTHROPLASTY (THA) WITH UNCEMENTED TANTALUM (HEDROCEL) IMPLANTS

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Bone mineral density (BMD) around acetabular implants may have important implications for long-term survival of the prosthetic-bone interface and therefore the longevity of the implant itself. We developed a technique using DXA for measuring periprosthetic BMD superior to uncemented acetabular tantalum (Hedrocel) implants following primary THA that discriminates between the operative hip and the contra-lateral non-operative hip.

Seventeen patients with a mean age of 64 were scanned 6-60 months following primary THA using the GE Lunar Prodigy with Orthopedic analysis using the encore software platform. BMD scans were performed in duplicate and bone edges were automatically placed on the operative hip utilizing the orthopedic software without manual manipulation. We utilized five identical custom regions of interest (ROI) extending 2.5 cm in 0.50 cm increments from the superior border of the implant/subchondral bone interface. Bone edges of the non-operative hip required manual manipulation utilizing the software 'paint' function so that the head of the femur was excluded. The SmartScan feature was disabled to avoid premature termination of the hip scan.

The mean and standard deviation of each site were calculated and p-values were determined for each zone in the operative compared with the non-operative side using SPSS 11.0 statistical software. Significant BMD differences ($p = .036$) were found using a repeated measures design with both hip (operative vs. non-operative) and zone as the repeated measure. DXA was able to discriminate the non-operative hip, which was significantly more dense across the five zones, from the operative hip. DXA may be a useful tool for evaluation of periprosthetic bone following THA with uncemented implants

P305 F

A COMPARISON OF DUAL-ENERGY X-RAY ABSORPTIOMETRY (DXA) WITH COMPUTERIZED TOMOGRAPHY (CT) IN EVALUATING PERIPROSTHETIC BONE MINERAL DENSITY (BMD) OF THE ACETABULUM FOLLOWING TOTAL HIP ARTHROPLASTY (THA)

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We describe a new technique for evaluating periprosthetic bone mineral density surrounding an uncemented acetabular implant following primary THA with DXA, and compare results with CT. We evaluated the operative and non-operative hip in 29 patients of a single surgeon 6-60 months following primary THA with tantalum (Hedrocel) implant using both techniques. DXA examinations were performed using the GE Lunar Prodigy with Orthopedic analysis using the enCORE software platform. BMD scans were performed in duplicate and bone edges were automatically placed on the operative hip utilizing the orthopedic software without manual manipulation. We utilized five identical custom regions of interest (ROI) extending 2.5 cm in 0.50 cm increments from the superior border of the implant/subchondral bone interface. CT scans were performed on a GE LightSpeed scanner and manually mapped for areas of cancellous bone (manually subtracting cortical bone) and volumetric calculations were performed for each ROI.

Precision of both scanning techniques was calculated for the entire sample using the coefficient of variation (CV). There was no significant difference in precision between DXA and CT ($p = 0.178$). In addition, the average correlation was 0.57, suggesting that DXA and CT measure similar bone mass in acetabular bone adjacent to a press-fit tantalum (Hedrocel) prosthesis following THA.

DXA affords an opportunity for measuring periprosthetic bone density superior to acetabular implants, which may be important in evaluating patterns of bone ingrowth and risk for implant failure. Longitudinal studies with DXA are needed for further validation of this potentially useful technology.

P306 S

MEASUREMENT OF GRUEN ZONE BMD IN ORTHOPEDIC SCANS USING THE GE LUNAR PRODIGY

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We evaluated the precision of bone mineral density (BMD) measurements from orthopedic scans using the Lunar Prodigy. Low precision error is important for measuring changes in periprosthetic bone density with confidence. Total hip replacement, a popular procedure for treating patients with hips damaged by disease or injury, alters the normal stresses applied to weight-bearing bone of the proximal femur. Subsequent redistribution of bone through remodeling, coupled with cell damage caused by wear debris shed from the implant, often results in substantial bone loss surrounding the implant and implant loosening. Both outcomes have a negative impact on prosthesis longevity and can cause difficulties for revision surgery. Dual-energy x-ray absorptiometry (DXA) precisely quantifies bone mineral density (BMD) adjacent to femoral prostheses in vivo, and is useful for measuring bone remodeling changes and bone loss longitudinally.

Twenty-five subjects with total hip implants were studied. Orthopedic scans were performed twice with interim repositioning with the Lunar Prodigy, a narrow-angle, fan-beam densitometer (GE Medical Systems). Orthopedic analysis software automatically positioned regions-of-interest at the seven Gruen zones around the implant to assess periprosthetic bone loss. Precision of Gruen zone BMD was calculated as the standard deviation (SD) and coefficient of variation (CV). The number of months required to detect a significant change in BMD was also calculated, assuming a conservative loss of 10% over 6 months.

Precision of 1% to 3% at all Gruen zones with the Lunar Prodigy was as good as or better than precision of 2% to 5% shown previously with pencil-beam systems [1,2]. Precision errors were considerably less than the BMD loss of 11% to 21% typically seen during the first six months following implantation [1], allowing rapid detection of bone loss in 2-5 months in this at-risk population.

1. J. Arthroplasty 1996;11:184-193. 2. Clin Orthop Res 1998; 352:66-74.

Gruen Zone	Mean BMD (g/cm2)	SD	CV	Time to Detect BMD Change
1	1.24	0.03	2.7%	4.5 months
2	2.11	0.03	1.6%	2.7 months
3	2.21	0.03	1.5%	2.6 months
4	2.08	0.03	1.3%	2.2 months
5	2.12	0.04	1.7%	2.8 months
6	1.83	0.04	2.0%	3.4 months
7	1.22	0.04	3.2%	5.4 months

P307 W

SPINE BMD MEASUREMENTS WITH AND WITHOUT CONVENTIONAL LEG ELEVATION

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Current procedure for measuring lumbar spine bone mineral density (BMD) using DXA requires the legs to be elevated to flatten the lower spine, with the intent of yielding more accurate and precise BMD values. We investigated whether not elevating the legs has a significant impact on spine BMD accuracy and precision.

Forty subjects were scanned six times at the lumbar spine (Lunar Prodigy, GE Medical Systems). Three scans used standard positioning with the legs elevated on the spine block positioner supplied by the manufacturer. The other three scans were done without the spine positioner, with the legs flat on the scan table and feet secured with the dual femur positioner. Subjects were repositioned between the scan positions. The influence of leg position on L1-L4 BMD was compared using ANOVA, and the precision error (%CV) was calculated for both positions.

There were no appreciable visual differences between the scans performed with the legs up or down; disc spaces and vertebral bodies appeared identical. There was a small, but statistically significant, difference of 1.5% in L1-L4 BMD between legs up and legs down values ($p < 0.01$). In most subjects, BMD values were higher with the legs down. Precision was excellent in both positions (0.96% CV vs. 0.92% CV, legs up and down, respectively).

Linear regression analysis confirmed that BMD measurements with legs up and down were highly correlated ($r = 0.99$), with slope near unity (0.93), an intercept near zero (0.07) and an SEE of 0.018 g/cm2 (1.8%). Because of the high correlation between BMD values, mathematical adjustments were determined to produce comparable T-scores between the two measurements. After adjustment, T-scores were virtually identical in scans performed in the two positions.

We conclude that differences in BMD from measuring the spine with legs positioned for a femur scan are small (1% to 2%), and there is no effect on precision. With appropriate adjustment, T-scores for spine scans measured with the legs down are equivalent to those obtained with legs up. Use of a legs-down position for spine scans would result in considerable time saved for both patient and clinic.

P308 F

AUTOMATED SCAN ANALYSIS: COMPARISON OF TWO FAN-BEAM BONE DENSITOMETERS

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The time involved in acquiring and analyzing bone densitometry scans has been reduced from as much as 30 minutes with first generation densitometers to just a few minutes with current fan-beam densitometers. Automated scan analysis is a significant factor in decreasing user intervention, and increasing precision and throughput. The success of automated scan analysis can be measured in the number of user interventions required to complete an analysis.

In this study, we quantified the number of user interventions required by a trained technologist to complete an automated analysis of lumbar spine scans with two fan-beam densitometers, the Lunar Prodigy (GE Medical Systems) using 'SmartScan and AutoAnalysis' and the Delphi (Hologic) using 'One Time AutoAnalysis'. A user intervention was defined as any action other than a visual review taken by the technologist during a spine analysis. Examples of user interventions included adjustment of intervertebral space markers, adjustment of vertebral body edges, and correction of vertebral body labels. The presence or absence of user intervention was recorded for 21 subjects scanned with Prodigy and 33 different subjects scanned with the Delphi.

User intervention was required in 14% (3 of 21) of Prodigy scans and 47% (15 of 32) of Delphi scans. Post hoc analysis showed that there was no significant difference in patient heights and weights between scan groups, but patients scanned with the Prodigy were older (70 years vs. 61 years of age) than those scanned with the Delphi. There was, however, no difference in age between subjects whose scans required intervention and those that required no intervention, indicating that age was not a factor in user interventions.

We conclude that automatic spine scan analysis with the Prodigy requires less user intervention than automatic analysis with the Delphi. Increased automation of scan analysis could provide more accurate and consistent results when new or multiple technologists perform the analysis.

P309 S

PATIENT-SPECIFIC DXA BMD INACCURACIES: THE QUANTITATIVE EFFECTS OF NON-UNIFORM EXTRA-OSSEOUS FAT DISTRIBUTIONS

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Patient-specific dual-energy X-ray absorptiometric (DXA) in vivo bone mineral areal density (BMD) measurements have been demonstrated to be inherently inaccurate even when extra-osseous fat (F) and lean muscle tissue (L) are uniformly distributed throughout the scan region of interest (ROI). The present work has quantitatively evaluated the extent to which clinically realistic soft tissue inhomogeneities external to the bone within the DXA scan ROI affect patient-specific in vivo BMD measurement inaccuracies. The results are particularly relevant to patient-specific lumbar vertebral and some proximal femoral sites. Actual Norland, Hologic, and Lunar DXA scans and corresponding dual-energy X-ray absorptiometric simulation studies of the same set of 225 different phantom arrays that were absorptiometric replications of bone mineral material (B), red marrow (RM) and yellow marrow (YM) mixtures, and extra-osseous F and L combinations spanning the anthropometric range encountered clinically. It was found that only relatively small extra-osseous soft tissue inhomogeneities within the ROI of DXA BMD scans can increase substantially the already sizable BMD inaccuracies shown earlier to pertain for uniformly distributed extra-osseous soft tissues. The extent of these in vivo BMD inaccuracies is shown to depend upon the marrow thickness and its specific composition, the mean extra-osseous F-to-L areal density ratio and its degree of non-uniformity within the local bone scan ROI, and the actual bone mineral areal density present in any given case. It was found that patient-specific DXA-measured in vivo BMD inaccuracies can, in many cases, exceed 30%-50% clinically, particularly for osteopenic, osteoporotic, and elderly patients, the very individuals for whom it is most important for DXA to assess BMD accurately. As these DXA in vivo BMD inaccuracies are unavoidable and exceed considerably the relatively small DXA precision errors (~1%), they can confound patient-specific diagnoses, evaluations of fracture risk, assessments of the efficacy of prescribed antiresorptive therapies, and, in prospective studies, mask or exaggerate clinically significant true changes in bone mineral areal density of a given patient. It is concluded that DXA-measured and actual BMD values are not necessarily synonymous and that diagnoses/prognoses of bone fragility and evaluations of bone responsiveness to treatment of individual patients based mainly on DXA in vivo BMD measurements are subject to substantial uncertainty.

P310 W

BONE MINERAL DENSITY ORDERING PATTERNS IN FEMALE AND MALE PRIMARY CARE PHYSICIANS FROM A SINGLE MANAGED CARE ORGANIZATION IN THE MIDWEST

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We provide osteoporosis care for patients in a managed care system of three hospitals and 9 primary care clinics in St. Paul, MN. The population served includes 26,000 women age 45 or greater and 6,022 men age 65 or greater. Despite satisfactory insurance coverage, accessibility and system-approved guidelines, the bone mineral density (BMD) service is underutilized.

We sought to identify the BMD ordering patterns by individual physician and physician gender in our group of 66 primary care physicians. We hypothesized that female physicians were ordering more BMD tests than their male partners.

There were 26 female physicians practicing Family Practice (FP) (n=21) or Internal Medicine (IM) (n=5) and 40 male physicians (25FP, 15IM). Data was collected for the period September 1, 2001 through August 31, 2002. Female physicians saw a total of 9,666 female patients >45 years and 1048 male patients >65 years. Male physicians saw a total of 14,198 female patients >45 years and 4,994 male patients >65 years. There were 5,205 patients who saw both male and female physicians during the study period.

Physicians ordered 2,236 BMD tests during the test period. Female physicians ordered 133.5 DXA scans per 1000 female patients, while male physicians ordered 60.6 DXA scans per 1000 female patients ($p<.001$). Female physicians ordered 20 DXA scans per 1000 male patients and male physicians ordered 12.8 DXA scans per 1000 male patients ($p=.071$). There was a trend for a small number of physicians to be high volume users of DXA and the remainder of physicians to be low volume users. The top 3 (14%) ordering female physicians and 5 (12%) male physicians ordered 33% of all BMD tests ordered by their gender group.

This data suggests that female FP and IM physicians order significantly more DXAs for their female patients than do their FP and IM male counterparts. There was a non-significant trend for female physicians to order more DXAs for their male patients. Both physician gender and unknown physician-specific factors influence DXA ordering practice. There are barriers contributing to resistance in ordering BMD testing in both genders that need further clarification.

P311 F

COMPARISON OF PQCT AND DXA ANALYSIS FOR ESTABLISHMENT OF OSTEOPOROTIC MODEL IN PROXIMAL FEMUR OF MATURE OVARIECTOMIZED RATS

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Aim: To compare pQCT with DXA in establishment of osteoporosis induced by ovariectomy(Ovx) in proximal femur(PF) of mature ovariectomized rats.

Methods: Sixteen 8-month-old Wistar rats were randomized into Ovx and Sham group. All rats were euthanized 3 months after surgery. The left femur of each rat was excised for the following evaluations at the PF: 1)To compare the reproducibility(precision error) of the repeated measurements of the PF between pQCT(volumetric-densitometer) and DXA(areal-densitometer); 2)to compare the differences in the measurements of the PF between Ovx and Sham group, including BMC, BMD, bone geometry and their correlations.

Results: (1) The precision error of pQCT-total volumetric BMD(vBMD) and trabecular vBMD were 2.27% and 2.00% respectively, while that of DXA-areal BMD(aBMD) was 3.36%. (2) The pQCT-total and trabecular vBMD in Ovx rats was 8.2% and 15.0% lower than that in Sham rats($p<0.01$), while the aBMD measured by DXA in Ovx rats was only 3.0% lower than that in Sham rats ($p>0.05$). The pQCT-total BMC in Ovx rats was only 3.7% lower than that in Sham rats($p>0.05$), whereas the pQCT pure trabecular BMC in Ovx rats was 11.4% significant lower than that in Sham rats($p<0.05$). (3)The correlation of total BMC measured by DXA and pQCT was high($r=0.82$, $p<0.001$). The correlation was found between DXA- projectional area(PA) and pQCT-volume($r=0.52$, $p<0.05$), whereas no correlation was found between DXA-aBMD and pQCT-vBMD. A positive correlation was found between DXA-BMC and aBMD($r=0.72$, $p<0.05$), whereas no correlation was found between DXA-PA and aBMD. A negative correlation was found between pQCT-volume and total vBMD($r=-0.57$, $p<0.05$), whereas no correlation was found between pQCT-total BMC and total vBMD.

Conclusion: This study revealed that pQCT had higher measurement reproducibility than DXA. pQCT demonstrated its capability in detecting faster bone loss induced by Ovx, particularly more significant in the pure trabecular bone compartment.

P312 S

IMPACT OF DEGENERATIVE RADIOGRAPHIC ABNORMALITIES ON BONE MINERAL DENSITY OF LUMBAR SPINE IN ELDERLY WOMEN

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Degenerative diseases of lumbar spine commonly occur among elderly persons, which may possibly affect the accuracy of bone mineral density (BMD) of lumbar spine. The aim of this study is to determine whether BMD of lumbar spine is related to the degree of degenerative diseases. This study included six hundred and thirty women aged 60 or above (mean age: 73.3 ±6.9 years) visiting the Osteoporosis Outpatient Clinic in Tokyo Metropolitan Geriatric Medical Center. Subjects underwent anteroposterior and lateral X-ray of the lumbar spine including L1 to L5. The radiographs were read to identify the presence and severity of osteophyte (Nathan classification), osteoarthritis (Kellgren method), bone sclerosis, joint space narrowing and spondylolisthesis (Meyerding method) involving L1-2 through L4-5 interspaces. Within one month after taking X-ray, BMD of L2-L4 anteroposterior lumbar spine and femoral neck were measured. The relation between BMD and the score of each item was assessed by regression analysis. Among 630 subjects, 619 (98.3%) had degenerative diseases of lumbar spine. BMD of femoral neck was correlated with age, however, BMD of lumbar spine was not correlated with age. The scores of osteophyte, osteoarthritis, bone sclerosis, joint space narrowing and spondylolisthesis were positively correlated with BMD of lumbar spine, however, they had no correlation with BMD of femoral neck. In multiple regression analysis with age, BMI and all items of degenerative diseases, only BMI, osteophyte, bone sclerosis and joint space narrowing were independently correlated with BMD of lumbar spine. According to the result of the multiple regression analysis, the adjusted lumbar spine BMD of the subjects who had mean score of degenerative diseases was 0.122mg/cm^2 lower than observed BMD. Unlike observed BMD of lumbar spine, the adjusted BMD of lumbar spine was correlated with age. This study suggests that degenerative diseases of lumbar spine are important sources of BMD overestimation at this site, contributing to misdiagnosis. We conclude that BMD of lumbar spine was related to the degree of degenerative diseases.

P313 W

HIGH PREVALENCE OF VITAMIN D DEFICIENCY AND REDUCED BONE MINERAL DENSITY IN MULTIPLE SCLEROSIS

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Objective: Multiple sclerosis (MS) is a chronic disease and a major cause of disability in young adults. MS patients are at high risk for osteoporosis because of disease associated immobility, low sun exposure and corticosteroid (CS) use. This study proposed to assess bone mineral density (BMD) at various sites and vitamin D (vit-D) status in MS patients compared to healthy age-and sex-matched controls.

Methods: Thirty-one patients (19F,12M, mean age 38.2) with MS and 30 (20F, 20M, mean age 36.4) matched healthy controls included into the study. Kurtzke Expanded Disability Status Scale (EDSS) in patients and Nottingham Health Profile (NHP) in both groups were used to scale disability and quality of life. Cumulative steroid dose (CSD) was calculated for every patient equivalent to prednisolone in mg. Serum 25(OH)vit-D levels were measured with ELISA using appropriate kits. BMD of lumbar spine, femoral neck, Wards, and trochanter regions were measured using DXA methodology on a Lunar DPX bone densitometer.

Results: MS patients had significantly lower BMD at lumbar spine (L2-L4, 1.08 vs 1.18 g/cm^2, $p=0.006$), femur trochanter (0.93 vs 0.99 g/cm^2, $p=0.03$) compared to matched controls. BMD of the lumbar spine was nearly 1 SD (mean -0.82 in F, and mean -1.2 in M) lower in MS patients compared with the healthy reference population (Z scores). MS patients had significantly lower vit-D levels (17.3 microg/l vs 43.1 microg/l, p less than 0.001) compared to controls and 19 patients (61 %) had a serum level of vit D less than 20 microg/l which was considered a deficient status. EDSS scores were inversely correlated with femoral BMD (femur neck,r:-0.66, Wards r:-0.60, and trochanter r:-0.54, p less than 0.001) but not with spinal BMD in patients. There was a negative correlation with CSD and BMD only for femur trochanter BMD (r:-0.38, p=0.04).

Conclusion: BMD was significantly reduced and vit D deficiency is prevalent in MS patients. There is a close relationship between patients' disability or mobility status with femoral BMD. Vitamin-D supplementation and rehabilitation programs might have crucial importance in preventing osteoporosis and fractures in MS.

P314 F

THE ROLE OF DUAL FEMUR BONE DENSITY MEASUREMENT IN LOW IMPACT FRACTURES

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There is high correlation documented between the left and right femoral bone mineral densities in the normal population. This suggests that dual femur measurements are not justified in clinical practice. This study evaluates whether this premise holds for subjects who have lost bone mass and have sustained fractures with minimal trauma. 78 women aged 31-81 years (mean=66 years) with previous low impact fractures had both proximal femora measured using dual energy x-ray absorptiometry.

There was significant correlation between values in the left and right total hip (TH) ($r=0.95$; $p<0.05$) and left and right femoral neck (FN) ($r=0.90$; $p<0.05$). The mean differences between the left and right TH and FN densities were not significant. However, the range of the limits of agreement for the TH (-0.074 to 0.086g/cm2) and FN (-0.115 to 0.105g/cm2) are greater than the 95% confidence interval for true change for the TH (0.05g/cm2) and FN (0.07g/cm2). Any longitudinal BMD assessment, therefore, needs to measure the same proximal femur to get a reliable comparison. A one-tail analysis shows 7.5% of subjects with a T-score discordance greater than or equal to 0.5 and 0.5% greater than or equal to 1 for the TH. For the FN, 9% have a T-score discordance greater than or equal to 0.5 and 2.5% greater than or equal to 1.

The use of dual femur measurements increases the diagnostic yield by about 10% in subjects with prior minimal trauma fractures.

P315 S

WITHDRAWN

P316 W

BONE MINERAL DENSITY IN HEMIPLEGIA

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The risk of hip fracture after stoke is 2 to 4 times as high as that in a reference population. these fractures usually occur relatively late after stroke onset and affect the paralyzed side. disuse osteoporosis may be an important factor predisposing to fracture in stroke patients. The objectives of this study were to determine whether there are differences in bone mineral density(BMD) between paralyzed and non-paralyzed sides in hemiplegia and to determine whether the difference in activities of daily living(ADL) levels result in a difference in BMD

We studied thirty stroke patients with hemiplegia. patients were divided into two groups according to the ADL level. BMD was measured at the blateral sides of femoral neck with Dual energy x-ray absorptiometry(DEXA).

BMD was significantly less on the paralyzed the non-paralyzed side of stroke patients with hemiplegia, especially in low ADL group.

The percentage difference in BMD between paralyzed and non-paralyzed side was significantly greater in low ADL group than in low ADL group.

We concluded that increasing the level of ADL through the earlier rehabilitation program would reduce the progression of secondary osteoporosis in stroke patients with hemiplegia.

P317 F

WHICH BONE DENSITOMETRY IS CLINICALLY USEFUL FOR MONITORING THE BONE MASS?

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Long-term precision is more important for monitoring bone mineral density (BMD) in response to aging or therapy than short-term precision. The long-term precision error for the individual subject is estimated by standard error of estimate (SEE), and it can be expressed as follows: long-term CV=SEE/mean x 100 (%). Since the sensitivity is important as well as the reproducibility for the monitoring, the long-term precision should be taken into the consideration with the range of bone loss, which was expressed as standardized long-term precision (SCV). SCV was estimated as simple long-term CV divided by annual bone loss rate. To investigate which bone densitometry is clinically useful for monitoring osteoporosis, we evaluated the SCV of several BMD measurements in healthy Japanese women. Totally 83 women, including 8 pre-, 22 peri-, 26 early post-, and 27 late post-menopausal women, visited our institute for annual measurement of BMD, at an exact interval of 12 months, for five or six times. Follow-up measurements of DXA on the lumbar spine and radius including ultradistal (UD) and 33% distal (33%) (EXP5000) and calcaneus (HeelScan), that of pQCT on the radius (Densiscan1000), and that of quantitative ultrasound (QUS) on the calcaneus (Achilles+) were obtained. Based on the simple long-term CV of spinal DXA, those of SOS and the diaphyseal pQCT were significantly greater, while those of the radial UD DXA and heel DXA were significantly poorer. The simple long-term CV of other measurements were statistically the same as that of spinal DXA. The spinal DXA (-1.68 %/year), the radial UD DXA (-2.16) and pQCT at both the distal metaphysis (-3.07) and diaphysis (-1.72) showed high rate of annual bone loss. The annual bone loss rates of QUS (-0.06~-0.39) and of heel DXA (-1.04) were significantly lower than that of spinal DXA. The SCV on the spinal DXA (1.20), and pQCT of the radial trabecular bone (0.96) and diaphysis (0.66) by Densiscan were great enough to monitor bone mass in comparison with radial DXA (UD:1.47, 33%:1.53), heel DXA (2.38), stiffness (3.87), SOS (5.17), and BUA (4.69).

P318 S

BONE MINERAL LOSS IN EARLY AND POST MENOPAUSAL INDIAN WOMEN

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Osteoporosis is a chronic, progressive condition associated with microarchitectural deterioration of bone tissue that results in low bone mass leading to bone fragility that consequently increases the susceptibility to fractures. With increasing population of elderly women, the assessment and treatment of postmenopausal osteoporosis has become an important problem in clinical gynecology. Postmenopausal osteoporosis develops asymptomatically and leads to fractures in the femoral neck, vertebral bone or distal end of radius and impairs the quality of life of elderly women.

The objective of the present work is to evaluate the bone mineral loss (BML) in postmenopausal women and to study the effect of early menopause and number of years since menopause (YSM). Bone mineral density (BMD) measurement in the lumbar spine (L2-L4) is carried out by dual energy x-ray absorptiometry (DEXA) technique using Lunar DPX bone densitometer at Chennai Osteoporosis Centre. BMD is measured in 42 premenopausal women (mean age 37.5 ±7.5), 52 postmenopausal women (mean age 60.8 ±8.0) and 15 women who had early menopause (mean age 58.3 ±11.2). The data is statistically analysed by simple linear regression and Student's t test. Regression analysis between BMD and age shows a moderately weak negative correlation in premenopausal women ($r = -0.42$, $P<0.001$) with about 5-6% of BML per decade, moderate negative correlation in postmenopausal women ($r = -0.62$, $P<0.001$) indicating 9-10% BML per decade, and strong negative correlation early menopause women ($r = -0.78$, $P<0.001$) with a BML of 11-12% per decade. Average T score is very low in early menopause women (-3.115) compared to pre and postmenopausal women (-0.69 and -1.981 respectively). To study the effect of YSM on BMD, postmenopausal women are grouped in to three categories based on YSM; Group 1 (YSM 1-10), Group 2 (YSM 11-20) and Group 3 (YSM>20). Regression analysis between BMD and YSM shows moderately negative correlation in group 1 ($r = -0.46$), weak negative correlation in group 2 ($r = -0.24$) and no correlation in group 3 ($r = 0.14$).

It is therefore concluded that, women who had early menopause are at a greater risk of developing osteoporosis fracture in subsequent years than other menopausal women and bone mineral loss in the first 5-10 years after menopause is seen to be high.

P319 W

RELATIONSHIP AMONG JAPANESE OSTEOPOROSIS QOL SCORES, BONE MINERAL DENSITY AND PHYSICAL FITNESS LEVEL OF THE AGED IN JAPANESE SUBURBAN COMMUNITY

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The purpose of this study was to investigate the aged persons' properties of the Japanese osteoporosis quality of life (JOQOL) scores, bone mineral density (BMD) and physical fitness level (PFL) at a suburban community in Japan. Subjects were 419 male and 620 female (1039 in total) whose age were over 65 years. They were measured the muscle strength (knee extension, hand grip, numbers of sit up), flexibility (forward reach in the long sitting position), one leg standing time (sec.) and walking capacities (stepping over obstacles and endurance). BMD was measured at the calcaneus by using an ultrasound instrument. The JOQOL was used to assess QOL for 119 females whose BMD were less than 1 SD of YAM. The correlation between BMD and PFL factors was analyzed by multiple correlation. The correlation between JOQOL and PFL factors was also analyzed. The JOQOL consists of 7 categories, such as pain, activities of daily living (ADL), social activities, well being, postural change, fear of falling and psychological factors and family support, and evaluates using the total scores of these categories. The results indicated that BMD was significantly

correlate to knee extension strength (r=0.2557, p=0.000), hand grip (r=0.1972, p=0.000), sit up (r=0.0827, p=0.026), flexibility (r=0.1247, p=0.001), one leg standing time (r=0.2594, p=0.000), stepping over obstacles (r=0.3104, p=0.000) and endurance (r=0.3134, p=0.000). Total score of the JOQOL was significantly correlate to knee extension (r=0.2806, p=0.024) and sit up (r=0.2621, p=0.035). In categories of the JOQOL, pain was significantly correlate to hand grip (r=-0.2725, p=0.025). ADL was significantly correlated to knee extension (r=0.31690, p=0.009), sit up (r=0.2506, p=0.039), one leg standing (r=0.3918, p=0.001), stepping over obstacles (r=0.3841, p=0.001) and endurance (r=0.2902, p=0.016). Social activities were significantly correlated to endurance (r=0.2787, p=0.021). Well-being was significantly correlated to flexibility (r=-0.3027, p=0.012). Fear of falling and psychological factors was significantly correlated to hand grip (r=-0.2449, p=0.046) and sit up (r=0.2533, p=0.039). In conclusion, BMD and QOL relate to muscle strength, flexibility, one leg standing time and walking capacities. Therefore, to keep and improve those PFL factors is important to prevent osteoporosis and to maintain QOL.

P320 F

LUMBAR SPINE BONE MINERAL CONTENT MEASUREMENT IS MORE RELIABLE THAN BONE MINERAL DENSITY: A STUDY ON 594 GIRLS WITH ADOLESCENCE IDIOPATHIC SCOLIOSIS

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Objectives: Osteopenia has been reported in girls with adolescent idiopathic scoliosis (AIS). However, the projectional area of the spine measured by dual energy X-ray absorptiometry (DXA) could be affected by the associated rotational deformity in scoliosis and thus the bone mineral density (BMD) value. The aim of this study was to evaluate the bone mineral status of in AIS patients by measuring the rotation independent BMC of the lumbar spine and compare the results with the BMD data and normal controls.

Methodology: 600 AIS girls, aged 11-16, with at least 10 deg Cobb's angle radiographic measurement in the coronal plane, were included in this study. BMC and BMD of lumbar spine (L2- L4) were recorded with Norland XR-36 DXA machine. Age and sex-matched normal reference range was used for comparison.

Results: Z-score BMC and BMD, standardized with age-matched control, of the lumbar spine were used for the analysis. 23.2% of AIS were classified as low bone mass, i.e. BMC z-score below minus 1SD and 1.4% of AIS were found to have extremely low bone mass, with BMC z-score below minus 2.5SD. 29.6% of AIS were found to have BMD z-score below minus 1SD and 2.9% of them below minus 2.5SD.

Discussion and Conclusion: The present study showed the overestimated percentage of osteopenia cases if BMD were used as diagnostic criteria instead of BMC (29.6% for BMD vs. 23.2% for BMC). BMC should be recommended for the measurement and monitoring of the bone mineral status of patients with scoliosis as it not affected by spinal rotation in projectional DXA BMD measurement.

P321 S

COMPUTER ASSISTED DENSITOMETRY: AUTOMATED ASSESSMENT FOR DXA LUMBAR SPINE ANALYSIS

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Bone densitometry systems automatically analyze lumbar spine scans and calculate bone mineral density (BMD). However, some vertebrae with unusual BMD characteristics due to osteoarthritis, fracture, or other conditions, should be excluded from analysis. Computer Assisted Densitometry (CAD) provided by the Lunar Prodigy (GE Medical Systems) helps the user determine when to review a scan and exclude vertebrae from analysis and diagnosis. We studied various criteria for the assessment of lumbar spine scans.

Visual examination of 231 female spine scans measured with Prodigy found confounding conditions that could influence results: focal areas of unusually high BMD (n=63), high density due to end-plate calcification (n=11), collapsed or crushed vertebrae (n=8), unusual spinal curvature (n=2). Exclusion criteria proposed recently include: a) focal structural abnormalities, b) failure of BMC or area to increase from L1 to L4, c) T-score differences >1 SD between adjacent vertebrae [1]. We studied the ability of three criteria to identify vertebrae with confounding conditions: 1) T-score differences between adjacent vertebrae, 2) T-score differences between L1-L4 average and individual vertebrae, 3) increase in BMC and Area with each vertebral level.

Results showed the best criterion for identifying vertebrae with confounding conditions was a T-Score difference >0.9 SD between L1-L4 mean and individual vertebrae, with specificity and sensitivity of 82%. We conclude that a criterion based on T-score differences provides the best method to identify spine scans for review. CAD programs with sensitive exclusion criteria should improve accurate clinical diagnosis of osteoporosis.

1. J Clin Densitometry 2002:5 (Suppl) S11-S17.

	Adjacent T-Score Differences			**L1-L4 vs. Individual T-Score Differences**			
	T>1.0	**T>1.5**	**T>2.0**	**T>0.7**	**T>0.8**	**T>0.9**	**T=>1**
Specificity	64%	93%	98%	61%	72%	82%	91%
Sensitivity	83%	56%	21%	89%	81%	82%	68%
	L1 to L4 Trend						
	BMC up-up-up		**Area up-up-up**	**BMD up-up-up**		**BMD up-up-down**	
Specificity	68%		69%	26%		43%	
Sensitivity	43%		38%	70%		65%	

Table 1: Sensitivity of exclusion criteria based on t-score differences and on l1 to l4 trend

P322 W

PRELIMINARY STUDY FOR DIAGNOSIS AND TREATMENT OF CORTICOSTEROID-INDUCED OSTEOPOROSIS IN JAPANESE PATIENTS

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[Objective] To clarify diagnostic criteria and efficacy of treatment for corticosteroid-induced osteoporosis in Japanese patients.

[Subjects and Methods] One hundred and ninety three patients (167 females and 26 males) with connective tissue diseases who took corticosteroids more than 5mg/day (equivalent to prednisolone) were subjected in this longitudinal study for 2 years. The mean of age, daily and total corticosteroid dosage were 50 years old, 9.3 mg/day, and 21.9g, respectively. Bone mineral densities (BMD) of the lumbar spine were measured by a dual energy absorptiometry (DXA), and an incidence of vertebral compression fracture was analysed by morphometric measurements with X-ray of the thoratic and lumbar spines. Selection of therapeutic agents for osteoporosis was non-randomized.

[Results] 1) A diagnostic value of BMD was analysed in patients without therapeutic agents for ospteoporosis (n=99). An incidence of the morphometric fracture in 2 years was 40%. A relative risk for the incidental fracture was increased to more than 2 in patients with the YAM of 71 to 80% compared to those with 91 to 110%. An abosolute risk for the latter group, however, was very high (19%). ROC analysis revealed 77% of the YAM as an most effective cut-off value for the prediction of the incidental fracture. In patients with more than 10mg/day of prednisolone, the cut-off of 90% of the YAM was more accurate. 2) Logistic regression analysis in all subjects showed that independent risk factors for the incidental fracture were the age (every 10 years old increase, odds ratio (OR) 1.86, p=0.0007), the corticosteroid dosage (every increase of 5mg/day, OR 1.96, p=0.0006), BMD (every 5% decrease in YAM, OR 1.27, p=0.004), deformities of the spine at the entry (OR 7.92, p=0.0009), and male (OR 3.52, p=0.04), and that independent protecitive factors were some of the therapeutic agents (Menatetrenone, a synthetic vitamine K analog, OR 0.04, p=0.0002; Etidronate, OR 0.02, p=0.0001).

[Conclusions] In Japanese, corticosteroid-induced osteoporosis could be diagnosed in patients with less than 80% of the YAM. Increased dosages of corticosteroids, however, might increase the cut-off value. Etidronate and Menatetorenone would be effective for the treatment of corticosteroid-induced osteoporosis.

P323 F

THE EFFECT OF BONE STRUCTURE ON PATIENT-SPECIFIC DXA IN VIVO BMD INACCURACIES

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An extended exposition is developed of the effects of bone structure on the form and extent of systematic inaccuracies in planar dual-energy X-ray absorptiometry (DXA) in vivo bone mineral density (BMD) measurement methodology. It is shown that sizeable and unavoidable patient-specific DXA in vivo/in situ BMD inaccuracies are inherent to the methodology and arise directly from anthropometric and X-ray absorptiometric disparities among the different tissues present within the scan region of interest (ROI) of the interrogated bone site. Criteria governing these BMD inaccuracies are derived for the relevant absorptiometrically distinguishable extra- and intra-osseous soft tissues present in every in vivo /in situ bone site (lean muscle tissue, interposed and admixed fat, and red/yellow marrow combinations). Different magnitudes and patterns of BMD inaccuracies are shown to pertain for bone structures that are (i) purely trabecular, (ii) wholly cortical, and (iii) those containing both cortical and trabecular bone. Explicit expressions for absolute and percentage BMD inaccuracies are derived and delineated. It is also demonstrated that typical, clinically encountered ranges of anthropometric and X-ray absorptiometric disparities between the extra-osseous soft tissues and the ubiquitous marrow within the bone structure lead to patient-specific BMD inaccuracies that can readily exceed 20% of the actual value of BMD present in the scan ROI. Due to the trend of natural anthropometric changes

in post-menopausal women, and older men/women, these BMD inaccuracies can be even more severe, and for many osteopenic/osteoporotic individuals (the very patients it is most important for DXA to assess correctly) rise into the range of 30% - 50%. The effects of bone structure on these BMD inaccuracies are found to further confound and complicate the clinical situation in ways not previously elucidated. The present findings demonstrate that a clear clinical distinction must be made between DXA-measured and actual BMD, as the two are far from being synonymous in any given in vivo/in situ case. These inherent, systematic, substantial inaccuracies in patient-specific DXA BMD methodology readily conduce to conclusions (diagnoses, prognoses, prospective results, etc.) that can be flawed and seriously misleading.

P324 S

AN ASSESSMENT OF OSTEOPOROSIS FOLLOWING STROKE

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Background: Hip fracture after stroke is a major concern because of the associated morbidity and mortality. Reduced bone mineral density (BMD) and increased risk of falls may have important predisposing factors. Immobilization due to hemiplegia leads to hemi-osteoporosis, but few studies have been carried out to identify the patients who are already at risk of osteoporosis and fracture prior to stroke rehabilitation.

Aim: To find out the proportion of patients who were already at risk of osteoporosis when they transferred to the rehabilitation ward following stroke, by measuring bone mineral density (BMD) and bone resorption.

Subjects & Methods: All stroke patients consecutively admitted the rehabilitation ward, at the rural hospital, Japan between September 2000 and November 2001 were included if they met inclusion criteria. Data were collected retrospectively from patient's hospital records. BMD was measured using dual-energy X ray absorptiometry (DEXA) (Hologic, QDR 4500) at both hips (paretic side and non-paretic side) and lumbar spine. In addition to routine biochemical analysis (e.g. Ca, P, ALP), urinary deoxypyridinoline (Dpd) was assessed as a bone resorption marker.

Results: 81 stroke patients (38 male and 43 female) with a mean age of 66.2 (range 31-88, SD=11.4) years were eligible for the retrospective study. Mean time from onset of stroke to investigation was 42.2 days. Approximately 40% of all stroke patients were osteoporosis with a T score of hip BMD of -2.5 SD or more below. The level of hip BMD was decreased with age in both male and female patients. There were no differences in BMD between paretic side and non-paretic side. With regard to bone resorption, the mean Dpd was 10.9 nmol/mmol Cr in male (normal range 2.1-5.4) and 19.8 in female (normal range 2.8-7.6) patients.

Conclusion: There were many stroke patients who were already osteoporosis. Increased bone resorption was seen in both male and female patients. This descriptive study suggested the need for attention to osteoporosis in sub-acute stroke patients. In addition to ordinary stroke rehabilitation, extra approaches such as falls prevention and pharmacological therapies may be needed for such patients in order to prevent post-stroke fracture.

P325 W

POSSIBILITY OF PERFORMING BMD USING CONVENTIONAL CT SCANNERS

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Introduction: Importance of the early diagnosis of osteoporosis leads to the establishment of several methods in this field. One of the recent methods is peripheral Quantitative Computed Tomography (pQCT), in which, axial images of radius and ulna are used for quantitative determination of bone density. In contrast with Dual Energy X-Ray Absorptiometry (DEXA), in this method trabecular and cortical bone can be evaluated separately. The advantage of this method is the possibility to show early stages of metabolic change in bone. Since pQCT scanners are not available in most undeveloped countries, the most available system is DEXA. QCT can be used for lumbar spine Bone Mineral Densitometry (BMD), in which a high radiation effective dose (250 μSV) is delivered to patient.

Materials & Methods: The aim of this work was to study the possibility of doing pQCT using conventional CT Scanner. For this propose, a radius-ulna phantom was constructed using Plexiglas, Aluminum and K_2HPO^4 solution, cortex and trabecular section of radius and ulna were contracted using 0.5 mm thick aluminum plate and 100 mg/cc K_2HPO^4 solution respectively, on which 43 axial computed tomography were performed by a GE 9800HR CT scanner.

Result: Our results showed that the coefficient variation (CV) and the accuracy of the technique were 1.5% and 1.2% respectively. The slope of scanner at 80kVp, 20mA and scan time of 3 seconds was to be within the concentration rang of 10 to 400 mg/cc was obtained to be 2.01 for the K_2HPO^4 solution.

Discussion: Our experimental study showed that conventional CT Scanners are potentially suitable for doing pQCT. However further in vivo study is needed for clinical approval.

P326 F

MUSCLE-BONE RELATIONSHIPS AS ASSESSED BY DEXA IN THE WHOLE BODY AND LIMBS OF 1,800 NORMAL MEN AND PRE- AND POST-MENOPAUSAL WOMEN

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In whole-body studies with DXA [Ferretti et al; Bone 22:683,1998, n = 1,450] we had shown that the densitometric mineral mass, either crude (BMC) or statistically adjusted to fat mass (FA-BMC) in order to avoid any fat interference with its determination, correlated linearly with the lean mass (LM) showing similar slopes but decreasing intercepts in the order: pre-MP women > men > post-MP women > children. This evidenced 1. the homogeneous control of bone status by muscle strength in the species through the bone mechanostat, and 2. the interaction of sex hormones with that regulation. Now we aim to expand that evidence by studying 1,900 normal Hispanic adults (60men, 600 pre-menopausal women, 1,800 post-menopausal women), including also the same determinations in the upper and lower limbs.

In all the studied regions the slopes of the BMC or FA-BMC vs LM relationships were always parallel. However, interesting region-related differences were found between the intercepts of the curves. In the whole body, the crude-BMC/LM relationships showed the same intercept differences as previously observed (pre-MP women > men > post-MP women). In the lower limbs the variance of the data was substantially reduced, and those differences were highly significant but lesser in magnitude, showing the order: pre-MP women > men = post-MP women. In the upper limbs 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 maintained in all cases.

The parallelism of the curves suggests a common biomechanical control of bones by muscles in the species. Assuming that LM is proportional to muscle mass, results suggest that the sex-hormone-induced differences in the DXA-assessed muscle-bone proportionality in humans would vary according to the region studied. Assuming also that BMC adjustment reduced the influence of fat on body weight, this could be related to the different weight-bearing nature of the musculoskeletal structures studied. The study design did not account for some gender-related aspects of hand/foot skeletal morphometry which could also help to explain those differences.

P327 S

POSSIBILITY OF SCREENING FOR SYSTEMIC OSTEOPOROSIS USING JAW BONE DATA

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[Introduction] It is very important to screen severe osteoporosis patients before they suffer bone fractures. Recently, it has been reported that osteoporotic fractures relate significantly to certain radiographic findings in the mandible. However, the real bone structural changes in the mandible have not yet been elucidated. In this study, we analyzed ovariectomized monkey mandibles, and compared them with the vertebrae and radii to clarify the relationships among them.

[Materials and Methods] Fourteen adult (9-years-old or older) female cynomolgus monkeys were divided into 2 groups of 7 subjects each. The test group was ovariectomized (OVX), and the controls received sham surgery (Sham). After surgery, periodic measurements were made of serum estrogen and lumbar bone mineral density (BMD) using DEXA. 76 weeks after surgery, all subjects were sacrificed and their mandibles and right radii were excised. Mandibular panoramic radiographs were taken, and the cortical and trabecular BMDs in the right half of the mandibles, as well as the BMD of the proximal radius were measured using pQCT. The bone structures of the mandibles and radii were also observed with micro CT.

[Results] OVX showed a significantly lower percentage change in lumbar BMD (BMD at end of the experiment/ BMD at start of the experiment*100%) than Sham. Mandibular cortical BMD was also significantly lower in OVX than in Sham. Mandibular trabecular and cortical BMDs correlated significantly with lumbar BMD in OVX.

The animal which had the highest lumbar BMD also showed the highest mandibular BMDs. On the other hand, the subject whose lumbar BMD was lowest also showed the lowest mandibular BMDs and had porous mandibular and radial cortices. These pores were clearly seen in the panoramic radiographs as striped shadows in the medio-distal direction inside the mandibular lower cortex. Furthermore, this subject's mandibular and radial trabecular bone was equally porous.

In conclusion, the ovariectomized monkey mandibles showed porotic changes in the cortical and trabecular bone. Mandibular cortex data in particular correlated closely with lumbar porosis. Therefore, information on the mandibular cortical bone seems useful in screening severe osteoporosis patients.

P328 W

PREVALENCE OF OSTEOPENIA AND OSTEOPOROSIS IN 11975 POSTMENOPAUSAL ARGENTINE WOMEN

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Taking into consideration the methodological limitations of the prevalence studies and the lack of information of this issue in our country, we describe the prevalence of Osteopenia/Osteoporosis in postmenopausal women (50 years old or older) participating in a free detection survey with Lumbar Spine (LS) and Femoral Neck (FN) DEXA (OMS Criteria), taking as diagnostic the lowest density region).

After the inclusion/exclusion criteria, were selected 11975 women, mean age: 67 years (50-94), mean menoage: 48 years, and mean BMI: 28.8 Kg/mt2.

Osteopenia prevalence was 65.46 % (n: 7839); of that, 27.9 % was mild (TS: -1/-1.49), 38.3 % was moderate (TS: -1.5/-1.99), and 33.8 % was severe (TS: -2/-2.49).

Osteoporosis prevalence was 15.91 % (n: 1905), and the discrimination by diagnostic region, showed that 52.23 % (n: 995) was only affected at LS, 17.27 % (n: 329) only at FN, and 30.5 % (n: 581) at both regions. This pattern presented some differences between decades of age, but confirm the diagnostic utility of FN in women younger than 65 years old, and for the LS in older women, even after 80 years old.

The prevalence of self-reported clinical fractures (wrist, hip, vertebral) was similar between osteoporosis and severe osteopenia patients (29.73% vs 25.36% p: 0.268)

In conclusion; we found some differences between our prevalence of osteopenia/osteoporosis and the findings of the references studies (probably due to multiple factors; technological, racial, geographic, etc). We reinforce the convenience of the diagnostic evaluation of both densitometric regions (LS/FN) at all ages. We remark the potential mistake of underestimate the fracture risk of severe osteopenia

P329 F

PREDICTORS OF LOW BONE MASS IN YOUNG CHINESE WOMEN

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Introduction: Low peak bone mass is a major risk factor for postmenopausal osteoporosis. Identifying premenopausal women with low bone mass is a cost-effective approach towards prevention of osteoporosis and related fractures in later life.

Method: Demographic information and clinical data were obtained from 544 healthy pre-menopausal Chinese women, aged 18-39 years, who were recruited from the community. Predictors of bone mass were assessed using a standardized questionnaire. Bone mineral density (BMD) was assessed using dual-energy X-ray absorptiometry (DXA) at the femoral neck and lumbar spine. Bone mass was considered low if the T-score was <-1.00, i.e., 1 standard deviation below the peak young mean for the local population.

Results: The mean age of the cohort was 31.9±5.7 years. 19% and 26% of our cohort were classified to be low bone mass at lumbar spine and femoral neck respectively. Multivariate logistic regression model revealed that low body weight was the only independent predictor for low bone mass at both the spine (Odds ratio 5.3, confidence intervals 3.3-8.7, p<0.0001) and femoral neck (Odds ratio 4.4, confidence intervals 2.9-6.8, p<0.0001) while daily weight bearing time of less than 1 hour was an additional risk factor for low bone mass at lumbar spine (Odds ratio 4.0, confidence intervals 1.1-14.2, p=0.03). All other factors including height, menarche age, calcium intake, family history of fracture, use of calcium supplement, contraceptive pill use and smoking or drinking habit were not predictive of peak bone mass in these young women.

Conclusion: Low body weight and lack of weight bearing exercise are the two most important risk factors for low peak bone mass in Chinese women. Early intervention in this group of women may reduce the risk for osteoporosis in later life.

P330 S

COMPARISON OF CHINESE MALE AND FEMALE PHALANGEAL BONE MINERAL DENSITY USING RADIOGRAPHIC ABSORPTIOMETRY

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INTRODUCTION: Assessing Bone Mineral Density(BMD) at the Phalangeal bones using Radiographic Absorptiometry(RA)is a simple and inexpensive procedure.Phalangeal BMD using RA metod has been shown to predict future fracture risk of the hip and spine[1-3].

A atudy was conducted to investigate this method in a normal Chinese population and to aompare the results to those of a larger study using a different method.

METHODS: 583 Chinese population between the ages of 10 to 89 participated in the study. 23 were disqualified because of Diabetes or prolonged use of Corticosteroid and 279 male/281 female with an average of 35 volunteers per 10 year-span age group qualified for normal BMD measurement.

A two view standard x-ray was acquired for the non-dominant hand of each volunteer with an aluminum reference wedge placed near the hand.

Phalangeal BMD was measured using the automated system OsteoGram.

(CpmpuMed,Inc., Los Angeles, California,U.S.A)

RESULTS: The results show a BMD peak at the 25-35 age group for both genders.

Percentage losses from BMD peak were calculated the table showing that BMD loss took place with a higher rate in female. While before peak, female BMD was slightly higher.

The results correlate favorably(Pearson's correlation coefficients r=.89 to.98) with BMD results of the 40,000 Chinese population investigated in the late 80 years using forearm(radius and ulna) Single Photon Absorptiometry(SPA)[4].

Table: Radiographic Absorptiometry Bone Mineral Density results using OsteoGarm and comparison to SPA BMD results

MALE			RA		SPA	
Age	N	Mean	SD	% loss	Rad.	Ulna
10-19	39	89.4	14.4	19.6		
20-29	38	111.2	12.6	0.0	0.700	0.707
30-39	34	109.5	11.3	1.5	0.759	0.759
40-49	34	110.2	9.9	0.9	0.725	0.722
50-59	33	103.9	9.9	6.6	0.691	0.694
60-69	36	93.2	13.0	16.2	0.660	0.659
70-79	33	92.4	11.1	16.9	0.622	0.624
80-89	32	89.9	11.9	19.1	0.562	0.567
RA-SPA correlation:0.89 0.90						
FEMALE			RA		SPA	
Age	N	Mean	SD	% loss	Rad.	Ulna
10-19	35	97.1	17.0	17.1		
20-29	38	117.1	9.5	0.0	0.665	0.658
30-39	36	112.3	9.4	4.1	0.706	0.696
40-49	31	108.5	9.3	7.4	0.670	0.651
50-59	37	96.6	16.2	17.6	0.599	0.578
60-69	35	84.9	11.9	27.5	0.541	0.520
70-79	36	75.1	12.9	35.9	0.467	0.458
80-89	33	66.3	8.7	43.4	0.376	0.384
RA-SPA correlation:0.97 0.98						

P331 W

THE INCIDENCE AND DIAGNOSTIC CRITERIA OF PRIMARY OSTEOPOROSIS IN CHINA

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From over 10 years clinical practice and epidemiological studies, we realize that the appropriate diagnostic criteria are the most important thing among basic research, clinical studies of the prevention and therapy for osteoporosis. In this paper,we will discuss the following two problems for using dual energy X ray absorptiometry(DEXA).

Which part of the bone is the better place to be measured? We can easily find that the results of bone mineral density in different areas are different. One of the reasons is the effect of bone size,shape and the directon. Hip neck is considered to be the best place for BMD measurement and osteoporosis diagnosis,because it has less artificial influences from the analysis based on our data. And we can see that ward's area (early bone loss,>25% at age 50) can not provide the accurate data for osteoporosis diagnosis. For L_{2-4} one should consider that soft tissue calcification can cause artificial high reading.

In 1985, the WHO proposed the following osteoporosis diagnositic criteria: a T-score of 2.0 SD below the mean BMD value for a young adult of the same sex was characterized as osteoporosis.In 1994, Dr.John A.Kanis advanced the following osteoporosis diagnostic criteria:osteoporosis-a value for BMD or BMC more than 2.5 SD below the young adult average value. In Japan,Dr.Orimo and Japanese bone mineral society defined the ostoporosis diagnostic guidelines. If BMD is 70% below the mean value of young adult of the same gender,it is ostoporosis.We suggest the following criteria for Chinese people:the cumulated bone loss>25%than the mean value of young adult of the same gender in Chinese can be diagnosed having ostoporosis.

Based on the above-mentioned results,in China,women over age 60 years and men over age 75 years have osteoporosis. Based on the fifth population census,conducted in 2000, the population of women over 60 years old is 75.57 million; and that of men, over 75 years old is 12.69 million; the total osteoporosis is 88.26 million,with the ratio of men to women,1:6. According to the results of the recent 5-year DEXA survey in connection with the population census in 2000, patients with primary osteoporosis account for 6.97% of the total population.

P332 F

BONE MINERAL DENSTIY IN INDIAN PATIENTS WITH OSTEOPOROTIC FRAGILITY FRACTURES

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Aims: Fragility fractures are the hallmark of osteoporosis. There is large body of evidence from the west demonstrating lower bone mineral density (BMD) in patients with either hip, vertebral or distal radial fracture. The aim of the present study was to study the difference in the BMD in patients with fragility fractures and healthy Indian population.

Material & Methods: A total of 38 female patients with either hip (n=25), vertebral (n=6) or distal radial (n=7) fracture attending one of the authors institute were studied. In addition to demographic data, BMD data was obtained after analysis of the plain radiograph of the non-dominant hand using Digital X-ray Radiogrammetry (DXR) (Pronosco X-posure System V.2). Comparison were done between patients and matched females by decade without fractures. We also studied the difference in the mean BMD between patients and age matched normals and between patients with hip and other fragility fractures. The BMD is expressed as mean±SD in gm/cm^2.

Results: There was significant difference between BMD in patients with and without fractures (0.420±0.06 vs 0.469±0.03;p=0.01). In all the decades above 50 years, patients with fractures had lower BMD compared to healthy age matched norms (50-59 yrs- 0.47±0.05 vs 0.52±0.06; 60-69 yrs- 0.41±0.05 vs 0.48±0.04; 70-79 yrs- 0.39±0.05 vs 0.44±0.05;p=0.02). Patients with hip fracture had a lower BMD compared to those with vertebral or distal radial fracture (0.40±0.06 vs 0.44±0.04;p=0.04).

Conslusions: The results of the present study confirm that Indian patients with fragility fractures have lower BMD compared to healthy age matched patients without fractures. Patients with hip fracture have BMD lower than either vertebral or distal radial fracture. It also demonstrates the ability of DXR to pick up differences in BMD between patients with and without fragility fractures.

P333 S

MANDIBULAR EVIDENCE OF BONE DISTURBS IS A RISK FACTOR TO VERTEBRAL AND FEMORAL OSTEOPENIA AND OSTEOPOROSIS

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Considering the architectural characteristics of the alveolar bone tissue at the mandible,with trabecular and cortical bone constitution, should be expected to occur the same changes in mandibular DMO that aging and menopausa induce on vertebral and femoral bone.

Thirty-nine Caucasoid women, from 48 to 83 years old were studied, considering the correlation between mandibular, vertebral and femoral bone mass.

All of them were submitted to densitometric evaluation (DMO), by means of a DEXA-DPX Lunar equipment, at lumbar spine (L2L4), femoral neck and at three different sites of the mandible.

For each site, a correlation coefficient between the means from the t-scores results (Pearson equation) shows a significant and positive correlation between mandible DMO referred to spine and femur.

So mandibular evidence of bone loss should be used as a predictor factor of vertebral and femoral osteopenia and osteoporosis.

P334 W

PREVALENCE OF LOW BONE MASS IN HEALTHY INDIAN POPULATION

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Aims: Osteoporosis in now recognised as a major health care problem all over the world. There is evidence in literature that osteoporosis may be more common in the Indian population but this was not based on any of the currently available bone mineral density (BMD) assessment techniques. The aim of this study was to assess the prevalence of low bone mass in healthy Indian population based on data analysed using Digital X-ray Radiogrammetry (DXR)

Material & Methods: In an open cross-sectional setting, a total of 261 Indian women and 177 Indian men between 20 and 79 years of age were enrolled to establish the normative reference database for BMD using DXR. Subjects with diseases or those taking medications impacting on bone metabolism and those with history of immobilisation of the non-dominant forearm and hand 6 months prior to enrolment were excluded. A radiograph of the non-dominant hand was obtained under standardised conditions and analysed using the Pronosco X-posure System V.2.

Results: It was found that 29.9% of women and 24.3% of men between 20 to 79 years of age had low bone mass (osteopaenia or osteoporosis by WHO classification). Further analysis revealed that about 50% of women and 36% of men over 50 years of age had low bone mass.

Conclusions: The present study suggests that there is higher prevalence of low bone mass in the healthy Indian population compared to the western population. If one considers the number of women over 50 years (postmenopausal) with additional risk factor together with 50% with low bone mass, the population of elderly Indian women with osteoporosis will be much higher than the west.

P335 F

NORMAL VALUES OF FOREARM BONE BMD AND INCIDENCE OF PRIMARY OSTEOPOROSIS IN CHINESE WOMEN

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In this study we obtained the normative reference data of forearm BMD in Chinese women using a new computerised radiogrammetric analysis system (Pronosco X-posure system), which generates a BMD estimate (DXR-BMD) through scanning of a plain radiogaph of the hand and forearm. DXR-BMD data were obtained in 354 normal Chinese women aged 20-79 years. The peak BMD was found in women between 30-39 years of age (estimated peak BMD=0.5569, SD=0.027,occurring at age 30-39). The incidence of osteoporosis diagnosed on the basis of pcak BMD -2.0 SD in this study population was similar to the prevalence of primary osteoporosis in Chinese women reported by others using osteodensitometry. We conclude that the Pronosco X-posure system can can provide simple,accurate and inexpensive assessment of bone mineral status,making it valuable in the diagnosis of osteoporosis, especially in developing countries.

Age	No.	BMD(g/cm)^2 (mean ±SD)	T-score (-2.0SD) (No.)	Incidence of Primary Osteoporosis (%)
20-29	57	0.530 ± 0.030	4	7.0
30-39	56	0.556 ± 0.027	0	0.0
40-49	59	0.554 ± 0.0-29	1	1.7
50-59	61	0.500 ± 0.045	25	39.7
60-69	61	0.467 ± 0.049	38	62.3
70-79	60	0.429 ± 0.043	55	91.7
Total	354		123	34.8

P336 S

BONE MINERAL DENSITY IN CITIZENS OF BELGRADE

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In Institute of Rheumatology, Belgrade, we examined 667 citizens of Belgrade, randomly selected from population register to estabilsh normal value for bone mineral density (BMD). Acording to criteria of the 'Protocol for Estabilishment of reference Data for Bone Mineral Density' (Lunar Corporation), out of 667 citizens we selected 425 women, aged from 20 to 78 years(X=47,13 ± 11,92) and 82 male, aged from 20 to 77 years (X=46,00 ± 13,04) and subject were stratified per decade of age. BMD of spine was measured by dual-energy X-ray absorptiometry, using a Lunar DPX/L device. The obtained results are considered reference values for women of Belgrade (X=1,166 ± 0,177 gr/cm^2) and are similar with BMD of Caucasian women obtained in studies carried out in other countries of Europe and USA. The reference values for male (X=1, 189 ± 0,192 gr/cm^2) did not established because the number of male (per decade) was not satisfied acording the 'Protocol' .

P337 W

BONE MINERAL DENSITY(BMD) IN SOUTH INDIAN MEN: A STUDY

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Aim: Our study aims to explore the variation of the Bone Mineral Density(BMD) & Bone Mineral Content(BMC) in different age groups of South Indian men.

Method & Materials: 120 apparently healthy South Indian men in 40yrs-80yrs age group were studied.age, height, weight & body mass index (BMI) were recorded. Serum testosterone values were estimated for all the individuals. The bone mineral density (BMD) & bone mineral content (BMC) of spine (L1-L4) & hip were measured

using Hologic Dual Energy X-ray Absorptionmetry (DEXA). The percentage of men suffering from osteoporosis is detected & compared with women of the same age group.

Results: The variation of bone mineral density(BMD) in the different age groups of South Indian men are given in the table below. On comparing the percentage of osteoporosis with South Indian women of similar age groups the following results were obtained.

Age: 41-50years: 11.21% of male & 13% in females were osteoporotic.

Age: 51-60years: 4.76% of male & 8.16% in females were osteoporotic

Age: 61-70years: 9.5% of male & 39.51% in females were osteoporotic

Age: 71-80years: 11.21% of male & 26.31% in females were osteoporotic.

Conclusion: There is no significant difference in the Bone Mineral Density (BMD) among the different age groups of South Indian men.When compared with the similar age groups of South Indian women the prevalence of osteoporosis in men is less common.There is no correlation between the serum testosterone & osteoporosis in South Indian men.

	41-50yrs	51-60yrs	61-70yrs	71-80yrs
Hip	0.959	0.965	0.924	0.985
Spine	0.959	0.942	0.970	0.921

Bone Mineral Density(BMD)in different age groups of SouthIndian men

P338 F

NORMAL DATA OF HEALTHY MALE'S BONE DENSITY MEASUREMENT ON FOREARM 1/3, 1/6 AND 1/10 IN LANG FANG DISTRICT, BEIJING

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We conducted bone density measurement on forearm 1/3, 1/6 and 1/10 of the selected 316 male adults with the adoption of SPA. Result demonstrated that the bone density in forearm 1/6 is lower than forearm 1/3 and the 1/10 forearm has the lowest bone density. This can be explained by the fact that forearm 1/3 is made of cortical bone and forearm 1/6 is forearm diaphysis and epiphysis plate and forearm 1/10 is composed of spongy bone. We may draw the conclusion that the bone density of the three different parts of the forearm has something to do with the bone composition. The measurement of the normal data of the three parts provides clinical guidance to the establishment of osteoporosis diagnostic criteria (see table below).

Normal data of healthy male's bone density measurement on forearm 1/3, 1/6 and 1/10 in Lang Fang District, Beijing,China

		Radius			Ulna		
Age group	(No.)	1/3	1/6	1/10	1/3	1/6	1/10
20-30	58	0.918 ± 0.082	0.748 ± 0.105	0.641 ± 0.201	0.934 ± 0.096	0.723 ± 0.160	0.642 ± 0.162
30-40-	56	0.983 ± 0.143	0.787 ± 0.087	0.712 ± 0.183	0.986 ± 0.123	0.786 ± 0.158	0.705 ± 0.183
40-50	62	0.962 ± 0.110	0.765 ± 0.131	0.635 ± 0.087	0.944 ± 0.139	0.810 ± 0.162	0.652 ± 0.085
50-60	69	0.941 ± 0.105	0.698 ± 0.091	0.543 ± 0.165	0.948 ± 0.133	0.712 ± 0.087	0.612 ± 0.164
60-70	48	0.872 ± 0.167	0.632 ± 0.158	0.512 ± 0.133	0.873 ± 0.110	0.628 ± 0.093	0.582 ± 0.187
70-80	23	0.846 ± 0.123	0.583 ± 0.129	0.487 ± 0.186	0.814 ± 0.134	0.546 ± 0.083	0.476 ± 0.125

P339 S

ASSOCIATION BETWEEN RESPIRATORY FUNCTION AND BONE DENSITY AMONG ASIAN WOMEN

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A positive correlation between forced expiratory volume (FEV1) and bone mineral density (BMD) has been established in healthy women and women with respiratory diseases previously. In this study we examined the relationship between FEV1 and BMD in a group of women, who participated in a community-based osteoporosis study in Southern Sri Lanka.

204 women, who were free of bone active diseases, respiratory diseases and had not taken bone active drugs, were included in the study. FEV1 was measured by ventilometer while spine and hip BMD were measured with DXA. Correlation between FEV1 and BMD and comparison of mean BMD in four quartiles of FEV1 were done unadjusted and then after adjusting for age, weight and height.

The average age, weight and height of women were 55.4 (12.9) years, 48.3 (9.9) kg and 1.48 (0.06) m respectively. When age, weight and height were controlled, no significant correlation was found between FEV1 and spine, femoral neck and trochanteric BMD. Unadjusted mean spine BMD in the lowest through to the highest FEV1 quartiles were 0.727, 0.798, 0.809 and 0.944 g/cm2 ($p<0.001$). The corresponding mean values for femoral neck were 0.654, 0.720, 0.732 and 0.817 ($p<0.001$) and for trochanteric area were 0.521, 0.590, 0.582 and 0.659 ($p<0.001$). However when adjusted for age, height and weight, differences in mean BMD in all three sites became non-significant ($p>0.05$). No correlation between FEV1 and BMD was found in the regression analysis.

Although a positive correlation independent of age, weight and height has been demonstrated between FEV1 and hip BMD in previous studies, we were unable to demonstrate the same among our women. The positive correlation seen between FEV1 and crude BMD in our analysis was entirely due to differences in age and body measurements.

P340 W

THE EFFECT OF BREAST-FEEDING ON BONE MINERAL DENSITY: A COMMUNITY BASED STUDY IN ASIA

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Breast-feeding has shown variable effects on bone mineral density (BMD) in previous studies. We examined the association between breast feeding and BMD in a group of women from Southern Sri Lanka, where children are generally breast fed for longer period.

This community based osteoporosis study recruited volunteers who were free of bone active diseases and drugs. The height, weight, number of pregnancies and the duration of breast-feeding in each pregnancy were recorded. The BMD of hip and lumbar spine were measured by DXA. The maximum period of breast-feeding for one pregnancy was considered as 24months. Sum of duration of breast-feeding in all pregnancies was expressed as the total period of breast-feeding (TPBF). Based on the TPBF, the sample was divided into three groups. Group 1: TPBF=0 (n=42). Group 2: TPBF=1-48 months, mean= 34.3, SD = 15.7, (n=94). Group 3: TPBF= above 49 months, mean=102.8, SD =34.7, (n=68). Mean ages of groups 1,2 and 3 were 54.0, 51.9 and 61.6 years ($p<0.001$). Mean heights and weights of three groups were not significantly different.

After correcting for age, height and weight, mean femoral neck BMD in groups 1,2 and 3 were 0.706, 0.736, 0.694 ($p=0.036$), trochanteric BMD 0.576, 0.595, 0.552 ($p=0.006$) and spine BMD were 0.815, 0.820, 0.785 gm/cm2 ($p=0.245$) These associations persisted even after correcting for number of pregnancies. In the regression analysis, when corrected for age, height and weight, breast feeding for 12 months caused loss of bone density by 0.005 gm/cm2 in the lumbar spine ($p=0.05$), by 0.005 in trochanteric area ($p= 0.01$) and 0.004 in femoral neck ($p=0.54$). The percentages of osteporotics in group 1, 2 and 3 were 33, 31, and 63 respectively ($p<0.001$). When compared with group 1, women in group 3 had relative risk (corrected for age, weight and height) of 2.9 of developing osteoporosis (95% CI 1.22 and 7.03).

This study shows that women who breastfed up to 48 months, had higher BMD at spine and femoral trochanter than women who never breastfed or breastfed more than 48 months. These associations were independent of age, body measurements and number of pregnancies.

P341 F

NORMAL DATA OF HEALTHY FEMALE'S BONE DENSITY MEASUREMENT ON FOREARM 1/3, 1/6 AND 1/10 IN LANG FANG DISTRICT, BEIJING

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We conducted bone density measurement on forearm 1/3, 1/6 and 1/10 of the selected 450 female adults with the adoption of SPA. Result demonstrated that the bone density in forearm 1/6 is lower than forearm 1/3 and the 1/10 forearm has the lowest bone density. This can be explained by the fact that forearm 1/3 is made of cortical bone and forearm 1/6 is forearm diaphysis and epiphysis plate and forearm 1/10 is composed of spongy bone. We may draw the conclusion that the bone density of the three different parts of the forearm has something to do with the bone composition. The measurement of the normal data of the three parts provides clinical guidance to the establishment of osteoporosis diagnostic criteria (see table below).

Normal data of healthy female's bone density measurement on forearm 1/3, 1/6 and 1/10 in Lang Fang District, Beijing, China							
		Radius			Ulna		
Age group	No.	1/3	1/6	1/10	1/3	1/6	1/10
20-30	78	0.824 ±0.154	0.608 ±0.208	0.532 ±0.186	0.824 ±0.154	0.617 ±0.158	0.538 ±0.086
30-40	76	0.918 ±0.150	0.682 ±0.105	0.546 ±0.172	0.918 ±0.150	0.653 ±0.168	0.582 ±0.128
40-50	88	0.887 ±0.128	0.660 ±0.098	0.501 ±0.087	0.887 ±0.128	0.673 ±0.097	0.531 ±0.165
50-60	97	0.886 ±0.119	0.603 ±0.035	0.468 ±0.165	0.886 ±0.119	0.614 ±0.163	0.487 ±0.183
60-70	79	0.723 ±0.072	0.482 ±0.143	0.376 ±0.187	0.723 ±0.072	0.438 ±0.151	0.386 ±0.176
70-80	32	0.537 ±0.107	0.381 ±0.186	0.328 ±0.093	0.537 ±0.107	0.383 ±0.087	0.329 ±0.187

P342 S

DEVELOPMENT OF A JAPANESE OSTEOPOROSIS QOL QUESTIONNAIRE

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The Japanese Society for Bone and Mineral Research established a committee for developing QOL measure for postmenopausal women suffering from osteoporotic back pain in 1998. The committee developed a disease-specific QOL measures designed for postmenopausal women with osteoporosis suffering from back pain due to osteoporosis.

The JOQOL is consisted of 40 items distributed across 7 domains: Pain, Activities of Daily Living(ADL), Social Participation and Leisure, General Health , Body Image, Fear of falling and Family support. JOQOL was constructed on the basis of previous studies of QOL assessment using OPAQ and Quel-effo 41 in Japanese women with postmenopausal osteoporosis.

To assess the reliability and validity, we administered the Japanese Osteoporosis QOL Questionnaire (JOQOL) and the 36 item form of the Medical Outcome Survey instrument(SF36) to 545 women with postmenopausal osteoporosis.

Participants'ages ranged from 46 to 96 years (mean age:46.6 years, SD:8.7). Bone densitometry revealed a mean bone density in % YAM of 64.0 ± 9.5(range 25.2-108.0). One hundred and thirty women had at least 1 vertebral fracture, 40 women had 1 vertebral fracture, 31 women had 2 vertebral fractures, 22 women had 3, 13 women had 4 and 33 women had more than 5 vertebral fractures (mean±SD: 3.3± 2.5, range : 1-12). The means of height and weight were 148.8 ± 5.7(range 13.0-166.0), 47.1 ± 6.6(range 32.0-71.0), respectively. Two hundreds and eighty-one women lived in their home and 28 lived in institution. Three hundreds and three women (97.4%) were independent in their basic activities of daily living which include feeding, dressing, bathing and toileting.

Cronbach's alpha was used to assess internal consistency. Correlation coefficients were obtained between total score of JOQOL and both bone density and number of fractured vertebrae.

Cronbach's alpha for total score was 0.916. Exploratory factor analysis identified 7 separate domains. Correlation coefficients between each subscale of JOQOL and corresponding subscales of the SF36 were at the .05 level of significance.

P343 W

THE ASSOCIATION BETWEEN OSTEOPOROTIC FRACTURES AND HEALTH RELATED QUALITY OF LIFE (HRQL) AS MEASURED BY THE HEALTH UTILITIES INDEX MARK II AND MARK III SYSTEMS

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A total of 3394 women and 1122 men 50 years of age and older, who were recruited from the Canadian Multicentre Osteoporosis Study (CaMos), participated in this cross-sectional study. Lifetime fracture occurrences were classified into seven categories including hip, pelvis, spine, lower body (defined as upper and lower leg, knee, ankle and foot), upper body (defined as arm, elbow, sternum, shoulder and clavicle), wrist and hand (defined as forearm, hand or finger), and ribs. Given that two thirds of vertebral fractures are not recognized, participants with subclinical vertebral deformities were also examined. Spinal radiographs were used to confirm the existence of vertebral deformities for all participants. The health utilities index (HUI) mark II and III systems were used to assess HRQL. The Mark II and III systems assess current HRQL across six and eight attributes respectively. Multivariate linear regression analyses were performed to determine the association between various osteoporotic fracture types and HRQL, adjusting for possible confounders. We calculated regression coefficient estimates and 95% confidence intervals (CI) for all parameters. The regression coefficients for the fracture (yes/no for each fracture type) terms represent adjusted differences in HRQL scores between participants with and without fractures. Results indicated that the multi-attribute scores for the mark II system were negatively related to hip (-0.05; 95% CI: -0.09, -0.01), lower body (-0.02; 95% CI: -0.03, -0.000) and subclinical vertebral fractures (-0.02; 95% CI: 0.03,-0.00) for women. The multi-attribute scores for the mark III system were negatively related to hip (-0.09; 95% CI: -0.14, -0.03) and rib fractures (-0.06; 95% CI: -0.11, -0.00) for women, and rib fractures (-0.06; 95% CI: -0.12, -0.00) for men. Differences in the time since last fracture was not strongly associated with the HUI attributes. For the most part, the largest differences in HRQL were observed between the 0-1 year and 10+ years categories. In conclusion, this study demonstrates a negative association between past osteoporotic fractures and quality of life in both women and men. Moreover, this study shows that the association between minimal trauma fractures and quality of life depends on fracture type and gender.

P344 F

DEVELOPMENT AND VALIDITY OF JAPANESE OSTEOPOROSIS QUALITY OF LIFE QUESTIONNAIRE

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The Japanese Osteoporosis Quality of Life Questionnaire (JOQOL) was developed to assess disability in women with osteoporosis. The domains of JOQOL include: quantitative indices of pain (5 questions), activities of daily living(4), housework(5), transfer(7), leisure and social activity(5), general health perception(3), posture and body image(4), fear of fall(4), familial support and summary(3), with a total of 40 questions. The questionnaire contains generic, disease-targeted and cross-cultural questions. Each question was graded as 4~0 points, and a total score ranged between maximum 160 and minimum 0 points. The total score may be converted to the expression of 100-0 points. The higher score shows the better quality of life.

Out of 487 patients, 198 answered all questions of JOQOL and SF-36, simultaneously assessed, with BMD of lumbar spine by DXA, and lateral view of radiograms of thoracic and lumbar spines. The range of age was 46 to 95 years and a mean was 70.5 ±9.5 (SD) years.

Internal consistency of a total score in Cronbach's alpha was 0.808. Reliability of the JOQOL was assessed using test-retest methods in 83 patients in 4 weeks apart. Correlation of coefficient was 0.920.

A mean of deviation from Japanese standards of SF-36 was PF, 51.7; RP, 51.6; RE, 51.4; SF, 49.2; MH, 49.7; BP, 49.8; VT, 48.8; and GH, 48.5.

There was a significant difference of the score between those with the compression fracture of the vertebrae and without the fracture ($p<0.001$).

There was a regressive correlation between the total score and the age, but no significant correlation between the total score and BMD.

A revised questionnaire consisting of 38 questions was made excluding two questions as to familial support and summary, which showed no significant correlation with a total score.

(JSBMR QOL Evaluation Committee for Osteoporotic Patients)

P345 S

THE CHANGES OF QUALITY OF LIFE IN OSTEOPOROTIC PATIENTS WITH BACK PAIN

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Back pain, caused by osteoporotic vertebral fractures, leads to pain and decrease in quality of life. The aim of this study is to evaluate the changes of quality of life in osteoporotic patients as outcome in the treatment of back pain.

Fifty-three patients with back pain due to vertebral fractures in outpatient clinic participated in this study. Of those fifty-three patients, fifty-one patients (male: 2, female: 49, 51-91 years of age, avg. 72.6) filled out visual analogue scales (VAS) and Japanese Osteoporosis Quality of Life Questionnaire (JOQOL) at initial examination, after one month, and three months later. JOQOL consists of six domains for scoring (maximum scale of 152 points convert into 100 points): Pain, Activities of daily living, Leisure and social activities, General health, Posture, and Psycho general. Calcitonin and vitamin D were given for the treatment of osteoporosis, and non-

steroidal anti-inflammatory drugs were also given until the pain relived. VAS decreased with the months, 6.8 at initial, 4.1 at one month, and 2.8 at three months. JOQOL scores at initial, one month, three months are 58, 71, 68, respectively. The domain of Pain and Activities of daily living markedly improved with the decrease of VAS. On the other hand, Leisure and social activities, General health, Posture, and Psycho general did not improve in spite of the improvement of VAS.

These results indicated that the improvement of QOL in osteoporotic patients with back pain were required not only the relief of the back pain, but also social support.

P346 W

APPLICATION OF A LATTICE GAS MODEL FOR RESTORATION OF SUBPIXEL INFORMATION FROM MEDICAL IMAGES OF TRABECULAR BONE

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In the study an algorithm is proposed to recover subpixel data from input low-resolution grey level medical images of trabecular bone. Binary high-resolution images of trabecular bone are the output of the algorithm. The reconstruction is based on a diffusion process, defined on the lattice identified with the matrix of pixels. White pixels of a binary intermediate step images are identified with particles allowed to diffuse. Ising-like self-interaction and an interaction with an external field rule the diffusion. The interaction with an external field is defined as an attraction to the ridges of the trabecular structure. The diffusion is driven by standard Metropolis algorithm. The performance of the introduced algorithm is compared with standard thresholding techniques. The novel method introduced in the study produces output images the architectural properties of which are the same (within the error bars) as the properties of the original structures. Also the errors of the reconstruction are a few times less than the errors of threshold techniques.

P347 F

THE OSTEOPOROSIS SELF-ASSESSMENT TOOL FOR ASIANS (OSTA): VALIDATION IN MALAYSIA

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Background

Patients with low bone mineral density (BMD) have a high risk of future fractures, and should be actively considered for treatment. However, BMD measurements are not easily accessible in Malaysia because of lack of equipment and cost of BMD testing.

Simple risk assessment questionnaires have been designed to increase awareness of osteoporosis and encourage high-risk women to go for definitive BMD measurements, thereby avoiding the cost of measuring women at low risk.

Such studies have previously been done on both Asian and non-Asian women. Eight countries in Asia had participated in such a study to develop and evaluate the Osteoporosis Self-assessment Tool for Asians (OSTA) to identify high-risk women as defined by femoral neck BMD T-scores of <-2.5.

Objective

The purpose of this study is to evaluate/validate the ability of OSTA (using risk factors of weight and age) to identify women with osteoporosis (as defined by femoral neck T score) in a sample of post-menopausal Malaysian women.

Method

505 post-menopausal women (500 were aged 50 years and older) were recruited at the University Malaya Medical Centre and had femoral neck BMD measured using DEXA (Lunar Corp.). OSTA is calculated as 0.2*(weight in kg minus age in yr), truncated to an integer. We examined the proportion of women with osteoporosis in three OSTA categories defined and validated previously: low risk (OSTA > -1), medium risk (OSTA -1 to -4) and high risk (OSTA < -4).

Results

The proportion of women with osteoporosis as measured by BMD was 70% in the high-risk group, compared with 14% and 5% of the medium risk and low risk women. Of the women with osteoporosis, 85% (sensitivity) had OSTA values of -1 or lower, and 50% of the women without osteoporosis had OSTA values of 0 or higher (specificity).

Conclusion

OSTA performed well for classifying the risk of osteoporosis among post-menopausal Asian/ Malaysian women. It correctly identified 85% of women with osteoporosis for BMD testing, and would appropriately avoid BMD tests in half of the women without osteoporosis. OSTA is not meant to replace BMD tests and women should use it in their discussions with their doctors about their risk for osteoporosis.

P348 S

OSTEOPOROSIS RISK ASSESSMENT TOOL (ORAT) ASSISTS CLINICIANS IN SELECTING POSTMENOPAUSAL WOMEN FOR DXA SCANNING

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Introduction: The Florida Osteoporosis Board has developed an osteoporosis risk assessment tool (ORAT) designed to be filled out by patient or office staff prior to patient seeing clinician in order to flag patients that should be considered for DXA scanning.

Methods: The study was designed to validate the ORAT as a screening test for osteoporosis. One thousand seventy postmenopausal females over 45 years old (> 90% Caucasian) undergoing DXA BMD testing at 12 centers in Florida were asked 5 questions from the ORAT (age, height loss, weight, estrogen use, steroid use).

Results: Overall the actual prevalence of osteoporosis in the entire patient population was 34% (any site), 15% (T-hip), 24% (femoral neck) and 22% (LS spine). The ORAT screening tool identified ninety-five percent of the patients who, when DXA was preformed, did indeed have osteoporosis.

Conclusion: The ORAT, a free and simple risk assessment tool can help increase awareness among patients and encourage the appropriate use of DXA testing in order to diagnose osteoporosis before fractures occur. Postmenopausal women who present with fractures or other major risk factors should be evaluated with DXA even if their ORAT score does not indicate further testing. The ORAT is not intended to replace DXA or the practitioner's clinical judgment in determining a patient's diagnosis.

ORAT	FemNeck (T ≤ -2.5)	T-hip (T ≤ -2.5)	LS spine (T ≤ -2.5)	Any Site (T ≤ -2.5)
Sensitivity	94%	95%	83%	85%
Specificity	47%	44%	45%	48%
Accuracy	53%	48%	50%	55%

P349 W

PREVALENCE OF SPINE FRACTURES ASSESSED WITH LVA IN POSTMENOPAUSAL WOMEN

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This study determined the prevalence of spine fractures/deformities assessed with dual-energy Lateral Vertebral Assessment (LVA) in normal, osteopenic, and osteoporotic postmenopausal women. Measurements were performed with the Lunar Prodigy bone densitometer (GE Medical Systems) utilizing a digital, CZT detector. LVA defines fractures as vertebral heights and ratios greater than 3 SD (moderate) or 4 SD (severe) below expected values. Osteopenia and osteoporosis were defined using WHO guidelines. Bone mineral density (BMD) was measured at the spine and both femora in 231 subjects (mean age 65±11.9 years). Osteopenia was found in 46.7% and osteoporosis in 26.4 % of women at one or more sites. More than half (53.2%) of the 231 women had spine fractures. Fractures were found in 72.1% of women with osteoporosis and 49.1% of women with osteopenia at any of the three sites. Surprisingly, 41.9% of women with normal T-scores and 46.5% of women without osteoporosis had vertebral fractures by LVA. Women who were osteoporotic at the total femur were just as likely to have vertebral fractures as women osteoporotic at the lumbar spine. 40-45% of women who had normal T-scores at central DXA sites had a spine fracture/deformity. These women would be considered low risk based on BMD T-scores but regarded as high risk for future fracture based on LVA measurements. We conclude that LVA can play an important role in assessing fracture risk in women over 65, regardless of BMD T-score.

Percentage of women with fractures based on T-score diagnosis			
	Normal (T>=-1.0)	Osteopenia (-2.5<T<-1.0)	Osteoporosis (T<=-2.5)
All Sites	41.9%	49.1%	72.1%
Spine (L2-L4)	45.8%	51.1%	74.4%
Total Femur (Left)	41.4%	59.0%	76.0%
Total Femur (Right)	41.6%	57.8%	73.0%

P350 F

IMPLEMENTATION OF AN ELECTRONIC QUESTIONNAIRE FOR THE ASSESSMENT OF OSTEOPOROSIS RISK FACTOR IN DUAL X-RAY ABSORPTIOMETRY (DXA) CLINICAL PRACTICE: THE GENEVA EXPERIENCE

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Some 4500 patients undergo DXA in our division annually. Technicians and physicians used to complete a paper questionnaire investigating each patient's potential osteoporosis risk factors. Since the collected information was stored in the hospital archives, retrospective use of data for statistical analysis purposes was uneasy and slow. Our objective was to computerize the process by designing an electronic, improved version of the paper questionnaire with the support of a multidisciplinary team. The ultimate objective was to create a powerful database tool for future analysis.

The department of medical informatics provided the software development part. The questionnaire's content was reviewed and tested by the nuclear medicine medical team so as to address 381 variables known to influence bone metabolism such as patient's usual medication, family and personal medical history, tobacco, alcohol, diary products consumption, exercise status. A technical evaluation of the performed scan quality was also included. Both technicians and physicians received a 2 weeks-training session on the best and fastest way to capture data on-line.

During the first 5 months of implementation, 2294 patients underwent DXA measurements; for 58% of them, the original paper form had to be revived because of first release software defects (36%), network unexpected interruptions (29%) and lack of medical availability due to extra-workload (35%). However, 961 (42%) of the electronic questionnaires could be filled normally within an average time of 10 minutes each. Among those, 62% were fully completed. In the remaining 48%, some fields were left empty thus revealing the software's incapability to differentiate 'not-addressed' questions from 'not-applicable' answers. The feed-back of this first test phase lead us to request the development of a second 'user-friendly' release of the software in order to correct the bugs, improve ergonomics, facilitate data capture and stabilize network access.

Preliminary tests of the new version proved to satisfactorily enhance compliance. The next step will lead to test a portable questionnaire unit (using an infra-red organizer), as well as to automatically pool the database results with the patients' DXA values into the medical report. This will allow for a better assessment of absolute risk and potential intervention threshold.

P351 S

THE SIGNIFICANCE OF MONITORING PATIENT SATISFACTION WITHIN AN ISO 9002 QUALITY MANAGEMENT SYSTEM FOR DXA SERVICES: THE GENEVA EXPERIENCE

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DXA is a widely used technology involving numerous medical specialties, instrumentations, investigation techniques and analysis. In our division, DXA represents 4500 patients annually, corresponding to 30% of the division's clinical activity. As a DXA laboratory involved in more than 13 research protocols, we strive to satisfy 4 major customers: patients, referring physicians, students, industry companies. In order to provide them with a continuous high level of performance in such a complex environment, a quality management system based on ISO 9002 was designed and certified in September 1998. These client-oriented standards provided all partners with a unique language for quality assurance. 50 procedures were written and regularly monitored by internal audit. 18 procedures describe DXA techniques, 11 concern research, data management and radioprotection and 21 describe administrative tasks. A dedicated form (ODDAS) was elaborated to help collaborators provide feed-back from the procedures thus enabling corrective measures. A single-page, 10 questions, anonymous questionnaire was designed to address each step of patients' visits including 4 crucial quality criteria: information/communication, comfort, waiting time, relationship aspects.

To date, all procedures have been audited at least once internally and the division has undergone 5 external audits. Between August 1998 and September 2002, 220 ODDAS forms and 1280 patient questionnaires were analysed. Patient satisfaction and annual trends have been reviewed. Negative peaks correlated with special events have been identified. Over 96 months, 6 negative peaks have been observed for secretarial performance. Only 1 negative peak was noticed for technologists' activity. 7 negative peaks were associated with medical activity. Patient requirements identified were: need for information on tests/results, human relationships, comfort, environment, short delivery time of results. The weaknesses identified were: lack of information on test results, osteoporosis and possible treatments, lack of dialogue with physician. Some 470 corrective interventions have been performed by the full time dedicated quality manager.

All partners benefit from patient satisfaction. The ISO standards proved to be feasible both in clinical and research setting. The continuous survey enables to identify patients' needs, to monitor satisfaction, to identify area requiring improvement. It allows patients to express themselves. Finally, it motivates staff with positive feed-backs.

P352 W

NONINVASIVE MEASUREMENT OF DEGREE OF BONE MINERALIZATION WITH SOLID STATE PROTON AND PHOSPHORUS MRI

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Water and fat suppressed projection MRI (WFSPI) is designed to utilize the large difference between the proton T_2s of solid matrix (mostly collagen, short T_2) and the fluid components (mainly water and fat, long T_2) of bone to suppress the signal from fluid components while preserving the signals from the solid matrix. The magnetization of water and fat is flipped into the x-y plane by low power water and fat selective pi/2 pulses, and is subsequently dephased by gradient pulses, while the magnetization of collagen remains mostly in the *z*-direction. Additional water and fat selective pi pulses in alternate scans help to further cancel the residual magnetization. Following water and fat suppression, the signal of collagen is excited by a short hard pulse and acquired by a 3D projection method. WFSPI was implemented on a 4.7 T MRI system and tested on phantoms and bone specimens. Solid organic bone matrix was visualized for the first time in proton MR images. The bone matrix signal per unit volume of bovine trabecular specimens was measured by WFSPI and compared with those determined by chemical analyses. This method could be used in combination with bone mineral density measurement by solid state ^{31}P MRI to determine the degree or extent of volumetric 3D bone mineralization for the first time noninvasively.

P353 F

TURNER SYNDROME AND PURE GONADAL DYSGENESIS ARE IMPORTANT RISK FACTOR TO OSTEOPOROSIS IN A 38 PATIENTS STUDY

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Bone mineral density (DMO) was evaluated at lumbar spine and hip, through dual x-ray densitometry (DEXA), in 38 women with primary hipoestrogenism, due to Turner syndrome(TS) and Pure Gonadal Dysgenesis(PGD). The relationship between T-score values and body mass index(BMI), age, beguinning of estrogen treatment and length of treatment was analyzed.

-DMO showed to be reduced at lumbar spine (45%=osteopenia and 45%=osteoporosis)

L2L4 mean value = 0,83g/cm2

-DMO at the femoral neck was reduced too

(55%=osteopenia and 5%= osteoporosis)

Femoral neck mean value = 0,83g/cm2

Lumbar spine DMO was positively associated to the length of estrogen treatment (R=0,57) and femoral DMO with BMI(R=0,48). No correlation was found between DMO and the other parameters evaluated.

So,TS and PGD can be strong risk factors to osteopenia and osteoporosis.

P354 S

MATCHED CONTROL STUDY OF THE SENSITIVITY, PRECISION, ACCURACY BETWEEN QUANTITIVE CT AND DXA IN MEASUREMENT OF BONE MINERAL DENSITY

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Objective: From statistical analysis of the sensitivity, precision, accuracy of quantitive CT(QCT) and DXA(dual energy X-ray absorptiometry) in measurement of bone mineral density(BMD), the measuring value of QCT and DXA in BMD changes were investigated.

Methods: If there were no fracture or other malformation, the BMD of nearby vertebra would be getting close and the BMD of inferior vertebra would be higher than that of the superior. For had been confirmed with no malformations such as fracture by posteroanterior and lateral X-ray pictures, 75 Han postmenopause females had been measured the BMD of lumbar vertebra 2 to 4(L2-4) by QCT or DXA, among them, 32 cases were measured by QCT(the QCT group) and 43 cases by DXA(the DXA group). The average and standard deviation of BMD of L2, L3 and L4 in every individual by groups were calculated and it would reflect the vicariance degree in the three nearby vertebra and its regression was indirect of the precision of the two methods. The average and standard deviation of the two groups were calculated and the vicariance degree of the BMD between every individual would reflect the sensitivities of the two methods indirectly. The accuracy of QCT and DXA

were showed by the ratio of cases whose BMD L2<L3<L4 in two groups and compared with X2 test in statistics. Supposed the sensitivity, precision and accuracy of QCT was 100%, those of DXA would be calculated respectively.

Results: There were no difference in age, sex and race between two groups. The average BMD of L2 to L4 were 98.63±35.15(mg/cm3), 0.816±0.121(g/cm2) and the standard deviation of L2 to L4 BMD were 5.92±3.23, 0.0477±0.0314 by QCT and DXA respectively. Supposed the sensitivity, precision and accuracy of QCT was 100%, those of DXA were 40.8%, 82.8% and 82.8%.There was no difference in accuracy by QCT or DXA.

Conclusions: For measuring the BMD of lumbar, the sensitivity of QCT was higher markedly than that of DXA, the precision and accuracy of QCT were a little higher than those of DXA also. For reflecting the change of BMD, QCT was more sensitive than DXA.

P355 W

STUDY OF BONE MINERAL DENSITY (BMD) IN POSTMENOPAUSAL WOMEN

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Aim: The aim of this study was to determine the Bone Mineral Density (BMD) of the South Indian postmenopausal women.

Methods & Materials: 250 women who were at least five years postmenopausal were studied. Basic parameters like age, height, weight, body mass index s(BMI) were recorded. Women on hormone replacement therapy (HRT) were excluded.

The bone mineral density (BMD) & bone mineral content (BMC) of spine (L1-L4) & hip were measured using Hologic Dual Energy X-ray Absorptionmetry(DEXA).

Results: The variation Bone Mineral Density (BMD) in the different age groups of South Indian postmenopausal women the prevalence of women suffering from osteoporosis are shown in the table below.

Conclusion: Prevalence of osteoporosis in South Indian post- menopausal women is very high when compared to Caucasian population.

	51-60 yrs	61-70 yrs	71-80 yrs
spine	0.7379	0.7342	0.7204
hip	0.7542	0.7292	0.7135
osteoporosis	8%	39.51%	27.03%

Bone Mineral Density (BMD)and Prevalence of Osteoporosis in Post-menopausal South Indian Women

P356 F

SEX DIFFERENCE IN AFFECTION OF GEOMETRIC AND MATERIAL PROPERTIES ON THE STRUCTURAL PROPERTY IN TIBIAL DIAPHYSIS OF CYNOMOLGUS MONKEYS

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Both geometric and material properties are necessary to assess the whole bone strength. This study was performed to investigate the sex difference in affection of geometric and material properties on the structural property in the long bone of Cynomolgus monkey. The right tibial diaphysis obtained from ten normal males (age:17.22 ±1.75 years, body weight:6.81 ±1.92 kg) and sixteen females (age:16.81 ±2.70 years, body weight:4.68 ±1.10 kg) were scanned by pQCT (XCT Research SA plus, Stratec, Pforzheim, Germany) to analyze cortical bone density, area moment of inertia and Strength Strain Index (SSI). Thereafter, mechanical test was performed by three - point bending method. In order to obtain the material properties such as ultimate stress and elastic modulus, the structural property was normalized by area moment of inertia obtained by pQCT. There were significant differences in the failure load, elastic modulus, cortical bone density, area moment of inertia, SSI and body weight between males and females, while there were no significant differences in the ultimate stress and the age. The significant correlation between the failure load and the area of moment of inertia (r=0.908, p=0.0003) as a geometric property was found in males, however, in females, the failure load was significantly correlated with cortical bone density (r=0.610, p=0.0121) and elastic modulus (r=0.514, p=0.0417) as a material property. These values were not significantly correlated with the age of both sexes. These findings suggest that the structural property in tibial diaphysis of females is involved in the material property rather than geometric property, while that in males is affected by geometric property rather than material property in the Cynomolgus monkey.

P357 S

NEW RADIOLOGICAL BONE MORPHOMETRIC ANALYSIS FOR EVALUATION OF THERAPEUTIC EFFICACY ON BONE TRABECURAL STRUCTURE IN OSTEOPOROTIC PATIENTS.

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The new radiological bone morphometric analyzing system to evaluate bone trabecular structure in digital images of femur and lumbar vertebral bone was developed. This system consists of digital imaging system, morphological filter based on mathematical morphology, bone morphometric analytical method and median or selective line skeleton filter. Computed radiography, which has been generally used in clinical field, was used for obtain digital image data of femur and lumbar vertebra. The skeletal binary images were extracted from these digital image data using median filter and morphological filter. The star volume, node strut analysis and bone histomorphometric analytical methods were applied to the quantification of skeletal binary images which eliminated isolated skeletal components using selective line skeleton filter.

The feature of this system were as follows:

1.The method may enhance the ability of visually and quantitative interpreters to achieve accuracy, in those patterns are extracted in a more impact.

2.By using skeleton operation for extract the skeletal patterns, instead of performing thinning processing, widely spread trabecular structures could be extracted. Furthermore, reversion to the original images (reversible preservation of the data) was possible.

3.Random noise could be eliminated by median filters.

4.By changing the skeleton operation number, any particular size of the target trabeculae could be arbitrarily selected.

5.During processed skeletal binary images, the thresholds level could be fixed.

6.It was possible to obtain 36 types of parameters for bone morphometrical measurement.

7.By performing selective line skeleton operation after morphological filter processing, connectivity, furcated, or isolated skeletons could be selectively extracted.

8.By the use of devised the mapping sheets for assessment of bone quality, the dimension of each parameter was unified, and the changes in each bone structure could be mutually compared in the same dimension.

This new system may be a useful tool for computer-aided bone trabecural structure analysis, in for example determining the prognosis following evaluating the therapeutic efficacy in the patients of osteoporosis.

P358 W

IMAGE ANALYSIS OF RADIOGRAPHS OF PROXIMAL FEMORAL CORES FROM CADAVERS YIELDS VALUES FOR TRABECULAR STRUCTURAL PARAMETERS THAT CORRELATE WELL WITH MEASUREMENTS OF BONE STRENGTH

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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 influence fracture risk. Key among those factors is alterations and disruptions of trabecular structure. We developed automated hip radiographic imaging technology that measures trabecular parameters similar to those used in bone histomorphometry. The technology uses routine proximal hip radiographs. In general, it involves x-ray digitization, identification of regions of interest, background subtraction, trabecular extraction 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. Core specimens were harvested from cadaveric proximal femurs. Specimen radiographs were obtained and 2D structural parameters were measured on the radiographs. Cores were then subjected to biomechanical testing. Core failure loads and stiffness were correlated with the 2D structural parameters trabecular area ratio (AR), interconnectivity index (ICI), trabecular length (L), trabecular thickness (T), normalized number of nodes (N) and normalized number of end nodes (EN). Pearson's correlation coefficients (r) between 2D structure parameters and biomechanical failure load and biomechanical stiffness, respectively, are listed in the table below.

	AR	ICI	L	T	N	EN
Failure load	0.87	0.75	0.84	0.86	0.74	-0.76
Stiffness	0.81	0.91	0.78	0.94	0.76	-0.77

We conclude that measurements of bone structure on hip radiographs correlate highly with biomechanical failure loads and bone stiffness. These results suggest that inexpensive proximal femoral trabecular structural analysis from hip radiographs may yield vastly improved diagnostic assessment of osteoporosis and estimation of fracture risk.

P359 F

THE USE OF PLAIN RADIOGRAPH OF THE CALCANEUM FOR EVALUATION OF OSTEOPOROSIS?

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Aims: To evaluate the performance characteristics of a modified grading system of osteoporosis based on calcaneal trabecular pattern and to study its correlation with current measures of bone status assessment.

Material & Methods: Lateral calcaneal radiographs were obtained on 253 women participating in a clinical trail in osteoporosis. Bone mineral density (BMD) measurement was done at the hip and distal radius in addition to ultrasound assessment of the calcaneum. The radiographs were graded by three clinicians according to a previously described Calcaneal Index (V grades). After critical analysis, a modified III grade system was developed. The performance characteristics and correlation with the current bone status assessment devices was studied for both the Calcaneal grading systems

Results: The best intra- and inter-observer reliability with the modified grading system was k=0.45 and 0.40 respectively. The correlation with other measures was moderate but statistically significant (Hip BMD r=0.31, Distal Radius BMD 0.28, Calcaneal Speed of sound 0.20 and Broadband Ultrasound Attenuation (BUA) 0.36; $p<0.005$). There were statistically significant differences in hip BMD and BUA between the three grades on the modified grading system (Kruskal-Wallis 1-way ANOVA;$p<0.0001$).

Conclusions: The modified III grade system may provide important information about bone structure and skeletal strength. After further reliability testing, it may have a role as a screening tool in osteoporosis at places where advanced modalities for assessment of bone status are not available.

P360 S

VERTEBRAL BODY BONE ARCHITECTURE MEASUREMENT USING 3D ANAGLYPHS

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Macerated sagittal slices of T12 and L1 vertebral bodies from 15 subjects were examined in a scanning electron microscope. These individuals (8 men and 7 women) had no clinical history of crush fractures and were chosen to represent each decade of adult life. 3D anaglyphs were created of nine contiguous fields per slice by recording two digital images (the second tilted 5^o). The anaglyphs were viewed through red/green stereo glasses and the length ($Tb.Le_{rods}$) and thickness ($Tb.Th_{rods}$) of trabecular rods measured using an image analyser.

1884 rod thickness measurements were made with a mean $Tb.Th_{rods}$ of 118 ±SD 31 microns, with male rod thickness (122 ±SD 32 microns) significantly greater than female (112 ±SD 29 microns, $p<0.001$). 1547 rod length measurements were made with a mean $Tb.Le_{rods}$ of 565 ±SD 168 microns, and male rod length (575 ±SD 164 microns) was significantly greater than female (555 ±SD 170 microns, $p<0.02$).

Age related changes showed that $Tb.Th_{rods}$ changes significantly with age in the males, with thicker rods in the younger men declining to rods of similar thickness to women in older age. Conversely, $Tb.Th_{rods}$ in women showed a trend to increase with age, but this trend was not statistically significant. $Tb.Le_{rods}$ increased significantly with age in females, with shorter rods in younger women increasing to rods of similar length to men in older age. A load-to-buckling index (based on the Euler buckling formula) shows decrease with age for males concomitant with $Tb.Th_{rods}$ decrease. However, in females load-to-buckling was constant through out life despite an increase in rod length. This implies an increase in $Tb.Th_{rods}$ in older women and demonstrates that, since $radius^4$ is used in the index calculation, small (not significant) increases in rod thickness can result in large changes in rod strength.

Age-related changes to trabecular bone architecture in the vertebral body may influence the tendency for cancellous bone failure due to trabecular buckling. These alterations in bone quality may increase the risk of crush fracture. Direct measurements from 3D anaglyphs provide 'real' dimensions for trabecular structure. The determination of ranges for rod thickness and length will allow more accurate finite element modelling and better estimates of fracture risk.

P361 W

SERUM TARTARATE RESISTANT ACID PHOSPHATASE 5B IS NOT A BETTER MARKER OF BONE RESORPTION THAN ANY OTHER BONE MARKERS

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We present results of our examination of a group of 224 probands, who were referred for suspect osteoporosis (mean age of 63.1 years, 196 women, 28 men; 34 probands with normal BMD, 81 probands with osteopenia, 109 probands with osteoporosis). All patients were subjected to osteodensitometry (DeXa). We collected blood from each patient for determination of Ca, creatinine, Mg, PTH, b-ALP, PICP, osteocalcin, for assessment of activities of TRAP and for measurement of activity of acid phosphatase bone isoenzyme 5b (ELISA, test Bone TRAP Assay). Each patient also provided urine samples which was used for analysis of free DPD and creatinine. First we evaluated data in the whole group, then we divided probands according to values of T score. The above defined groups differed significantly in many laboratory parameters and also in age, but no group was typical by a certain value of measured markers. Discrimination models classified the probands into correct groups only with 40-50% reliability. Activity of acid phosphatase 5b isoenzyme was not identified as predictive by the criteria in the model proposed by discrimination analysis.

The subsequent application of stepwise regression did not reveal any reliable way for prediction of osteoporosis; according to stepwise regression the activity of isoenzyme 5b was not important for prediction in any group we had under study. Our study indicates that the known activity of acid phosphatase isoenzyme 5b cannot be used for mathematical assessment of bone density. On the basis of the results obtained we suggest that a single examination of activity of acid phosphatase isoenzyme 5b is not more advantageous for assessment of osteoresorption or a single diagnosis of patients with osteoporosis (osteopenia) than any other, commonly recommended marker of bone remodelation.

P362 F

EVALUATION OF A NOVEL IMMUNOASSAY FOR SERUM TARTRATE-RESISTANT ACID PHOSPHATASE TYPE 5b ACTIVITY IN HORMONE REPLACEMENT THERAPY

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Tartrate-resistant acid phosphatase type 5b(TRACP 5b) is typically expressed in osteoclasts. Therefore, serum TRACP 5b has been regarded as a good marker for bone resorprion. We developed a novel immunoassay kit for determination of serum TRACP 5b activity. The aim of this study is to evaluate the new immunoassay kit in hormone replacement therapy (HRT) and compare with other bone resorption markers.

Method

The TRACP 5b was purified from human bone as antigen to develop a monoclonal anti-TRACP 5b antibody. The kit uses two new monoclonal antibodies(Trk62, Trk49), and bound TRACP 5b activity was measured by 2-chloro-4-nitrophenyl phosphate(CNPP) as a substrate at pH6.4. This assay can be specific detection for TRACP 5b activity. Serum and urine NTX(sNTX and uNTX) was measured by OSTEOMARK NTx Serum and Urine (Ostex International, Inc., Seattle, USA), respectively. Urine deoxypyridinoline(uDPD) was measured by Osteolinks-DPD(Sumitomo Pharmaceuticals Co., Ltd., Osaka, Japan). Serum and urine samples were collected from 14 patients at baseline and at 6 months after HRT.

Results

We found that all markers were decreased significantly after HRT ($p<0.001$). Mean values of each markers were: TRACP 5b 5.1 ±1.6 vs 2.7 ±1.3 U/L, sNTX 20.1 ±4.5 vs 15.5 ±3.1 nmolBCE, uNTX 71.0 ±24.4 vs 41.7 ±18.5 nmolBCE/mmol cre and uDPD 9.0 ±3.7 vs 6.8 ±3.2 micromol/mol cre. The decreasing rates were: TRACP 5b 46.7%, sNTX 22.4%, uNTX 41.2%, and uDPD 24.3%.

These results strongly suggest that serum TRACP 5b can be most effective for monitoring of bone resorption in HRT. Not only the effective results but also serum sample method offers advantage over the other urine methods that require creatinine-adjustment.

P363 S

ACCURACY OF BIOCHEMICAL BONE MARKERS IN PREDICTING EARLY RESPONSE TO ANTIRESORPTIVE TREATMENT IN POSTMENOPAUSAL OSTEOPOROSIS

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The ability of biochemical bone markers to predict long-term prevention of bone loss by antiresorptive treatment in postmenopausal osteoporotic women is well established.

The aim of this study was to explore the accuracy of short-term measurement of markers of bone resorption in predicting early responses (1 year) to antiresorptive treatment.

We studied 37 postmenopausal osteoporotic women who received either alendronate 10 mg/day (n=17) or raloxifene 60 mg/day (n=20) for 1 year. Urinary immunoreactive free deoxypiridinoline (DPD), type I collagen alpha helicoidal peptide 620-630 (HePe) and serum crosslaps (sCTX) were measured at baseline and after 3 months of treatment. BMD at lumbar spine (LSBMD) and femoral neck (FNBMD) were measured by DXA at baseline and month 12. Least significant change (LSC) for each bone marker was calculated using short-term coefficient of variation from 2 repeated measurements in a control group of 30 postmenopausal osteoporotic women. LSC for BMD measurements was calculated from values in 11 subjects of the control group who underwent 2 BMD measurements over 1 year.

The resulting cut-off values were 17.8% for sCTX, 21.8% for DPD, 15.8% for HePe, 3.88% for LSBMD and 5.57% for FNBMD. A change from baseline at month 3 in bone markers predicted a gain in BMD above the cut-off values with sensitivities ranging from 66.6% to 100%, and specificities ranging from 7.1% to 76.9%. The positive predictive values indicated a probability of 13.3-57.1% that BMD increases if markers decreased below the cut points. In contrast, negative predictive values indicated a probability of 50-91.7% that BMD will not increase if markers did not decrease below the LSC.

As a difference from previous studies, we focused on early responses to treatment. Also, an increase in BMD above LSC was considered to define response to treatment instead of just prevention of bone loss. Under the conditions of our study, the short-term measurement of bone resorption markers showed a poor level of accuracy to predict positive response to treatment. However, bone markers provided early identification of lack of response to treatment with excellent levels of accuracy.

P364 W

GLYCEMIC CONTROL AFFECTS BONE MARKERS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Although type 2 diabetes mellitus seems to be associated with a high or normal bone mass, recent studies have indicated that it is associated with a higher risk of fractures. Fracture prevention efforts are a consideration in the treatment of diabetes. However, little is known about the influence of changes in glycemic control on bone turnover. We assessed the effect of glycemic control on bone turnover for a short term (2-3 weeks) in Type 2 diabetes patient. Thirty-one patients were enrolled into this study. The ages of patients ranged from 28 to 83 years (16 men and 14 women). The mean body mass index was 23.5 kg/m2. Glycemic control (Glycoalbumin 27.3 -> 21.5%) increased urinary crosslinked N-telopeptide of type I collagen (NTx) levels(p = 0.05) but did not significantly affect serum alkaline phosphatase (BALP) levels. Improvement in glycemic control caused a reduction in urinary calcium (Ca) and phosphate (IP) level. There was no significant difference in serum Ca and IP level. The improvement in glycemic control increases NTx, bone resorption maker. It is possible that glycemic control alters a low bone turnover state and protect type 2 diabetes mellitus patients from bone loss and fractures.

P365 F

THE EFFECTS OF PREGNANCY AND LACTATION ON HORMONAL STATUS AND BIOCHEMICAL MARKERS OF BONE TURNOVER

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Introduction: Biochemical markers of bone turnover are reliable indices for measuring changes in bone formation and bone resorption. Due to limitations in the use of bone densitometry during pregnancy biochemical markers of bone turnover provide an excellent alternative to examine the state of the skeleton during this physiologic state.

Study Design: We performed a prospective study in 20 women, during their first full term pregnancy until 12 months postpartum, intending to breast feed for 12 (mean (range) = 9.1 (7-12)) months postpartum. Morning blood and urine samples were obtained for laboratory tests: within 3 months before conception (baseline); between the 22nd and 24th gestational weeks; after delivery; and 6 and 12 months postpartum. Serum 25-hydroxyvitamin D (25-OH-D), parathyroid hormone (PTH), bone specific alkaline phosphatase, osteocalcin, procollagen I carboxypeptides, calcium, phosphate and creatinine in addition to urine deoxypyridinoline crosslinks and calcium were measured.

Results: There was no significant difference in the values of urinary calcium/creatinine and serum calcium, phosphate and 25-OH-D between the different visits during the study. In our patients there was a significant increase in PTH levels at 12 months postpartum as compared to baseline, although the mean values remained in the PTH reference range. All bone turnover markers increased during pregnancy and failed to reach baseline level even 12 months postpartum.

Conclusion: The high maternal bone turnover may suggest that the calcium needed for infant growth during pregnancy and lactation may be drawn at least in part from the maternal skeleton.

P366 S

DETERMINATION OF A LOCAL REFERENCE RANGE FOR SERUM OSTEOCALCIN LEVELS FOR A HEALTHY POSTMENOPAUSAL FEMALE POPULATION

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Introduction and aims: Osteocalcin is a small, vitamin K-dependent protein with the molecular mass of 5.8 kDa, consisting of 49 amino acids. It is synthesized exclusively by osteoblasts and odontoblasts and is one of the most abundant non-collagenous proteins of the bone matrix. It is therefore considered to be a highly specific marker for osteoblast activity in various disturbances of bone metabolism. Knowing that reference ranges for markers of bone turnover can be influenced by circadian rhythm, sex, menopausal status, ethnic and geographical differences, we determined the reference range for serum osteocalcin for healthy postmenopausal female population of our region.

Material, methods and patients: We determined the serum osteocalcin values using the N-MID Osteocalcin (Osteometer Bio Tech A/S, Herles, Denmark) electrochemiluminiscence immunoassay using the Elecsys 1010 instrument (Roche Diagnostics, Basel, Switzerland). The assay measures both the intact and the N-MID fragments. Morning blood samples were collected from 250 women. They were all out patients and further scrutinized using the exclusion criterias: premenopause, chronic warfarin use, disease of liver, kidney and bone metabolism, known endocrine, rheumatoid or metabolic disease, treatment that could influence bone metabolism, abnormal values for serum calcium, inorganic phosphorus and alkaline phosphatase.

Results and conclusion: In total 81 (age, years (mean, range) = 58.4 (45 - 78)) postmenopausal women qualified for the study. The demographic characteristics of the participants were not statistically different from those whose results were excluded from the study. The median (5th - 95th percentile) osteocalcin value was 23.2 (14 - 42) microgram/L. The median (reference range) provided for post menopausal women (n = 22) by the manufacturer was 27 (20 - 48) microgram/L. In conclusion, we derived a reference range for a healthy postmenopausal female population, as such providing a tool better suited to our region.

P367 W

BIOCHEMICAL MARKERS OF BONE TURNOVER IN RURAL AND URBAN WOMEN. EPIDEMIOLOGICAL STUDY OF THE LUBLIN REGION (EAST POLAND)

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Aims: The aim of the study was determination the reference values of biochemical markers of bone turnover for women from Lublin Region (East Poland).

Methods: Subjects of the study were 188 normal women aged 30-79, all residents of Lublin Region. Analysed markers of bone turnover were: Osteocalcin (BGP), C-terminal telopeptide of collagen type I, Bone Alkaline Phosphatase. BGP and CTX were assessed using ELISA method (Osteomark, Denmark). All blood samples were taken and analysed at Clinical Chem. Laboratory and Patomorphology Department at the Institute of Agricultural Medicine in Lublin in 2000.. The lumbar spine (L2-L4) of all subjects was examined in a-p position using the dual X-ray absorptiometry- DEXA (LUNAR Corp.) at the Densitometric Lab. of Institute of Agricultural Medicine.

Results and Conclusion: 1. Serum levels of BGP, CTX, bALP in women in every age range are different, generallyrising with age (Table 1). 2. Serum levels of BGP, CTX,bALP in women strongly depend of both: menopausal status and bone mineral density (Table 2).

Table 1. Reference values of bone turnover markers in five-year intervals for Lublin Region women (East Poland).

	BGP (ng/L)	CTX (pM)	bALP (U/L)	%bALP (%)	BGP (ng/L)	CTX (pM)	bALP (U/L)	%bALP (%)
Age	Min	Max	Min	Max	Min	Max	Min	Max
≤ 44	6.2	18.0	1285	3796	11.3	33.4	19.9	48.8
45-49	8.4	21.8	1516	4273	10.2	45.3	19.3	60.3
50-54	8.6	22.8	1724	6147	11.5	49.0	19.1	50.1
55-59	4.9	39.0	1796	6560	16.1	41.7	21.3	44.0
60-64	11.4	36.7	2544	7683	17.1	52.1	15.8	51.8
≥ 65	0.0	52.1	819	8534	15.6	40.4	19.5	37.4
Total	5.4	28.9	1419	5730	12.2	44.0	18.9	51.4

Table. 2 Reference values of bone turnover markers for Lublin Region women (East Poland) according to the bone mineral density (*T-score*) and menopausal status.

	BGP (ng/L)	CTX (pM)	bALP (U/L)	bALP (%)	BGP (ng/L)	CTX (pM)	bALP (U/L)	bALP (%)
	Min	Max	Min	Max	Min	Max	Min	Max
Before menopause (BMD L_2–L_4 > -1 SD)	7.0	19.0	1411	4228	10.3	38.2	19.4	51.1
Before menopause (BMD L_2–L_4 ≤ -1 SD)	8.4	21.1	1691	4345	10.2	37.4	17.3	59.1
After menopause (BMD L_2–L_4 > -1 SD)	6.6	34.7	1981	5759	12.4	50.8	17.1	51.5
After menopause (BMD L_2–L_4 < -1 SD i > -2,5 SD)	6.6	32.0	1598	7311	11.3	53.3	19.7	49.6
After menopause (BMD L_2–L_4 ≤ -2,5 SD)	5.4	46.1	1904	9100	18.3	44.7	21.9	49.5

P368 F

A PROPOSAL OF INCLUSION CRITERIA FOR CLINICAL TRIALS TO EVALUATE A NEW THERAPY ON THE POSTMENOPAUSAL OSTEOPOROSIS USING THE WEIBULL ACCELERATED MODEL

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We confirmed the effect of covariates for the new vertebral fracture in the cohort study after 3.1 years follow-up period using the Weibull accelerated model including nine covariates observed in common. Six hundred ninety-one postmenopausal women (mean [sd] of initial covariates: Age, 62.9[10.6]; BMD (DPX), 0.97[0.19]; BMI, 22.6[3.0]; U-DPD, 7.0[2.3]; and Pre-Fr (+), 16.1%) were observed in the cohort study at the Research Institute and Practice for Involutional Diseases. The ninety-three women had new vertebral fracture(s) during the follow-up period. Five covariates had significantly effects on new fracture(s), their ratio of fracture time [95% CI] in the Pre-Fr (+/-), BMD (0.1g/cm/cm increase), Age (5 years increase), BMI (1Kg/m/m increase) and U-DPD (1nM/mMCr increase), respectively, estimated 0.607[0.453-0.814], 1.188[1.085-1.300], 0.872[0.799-0.953], 0.913[0.874-0.953], and 0.904[0.856-0.954]. The phase III trials with new vertebral/nonvertebral fractures as the usual primary endpoint have been, over the past few years, conducted for the prevention and treatment of postmenopausal osteoporosis patients who were included based on BMD value and prior fracture. These findings imply that the conventional inclusion criteria are not appropriate because high-risk patients with new fracture are not well defined in clinical trials . We demonstrated that the Weibull accelerated model adequately fit the above cohort data. The accelerated model that can estimate the predicted incidence with new fracture is feasible to estimate the inclusion criteria and to calculate the sample size. Contrarily, the proportional hazard model usually used in clinical studies is unsuitable for estimating survival time, whereas it is robust for evaluating the effect of covariates. We conclude that a certain statistical model incorporating influential covariates should be used for including patients into the clinical trial. Its criteria should be determined as the predicted fracture incidence at a terminal period calculated on the statistical model incorporating individual initial values of covariates. We show its example also for the sample size calculation of clinical trials. Similarly, these statistical model based concept is valid to determine the criteria of initiating treatment. We show also another example of the cut-off value of predicted fracture incidence for the initiating treatment.

P369 S

CLINICAL STUDY ON BONE RESORPTION BIOCHEMICAL MARKER OF SECOND GENERATION-SERUM TARTRATE-RESISTANT ACID PHOSPHATASE(TRACP)5B

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Osteoporosis is the major cause of morbidity and mortality in the elderly population. Early diagnosis of osteoporosis are concerned by scientists. A problem with the first generation markers of bone resorption is that their normal levels vary a lot between individuals. Although these markers are useful in selecting and mornitoring antiresorptive treatment and in the prediction of osteoporotic fractures, it is difficult to be a biomarker of osteoporotic diagnosis. The novel study showed that tartrate-resistant acid phosphatase(TRACP) 5b maybe a good second generation biomarker of osteoporosis.

The serum of 437 person were detected with Bone TRAP kits in several hospitals of China. Our data implied that serum TRACP5b level of osteoporosis patients and older person are higher than normal. It will be a more convinience, reliable and economical new method of early diagnosis on osteoporosis.

Our data showed that the serum TRACP5b level were lower in men before 64 and in women before 49 years old respectively. But their TRACP5b level and bone resorption, especially in osteoporotic patients, were increased climacterum later, and there are markedly differentiation compared with adult and control groups in statistics. These results implied that the serum TRACP5b would be a specificity marker in mornitoring bone resorption and osteoporosis diagnosis.

	Age	(No.)	TRACP5b value	t-test	
			X ± SD		
Normal male	20-64	87	3.76 ±1.35		
Normal male	> or =65	79	4.66± 1.50	*p<0.01	
osteoporotic male	> or =60	58	5.69± 1.86	*p<0.01	**p<0.01
Normal female	20-49	73	2.89±1.28		
Normal female	> or =50	115	4.81± 1.54	#p<0.01	
osteoporotic female	> or =45	61	5.24 ± 1.65	#p<0.01	##p<0.05

*:compared with 20-64 group;
**:compared with >or=65 group;
#:compared with 20-49 group;
##:compared with > or =50 group

P370 W

THE UTILITY OF QUANTITATIVE ULTRASOUND OF THE CALCANEUS TO IDENTIFY OSTEOPOROTIC AND OSTEOPOROTIC OR OSTEOPENIC WOMEN

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To assess clinical utility of a gel-coupled quantitative ultrasound (QUS) device to diagnose osteoporosis and/or low bone mass, 760 women aged 30-80 years were examined for bone status by dual-energy X-ray absorptiometry (DXA) at the spine and hip and by QUS of the calcaneus.We performed three receiver operating characteristic analyses to accurately identify osteoporotic (T-score below -2.5) and osteoporotic or osteopenic subjects (T-score below -2.0 and -1.5) diagnosed by DXA at the femoral neck, total hip, and spine by determining the sensitivity and specificity of various cut-off points for Quantitative Ultrasound Index (QUI,a parameter that combines BUA and SOS measured by the QUS device). Positive (PPV) and negative predictive values (NPV) for osteoporosis and/or osteopenia for each cut-off value were calculated. While low QUI (57, 58, and 60 for total hip, femoral neck, and spine) was highly suggestive of osteoporosis with the specificity of 95%, the sensitivity (25%-30%) was poor. PPVs varied between 33% and 63%, indicating a large number of false negatives. The inclusion of osteopenic subjects resulted in higher PPVs (varying between 74% and 85%). On the other hand, at a QUI of 92, 99, and 100, QUS had a 95% sensitivity for the diagnosis of osteoporosis at the total hip, femoral neck, and spine, respectively. Very high NPVs (99%, 96%, and 93%) suggested that QUS may reliably screen out women unlikely to have osteoporosis. However, poor corresponding specificities (32%, 23%, and 25%) preclude an inference that a woman with a lower QUI has osteoporosis. Therefore, we cannot decide that a person has osteoporosis based on QUS measurements, but we can reliably think that a person does not have osteoporosis. In conclusion, the results of our study indicate that QUS may be used as a prescreening tool to rule out the diagnosis of osteoporosis in women in order to minimize the number of subjects for DXA referral. It should be noted that women with low QUS measurements do require DXA measurements to confirm the

presence of osteoporosis. The use of information obtained from QUS in clinical decision making for initiation of therapy for osteoporosis cannot yet be recommended. We believe that the cut-off values presented in this study will serve as a valuable information for clinicians to aid their decisions for DXA referral.

P371 F

NON-INVASIVE EVALUATION OF TRABECULAR BONE DENSITY AND STRENGTH USING SCANNING ULTRASOUND

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Musculoskeletal complications induced by age-related diseases like osteoporosis, and in long-term disuse osteopenia such as a lack of microgravity during extended space missions, represent a key health problem. Such a skeletal disorder changes both the structural and strength properties of bone, and the latter plays a critic role in ultimately leading to fracture. Early diagnosis of these skeletal disorders (i.e., osteoporosis), leads to prompt treatment and could dramatically reduce the risk of complications. Using our newly developed scanning confocal acoustic navigation (SCAN) system, we evaluated the ability of quantitative ultrasound to non-invasively predict trabecular bone quality on 63 sheep bone samples (1x1x1 cm). The structural and strength of bone were validated using micro-CT and mechanical testing in three orthogonal directions, i.e., longitudinal (LG) (animal's weight-bearing direction), anteroposterior (AP), and mediolateral (ML). The ultrasound measurement consisted of confocal scanning of acoustic beam through the central region (2-D plane) of the sample with a resolution of 0.5 mm pixel size. These waveforms were processed to calculate the energy attenuation (ATT) (dB), the broadband ultrasound attenuation (BUA) (dB/MHz), the slope of the frequency-dependent attenuation at bandwidth 300-800 kHz, and the ultrasound velocity (UV) (m/s). An acoustic image in the region of interest (ROI) was then determined from derived ultrasound parameters. While there are fair correlations between broadband ultrasonic attenuation and micro-CT determined parameters such as bone volume fraction (BV/TV) (R=-0.68), as well as tissue bulk modulus (R=-0.31), strong correlations exist between ultrasound velocity and bone strength and structural parameters such as bulk modulus (R=0.82), and BV/TV (R=0.93). The correlations between SCAN prediction and bone quantity and quality parameters were improved by using a parameter to combine BUA and UV in a linear regression analysis, yielding R=0.96 with bone volume fraction (BV/TV), R=0.82 with tissue stiffness (bulk modulus), R=0.93 with yield strength and R=0.94 with longitudinal ultimate failure stress. These results suggest that SCAN has the capability to non-invasively assess bone mass and strength. Considering the complex architecture of trabecular bone, combining BUA and UV can provide a better prediction of bone's quality for determining osteogenic conditions.

P372 S

THE TRIAL TO ESTABLISH THE DIAGNOSTIC CRITERIA OF QUANTITATIVE ULTRASONOMETRY OF CALCANEUS

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Quantitative ultrasonometry (QUS) is suggested as one of the predictors of hip fractures, but it is not utilized for the diagnosis of osteoporosis, because of the lack of diagnostic criteria. AS QUS is one of the most widely utilized tools for the evaluation of osteoporosis in out patient's clinics, and the diagnostic criteria has been expected to be clarified. The aim of this investigation was to suggest the diagnostic criteria of osteoporosis using QUS in postmenopausal women. We measured both lumbar BMD by DXA and Osteo-Sono assessment Index (OSI) of os calcaneus by QUS, and evaluated the efficacy of QUS for the diagnosis of osteoporosis and risk evaluation of spinal fracture(s). The normal values of OSI were obtained from 19,306 residents after eliminating residents who showed over or less than 2.0 SD. <Results> Among the 1064 patients evaluated by both DXA and QUS, 459 patients were found having osteoporosis (lumbar BMD < -2.0 SD of young adult mean(YAM)) by DXA. The percentage of patients who showed lower OSI than -2.5SD of YAM, and -1.5SD were 20.7%, and 77.8% respectively among the osteoporotic women. If cut point of OSI is defined at -2.2 SD(80.8% of YAM), ROC analysis showed both sensitivity and specificity were 60% if cut point was set at -2.2 SD. Among 294 women who were evaluated their spinal X-ray examination, 27.6% had spinal fracture(s). If the cut point was defined at -2.37 SD below YAM, ROC analysis showed AUC of OSI was 0.615, and that of DXA was 0.545, respectively. The sensitivity and specificity at the cut off level were both 60%. <Conclusion> Judging from spinal fracture(s) and consistency of the diagnosis of osteoporosis by lumbar BMD, OSI of 2.2-2.4SD below the YAM is suggested as the diagnostic criteria of osteoporosis.

P373 W

AN ENHANCED QUS COUPLING AGENT: PERFORMANCE OF A FOURTH-GENERATION IMAGING ULTRASONOMETER

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Ultrasonometry of the os calcis is increasingly accepted as an effective low-cost method to assess osteoporotic fracture risk. The Lunar Achilles InSight (GE Medical Systems) is a fourth-generation system incorporating a 2-D solid-state receiving array. It eliminates 'blind' measurement by displaying a real-time heel image prior to and during measurement. A Region-of-Measurement circle on the image shows the measurement location. Historically, ultrasonometers have used water, ultrasound gel or mineral oil as the coupling medium to transfer the ultrasound signal from the transducers through the heel. Oil-based agents create difficulties in clean up and staining of clothes. Gel is water-soluble but must be cleaned from the transducers and heel. We evaluated a new coupling agent, isopropyl alcohol (70%) that was selected for its recognized safety in external application.

In vivo performance was compared between the Achilles InSight and the Achilles Express (GE Medical Systems), a previous generation ultrasonometer. Volunteers (n=29, including 22 older women) with a wide range of heel densities were measured on two days, using gel one day and alcohol the other. The combined data were subjected to regression (alcohol on gel values) and paired t-test analysis. Stiffness Index was highly correlated between alcohol and gel (r=0.983). Slope was almost identical to 1 while offset equaled 1.5, not significantly different from zero (alpha = 0.05).

Measurement convergence time of Stiffness Index was evaluated on 22 subjects who were measured with gel and isopropyl alcohol using a fixed acquisition time of 3 minutes. Alcohol-coupled signals converged more rapidly and BUA and SOS values stabilized faster as compared to gel.

Precision (%CV) was calculated for alcohol and gel measurements made on the same foot on separate days. Ten subjects were measured fifteen times in succession with repositioning between measurements. Alcohol had better repeat precision (lower variance). Results were highly significant ($p<0.001$) by F-test for Stiffness Index (CV of 1.95% Vs. 2.55%), BUA and SOS.

Isopropyl alcohol as a coupling agent gives Stiffness Index results nearly identical to coupling gel. Alcohol provides significantly shorter measurement time, superior stability and improved precision while eliminating the problems of messy oils or gels.

P374 F

JAPANESE PEDIATRIC REFERENCE VALUES USING THE LUNAR ACHILLES BONE ULTRASONOMETER

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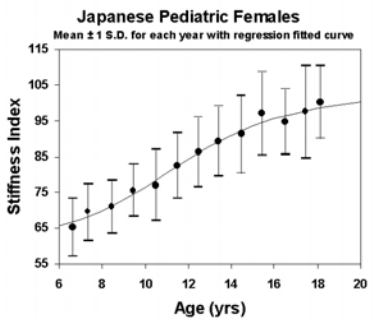
Quantitative ultrasonometry of the os calcis allows evaluation of pediatric bone status without exposing children to ionizing radiation. This study was undertaken to survey Japanese pediatric Stiffness Index values using the Lunar Achilles (GE Medical Systems). Age-matched population mean values are necessary for the interpretation of patient measurements.

Nine Lunar Achilles and Achilles+ units were used to measure 610 female and 538 male Japanese subjects ages 6 though 18 years. Subjects known to have diseases that affect bone metabolism (e.g. renal, thyroid, adrenal, chronic gastrointestinal, liver, cancer, diabetes, Paget's) were excluded from the study. Sets of shims were used to adjust the heel position to compensate for small foot sizes. The sample size and Stiffness Index mean values and standard deviation are shown for each year of age.

The relationship of Stiffness Index to age was determined by logistic dose response equations using regression weights based on variance. Stiffness Index for females followed age-related changes associated with childhood growth, with gradual increase from age 6 through 8, increased growth from age 9 to 15, followed by a plateau with little additional growth after age 16. Males followed a similar pattern with the exception that Stiffness Index increased most rapidly at about 10.5 years of age for girls and 13 years of age for boys. Results can be used as reference data for Japanese pediatric subjects. The age-matched, mean Stiffness Index values from the regressions are:

Females: $64.07 + 39.51 / [1+(age/11.85)^{-4.54}]$
Males: $71.99 + 37.15 / [1+(age/13.19)^{-6.88}]$

Japanese Pediatric Stiffness Index Values														
Age (yrs)		6	7	8	9	10	11	12	13	14	15	16	17	18
Female	N	21	31	52	38	42	42	64	50	57	47	58	80	28
	Mean	65	70	71	76	77	82	86	89	91	97	95	98	100
	SD	8.2	8.0	7.4	7.3	9.9	9.0	9.7	9.6	10.9	11.6	9.1	12.9	10.4
Male	N	23	35	32	39	44	42	36	10	47	70	66	64	30
	Mean	74	70	75	76	79	81	86	92	97	100	102	105	106
	SD	9.9	8.4	7.6	8.6	10.2	8.4	9.9	10.9	11.7	14.3	14.5	15.5	17.4



P375 S

SEX & AGE DIFFERENCE IN CORRELATION BETWEEN ULTRASOUND MEASUREMENT OF CALCANEUS WITH DXA MEASUREMENT IN CHINESE

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Aim: To access the association between the ultrasound measurement of calcaneus (Sahara, Hologic Inc.) and DXA measurement in hip and spine in elderly men, and young and elderly women.

Subject and Method:

Study subjects were 1446 elderly men (age above 65), 330 elderly women (age above 65) and 214 young women (age 20 to 35) who come to our centre for DXA measurements.

Both quantitative ultrasound (QUS) and DXA measurement were performed in the same visit. Broadband ultrasound attenuation (BUA, dB/MHz) and speed of sound (SOS, m/s) on the right heel were performed on a Sahara Bone Sonometer (Hologic Inc), which combine and provide Quantitative Ultrasound Index (QUI). BMD of lumbar spine and hip were measured by DXA Delphi 4500 (Hologic, Inc). The association between the QUS parameters and the various DXA measurements in each subject groups was determined by the calculation of Pearson correlation coefficients. Significant correlations were defined as those with $p < 0.05$.

Result:

QUS and DXA measurement were only weakly to moderately associated, ranging from 0.131 to 0.458. In all groups, highest correlation coefficients were found between the BUA and the DXA measurement. The correlation coefficients between BUA and QUI with DXA measurement were listed in table 1.

Discussion

In general, the correlation between QUS and DXA measurement were similar to other studies(1). Our study shows discrepancy between the correlation of QUS & DXA measurement in difference sex and in different age group. Correlation between QUS & DXA measurement parameters was better in elderly men, suggested that QUS measurement for predicting BMD values will be more reliable in men than in women. Correlations were poor in young women compared with elderly women, suggesting QUS measurement should not be used as prediction of BMD in younger age group.

Conclusion

Sex and age difference should be considered in deciding if QUS can be used to predict BMD values.

1. Faulkner KG et al (1994) Quantitative Ultrasound of the Heel: Correlation with Densitometric measurements at different skeletal site. Osteo Int 4:42-47

	Elderly Man		Elderly Women		Young Women	
	BUA	QUI	BUA	QUI	BUA	QUI
Total Hip	0.457	0.444	0.349	0.317	0.277	0.241
Trochanter	0.458	0.452	0.350	0.307	0.292	0.251
Intertrochanter	0.436	0.418	0.340	0.313	0.270	0.229
Femoral Neck	0.411	0.398	0.269	0.245	0.250	0.236
Lumbar Spine	0.402	0.390	0.282	0.250	0.214	0.192
All correlation listed are significant, with p<0.001						

P376 W

QUANTITATIVE ULTRASOUND PARAMETERS OF THE CALCANEUS PROFESSIONAL SOCCER PLAYERS: INFLUENCE OF PREFERRED VERSUS NON-PREFERRED LEG

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Objective: Physical activity appears to play an important role in the development and maintenance of bone mineral density (BMD). A lot of studies have reported higher BMD in professional athletes compared with the normal population. Quantitative ultrasound (QUS) may provide information related to the structural properties of bone. The aim of this study was to assess QUS measurement of calcaneus and to compare referred to non-referred side broad band ultrasound attenuation (BUA) and speed of sound (SOS) parameters in professional soccer players versus controls.

Methods: We measured bilateral calcaneus QUS -both BUA and SOS- using a Hologic Sahara clinical bone sonometer in 16 male professional soccer players and 20 healthy age and sex matched control. Professional soccer players had a high physical condition, training at least three times in a week for 10 or more years. The dominant or referred side was right in all of the subjects.

Results: Football players had significantly higher BUA and SOS with respect to controls. On the other hand there was no significant difference between referred and non-referred calcaneus BUA and SOS in both groups.

Conclusion: Regular sports activities have beneficial effects on bone status. However the positive effect of these long-term physical activities may be unrelated to dominant or non-dominant side.

Bilateral calcaneus QUS parameters of both groups			
	Professional soccer players	Controls	p
Age	25.0	27.3	NS
SOS right (m/s)	1628	1555	0.001
SOS left (m/s)	1632	1557	0.001
BUA right (dB/MHz)	116	82	0.001
BUA left (dB/MHz)	117	81	0.001

P377 F

THE MEASUREMENT OF SPEED OF SOUND USING BY QUANTATATIVE ULTRASOUND SONOGRAPHY IS USEFUL IN SCREENING FOR OSTEOPOROSIS

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Objective

The aim of our study is to evaluate whether the measurement of speed of sound(SOS) by using quantitative ultrasaound(QUS) is useful in screening for osteoporosis.

Design

The study group comprised 1030 women 30 to 75 years of age (mean, 54.5 years), consecutively enrolled at our climacteric outpatient clinic for 13 months(from January 2001 to January 2002).SOS was measured in right os calcaneus by using the handy QUS device (FURUNO CM-100).

Bone Mineral Density(BMD) was measured in the radius of the nondominant arm(super distal) by a DXA-70 bone densitometer(Matsushita Electrical Company), and in the lumbar spine(L2-4)by an XR-36 bone densitometer(Norland Corp.).

Results

We found that

(1) L2-4BMD had a tendency to decrease since 45-49 years old ,especially the rate of decrease from 51-54 years group to 55-60 years group was the highest.

(2) SOS had a tendency to decrease since 40-44 years old ,especially the rate of decrease from 51-54 years group to 55-60 years group was the highest, the same as L2-4BMD.

(3)The BMD of super distal radius had a tendency to decrease since 40-44 years old ,especially the rate of decrease from 51-54 years group to 55-60 years group was the highest, the same as L2-4BMD.

(4) There was a positive correlation between L2-4BMD and calcaneus SOS($r = 0.415$, $p<0.0001$), but the correlation between L2-4BMD and radial BMD was stronger than this .

Conclusion

Although the correlation between L2-4BMD and calcaneus SOS was not higher than that between L2-4BMD and radial BMD, Our results show that the measurement of SOS using by the handy QUS device (FURUNO CM-100)is useful in screening for osteoporosis.

P378 S

RELATIONSHIP OF SPEED OF SOUND AND BONE MINERAL DENSITY OF THE HUMAN CADAVERIC FEMORAL CORTEX

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PURPOSE: Cortical bone can be analyzed by both QUS (Quantitative ultrasonometry) and DXA (Dual X-ray absorptiometry). The aim of this study is to assess the relationship of QUS parameter and bone mineral density (BMD) from DXA at human femoral diaphysis.

METHODS AND MATERIALS: Twelve femoral cortical bone specimens from eight human cadavers were included. Measurement of SOS (Speed of sound) in the anterior and posterior cortex using Omnisense (Sunlight Ltd, Israel) was performed twice each after repositioning of the small probe (5 cm in length). The Omnisense combines the axial transmission mode and the critical angle concept which influenced by the mechanical properties of bone determined by cortical material and structure. It has shown great feasibility for multi-site cortical bone measurement. DXA measurement was done using Hologic QDR 4500 A at the matched site with anterior and lateral projection. Cortical diameters were measured from plain specimen radiographs.

RESULTS: Overall precision of SOS from duplicate measurements of each specimen was 0.013% (as root mean square CV). Correlation coefficient (R) between mean SOS and BMD was 0.66 in this study. SOS of the posterior cortex is higher than that of the anterior cortex in nine femoral specimens.

CONCLUSIONS: SOS in cortical bone is moderately correlated with projectional BMD in the femoral cortex. In spite of some discrepant factors including positioning problem of region of interest inherent to the QUS and DXA, we can assume other important factors other than BMD may contribute to SOS in the cortical bone. Further study with micro-CT and histomorphometry of the specimens is warranted.

P379 W

A COMPARISON BETWEEN ULTRASOUND AND DXA DETERMINATION OF BONE MASS DENSITIY FOR PREDICTION OF THE RISK FOR OSTEOPOROSIS

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The aim of the study was to compare the predictive value for osteoporosis of quantitative ultrasound (QUS-heel) with DXA (spine) determination for bone mass density.

Material and method: 150 women with the mean age of 51.62 years were assessed for their BMD by both quantitative ultrasound and DXA method. 109 women were in postmenopausal period (mean age for the last period= 44.72 years) and 41were in their perimenopausal years.

Results: Using DXA as golden standard 43 women had osteoporosis (Tscore minus 2.5), 71 had osteopenia (Tscore between minus 0.1 and minus 2.5) and 36 were normal (Tscore over minus 1). In 43 women who had osteoporosis by DXA examination: average DXA Tscore was -3.17 - 0.55 and average QUS T was -1.7 - 0.82. In this group of women 13.95 % had QUS Tscore over minus 1 (normal) that represent false negative results, 65.11 % had QUS Tscore up to minus 2.5 and 20.9 % had QUS Tscore below minus 2.5. In 71 women with osteopenia the average DXA Tscore was min. 1.7 - 0.43, the average QUS Tscore was minus 1.3 - 0.93, and 26.75 % of cases had QUS T score between 0 and minus 1, false negative for the risk for osteoporosis. In 36 women with DXA Tscore between minus 1 and plus 1 (normal range) 33 % had QUS Tscore between minus 1 and minus 2.5 and 5.55 % had QUS T score below minus 2.5. As compared with DXA Tscore values, QUS T score values had the trend to be higher for the interval of values which is predictive for osteoporosis by DXA standards and lower, thus predictive for the risk for osteoporosis, in cases in which DXA values are in the range for osteopenia or are normal.

Conclusions: as compared with DXA (spine) method for BMD assessment and prediction of the risk for osteoporosis, QUS (heel) examination is less accurate and should be kept only as a screening method.

P380 F

IN VIVO ASSESSMENT OF BONE QUALITY IN THE CLINIC WITH A SECOND GENERATION ULTRASOUND CRITICAL-ANGLE REFLECTOMETER

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With many skeletal disorders, changes in the micro-architecture and relative constituents of bone lead to altered mechanical and functional properties that may or may not be symptomatic. Current clinical bone mineral densitometry (BMD) and ultrasound broadband attenuation techniques (QUS/BUA) provide data on bone mass and its distribution, but not on its mechanical properties. Commercial ultrasound devices used to measure some mechanical properties in vivo are also available (Soundscan, Myriad Ultrasound Inc, Israel); however they lack the ability to assess these properties fully, and in particular neglect bone anisotropy, an important factor of the strength of both cortical and cancellous bone. The strength of bone determines its ability to fulfill its structural function, and a clinical determination of strength would be vital in assessing bone health and quality in numerous circumstances, including the effects of inactivity, the absence of gravity, the severity and prognosis of a fracture, the progression of a metabolic disorder, and the efficacy of treatment and countermeasures.

Ultrasound Critical Angle Reflectometry (UCR) is a relatively recent method that has been introduced to measure both the bone elasticity and its anisotropy. In the complex interaction between the ultrasonic beam and bone, the reflected acoustic field is modulated by the material properties with a dependence on angle of incidence and relation to material principle axes. The ability of UCR to sample the complete material elasticity matrix is unique when compared to other methods of assessing bone quality. We present here clinical results from a second generation UCR system based on new technologies developed by us, including large aperture, focused transducers for simultaneous illumination at multiple angles of incidence and multi-element receiver arrays coupled to a scalable, multi-channel data acquisition system for angular spectrum analysis of the reflected beam. In particular, we present bone quality measurements in the calcaneus (as determined by UCR assessment of material properties, including anisotropy) for a blinded, cohort study of osteoporotic patients and normals. UCR assessment of bone quality in the calcaneus is also compared to traditional bone mineral densitometry using a clinical BMD system for the heel.

P381 S

ULTRASONOMETRIC BONE PATTERN IN 32000 BRAZILIAN POSTMENOPAUSAL WOMEN: EVALUATION OF GEOGRAPHIC AND ETHNIC VARIANTS

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The frequency and distribution of ultrassonometric bone (calcaneous)values (BUS)were evaluated in 32000 Brazilian women, with 45 years old and more, at the nine main cities of the five different great geographic regions.

Variants as age, race, geographic origin and body index mass (BMI) were related to BUS values (using a LUNAR Achiles ultrassonometric portable unit). Mean square estatistic model for multiple comparators (Tukey test) was performed just in 12 842 women.

'Ultrassonometric osteopenia' was found in 12,2% and 'ultrassonometric osteoporosis in 1,1% ($p<0,0001$).

Conditions that interfered with these results were: mainly age and BMI. Geographic and ethnics variants seems not to be closely related to BUS values in this study. However, areas with European colonization and lower sunlight exposition showed to have more intense bone alterations at BUS.

Osteoporosis: Epidemiology

P382 W

EFFECT OF MILK SUPPLEMENTATION ON ALLEVIATING DEPRESSION AT METAPHYSES OF PHALANGES IN CHINESE ADOLESCENTS

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While depression at metaphyses of phalanges shown on plain hand X-ray is usually regarded as a normal variation in growing children, this X-ray sign has been reported as one of abnormalities seen in young patients with endemic Kashin-Beck disease in China, with chondronecrosis and impaired bone formation in pathological examinations. To investigate the relationship between this depression X-ray sign and nutrition, hand X-ray photos of 676 healthy Beijing girls who participated in a 2-year milk supplementation trial and had both baseline (10 years old) and end-trial X-rays taken were used. The subjects were from 9 primary schools in Beijing and randomly assigned into supplemented (n=437, averaging 144 ml milk with 245 mg calcium daily over 24 months, half of the girls with additional 3.33 microg vitamin D daily) and control groups (n=237, normal diet). Depression at metaphyses of phalanges was classified as either present (positive) or absent (negative).

Preliminary results showed that at baseline, prevalence rates of depression in the supplemented and control groups were the same at 43.5%, whereas at end-trial the supplemented group had a significantly lower rate than the control (34.4% vs 42.2%, P = 0.045). Additional vitamin D supplementation did not cause significant difference in the prevalence rate and therefore both groups were pooled into one supplemented group in the analysis. Logistic regression showed that risk of depression in the control group was 1.39 times that in the supplemented group (95CI: 1.01-1.93). Compared with controls, supplemented girls had a significantly higher calcium (at the end-trial, total dietary intake: 453 vs 749 mg) phosphorus (813 vs 947 mg), and protein intakes (55 vs 59 g). When stratified by supplemented/control, girls with depression signs had significantly lower bone age (baseline), delayed breast and pubic hair development, a lower percentage increase in total body bone mineral density, but higher percentage increase in body height at end-trial than their counterparts without depression signs. The results demonstrated that the depression at metaphyses of phalanges in Chinese adolescents was nutrition-related, and that milk supplementation reduced the prevalence rate. The long term biological significance of the depression change in growing girls needs further investigation.

P383 F

SMOKING AND POSTMENOPAUSAL OSTEOPOROSIS

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Introduction. Postmenopausal osteoporosis is still represents an important health challenges in our country. Although, it is well known that smoking plays an important role in increasing osteoporosis in postmenopausal women, but its long-term risks remains unclear. Several studies have demonstrated that long-term smoking aggravates osteoporosis in women.

Aim. The importance of this study emerges, as it is the first one to reveal the influence of smoking on osteoporosis in postmenopausal women.

Methods. We studied the effect of smoking 10 cigarettes or more per day in women compared with non smoker women on bone mineral density in 220 postmenopausal osteoporosis women between 60-75 years of age. Bone mineral density of the lumbar spine measured annually for two years by dual-energy x-ray absorptiometry.

Results. At the end of two years, the mean differences in bone mineral loss between the women who smoke 10 cigarettes or more per day and those who do not smoke, were 6.6% in the spine more in smoker women. It was noticed that smoking was associated with new vertebral fractures (6.3%, vs. 3.3% in the non-smoker group), an increased progression of vertebral deformities (42%, vs. 31% in the non-smoker group), and a reduced loss of height.

Conclusion. Smoking progressively decreases the bone mass in the spine, and increases the incidence of vertebral fractures, the progression of vertebral deformities, and height loss in postmenopausal women with osteoporosis.

P384 S

ESTIMATED BONE MINERAL DENSITY, DIET AND EXERCISE IN FEMALE STUDENTS AT THE UNIVERSITY OF SHARJAH, UNITED ARAB EMIRATES

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Values for reference populations of bone mineral density in young adults have been collected into databases for different ethnic groups, but until now not for the United Arab Emirates population. However, studies have shown that in other Arab countries bone mineral densities are statistically less than in their Caucasian counterparts.

Strategies for prevention of osteoporosis focus on attainment of optimum peak bone mass and maintenance of bone health from childhood onwards. Lifestyle factors such as low dietary calcium, insufficient weight bearing exercise, smoking, caffeine consumption and lack of exposure to the sun are known to reduce bone density.

This paper will present research being carried out to estimate, using peripheral quantitative ultrasound of the heel, bone mineral density in healthy, young adults at the University of Sharjah. The study addresses knowledge levels in the areas of diet, exercise and age related factors.

P385 W

EPIDEMIOLOGY OF OSTEOPOROSIS IN CHINA

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This article mainly described the study on osteoporosis epidemiology issued by China National 95 Priority Science and Technology Program. The study used the three manufactures of Dual Energy X-ray Absorptiometry (DEXA) equipments. Bone mineral density (BMD) was taken for 7,182 people aged above 20 years and questionnaires were taken for 48,943 people aged above 40 years in five administrative area of China by the stratified-multi-steps-cluster sampling method; 230 variables were analyzed by the non-conditional logistic regression. We found out the standardized bone mineral density among the general population in China health Han nationality, peak bone values, references of BMD diagnosis and treatment for osteoporosis from peak bone values in lumbar spine and proximal femur by sex and age difference; the total standardized prevalence rate of osteoporosis in the population aged over 60 years old was 22.6%. Of them, the standardized prevalence rate of male was 15.0% and that of female was 28.6% (P<0.01); there were also osteoporosis prevalence differences among cities, age groups and gender groups; but there were no osteoporosis prevalence differences between urban and rural areas. We screened 47 factors that influence the osteoporosis including the age, the chronic disease in respiration, the stomach trouble and the age of stop smoking which are common risk factors and house works, taking calcium, taking fruits, taking vegetables, vertical jumping up and riding bicycle which are common protect factors both in male and female; We concluded that the prevalence rate of osteoporosis in females was higher than that of female and increased with age. The prevention and treatment of osteoporosis are very important to the female but should not be ignored in male; there are common influencing factors and there are some certain variances of the risk factors and exposed level in male and female in China.

P386 F

THE HUNGARIAN OSTEOPOROSIS RISK ASSESSMENT (HORA) STUDY

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Background. It is widely accepted that race and geography significantly affect the risk for osteoporosis. However, most of our knowledge on risk factors was derived on populations from Western-Europe and the United States. Much less is known about similar associations in Eastern European people.

The aim of our present study was to describe the frequency and risk factors for osteoporotic fractures and osteoporosis in a female population in a cross-sectional, multi-center study performed under the auspices of the Hungarian Society for Osteoporosis and Osteoarthrology.

Patients and methods. From five randomly selected regional and 5 local osteoporosis centers, altogether 2606 women over 18 years of age, referred to the given center with any osteoarthrological reason, participated. During the office visit, detailed risk factor assessment questionnaire was filled in and blood pressure, weight, height and bone mineral density (BMD) were measured.

Results. Using the results of a univariate analysis the following variables were made available for further examination: older age, longer duration since menopause, lower femoral T-score, positive family history of bone fracture, less physical activity, fall in the previous year, lower diastolic blood pressure and smoking habit. Using multiple regression analysis, only older age, lower diastolic blood pressure, family history of bone fracture, fall in the previous year and lower T-score were independently related to fractures ($p < 0.05$). In comparison the univariate risk factors for femoral osteoporosis were higher age, lower weight, lower BMI, positive family history of bone fracture, fall in the previous year, glucocorticoid treatment, lower parity, lower diastolic blood pressure, longer duration since menopause and less physical activity. The multiple regression analysis revealed the following independent associates ($p < 0.05$) from the previous list: older age, lower weight, family history of bone fracture, less physical activity, fall in the previous year and glucocorticoid treatment.

Conclusion. Our study is the first large-scale epidemiological survey describing risk factors of osteoporosis and fractures in a Hungarian female population. Our data suggest that lower diastolic blood pressure might be a new risk factor related to osteoporotic fractures; however, it needs further examinations.

P387 S

OSTEOPOROSIS, VERTEBRAE FRACTURE AND 25-OH VIT D IN RUSSIAN POSTMENOPAUSAL WOMEN

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Aim: To study the prevalence of osteoporosis in lumbar spine and vertebrae fractures among women 55 and elder.

Material and methods: cross-sectional study of 2155 women 55 and elder using standardized questionnaire.Bone mass measurements of lumbar spine (L1-L4) were performed by DEXA (Hologic 4500A), X-ray examination of thoracic and lumbar spine of 700 patients with morphometric analysis of images. In sample of 80 random postmenopausal women with osteoporosis (mean age 68+6 years) 25-OH Vit D was measured.

Results: The prevalence of osteoporosis in lumbar spine was 29,8% and increased with age from 19,6% in 55-60 years to 32,1% in 75-79 years. Spine osteoporotic fractures were revealed in 15,5% by morphometric analysis of x-ray images. 80 postmenopausal women with osteoporosis, among them 42 (52,5%) with osteoporotic vertebrae deformaties, had normal level of 25-OH Vit D (mean 68,35+16,77 nmol/l).

No correlations between 25-OH Vit D level and BMD value of lumbar spine and femoral neck was found. 25-OH Vit D level not correlated with presence of osteoporotic vertebrae fractures.

CONCLUSION: our study revealed prevalence of osteoporosis and vertebrae fractures among postmenopausal Russian women and absence of vitamin D insufficiency in osteoporotic women.

P388 W

SEASONALITY AND INCLEMENT WEATHER AND THE RISK OF HIP FRACTURE

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Introduction: An association between inclement weather and hip fractures has been documented in cities with cold weather.

Objectives: To analyse if in our zone (lat 40) exists seasonal variation in the incidence of hip fracture. To study how climatologic variables influence in the incidence of hip fractures.

Methods: We obtained information that included hospitalisation data of all hip fractures in our city from Oct-1998 to Sept-2001 (3 years), and meteorologic data of all the days of this period was asked to the National Institute of Meteorology of our country. We used a cross-level design to examine the association between the rate of hip fractures and the meteorologic conditions on the day of the accident in both sexes and two age strata. For the comparative analysis the density of incidence has been used (n of cases by 100,000 person-days). The relative risk has calculated (RR) and its interval of confidence 95% (IC), between climatologic stations and for the different studied climatologic variables.

Results: There were a total of 704 hip fractures over 1,095 day study period. The overall incidence was 1,08 per 100,000 persons-days. Among women, the risk of hip fracture was increased during winter versus spring (RR 1.33, IC 1,06-1,68), and versus summer (RR 1.34, IC 1,06-1,69). In older individuals (aged > 75 years) the risk of hip fracture was increased during winter versus spring (RR1,29, IC 1,03-1,62), and versus summer (RR 1,26, IC 1,01-1,58). We do not observed this pattern among men nor younger individuals (aged < 75 years). The meteorologic conditions associated with increased rates of hip fracture were snow (RR 2,15, IC 1,18-3,90), fog (RR 1,63, IC 1,21-2,20) and strong wind (RR 1,24, IC 1,05-1,47). We do not find association with rain, intense rain, storm, hail and lower temperatures.The association between inclement weather and hip fractures was stronger among older persons and women.

Conclusions: We confirmed the seasonal variation of the hip fracture, in our zone. Some climatologic variables (snow, fog and wind) explain part of this increase.

P389 F

INFLUENCE OF FLUORIDE CONCENTRATION IN DRINKING WATER AND BRICK-TEA WATER ON BONE MASS IN HEALTHY INNER MONGOLIAN YOUNG WOMEN

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BACKGROUND: It has been found that the concentrations of fluoride in drinking water and brick-tea are high in the grassland area of Inner Mongolia, China. We undertook a cross-sectional study to evaluate the influence of drinking water and brick-tea water fluoride levels on bone status.

SUBJECTS AND METHODS: We studied 38 and 46 healthy Mongolian women with brick-tea drinking habit in a grassland area and without in an urban area respectively, aged 20 to 34. Speed of sound (SOS), broadband ultrasound attenuation (BUA) and stiffness index (SI) were measured at the calcaneus using quantitative ultrasound (QUS) analysis, while the second metacarpal bone mineral density (BMD) and metacarpal cortical index (MCI) of dominate and non-dominate hands were measured using computed X-ray densitometry (CXD). We analyzed fluoride contents in well water and brick-tea water from the household of each subject in the grassland area and in city water from one source in the urban area.

RESULTS AND DISCUSSIONS: Fluoride concentrations in drinking water were remarkably higher in the grassland area (1.14 -5.39 ppm, mean= 2.69 ppm, well water) than that in the urban area (0.31 ppm, city water). Fluoride concentrations in brick-tea water were 2.61-10.87 ppm (mean= 5.14 ppm). Compared with women in the urban area, those in the grassland area were found to have significantly lower bone mass in SOS ($p<0.01$), non-dominate-hand MCI ($p<0.05$) and dominant-hand MCI ($p<0.05$), after adjusted by age and body mass index. Brick-tea water fluoride concentration indicated significant correlations with dominate-hand MCI ($r=-0.49$, $p<0.01$) and non-dominate-hand MCI ($r=-0.49$, $p<0.01$) in the grassland area, but not with SOS. No correlations between drinking water fluoride and bone mass indexes were found in the grassland area. These results suggest that excessive intake of fluoride may decrease bone mass, and that intake of fluoride from brick-tea may play an important role in this grassland area's low bone mass. To further clarify the influence of fluoride exposure on bone, correlations of fluoride exposure and bone metabolism are under investigation.

CONCLUSION: This study shows that low bone mass in the grassland young women is related with high fluoride concentration in brick-tea water.

P390 S

RISK FACTORS FOR FRACTURES OF THE DISTAL RADIUS AND PROXIMAL HUMERUS AMONG JAPANESE POPULATION

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Objectives: Although risk factors for hip fractures among Japanese have been described, no study has examined risk factors for fractures of the distal radius and proximal humerus. We conducted a case-control study to identify risk factors for these fractures.

Patients and Methods: Subjects were selected from patients aged 35 years old and over with distal radius and proximal humerus fractures which occurred by minor trauma and were treated at 6 hospitals in Tottori Prefecture from 1999 to 2000. Two age (± 3yrs) and sex-matched controls for each case were selected from patients who subsequently visited the same hospital for treatment other than for fracture. Questionnaires concerning body weight, body height, place of residence, smoking, alcohol drinking, milk intake, life style, physical activity, eyesight and previous diagnosis were sent by mail to both subjects and controls.

Results: Distal radius fractures: A total of 213 subjects (31 men and 182 women) aged from 35 to 98 years old (mean 66.2 years) and 426 controls were selected and 177 (83.1%) subjects and 346 controls (81.2%) responded. Compared with the control group, patients with distal radius fracture were less likely to have difficulty in walking (O.R. 0.30, 0.14-0.64, $p<0.001$), have difficulty in going up stairs (O.R. 0.45, 0.23-0.05, $p<0.03$). Futon use (not bed use) recently but before the fracture (O.R. 0.59, 0.37-0.94, $p<0.03$) had a significant association with reduced risk of distal radius fractures. Smoking, alcohol drinking, milk intake and body mass index showed no significant association with fracture risk. Proximal humerus fractures: A total of 57 subjects (6 men and 51 women) aged from 37 to 93 years old (mean 75.9 years) and 114 controls were selected and 45 (78.9%) subjects and 97 controls (85.1%) responded. Compared with the control group, those with proximal humerus fracture were more likely to have difficulty in walking (O.R. 8.92, 1.05-75.51, $p<0.03$). Futon use (not bed use) recently but before the fracture (O.R. 0.40, 0.16-0.99, $p<0.05$) had a significant association with reduced risk of proximal humerus fractures.

Conclusion: Different risk factors were associated with distal radius and proximal humerus fractures; however, futon use was a protective factor for both fractures.

P391 W

SKELETON EFFECTS OF AMENORRHEA

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The association between postmenopausal oestrogen deficiency and osteoporosis has been clearly established for many decades. The deleterious effects of acquired functional oestrogen deficiency on bone metabolism in young women has only more recently been recognised.

Estrogen deficiency during puberty can impair the attainment of normal peak bone density; therefore, the skeleton effects will change in accordance with the age- onset of oestrogen deficiency. The persistent amenorrhea is the principal alarm signal.

In this study we have evaluated 2.509 women with a mean age of 51.7±4.5yr (range: 40-59 years) BMI 25±4.3 and BMD 1.104±0.165 g/cm2 , arrived for the first time at our centre to effect a bone densitometry (DPX Lunar V 3.61, Madison, Wisconsin) at lumbar spine (L2-L4). We have excluded women with well known risk factors for osteoporosis and in previous treatment with antiresorptive drugs. We have selected 570 amenorrheic women and 1232 controls. Table I shows the characteristics and BMD of the subjects.

Our data show a significant reduction of BMI and BMD in amenorrheic women in comparison to controls.

The reduction of BMI can be due to younger age of amenorrheic women.

The Amenorrhea is to consider an important risk factor for osteoporosis also in relation to younger age of amenorrheic group.

These data suggest the critical importance of HRT among premenopausal women.

Groups	n	Age	BMI	BMD L2-L4
Amenorrhea	570	50.6±4.6	21.4±1*	1.069±0.152*
Controls	1232	52.0±4.5	25.9±1.8	1.108±0.159
(*p<0.001 vs controls)				

P392 F

ASSOCIATION OF MUSCULOSKELETAL SYMPTOMS AND OSTEOPOROSIS IN POSTMENOPAUSAL WOMEN

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Background: Menopause has been characterized as the state of potential musculoskeletal changes. Osteoporosis and fracture are one of the most debilitation conditions which can be prevented during the early stages of postmenopause. Objectives: This study is to define the association between imposed musculoskeletal symptoms, osteoporosis and consequent spinal fracture Study design: 1,500 women who seek for health maintenance checkup, aged between 28 to 76 years old were included in the study. The risk of osteoporosis and spinal fracture associated with menopause and skeletal symptoms were evaluated. Results: The most frequently complained musculoskeletal symptoms by these women were lumbago, small and large joint pain, stiffness and numbness. Large joint pain was the only musculoskeletal symptoms significantly more frequent due to the changes in menopause state. Prevalence of radiological spine compression fracture in randomly selected 500 women were 9% (n=45) of which N=39 were postmenopausal women. Lumbago and large joint pain was significantly associated with spinal fracture prevalence. Spinal fracture was significantly inversely associated with all BMD except the femur total BMD. The prevalence of osteoporosis in postmenopausal women, estimated by WHO criteria with DXA spine L2-L4 mean, spine lowest, femoral neck, wards triangle, and femur total were 18%, 41%, 4.4%, 30% and 1% respectively. Large joint pain did not correlate with BMD. Bone turnover estimated by serum osteocalcin, alkaline phosphatase and urinary deoxypyridinolin were largely dependent on the state of menopause and were statistically significantly higher in postmenopausal women ($p<0.05$). And spinal fracture was positively associated with serum ostocalcin and alkaline phosphatase. Conclusions: These findings indicate that skeletal symptoms were significantly associated with consequent skeletal changes in postmenopausal women and the extent to urge the physicians to take appropriate measures for early diagnosis boneloss and management must be provided as early as possible.

P393 S

REFERENCE INTERVALS FOR QUANTITATIVE IDENTIFICATION OF PREVALENT VERTEBRAL DEFORMITIES IN SAMPLE WITH HIGH PREVALENCE OF DEFORMITY

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Reference intervals (mean and SD) for quantitative vertebral morphometry can be derived using a trimming approach. However, this can be problematic if the reference population has a high prevalence of deformity. The aim of this study was to develop a robust method to obtain reference intervals from a clinic-based sample by searching for the best measure of central tendency, and searching for the best approach for setting critical values.

We studied 80 women from metabolic bone clinic, ages 48 to 87. We also studied 372 women from three general practice populations in the same city as the clinical sample, ages 50 to 85. Lateral spinal radiographs were marked for T4 to L5 (Hp was marked at the base of the uncinate process). Four heights were measured (Ha, Hm1, Hm2, and Hp). Four height ratios were calculated (Ha/Hp, Hm/Hp, and Hp/Hp+-). Mean and SD, median and mode (the most frequent value) of ratios were calculated for the two samples.

Means derived from clinical sample were generally lower than those from population sample, especially at vertebral levels with high fracture prevalence (T8, T11-L2), while SDs were much higher for the clinic-based sample. Median and mode derived from the clinic-based sample were higher than the mean, and close to the population mean, with mode more closer to the population mean at T8, T11-L2 than median. In the population sample, median and mode were very similar to mean at all vertebral levels.

We conclude that mode could replace mean in samples with a high prevalence of deformity. The SD in reference interval should be derived from a population sample with the same method of marking.

Table: Mean and SD, median and mode for Hm/Hp of T8, T11-L2

		T8	T11	T12	L1	L2
Clinical	Mean	0.84	0.88	0.85	0.82	0.88
sample	SD	0.13	0.10	0.14	0.16	0.10
	Median	0.89	0.92	0.91	0.88	0.92
	Mode	0.96	0.99	0.96	0.96	0.97
Population	Mean	0.97	0.97	0.96	0.99	0.99
sample	SD	0.03	0.03	0.03	0.02	0.02
	Median	0.98	0.97	0.97	0.99	0.99
	Mode	0.98	0.96	0.97	0.98	0.99

P394 W

PREVALENCE OF OSTEOPOROTIC VERTEBRAL FRACTURES: COMPARISON OF MORPHOMETRIC RADIOGRAPHY (MRX) AND MORPHOMETRIC X-RAY ABSORPTIOMETRY

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The purpose of our study is to compare MRX and MXA for the identification of vertebral fractures. On 150 postmenopausal women (ages 45-80 years)referred to our Osteoporotic Centre, were acquired spinal lateral radiographs for MRX and scans with dual energy X-ray absorptiometry (DXA) high definition for MXA, using the Hologic QDR 4500A. For gold standard identification of vertebral fractures we used a consensus reading of the radiographs by two experienced radiologists (D.D. and E.T.) using Genant's semiquantitative (SQ) method.MRX and MXA identification of vertebral fractures was performed using the Mc Closkey algorithm.

SQ identified a total of 69 vertebral fractures in 45 subjects; MRX and MXA correctly identified 93% and 80% of vertebral fractures identified by SQ, respectively. Agreement between morphometric methods (MRX and MXA) and semiquantitative (SQ) radiological assessment was slightly better for MRX (k=0.88; 95%CI=0.83-0.91) than for MXA (k=0.75; 95%CI=0.69-0.77). Both MRX and MXA were less effective than SQ in detecting mild endplate deformities. Nevertheless in our study only 9 vertebral fractures were missed by MXA because of vertebrae excluded from analysis, correctly identified by MRX. In conclusion, acquisition of spinal scans with DXA high definition, improving the image resolution, allows to visualize sufficiently for morphometric analysis the upper thoracic spine. Therefore MXA is a useful, low-radiation technique to identify prevalent vertebral fractures.

P395 F

EPIDEMIOLOGY OF HIP FRACTURE IN NORTH OF PERU

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The objective of the present study is to know the incidence and the mode of presentation of hip fractures in to population in the majority half beed of the north of Peru

Material and methods: We study the population of direct covering referred to the HNAAA (365,000 social security people). 17.6% is oldest than 50 years. The regional total population is of 1 980243. The difference for sex is that the women in population are 51% and in insureds and they are 49%. Traumatology surgery receives practically all the total of hip fractures happened in insureds. This is the result to revises the registered revenues among January 1993 and December 2001.

The epidemiology of 750 hip fractures happened in that period is analyzed. The incidence average of the 213x100000 in women and of 136x100000 in man $p < 0.05$. The general half age was 78.1 years, in women was 77.6.6 and in man 79.1 p=0.2. The localization interchocanteric: 63% and Neck femoral 27% $p < 0.001$. In women the neck it fractures was 41% and in man 27% $p < 0.001$

The annual variation of incidence had constant increment to year exception 2001. The presentation age in women increase in the latest 3 years.

Discussion: we confirm similar incidence to other countries of Latin America with fractures age on the 77 years and bigger frequency and prematuridad for the female. The localization interchocanteric is bigger in both sexes but rate of fractures of femoral neck is significantly predominant in feminine sex The measured of prevention of the last years seemingly have not been successful, for its bigger increment of incidence over the populational vegetative growth, but there is tendency to fractures in the woman at more age. This study is a bigger casuistry in the nation and it have an additional objectives in relationship rates of mortality and factors of risk to neck in female.

P396 S

PATIENT CHARACTERISTICS THAT PREDICT THE TIME TO FIRST CLINICALLY RECOGNIZED VERTEBRAL DEFORMITY IN POSTMENOPAUSAL WOMEN ENROLLED THE CANADIAN MULTICENTRE OSTEOPOROSIS STUDY (CAMOS)

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Osteoporosis is a silent disease, until the first fracture occurs. Given that bone densitometry screening of all women is not practical to identify individuals who will suffer a fracture, other characteristics are required to estimate fracture risk. Utilizing participants from CaMos, a national, random sample of the population, we performed a 3 year prospective cohort study in community dwelling postmenopausal women to

examine the association between various patient characteristics and clinically recognized incident vertebral deformities. Participants were classified into two groups according to their incident fracture status; those without incident fractures during the study period (n=4829), and those with an incident vertebral deformity (n=34). Fractures due to major trauma such as motor vehicle accidents were excluded from the analysis. Follow-up was calculated as the time from baseline examination to the date of the incident deformity or the date of last follow-up examination. Characteristics examined in the Cox multivariate survival analysis included age, prevalent vertebral deformity status, change in height, current height, change in weight, current weight, body mass index, SF-36 physical component summary score, and lumbar spine and femoral neck bone mineral density (BMD). Relative risk and 95% confidence intervals (CI) were calculated. The mean (standard deviation) age, height and weight of participants was 66 (10) years 159 (6) cm and 69 (14) kg for those without incident fractures and 74 (10) years, 156 (7) cm and 59 (11) kg for those with an incident vertebral deformity. The relative risk of sustaining an incident vertebral fracture was associated with the SF-36 physical component summary score (0.959; 95% CI: 0.924, 0.996) and femoral neck BMD (0.002 95% CI: 0.00, 0.506). A prevalent vertebral deformity (2.337; 95% CI: 0.897, 6.088) and a change in height (1.075; 95% CI: 0.970, 1.193) tended to be associated with incident fracture status but further evidence will need to be collected to verify these findings. Patient characteristics including quality of life and femoral neck BMD are important factors associated with incident vertebral deformities. The identification of high-risk patients is essential to effectively use the growing number of available osteoporosis therapies.

P397 W

RISK FACTORS THAT PREDICT THE TIME TO FIRST NON-VERTEBRAL FRACTURE IN POSTMENOPAUSAL WOMEN RECRUITED FROM THE CANADIAN MULTICENTRE OSTEOPOROSIS STUDY (CAMOS)

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A number of factors have been found to be associated with previous non-vertebral fragility fractures; they include advanced age, height, existing fracture, and propensity to falls. Unfortunately, our understanding of risk factors related to incident non-vertebral fractures is still inadequate. Thus, the purpose of this study was to examine the association between various potential risk factors and incident non-vertebral fractures in a 3 year prospective cohort study of postmenopausal women who were enrolled in CaMos. CaMos is a national, randomly selected sample of the Canadian population that provides substantial data regarding osteoporosis and potential risk factors. Participants were classified into two groups according to their incident fracture status; those without incident fractures during the study period (n=4829), and those with an incident non-vertebral fracture at the wrist, hip, humerus, pelvis or ribs (n=163). All incident fractures were a result of minimal trauma. Follow-up was calculated as the time from baseline examination to the date of the incident fracture or the date of last follow-up examination. Information collected at cohort entry was extensive and included data from questionnaires and a number of physical measurements. Potential risk factors included in the Cox multivariate survival analysis were anthropometric, fracture history and family history of osteoporosis, current medications use, comorbid conditions, caffeine and alcohol intake, leisure and occupational physical activity levels; tobacco use, bone density and quality of life measures. Relative risk and 95% confidence intervals (CI) were calculated. The mean (standard deviation) age of the participants was 66 (10) and 71 (10) years for those without and with incident non-vertebral fractures. Results indicated that important risk factors that modified relative risk include previous minimal trauma forearm fracture after the age of 50 years (3.626; 95% CI: 1.876, 7.008), and other minimal trauma fractures (1.957; 95% CI: 1.082, 3.540), SF-36 physical component summary score (0.965; 95% CI: 0.939, 0.991), femoral neck bone density (0.036; 95% CI: 0.001, 0.937) and inflammatory bowel disease (2.207; 95% CI: 1.091, 4.465). In conclusion, several important risk factors are associated with incident non-vertebral fractures. These factors should be evaluated as a first step in osteoporosis management.

P398 F

THE ASSESSMENT OF RISK FACTORS FOR GLUCOCORTICOID-INDUCED OSTEOPOROSIS IN WOMEN CHRONICALLY TREATED WITH ORAL GLUCOCORTICOIDS

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The purpose of our study was to determine main factors influence on bone mineral density (BMD) in premenopausal and postmenopausal women chronically treated with oral glucocorticoids (OGC). The study comprised 50 women with bronchial asthma aged 41-66 years (54.5±6.7 (M±s)) received OGC for 1-34 years. Control group consisted of 129 women 41-66 years old (53.3±8.3) hadn't ever received any OGC therapy. BMD was assessed utilizing DEXA technique. BMD in lumbar spine (L2-L4), proximal femur (femoral neck, Ward's triangle and trochanter) was measured by Lunar DPX absorptiometer, BMD in distal forearm was measured by Osteometer DTX-200. Incidence of vertebral deformities was assessed using lateral thoracic and lumbar spine X-rays. Vital and gynecological anamnesis, body weight, average daily calcium intake, standardized average daily dose of OGC (tablets) and duration of OGC therapy were assessed. Results: Premenopausal women used OGC (n=18) had no difference in vertebral end non-vertebral fracture rates vs. premenopausal controls (n=52), but incidence of vertebral deformities was 64% in postmenopausal ones vs. 42% in postmenopausal controls ($p<0.05$). There was no difference in BMD in premenopausal and postmenopausal groups vs. age-related controls, but BMD (T-score, SD) was lower in all women treated OGC vs. all controls (-1.25±1.5 vs. -0.71±1.5 in lumbar spine and -0.45±1.3 vs. 0.04±1.3 in total proximal femur, $p<0.05$). Asthmatic women with osteoporosis vs. normal ones were older (60.6±4.0 vs. 51.9±6.2 years, $p<0.001$), had more postmenopausal age (11.5±3.9 vs. 4.49±5.6 years, $p<0.05$), duration of OGC treatment (15.6±11 vs. 8.83±6.4 years, $p<0.05$) and lower body weight (68.1±12 vs. 77.9±10 kg, $p<0.05$. Only body weight showed significant correlation with BMD (g/cm2) in all skeletal sites (r=0.54-72, $p<0.05$) in premenopausal asthmatic women. Conclusions: Prolong OGC therapy increases risk of osteoporosis, and increases risk of vertebral deformities in postmenopause. In women chronically treated with OGC BMD depends on body weight in premenopause, and on age, postmenopausal age, duration and dose of OGC therapy, body weight and daily calcium intake in postmenopause

Factors	BMD (g/cm^2)				
	L2-L4	Femoral neck	Ward's triangle	Trochanter	Forearm
Age, years	-0.41*			-0.41*	
Postmenopause, years		-0.47*	-0.41*	-0.54**	-0.37*
Duration of OGC therapy, years	-0.34*	-0.41*	-0.39*	-0.37*	-0.56**
Standardized daily dose of OGC, tabs	-0.43*				
Daily calcium intake, mg	0.41*	0.4*	0.34*	0.45	
Body weight, kg	0.55**	0.45*	0.47*	0.43*	0.48**
Correlation of BMD with risk factors in postmenopause (*p<0.05, **p<0.01).					

P399 S

WITHDRAWN

P400 W

APPENDICULAR MUSCLE STRENGTH IN OSTEOPOROTIC VERSUS NORMAL SUBJECTS

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Both sarcopenia and osteoporosis progress with age. Sarcopenia is associated with loss of strength and may result in impaired mobility and increased risk of falls. In order to maintain function in patients with osteoporosis, it is necessary to adequately assess strength deficits. This study was initiated to determine whether there is a significant difference in lower extremity strength between normal and osteoporotic individuals. Knee extensor strength (KES) was measured in a cohort of 64 adult females with radiographic evidence of osteoporosis. KES was recorded as the maximal weight lifted through a full arc for 10 repetitions. Subjects were stratified by decade of life. The 95% confidence intervals for osteoporotic subjects' mean strength were compared with published means for normal subjects (controls). Means for height and weight of normal subjects within each decade were within the respective confidence intervals for osteoporotic patients, demonstrating no significant demographic differences. Osteoporotic mean KES was statistically significantly weaker than that of normal subjects in the fifth through the eighth decades. At the fifth decade, mean right KES was 2.24 times less in osteoporotic subjects compared with age-matched controls. By the ninth decade, though osteoporotic mean KES was weaker than that of normal subjects, it was not statistically significant. The variable results in the oldest group may be due to the smaller available sample (N=6 for osteoporotic subjects). Further study is warranted, in which strength in a larger sample of osteoporotic patients can be compared to known normal values to better characterize the need for strengthening interventions in osteoporosis patients to help reduce the risk of falls.

P401 F

THE EFFECT OF PRE-EXERCISE MOOD ON BONE METABOLISM DURING AEROBIC SESSIONS

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[Aim] Exercise is widely performed for health promotion in the world. However, it is not known the effect of pre-exercise mood on bone metabolism, which directly affect the health promotion. Therefore, we studied the effect of pre-exercise mood on bone metabolism during aerobic sessions, through measurement of osteocalcin (OC) and deoxypyridinoline (DPD).

[Subjects and Methods] We enrolled 31 normal volunteers (5 males and 26 females) who are healthy enough to perform aerobic exercise sessions. Their mood status was evaluated using the Profile of Mood Status (POMS) inventory, and the subjects were divided into two groups according to the T-scores of Depression-Dejection scale (DD) and Tension-Anxiety scale (TA). Their characteristics were shown in a table. The bone metabolic state was evaluated by measurement of serum intact OC and urine DPD, both pre- and post-exercise. One aerobic session consisted of a 20-min pre-load exercise, a 50~70-min aerobic exercise, a 20-min weight machine training and a 15-min cooling down. The subject had continued the exercise session twice a week for 3 months.

[Results] Pre-exercise OC levels did not differ according to the scores of DD nor TA. OC level increase in low DD group (pre: 7.21±2.71, post: 8.64±3.36) and in low TA group (pre: 7.09±2.61, post: 8.38±3.30) significantly ($p<0.05$, paired t-test), while not in high DD group (pre: 6.76±3.09, post: 6.23±2.11) nor high TA group. DPD in urine did not differ according to the scores of DD nor TA, and did not change after exercise.

[Conclusion] These data suggest that pre-exercise mood, such as low depression and/or low tension, affect on the OC levels, which may lead to the influence on future bone formation.

	High DD(>50)	Low DD(<50)	High TA(>50)	Low TA(<50)
T-score	50±6	45±3	56±5	43±3
M/F	2/10	3/16	2/8	3/18
mean age	45	52	46	51
range	20-72	21-67	20-72	21-67
OC(pre)	6.76±3.09	7.21±2.71	6.93±3.39	7.09±2.61
OC(post)	6.23±2.11	8.64±3.36	6.30±2.31	8.38±3.30
DPD(pre)	6.76±1.86	5.84±2.02	6.74±2.05	5.94±1.94
DPD(post)	6.89±2.29	6.02±1.93	6.86±2.44	6.12±1.91

P402 S

IS EDUCATIONAL LEVEL ASSOCIATED WITH LIFE STYLE OF OSTEOPOROTIC WOMEN AND ATTITUDES TOWARDS OSTEOPOROSIS MANAGEMENT?

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Data on a possible association between socioeconomic status (as assessed by the educational level) and osteoporosis have been conflicting due to several reasons. The aim of this study was to identify the impact of educational level on lifestyle, and other parameters related to the management of osteoporosis, e.g. choice of physician by women with primary osteoporosis in Greece.

For this purpose 255 osteoporotic women (as defined by WHO criteria) from an Osteoporosis Center were studied. Educational level was classified as low (elementary school), intermediate (high school) and high (University). Mean age was 60±0.5 years and mean age of the diagnosis of osteoporosis was 55±0.5 years. Menopause appeared automatically in 192 women (75,29%) and postsurgically in 63 (24,71%). Postmemopausal symptoms were referred by 154 women (60%) and specifically, by 50% of the low, by 85.96% of intermediate and by 60.34% of high educational level ($p<0.001$).

Calcium intake before the age of 25 years was associated to educational level ($p=0.002$) while present calcium and caffeine consumption and smoking habits were not different among the groups.

Physical activity was related to educational level ($p=0.025$). Of the 58 patients that reported no physical activity 41.38%, 24.14% and 34.48% belonged to the low, intermediate and high educational levels respectively.

Diagnosis, in most of the cases and in all groups, was established by an orthopedist, followed by a rheumatologist in women of lower and intermediate educational level (27.34% and 26.32%) and by a gynecologist (18.97%) in women of higher educational level. Mean age of diagnosis was also different among the three groups (56, 54 and 53 years respectively; $p=0.021$). Surprisingly, clinical fractures, were significantly higher in the lower educational group (72.6%), compared to the other two groups (13.7% for both ;$p=0.002$)

Current medical treatment was mainly by bisphosphonates in the first group (57,76%) and HRT in the other two (38.6% and 27.59%; $p<0.001$)

In conclusion, educational level and in extension the socioeconomic status appears to be involved in many parameters of osteoporotics' life style. The choice of the specialist by osteoporotics may also be influenced by educational level as well as medical management of their disease.

P403 W

OSTEOPOROSIS IN NON-CAUCASOID (AFRO-BRAZILIAN) PATIENTS: ASSOCIATION WITH DIABETIS MELITUS (DM) AND HYPERPARATHYROIDISM

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AIM:To analyze the risk factors, clinical and laboratory features of osteoporosis in (non-caucasoid) afro-brazilians.

PATIENTS AND METHOD: The files of 35 afro-brazilian patients, from the Osteoporosis Unit in Rheumatology Division of the State University of Campinas (UNICAMP) were reviewed. Mean body index mass, age, age at menarche and menopause, drugs, associated diseases,habits as smoking and drinking, and abnormalities in calcium balance were considered as risk factors. Evaluation of bone mineral density (DMO) was performed at lumbar spine and femoral neck (DEXA-Lunar equipment).

RESULTS: Mean age of the affected patients was 64,6 years (DP=2,3) and mean body index mass was 24,8 kg(DP=1,3). Mean DEXA values were at lumbar spine(L2L4)= -,47(DP+0,36) and at femoral neck = -2,3 (DP=0,006). Osteoporosis was found in 15 patients (42,8%), related mainly to DM (RR=5), hyperparathyroidism (RR=6) and osteoporosis family history(RR=2,1).

CONCLUSIONS: Although osteoporosis could be a rare disorder in non-caucasoid, in this afro-brazilian population, commom risk factors as hyperparathyroidism, DM and family history for osteoporosis can justify a higher prevalence in afro-offsprings.

P404 F

OBESITY AND BONE MASS

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It is well known that the obesity is responsible for cardiovascular, metabolic, hormonal, rheumatic, pulmonary complications and for malignant tumors. Epidemiological data would seem to have documented a protective role of the obesity towards the osteoporosis.

In this study we have evaluated 2.411 women with age range of 30 to 59 arrived for the first time at our center to effect a bone densitometry at lumbar spine(L2-L4). The whole sample has been divided in 3 groups of age. Women with well known risk factors for osteoporosis and in previous treatment with antiriabsorbitive drugs have been excluded from the statistical analysis.

In the table I the general and middle characteristics of the three groups of apparently healthy women, the average of the BMD of the control groups for analogous age strips of our densitometry (DPX LUNAR V 3.61) and the number of women with risk factors.

Since women with age range of 50 to 59 years had a significant higher BMD vs controls with the same age range of DPX Lunar, we have excluded 148 women with BMI >30 from our sample.

The others 716 women had a bone mass like controls DPX (BMD 1.091±0.164 vs 1.081 g/cm2; $p=0.1$). The exclusion of 9 and 52 thirty and forty-years-old women has not determined, perhaps because of short number of subjects, significant difference vs control groups (DPX).

Our data show that:

1. a higher BMI determines an increase of the bone mineral density, probably because of high levels of dependent adipose tissue estrogens and/or arthrosis.

2. Healthy women of middle Italy (Adriatic cost) and controls of DPX Lunar V 3.61 have the same BMD at lumbar spine.

Groups of age	n	Age	BMI	BMD L2-L4	BMD L2-L4 controls	n women with risk factors
30-39	59	35.5±2.4	25.2±5.2	1.198±0.137	1.198±0.12	71
40-49	439	46.4±2.2	25.0±4.3	1.146±0.153	1.170±0.12	315
50-59	864	54.6±2.5	26.3±4.3	1.101±0.167*	1.081±0.12	663
(* $p < 0.001$ vs gruppo controllo DPX Lunar)						

P405 S

THE PREVALENCE OF OSTEOPOROSIS AND VERTEBRAL FRACTURES IN JAPANESE PATIENTS WITH RHEUMATOID ARTHRITIS

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Complications of osteoporosis(OP) increase the risk of vertebral fractures (VFs) which could reduce daily activities in patients with rheumatoid arthritis (RA). Though patients suffering from OP and VFs are not uncommon in RA, the rate of their occurrence in Japan remains unclear. In this study, we investigated the prevalence of OP and VFs in Japanese patients with RA.
Methods: We measured bone mineral density (BMD) of lumbar spine(LS) and of proximal femur by dual energy X-ray absorptiometry(DXA), and checked thoracic and lumbar VFs by roentgenograms. The patient, who has fragile fractures, or whose BMD of any portions, LS, femoral neck(FN), or total hip(TH), is less than 70% of young adult mean (corresponding T score is -2.6 in LS, -2.2 in FN, and -2.4 in TH, respectively) was diagnosed as OP. We examined 623 patients with RA (62 male , 75 premenopausal, and 486 postmenopausal female). All patients had never been treated with any bisphosphonates and estrogen. Four hundred thirty patients out of all were under treatment with glucocorticoid (GC).
Results: Three hundred thirtythree patients out of all (53.5%) were diagnosed as OP, and the occurrence of OP in female patients with RA (56.3%; 316 out of 561) was approximately twice as high as that of primary OP in Japanese age-matched population. Seventeen out of 62 male patients (27.4%) had OP, however, its occurrence was significantly less frequent than in female patients. VFs were observed in 114 out of 623 patients (18.3%) and, among them, 56 patients (9.0%) had two or more fractured vertebrae. The occurrence of OP and VFs were more frequent in patients who are older and have longer disease duration of RA, more severe destruction of joints, and lower daily activities. Compared with the patients without use of GC, those treated with GC had higher rate of complication of OP ($p<0.001$) and of VFs. Conclusion: Osteoporosis in RA was more frequent than primary OP in Japan, and these patients with RA are likely to develop VFs. It is important to extract risk factors and take preventive measures in the early stage.

P406 W

TOOTH LOSS AND BONE MINERAL DENSITY IN ASIAN WOMEN: A COMMUNITY BASED STUDY

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A negative association between bone mineral density (BMD) and tooth loss has been reported in previous studies. We studied the association between BMD and tooth loss among 191 women, who participated in a community based osteoporosis study in Sri Lanka.

These women were free of bone active diseases and medications. The total number of teeth lost due to any cause was recorded (the number of remaining teeth were counted to make sure the accuracy) and the BMD of lumbar spine and hip was measured by DXA.

Mean age, height and weight of the sample were 55.6 (±12.9) years, 1.48 (±0.059) meters and 48.8 (±4.3) kg. Total tooth loss (TTL) had a range of 1 to 32 with a mean of 16.2 (±10.2). The average numbers of teeth lost in four quartiles were 3.5, 10.9, 21.1 and 29.6. Partial correlations between TTL and BMD (controlling age and body mass index) showed a significant negative association in the lumbar spine (r=minus 0.16, p=0.02) and trochanteric area (r=minus 0.16, p=0.03). Correlation between TTL and femoral neck BMD was not significant (r=minus 0.10, p=0.16). Mean spinal BMD (corrected for age and BMI) of the lowest through to the highest quartiles of TTL were 0.841, 0.800, 0.770 and 0.784 g/cm2 respectively (p=0.05). The corresponding mean values for trochanteric area were 0.596, 0.586, 0.579, 0.539 g/cm2 (p=0.02) and for femoral neck 0.737, 0.719, 0.707 and 0.693 g/cm2 (p=0.31). The prevalence of osteoporosis (as defined by WHO) was 20%, 34%, 47% and 73% in the lowest to the highest quartiles of TTL (p=<0.001). When compared with the lowest quartile, women in the highest quartile of TTL had a relative risk of 3.1 for developing osteoporosis (95% confidence intervals 1.3 to 7.2).

A significant and inverse relationship between tooth loss and BMD was seen in our women. When compared to women with lesser tooth loss, women with greater tooth loss had a high risk of developing osteoporosis.

P407 F

HEIGHT LOSS, WEIGHT LOSS AND BONE LOSS: THE MIYAMA STUDY

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The objective of this study was to clarify the association between rate of bone loss and the change of anthropometric factors, especially height and body weight, among general inhabitants of Miyama, a rural Japanese community.

A cohort of 1543 inhabitants aged 40-79 years was established using resident registration in 1989. Fifty men and 50 women each in 4 age strata between 40 and 79 years, totaling 400 participants, were selected and completed a self-administered questionnaire and anthropometric measurements. In 1990, the baseline BMD of lumbar spine and proximal femur was measured using Dual energy X-ray absorptiometry (DXA). BMD was measured on the same participants in 1993, 1996 and 2000.

The rates of changes of lumbar spine BMD during 10 years in men in their 40s, 50s, 60s and 70s were 1.7%, 5.5%, 0.1% and -1.6%, respectively, and those in women were -8.7%, -8.4%, -4.8% and -4.8%, respectively. Thus in men, BMD at the lumbar spine increased in all age strata but the oldest, when it decreased, while in women, it decreased in all age strata.

The change of height during 10 years in men in their 40s, 50s, 60s and 70s were -0.7cm, -0.5cm, -1.2cm and -1.5cm, respectively, and those in women were -0.7cm, -1.4cm, -2.1cm and -3.6cm, respectively. The change of weight during 10 years in men in their 40s, 50s, 60s and 70s were -0.2kg, -0.8kg, 3.0kg and -3.0kg, respectively, and those in women were -0.3kg, -1.7kg, 2.4kg and -3.1kg, respectively. Among men, there was significant positive relation between weight change and the rate of change of BMD at the lumbar spine after adjustment for age ($p<0.01$). By contrast, among women, there was significant positive association between the change rate of BMD at the lumbar spine and height and weight change after adjustment for age ($p<0.05$). We concluded weight loss was significantly associated with bone loss at the lumbar spine in both men and women. Also, height loss were significantly related to bone loss at the lumbar spine in women.

P408 S

RISK FACTORS FOR THE PROGRESSION OF OSTEOPENIA TO OSTEOPOROSIS

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Aim: Since osteopenia defined by moderate loss of bone mass (-1.5>T score>-2.5), has not been well characterized, there is no guideline how to manage this. We have attempted to investigate the fate of osteopenia prospectively. Subjects: A total of 207 postmenopausal women (mean age was 63 yo) who's lumbar BMD were ranged between -1.5 and -2.5 T score, were followed their lumbar BMD and the occurrence of fragility fractures for 1 to 8 years (mean duration: 3.2 years) without any intervention for the loss of bone mass. The baseline evaluation of bone turnover was also assessed in all subjects. Results: The lumbar BMD in 46 subjects decreased below -2.5 T score and 30 subjects occurred fragility fractures at vertebrae (n=22), Colles fracture (n=3), femoral neck fracture (n=2) and other types of fracture (n=3). As a result, a total of 76 subjects (36.7%) went to have osteoporosis. The baseline characteristics were compared between those 76 subjects (progress group) and the remaining 131 subjects (control group). The age and body size were not different between the groups. The initial BMD, urinary excretion of DPD (HPLC) and the rate of bone loss were significantly different between the groups. The multi-regression analysis revealed that those three parameters were independent risks for the progression to have osteoporosis. Conclusion: Those results indicated that the osteopenic patients with high bone resorption, relatively low bone mass and high rate of bone loss would be introduced to the preventive intervention.

P409 W

EFFECT OF SPINAL DEFORMITIES ON QUALITY OF LIFE IN PATIENTS WITH OSTEOPOROSIS

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It is well known that osteoporosis causes spinal deformities which induce chronic back pain and disability in physical activities. However, it is still unknown whether spinal deformities disable their quality of life (QOL) or which type of spinal deformity most commonly impairs their QOL. To answer these questions, we evaluated the effect of spinal deformities on QOL in patients with osteoporosis. A total of 132 postmenopausal women with osteoporosis aged over 60 years were divided into the following five groups according to their spinal deformities: round back (RB, n=39), hollow round back (HRB, n=29), whole kyphosis (WK, n=34), lower acute kyphosis showing localized kyphosis with straight thoracic spine (LAK, n=14), and normal posture (NP, n=16). QOL was evaluated using the Questionnaire for QOL Evaluation in Osteoporosis (QOL Evaluation Committee, Japanese Society for Bone and Mineral Research, 2000 Edition). This questionnaire consists of items on pain (20 points),

activity of daily life (ADL, 64 points), recreation and social activity (20 points), general health (12 points), posture and figure (16 points), and falls and physiological factors (20 points). The full score is 152 and a higher score indicates a higher QOL. Total QOL score and scores of each item were compared between the groups. Total QOL score, items on ADL, and items on posture and figure in RB, HRB, WK, and LAK groups were significantly lower than those of NP group ($p<0.05$). Furthermore, total QOL score and items on ADL were significantly more impaired in WK group than other groups ($p<0.05$). There were no significant difference between the groups in items on pain and items of recreation and social activity. General health score was significantly lower in WK and LAK groups than in NP group ($p<0.05$). The scores in falls and physiological factors were significantly lower in WK group than in NP group ($p<0.01$). Significant positive correlations were observed between all items in total of 132 patients ($0.23<r<0.59$, $p<0.05$). We concluded that QOL in patients with osteoporosis was impaired by spinal deformities, especially by whole kyphosis.

P410 F

DIMORPHISM IN AXIAL AND APPENDICULAR SEGMENT SIZE AND MASS: IMPLICATIONS CONCERNING DIFFERENCES IN FRACTURE RISK BY RACE AND SEX

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To examine the structural basis for race- and sex- specific incidence of fractures we studied 656 Chinese (429 females) and 1244 Caucasians (850 females) 18-92 years old. In young adulthood, Chinese had shorter stature than Caucasians due to shorter leg length; sitting heights were similar. Chinese had smaller vertebral body (VB) due to smaller vertebral body CSA, VB height was only slightly less (NS). Volumetric BMD in young adulthood was higher than Caucasians. Peak stress (load/area) was no different by race because the smaller VB in Chinese was loaded by a smaller upper body mass. However, peak vertebral Fracture Risk Index (FRI) was lower in Chinese because the higher volumetric BMD. From youth to old age, VB CSA increased more in Chinese than Caucasian women while VB volumetric BMD decreased similarly by race. In young adulthood, peak FN width and cortical thickness were 10-16% less in Chinese than Caucasians but Chinese women had a relatively thicker cortices in the more slender FN. There was no racial difference in peak FN volumetric BMD. In Chinese, the more slender FN resulted in a high risk of fracture in bending (lower section modulus) but the relatively thicker cortex in the more slender FN resulted in a lower risk of structural failure by local buckling than in Caucasians. Across age, FN diameter increased more in Chinese than Caucasian women, but similarly in Chinese and Caucasian men. If so, the relatively wider femoral neck with a narrower cortex in Chinese should increase the buckling ratio more in Chinese. The changes on the external (periosteal) and internal (endosteal) envelopes of bone during growth and ageing are race and sex specific. Greater periosteal apposition in Chinese than Caucasians, and in men than women, is likely to contribute to a lower incidence of fractures while higher peak VB vBMD may protect against the similar loss of bone.

Osteoporosis: Pathophysiology

P411 S

THE DEVELOPMENT OF BMD COMPOSITE INDEX SCORES TO CORRELATE BONE GROWTH INDICES IN ADOLESCENTS

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OBJECTIVE: Condensation of multiple skeletal-sites BMD measurements into a single composite-index helps simplify data-analysis and data-interpretation. This study describes the development of BMD composite-index-scores (BMD-CISs) to correlate with bone growth determinants in adolescents.

METHODS: BMD measurements using DXA: Norland-XR36 (spine, femoral-neck, trochanter & Ward's triangle) and pQCT: Denisiscan-2000 (distal-radius and tibia) were obtained from 101 healthy girls aged 12-15-y to develop the BMD-CISs. Adolescent bone-growth predictors (weight, height, pubertal-status, weight-bearing physical-exercise and bone-turnover markers) were evaluated to correlate with BMD-CISs.

Factor and principle-component analyses were used to examine the internal-structure of 11 BMD- variables and to create new BMD-CISs which summarizes characteristics of parent BMD-variables. Correlations between the BMD-CISs and parent BMD-variables were done. Univariate- and multivariate-analysis were performed to associate BMD-CISs with bone-growth predictors for examining the strength of correlations and predictions when compared with the original BMD-variables.

RESULTS: Two independent DXA-generated-BMD-CIS and pQCT-generated-BMD-CIS were obtained to summarise the 11 original BMD-variables. Each BMD-CIS correlated highly with respective DXA or pQCT variable ($r=0.32$-0.92; $P<0.05$ - $P<0.001$). DXA-generated-BMD-CIS ($r=0.24$ - 0.73; $P<0.05$-$P<0.001$) and pQCT-generated-BMD-CIS ($r=0.24$ - 0.39; $P<0.05$ - $P<0.001$) were significantly correlated with most bone-growth predictors. In multiple-regression-analysis, R-square of DXA-BMD-CIS (66.4% vs. 36.3% - 66.1%) and pQCT-BMD-CIS (25.1% vs. 12.2% - 42.2%) have similar predicting values when compared with parent BMD-variables demonstrated that the two new BMD-CISs summarized well the characteristics of the parent BMD-variables.

CONCLUSIONS: The newly developed BMD-CISs correlated well with original variables and have similar predicting powers when compared with the original BMD-variables. The use of BMD-CISs would reduce load of data-analysis and simplify correlation and comparisons between multiple BMD measurements and other predicting variables.

P412 W

CORTICAL BONE MINERAL DENSITY AND OSTEONAL ACTIVITY IN MID-DIAPHYSIS OF LONG BONES IN FEMALE BEAGLE DOGS

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Bone mineral density (BMD) is widely used as a parameter to estimate bone fracture risk. The BMD of long bones (tibia, femur and humerus) that the body weight burden is different, larger on the fore limbs than the hind limbs, were measured to examine whether BMD is reliable as a parameter to estimate the risk of bone fracture. The long bones labeled with tetracycline were obtained from female beagle dogs aged from 2 to 16 years. The cortical BMD and related parameters such as cortical area, thickness and strength strain index (SSI) in the mid-diaphysis of long bones were measured by pQCT. Thereafter, the undecalcified bone section was obtained to observe the bone activity by differentiating tetracycline-labeled, and eroded osteons in the anterior and posterior regions. The cortical BMD of mid-diaphysis was not different among three bones and decreased after 7-8 years. The cortical area of humerus was larger than those of tibia and femur, and the cortical thickness of humerus as well as tibia was wider than that of femur, and both values decreased after 7-8 years. The SSI of humerus was higher than femur and tibia after 7-8 years. In the mid-diaphysis of tibia, the tetracycline-labeled osteons were observed in the anterior region more than in the posterior region and decreased with age. The osteons with erosion was frequently in the posterior regions with increasing age. The results indicate that the bone strength (SSI) depends on the shapes such as cortical area and thickness rather than the BMD and the alteration of osteonal activity and morphology.

P413 F

THE EFFECTS OF $1,25(OH)_2D_3$ AND PHYTASE SUPPLEMENTATION TO THE DIET WITHOUT VITAMIN D_3 ON STRENGTH OF BONES IN BROILER CHICKENS AT DAY 49 OF LIFE

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About two-thirds of phosphorus in feed of vegetable origin is in the form of phytates. Poultry utilize phytate phosphorus to a small degree because of lack of phytase, an enzyme capable of hydrolyzing phytates.

Aims: The purpose of this study was to investigate the effects of microbial phytase and $1,25(OH)_2D_3$ administered to the low-calcium and low-phosphorus diet without vitamin D_3 on mechanical properties of broiler chickens bones.

Materials and methods: Experiment was carried out on broiler chickens divided into four groups: control positive (2500 IU D_3/kg feed), control negative (without vitamin D_3) and experimental group fed on the diet without vitamin D_3but supplemented 0,003 mg $1,25(OH)_2D_3$and phytase 500 PTU/kg feed from the first day of life until day 21 and 49 (PTU - unit of phytase, activity which liberates one micromole of inorganic phosphorus per minute from 0,0015 m Naphytate). On the 49th day of life, 10 birds from each treatment were sacrificied. Femora were isolated for measurement of physical and geometrical parameters of bones. The three-point

bending test (Instron Testing Machine) was used to determine mechanical bone properties: maximum strength and maximum elastic force. Cross sectional area, second moment of inertia, mean relative wall thickness, cortical area, cortical thickness, cortical area index and cortical index were measured as well.

Results: After 21 and 49-d $1,25(OH)_2D_3$ and phytase supplementation to the diet without vitamin D_3 strength of bones in experimental groups was greater then positive and negative controls. The values of physical parameters were not significantly different in both experimental groups fed on the diet with combined addition of $1,25(OH)_2D_3$and phytase during 21 or 49 days. Structural parameters in experimental groups were similar to positive and greater than negative control.

Conclusion: $1,25(OH)_2D_3$and phytase combined supplementation to the diet without vitamin $D_3$was more effective on mechanical properties of bones than adequate level of vitamin D_3. The lack of significant differences of physical parameters of bones between both experimental groups suggests that supplementation of 0,003 mg $1,25(OH)_2D_3$and 500 PTU phytase/kg feed during first 21 days provided normal growth of bones of broiler chickens in the subsequent period.

P414 S

MENINGES PLAY AN ESSENTIAL ROLE IN SKULL DEVELOPMENT

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The skull vault (calvaria) consists of several membrane bones connected by sutures made of fibrous connective tissue. Growth of the skull takes place mainly in the sutures, whose function is therefore essential for expansion of the growing brain. The meninges that underlie the skull comprise three layers of membranous tissue: dura mater, arachnoid mater and pia mater. The dura mater has been experimentally demonstrated to play an important role in maintaining the suture in a functional state.

This study was designed to gain some insight into the role of TWIST in skull development. TWIST is a basic helix-loop-helix transcription factor that is associated with the maintenance of cells in an undifferentiated condition. Loss-of-function mutations in human TWIST result in premature obliteration of the coronal suture. In the mouse, Twist is ubiquitously expressed in undifferentiated mesenchyme in the head, including the meninges, on embryonic day 14 (E14), the stage of onset of calvarial osteogenesis. At E17, Twist expression disappears from the meninges except for a specific area subjacent to the coronal suture. We also find that expression of an osteogenic inducing factor, bone morphogenetic protein 2 (Bmp2), is expressed in the osteogenic layer, while Bmp7 transcripts are present in meninges at E14. At E17, Bmp2 maintains the same expression pattern as at E14, whereas Bmp7 expression is observed not only in the meninges but also in osteoblasts of developing bone.

We injected an adenoviral vector carrying Twist cDNA under the control of the CMV promoter subcutaneously into the E13.5 mouse head by ex-utero surgery. Twist expression was maintained in the meninges. Domains of expression of osteogenesis-related genes were smaller on the injected side than on the control side 96 hours after the injection, suggesting inhibition of osteogenesis. In addition, Bmp7 expression in the meningeal layer was lost.

From these results, we suggest that Bmp7 secreted by the meninges promotes osteogenesis in the overlying osteogenic layer, and that the normal mechanism of sutural maintenance by Twist involves inhibition of Bmp7 expression and hence inhibition of osteogenesis in the overlying skeletogenic tissue.

P415 W

SILICON INTAKE IS A MAJOR DIETARY DETERMINANT OF BONE MINERAL DENSITY IN MEN AND PRE-MENOPAUSAL WOMEN OF THE FRAMINGHAM OFFSPRING COHORT

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Silicon is a major nutrient in the human diet and recent cellular and animal models have demonstrated an important role for silicon (Si), in the form of orthosilicic acid $[Si(OH)_4]$, in osteoblast stimulation and differentiation, collagen maturation, and bone formation. Data in humans, however, are lacking. Recently we defined the major dietary sources of silicon and demonstrated the metabolism of orthosilicic acid. Here, we have created the first dietary silicon database and regressed silicon intakes against the bone mineral density (BMD) at the hip sites (total hip, trochanter, Ward's area and femoral neck) and lumbar spine of 1251 men and 1596 women (aged between 30 and 87 years) in the Framingham Offspring cohort (Framingham, MA, USA). In a simple linear model (in PC SAS for Windows; version 8.1), adjusted for all potential confounding factors, silicon intake (mg/d) correlated positively with BMD (g/cm^2) at the four hip sites in men and pre-menopausal women ($P < 0.05$), but not in post-menopausal women. Analysis by quintiles of silicon intake supported these findings and showed large differences in BMD (4-9%; $P < 0.05$) between the highest (> 32 mg Si/day) and lowest (< 17 mg Si/day) quintiles of silicon intake. Even with the use of a more rigorous energy-adjusted model, the effect of silicon on BMD was as great as that reported for any other nutrient intake. Further analyses indicated that some of the positive effect seen for moderate alcohol consumption on BMD may be attributed to silicon, which is found at especially high levels in beer. The finding that dietary silicon is associated with BMD in men and pre-menopausal women, but not post-menopausal women, is consistent with its role in bone formation rather than in preventing bone resorption. Orthosilicic acid appears to be an important nutrient with anabolic affects on bone.

P416 F

COMPARISON OF THE EFFECTS OF ENDOCHONDRAL BONE MATRIX GELATIN (EC BMG) WITH AUTOGENOUS BONE GRAFTS IN THE RECONSTRUCTION OF BONE DEFECTS: AN EXPERIMENTAL STUDY IN RABBITS

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Purpose: The use of autogenous bone grafts is usual for the reconstruction of bone defects in routine practice. The problem of producing and use of grafts have encouraged investigators to search for a suitable alternative, of which, Bone Matrix Gelatin (BMG) is the most popular material. In order to evaluate and compare the effect of BMG and autograft on the reconstruction of bone defects, this study was conducted in rabbits.

Materials and Methods: The design of the study was experimental and data was collected using a questionnaire and microscopic observation. Male white New Zealand rabbits were used for this study. Using Urist technique, BMG was prepared and grafts were also removed from rabbits left iliac bone. The grafts were placed on maxilla right defect and BMG was applied to fill left defect. The rate and amount of reconstruction were compared at 7th. 14th, 24th and 60th days after operation according to following items: 1- Type of osteogenesis 2- Cartilage formation 3- Rate of bone formation 4- Inflammation types.

Results: The results showed that through time the inflammation degree was reduced and bone formation was increase in two groups.

Conclusion: This indicates that Ec BMG, like autograft, can be useful in the reconstruction of rabbit's maxillofacial bones defects.

P417 S

IS MAGNESIUM NECESSARY FOR OPTIMAL GROWTH AND CALCIUM ABSORPTION: AN INVESTIGATION IN THE GROWING MALE RAT

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Magnesium plays an integral role in maintaining bone health, both via regulating active calcium transport and incorporation into bone. Whether magnesium is of importance when the diet is replete in calcium is not clear. In this study this issue was addressed by comparing the effect of magnesium at the RDA and at 150% and 300% of the RDA for the growing rat.

Male Sprague Dawley rats (age 4 weeks) were randomised into three groups (n=15 per group) onto a semi-synthetic diet containing 0.5% calcium and 0.05% magnesium (rat RDA). After one week on the base diet one group remained on the base diet, while two groups were fed either 0.075% (150%) or 0.15% (300%) Mg respectively. The animals were fed for 8 weeks and their weight was monitored weekly. After 4 weeks on the diets a calcium and magnesium balance was done. At week 8, all animals were sacrificed and femoral and spine bones harvested and bone mineral density measured. Plasma parathyroid hormone (PTH) levels were also measured.

Body weight was not significantly different between groups but at age 6 weeks the control group had a reduction in growth which indicated a transient suppression of growth possibly due to limited magnesium. Calcium absorption was significantly increased in both Mg supplemented groups compared to control. Overall absorption of magnesium was not significantly different between groups. PTH was significantly elevated in the 300% Mg group. Bone mineral density was higher in the 150% group but not significantly (p=0.08).

We conclude that Mg seems to be important for normal growth in the young male rat. Raising magnesium intake when calcium intake is sufficient, does increase calcium absorption significantly. This increase in absorption may be beneficial to bone mineral content and density over a longer period of time.

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P418 W

CONNECTIVE TISSUE GROWTH FACTOR REGULATES THE FIRST BRANCHIAL ARCH MESENCHYMAL CELLS CONDENSATION AND THE INITIATION OF SKELETAL DEVELOPMENT

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Connective tissue growth factor (CTGF) is a potent cytokine which has important roles in cell proliferation and differentiation. In this study, we analyzed whether CTGF is involved in Meckel's cartilage development and what roles it may have. In situ hybridization and immunohistochemistry revealed that CTGF was strongly expressed at the mesenchymal condensation stage, decreased during differentiation of type II collagen positive chondrocytes, and was again increased in chondrocytes at late embryonic stages. At each stage analyzed, strong CTGF expression characterized perichondrium. When mesenchymal cells from E10 mouse first branchial arch were isolated and micromass-cultured in DMEM containing 10% serum, the cells aggregated and formed CTGF positive cartilage nodules by 4-8 day. Interestingly, when the cells were cultured in the presence of recombinant CTGF at 10, 100 or 1000 nanog/ml, a dose dependent increase in cartilage nodule formation was observed. In good agreement, nodule formation was inhibited by treatment with neutralizing CTGF antibodies. To clarify the mechanisms of endogenous CTGF action in nodule formation, mesenchymal cells from first branchial arch were reared in micromass-culture in DMEM containing 10% serum for 48 hrs and then switched to DMEM containing 0.5% serum for 24 hrs. The cells were then treated with different concentrations of recombinant CTGF. We found that the recombinant CTGF treatment increased fibronectin mRNA levels, a matrix molecule which is known to be up-regulated during chondrogenic cell aggregation and differentiation. The effect was consistent and was similar to seen after treatment with TGF-beta. Taken together, our data demonstrate for the first time that CTGF is expressd during Mechel's cartilage development. The possible role CTGF appears to play in this process is to promote mesenchymal cell aggregation via stimulation of fibronectin gene expression and cell-cell interactions.

P419 F

MILK BASIC PROTEIN (MBP) PROMOTES BONE FORMATION AND BONE GROWTH IN WEANING RATS

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Purpose: Milk is an excellent source for calcium supplementation to preserve skeletal health. Milk also contains various proteins that may be important for skeletal growth. Previously, we found that milk whey protein promoted bone formation and suppressed bone resorption from in vitro study. Active components in this protein localized to milk basic protein (MBP). In OVX rats (fifty-five-weeks-old), MBP prevented especially bone loss of femoral metaphysics of bone mineral induced by ovariectomy. The purpose of this study is to investigate the effect of MBP on bone formation and bone growth in early stage of weaning.

Methods: Fourteen-day-old female SD rats were divided into four experimental groups. Rats were fed with the AIN-76 diet ad libitum for 3 weeks. MBP was given by oral administration at 20 mg/kg, 40 mg/kg, 80 mg/kg. Bone mineral density (BMD), bone mineral content (BMC), and the bone strength (breaking strength and energy) of the femur were measured. Moreover, we observed the width of the cartilage growth plate and trabecular bone of tibia.

Results: In young weaning rats, MBP increased the BMD and BMC in the 40 and 80 mg/kg MBP administration groups. The breaking strength for the 40 and 80 mg/kg MBP administration groups was significantly higher than that of control group. The trabecular bone volume of 40, 80 mg/kg MBP groups was increased compared with that of the control group. In addition, the width of growth cartilage plate of 20, 40 and 80 mg/kg MBP groups were significantly wider than that of the control group. Osteoblast and chondroclast numbers below the growth cartilage plate were also increased in the 40 and 80 mg/kg MBP administration groups.

Conclusion: These results suggested that MBP was helpful for bone health by promoting bone formation and bone growth in weaning period.

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AXIAL AND LOCAL BONE MINERAL DENSITY AND METABOLISM IN MENOPAUSAL WOMEN WITH MILD KNEE OSTEOARTHRITIS

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A negative association between osteoporosis (OP) and osteoarthritis (OA) has been described. OA has been related to an increased subcondral bone mineral density (BMD).

Our aim was to assess axial (lumbar spine (LS) and femoral neck (FN)) and local tibial (tibial spine (TS) and internal condylus (IC)) BMD in menopausal women with mild knee OA (kOA).

Patients & methods: 77 consecutive menopausal women, aged 61±5 years, BMI 27.7±3.8 kg/m2, were studied. 81.8% had mild spine OA (sOA) and 51.9% mild kOA (Kellgren-Lawrence scale, KLs). 10 women without OA were used to calculate local tibial z-scores (TS and IC). BMD was measured by DXA (Hologic QDR1000w). Bone turnover markers (BGP, total and bone ALP, NTx & Pyr) and calciotrophic hormones were measured (PTHi, 25(OH)D, IGF-I and estradiol).

Results: Control and OA groups were comparable in age, weight, years since menopause, calciotrophic hormones and bone markers except for BGP, lower in OA patients (p=0.003). Local tibial BMD was increased in kOA patients (z-score): IC: 2.653 ±5.359; TS: 0.735 ±1.150 (p<0.003 vs 0). Local tibial BMD correlated with radiographic rating scale of OA, and more closely with axial BMD. 52.6% of patients showed densitometric OP. Patients with kOA showed higher age (62 ±5 vs 60 ±5; p=0.02) and weight (71.4 ±10.9 vs 66.0 ±8.9; p=0.02) and lower BGP (7.2 ±8.1 vs 13.3 ±8.6; p=0.004) and 25(OH)D levels (18.3 ±7.1 vs 23.5 ±11.1; p=0.023). Osteoporotic patients had lower 25(OH)D (17.4 ±7.7 vs 25.2 ±10.1; p=0.001) and higher BGP levels (12.3 ±9.2 vs 7.9 ±7.9; p=0.042).

Conclusions: Local tibial BMD is increased in menopausal women with mild knee OA and this increase is related to radiological score. Local tibial BMD and axial BMD are closely related. The prevalence of OP in these women is very high. OP and OA are associated with low vitamin D levels but BGP levels have opposite changes.

P421 W

BONE MINERAL DENSITY AT THE FEMORAL NECK IS ASSOCIATED WITH TYPE OF EXTRACAPSULAR HIP FRACTURE

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Although some studies have shown the relationships between bone mineral density (BMD) and type of the hip fracture, intracapsular or extracapsular, whether BMD can be a determinant of the type of the extracapsular fracture is not be clearly represented. To resolve this question, we assessed BMD at the unaffected femoral neck and fracture type in 48 Japanese elderly (60 years and over) patients (41 female and 7 male, mean age 80.6 years) with extracapsular hip (trochanteric and subtrochanteric) fractures. The AO and Evans-Jensen classification systems were used for identification of type of the fracture and relationships between the fracture type and BMD were analyzed.

The BMD was significantly higher in patients with sbutrochanteric and reversed obliquity type fracture (0.543 ±0.074 g/cm^2) compared with in those with other types of trochanteric fractures (0.462 ±0.083, p<0.05). BMD was significantly correlated with type in both AO (r=0.32, p<0.05) and Evans-Jensen (r=0.30, p<0.05) classifications; the patients with low femoral neck BMD tend to have less comminuted fractures, whereas the fracture was likely to severely comminuted in higher BMD patients. We conclude that BMD, as well as the leg position at injury may be a determinant of extracapsular hip fracture type.

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TRABECULAR MINIMODELING IN HUMAN ILIAC BONE

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In adult human beings, remodeling creates nearly all of new bone tissue. However, Frost hypothesized that modeling can go on in trabeculae throughout life. As this hypothesis has not been verified, we looked for histologic evidence of trabecular modeling (minimodeling) during bone histomorphometry of transiliac bone biopsy specimens obtained from 34 patients (age range, 38 - 81 years; mean age, 58.4 years; female, 31 / 34) at the time of total hip arthroplasty. Before the bone biopsy study, we confirmed that the bone biopsy site was not affected by ipsilateral hip joint disease. Patients who had metabolic bone diseases or who had taken medications known to

affect bone metabolism were excluded from the study. During modeling where bone formation and bone resorption are not coupled, bone formation can occur on quiescent bone surfaces without preceding bone resorption and create smooth cement lines. Therefore, the combination of fluorochrome labeling and a smooth cement line without interruption of surrounding collagen fibers was regarded as evidence of minimodeling. Histologic evidence of minimodeling was detected in 21 of the entire 34 specimens (62%) and 17 of 27 specimens obtained from postmenopausal patients (63%). Bone volume of minimodeling sites was less than 1% of the trabecular bone volume, and these sites accounted for less than 2% of the entire bone surface on average. However, osteoid volume of minimodeling sites comprised approximately one tenth of the entire osteoid volume, and their labeled surface constituted one fourth to half of the entire labeled surface on average. Therefore, when performing bone histomorphometry of adult cancellous bone, minimodeling should be taken into account when dealing with parameters related to osteoid volume and mineralization. A comparison of specimens with and without minimodeling demonstrated that the presence of minimodeling was correlated with smaller physique of patients, accelerated mineralization (as indicated by the higher mean MS/BS and MAR values and the shorter mean Omt), and higher metabolic turn-over of bone (as indicated by the higher mean BFR/BV value). Although the findings still need to be verified in a larger number of normal subjects without hip joint disease, they support Frost's hypothesis that minimodeling can continue throughout human life.

P423 S

GEOMETRY OF ARTIFICIAL EXTRACELLULAR MATRIX: CREATION OF THREE-DIMENSIONAL BONDING BETWEEN BONE AND TITANIUM BY THE WEB OF 50-MICRONS TITANIUM FIBERS

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So far almost all the trials of bonding between bone and titanium have been limited their interfaces essentially to the two-dimensional, plain-to-plain bonding. These methods crucially limited the space for osteoblasts to exert active bone formation, leading long time for formation of bone-titanium bonding. We proposed that geometrical function of artificial ECM is crucially important for bone formation, together with physical, chemical, and biochemical functions (J Bone Joints Surg, 83-A, S1-105-115, 2001). Based upon this hypothesis, here we report an entirely new method of bone-titanium bonding, in that both substances coexist three-dimensionally in the 'collaboration zone', mediated by titanium fibers of 50-microns diameter. The titanium fibers were prepared by the method of Yanagisawa (US patented), and fabricated into a form of unwoven sheet (titanium web) with a thickness of 0.5 mm. With the titanium web, titanium rod with diameters of 1.5 - 2.0 mm was wrapped tightly and vacuum-sintered at 1000-1100 centigrade for 4 hrs. In the product, titanium web-equipped titanium rod (TWT), the fibers within the web were strongly fused together, and the web layer was on the surface of the rod as well. The TWT was cut into a cylindrical form (3 x 3 mm), coated with hydroxyapatite by the method of Osaka (US patented) and implanted into the cranial bone of rabbit, on which holes (3 mm in diameter) were prepared beforehand. At 4 weeks, histological observation showed that new bone developed from surrounding calvaria into the entire width of titanium web, reached to the surface of titanium rod. Density of bone in the web was slightly lower than the surrounding calvaria at 4 weeks, but it became equivalent at 6 weeks. In the control experiment of a conventional device of titanium beads-attached titanium rod, bone development reached only on the surface of beads-layer and did not enter the spaces between the beads at 4 weeks. It was concluded that rapid and effective bone-titanium bonding in TWT was due to the three-dimensional spaces provided by the geometry of the web, which was more feasible environment for osteoblasts than those of conventional devices.

P424 W

CHANGE OF TRABECULAR BONE MICRO-ARCHITECTURE OF ADOLESCENT IDIOPATHIC SCOLIOSIS WITH DIFFERENT CURVE SEVERITY

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Introduction: In our previous study on bone mineral status of adolescent idiopathic scoliosis (AIS), the AIS patients had lower bone mineral density (BMD) than the healthy normal control. With the recently development of micro-computered tomography (microCT), we are capable to study the micro-architecture of trabacular bone in three dimensions. In this study, we characterized the micro-architecture of AIS trabecular bone at the spinous process with different curve severity.

Methods: Biopsies of spinous process were collected from 9 AIS patients who had Cobb's angle greater than 40 degrees and required corrective surgery with instrumentation and spinal fusion. The biopsies were obtained after consent had been obtained from the patients. Before surgery, the bone mineral density of L2 and L4 of the patients were evaluated by DEXA. The biopsies obtained during the surgery were fixed in alcohol and scanned by high-resolution microCT. Three-dimensional histomorphometry was generated automatically by the microCT workstation.

Results: The average Cobb's angle of 9 AIS patients (mean age: 15.8 yrs) was 56.8 deg (Range:50-65 deg). While bone fraction (BV/TV) in spinous process did not show strong correlation with Cobb's angle, the bone surface area increased as the Cobb's angle increased. Using the well-established structure model index to study the trabecular bone morphology, the trabecular bone changed from plate-like to rod-like structure while the Cobb's angle increased. When the 3-D histomorphometry was compared with the DEXA measurement, the Cobb's angle had a negative relationship with the lumber spine BMD. However, the higher the lumber BMD, more plate-like trabecular bone was present in spinous process. This agreed with the relationship between BV/TV and structure model index that the trabecular bone in the spinous process became more plate-like when the BV/TV increased.

Conclusion: Our previous study had shown that there was abnormality in bone mineral status of AIS patients. The present study showed that the curve severity of the AIS patients might be related to the change of trabecular bone micro-architecture. This implied that abnormality in trabecular bone micro-architecture might present also in AIS patients.

P425 F

THE EFFECT OF EDUCATIONAL LEVEL ON BONE MINERAL DENSITY IN POSTMENOPAUSAL WOMEN

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This study describes the influence of educational level on bone mineral density (BMD) and investigating the relationship between educational level and bone mineral density at four sites in postmenopausal women.

A total of 509 postmenopausal women, from 45 to 86 years of age (mean age of 60.85+7.53 years) were included in this study. A standardized interview was used at the follow-up visit to obtain information on demographic, life-style, reproductive and menstrual histories such as age at menarche, age at menopause, number of pregnancies, number of abortions, duration of menopause, duration of fertility, and duration of lactation. Patients were separated into four groups according to the level of education, namely illiterate (Group 1 with 209 patients), elementary (Group 2 with 222 patients), high school (Group 3 with 49 patients), and university (Group 4=29 patients).

The mean ages of groups were 59.75+7.29, 61.42+7.50, 59.27+7.80, and 57.80+7.70, respectively. There was no significant difference between group 3 and 4 in terms of number of pregnancies and duration of lactation, whereas the same was not true for the other groups ($p<0.05$). The number of pregnancies and duration of lactation were found to be the highest in Groups 1 and 2. The respective values for number of pregnancies and duration of lactation were 7.11+3.38 and 133.23+54.34 months for Group 1 and 4.93+3.61 and 93.62+50.66 month for Group 2. Spine BMD was significant lower in Group 1 than that of Group 2, 3 and 4 ($p<0.05$). Trochanter and ward's triangle BMD were the highest in Group 4 and there was a significant difference between Group 1 and 4 ($p<0.05$). Additionally, there was a significant correlation between educational level and spine BMD ($r=0.20$, $p<0.01$), trochanter BMD ($r= 0.13$, $p<0.01$), and ward's BMD ($r=0.14$, $p<0.01$).

The results of the study suggest that educational level has a considerable affect on BMD. Losses in BMD for women of lower educational level tend to be relatively high and, losses in spine and femur BMD showed a decrease with increasing educational level.

P426 S

EFFECTS OF PHYSICAL TRAINING ON CORTICAL BONE AT PROXIMAL TIBIA ASSESSED BY PQCT

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In contrast to DXA, peripheral quantitative computer tomography (pQCT) measures the volumetric bone mineral density (vBMD), and can determine bone geometric properties that closely related to bone strength in addition to vBMD. The purpose of the present study is to evaluate effects of long-term exercise on vBMD, bone geometric properties and bone strength index of jumpers as high impact loading group, and swimmers as non-impact and active loading group, compared to non-athletic controls by using pQCT. The study population complies 25 jumpers (13 females), 30 swimmers (15 females) and 25 controls (15 females), aged 18-23.

The cortical vBMD of female athletes was lower than that of the controls (1.90 ±0.08, 1.92 ±0.12, and 2.00 ±0.05 g/cm ^{3}, respectively for swimmers, jumpers, controls). On the other hand, periosteal areas of female athletes and male jumpers were larger than that of controls (3.41 ±0.73, 3.78 ±0.75, and 2.83 ±0.52; 4.83 ±0.46, 5.12 ±0.55, and 4.60 ±0.50 cm ^{2}, respectively for both female and male swimmers, jumpers, and controls). The endocortical area of only female swimmers was significant larger than that of controls (1.48 ±0.52, 1.35 ±0.54, and 1.03 ±0.29 cm ^{2}, respectively for swimmers, jumpers, controls). The polar moment of inertia of jumpers in both sexes and female swimmers were greater than that of controls (11062 ±1731, 12729 ±2075, and 10396 ±1662; 5789 ±2179, 7661 ±2397, and 3920 ±633 mm ^{4}, respectively for both male and female swimmers, jumpers, and controls). We conclude that : (1) an improvement of the mechanical properties of a young athlete's bone in response to long-term physical exercise is related to geometric adaptation and not to vBMD, (2) in female swimmers, physical training started in the earlier part of puberty (7.6±1.0 yr) may contribute to enlarged endocortical area. Therefore, question remains to be settled, whether the physical exercise before puberty accelerates the expansion of endocortical area.

P427 W

CHARACTERISTICS OF REGIONAL BONE MINERAL DENSITY AND SOFT TISSUE COMPOSITION IN PATIENTS WITH STEROID-INDUCED OSTEOPOROSIS

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We studied the effects of oral glucocorticoids (OGCs) on bone mineral density (BMD) and soft tissue composition in Japanese women.

Fifty-seven women, 29 to 74 years of age, were divided into two groups: women receiving at least 7.5 mg of oral prednisone daily (OGCs group, n=27) and healthy women (control group, n=30). The BMD of the 2nd to 4th lumbar vertebrae (L2-4BMD), head, arms, legs, ribs, thoracic vertebrae, lumbar vertebrae and pelvis as well as the lean mass and fat mass of the head, arms, legs, and trunk were measured by dual energy X-ray absorptiometry. L2-4BMD and BMDs of the lumbar spine, thoracic spine and pelvis of the OGCs group were significantly lower compared to the control group ($P < 0.001$). The fat mass of the trunk and head was significantly higher in the OGCs group than in the control group ($P < 0.001$); whereas, there was no significant difference regarding lean mass between the two groups. The results showed that prolonged treatmen with OGCs was associated with a decrease of the BMDs of spine and pelvis and an increase of the fat mass of the trunk and head. We conclude that OGCs affects weight-bearing and axial bone which is rich in cancellous bone, and that OGCs facilitates the proliferation of adipose cells in the head and trunk, changing thereby the distribution of adipose tissue in women under prolonged daily treatment with OGCs.

P428 F

DETERMINANTS OF BONE MINERAL DENSITY IN PREMENOPAUSAL PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS ON CORTICOSTEROIDS

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Aims: The aim of this study was to assess the bone mineral density (BMD) of premenopausal patients with systemic lupus erythematosus (SLE) on corticosteroids and to determine if there were any other factors influencing BMD.

Methods: Premenopausal SLE patients were recruited from outpatient clinics in 2 teaching hospitals in Kuala Lumpur, Malaysia. They all fulfilled the ACR criteria for the diagnosis of SLE. A short questionnaire on risk factors for osteoporosis was administered and BMD was measured using a LUNAR DPX machine.

Results: 76 patients were recruited into the study. There were 43 (56.5%) Chinese, 26 (34.2%) Malay and 7 (9.2%) Indian patients. All patients had premenopausal range FSH and LH levels. Mean age was 30.21 ± 7.76 years. Mean age at diagnosis of SLE was 24.8 ± 7.49 years. Mean duration of SLE was 63.75 ± 67.94 months. Mean dose of prednisolone at time of BMD measurement was 18.63 ± 10.15 mg daily. Mean duration of corticosteroid use was 4.87 ± 5.14 years. Median SLEDAI score was 9 (range 0-29). Overall, 6 patients (7.9%) had osteoporosis, 23 (30.3%) had osteopenia and 47 (61.8%) had normal BMD on WHO classification. Taking a cut-off of T -1.5, 23 (30.3%) patients were potential candidates for treatment for corticosteroid-induced osteoporosis. No patient had prevalent vertebral fractures on x-ray. There was a significant correlation between age and lumbar spine (LS) BMD ($p = 0.003$) but not with femoral neck (FN) BMD. FN BMD was significantly correlated with duration of corticosteroid use ($p = 0.033$) but not LS BMD. There was no correlation between BMD and race, SLEDAI score, self-reported calcium intake, self-reported exercise, duration of SLE and cumulative corticosteroid use.

Conclusions: Only 61.8% of these premenopausal Asian SLE patients had normal BMD. Almost one third (30.3%) had osteopenia and a significant minority (7.9%) had osteoporosis. However, there was no obvious correlation between BMD and the commonly assessed risk factors for osteoporosis. These findings would suggest that BMD should be measured, even in premenopausal SLE patients on corticosteroids, to fully assess their osteoporosis risk.

P429 S

STUDY OF BONE MINERAL DENSITY(BMD) IN SOUTH INDIAN TYPE 2 DIABETICS

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Aim: Osteoporosis is one of the most common problem faced in geriatrics clinics nowadays.This study has been conducted to asses the impact of diabetes Type 2 in the bone mineral density (BMD) & bone mineral content (BMC)of the aging population.

Methods & Materials: 40 diabetic & 60 non diabetic men, 90 diabetic & 160 non-diabetic women in the age group of 51-80 years were studied. Age & sex match were done.

Other parameters like diabetic duration, medications, menopausal years for women were recorded. Women on hormone replacement therapy were excluded.

The bone mineral density(BMD) & bone mineral content(BMC) of both spine(L1-L4) & hip were measured using hologic Dual Energy X ray absorptionometry(DEXA).

Results: The Bone mineral density (gm/cm^2) in men of different age groups are;
51-60yrs-Diabetic: 0.957, 0.9819 (spine, hip)
Nondiabetic: 0.931, 0.9521(spine, hip)
61-70yrs- Diabetic: 0.9872,0.910 (spine, hip)
Nondiabetic: 0.954, 0.935 (spine, hip)
71-80yrs- Diabetic: 1.048, 1.048 (spine, hip)
Nondiabetic: 0.9501,0.9034 (spine, hip)

The prevalence of osteporosis in diabetic and non diabetic women are shown in the table below.

Conclusion: There is no significant difference between the bone mineral density(BMD) of diabetic & non diabetic South Indian men. Osteoporosis is more common in the diabetic than the non diabetic post menopausal South Indian women.

Age	% of osteoporosis in Nondiabetic	% of osteoporosis in Diabetic
51-60yrs	3.13%	17.65%
61-70yrs	36.76%	53.85%
71-80yrs	21.74%	35.71%
Prevalence of osteoporosis in diabetic & non diabetic women		

P430 W

WITHDRAWN

P431 F

COMBINED HORMONAL IMPACT AND RESPECTIVE INVOLVEMENT OF NUTRITIONAL STATUS IN LEAN ANOREXIA NERVOSA BONE UNCOUPLING

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In anorexia nervosa (AN), osteoporosis occurs as a result of uncoupling of bone turnover, with decreased bone formation and increased bone resorption. Conversely, refeeding and weight gain induces a recovery of this imbalance. The aim of this study was to evaluate the respective impact of both hormonal and nutritional abnormalities on bone remodeling.

In a group of 69 AN patients (mean BMI=14.3 kg/m^2) and 21 age-matched controls (mean BMI=20.2 kg/m^2), the following parameters were determined: bone markers (serum osteocalcin and CTX), nutritional markers (BMI, fat and lean mass), hormones (FT3, FT4, LH, FSH, 17beta estradiol, DHEAS, IGF-I, GH and cortisol), and calcium metabolism parameters (PTH, Ca, vitamin D).

Osteocalcin positively correlated with CTX in controls and not in AN suggesting independent regulation of these markers in AN. Osteocalcin levels strongly correlated with FT3, IGF-I, cortisol and GH levels while CTX correlated with BMI, fat mass, IGF-I, mean GH, FSH, LH, 17beta estradiol, and cortisol. In stepwise regression models, up to 59% of osteocalcin variance was due to FT3, cortisol and IGF-I. Up to 30% of CTX variance was determined by BMI and 17beta estradiol.

In conclusion we found that osteoclastic resorption was driven primarily by undernutrition and estrogen deficiency, while osteoblastic formation seemed to be more influenced by circulating hormones: FT3, cortisol and IGF-I.

P432 S

HIGH PREVALENCE OF HYPOVITAMINOSIS D IN YOUNG FINNISH MEN: EFFECT ON PEAK BONE MASS

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Objective

To evaluate the prevalence of hypovitaminosis D {serum 25-hydroxyvitamin D [25(OH)D] < 37.5 nmol/l} and the relationship between vitamin D status and peak bone mass among young Finnish men.

Design

Cross-sectional study of determinants of peak bone mass with data on lifestyle factors collected retrospectively. Serum 25(OH)D concentrations and biochemical markers were followed prospectively for one year.

Setting

Southern Finland, District of Uusimaa

Participants

220 young men, aged 18 to 20 years. 170 men were recruits of the Finnish army, and 50 men of similar age, who have postponed their military service for reasons not related to health. For this study the groups were combined.

Main outcome measures

Prevalence of hypovitaminosis D over one year, relationship of serum 25(OH) concentration to bone mineral density (BMD) measured in lumbar spine and upper femur by dual-energy X-ray absorptiometry (DXA), and to serum parathyroid hormone concentration (iPTH) and bone turnover markers.

Results

In July 2000 59 out of 220 men (26.8%) had hypovitaminosis D. Six months later on wintertime the respective percentage was 94.6% (158 out of 167), and during summer 2001 29.8% (28 out of 94 men). After adjusting for age, height, weight, exercise, smoking, calcium and alcohol intake there existed a positive correlation between serum 25(OH)D and areal (but not volumetric) lumbar spine (P=0.0347), femoral neck (P=0.0611), trochanter (P=0.0563), and total hip (P=0.0679) BMD. Serum iPTH correlated negatively (r=-0.24, P=0.0007) with 25(OH)D at baseline and exhibited a seasonal variation inverse to that in serum 25(OH)D. Bone turnover markers declined over time.

Conclusion

Hypovitaminosis D is very common in Finnish young men, especially during wintertime, and it may have detrimental effects on the acquisition of maximal peak bone mass. Since in Finland vitamin D supplementation to infants is now stopped at the age of 3 years, it can be asked, whether it at our latitude should be continued from that age onwards, no more for the prevention of rickets but as the first prophylaxis of osteoporosis.

P433 W

BONE FORMATION BY NANO-SECOND PULSED LASER IRRADIATION IN UNLOADING AND LOADING RATS

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It is well known that bone formation is accelerated by the physical stress. The stresses as mechanical loading, low-intensity ultrasound and low-intensity laser light etc, apply to the study of bone formation. Nano-second pulsed laser irradiation which has a high-intensity laser light can produce stress waves in the target surface by rapidly heating the tissue. We considered that the stress waves induced by nano-second pulsed laser irradiation accelerate bone formation. To clarify whether nano-second pulsed laser irradiation accelerates bone formation, we investigated bone formation in the irradiated femur of the rat using histomorphometric analysis. Moreover, the effects of laser irradiation against the bone cells were examined by histochemical analysis. Rat femurs were irradiated with a Q-switched Nd: YAG laser, which has a wavelength of 1064 nm, under conditions following as two times every 12 hours setting an average fluence rate of 50 mW/cm2 in unloading rats and 500 mW/cm2 in loading rats. In unloading rats, the laser irradiation has influence on the bone formation of trabecular bone and metaphyseal endosteum. The means of bone volume and mineral apposition rate of trabecular bone in irradiated group were significantly higher than those of the non-irradiated group (control). These values were about 1.52 and 1.25-fold those of the control, respectively. In loading rats, the effects of laser irradiation appeared on diaphyseal periosteum, in addition to trabecular bone and metaphyseal endosteum. Those values of bone volume on trabecular bone and mineral apposition rate on diaphyseal periosteum were about 1.27 and 1.82-fold those of control group, respectively. Moreover, histochemical analysis was clearly that the number of osteoclast was decreased and the proliferation of bone marrow cells was hastened by laser irradiation. Thus, this study indicated that nano-second pulsed laser irradiation accelerates bone formation on metaphysis and that the increment of bone volume is caused by inhibition of bone resorption and acceleration of formation. These bone formations induced by nano-second pulsed laser irradiation might be due to laser-induced stress waves.

P434 F

EFFECT OF BODY MASS INCREASE WITH RESISTANCE TRAINING ON BONE MINERAL DENSITY AND CONTENT IN YOUNG MALES

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Purpose: To determine the effect of body mass increase with resistance training on bone mineral density (BMD) and bone mineral content (BMC) in young males.

Methods: Subjects were 25 university males (mean age 18.6 ±0.6yr) who started playing American football recently. During an 8-month training period, they practiced 2h/d for 6d/wk. The subjects were instructed to perform a variety of resistance training activities including bench press, arm curls, squats, and leg curls, at 70-80% of their one repetition maximum. They consumed at least 3000kcal/d and at least 2g protein/d/kg body mass. Body composition and BMD were measured by dual energy X-ray absorptiometry (DPX-L, Lunar) at baseline, month 4 and month 8.

Results: Sixteen subjects completed all measurements. Over the training period, the mean body mass increased from 72.8 ±12.5kg to 79.9 ±12.6kg. This increase in body mass (7.1 ±3.3kg) was consisted of 4.1 ±2.8kg in fat mass, 2.9 ±2.0kg in lean mass, and 0.08 ±0.10kg in total BMC. These changes were all significant ($P < 0.05$). While total BMD was maintained (+0.004 ±0.024g/cm^2), lumbar spine BMD (+0.025 ±0.038g/cm^2) and radial BMD (+0.019 ±0.020g/cm^2) increased significantly. Correlation analysis indicated that the change in total BMC was significantly related to the change in lean mass.

Conclusion: This study suggests that an increase in lean mass associated with resistance training may contribute to an increase in regional BMD and total BMC.

P435 S

BONE MASS AND SKELETAL MUSCLE PUMP ACTIVITY IN ELDERLY PERSONS

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The importance of transcortical fluid flow in the maintenance of bone mass has been demonstrated in numerous animal models. Previously, in a small cohort (N=12) of elderly women, we showed a strong positive correlation between bone mineral density in the hip and postural sway. Our interpretation of this result was that the skeletal muscle activity associated with the postural swaying serves to reduce both venous and interstitial pressures, thereby maintaining substantial transcortical pressure gradients in the lower appendicular skeleton during orthostatic stress.

To address whether postural sway in the elderly could play a significant role in maintaining fluid flow in bone, we assayed skeletal muscle pumping indirectly by recording the cardiovascular response to orthostatic stress (quiet standing), and correlated these changes to the subject's postural sway recorded during quiet standing. In addition, we investigated the ability of daily exposures to plantar-based vertical vibration to improve the subjects' cardiovascular response to orthostatic stress.

Twenty-four women, aged 80-92 years, residing in a continuing care retirement community, were recruited into a six month study. The cardiovascular response of each subject to orthostatic stress was obtained by comparing mean arterial blood pressure (MAP) following three minutes of quiet standing to their MAP in the seated position. Medial-lateral postural sway was obtained with the subject standing quietly on a balance platform for two minutes. Finally, one-half of the subjects underwent 10 minutes/day of a 30 Hz, 0.3g plantar surface vibration stimulation, while one-half experienced a placebo treatment. Measurements were obtained at the start of trial, and at one, three and six months.

Mean arterial pressure at baseline (N=24) was found to drop by an average of 8 mm Hg (negative 20 to plus 5) following three minutes of quiet standing. Consistent with the hypothesis, increased postural sway in this population was associated with significantly lower drops in blood pressure during standing (p=0.04). Twenty-one subjects (10 active, 11 placebo) completed the six month vibration protocol, and daily exposure to the foot based vibration resulted in improved tolerance to orthostatic stress (p=0.08; paired t-test) in the active group, with no significant effect (p=0.15)observed in the placebo group.

P436 W

BIOPHYSIC STUDY ON THE EFFECTS OF IMMOBILIZATION ON RAT FEMUR FOLLOWING SCIATIC NERVE NEURECTOMY-APPLICATION OF FOURIER TRANSFORM INFRARED SPECTROSCOPY AND CALORIMETRIC ANALYSIS

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We studied the morphological and biophysic effects of disuse on rat femur following unilateral sciatic nerve neurectomy (USN), Fourier transformed infrared spectroscopy (FTIR) and calorimetric analysis in 15 growing Wistar-derived rats, 5 weeks of age. The rats were divided into two groups: operated group (right femur) and non-operated group (left femur). The mineral /matrix ratio was evaluated by FTIR. The denatured temperature (Tm), half peak breadth (HPB) and transition temperature (TT) (mj/mg) of type I collagen of the femoral shaft were measured by calorimetry. The mineral/matrix ratio of the cortical bone did no differ significantly between the operated and non-operated groups. The Tm and TT of the operated group were significantly higher than those of the non-operated group ($p<0.05$), whereas the HPB of the operated group was significantly lower than that of the non-operated group. We conclude that immobilization affected by USN has no significant effect on the relative amount of mineral and matrix content in rat femurs determined by Fourier transform infrared spectroscopy, and that immobilization increases chemical stability of type I collagen.

P437 F

MECHANICALLY INDUCED BONE REMODELING DURING TOOTH MOVEMENT IN OSTEOPONTIN PROMOTER DRIVEN GFP-EXPRESSING TRANSGENIC MICE

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Osteopontin (OPN) is one of the major noncollagenous bone matrix proteins. OPN plays important role for triggering bone remodeling caused by mechanical stress during tooth movement. To elucidate the molecular mechanism of the enhancement of osteopontin gene expression induced by mechanical stress, we investigated in vivo osteopontin promoter activity in alveolar bone cells during tooth movement. The transgenic mice of 10-12 weeks old, which carrying green fluorescent protein (GFP) gene under the control of 5.5-kilobase (kb; Op5.5GFP), 2.5-kb (Op2.5GFP), 1.5-kb (Op1.5GFP) and 0.9-kb (Op0.9GFP) mouse osteopontin promoter fragment, were generated and used in this study. Orthodontic closed coil springs were bonded to maxillary first molars and incisors for the experimental tooth movement and maxillary first molars were mesially moved by continuous force (approximately 10g). Expressions of endogenous osteopontin and GFP were examined by in situ hybridization and immunohistochemistry and compared with each other. OPN mRNA-expressing cells were detected in a population of osteocytes, osteoclasts and osteoblasts on the alveolar bone surface after 72 hours of treatment in wild-type (non-transgenic) mice. The localization of GFP in Op5.5GFP mice was consistent with that of endogenous osteopontin during tooth movement. On the contrary, the localizations of GFP-expressing cells in Op2.6GFP and Op1.5GFP were dfferent from that of Op5.5GFP mice. Moreover, expression levels of GFP were decreased in Op2.6GFP and Op1.5GFP transgenic mice and were undetectable in Op0.9GFP transgenic mice during tooth movement. Taken together with the results obtained by the analysis of OPN-knockout mice, our experimental system and result may provide us a clue for the elucidation of the molecular mechanism of bone remodeling caused by mechanical stress.

P438 S

THE IDEA THAT STRESS INDUCED FLUID FLOW CAN PROVIDE SUFFICIENT NUTRIENT TRANSPORT IN OSTEONS COULD BE AN ILLUSION

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It was argued from the results of marker migration experiments that mechanical loading of cortical bone in vivo increases marker penetration into the osteon. However, it is not obvious that it is result of passive stress induced fluid flow, as Knothe Tate and Niederer assume (1998), or of stress induced stimulation of an existing active transport process.

In fact, the ratio between the dilated lacunar volume and its reference volume is smaller than order of 0.0001. This means that the number of cycles necessary to create a concentration of nutrients within lacunae of the same order as in blood should be of order of 10 000. But at normal physiologic loading such a number of cycles would an elapsed time period of order of 10 000 sec. However according the experimental study described by Wang et al. (2000) the increase of tracer penetration was fixed after only 20 seconds at 1Hz cyclic loading immediately after injection of horseradish peroxidase solution.

In the present study, to avoid the problems with the unknown canalicular geometry and fluid viscosity we did analysis for dilatation of an empty lacuna, which, from mechanical point of view, represents an upper limit for dilatation of a real fluid filled lacuna. Although the calculated value of the rate of delivery of glucose is 3 orders less than the suspected value of glucose necessary to sustain osteocyte vitality. Result strongly suggests that the stress induced fluid flow within the lacunar-canalicular system should be ineffective as the nutrient transport mechanism. Perhaps an active transport mechanism should be involved.

P439 W

A STRESS-ADAPTIVE BONE REMODELING MODEL BASED ON REACTION-DIFFUSION SYSTEM

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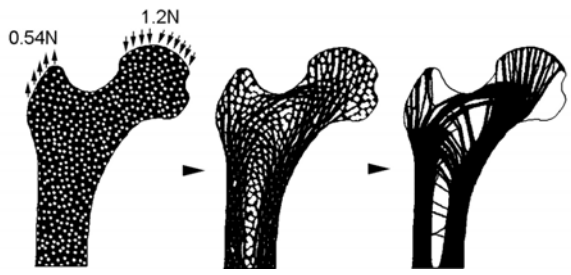
Bone is a highly specialized form of connective tissue consisting of organic and inorganic materials and is continuously remodeled by bone-forming osteoblasts and bone-resorbing osteoclasts. In terrestrial vertebrates, the activities of these 2 types of cells are strictly balanced and adapt the shape of the bone to mechanical stress with limited calcium intake from foods. Recent studies suggest that the osteocyte network embedded in the bone matrix acts as a mechanical sensor providing stress information to osteoblasts and osteoclasts. However, the mechanism underlying this cooperative shape optimization system is not known. Therefore, it would be very useful to construct a model that emulates the behaviors of these cells to examine better the function and structure of bone tissue. So we made a computer model of bone remodeling (iBone) by coupling the bone formation and resorption based on a reaction-diffusion system influenced by local stress. When an external mechanical stress was applied, stimulated bone formation and subsequent activation of bone resorption caused efficient adaptation of the shape of the model bone to a given stress. In macroscopic models, iBone could demonstrate major structures of the human femoral neck (figure) and deformation of it during osteoporosis caused by imbalanced bone formation and resorption. Here we propose a computer model to understand how bone cells communicate and form a cooperative system that adapts the micro and macro structures of bone to voluntary mechanical loads.



P440 F

MECHANICAL TENSION FORCE INHIBITS OSTEOCLASTOGENESIS VIA OSTEOPROTEGERIN PRODUCTION FROM PERIODONTAL LIGAMENT CELLS

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Orthodontic tooth movement occurs during sequential bone remodeling induced by therapeutic mechanical stress. When a unidirectional force is applied to teeth, bone resorption occurs specifically on the compressed side of alveolar bone, and bone formation occurs on the tension side. Previously, we reported that compressive force upregulates the receptor-activator of NF-kappa B ligand (RANKL) in periodontal ligament (PDL) cells, increasing osteoclastogenesis, while there is no change in osteoprotegerin (OPG) expression. Since osteoclastogenesis is not upregulated on the tension side during orthodontic tooth movement, we hypothesized that mechanical tension upregulates OPG expression in PDL cells, inhibiting osteoclastogenesis. This study examined the effects of a cyclic tension force on OPG production by human PDL cells and on osteoclastogenesis.

PDL cells were precultured until confluent in flexible-bottomed culture plates, with bottoms made of silicon membrane coated with type I collagen. PDL cells were subjected to a cyclic tension force (15% elongation, 1 s stretch / 1 s relaxation) using a Flexercell Strain Unit. The conditioned medium was collected and total RNA was extracted for semi-quantitative RT-PCR analysis.

Peripheral blood mononuclear cells (PBMCs) were cultured with $1\alpha,25\text{-}(OH)_2D_3$ (10^{-8}M) in the presence of conditioned medium from PDL cells stimulated by the tension force or from non-stimulated controls. TRAP-positive multinucleated cells

differentiated from PBMCs after three weeks. The number of TRAP-positive multinucleated cells cultured in the presence of conditioned medium from stimulated PDL cells was significantly lower than in that from controls. Tension force inhibited osteoclastogenesis by PBMCs. The amount of OPG in the conditioned medium from PDL cells was measured using ELISA. Tension force increased OPG protein production by PDL cells. Furthermore, tension force up-regulated OPG mRNA expression in PDL cells.

In conclusion, these results suggest that tension force increases OPG production by PDL cells, inhibiting osteoclastogenesis.

P441 S

WHY DO BONE RESORPTION INHIBITORS CAUSE AN INCREASE IN BONE MASS? IN SILICO EXPERIMENTS ON MECHANOSTAT SIMULATOR

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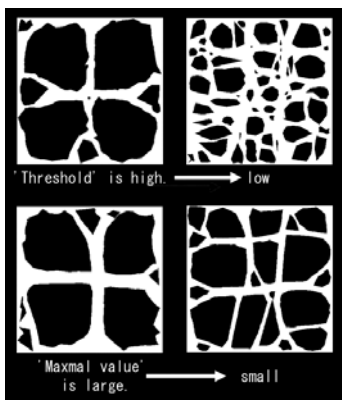
'Mechanostat Theory' is a well-known hypothesis that explains the mechanism of bone remodeling responding to local mechanical stress. We have developed a computer simulation program of bone metabolism, based on Mechanostat theory and finite element method. Using this program we could construct virtual architectures similar to several types of bone structure, such as trabecular bone, cortical bone, trabecular osteon and cortical osteon. Moreover, in this program, we can perform experiments of bone metabolism in silico by freely changing various parameters that define bone resorption, formation and external force.

Most currently used drugs for osteoporosis are thought to increase bone mass through suppressing osteoclastic bone resorption. However, it is not clear why bone mass increases when bone resorption is suppressed. Therefore, we tried to suppress bone resorption on this mechanostat simulator, and considered the change of 'bone mass and structure' theoretically.

In this program, we hypothesized that response curve of bone resorption to local mechanical stress could be described as a sigmoid curve. A sigmoid curve is determined by four parameters, those are threshold value, maximal value, minimal value and change rate on the threshold. We thought that suppression of the threshold value or the maximal value could suppress bone resorption. Then, we made various trabecular bones by changing these two parameters under the same condition of external force, and observed bone mass and structure.

When we lowered threshold setting for bone resorption, bone volume (BV) and trabecular number (Tr.N) remarkably increased but trabecular thickness (Tr.Th) didn't change. When we reduced maximal value of bone resorption, unexpectedly BV didn't change, Tr.N increased and Tr.Th decreased (Figure).

These results indicate that suppression of bone resorption may not always increase bone mass. It is suggested that reduction of threshold in mechanical stress that causes bone resorption is critical to increase bone mass.



P442 W

EFFECT OF ROTATING MAGNETIC FIELD ON CALCITONIN, PARATHOID HORMONE AND ALKALINE PHOSPHATASE IN SERUM OF POSTMENOPAUSAL OSTEOPOROSIS WOMEN AND OVARIECTOMIZED RATS

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OBJECTIVE: The effect of magnetic fields on organisms is an important problem being paid much more attention at present. According to ancient Chinese famous medical books, magnetic fields have been employed in treating human disease since long ago. We have designed and built rotating-high magnetic field therapeutic apparatus. Our previous clinical work indicated that magnetic field can make bone density and intensity increase in osteoporosis patients. Our present experiment was undertaken to investigate endocrinology of calcium metabolism under action of rotating magnetic field.

METHODS: WMG group 15 voluntary osteoporosis postmenopausal women were given oral gluconate calcium (60mg/kg/day) and rotating magnetic treatment (0.6 T, 8Hz) daily during 4 weeks, but WM group 15 only were given rotating magnetic treatment. 30 Adult healthy female SD rats were divided randomly into 3 groups (n=10): ovariectomized+magnetic field (OTM) group, shamoperated+magnetic field (SOTM) group, ovariectomized +magnetic field+oral gluconate calcium (100mg/kg/day) (OTMG) group. The plasma specimen from above groups were collected before and after they take in rotating magnetic treatment. Calcitonin (CT) and parathyroid hormone (PTH) were measured by RIA, ALP was assayed by ELISA.

RESULTS: In WM, WMG group, CT levels of serum were elevated by the rotating magnetic treatment markedly ($p<0.01$), it reached the highest level during 2-3 week ($p<0.01$), and decreasing slowly but still remaining higher than the control level at 4 week ($p<0.05$), returning to the control level by 6 week (2 week after magnetic treatment finished); change of ALP resembled that of CT basically. PTH increased little. There is no significant difference in bone density between WMG group and WM group. It suggest that content of calcium in present Chinese food is enough for need of body. In OTM and OTMG group, change of CT levels were completed within 3 week.

CONCLUSIONS: Our work supports such conclusion that increasing bone density under action of rotating magnetic field results from endocrinological change of calcium metabolism. High level secretion of CT maybe play an important role in rotating magnetic treatment of osteoporosis. The further research of magnetic treatment osteoporosis is being made.

P443 F

BONE MINERAL DENSITY SEEMS NOT TO BE AFFECTED BY DIFFERENT SUBTYPES OF SYSTEMIC SCLEROSIS

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In a prospective study, the bone mass (DMO) of 74 female patients, with 18 years old and more, affected by Systemic Sclerosis (SSc), was evaluated through a DEXA Lunar-DPX unit, in order to consider variants (subsets of SSc, race, treatment with cyclophosphamide and penicillamine, menopause) effects on T-score at lunbar spine and femoral neck.

Thirty-four patients presented normal values of DMO, 26 showed osteopenia and 14 densitometric osteoporosis.

At lumbar spine (L2L4) reduced T-score has a positive association with time of menopause (p=0,001) mainly over 10 years.

The same with femoral neck T-score values (p=0,001) and the ward triangle (p=0,001).

It was not found statistical association between BMD and other SSc variants as SSc clinical subsets, time of disease ,and previous use of cyclophosphamide and d-penicillamine.

P444 S

PREFERENTIAL BONE LOSS IN PARAARTICULAR DISTAL RADIUS AND CALCANEUS IN EARLY STAGE RHEUMATOID ARTHRITIS

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Rheumatoid arthritis (RA) is a major cause of secondary osteoporosis and is frequently associated with both paraarticular and generalized bone loss. The present study was designed to investigate the preferential sites of bone loss particularly in the early stage of RA, with special emphasis on the differential effect of RA on bone loss in trabecular and cortical components. The subjects (30 RA patients and 26 healthy subjects) were all female with disease duration of less than one year. Bone mineral density (BMD) in the radius and lumbar spine were measured by peripheral quantitative computed tomography and dual X-ray absorptiometry, respectively, and the osteo-sono assessment index (OSI) of the calcaneus by ultrasound. RA patients lost bone mass preferentially in calcaneus and the trabecular component of the radius (4%), but not in the total radius. Furthermore, the radius (20%) (midshaft) and lumbar spine did not show significant loss of bone mass compared to age-matched normal controls. BMD in the radius (4%) exhibited a negative correlation with serum CRP, ESR, and RF, and calcaneus OSI with M-HAQ score, suggesting disease activity of RA and impairment of daily physical activity as significant determinants of paraartciular bone loss and calcaneus bone loss, respectively, in early-stage RA patients.

In conclusion, it was demonstrated that early-stage RA patients lose bone mass preferentially in paraarticular bone and the calcaneus, probably by distinct mechanisms.

P445 W

CHRONIC ANTICONVULSIVANT THERAPY CANNOT BE A RISK FACTOR TO OSTEOPOROSIS IN TROPICAL COUNTRIES

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Bone mineral density(BMD) of 69 outcome patients with epilepsy on chronic anticonvulsivant therapy was assessed (DEXA-lUNAR equipment),and the values of

T-score of lumbar spine and femoral neck were related to alterations of calcium and vitamin D metabolism.

They have been taking phenobarbital phenytoin and carbamazepine in single or combined-drugs for at least 5 years, and compared to normal controls.

Lab procedures: serum levels of calcium, ionezed calcium, albumine, PTH, alkaline phosphatase, 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D.

No differences in BMD, ionized calcium, 25-hydroxy-vitamin D, 1-25-dihydroxy-vitamin D and intact-PTH were observed between patients and controls,however the mean serum calcium concentration was lower (p,0,05) and the mean serum ankaline phosphatase concentationwas higher (p<0,05) in patientes than in controls.

These results suggest that patients on chronic anticonvulsivant therapy, living at sunny places could not have low serum vitamin D levels. Light serum calcium levels can be due to lower calcium intestinal absortion, but this is unable to determine secondary hyperparathyroidism and BMD decrease.

P446 F

EFFECT OF HIGH-GLUCOSE CONDITION ON AROMATASE ACTIVITY IN MOUSE BONE MARROW STROMAL CELLS

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[Purpose] Aromatase synthesizes estrogen from androgen, and is found on osteoblasts. In order to clarify the onset mechanisms of diabetic bone changes, this study investigated the effects of high concentrations of glucose on aromatase activity on osteoblasts.

[Methods] Mouse bone marrow stromal cells were collected from the femurs of 6-week-old male ddY mice and incubated in 10%FCS and alpha MEM containing L-glutamine, ascorbic acid, beta-glycerophosphate, dexamethasone, 4-androstene-3,17-dione, and 100-400mg/dl D-glucose. Culture medium was changed every 3 days, and at 24 hours after the last change of medium, levels of estradiol in the medium were measured. Aromatase activity was determined based on amounts of estradiol released into culture medium. At the end of incubation, total RNA was extracted, and the expression of each exon of aromatase under the various experimental conditions was measured using semi-quantitative RT-PCR. Amount of aromatase under various experimental conditions were also measured at the end of incubation, using Western blotting.

[Results] The aromatase activity of the bone marrow stromsl cells were inhibited significantly by the D-glucose more than 200 mg/dl. Inhibition of the aromatase activity wasn't detected with L-glucose and mannitol. Amount of the aromatse of bone marrow stromal cells were inhibited significantly by the D-glucose more than 200 mg/dl. The aromatase mRNA expression of bone marow stromal cells were inhibited significantly by the D-glucose more than 200 mg/dl. The expression of each nine exon of aromatase was compared. The expression of exon 2 was inhibited significantly.

[Discussion] Aromatase deficiency in men causes a marked decrease in bone mineral density. Estrogen supplementation improves clinical symptoms. In aromatase KO mice, both male and female mice exhibit reduced cancellous bone and increased osteoclasts, suggesting that estrogen is essential for bone metabolism in both males and females. In this study, when mouse bone marrow stromal cells were incubated under high concentrations of glucose, aromatase activity on osteoblasts decreased significantly, probably due to the inhibition of exon 2 transcription. These findings suggest that, as one cause of diabetic bone changes, a decrease in aromatase activity could locally lower estrogen levels.

P447 S

LIPOPROTEINS AND POSTMENOPAUSAL RISK OF BONE LOSS

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Bone metabolism may be influenced by lipid metabolism; are they key factors in the pathophysiology of postmenopausal bone loss?

Methods. In a prospective two-year study 70 healthy postmenopausal women with an intact uterus and without oophorectomy, aged 45 to 54 years, on average two years after natural menopause (not taking hormones, body mass index < 30 kg/m^2), were measured by peripheral quantitative computed tomography (thin- and multislice 3D-pQCT), quantifying in mg/cm^3 the bone loss in the pure trabecular tissue (without corticalis) at ultradistal radius.

Results. The values of apolipoprotein B or of LDL cholesterol are positively correlated to the trabecular bone mass, also under controlling for body mass index and annual bone loss rate ($P < 0.001$). Regarding the association between lipid parameters and annual change of bone mass (range: -19 to +2.6 %), the correlations adjusted for body mass index and trabecular bone mass, show: the higher the trabecular bone loss, the higher is the apolipoprotein B ($P < 0.02$) or, inversely, the lower the trabecular bone loss, the higher is the apolipoprotein A-I ($P < 0.05$) or the HDL cholesterol ($P < 0.02$). Consequently, the higher the ratio of apo B to A-I, but the lower the trabecular bone mass and (independently) the lower the number of pregnancies, the greater is the probability that the particular woman is a fast bone-loser (trabecular bone loss > 3.8 % per year; prevalence 34 %). Lifestyle factors, such as exercise, calcium intake and smoking affecting bone density, are unable to discriminate the fast from the low bone-losers.

Conclusions. During the years of fertility, apolipoprotein B and LDL cholesterol sustain the development of trabecular bone mass. After menopause, apolipoprotein A-I may have protective effects on bone loss. The atherogenic risk indexes (ratios of apo B to A-I or of LDL to HDL) are strongly associated not only with coronary heart disease, but also with postmenopausal osteoporosis. Women, having a proatherogenic risk index without above-average trabecular bone mass, are at high risk.

P448 W

SOLUBLE CD30 SERUM CONCENTRATIONS INVERSELY CORRELATE WITH HAND BONE MINERAL DENSITY IN PATIENTS WITH ACTIVE RHEUMATOID ARTHRITIS UNDER THE TREATMENT OF LOW-DOSE METHOTREXATE

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Objective: Rheumatoid arthritis (RA), a chronic inflammatory disease, principally affects the synovium, leading to lesions in articular cartilage and subchondral bone. Measurement of hand BMD using DXA has been reported as an accurate, reproducible, and sensitive method to quantify hand BMD in RA (CV 0.8%) and considered to reflect inflammation induced periarticular bone loss. sCD30 is released by CD30+ T cells (Th2/0 phenotype) present in rheumatoid synovium and its real role in the pathogenesis of RA is unclear. This study proposed assess relationship of total right hand and right metacarpophalangeal BMD (p-BMD) with acute phase response and soluble CD30 (sCD30) levels in a group of RA patients under the treatment of low-dose (7.5-12.5 mg/wkly) methotrexate (MTX).

Methods: Thirty-five patients (31F, 4M, aged 51.0±13.1) with a disease duration of 7.4 years and twenty normal controls (17F, 3M, aged 52.1±13.1) included into the study. Eighteen patients had active disease/partial remission (group A) and 17 patients were inactive (group IA). Serum levels of sCD30 and IFN-gamma were evaluated by commercially available ELISA kits. Acute phase proteins, alpha-1 antitrypsin (AAT), haptoglobin (H), tranferrin (T), and CRP were evaluated by nephelometric/turbidimetric methods. Total hand (h-BMD), p-BMD, AP spine, and femoral BMD of both groups were measured using a DXA methodology on a Lunar-DPX densitometer.

Results: Patients had significantly higher levels of sCD30 (50.7 vs 21.6 U/ml, p less than 0.001), and lower levels of IFN-g (0.6 vs 1.4 pg/ml, p less than 0.001) than controls. There was not a significant difference in BMD measurements between overall patients and controls. Patients in A group had higher sCD30 (64.4 vs 36.1 U/ml, p=0.001) concentrations than IA patients. sCD30 concentrations in group A patients inversely correlated with total hand and p-BMD (r: -0.59 and r: -0.59, p=0.01, respectively), femur-neck and trochanter-BMD (r:-0.60, p=0.009 and r:-0.58, p=0.01, respectively). sCD30 in the group A patients also correlated with CRP levels (r:0.89, p less than 0.001).

Conclusion: sCD30 serum concentrations in MTX treated RA patients with active/partial remission disease inversely correlate with periarticular bone loss and may reflect a shift to Th2 response in an attempt to downmodulate inflammation.

P449 F

THREE-DIMENSIONAL TRABECULAR BONE MICROSTRUCTURE IN TYPE 2 DIABETIC RATS: THE ANALYSIS USING MICRO-CT

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Unlike type 1 diabetes, type 2 diabetes has a low frequency of low bone mass, and it has been questioned as a cause of osteoporosis. However, it has recently been reported that type 2 diabetes is related to the increase in a fracture risk, and the existence of the factor except for low bone mass is suggested as the mechanism. In this study, the change of trabecular bone microstructure in diabetes mellitus was studied using type 2 diabetic rats, and its significance in the pathophysiology of the diabetic osteoporosis was examined.

Type 2 diabetic rats of 14, 28, 42 and 56 weeks old (OLETF, n=10 for each group) and control rats of the same week old (LETO, n=10 for each group) were used for the study. After the sacrifice, fifth lumbar vertebra (LV), proximal tibial metaphysis (PT), and distal tibial metaphysis (DT) were scanned by microCT in the slice thickness of 14.1-18.6 microm and the pixel size of 17.6-23.2 microm. Three-dimensional image

data were analyzed by the image analysis system to obtain bone mass, trabecular thickness, number, separation, connectivity, trabecular bone pattern factor, structure model index, and degree of anisotropy.

In all week groups, body weight was higher in OLETF. OLETF rats showed higher values of bone mass in LV at 14 weeks old and in DT at all week groups. On the other hand, at 56 weeks old OELTF rats showed lower bone mass in LV and PT. The trabecular microstructure of LV in OLETF rats was rod-like at 28, 42 and 56 weeks and less connected at 28, 42 and 56 weeks old, whereas DT in OLETF rats showed plate-like and more connected structure at all week groups.

These results suggests that the bone change in diabetes mellitus greatly varies among the skeletal sites and in the mechanically less loaded site such as lumbar vertebra and proximal tibia, the deterioration of trabecular microstructure precedes the decrease in bone mass.

P450 S

SPONTANEOUS FRACTURES OF LONG BONE IN BEDRIDDEN ELDERLY PATIENT: CLINICAL FEATURES AND PROGNOSIS

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Fractures of long bone as the consequence of falls in the elderly are well known. However, reports on spontaneous fractures in bedridden elderly are limited. We report 13 spontaneous fractures over a 5-year period in permanent bed-ridden inpatients. (Subjects) Twelve women and one man with spontaneous fractures were studied. Their mean (±S.D.) age was 87.9 ±9.2 years old and the mean period for the bedridden status was 8.8 ±6.9 years. Fractures included 7 supracondylar fractures, 1 intertrochanteric fracture of femur, 2 surgical neck fractures, 2 shaft fractures, and 1 supracondylar fracture of humerus. Eight patients were suffered from fractures for the first time, although 4 patients were suffered for the second time, and 1 patient for the third time on the same side of their previous fractures either of traumatic or nontraumatic origin. All fractures occurred near by the articular contracture at the same extremity of patients and were tend to be occurred at the hemiplegic side (4 cases out of 5 hemiplegic patients). The nutritional status of these patients were poor, evaluated by BMI, serum albumin level and blood lymphocytes counts. Four patients were suffered simultaneously from aspiration pneumonia and 3 patients were suffered from decubitus. These patients were treated with either plaster bandage, splint bandage or adhesive plaster bandage. One patient past away due to the worsening of aspiration pneumonia after 1 month and the rest 12 patients recovered after approximately 2 months of fixation bandage. (Summary) Long-termed bedridden patients have high risk of suffering from spontaneous fractures, especially in the patients with a past history of previous traumatic or nontraumatic fractures, articular contracture and hemiplegia. Although most patients with spontaneous fractures can be satisfactorily treated with the conservative treatment. However, patient with severe complication might result in poor prognosis. In order to prevent the fractures, new methods of transferring and turning techniques and prevention of articular contracture should be developed. Meanwhile, any audible crack sound, swelling or deformity during transfer or turning should not be ignored.

P451 W

FEMORAL FRACTURE HEALING IN THE RAT: CHANGE IN EXPRESSION OF NERVE-RELATED GENES WITH AGE

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The formation of bridging callus following femoral fracture slows with age in both humans and rats for unknown reasons. Abnormalities in the innervation of the fracture site will slow skeletal healing clinically. We explored whether abnormal nerve behavior in the older rats might contribute to this slowing of skeletal repair. Simple, transverse, mid-shaft, femoral fractures with intramedullary rod fixation were induced in female Sprague-Dawley rats at 6, 26 and 52 weeks of age. At 0, 0.4, 1, 2, 4, and 6 weeks after fracture a bony segment, one-third the length of the femur, centered on the fracture site, including the external callus, cortical bone, and marrow elements, was harvested from each subject. Total RNA was extracted from each segment. RNA was pooled between two rats of the same age and time after fracture. cRNA was prepared and hybridized to 18 Affymetrix U34A microarrays (1/age/time point). Of the 8,700 genes on each array, an average of 3,300 were scored as present. 240 genes were related to neural function. The mRNA expression of most of these responded to fracture. About 70 were increased by fracture, such as galanin (J03624), pleiotrophin (AI102795), and glia maturation factor B (Z11558). 52 decreased after fracture at all three ages, such as synuclein (AF007758), neurogranin (L09119), and neuropeptide Y (M15880). There was some differential effect with age. A few genes, 12, had a stronger response to fracture in young rats than in older rats, such as PCTAIRE 2 (AB005540) and 3 (AB005541) and synaptic scaffolding molecule (AF034863). More genes, 42, had greater change with age in the older rats than in the younger, such as glial-derived neurotrophic factor (L15305), thrombospondin 4 (X89963), and postsynaptic density protein (PSD-95) (M96853). In conclusion, mRNA of genes related to neuronal function is found in bone and in the fracture callus. Most of these genes respond to fracture with altered mRNA gene expression. Differential expression with age may reflect altered nerve cell function at the fracture site that may be related to the slowing of fracture healing.

P452 F

BONE MINERAL DENSITY AND ORTHOSTATIC INTOLERANCE IN THE PERIMENOPAUSAL POPULATION

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Perimenopausal bone loss represents the highest rate of bone loss most women will experience during their lifetime. Starting approximately three years prior to the menopause, and continuing for two to three years past the menopause, bone loss can exceed 2-3% per year. Prevention of bone loss during this relatively brief period of time could, therefore, have a significant impact on the incidence of osteoporosis. Previously, we have shown that in elderly women the inability to maintain normal blood pressure during orthostatic stress (i.e. orthostatic intolerance) is associated decreased bone mineral density. In the elderly population, orthostatic intolerance develops concomitantly with the loss of skeletal muscle mass and activity, and therefore skeletal muscle pump activity. In the perimenopausal women, muscle activity is not expected to be as severely compromised as in the elderly woman, nonetheless, the abrupt changes in estrogen levels during this phase of life may well affect muscle tone, and therefore veno-constriction and skeletal muscle pump activity, leading to orthostatic intolerance and subsequent bone loss.

To address this hypothesis, we recruited 150 perimenopausal women (age 47-53 years) from which we obtained hip bone mineral density using dual energy x-ray absorption (DEXA), as well as the change in mean arterial pressure following three minutes of quiet standing after rising from a seated position. Individuals diagnosed as osteoporotic (i.e. t-scores less than -2.5) or diagnosed as orthostatic hypotensives (i.e. systolic blood pressure drop greater than 20 mm Hg upon standing) were excluded from the study.

Change in mean arterial pressure (MAP) following standing ranged from -15 mm Hg to +16 mm Hg in this population, and hip t-scores ranged from -2.4 to +3.2. Hip t-scores were correlated to the changes in MAP during orthostatic stress (quiet standing) (p=0.001) and was able to explain over 11% of the total variation in the hip t-scores. In a multiple regression analysis, change in MAP was able to independently explain as much of the variation in hip bone mineral density in this group of women as body weight, such that the two combined accounted for over 23% of the variation in hip t-scores.

P453 S

SENILE OSTEOPOROSIS: BONE MASS AND GONADAL HORMONE LEVELS

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Aim: To assess the possible relation among levels of gonadal and calciotropic hormones and bone mineral density (BMD) in women older than 65 years. Patients and methods: Patients were referred to our Division to assess BMD. Lumbar and hip densitometry were performed with the bone densitometer analyzer Hologic QDR 1000, (Waltham, MA, USA). Serum levels of FSH, LH, estradiol, estrone, i-PTH, 24-h urine calcium excretion and (25OH) vitamin D were obtained. Anthropometric data as weight (kg) and height (cm) were assessed and bone mass index (BMI) was figured out. Age of menarche and menopause were recorded, and the years since menopause were calculated. Calcium intake (mg/day) was evaluated through a semiquantitative questionnaire. Statistical analysis was performed. Results: 265 women were included. Age of menopause (49 years); Calcium intake: (mean: 800 mg / day). None of these data were correlated with BMD. BMI (mean 27.8) was positively correlated with lumbar and femoral neck BMD (r: 0.292, p= 0.002 and 0.282; p= 0.001, respectively). Age (mean 72 years) was negatively correlated with BMD at all bone sites. 25 OH Vitamin D levels were not correlated with BMD. i-PTH levels were correlated with lumbar BMD (r: 0.174 p=0.033). LH was related to femoral neck (r: 0.196; p=0.034) and trochanteric BMD (r=0.312; p=0.001); and estrone levels with trochanteric BMD (r=: 0.208; p=0.027). Conclusions: BMD in women older than 65 years seems to correlate more with residual gonadal status than with alterations in calcium intake and its absorption.

P454 W

ESTROGEN-DEPLETION RELATED TIME-COURSE CHANGES IN MUSCLE AND BONE OF THIGH - AN AGED OVARIECTOMIZED RAT MODEL

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To characterize ovariectomy-induced time-course-changes of Myosin Heavy Chain-I (MHC-I) mRNA of quadriceps femoris, volumetrical bone mineral density (vBMD) and geometry in cross-section of proximal femur (including femoral neck(FN) and intertrochanteric region(IT)) in aged rats after ovariectomy.

Thirty-five 12 month-old female Wistar rats were randomized into five weight-matched groups(n=7/group). On the day of surgery(day0), seven rats were euthanized as baseline(BSL). The remaining rats were subjected to either bilateral sham-operation(CON) or ovariectomy(OVX). CON and OVX rats were both sacrificed at 45 days and 90 days postsurgery. MHC-ImRNA was determined by RT-PCR. Degree of bone mineralization (ratio of calcium and matrix=Ca/M) was determined by chemical assay. Amount of mineralized bone in FN/IT (total/trabecular vBMD=vBMD-TOTA/vBMD-TRAB), geometry in FN/IT (cross-sectional area of tissue/marrow/bone=CSTA/CSMA/CSBA and cross-sectional moment of inertia=CSMI) were all determined by pQCT analysis.

MHC-ImRNA, indices of material (Ca/M, vBMD-TOTA(TRAB)-FN(IT)) and geometry in CON rats always remained relatively constant in comparison with those in BSL during the course of the study. In OVX rats, MHC-ImRNA immediately started to hardly reach the detectable level of the study at 45 days postovariectomy. Dissimilarly, a slight decrease at 45 days postovariectomy in OVX rats compared with CON rats was only observed in indices of material, indices of geometry such as CSBA-FN(IT) and CSMI-FN(IT), whereas a significant decrease at 90 days postovariectomy was just observed in those. On the contrary, CSMA-FN(IT) in OVX rats compared with CON rats was slightly increased at 45 days postovariectomy and further significantly increased at 90 days postovariectomy, but CSTA-FN(IT) in OVX rats was only moderately increased at all the time point of the study in comparison with that in CON rats.

MHC-ImRNA level of muscle was an earlier indicator of estrogen-depletion induced alteration than bone parameters. High bone turnover results in decrease of degree of bone mineralization, vBMD and alterations in distribution of cross-section.

P455 F

ESTRADIOL MAY ACTIVE FOR DETERMINING BONE DENSITY IN BOYS AT VERY LOW CONCENTRATION

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Importance of estradiol in bone maturation was proved by the analysis of patients with estrogen receptor mutation or aromatase deficiency. However, the importance of estradiol on bone in boys was not analyzed because of low serum estradiol concentration in childhood. In the present study, the relationship between estradiol and bone was analyzed by using newly developed sensitive assay for estradiol.

PATIENTS AND METHODS: Fifty two growth retarded male patients (age 4-14y: bone age 2y to12y6m) joined this study. Serum estradiol concentration was assayed by ultrasensitive assay (ESTR-US-CT, CIS bio international, range:1.4-500 picogram/microliter). Testosterone and other pituitary hormones were also analyzed by provocative tests.

RESULTS: Bone density was measured by dual-energy x-ray absorbtiometry, and values were analyzed by volumetric bone density (vBMD).

Serum estradiol level in patients were remained below 10 picogram/microliter which was lower detection limit of conventional method. Significant relationship was determined between vBMD and serum estradiol concentration or body mass index (r=0.509 (p=0.015), r=0.035 (p=0.035)). Estradiol concentration in patients was related to the concentration of testosterone and follicle-stimulating hormone which was substrate and inducer of aromatase.

DISCUSSION: Serum estradiol concentration was extremely low in boys, however newly developed method enabled assessment of estradiol concentration. This pilot study for the assessment of estradiol function at low concentration revealed significant relationship between bone density. According to this result, even mild hypogonadism or delayed adolescence may be risk factor for lacking sufficient bone density in adult life. Furthermore, the possibility of involvement of follicle-stimulating hormone-aromatase system for determining estradiol concentration means the importance of further investigation of aromatase regulating system including adipose tissue.

P456 S

BMD AND TESTOSTERONE LEVELS CORRELATIONS IN ARGENTINE MEN

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Osteoporosis factors and pathogenesis in men is still controversial. We conducted an open study in male population (216 healthy men) during year 2001, age between 29 to 95 (average: 57) not suffering from severe diseases involving bone metabolism. We performed BMI, testosterone levels, SHBG, osteocalcine, urinary Deoxipyridolines, DHEA-S, Alkaline Phosphatase, Calcium levels ,an autotest questionnaire looking for lifestyle ways (smoking, drinking, sedentarism, etc.) and BMD using pQCT. General characteristics of population was: age: 55±8.3 years, BMI(kg/m2): 25.5±3.9; osteocalcine(ng/ml): 7.0±3.7, SHBG(nmol/l): 34.9±15.6; testosterone(ng/dl): 520.2±10.1; DPD(nMPD/mMore): 5.0±1.8; FTI(%): 16.0±0.8; BMD(mg/m3): 374.2±70.1

Results: data shows no correlations with testosterone or abnormal BMD (ANOVA test); but does correlate with age(r:0.40;p<0.01), SHBG(r:0.21,p<0.01), osteocalcine(r:0.12, p<0.05), DPD(r:0.14, p<0.01) and with FTI(r:0.023, p<0.01).

P457 W

ANDROGEN ACTION IS ESSENTIAL FOR MAINTENANCE OF BONE METABOLISM

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Androgens have important effects on the human skeleton in both males and females. Hypogonadism in men is associated with increased bone turnover and bone loss, which is reversed after treatment with androgens. Clinical studies suggested that combined therapy of estrogens plus androgens may enhance bone mineral density and bone mass to a more significant degree than estrogen therapy alone in postmenopausal women. However, the mechanism of androgen action on bone metabolism remains controversial. By using a cre-lox conditional knockout strategy, we report here the generation of androgen receptor knockout (ARKO) mice. Histologic analysis of bone sections from 8-week-old ARKO mice shows that cancellous bone volumes are lower in the ARKO mice than in both female and male wt littermates. The decrease in bone volume is seen readily in the metaphyses of the femora and tibiae. Osteoclast numbers in the femoral metaphyses are higher in ARKO than in wt mice. However, the number of osteoclasts formed in vitro from spleen cells from ARKO mice is similar to that in wt mice. Mean values for mineral apposition and bone formation rates in the femoral metaphyses are higher in the ARKO mice than in the wt mice. These data indicate that in the absence of the AR, mice develop osteopenia. Because osteoclast numbers and bone formation are both increased in these mice, the osteopenic phenotype most likely is because of bone resorption being increased beyond that of the increase in bone formation. Thus, it is possible that osteoclasts are not only increased in number in ARKO mice, but that they also live longer than normal because of reduced testosterone concentrations, and therefore resorb more bone than wt cells. These possibilities are currently under investigation. The increased bone mineral apposition and bone formation rates coupled with increased osteoclast numbers and low bone volumes observed in the mice is a phenotype that is seen in sex steroid deficiency and is consistent with the low T concentrations in the mice. Taken together, the osteopenic phenotype of ARKO mice strongly supports an important role of AR signaling in bone metabolism.

P458 F

PROSTAGLANDIN E2 ADMINISTERED BY CONTINUOUS INFUSION CAUSES HIGH TURNOVER REMODELING-INDUCED CANCELLOUS BONE LOSS AND HIGH TURNOVER MODELING-INDUCED NEW CORTICAL BONE

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The effects of prostaglandin E2 (PGE2) given by continuous infusion or by daily subcutaneous (sc) injection were analyzed on proximal tibial metaphysis (PTM), tibial shaft (TX), and lumbar vertebral body (LVB) by histomorphometry. Six months old intact female rats were treated with 1 or 3 mg PGE2/kg/day via infudisc continuous infusion or daily sc injection for 21 days. In the cancellous bone of the PTM and LVB, PGE2 continuous infusion lowered cancellous bone area, decreased trabecular architecture (decreased width and number and increased separation) and increased osteoid and bone formations, bone resorption, bone turnover with changes in bone resorption exceeding bone formation. In contrast, when administered by sc injection,

PGE2 increased cancellous bone area, trabecular width, osteoid and bone formations, bone resorption and bone turnover with changes in bone formation greater than bone resorption. In addition, PGE2 decreased the resorption period and increased the formation period during bone remodeling. In cortical bone of the TX, PGE2 continuous infusion had no influence on cortical bone or medullary areas, but the treatment increased intracortical porosity, stimulated massive periosteal formation drift partially compensated by endocortical resorption drift (modeling), which resulted in a slightly increased cortical thickness composed mainly of woven and new lamellar bone. In contrast, when administered by sc injection, PGE2 increased cortical area and reduced medullary area, increased periosteal and endocortical bone and endocortical osteoid formations. Thus, continuous infusion resulted in no change in cortical area and medullary cavity, but the cortical bone was composed of woven and new lamellar bone from massive periosteal formation drift. The findings of decreased cancellous bone mass and poorer cortical quality (woven bone and new lamellar bone) suggest that continuous infusion treatment may reduce bone strength. These results indicate that continuous infusion is not a valid means of delivery of PGE2 to increase bone mass and strength.

P459 S

NITRIC OXIDE MODULATES BONE MINERALIZATION IN GROWING RATS

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We examined the effects of chronic nitric oxide (NO) blockade on bone mineral status in growing rats. Oral administration of NG-nitro-L-arginine methyl ester (L-NAME) for 4 wk caused hypertension and a significant reduction in urinary NO2- and NO3- excretion. Four-week oral aminoguanidine (AG, 400 mg/dl of drinking water) did not alter blood pressure but caused a significant decrease in urinary NO2- and NO3-. Rats treated with L-NAME at doses of 20 and 50 mg/dl had normal bone mineral mass in the lumbar spine, but the highest dose (80 mg/dl) caused a slight decrease in bone mass. Chronic AG induced a significant spine osteopenia. This effect of AG was abolished by the simultaneous administration of L-arginine (2.0 g/dl). AG-induced osteopenia was associated with a significant increase in urine excretion of collagen cross-links with normal serum osteocalcin. These findings indicate that chronic AG administration can cause an imbalance between bone resorption and formation, resulting in a decrease in bone mass in growing rats, and suggest that NO produced by inducible NO synthase plays an important role in basal osteoclast bone degradation activity in vivo.

P460 W

THE INFLUENCE OF ALPHA-KETOGLUTARATE (AKG) ON DEVELOPMENT AND MINERALIZATION OF THE SKELETAL SYSTEM DURING THE POSTNATAL LIFE IN THE PIG INVESTIGATED ON THE FEMUR AND RIBS MODEL

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The recent study revealed that enteral treatment of alpha-ketoglutarate (AKG) increases the portal net apperance of proline and may increase the supply of this indispensable amino-acid for bone collagen synthesis, mainly type I, in the developing skeletal system in the new-born piglets. However, the exact mechanism of AKG influence on skeletal system, via digestive tract, is still poorly known and requires further research. The purpose of the study was to investigate the effect of exogenous AKG on the bone mechanical and geometrical properties and also on bone mineral density (BMD) during the postnatal life in the pigs.

Material and methods: Experiment was carried out on piglets divided on two groups, a control (C), which was administered physiological saline (PhS) per os and experimental (E), which was administered AKG solution in a dose 0.4 g/kg b.w./day. Both femora and twelve ribs, from the fourth to ninth rib of every side, were isolated from every piglet for measurements of the geometrical parameters, like cross sectional area (A), second moment of inertia (Ix) and mean relative wall thicknes (MRWT). The three-point bending test and INSTRON 4302 apparatus was used to determine bone maximum elastic strength and bone ultimate strength. Bone mineral density was determined by DEXA method. Osteocalcin (OC), as bone formation marker, in the blood plasma was also assayed.

Results: The values of the mechanical and geometrical parameters of both, femora and ribs were significantly higher in experimental group in comparison to control values during the whole period of observations. Moreover, BMD increased after AKG administration in comparison to control. A positive correlation was observed between increase of BMD and OC level in blood plasma of experimental piglets.

Conclusion: Positive results of AKG administered orally indicate on the possibility of existence of the digestive tract-skeletal system axis mediated by enteral AKG as a nutrient or precursor of proline affecting growth and mineralization of the bones. Not only femur but ribs may be used as suitable model bones for research of the developmental changes in the mechanical and geometrical parameters during the postnatal life in the pig.

P461 F

INTRAVENOUS ADMINISTRATION OF ZOLEDRONIC ACID PREVENTS THE BONE LOSS AND REDUCTION OF MECHANICAL PROPERTIES INDUCED BY AROMATASE INHIBITION OR SURGICAL OVARIECTOMY IN RATS

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Aromatase inhibitors (AIs) are being evaluated in adjuvant endocrine therapy of post-menopausal (PM) women with hormone-dependent breast cancer. AIs induce estrogen deprivation. One of the expected consequences is increased bone loss as was recently reported following anastrozole therapy in the ATAC trial (Lancet 359: 2131-2139 2002). Zoledronic acid (Zol), a new and potent bisphosphonate (BP), has been shown to exert a bone protective effect in PM women (NEJM 346: 653-661 2002). We investigated whether bone loss and the reduction of mechanical properties in rats as induced by either ovariectomy (OVX) or the AI letrozole (Let) could be prevented by Zol.

Eight-month-old female Wistar rats were assigned to the following treatment groups (10 animals/group): Sham (vehicle), OVX, Let-treated, OVX + Zol and Let + Zol. Zol was injected into the tail vein as a single dose of 0.8, 4 or 20 microg/kg either before OVX or initiating daily oral dosing of Let (1 mg/kg) for 24 weeks. Changes in bone parameters in the proximal tibial metaphysis (PTM) were monitored by pQCT at 0, 2, 4, 8, 12, 16 and 24 weeks and mechanical properties of the 4th lumbar vertebral body (LVB) determined.

BMD of the PTM decreased rapidly after OVX, slowing at 12 weeks to plateau at around 20 weeks (-16%). A single i.v. injection of 0.8, 4 or 20 microg/kg Zol delayed bone loss significantly for up to 8, 20 and 24 weeks respectively. Total BMD also decreased slowly after administration of Let to reach a plateau at 12 weeks (-13% at 24 weeks) (P<0.01 at all time points vs sham). In Let-treated rats, a single i.v. injection of 0.8, 4 or 20 microg/kg Zol delayed bone loss significantly for 4, 24 and 24 weeks respectively. Zol administration led to a dose-dependent improvement in mechanical parameters in both Let-treated and OVX animals in LVB compression tests.

Zol dose-dependently protects against the bone loss and reduction in mechanical properties in rats induced by daily oral Let or OVX with full protection being achieved at 20 microg/kg for at least 24 weeks. These data support the use of Zol in preventing bone loss not only in otherwise healthy PM women but also in PM women undergoing adjuvant treatment with AIs.

P462 S

GLUCOCORTICOID-INDUCED OSTEOPOROSIS IN ADULT CYNOMOLGUS MONKEYS: EFFECTS ON BIOCHEMICAL MARKERS OF BONE TURNOVER

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Glucocorticoid exposure has been demonstrated to induce osteoporosis though several issues regarding the skeletal effects of glucocorticoids still remain uncertain. The use of nonhuman primates as animal models for glucocorticoid-induced osteoporosis has not been extensively explored despite the close phylogenetic association of nonhuman primates to man and their particular similarities in bone architecture and responsiveness to hormonal influences. The objective of this study was to investigate the changes in bone turnover in response to two-week alternate-day intravenous glucocorticoid administration in monkeys. Adult cynomolgus monkeys were divided into three groups, each comprising 3 males and 3 females: one group received intravenous injections of 1 mg/kg dexamethasone phosphate while a second group was treated intravenously with methylprednisolone succinate at 4 mg/kg. The placebo group was injected with distilled water. Serum samples obtained at baseline and on days 2, 4, 8 and 14 were tested for osteocalcin (OC), bone-specific alkaline phosphatase (B-ALP) and type I collagen cross-linked N-telopeptide (NTx). Cumulated 24-hour urine collected at baseline and on days 2, 4, 8 and 13 were analyzed for NTx. Bone mineral density (BMD) changes were evaluated by dual energy x-ray absorptiometry done before and immediately after the two-week administration period.

Significant reductions in serum OC levels were observed early and throughout the course of dexamethasone treatment (29.61, 47.53, 56.69 and 56.27% decline on days 2, 4, 8 and 14, respectively, at p=0.01) compared to the placebo-treated group. In contrast, dexamethasone caused significant increases in serum (92.54, 60.19 and 81.41% on days 4, 8 and 14) and urinary NTx (78.73% on day 14). Similar effects were not associated with methylprednisolone. No effects on B-ALP and BMD were observed.

The study showed that dexamethasone administration inhibited bone formation and increased bone resorption in cynomolgus monkeys as manifested by decreased levels of the serum osteoblast protein, osteocalcin, and increased levels of the serum and urinary type I collagen degradation product, NTx. These data were consistent with previous findings in humans demonstrating that the cynomolgus monkey model can be used effectively in evaluating the skeletal actions of glucocorticoids.

Osteoporosis: Treatment

P463 W

EFFECTS OF FLUOR SALTS, HORMONE REPLACEMENT THERAPY AND CALCITONIN ON THE CONCENTRATION OF BONE MATRIX GROWTH FACTORS IN PATIENTS WITH OSTEOPOROSIS

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Data from cell culture experiments suggest that local insulin-like growth factor (IGF)-I, IGF-II and/or transforming growth factor(TGF)-beta1 may mediate the positive effects of estrogen, calcitonin or fluor ions on the skeleton. In order to assess the in vivo relevance of in vitro reports, the effect of fluor salts, hormone replacement therapy (HRT) and calcitonin, respectively, on the concentration of IGF-I, -II and TGF-beta1 in bone matrix extracts from the iliac crest was evaluated in 170 patients (76 men and 94 women) with primary osteoporosis aged 55.5 ±0.8 years (range 27-88 years). Bone matrix extraction was performed based upon a guanidine-HCL/EDTA method.

No influence of long term therapy with fluor ions or calcitonin on the bone matrix concentration of IGF's or TGF-beta1 was noticed. Postmenopausal women with osteoporosis on HRT had significantly lower skeletal IGF-I but not IGF-II levels as compared to age- and body mass index-matched non-users. However, the lower rate of bone turnover in women using HRT may account for this difference, since the significance was lost after adjustment for alkaline phosphatase levels. Likewise, a tendency towards lower TGF-beta1 levels was observed in HRT-users as compared to non-users but was lost after adjustment for the rate of bone turnover. The concentration of growth factors in the skeleton was not related to the lenghth of therapy in either group. None of the therapies influenced the serum levels of growth factors when patients receiving continous therapy for at least one year before bone biopsy were considered.

Our data suggest no direct effect of fluor therapy on skeletal IGF's or TGF-beta1 levels. In addition, neither hormone replacement therapy nor calcitonin appeared to exert any significant influence on serum or local growth factors levels.

P464 F

VASCULAR BUNDLE INSERTION ENHANCES BONE FORMATION IN BONE MORPHOGENETIC PROTEIN-2-BASED TISSUE-ENGINEERED BONE

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Recently, growing attentions have been paid on tissue engineering technique for bone and cartilage regeneration. It has been suggested that blood supply is important for bone tissue engineering but no experimental evidence has been reported to date. The aim of the current study is to investigate if it is possible to build a porous hydroxyapatite (HA) ceramics integrated with capillary vessel network by inserting a vascular pedicle, and whether this procedure enhances new bone formation in bone morphogenetic protein-2 (BMP-2) -based tissue engineered bone. First, a synthetic interconnected porous HA, IP-CHA, that we developed recently, was implanted subcutaneously into rat groin with or without inserting superficial inferior epigastric vessels that were raised as a vascular pedicle. On histological examinations at six weeks after implantation, thick fibrous connective tissues with a number of large-sized blood vessels that seemed to derive from the inserted vascular bundle were observed in the pores of IP-CHA with vascular bundle insertion. India ink perfusion from heart revealed that those blood vessels developed in the pores were effectively connected to host circulation system. However, the fibrous tissues seen in IP-CHA without vascular bundle were markedly looser and large blood vessels were hardly found. Next, IP-CHAs loaded with recombinant human BMP-2 using synthetic copolymer PLA-DX-PEG (poly-D, L-lactic acid-p-dioxanone polyethylene glycol block copolymer) as a carrier were implanted. When a vascular bundle was inserted, abundant newly formed bone was observed in the pores at deep portion close to the inserted vessels three weeks after the implantation. On the other hand, without vascular bundle, small amount of new bone was seen only in the pores close to the surface but not in the deep portion. Histomorphometric analysis for the area of newly formed bone matrices demonstrated that the vascular bundle insertion significantly increased new bone formation in BMP-2-based tissue engineered bone. We believe that our system integrating vascular network into interconnected porous HA is a useful and essential technique for bone tissue engineering as a treatment for challenging orthopedic conditions.

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P465 S

HIGH PROTEIN DIETS HAVE AN INFLUENCE ON BONE MINERAL STATUS IN RATS

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In this study we directed our attention towards mineral status of bones in rats fed standard and semipurified diets with different protein levels.

Animals were divided in three experimental groups: LCP- diet with 13.5% crude protein in DM (5% of gluten, 10% of casein), HCP- diet with 21.2% CP in DM (8% of gluten, 10% of casein) and LSM- diet it was standard diet based on grain meals and meat-bone meal with 21%CP in DM.

After 28 days animals were killed by cervical dislocation and femur bones were collected, weighed and kept frozen until analyses.

Generally diets with 21% protein significantly increased weight and mineral content in femur bone, for Ca, Mg, Zn, and Cu. The content of mineral elements was related to greater relative (%) bone weight. Despite the high fibre content (5.6%), LSM diet increased both absolute and relative values of bone weight. This might be related to the higher mineral content and/or multiple protein sources in the LSM diet.

It can be concluded that level of dietary protein and/or its quality can modify absorption of mineral elements and influence bone mineral status. Further studies on this topic should be performed to explain mechanisms of protein action on mineral metabolism.

Item	Diets		
	LCP*	HCP**	LSM***
Characteristics of the rat diets			
Dry matter (g/kg)	959	957	881
Dry matter basis (g/kg)			
Crude protein	134.8	212.1	210.0
Crude fibre	9.3	7.3	56.0
Ash	33.8	33.6	71.0
Ether extract	99.1	77.3	34.0
N-free extractives	723	670	629
Ca (g/kg)	5.92	5.72	13.00
Mg (g/kg)	0.375	0.371	1.309
Fe (mg/kg)	48.2	56.5	281.6
Zn (mg/kg)	42.1	41.7	80.3
Cu (mg/kg)	7.69	6.61	22.21
Cr (mg/kg)	ND	ND	ND
Bone weight			
mg	265 ±21	416 ±19	389 ±26
‰ of body mass	1.14 ±0.08	1.76 ±0.07	1.80 ±0.12
Bone mineral content			
Ca (g/kg)	11.4±0.5	17.4±1.1	15.7±1.1
Mg (g/kg)	2.02±0.04	2.93±0.14	2.72±0.15
Fe (mg/kg)	68.4±3.4	79.6±4.3	76.0±7.3
Zn (mg/kg)	145.1^{a}±2.9	173.0^{b}±8.5	178.7^{b}±7.9
Cu (mg/kg)	3.32^{a}±0.04	5.23^{b}±0.29	4.53^{b}±0.25
Cr (mg/kg)	1.48^{a}±0.05	1.79^{a}±0.19	2.35^{b}±0.14

*- low protein diet; **- high protein diet; ***- standard natural diet
a-b- means in rows tagged with a different letter differ at P ≤ 0.05.

P466 W

MUSCULOSKELETAL EFFECTS OF HUMAN RECOMBINANT GROWTH HORMONE (HGH) IN HYPOPHYSECTOMIZED RATS

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This paper describes the musculoskeltal effects of hGH (kindly provided by BioSidus, Buenos Aires) on femoral diaphyses (pQCT, 3-point bending tests), tibial metaphyses (dynamic histomorphometry) and gastrocnemius muscles (weight) of intact (n=9) or hypophysectomized rats (Hx, performed at 15 days of age, 20), treated or not (4) since 15 days later with 30 or 150 mUI/d sc hGH (8, 8) for 45 days.

Hypophysectomy limited both muscle (-60%) and femoral development [cross-sectional area and moment of inertia (CSMI, -50 and -75%), volumetric cortical BMD (vCtBMD, -17%)], and reduced the tibial mineral aposition rate and osteoid surface ($p<0.01$). The hGH neutralized 20-50% of those changes, following a dose-response pattern. Hormone effects on muscles were proportionally larger than those exerted on bones. Strikingly, despite of reducing mineralization, hGH enhanced as much as 70% the intrinsic stiffness of cortical tissue (elastic modulus E, proportional to calcification; $p<0.001$). This stiffening effect, a dangerous inductor of tissue brittleness, reduced 50% the diaphyseal plastic resistance (BPR, cortical tissue resistance to microcrack progress) and fracture load (FL, both $p<0.001$). Our Bone Strength Index (BSI = vCtBMD x CSMI), which does not capture the (non-measurable) microstructural determinants of bone matrix stiffness, failed to predict FL. Treatment did not affect E, BPR, or FL.

This suggests that Hx 1. affected musculoskeletal development and bone mineralization, and 2. paradoxically stiffened bone material through some unknown mechanism, perhaps affecting its microstructural determinants, thus facilitating microcrak prsgress (lower BPR), and hence reducing FL. Larger hGH effects on muscles than bones suggest either a common anabolic effect with a greater delay on bones than muscles. However, some indirect effect on bones of the positive effects on muscles could also be proposed. The assayed hGH doses would have (partially) prevented only the first group of Hx-derived effects. It is not known why hGH failed to prevent the other Hx-induced alterations and their biomechanical consequences on bones. Further investigation is required to evaluate the effects of higher hGH doses on the same or a similar model, and to describe the eventual interaction of the lack of other hypophyseal hormones with those inedit results.

P467 F

THE EFFECT OF ALENDRONATE ON BONE MINERAL DENSITY OF THE DISTAL FEMUR AND PROXIMAL TIBIA AFTER TOTAL KNEE ARTHROPLASTY

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Background and Purpose: Mechanical failure due to decreased bone mineral density (BMD) may contribute to prosthetic migration and periprosthetic fractures after total knee arthroplasty (TKA). We hypothesized that alendronate may improve the BMD after TKA. The purposes of the study were to investigate the changes of BMD in the proximal tibia and distal femur after TKA, and to evaluate the effect of alendronate on BMD changes after TKA.

Materials and Methods: Ninety-six women with an average age of 70±8 years undergoing TKA were included in this study. The patients were randomly divided into two groups with 48 patients each in the study group and the control group. Patients in the study group received oral alendronate 10 mg per day for 6 months, whereas patients in the control group received no medication. The BMD was performed preoperatively, and at 6 and 12 months postoperatively.

Results: In the control group, the BMD values decreased 13.8% and 7.8% in the distal femur, and 6.5% and 3.6% in the proximal tibia in 6 and 12 months respectively; and the decreases of BMD were statistically significant. There was a faster rate of BMD decrease in the first 6 months after TKA. In the study group, the BMD values increased 10.0% and 1.9% in the distal femur, and 9.4% and 5.4% in the proximal tibia in 6 and 12 months respectively; and the increases of BMD were statistically significant. The difference of the overall BMD changes of the distal femur and proximal tibia between the study and control groups was statistically significant.

Conclusion: The BMD values of the distal femur and the proximal tibia decreased significantly after TKA. Administration of 6 months of oral alendronate therapy to women who had undergone total knee arthroplasty significantly improved the bone mineral density in the distal femur and proximal tibia versus a control group. This improvement may contribute to better component fixation and prosthesis survivorship, and may reduce the risk of periprosthetic fractures after TKA.

P468 S

ACUTE SIDE EFFECTS OF ZOLEDRONIC ACID TREATMENT IN CHILDREN

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Background: The bisphosphonate Pamidronate has been successfully used in children for the intravenous treatment of severe osteoporosis, in particular osteogenesis imperfecta. Zoledronic acid (ZA) is a highly potent bisphosphonate used in the treatment of hypercalcemia of malignancy in adults. While the acute side effects of Pamidronate have been well characterised, there is no such information available on the more potent ZA.

Objective: To review the frequency of hypocalcemia and other acute side effects of ZA in children.

Subjects and Methods: Twelve children at a mean age of 12.2 years (range 9-17) received IV ZA in doses between 0.02 to 0.05 mg/kg. Eleven children were suffering from osteoporotic conditions and one was treated for malignancy induced hypercalcemia. Clinical side effects and changes of the calcium levels were monitored in the first 48 hours post infusion. To assess whether the frequency of side effects are dose related, we compared patients between low (0.02 to 0.025 mg/kg), medium (0.04 mg/kg) and high dose (0.05 mg/kg) categories.

Results: Eight patients developed fever with flu-like symptoms, five had nausea and two vomited within the first 48 hours. In the 11 osteoporotic patients, calcium levels decreased from 2.35 mmol/L (SD 0.1) at baseline to 2.13 mmol/L (SD 0.17) at 24 hours ($p=0.003$) and 2.06 mmol/L (SD 0.2) at 48 hours ($p=0.012$). The mean drop in serum calcium was 0.33 mmol/L (SD 0.17) within the first 48 hours. The greatest decrease was seen in the hypercalcemic patient (3.15 mmol/L). Six of the 12 patients experienced calcium levels of less than 2.0 mmol/L, three of whom experienced associated clinical symptoms with calcium levels between 1.61 and 1.83 mmol/L. The frequency of side effects and hypocalcemia were not significantly different between dosage groups, but hypocalcemia was more common if oral intake was compromised.

Conclusion: Acute side effects were found in nearly all patients treated with ZA with hypocalcemia being a frequent complication. Dose ranging studies are required to determine the benefits and risks of ZA in children.

P469 W

EFFECTIVENESS OF ETIDRONATE AND ALENDRONATE IN THE TREATMENT OF OSTEOPOROSIS IN MEN REGISTERED IN THE CANADIAN DATABASE OF OSTEOPOROSIS AND OSTEOPENIA (CANDOO)

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Although osteoporosis is regarded as a disease of women, this condition is associated with disability and death in men. Nonetheless, relatively few studies have examined osteoporosis in males and the optimal treatment for this disease in men is uncertain. Thus, the purpose of our prospective one year cohort study was to determine the effectiveness of etidronate and alendronate to increase bone mineral density in men who were registered in CANDOO. The database was searched for men receiving daily etidronate (ETD), alendronate (ALD) or calcium and vitamin D (CON) therapy. Patients were excluded if they did not start osteoporosis therapy on or after their first clinical visit and did not have at least one follow-up bone density visit after initiating therapy. Furthermore, patients were excluded if they had been treated with calcitonin, fluoride or another bisphosphonate at any point during the two years preceding the study. A total of 269 men meet the inclusion criteria and were enrolled in the study. Of these men, 101, 42 and 125 were taking daily ETD, ALD, and CON therapy. Our primary outcome measures were differences between treatment groups in the percent change in lumbar spine and femoral neck bone mineral density from baseline to one year follow-up. Multivariable regression analysis was conducted. Regression coefficient parameter estimates as well as 95% confidence intervals were calculated while controlling for possible confounding effects of the other variables. The coefficient estimates represent percent differences between groups in bone mineral density. Multiple imputation was used to impute missing data. Results are shown in table. Differences between the CON and the bisphosphonate groups were observed at the lumbar spine but not at the femoral neck. In conclusion, ETD and ALD therapy appear to be effective in the treatment of osteoporosis in men, particularly at the spine.

Location	ETD vs. CON*	ALD vs. CON*	ETD vs ALD*
Lumbar spine	2.26 (0.67, 3.85)	5.23 (2.98, 7.47)	-1.94 (-3.91, 0.02)
Femoral neck	0.068 (-1.17, 2.53)	2.19 (-0.52, 4.92)	-1.21 (-3.82, 1.39)
* reference group			

P470 F

INCADRONATE DISODIUM DELAYS AND INHIBITS PERIARTICULAR BONE ATROPHY AND JOINT DESTRUCTION IN RAT ADJUVANT ARTHRITIS

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Purpose: Rheumatoid Arthritis (RA) is accompanied with periarticular bone loss. Bisphosphonates have been used as agents for osteoporosis treatment. In this study we investigated whether bisphosphonate can prevent or delay joint destruction and periarticular bone loss in RA by using rat adjuvant-arthritis (AIA) model.

Materials and Methods: Arthritis was induced by injecting 0.1millig of heat-killed mycobacterium butyricum into the base of tail of female Lewis rats (7 weeks age). Bisphosphonate (incadronate disosium : YM-175) was administered subcutaneously 3 times per week at a dose of 10,100microg/kilog/day. Body weight and hindpaw volume were checked every week. 2,4,6,and 10 weeks later, rats were sacrificed and both hindpaws were excised and used as specimens for evaluation of joint destruction, radiologically and histologically. Radiography of ankle joint were taken by using soft X-ray system. Decalcified saggital sections were cut from ankle joint, stained with toluidine blue and tartarate-resistant acid phosphatase(TRAP). Destruction of articular cartilage and subchondral bone and osteoclasts infiltration were evaluated by using histomorphometry.

Results: In arthritic rats, body weight did not increase well and hindpaws swelling appeared from 1 week. At 2 weeks, no changes were seen both radiologically and histologically. At 4 weeks, radiographically, widespread and severe destruction was observed in tibio-tarsal joint and tarsal bone in arthritic control group, while bone destruction was milder in YM-175 treated groups. Histologically, severe destruction of articular cartilage, numerous osteoclasts infiltration and marked osteopenia of subchondral bone were found in arthritic control group, while these changes were reduced dose-dependently by YM-175 treatment. At 6 and 10 weeks, further progressed destruction was observed both radiologically and histologically and it was not reduced by YM-175 effectively.

Discussion: These results suggest that YM-175 could not prevent occurrence of arthritis itself but delayed progression of arthritis, thereby inhibited joint destruction (articular cartilage destruction and periarticular bone atrophy) at early time in AIA.

P471 S

EFFECT OF PAMIDRONATE ON BONE REMODELING IN ADULT PATIENTS WITH BETA-THALASSEMIA MAJOR AND OSTEOPOROSIS

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Patients with thalassemia major are susceptible to osteoporosis due to several factors leading to an unbalanced bone turnover with an increased resorptive phase. Pamidronate has been proven to be an effective and safe treatment for osteoporosis. In this randomized trial, we compared the effect of two different doses of pamidronate on bone remodeling in patients with beta-thalassemia and osteopenia/osteoporosis. Twenty-six patients (6M/20F, median age: 35.5years) were studied. Sixteen patients were randomized to receive 30mg of pamidronate (group I), while 10 patients received 60mg of pamidronate (group II). Pamidronate was given intravenously every month. Bone mineral density (BMD) of lumbar spine, femoral neck and forearm was determined using DEXA-scan before and 12 months after treatment. Biochemical parameters of bone resorption [Í-telopeptide of collagen type-I (NTX), tartrate-resistant acid phosphatase type-5b (TRACP-5b)], bone formation [bone alkaline phosphatase (bALP), osteocalcin (OC)], and osteoprotegerin (OPG) were evaluated at baseline and then monthly for 12 months. The above biochemical parameters were also evaluated in 45 healthy individuals. This is the first time that pamidronate has been given in beta-thalassemia, and TRACP-5b and OPG have been measured for monitoring bone disease in such a cohort of patients. Patients of both groups had elevated mean baseline NTX, TRACP-5b, bALP and OC values and decreased OPG levels compared with controls. In both groups, the administration of pamidronate produced a dramatic decrease of TRACP-5b, from the 2nd month of treatment ($p<.02$, and $p<.04$, for group I and II, respectively). A significant reduction of NTX, OPG, and OC was also noticed after the 4th month of treatment. These changes persisted throughout the 12-month follow-up period. There was no statistical difference between the two dosages of pamidronate in terms of percentage change of biochemical markers from baseline. Pamidronate, irrespective of the dosage, dramatically improved the BMD in lumbar spine ($p<.003$), but not in other sites. A significant correlation was observed between TRACP-5b, NTX levels and z-score of the lumbar spine, both at baseline and after 12 months of treatment. This study suggests that 30mg of pamidronate is an effective treatment for thalassemia osteoporosis, reducing bone resorption and increasing BMD in these patients.

P472 W

OSTEOPOROSIS: TREATMENT WITH ETIDRONATE AND ALENDRONATE-COMPARATIVE STUDY ON BONE MINERAL DENSITY AND FRACTURE

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Bisphosphonates have an established role in the treatment of osteoporosis and fracture prevention. Fracture rate is inversely related to the duration of the treatment. The cost implications have not been taken into account. The first generation of bisphosphonates are relatively less expensive.

Material and Methods: Criteria for entry to the study comprised normal haematology and biochemistry and Lumbar spine or hip Bone Mineral Density more than 2.5 SD below the sex matched reference value at the age of peak density i.e. T score <-2.5. BMD measurements were made using HOLOGIC 4500 DEXA. The patients were randomly allocated to the two regime of bisphosphonates. The Didronel was administered cyclically at a dose of 400mgms. daily for 14 days one hour before breakfast on an empty stomach taken with water followed by 500 mgms elemental calcium for 76 days. Fosamax 10 mgms was taken daily 1 hour before breakfast with glass of water making sure patients remained upright,supplemented with 500 mgms of calcium in the evening. A questionnaire at base line and 12 months relating to the incidence of adverse reactions and fracture was recorded.

Results: There were 59 females and 9 males in the Fosamax group and 65 females and 7 males in the Didronel group. The mean +SD age at base line was 65.17±7.87 and 67.96±9.94 the groups were well matched for gender and time lapsed between the scans.

There were no new fractures in either group. However there were 5 patients in the Fosamax group who had gastric discomfort.The increase in BMD in the 2 groups was statistically significant in favour of Alendronate.

Conclusion: This first head to head randomised study showed significant difference in the increase in the BMD in favour of the former drug. However there was no difference in the fracture rates. Long-term studies to elucidate if the results obtained in this study continue to be reflected in view of Didronel being cost effective by half compared to Fosamax.

P473 F

IDIOPATHIC OSTEOPOROSIS IN MALE PATIENTS: ASSESSMENT AND TREATMENT

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It is now well established that osteoporosis in men is often caused by an underlying condition, such as, hypogonadism, medications, or lifestyle factors. However, in those patients with no obvious cause ('idiopathic'), the treatment choice remains to be clearly established. The aim of this study was to assess the effectiveness of alendronate therapy in male patients with idiopathic osteoporosis.

Seventeen men with idiopathic osteoporosis (T-score at lumbar spine or hip < -2.5 SD, serum concentrations of free testosterone, Ca, P, Mg, and alkaline phosphatase (ALP) within normal limits) were enrolled in a prospective study between March 1999 and September 2002. Patients were 35 to 66 years old (mean: 56 years). They were treated with alendronate sodium (10 mg/d, last year 70 mg/w), in combination with 500-mg elemental calcium. The BMD of the lumbar spine (L1 -L4) and left hip was measured in all patients using DXA densitometry (Hologic QDR 2000+) at the start of the treatment and 24 months after initiation of the treatment. The serum levels of Hgb, Htc, WBC, Plt, testosterone, Ca, P, Mg, Na, K, Cl, ALP, AST, ALT, BUN, and creatinine were measured every 12 months.

Average baseline BMD was 0.762 g/cm2 (n=17, range 0.699 to 0.823 g/cm2) at the lumbar spine and 0.737 g/cm2 (n=17, range 0.697 to 0.793 g/cm2) at the hip. After treating patients for 2 years, the average BMD was 0.815 g/cm2 (n=17, range 0.729 to 0.899 g/cm2) at the lumbar spine and 0.790 g/cm2 (n=17, range 0.751 to 0.844 g/cm2) at the hip. BMD thus increased on average by 6 % at the lumbar spine and 7.5 % at the hip. Serum levels remained within normal limits throughout the treatment, with no adverse events observed during the study.

Results of our on-going study suggest that alendronate sodium can provide clinically relevant benefits in male patients with idiopathic osteoporosis. Alendronate sodium was well tolerated in all patients.

P474 S

THE EFFICACY OF ALENDRONATE IN JAPANESE POSTMENOPAUSAL OSTEOPOROTIC WOMEN

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Hormone replacement therapy (HRT) is essential for treating postmenopausal osteoporosis. But there are some populations who had not be received HRT because of complications (i.e. breast cancer, thrombosis etc.). In this study, we assessed the effects of HRT or Alendronate on bone mass and turnover in postmenopausal osteoporosis in Japanese population. Seventy four postmenopausal osteoporotic Japanese women (mean age, 64.7±8.6 years) were enrolled into this study. Thirty four women (mean age, 63.6±7.3 years) were treated with HRT (conjugated estrogens 0.625mg/day, medroxyprogesterone acetate 2.5mg/day; HRT group) and 40 women (mean age, 64.7±8.1 years) were treated with Alendronate (5mg/day ; A group). Lumbar spine bone mineral density (BMD) was determined at the start of the study (0 month) and every 6 months thereafter and biochemical indices, serum alkaline phosphatase (ALP), intact osteocalsin (IOC), urinary deoxypyridinoline (DPD), crosslinked N-telopeptide of type I collagen (NTx) and creatinine were measured at 0,1,3 and 6 months. BMD measurement was performed using dual energy X-ray absorptiometry (DXA, QDR2000, Hologic Comp.). There was no differences in age, years since menopause or BMI. Baseline lumbar BMD and biochemical indices did not show any substantial differences between two group. Four (10.0%) have dropped out because of adverse effects in A group, the most common was gastrointestinal events.There were significant differences in percent changes of BMD (HRT group; 2.6±2.3%, A group; 5.2±2.3%, A vs. HRT, $p<0.05$ at 6 months.) NTx and DPD significantly decreased at 3 months in each group from baseline ($p<0.03$). The magnitude of decrease was grater A group than HRT group (NTx: HRT;-34.1±11.5%,A;-46.4±9.5%). Furthermore, in A group, NTx and DPD significantly decreased at 1 month ($p<0.03$). In conclusion, 1) The bone spearing effect of Alendronate for treatment of postmenopausal osteoporosis appeared earlier and stronger than that of HRT.2) Biochemical markers of bone resorption were clinically useful in the prediction of future BMD in patients treated with HRT or Alendronate.

P475 W

EFFICACY OF ALENDRONATE 70MG ONCE-WEEKLY IN POSTMENOPAUSAL CHINESE WOMEN WITH OSTEOPORORIS

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Introduction: Oral bisphosphonates have been proven to be effective and well-tolerated in treatment of postmenopausal osteoporosis. Once-weekly dosing regimen improves drug compliance and reduces oesophageal irritation.

Method: We conducted an open-labelled 12-month prospective study on two different alendronate dosing regimens in healthy postmenopausal women with osteoporosis as defined by T-score lumbar spine bone mineral density (BMD) less than -2.5. 86 subjects were assigned to one of the three treatment groups: placebo (PLB, n=29), alendronate 10mg once-daily (ALN-10, n=28) and alendronate 70mg once-weekly (ALN-70, n=29). All subjects also received elemental calcium 500mg daily. BMD at baseline, 3, 6 & 12 months after treatment were measured. Blood samples for plasma total alkaline phosphatase (ALP) were collected at baseline, 3, 6, 9 & 12 months after treatment.

Results: Subjects in ALN-70 group were younger, had earlier menarche, shorter years since menopause & higher daily calcium intake than those in the other 2 groups. In both alendronate treatment groups, there were comparable percentage increases in BMD over the baseline (L1-4: 5.7% in ALN-10 and 6.0% in ALN-70 vs. 1.4% in PLB group; total hip: 3.4% in ALN-10 and 3.5% in ALN-70 vs. 0% in PLB group). Both alendronate regimes produced 25% and 30% suppression of ALP at 3 & 12 months, respectively. None of the subjects in ALN-70 group reported significant adverse effect but 2 gastric ulcers were documented in ALN-10 group.

Conclusion: Alendronate 70mg once-weekly dosing regimen is a more convenient, yet therapeutically equivalent alternative to daily dosing in Chinese. It may enhance compliance and long-term adherence to therapy.

P476 F

TEN-YEAR EFFICACY AND SAFETY OF ALENDRONATE IN THE TREATMENT OF OSTEOPOROSIS IN POSTMENOPAUSAL WOMEN

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Alendronate sodium* (ALN) inhibited bone resorption, reduced the risk of vertebral fractures and progressively increased BMD over 3 yrs in a study of 994 osteoporotic women. We now report the results for 247 women who entered a final 3 yr extension (Yrs 8-10). During Yrs 6-10, patients in the ALN 5 and 10 mg groups remained on the same doses. Patients in the ALN 20/5/placebo (A-PBO) group (20 mg for 2 yrs, 5 mg for 3 yrs) received placebo in Yrs 6-10. Significant increases in spine BMD of 2.25 % with ALN 10 mg and 1.60 % with 5 mg were found during Yrs 8-10, and prior increases in hip and total body BMD were maintained. Forearm BMD was stable with 10 mg but decreased slightly with 5 mg. Women in the A-PBO group who had not been treated with ALN since the end of Yr 5 showed no significant change in spine and total body BMD, but small decreases in hip and forearm BMD occurred during Yrs 8-10. Cumulative 10 yr spine BMD increases were 13.7 % with ALN 10 mg and 9.8 % with 5 mg. The safety and tolerability profiles of ALN 5 and 10 mg were similar to placebo during both Yrs 8-10 and Yrs 6-10. The 3 yr incidences of non-vertebral fractures during Yrs 8-10 were 8.1, 11.5, and 12.0 % in the ALN 10 mg, 5 mg and A-PBO groups. The 3 yr incidences in the original cohort during Yrs 1-3 were 8.5 % (pooled ALN) and 10.7 % (placebo). Neither stress fractures nor fracture malunion were reported. We conclude that ALN treatment is effective for 10 yrs and is generally well tolerated. Spinal BMD continues to increase over 10 yrs and other skeletal benefits are maintained. Non-vertebral fracture data indicate similar risk over time, and suggest that fracture risk reduction is maintained during continued treatment. Discontinuation of ALN after 5 years leads to bone loss at non-spine sites, and continued treatment with ALN through 10 years yields sustained skeletal benefits.

* Manufactured by Merck & Co., Inc., Whitehouse Station, NJ

P477 S

RESPONSE TO TREATMENT WITH ALENDRONATE OR CALCITRIOL IN PATIENTS WITH POSTMENOPAUSAL OSTEOPOROSIS IN MALAYSIA

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Aims: The aim of this study was to assess the response of Malaysian patients taking either alendronate (group A) or calcitriol (group C) as treatment for their postmenopausal osteoporosis.

Methods: The data was prospectively collected from the Osteoporosis Clinic at a teaching hospital. Patients with secondary causes of osteoporosis or on other treatments for osteoporosis were excluded. Patients were allocated to the treatments based on the attending doctor's judgment following bone mineral density (BMD) measurement. After 1 year on treatment, BMD was repeated. BMD was measured using a LUNAR DPX machine. The precision error of the machine is 2%.

Results: 94 patients of whom 65 were on alendronate and 29 on calcitriol were studied. There was no difference in the mean age of the patients in both groups: 63.67 ± 8.33 (A) and 65.79 ± 8.60 (C) years respectively. The years since menopause was similar in both groups: 14.59 ± 8.91 (A) and 17.07 ± 9.3 (C) years respectively. 16 patients (24.6%) in group A had previous osteoporotic fractures compared to none in group C. The mean T scores in group A was significantly lower (T -3.09 LS, -2.45 FN) compared to group C (T -2.38 LS, -2.01 FN). After 1 year, the mean change in lumbar spine (LS) BMD in group A was 5.56% ($p < 0.0001$ compared to baseline). There was no significant change in femoral neck (FN) BMD in group A over 1 year ($p = 0.31$). There were no significant changes in both LS and FN BMD ($p = 0.19$, $p = 0.97$ respectively) in group C over 1 year of treatment. There were no new fractures in either group while on treatment.

Conclusions: In this group of Asian patients, over 1 year, there was a significant gain in LS BMD (5.56%) in patients taking alendronate. There was no significant change in LS BMD in those on calcitriol. There was no significant change in FN BMD over 1 year in either group.

P478 W

EFFICACY OF FOSAMAX VS EVISTA COMPARISON TRIAL (EFFECT): RESULTS OF A RANDOMIZED, MULTICENTER STUDY

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Objective: To compare the efficacy of once- weekly (OW) alendronate (ALN) and daily raloxifene (RLX) in the treatment of postmenopausal osteoporosis.

Methods: We enrolled postmenopausal women with low bone mineral density (BMD) from 52 centers in the U.S. in a double-blind, active-controlled study for 12 months. Patients were randomized 1:1 to OW ALN 70 mg with daily RLX placebo or RLX 60 mg daily with OW ALN placebo. The primary endpoint is the mean percent change from baseline in PA lumbar spine BMD. Additional efficacy endpoints include change in hip BMD (total proximal femur and femoral trochanter), and the percent change from baseline in biochemical markers of bone turnover (urinary N-telopeptide (UTNx) and bone-specific alkaline phosphatase (BSAP)). We will also examine the safety and tolerability of both treatments, including the percent of patients reporting any upper gastrointestinal adverse events (AEs) or vasomotor AEs.

Results: This study enrolled 455 postmenopausal women, (92% Caucasian). The mean age was 64 years (range: 37 to 89). Their mean age at menopause was 47 years. The mean baseline PA lumbar spine T-score was minus 2.50. The mean baseline total hip T-score was minus 1.76. A total of 243 (53.4 %) patients reported a history of fracture of the hip, ribs, spine, wrist or other at baseline. This study is on-going with completion scheduled for 15/JAN/03. Efficacy and safety data will be available for presentation at this meeting.

Conclusion: This will be the first clinical study to provide a direct comparison of the efficacy, safety and tolerability of once-weekly alendronate and daily raloxifene in the treatment of postmenopausal osteoporosis.

P479 F

ONCE WEEKLY ALENDRONATE PRODUCES A GREATER INCREASE IN BONE MINERAL DENSITY THAN DAILY RISEDRONATE

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We report the 12-month Bone Mineral Density results of the first head-to-head trial designed to compare the efficacy of alendronate and risedronate for the treatment of osteoporosis. The 3-month, randomized, double-blind, multicenter international study with double-blind extensions for an additional 9 months (12 months in total), enrolled 549 postmenopausal women. Patients were 60-90 years old (mean, 69), with osteoporosis defined by low BMD T-score (either lumbar spine or total hip/femoral neck less than/equal -2.5, or less than/equal -2.0 at both sites). Patients maintained a calcium intake of at least 1000 mg daily through food and/or calcium supplements. Patients were randomized into three treatment groups: alendronate 70mg* once weekly using standard am dosing; risedronate 5mg daily dosed 2 hours after a meal and at least 2 hr before the next; or matching placebo for each. Results are based on a modified intention-to-treat approach of lumbar spine and hip BMD at month 12.

In this study, alendronate 70 mg once weekly produced significantly greater BMD increases over 12 months than did risedronate 5 mg daily, at the spine and all hip sites. These differences may be due to the superior anti-resorptive efficacy of alendronate 70 mg once weekly, reduced bioavailability of risedronate resulting from post-meal dosing, or both.

*Manufactured by Merck & Co., Inc., Whitehouse Station, NJ

Table. Percent Change From Baseline at Month 12 at Lumbar Spine, Femoral Neck, Trochanter, and Total Hip

BMD Site					
	LS Mean				
Treatment	N	Lumbar Spine	Femoral Neck	Trochanter	Total Hip
Placebo	99-101	0.1	-0.1 1.5 † 2.2 †	-0.7 0.8 * 3.2 †	-0.2 0.9 † 2.7 †
RIS 5 mg Daily	206	2.8 †			
ALN 70 mg OW	188-190	4.8 †			
Between-Treatment Comparison					
	LS mean Difference				
Treatment		Lumbar Spine	Femoral Neck	Trochanter	Total Hip
ALN 70 mg - PBO		4.7 †	2.4 †	3.9 †	2.8 †
RIS 5 mg daily – PBO		2.7 †	1.6 †	1.5 *	1.1¶
ALN 70 mg OW – RIS 5 mg Daily		2.0 †	0.8 *	2.4 †	1.7 †

† $p < 0.001$; ¶$p<0.01$; * $p \leq 0.05$; between-groups and within-group test of LS (least-squares) mean percent change.
ALN: Alendronate; PBO: Placebo; RIS: Risedronate; OW: Once-Weekly.

P480 S

NONVERTEBRAL FRACTURE RISK REDUCED AS SOON AS 6 MONTHS WITH RISEDRONATE THERAPY IN POSTMENOPAUSAL OSTEOPOROSIS

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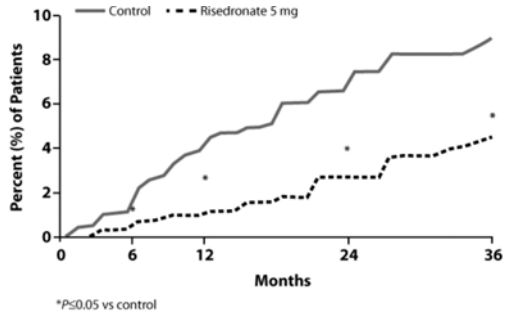
Osteoporosis-related nonvertebral fractures occur throughout the skeleton and account for a large proportion of all osteoporotic fractures. These fractures, especially those of the hip, are associated with significant morbidity and mortality. Therefore, rapid prevention of nonvertebral fractures is an important goal of osteoporosis therapy. We analyzed clinical trial data to determine the onset of effect of risedronate on nonvertebral osteoporotic fractures.

This analysis used data from 4 prospective, double-blind, placebo-controlled studies ranging in length from 12 months to 3 years. These studies enrolled postmenopausal women on the basis of low spinal bone mineral density (BMD) and/or the presence of vertebral fracture. Data for 1172 patients with lumbar spine T-scores <= minus 2.5 with or without a prevalent vertebral fracture were analyzed (42% had no fracture, 25% had 1 fracture, and 33% had >1 fracture). All patients received calcium 1 g/d and either placebo or risedronate 5 mg daily. The effect of risedronate on nonvertebral fracture risk was based on a combined endpoint of fractures of the hip, wrist, pelvis, clavicle, humerus, and leg. Radiographically confirmed nonvertebral fractures were collected at 3-month intervals as adverse events. Fracture incidence was determined using Kaplan-Meier estimates and treatment effect was calculated using the Cox proportional hazards regression model.

Risedronate significantly reduced the incidence of nonvertebral fractures in women with osteoporosis as early as 6 months (P=0.048), with a 74% reduction at 1 year (4.5% for control vs 1.2% for risedronate; P=0.001). This significant effect on nonvertebral fracture incidence was maintained for 3 years (59%).

In women with postmenopausal osteoporosis, risedronate 5 mg/d significantly reduces nonvertebral fracture risk as early as 6 months. These data, combined with reports of vertebral fracture risk reduction as early as 6 months, provide support for the rapid onset of effect of risedronate at both nonvertebral and vertebral sites.



P481 W

AN ANALYSIS OF POOLED DATA FROM 9 CLINICAL TRIALS TO EVALUATE THE UPPER GASTROINTESTINAL TOLERABILITY OF RISEDRONATE

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Some bisphosphonates have been associated with upper gastrointestinal (GI) adverse events. The objective of this study was to assess the upper GI tolerability of risedronate using pooled clinical trial data. The safety profile of risedronate 2.5 mg/d has been shown to be similar to 5 mg/d, so these analyses were based on 5 mg/d.

GI adverse event data from 9 multicenter, randomized, double-blind, placebo-controlled phase III studies of risedronate lasting up to 3 years were pooled and analyzed. Patients with a history of ongoing GI disease (peptic ulcers, esophagitis, heartburn, etc.), or taking H2-blockers, proton pump inhibitors (PPIs), antacids, nonsteroidal anti-inflammatory drugs (NSAIDs), or aspirin were not specifically excluded. Endoscopy was performed when clinically indicated as assessed by the investigator in patients with GI complaints and was evaluated by site (esophagus, stomach, duodenum).

10,068 patients were enrolled in the placebo (n=5048) and risedronate 5 mg/d (n=5020) groups. The percentages of patients taking NSAIDs and/or aspirin (56%) or H2-blockers and/or PPIs (12%) at baseline were the same in both groups, as were the percentages of patients with a history of (61%) or active (39%) GI disease. Upper GI adverse events were reported by 29.6% of patients in the placebo group and 29.8% of patients in the risedronate group. Similar proportions of patients in the placebo and risedronate groups withdrew because of adverse upper GI events (3.0% vs 3.3%). The incidence of upper GI adverse events was similar for placebo and risedronate recipients taking NSAIDs and/or aspirin and in those taking H2-blockers and/or PPIs. Risedronate treatment did not worsen the condition in patients with active upper GI disease, nor was there an increased incidence of upper GI adverse events in patients with a history of upper GI disease. Endoscopy (n=349) showed no statistically significant differences between the placebo and risedronate groups in percentages of patients with inflammation, erosion, or ulcer of the esophagus, stomach, or duodenum.

Results of this large pooled analysis of clinical trials indicate risedronate 5 mg/d is not associated with an increased incidence of upper GI adverse events relative to placebo, even among patients considered to be at high risk for these events.

P482 F

RISEDRONATE 5 MG/D RAPIDLY REDUCES CLINICAL VERTEBRAL FRACTURE RISK AS EARLY AS 6 MONTHS IN POSTMENOPAUSAL OSTEOPOROSIS

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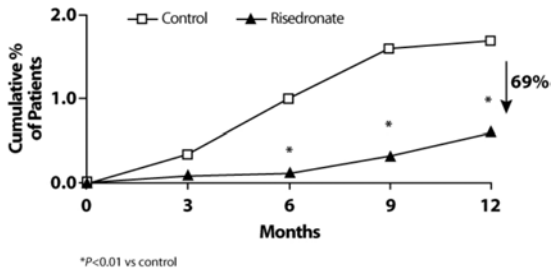
Clinical trials have shown that treatment with risedronate rapidly and significantly reduces morphometric vertebral risk by up to 65% in postmenopausal women with osteoporosis. In this analysis, data from 2 large trials in postmenopausal osteoporosis, the Vertebral Efficacy with Risedronate Therapy-North American and -Multinational trials (VERT-NA and VERT-MN, respectively), were assessed to determine the efficacy of risedronate in reducing clinical vertebral fracture risk.

VERT-NA enrolled 2458 women based on the presence of 2 or more prevalent vertrebral fractures or 1 prevalent vertebral fracture and lumbar spine bone mineral density (BMD) T-score <= -2 at baseline. VERT-MN enrolled 1226 women based on the presence of 2 or more prevalent vertebral fractures at baseline. All patients received either placebo or risedronate (2.5 or 5 mg) daily plus calcium 1000 mg/d and 500 IU/d of vitamin D if baseline levels were low. The 1-year morphometric analyses from VERT-NA and VERT-MN were prospectively conducted, using both asymptomatic and symptomatic (clinical) fractures. Clinical fractures were reported as adverse events, diagnosed by a physician and confirmed radiographically. The incidence of clinical vertebral fractures was calculated using Kaplan-Meier estimates of the survival function.

About one quarter (26%) of the morphometric vertebral fractures were also reported as clinical vertebral fractures, which is consistent with the literature (Lindsay et al., *JAMA*. 2001;285:320). As shown in the figure, a statistically significant 69% reduction in clinical vertebral fracture risk was observed after 1 year in the risedronate 5 mg/d group in the combined analysis (1.7% vs 0.6%; *P*=0.009). Risedronate 5 mg/d significantly reduced the risk of clinical vertebral fracture as early as 6 months compared with placebo (1.0% vs. 0.1%; *P*<0.01).

The results of this analysis showed that risedronate 5 mg/d statistically significantly reduces clinical vertebral fracture risk from as early as 6 months in postmenopausal osteoporosis.



P483 S

EFFECTS OF ALENDRONATE AND PROSTAGLANDIN E RECEPTOR (EP4) AGONIST ON OVARIECTOMIZED MONKEY MANDIBLE

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In order to examine the usefulness of Alendronate and prostaglandin E receptor (EP4) agonist in the prevention of mandibular osteoporosis, we assessed the effects of these two agents on ovariectomized monkey mandibles. Four rhesus monkeys were used as subjects for the study, one control (CONT) and three ovariectomized and injected either with a vehicle (OVX), or Alendronate (0.25mg / kg / 2 weeks) (OVX + ALN), or EP4 agonist ONO-4819 (3 microgram / kg / day) (OVX + EP4A). Thirty-two weeks after ovariectomy, the subjects were sacrificed and their mandibles excised and observed using micro CT and confocal laser scanning microscopy. Paraffin sections of decalcified specimens were stained with hematoxylin-eosin. Tartrate-resistant acid phosphatase was detected from the sections. (OVX): Estrogen deficiency caused trabecular bone loss and osteoporotic changes in the mandibular cortical bone. The interradicular septa showed porotic cribriform internal plates around the roots. Osteoclasts were also found on the alveolar bone surface facing the medial roots, resulting from the natural medial movement of the teeth. (OVX + ALN): The cortical bone seemed compact and showed no osteoporotic changes. The interradicular septa consisted of internal cortical plates; however, the periodontal ligament space became wider. Many osteoclasts appeared on the irregular surface of alveolar bone facing the medial roots, with some of them showing apoptotic changes. (OVX+EP4A): The cortical bone appeared as compact as those of OVX+ALN. In the interradicular septa, compact internal cortical plates were found around the roots and thick trabecular bone was observed between the plates. Similarly to CONT, a small number of osteoclasts were located on the alveolar bone surface facing the medial roots. Alendronate generally inhibits osteoclastic function and in this study also prevented osteoporotic estrogen-deficiency-induced changes in the jaw bones. However, by inhibiting natural resorptive processes, the drug also affected the alveolar bone metabolism caused by the movement of the teeth. On the other hand, EP4 agonist also seems to similarly prevent osteoporotic changes in the jaw bones, without inhibiting naturally-occurring resorptive processes, but rather by changing the bone-turnover from a bone-resorption-predominant condition to a bone-formation-predominant one.

P484 W

ANTIFRACTURE EFFICACY OF ORAL IBANDRONATE GIVEN WITH A BETWEEN-DOSE INTERVAL OF >2 MONTHS IN POSTMENOPAUSAL OSTEOPOROSIS

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Introduction: Simplified, less frequent oral bisphosphonate dosing schedules are predicted to promote adherence and optimise patient management in postmenopausal osteoporosis (PMO). Ibandronate is a highly potent nitrogen-containing bisphosphonate that can be administered in unique and simple oral and intravenous injection regimens with extended between-dose intervals. Here, we present the results of a phase III fracture study (BONE: oral iBandronate Osteoporosis vertebral fracture trial in North America and Europe), which investigated the fracture efficacy and safety of oral ibandronate administered either daily or intermittently with an extended between-dose interval of >2 months in PMO.

Methods: 2,946 women (aged 55-80 years, time since menopause >=5 years) with a bone mineral density (BMD) T-score below -2 in >=1 vertebra of the lumbar spine (L1-L4) and 1-4 prevalent vertebral fractures (VF) participated in this multicentre, double-blind, placebo-controlled study. At enrolment, participants were randomised to

placebo (n=982), oral daily (2.5mg; n=982) or intermittent (20mg every second day for 24 days every 3 months; n=982) ibandronate for 3 years. All patients received daily calcium (500mg) and vitamin D (400IU). The primary endpoint was the rate of patients with new incident VF after 3 years (intent-to-treat analysis).

Results: Oral daily and intermittent ibandronate significantly reduced the risk of radiologically confirmed VF by 62% and 50%, respectively, versus placebo. A significant reduction in clinical VF was also observed in both treatment groups. Ibandronate therapy produced significant increases in BMD and sustained decreases in biochemical markers of bone turnover. A reduction in non-VF (69%, $p<0.012$) was observed with the daily regimen in patients with low femoral neck BMD (T-score <=3). Oral ibandronate was well tolerated with no differences in upper gastrointestinal tolerability between ibandronate and placebo.

Conclusions: The results of this study demonstrate that oral daily and intermittent ibandronate are highly efficacious in reducing the incidence of fractures in patients with PMO. Moreover, ibandronate is the first bisphosphonate to prospectively demonstrate proven and robust anti-fracture efficacy when administered with a between-dose interval of >2 months. Ongoing studies are evaluating convenient oral ibandronate regimens, including once monthly, in order to further optimise patient management in PMO.

P485 F

EFFECT OF TEPRENONE (GGA) WITH ETIDRONATE(EHDP) ON LUMBAR BONE MINERAL DENSITY IN POSTMENOPAUSAL WOMEN WITH LOW BONE MINERAL DENSITY

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It has been reported that the inhibitory effect of Teprenone (Geranylgeranyl acetone: GGA) on bone resorption. GGA, an antiulcer drug, has almost the same chemical structure as that side chain of Menatetreone. Therefore, we investigated the effect of GGA with Etidronate (EHDP) on lumbar bone mineral density in postmenopausal women with osteoporosis and osteopenia. The baseline characteristics were not significant differences between the EHDP with GGA group (n=50) and the only EHDP group (n=144). The percent changes on lumbar bone mineral density from baseline in the EHDP with GGA were higher than the EHDP only group at 12 months to 30 months, but there were no significant differences between two groups. The percent changes on lumbar bone mineral density from baseline in the EHDP with GGA at 36 months ($p<0.05$), 42 months and 48 months ($p<0.01$) were significantly higher than the EHDP only group. Thus, GGA may be effective in not only preventing the gastric mucosa but also in the lumbar bone mineral density in postmenopausal women with EHDP.

P486 S

COMBINED USE OF RISEDRONIC ACID AND RALOXIFENE IN POSTMENOPAUSAL OSTEOPOROSIS

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Statement of purpose: determining if the combined risedronic acid and raloxifene HCL influences on bone mass loss in postmenopausal women.

Statement of method: we studied for 12 months 23 women who were 47 to 62 years old at base line, were within 3 and 10 years of menopause, and had a bone mineral density at the lumbar spine between 110 mg/cc and 50 mg/cc measured by the QBMAP system with a spiral CT Picker PQ-S densitometer at L2, L3, L4 and L5. Of all the women, 10 were assigned to combined use of risedronic acid 5 mg and raloxifene CLH 60 mg and 13 were treated with raloxifene CLH 60 mg. The SPSS programme was used for statiscal analysis.

Summary of results: The characteristics of the women recruited for both groups were similar. Mean mineral bone density at the lumbar spine was between 2.5 and 3 DS below the mean value for 30 years old normal premenopausal women. After a treatment statistically significant difference was found among the groups with and without risedronic acid as for the bone mineral density at the lumbar spine.

Conclusions: it is necessary to carry out a wider study but it seems that the combined risedronic acid and raloxifene HCL contribute advantages versus only raloxifene HCL to decrease the postmenopausal osteoporosis.

P487 W

SHORT TERM COMPLIANCE WITH ALENDRONATE 70 MG IN PATIENTS WITH OSTEOPOROSIS: THE ECMO 2 STUDY

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Fracture prevention efficacy of most anti-osteoporotic drugs is probably decreased due to low rates of adherence. There is also a lack of measures directed to overcome low compliance with established treatments. The ECMO 2 study was a program aimed to monitor adherence to weekly administration of alendronate 70 mg (Marvil, Elisium) and to see if decreased frequency of administration of the medication could improve compliance. Patients were included if they had primary osteoporosis diagnosed either by DEXA-WHO criteria (85%) or the existance of low energy spine fractures (15%). Three hundred and twenty five physicians (56% internal medicine) and 3042 patients from all over the country (98% post menopausal women and 2% men) participated in the program. Adherence was monitored through an independent telephone network. Treatment was never started by 109 (4%)patients and the most frequent reasons were the cost of the medication (36%) and personal reasons (30%). Of those who started treatment, 381 could not be adequately followed (12,5%). Of the 2552 that were adequately followed, 337 (13,2%)abandoned treatment before 6 months: 46% abandoned due to the cost of the medication, 25% for adverse reactions and 21% for personal reasons. Compliance proved to be only slightly higher than our previous report with alendronate 10 mg daily (15,4% in 1888 patients), suggesting that the simple measure of decreasing the number of administrations of the medication does not improve compliance in a significant way.

P488 F

BONE REMODELING AT THE ILIAC CREST CAN PREDICT THE CHANGES IN REMODELING DYNAMICS, MICRODAMAGE ACCUMULATION AND MECHANICAL PROPERTIES IN THE LUMBAR VERTEBRAE

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We recently demonstrated that suppression of bone remodeling allows microdamage to accumulate, leading to reduced bone toughness in dog bone. In this study, we evaluated the relationships between bone remodeling at the iliac crest and skeletal activation frequency, microdamage accumulation or biomechanical properties of lumbar vertebrae using the same dogs to determine whether bone remodeling at the iliac crest can predict damage accumulation and mechanical parameters of the lumbar spine following treatment with anti-resorptive agents. Thirty-six female beagles, 1-2 years old were divided into three groups. The control group was treated daily for 12 months with saline vehicle. The remaining two groups were treated daily with risedronate at a dose of 0.5 mg/kg/day, or alendronate at 1.0 mg/kg/day orally. The doses of these bisphosphonates were 5-6 times the clinical doses approved for treatment of osteoporosis in humans. After sacrifice, the right ilium and L2 vertebra were assigned to histomorphometry. The left ilium and L3 vertebra were used for microdamage analysis. L4 vertebra was mechanically tested to failure in compression. There were strong positive relationships for activation frequency (Ac.f) between ilium and lumbar vertebrae ($r^2 = 0.52$; $p < 0.001$). Reduced Ac.f at the ilium was significantly associated with increased microdamage accumulation ($r^2 = 0.37$, $p < 0.001$) and decreased toughness ($r^2 = 0.37$, $p < 0.0001$) in lumbar vertebrae. Additionally, increased microdamage accumulation in the ilium was positively related to microdamage accumulation in the L3 vertebra ($r^2 = 0.20$; $p < 0.01$). These results clearly indicate that bone remodeling data obtained from iliac crest biopsy could be used to monitor the activation frequency, microdamage accumulation and intrinsic mechanical properties in the vertebral column. This may be clinically important in assessing the potential risk of fracture in the vertebrae with prolonged treatment by pharmaceutical agents used to treat bone loss in osteoporosis.

P489 S

THE EFFECTIVENESS OF INTERMITTENT TREATMENT OF SALMON CALCITONIN IN GLUCOCORTICOID-INDUCED OSTEOPOROSIS (GIO)

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The aim of this study was to investigate effectiveness of intermittent treatment of salmon calcitonin and usefulness of bone turnover biochemical markers for assessment of response to calcitonin in GIO. Method: 15 postmenopausal women with GIO (age 55±8.2 years) received nasal spray of salmon calcitonin (Miacalcic, Novartis) in dosage 200 IU/day intermittently for 12 months. Control group consisted of 10 postmenopausal women with GIO did not receive any treatment for study period. BMD was measured by DEXA at baseline and after 12 months. Serum osteocalcin, total alkaline phosphatase (AP), cross-laps, parathyroid hormone, calcium, magnesium and inorganic phosphorus were measured at baseline and after 3 months. Results: There was a significant increase ($p<0.05$ vs baseline) in BMD of lumbar spine (in 3%), total proximal femur (in 4%), femoral neck (in 3.4%) and trochanter (in 4.3%) and no significant changes in distal forearm BMD in treated patients. We found a decrease in total magnesium from 0.81±0.05 (M±s) to 0.75±0.04 mmol/l ($p<0.01$), ionized magnesium from 0.58±0.04 to 0.54±0.03 mmol/l ($p<0.001$), osteocalcin from 28±19 to 24±18 ng/ml ($p<0.001$) and cross-laps from 0.53±0.33 to 0.45±0.30 ng/ml ($p<0.05$) in treated women. There were a decrease in osteocalcin from 30±19 to 25±19 ng/ml ($p<0.01$) and cross-laps from 0.51±0.29 to 0.40±0.26 ng/ml ($p<0.05$), and an increase in AP from 98±24 to 106±27 U/l ($p<0.05$) in

responders to calcitonin treatment by spinal BMD (n=9). There were no any significant changes in biochemical picture in non-responders. Conclusions: These results demonstrate that intermittent treatment of salmon calcitonin increases BMD in axial and peripheral sceleton and decreases bone remodeling. Osteocalcin, cross-laps and AP are sensitive biochemical markers for monitoring calcitonin efficacy in postmenopausal women with GIO.

P490 W

A RANDOMIZED TRIAL OF NASAL SPRAY SALMON CALCITONIN IN POSTMENOPAUSAL KOREAN WOMEN

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PURPOSE: We conducted a 3-year, Single-blind, randomized study to determine whether salmon calcitonin nasal spray reduced the bone loss in postmenopausal women with osteoporosis.

SUBJECTS AND METHODS: A total of 250 postmenopausal women were randomly assigned to receive , no tratment or, HRT, or HRT+ calcitonin nasal spray (200 IU) daily, HRT+ vitamin D3 or HRT+calcitonin nasal spray + vitamin D3. BMD were assessed with LUNAR Expert(Lunar, USA) on spine and femur. The primary efficacy endpoint was the % gain of BMD in the salmon calcitonin nasal spray 200-IU group compared with the control and other treatment groups.

RESULTS: During 3 years, 250 participants had at least one follow-up radiograph. A total of 228 women completed 3 years of treatment. Musculoskeletal symptoms (lumbago, small and large joint pain, joint stiffness and numbness) were significantly improved (10% improvement vs 50 to 60%) in treated patients compared to control. Joint stiffness was significantly improved in patients with nasal calcitonin treatment(10% in control vs 67% in HRT+ nasal calcitonin group $p<0.01$). The relative risk of new vertebral fracture in treated patients was (RR) = 0.13, 95% confidence interval (CI): 0.08- to 0.33, P = 0.03]. Lumbar spine bone mineral density increased significantly from baseline (1% to 5.3%, $P<0.01$) in all active treatment groups. The magnitude of spine BMD increase was significantly higher in calcitonin added groups (-3.4% in control vs 1.8% in HRT only vs 5.3% in HRT+calcitonin, $P<0.01$). The magnitude of femur neck BMD was significantly higher in calcitonin added groups (0.5% in control vs 2.9% in HRT only vs 5.4% in HRT+calcitonin, $P<0.01$). The femur wards triangle BMD was significantly higher in calcitonin added groups (-3.5% in control vs -1.0% in HRT only vs 3.1% in HRT+calcitonin, $P<0.01$). Bone turnover markers; serum osteocalcin, alkaline phosphatase, and urinary deoxypyridinolin were inhibited but was not statistically significant compared with the control. CONCLUSION: Addition of Salmon calcitonin nasal spray at a dose of 200 IU daily in postmenopausal women on HRT significantly improve musculoskeletal symptoms and increase BMD on all sites and reduces the risk of new vertebral fractures in postmenopausal women with osteoporosis.

P491 F

VOLUMETRIC STUDY OF CALLUS IN HYPOGONADAL MALE RATS TREATED WITH SCALCITONIN

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Aim: The aim of this study is to investigate the volumetric changes in the callus after osteotomy of the femur in normal male Wistar rats, normal rats treated with sCalcitonin (sCT), orchidectomised rats and orchidectomised rats treated with Sct.

Material and Methods: we used 112 male Wistar rats divided into 8 groups of 14 each. The 56 rats were orchidectomised at the age of 2 months and all the animals were undertaken hemiosteotomy (OT) in the middle of their right femur at the age of 3 months. The animals were divided in 8 groups as follow:

Group Sacrifice at Group Sacrifice at
a 2 weeks A 4 weeks Normal
b 2 weeks B 4 weeks Normal + sCT
c 2 weeks C 4 weeks Orchidectomised
d 2 weeks D 4 weeks Orchidectomised

The animals of b,B,d and D groups immediately after the OT were given 5IU of sCT subcutaneous daily. After the sacrifice of the animals we studied the total bone content, the total bone density and the total bone area of the callus by pQCT (STRATEC XCT 960).

Results were as follow:

Total BMC (mg/cm2) Total BMD (gr/cm3) Total area (cm2)
a compared to b: ns a compared to b: ns a compared to b: ns
A compared to B: ns A compared to B: B>A(p=0.02) A compared to B: ns
c compared to d: ns c compared to d: ns c compared to d:d>c(p=0.02)
C compared to D: D>C($p<0.0005$) C compared to D: D>C(p=0.02) C compared to D: D>C($p<0.01$)

Conclusion: The volumetric parameters of the callus in normal rats given sCT do not demonstrate any changes, with the exception of total BMD, whereas in orchidectomised rats treated with sCT these parameters demonstrate statistically significant changes, especially after 4 weeks of administration, thus suggesting a possible positive effect of salmon calcitonin on fracture healing in the hypogonadal animals.

P492 S

TWO-YEAR TREATMENT WITH RALOXIFENE PLUS CALCIUM AND VITAMIN D3 SUPPLEMENTATION CONTRIBUTES TO INCREASED BONE MINERALIZATION IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS

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The selective estrogen receptor modulator (SERM) raloxifene has been shown to increase BMD and reduce the risk of vertebral fracture in postmenopausal women with osteoporosis (Delmas, JCEM 2002). In this study, we report the results of the first prospective longitudinal study to evaluate the mean degree of mineralization of bone (MDMB) in a subpopulation of patients enrolled in the Multiple Outcomes of Raloxifene Evaluation (MORE) trial who had consented to participate in the biopsy study (Ott, JBMR 2002). Patients were randomly assigned to one of three treatment groups: placebo (n=24), raloxifene 60 mg/day (RLX60, n=22) or raloxifene 120 mg/day (RLX120, n=18), and all patients received calcium (500 mg) and vitamin D3 (400-600 IU) supplementation for the duration of the study. Iliac crest biopsies were taken at baseline (prior to the initiation of the study) and following two years of treatment.

Quantitative microradiography (Boivin & Meunier, CTI 2002) was used to analyze the biopsy specimens, revealing a mean percent increase in MDMB following treatment with RLX60 of 7.6%, 6.5% and 7.0% for cortical, trabecular and total (cortical + trabecular) bone respectively, compared to baseline. A similar increase in MDMB was observed following treatment with RLX120. However, the numerical increases in MDMB following raloxifene treatment were not found to be significantly different from the calcium and vitamin D3 supplemented placebo group. The mean percent increase in MDMB for the placebo group was 4.9%, 5.4% and 5.0% for cortical, trabecular and total bone respectively, compared to baseline. When compared to placebo, raloxifene treatment shifted the sample distribution to higher values of mineralization. RLX60 increased the mineral content of cortical, trabecular and total bone by 36%, 16%, and 29% respectively. Estimated increases in mineral content were determined using an ANCOVA model adjusted for differences in baseline mineralization. There are data to support that the increase in distribution to higher levels of MDMB, and the overall percent increase in MDMB compared to baseline following RLX treatment, closely resembles the degree of mineralization of physiologic premenopausal bone.

P493 W

EFFECT OF TWO YEAR TREATMENT WITH HORMONE REPLACEMENT THERAPY (HRT) ON BONE MINERAL DENSITY (BMD) IN POSTMENOPAUSAL WOMEN WITH SYSTEMIC LUPUS ERITEMATOSO (SLE)

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Aim: To determine the effect of HRT on BMD in postmenopausal women with SLE. Double-blind placebo controlled clinical trial.

Patients and Methods: Seventy-six of 106 postmenopausal SLE women participating in a clinical trial designed to test the effect of HRT on SLE disease activity, had a lumbar spine and hip BMD measurement at baseline, 12 and 24 months with dual energy x-ray absorptiometry (DEXA). Women were randomly assigned to equine conjugated estrogens 0.625 mg/day and cyclic medroxyprogesterone acetate 5 mg/day/10 days/ month or identical placebo. All women received 1200 mg/day of calcium carbonate. At baseline, a questionnaire for osteoporosis risk factors was applied. Use/dose of prednisone and SLE disease activity (SLEDAI) were registered at each study visit. The accumulated dose of prednisone throughout SLE duration was calculated from the medical chart.

Results: 34 women were assigned to HRT and 34 women to placebo. Mean age of women was 47.2 and 51.4 yr. (p=0.02), time since menopause 6.4 and 7.1 yr. (p=ns), BMI 27.4 and 26.6 (p=ns), SLE duration 9.5 and 9.9 yr. (p=ns) accumulated dose of prednisone 31381.4 and 35820 mg. (p=ns); SLEDAI scores, and use/dose of prednisone were similar at baseline and throughout the study in the HRT and placebo groups. At baseline: lumbar spine BMD was 0.889 and 0.862 gr/cm2 (p=0.41), and t score was minus 1.04 and minus 1.21 (p=0.40); hip BMD was 0.789 and 0.766 gr/cm2 (p=0.48), and t score was minus 1.88 and minus 2.04 (p=0.53) in the HRT and the placebo groups. At 12 months: lumbar spine BMD was 0.917 and 0.855 gr/cm2 (p=0.03), and t score minus 0.87 and minus 1.257 (p=0.054). At 24 months: lumbar spine BMD was 0.926 and 0.852 gr/cm2 (p=0.013) and t score minus 0.765 and minus 1.274 (p=0.01) in the HRT and placebo groups. The percentage of change in lumbar spine at 12 and 24 months was 3.1% (95% CI 2.7-3.5%) and 4.2% (95% CI 3.7-4.7%) respectively. In the hip, no significant difference was observed in the BMD and t-score at 12 and 24 months. Bone turnover markers were taken.

Conclusion: HRT improves lumbar spine BMD in postmenopausal women with SLE.

P494 F

EFFECTS OF RALOXIFENE ON THE RISK OF VERTEBRAL FRACTURE IN POSTMENOPAUSAL WOMEN WITH AND WITHOUT PRIOR USE OF HORMONE REPLACEMENT THERAPY (HRT)

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The objective of this analysis is to determine the effects of raloxifene on the risk of vertebral fracture (VF) in postmenopausal women with osteoporosis, with or without prevalent VF, who used HRT prior to enrollment in the 4-year Multiple Outcomes of Raloxifene Evaluation (MORE) study. Of the 7682 women who reported their status of prior HRT use, 2235 women used HRT before enrolling in MORE. Separate logistic regression models analyzed the relationships between prior HRT use and the risk of VF. At baseline, women who previously used HRT were younger and more likely to have a family history of osteoporosis than women who did not use HRT. Raloxifene 60 mg/d significantly decreased VF risk in women who did [RR 0.46 (95% CI 0.32-0.67)] and who did not [RR 0.71 (95% CI 0.58-0.86)] previously use HRT. The interaction between prior use of HRT and VF risk reduction was significant (P=0.05), suggesting a possible differential effect of raloxifene on VF risk in women with and without prior HRT use. In women who had used HRT prior to enrollment, raloxifene 60 mg/d reduced VF risk by 71% in those without prevalent VF [RR 0.29 (95% CI 0.13-0.63)] and by 46% [RR 0.54 (95% CI 0.36-0.81)] in those with prevalent VF. In women who did not use HRT before enrollment, raloxifene 60 mg/d reduced VF risk by 38% [RR 0.62 (95% CI 0.41-0.95) in those without prevalent VF, and by 29% [RR 0.71 (95% CI 0.57-0.88)] in those with prevalent VF. In summary, raloxifene 60 mg/d significantly reduces VF risk in postmenopausal women with osteoporosis, irrespective of prior HRT use. However, women who had previously used HRT may experience further reductions in VF risk.

P495 S

PHYTOESTROGEN-RICH HERBAL FORMULA XLGB IN PREVENTION OF OVX INDUCED BONE LOSS IN RATS

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Aim: This study investigated the effects of individual and combined therapy with a phytoestrogen-rich herbal formula-XLGB (Genistein 510μg/g, Daidzein 2500μg/g, Icariin 9200μg/g, Psoralen 930μg/g and Isopsoralen 950μg/g) and Calcium-Citrate supplementation for prevention of OVX-induced bone loss in old rats.

Methods: Forty-five 12-month-old female Wistar rats were randomly divided in following groups: one sham-operated and four OVX subgroups, i.e. OVX with vehicle, OVX with XLGB, OVX with calcium, and OVX with XLGB and Calcium-Citrate. Daily oral administration of XLGB(250mg/kg) and/or Calcium-Citrate(65mg/kg) started immediately after OVX for 12-week. Left femur was collected for BMD measurements at proximal femur using both DXA(Norland-XR36) for measuring areal BMD(aBMD) and pQCT(Scanco-Densiscan2000) for measuring volumetric BMD(vBMD) of both integral BMD (iBMD: vBMD within total bone volume) and trabecular BMD (tBMD, vBMD within core 50% of the bone volume). Serum and urine were also collected for studying biochemical markers, including serum osteocalcin and PTH as well as urine DPD.

Results: Two-way-factorial-ANOVA(Table 1) showed that XLGB increased DXA-aBMD ($P<0.01$) and pQCT-vBMD of both iBMD and tBMD ($p<0.01$ for both). XLGB treatment did not affect osteocalcin level but reduced PTH and DPD/Cr ($P>0.05$, $P<0.05$, and $P<0.01$, respectively). Calcium-Citrate increased iBMD and tBMD measured by pQCT ($p<0.01$ for both) but not areal BMD measured by DXA ($p>0.05$). Calcium supplement reduced Osteocalcin, PTH and DPD/Cr level ($p<0.01$, $p<0.05$, and $p<0.01$, respectively). No significant two-way interaction was found between the individual effects of XLGB and Calcium-Citrate on prevention of vBMD, Osteocalcin and DPD/Cr, except areal BMD and PTH.

Conclusions: These data suggested that phytoestrogen-rich XLGB was able to prevent OVX-induced bone loss, possibly by both anti-bone resorption and promoting bone formation. Accordingly, OVX-induced bone loss was also effectively retarded by calcium supplementation, mainly attributed to its effects on anti-bone resorption. Combined therapy of XLGB and Calcium-Citrate showed maximum preventive effects as compared with either treatment alone.

Table 1 The effects of individual and combined therapy with XLGB and calcium supplementation on BMD of proximal femur and serum/urine bone biochemical markers in aged OVX rats (Data in mean±SD, n=7-9)

	Sham	OVX	XLGB	Ca	XLGB+Ca			
						Two-way Factorial ANOVA		
						Main effects		Interactions
						XLGB p	Ca p	XLGB*Ca p
DXA-aBMD (g/cm^2)	0.19 ±0.01*	0.17 ±0.01	0.18 ±0.01	0.18 ±0.01	0.19 ±0.004	0.000	0.089	0.140
pQCT-iBMD (g/cm^3)	1.21 ±0.07*	0.91 ±0.08	1.20 ±0.04	1.10 ±0.08	1.21 ±0.08	0.000	0.002	0.003
pQCT-tBMD (g/cm^3)	1.20 ±0.07*	0.89 ±0.07	1.19 ±0.12	1.09 ±0.09	1.20 ±0.07	0.000	0.004	0.010
Osteocalcin (nmol/L)	5.47 ±0.22*	6.29 ±0.25	6.20 ±0.23	5.80 ±0.20	6.09 ±0.21	0.222	0.001	0.030
PTH (pg/ml)	20.93 ±8.39	26.98 ±4.45	17.31 ±6.95	17.39 ±6.98	16.37 ±6.57	0.035	0.037	0.083
DPD/Cr (nmol/mmol)	63.20 ±25.38*	85.94 ±9.31	67.25 ±11.09	78.06 ±1.81	43.79 ±8.09	0.000	0.000	0.021

P496 W

EFFECTS OF SOYBEAN ISOFLAVONE ON BONE MARROW LYMPHOPOIESIS AND BONE LOSS IN CASTRATED MALE MICE

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Soybean isoflavones exhibit selective effects on bone metabolism in postmenopausal women as well as in ovariectomized animals. Recently the role of estrogen in the bone metabolism in men has also received attention, since a man with a mutated estrogen receptor alpha gene exhibits osteoporotic phenotypes. To examine the possible role of genistein, a soybean isoflavone, in bone marrow hemopoiesis and bone metabolism in males, male mice were orchidectomized (ORX) and treated with genistein (0.4-0.8 mg/day) or 17 beta-estradiol (E2: 0.03 microg/day) subcutaneously for 3 weeks. In ORX mice, seminal vesicle weight markedly decreased, and it was not affected by the administration of genistein or E2. The number of bone marrow cells was markedly increased after ORX, and the majority was B-220-weakly positive pre-B cells. The increased B-lymphopoiesis was completely restored by E2 or genistein administration. In ORX mice, bone mineral density of the femur markedly decreased, and this bone loss was significantly prevented by treatment with genistein as well as E2. Histomorphometry showed that the trabecular bone volume in the femoral distal metaphys was markedly decreased by ORX and genistein and E2 prevented this bone loss. Furthermore, combined intervention of moderate exercise and a low-dose of genistein administration showed an additive effect in preventing bone loss in ORX mice. These results suggest that intake of soy isoflavone or combination with exercise can prevent bone loss due to androgen deficiency in males.

P497 F

RALOXIFENE IMPROVES THE SKELETAL EFFICACY OF TERIPARATIDE [RHPTH (1-34), TPTD] AT CLINICALLY RELEVANT DOSES

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Raloxifene (RLX) was evaluated to ascertain if it could significantly augment the skeletal efficacy of teriparatide [recombinant human parathyroid hormone (1-34), rhPTH(1-34), TPTD] in vivo. Teriparatide was shown previously to be efficacious in the human skeleton; therefore, assuming an average body weight of 60 kg for women, we calculated that a clinically relevant 20 ug dose (Neer, NEJM 2001) would correspond to approximately 0.3 ug/kg sc in rats. Similarly, a clinically relevant 60 mg dose of raloxifene would correspond to approximately 1 mg/kg po in rats. Raloxifene was evaluated alone and in combination with teriparatide (TPTD 0.3 ug/kg, TPTD 1 ug/kg, TPTD 3 ug/kg) in osteopenic ovariectomized rats that were permitted to lose bone for 1 month before dosing for 3 months with RLX, TPTD 0.3, TPTD 1, TPTD 3, or combinations thereof (RLX/TPTD). After necropsy, the proximal tibial metaphysis, vertebra, femoral midshaft and proximal femur were evaluated by QCT, biomechanical testing and histomorphometry. The rank order of skeletal efficacy of agents in this model was TPTD 3>TPTD 1>TPTD 0.3>RLX. Interestingly, the RLX/TPTD combinations had BMD, BMC, trabecular bone area, trabecular number, bone formation rate (BFR/TV), vertebral strength (peak load), and midshaft strength (ultimate stress) that were greater than either TPTD or RLX alone. Osteoclast number, serum cholesterol levels, and body weight were reduced for the RLX/TPTD combinations compared to respective TPTD groups. Therefore, RLX/TPTD combinations significantly improved bone mass, skeletal architecture, and strength compared to respective TPTD groups. In additional studies, raloxifene had no effect on the skeletal efficacy of 80 ug/kg teriparatide in ovariectomized rats, which increased BMD and BMC of the proximal tibial metaphysis by 25% and 24%, respectively, after 5 weeks of treatment by itself (TPTD 80). These data taken together

show that raloxifene can improve the skeletal efficacy of teriparatide at clinically relevant doses, but not at higher doses of teriparatide (80 ug/kg) where the antiresorptive effects of raloxifene were overwhelmed by the teriparatide stimulation of new bone formation.

P498 S

PHITOESTROGENS EFFICACY ON BONE LOSS RISK

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The first choice for prevention of Osteoporosis in Perimenopausal women with normal bone mass or Osteopenia would be HRT, because it has so many other beneficial effects for women of this age.

However new prospects for overcoming this choice to take advantage of the skeletoprotective actions have opened up with the development of the efficacy studies on Phitoestrogens(Pe) treatments.

The controversy over hormone replacement therapy as been fuelled as much by myths and fears as by scientific data. In a debate still clouded by uncertainties about cardiovascular benefits versus cancer risks, at least remain the alternative option of Pe.

We have made a revue on Phitoestrogen therapy and his efficacy about Osteoporosis risk and we support with our personal studies on Pe against HRT after two years on treatment with a dosage of soy isoflavones + calcium and vit.D 3 + equisetum and lactobacillus (± 120 mg/die) against HRT (E2 2mg./die + 0.5 mg. trimegestone).

P499 W

EFFECTS OF EXERCISES ON BONE MINERAL DENSITY AND PHYSICAL STRENGTH IN ELDERLY WOMEN: A 6-YEAR FOLLOW-UP

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Several studies reported that exercises were important for therapy and prevention in osteoporosis. However it was not clear which kind of exercise could effect on bone mineral density (BMD), and how about long-term efficacy of exercises. The aim of this study was to investigate the long-term effects of exercises on BMD and physical strength in elderly women. We designed the exercise protocol for elderly women including *stamp, clapping hands*, which were come from *sumo wrestling*, squat, sit up and gripping. These exercises consisted of high-impact exercises for bone and combined balance and muscle strength training. We studied 34 elderly women over 10 years after menopause (mean age 62.2 years) to determine the effects of exercises (Ex-group: N = 17) and non-exercise (Cont-group: N = 17) for 6 years. Ex-group continued our program for 90 min twice a week (one was group with a trainer, and the other in person). The profile of general conditions in age, height, weight between the two groups showed no significant differences. Every woman had no evidence of disease and drugs known to influence on bone metabolism. BMD was measured using DXA (QDR-1000, Hologic) before and after every 1 year until 6 years. The sites assessed were the lumbar (L2-L4), the hip (total proximal femur) and the radius (33%distal). Lumbar BMD decreased significantly after 4 and 6 years in Cont-group. However, lumbar BMD of Ex-group maintained for 6 years. Hip BMD decreased significantly after 2 and 3 years in Cont-group, but decreased significantly after 5 years in Ex-group. Radius BMD decreased significantly after 2 years in both of groups. On the aspect of physical strength, Ex-group had three patterns of change for 6 years. 1) Grip power and leg extensor strength increased short-term (1 or 2 years) but gradually decreased for 6 years. 2) One leg standing time, squat and sit up times gradually increased for 6 years. 3) The balance increased significantly after 5 years. In conclusion, our designed exercises have effect on maintenance of lumbar and hip BMD in elderly women for 6 years, but have few effects on radius BMD.

P500 F

FRACTURE, CARDIOVASCULAR, AND BREAST CANCER EVENT RATES AT 3 YEARS IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS FROM THE PLACEBO GROUP OF THE MULTIPLE OUTCOMES OF RALOXIFENE EVALUATION (MORE) STUDY

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Osteoporosis, cardiovascular events, and breast cancer are 3 major postmenopausal health concerns. Treatment selection should weigh the relative occurrence rates for these diseases. This analysis examines the number of women with at least one new fracture, cardiovascular (CV), or breast cancer (BC) event at 3 years in the placebo group of MORE. Women with prevalent vertebral fracture (VF) [n=938, mean age 69 ±6 (SD) years] and without prevalent VF [n=1627, mean age 66 ±7 (SD) years] were considered separately. All women were given daily calcium and vitamin D supplements. Spinal radiographs and mammograms were used to determine VF and BC, respectively, at 2 and 3 years. Subjects were asked at each clinic visit if they had any symptoms suggestive of fracture or diagnoses of CV or BC events, which were recorded as adverse events. Adjudication boards confirmed the radiographic diagnoses and self-reports to determine new cases of fracture, CV, and BC events, which are reported as rates per 1000 patient-years (Table).

The occurrence of any fracture was the most common event, as expected. In women with prevalent VF, the rate of vertebral or clinical VF was greater than for hip fracture, CV, or BC events. Furthermore, the likelihood of any CV event was approximately 3 times greater than hip fracture, even in these women who were at high risk for subsequent fracture. In women without prevalent VF, any fracture and VF were the most common events, while coronary and BC events occurred at similar rates, and the hip fracture rate was very low. In conclusion, the relative importance of clinically significant skeletal and extra-skeletal events should be considered when choosing an agent for postmenopausal health maintenance. These data would be useful in formulating decisions regarding prevention and treatment strategies for this population.

Event	Prevalent Vertebral Fractures	
	With	Without
Any fracture (vertebral or nonvertebral)	117.4	45.4
Vertebral fracture	77.1	15.2
Any cardiovascular (coronary or cerebrovascular)	15.1	8.3
Breast cancer (all cases)	2.6	5.2
Coronary	7.1	5.2
Breast cancer (invasive)	1.9	4.6
Clinical vertebral fracture	25.7	4.7
Hip fracture	5.8	0.9

P501 S

EFFECTS OF RALOXIFENE, TAMOXIFENE AND BETA-ESTRADIOL ON MATRIX METALLOPROTEINASE 9 CONCENTRATION IN HUMAN UMBILICAL ARTERY ENDOTHELIAL CELLS STIMULATED WITH KAPPA-ELASTIN

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Raloxifene (RLX) - a selective estrogen receptor modulator (SERM) is approved by FDA as an agent for the prevention and treatment of osteoporosis. Many studies with bone mineral density as primary outcome have shown significant efficacy of RLX and hormonal replacement therapy (HRT). Observational studies have indicated a significant reduction of hip fracture risk in cohorts of women who maintained HRT therapy. RLX maximizes the beneficial effects of estrogens on bone and vascular wall and minimize or antagonize the deleterious effects on the breast and endometrium. According to the results of HERS and WHI studies hormonal replacement therapy is not recommended in prevention of cardiovascular diseases in high-risk patients and in overall population. According to the results of Barrett-Connor and al. analysis of high-risk group in MORE study, raloxifene reduced total risk of cerebrovascular events by 40% in high-risk population. Ardans and al. found that raloxifene increase matrix metalloproteinase-1 production by activated monocytes. Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases degrading extracellular matrix proteins. They are secreted by monocytes, macrophages and stimulated endothelial cells. MMP-9 is involved in inflammation, tissue remodeling, wound healing and mobilization of matrix-bound growth factors. Expression of MMP9 in atherosclerotic plaque is one of the causes of plaque destabilisation and rupture which leads to coronary artery occlusion and myocardial infarction. Elastin-derived petpitdes (EDPs) which circulating plasma level is increased in patients with atherosclerosis, diabetes and inflammatory diseases are responsible for induction and progression of experimental atherosclerosis.

The aim of this study was to evaluate the effects of beta-estradiol, raloxifene and tamoxifene on production of matrix degrading enzyme MMP9 by umbilical artery endothelial cells incubated with kappa-elastin.

Materials and methods: Briefly, we incubated endothelial cells obtained from human umbilical artery in medium containing kappa-elastin (0.5 microg/l) and examined drugs in two concentrations (1 and 10 microM/l) for 12h. As a control unstimulated cells and cells stimulated with kappa-elastin only were used. Total amount of MMP9 in culture supernatants was assayed using quantitative sandwitch enzyme immunoassay technique. Statistical analysis was performed using ANOVA test.

Results: Kappa-elastin didn't induce increased production of MMP9. In cells preincubated with kappa-elastin and beta-estradiol, raloxifene and tamoxifene levels of MMP9 were not statistically different comparing to control cells.

P502 W

SOYBEAN ISOFLAVONES AND RALOXIFEN IN POSTMENOPAUSAL WOMEN WITH BONE MASS LOSS

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Statement of purpose: determining if there are differences in using soybean isoflavones and raloxifen on bone mass loss in postmenopausal women.

Statement of method: we studied for 20 months 24 women who were 45 to 62 years old at base line, were within 1 and 10 years of menopause, and had a bone mineral density at the lumbar spine between 150 mg/cc and 50 mg/cc measured by the QBMAP system with a spiral CT Picker PQ-S densitometer at L2, L3, L4 and L5. Of all the women, 12 were assigned to therapy with soybean isoflavones 80 mg and 12 were treated with raloxifene HCL 60 mg. The SPSS programme was used for statiscal analysis.

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

Conclusions: it is necessary to carry out a wider study but it seems that raloxifene HCL contribute advantages versus isoflavones therapy to decrease the bone mass loss in postmenopausal women at least at lumbar spine.

P503 F

THE EFFECTS OF RALOXIFEN AND CIMICIFUGA RACEMOSA IN POSTMENOPAUSAL WOMEN

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Aims: The effect of combined therapy with raloxifen and Cimicifuga racemosa on osteoporosis and hot flushes in postmenopausal women with established osteoporosis and hot flushes.

Material & Methods: In this 1-year clinical trial we have evaluated the changes of hot flushes and T-score in 93 postmenopausal women. We asked about the number of hot flushes per day on one hand and used Norland DEXA scan on the other hand. Women were given 1 tablet raloxifen and 2 x 1 tablet Cimicifuga racemosa per day. PAP smear, colposcopy, bimanual gynaecological examination, vaginal ultrasound, mammography, safety laboratory exams were carried out in every patient both at the beginning and at the end of the study.

Results: From the first 3 cycles the treatment significantly decreased the mean daily number of hot flushes in most women. The treatment improved both lumbar spine and femoral neck bone mass. The drop-out number was 6.

Conclusions: We have found the two medicaments having been used together effective for the complex therapy of postmenopausal women with established osteoporosis still suffering from hot flushes. In our study the compliance of the patients were excellent.

P504 S

SHOULD SOY ISOFLAVONES BE TESTED IN THE PREVENTION OF BONE LOSS? A PHARMACOKINETIC STUDY

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About 40% of menopausal women in the US have at one point taken plant-derived estrogens for menopausal symptoms. Since phytoestrogens (PE) have sufficient estrogenic effects to alter the menstrual cycle in premenopausal women, they have the potential for protecting postmenopausal women from bone loss. Although PE are 1,000-10,000-fold less estrogenic than 17-beta estradiol, plasma concentrations are high in individuals consuming a traditional Asian diet (1000-fold higher than the levels of 17-beta estradiol in premenopausal women).

To investigate serum levels achieved with over-the-counter preparations we did two pharmacokinetic (PK) studies, testing two concentrations of soy isoflavone tablets (125 mg or 200 mg isoflavones daily) in healthy female volunteers 18 years of age or older. Serum levels were determined by LCMS. After an overnight fast, 5 women ingested a single dose of 200 mg total isoflavones. Blood was drawn at baseline and at 30, 60, and 90 minutes and at 2, 4, 6, 8, 24, and 48 hours after. Mean age was 23.6 years ± 1.14 (SD) and median weight was 125 lb (range 100 to 175). Daidzein and genistein demonstrated initial peaks at 2 hrs and second peaks, which comprised 85 to 95% of the area under the curve to last concentration (AUC0-t), including the maximum plasma concentration (Cmax) and time at Cmax (tmax). Because of the high isoflavone serum levels at 24 hrs, and the concern that daily dosing would result in accumulation of isoflavone, we conducted a 4 days multiple-dose study: 3 subjects received 200mg daily, and 2 subjects 125mg daily. Mean age was 36.0 years ± 7.3 (SD) and median weight was 168 lb (range 130 to 351). Multiple-dosing produced steady-state concentrations of isoflavones during the dosing period, as opposed to progressive accumulation. This finding was not predicted from the single-dose study, suggesting potential induction of metabolizing enzymes in the intestine, liver, or in microflora.

Thus postmenopausal women who consume 200 mg isoflavones/day (about 3 mg/kg body weight) will achieve peak serum concentrations of approximately 3 mM. Since sufficient serum concentrations can be achieved with these doses and with the increasing interest in the effect of isoflavones on menopause-related symptoms and diseases, trials are needed to determine their skeletal biological potency.

P505 W

LOW WEIGHT BEARING PHYSICAL EXERCISE IS ASSOCIATED WITH OSTEOPENIA IN PERIPUBERTAL GIRLS WITH ADOLESCENT IDIOPATHIC SCOLIOSIS

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INTRODUCTION: Adolescent Idiopathic Scoliosis (AIS) is a three-dimensional deformity of the spine. Systemic osteopenia are found in AIS patients. Whether physical-exercise (PE) is a risk factor for osteopenia in AIS unclear. This investigation attempted to study whether PE level is associated with bone mineral density (BMD) in AIS.

METHODS: 599 AIS patients and 307 healthy girls aged 11-16-y participated in the study. To assess the effect of PE on BMD, weight-bearing and non weight-bearing PE were evaluated by a physical-activity-questionnaire. Puberty was examined by Tanner's staging. Areal BMD (aBMD) and volumetric BMD (vBMD) were evaluated by Dual-energy-X-ray Absorptiometry (Norland-XR-36) and pQCT (Densiscan-2000) respectively.

RESULTS: Overall weight-bearing PE (1.63 vs.1.81 hrs/d, P=0.02) and high-impact weight-bearing PE (0.51 vs. 0.58 hrs/d; P=0.003) of AIS were significantly lower than those of controls. AIS at all ages had aBMD and vBMD significantly lower than those of controls. Systemic osteopenia was found in over 25% of AIS girls. Overall weight-bearing PE was significantly correlated with aBMD and vBMD (P=0.003). High-impact weight-bearing PE correlated significantly with aBMD and vBMD (P=0.002). Furthermore, medium-impact weight-bearing PE significantly correlated with aBMD & vBMD among AIS girls (P<0.05). However, this phenomenon was not found in the controls. Multivariate-analysis showed that weight-bearing PE was an independent predictor on vBMDs (P=0.006), femoral-neck BMD (P=0.044) after controlling for group (AIS/Controls), calcium intake, age, weight and height.

CONCLUSIONS: One-quarter of AIS were osteopenia in the present study. The amount of weight-bearing PE in AIS girls was very low for the age. Weight-bearing PE was an independent predictor on BMD (P=0.001). Further interventional studies with physical-training program is required to confirm the effect of weight-bearing PE on bone-mineral acquisition in AIS during peripubertal growth.

P506 F

WHOLE-BODY VIBRATION EXERCISE IN THE ELDERLY PEOPLE

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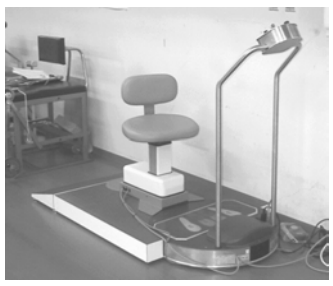
Most of femoral neck fractures in the elderly are caused by fall. Although exercise is considered to prevent fall by maintaining muscle power and balance and functional fitness, many old subjects are unable to exercise effectively. The aim of this study is to investigate the effects of the Whole body vibrations (WBV) in the elderly people. Twenty-one people aged 72.6 years old attending health program in local community were included in this study. Eleven carried out the exercise (Ex.) by low frequency oscillation loading device (Galileo 900, Novotec Pforzheim Germany, Fig.1) and the other ten did not (Cnt.). Ex. was exposed to a bout of the 20-30Hz vibrations standing on the platform 3 times a week. Calcaneal bone mineral density was measured using QUS (AOS-100, Aloka Japan), statical and dynamic balance test, functional fitness tests was performed before and after 6 months exercise program. Calcaneal bone mineral density did not differ between 1st and 2nd measurement, but balance-function improved significantly after 6 months exercise in Ex. These results suggest that WBV possibly prevents fall and femoral neck fracture by improving standing balance in elderly subjects.

Fig.1 Low frequency oscillation loading device (Galileo 900, Novotec Pforzheim Germany)



P507 S

EFFECT OF COMBINATION OF DIFFERENT INTENSITIES TREADMILL EXERCISE AND ESTROGEN ON BONE MASS IN EXPERIMENTAL OSTEOPOROSIS RATS

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The effect of combination of different intensities exercise and estrogen on bone mass in the proximal tibial metaphysis was investigaed in adult ovariectomized (OVX) rats by histomorphometric assessment. Seventy female SD rats were divided into 8 groups as follow: BASAL, SHAM, OVX, OVX+DRUG(estrogen administration), OVX+EX1(20 m/min), OVX+EX2(30 m/min), OVX+DR+EX1 and OVX+DR+EX2. Static and dynamic histomorphometry were measured on the concellous in the proximal tibial. The exercise rats were trained on a treadmill for 1h/day ,5 days/week for 8 weeks. The results showed that the bone mass reduced significantly in OVX rats with increasing bone turnouver. In OVX-EX1 rats moderate exercise suppressed increased bone turnover, while high intensity exercise did not affect the histomorphometric parameters significantly compared with OVX rats,also bone formation and resorption parameters is higher significantly compared with in OVX-EX1 rats.The two combination groups had the highest bone mass in the all OVX groups and similar to BASAL, but lower than SHAM. In conclusion that two combination groups can reduced the cancellous bone loss much more than either that of estrogen administration without exercise or exercise training without estrogen.

P508 W

COMBINED INTERVENTION OF SOY ISOFLAVONE AND A MODERATE EXERCISE PREVENTS BONE LOSS AND HYPERCHOLESTEROLEMIA IN OVERIECTOMIZED MICE

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Osteoporosis and hypercholesterolemia are both associated with estrogen deficiency following menopause. We have reported that the combined intervention of soy isoflavone and a moderate exercise exhibit cooperative effects on the prevention of whole body bone loss in the femur in ovariectomized (OVX) mice. In this study, we examined whether the both interventions exhibit cooperative effects on bone mass, body composition and lipid metabolism in OVX mice fed a high cholesterol diet (HC: AIN 93G + 3 g cholesterol and 0.5 g sodium cholate per kg diet). Eight wk-old female mice were assigned to six groups: (1) sham operated (Sham); (2) ovariectomized (OVX); (3) OVX, received a soy isoflavone diet (+ 0.16% pure isoflavone conjugates, OVX+ISO); (4) OVX, exercised on a treadmill for 30min/day, 6 days/wk at 12m/min (OVX+EX); (5) OVX, given with both isoflavone and exercise (OVX+ISO&EX); and OVX, treated with 17beta estradiol subcutaneously (OVX+E2). Bone mineral density (BMD) and body composition were estimated by dual-energy X-ray absorptiometry (DXA) and dynamic parameters at midshaft of the femur were evaluated histomorphometric analysis. After 6 wk interventions, the BMD of the whole body, lumbar spine and femur were significantly reduced by OVX, and the bone loss was suppressed by either intervention alone. Furthermore, the combined intervention of isoflavone and exercise restored completely the bone mass to the sham levels, even though at the lumber vertebrae loading less mechanical stress. Histological analysis revealed that the combined intervention increased bone formation rate/bone surface (BFR/BS) at the periosteum in the cortical bone of the femoral diaphysis. In regard to body composition, the whole body fat (%) was significantly higher in OVX group than that in sham group, and it was restored completely in OVX+ISO&EX group. However, the lean body mass in the whole body was increased by the combined intervention. Thus, high bone mass in OVX+ISO&EX group could be related to the increased lean body mass in whole body. Serum total cholesterol significantly suppressed in OVX+EX and normalized in OVX+ISO&EX, as in OVX+E2 group. In contrast, serum HDL-cholesterol was significantly elevated by the combined intervention. Thus, a combination of moderate exercise and the isoflavone supplementation may offer an effective regimen for the prevention of life-style related diseases such as osteoporosis and hypercholesterolemia in ovarian hormone-deficient women.

P509 F

THE EFFECTS OF SWIMMING AND RUNNING ON THE RECOVERY OF DISUSE OSTEOPOROSIS IN FEMUR

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Disuse osteoporosis occurs with reduced weight-bearing loading. Rehabilitation should be prescribed to subject the atrophied bone to an appropriate level of mechanical stress.

The purpose of this study was to compare the effects of two programs of training on bone development in rats . 3-month-old male SD rats were immobilizated for 21 days, only immobilizated on one side while the opposite side served as a control. 32 male SD rats were randomly assigned to one of four groups: control(CON) , run-trained recover (RR) , swim-trained recover (SR) or naturally recover (NR), After 3 weeks immobilization , the CON group were killed, the RR, SR and NR group were allowed to recover 3 weeks then killed .

The femur was extirpated for the measurement of bone mass, bone morphological index, biomechanical properties and biochemical index. BMD and BMC was measured by DEXA at the whole femur. Biomechanical properties of the femur are determined from three point bending tests. Maximal loads, deformation, stress and strain was measured.

In a long term, the measurement of bone mass is one of necessary methods to evaluate the condition of bone. However, the animal experiments and clinical research show that the quality of bone will not be improved with the increase of the mineral content of bone, sometimes it wil be decreased in inverse. Therefore, the measurement of bone mechanical properties become very vital step for the research of condition of bone.

The results of this research indicate that : (1)Under the situation of disuse osteoporosis and the influence of sports , body weight and the change of bone mass are not coincident , so it is not available to deduce bone mineral density according to Weignt=aBMDb . (2)In the early recovery of disuse osteoporosis, the change of serum BGP is consistent with that of bone maximal stress. (3)Simply recovery in bone mass or bone morphological index can not indicate the recover of bone biomechanical index directly. (4)The SR group do not produce a significant differ with the NR group. Running can have beneficial effect on bone .

P510 S

A PILOT STUDY TO DETERMINE DAILY COMPLIANCE OF ELDERLY WOMEN ENROLLED TO STAND ON A LOW-LEVEL VIBRATION PLATFORM SYSTEM TO TREAT OSTEOPOROSIS

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To reduce polypharmacy and medication side effects, non-pharmacologic strategies for the treatment of osteoporosis are ideal for elderly patients. One such potential treatment is the non-invasive delivery of low-level mechanical loading via high-frequency (30 Hz) low-magnitude accelerations (0.3g), an intervention shown to be anabolic in animal studies (1). A randomized, placebo-controlled, double-blinded 6-month pilot study was designed to test compliance for daily use of a 10-minute vibrating platform treatment in elderly female residents of a Continuing Care Retirement Community (CCRC). Compliance was calculated as the number of days attended divided by the trial's 182 days. The primary goal was 80% compliance by all women, and exit assessment of satisfaction with daily use of the device. A secondary aim was to examine the ability of the signal to influence bone quality, as assessed by quantitative ultrasound measures of the right calcaneus (Hologic, Waltham, MA). Of the 24 women enrolled (86 yrs, range 79-92), 21 completed the study, with 93% compliance for daily treatment over 6-months. Including the three women who withdrew from the study, 83% of total subjects had compliance of at least 80% (range 7-100%), with no difference in compliance evident between active vs. placebo treatment. 54% of days missed were for vacation, and 29% for illness. 95% of participants reported overall satisfaction with daily use of the platform, 57% preferred the platform vs. daily oral medications for prevention of bone loss, and 19% had no preference. No subjects complained of discomfort or uneasiness during or following the daily routine. In the short time frame for this study, ultrasound showed no statistical differences in change in bone density ($p > 0.20$) between groups. In conclusion, elderly women living in a CCRC showed high compliance and satisfaction with a daily non-pharmacologic treatment designed to inhibit bone loss. Larger, longer-term trials are required to determine the efficacy of the low-level mechanical

stimulus for preventing, or perhaps reversing, osteoporosis in the elderly. *1) Rubin et. al. (2001) Nature 412:603-604.* Supported by grant from the NIH with equipment provided by Exogen, Inc.

P511 W

BONE MINERAL DENSITY IN SEVERE AND REFRACTORY RHEUMATOID ARTHRITIS PATIENTS TREATED WITH INFLIXIMAB

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Objective: To determine the effects of anti-TNF therapy [infliximab (IFX)] on bone mineral density (BMD) in a large cohort of patients with severe rheumatoid arthritis (RA) refractory to DMARDs and glucocorticoid (GC) therapy. CTx and OPG were determined at baseline, week 6 and 52 in males only.

Materials and Methods : 78 RA patients (63 F, 15 M, mean age: 54.3 y) with active RA (mean disease duration: 10 y, mean number of previous DMARDs: 4, mean baseline CRP: 2 mg/dl) despite at least 15 mg weekly methotrexate or 2.5 mg/kg/day azathioprine therapy and low dose of GC (prednisone<10 mg/d) were treated with IFX (3 mg/kg at day 0, week 2, week 6 and every 8 weeks afterwards). BMD of the lumbar spine and of the total hip were measured by DXA using a QDR 4500 (Hologic) at baseline and after one year of therapy. Among the postmenopausal women (n=42), 20 were treated with stable hormonal replacement therapy. Administration of calcium and vitamin D were allowed during the study. The levels of CTx and OPG from 15 males were determined by ELISA.

Results: At baseline, 41 (52%) of the patients had osteopenia and 23 (29%) had osteoporosis at least at one site, according to the WHO rules. Lumbar BMD [mean (SD)] remained stable after one year [0.900 (0.130) vs [0.900 (0.120) at baseline]. In contrast, there was a significant decrease in BMD of the total hip after one year [0.790 (0.120) vs 0.805 (0.130), $p < 0.01$]. This decrease was more pronounced in males. In our population, some weight gain was observed [70.8 (16.0) vs 70.0 (17.0) kg before IFX], significant in premenopausal women only [58.7 (10.0) vs 57.0 (9.0) kg, $p < 0.05$). All clinical variables (HAQ, joint count, morning stiffness and CRP) improved. There was a non-significant trend to decrease in CTx and OPG at week 6 and 52 (mean baseline CTx 340 pg/ml, week 6, 302 pg/ml and week 52, 269 pg/ml and baseline OPG 6.1, week 6, 5.9 and week 52, 5.9).

Conclusion: Although lumbar BMD was maintained, the preliminary results obtained in this open study seem to indicate that IFX could not reverse osteoporosis nor slow down bone loss in severe and refractory RA population.

P512 F

EFFECTS OF CYCLICAL ETIDRONATE COMBINED WITH ALFACALCIDOL VERSUS CYCLICAL ETIDRONATE ALONE ON LUMBAR BONE MINERAL DENSITY, BONE RESORPTION, AND BACK PAIN IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS

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The purpose of the present study was to compare the effects of cyclical etidronate combined with alfacalcidol with those of cyclical etidronate alone on lumbar bone mineral density (BMD), bone resorption, and back pain in postmenopausal women with osteoporosis. Forty postmenopausal women with osteoporosis, 60-86 years of age, without any vertebral fractures in the lumbar spine, were randomly divided into two groups with 20 patients in each group: cyclical etidronate group (oral etidronate 200 mg daily for 2 weeks every 3 months) and cyclical etidronate combined with alfacalcidol group (cyclical etidronate plus alfacalcidol 1 microg daily). BMD of the lumbar spine (L1-L4) measured by DXA, urinary cross-linked N-telopeptides of type I collagen (NTX) level measured by enzyme-linked immunosorbent assay, and back pain evaluated by face scale score were assessed at baseline, 6 months, and 12 months. There were no significant differences in baseline characteristics including age, body mass index, years since menopause, lumbar BMD, urinary NTX level, and face scale score between the two treatment groups. Both treatment significantly reduced urinary NTx level and back pain. Cyclical etidronate combined with alfacalcidol significantly increased lumbar BMD with a more significant reduction in urinary NTX level than cyclical etidronate alone, but cyclical etidronate alone did not significantly alter lumbar BMD. Improvement of back pain was similar in both treatment groups. These results suggest that cyclical etidronate combined with alfacalcidol appears to be more useful than cyclical etidronate alone for increasing lumbar BMD by more markedly suppressing bone resorption in postmenopausal women with osteoporosis.

P513 S

COMBINATION THERAPY OF ALFACALCIDOL AND ALENDRONATE ON THREE-DIMENSIONAL TRABECULAR STRUCTURE IN OVARIECTOMIZED RATS

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The purpose of this study was to determine the effect of the combination therapy of alfacalcidol (ALF) and alendronate (ALN) in ovariectomized rats. 40-week-old female Winstar rats were subjected to OVX or sham operation, and were divided into 7 groups (n=5-7) at 12 weeks after surgery; sham-vehicle (sham), OVX-vehicle (OVX control), OVX-ALF 0.025microg/kg (low ALF), OVX-ALF 0.1microg/kg (high ALF), OVX-ALN 0.3mg/kg (low ALN), OVX-ALN 1.0mg/kg (high ALN) and OVX-ALF 0.025microg/kg in combination with ALN 0.3mg/kg (ALF+ ALN). Each drug was administered orally 5 times a week for 12 weeks. The whole body of the 5th lumbar spine and the proximal metaphysis of the left tibia were scanned using micro-CT (micro-CT20, SCANCO Medical) with 13 microm increment for 250 and 150 slices, respectively. The structural parameters such as bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), Trabecular Bone Pattern factor (TBPf), Structure Model Index (SMI), and Degree of Anisotropy (DA) were assessed. The BV/TV did not significantly increase in both the low ALF and the low ALN in comparison with the OVX-control. On the other hand, The BV/TV significantly increased in the ALF+ALN as well as in the high ALF and the high ALN in comparison with the OVX-control. The increase of the BV/TV was associated with the significant change of Tb.N and Tb.Sp in the tibia, and of Tb.N, Tb.Th, Tb.Sp, SMI and TBPf in the spine. In conclusion, the therapeutic administration of ALF as well as ALN effectively prevented the trabecular deterioration in dose dependent fashion. The combination therapy of the low dose of ALF and ALN had an additive effect that was equivalent with those of high dose of ALF or ALN.

P514 W

A TNF-ALPHA ANTAGONISTIC PEPTIDE INHIBITS BONE LOSS INDUCED BY ESTROGEN DEFICIENCY

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The WP9QY peptide which mimics the critical ligand recognition site on TNF (tumor necrosis factor) receptor (I) was designed as a TNF antagonist by using a molecular modeling technique (Nat. Biotech. 15: 1266, 1997). We have already shown that this peptide antagonist also works as a RANKL (receptor activator of NF-kB ligand) antagonist, inhibiting osteoclastogenesis induced by soluble RANKL and M-CSF in vitro. Surface plasmon resonance bioassay revealed that WP9QY bound to soluble RANKL in almost the same extent to TNF. In the in vivo study, WP9QY blocked the bone loss induced by the low Ca feeding for 2 days. This low Ca model is good for the drug screening to see the inhibitory effect on bone resorption, but we cannot see the long-term effect of WP9QY on bone resorption using this model. Here we showed the effect of WP9QY peptide on bone resorption using the ovariectomized (OVX) model. Female C57BL/6 mice (9 week-old) were ovariectomized (OVX group) or not sham operated (sham group), and were sacrificed 4 weeks after the operation. Half of the mice in OVX group received subcutaneous administration of WP9QY (2 mg/kg/day) by the osmotic pumps, another half of the mice in OVX group and all mice in sham group received the vehicle by the infusion pumps. The bone mineral density (BMD) at the tibial metaphysis, measured by DXA, was significantly reduced from 37.5 mg/cm^2 in the sham group to 20.9 mg/cm^2 in the OVX group, while WP9QY inhibited this reduced BMD (-43.3% in OVX mice vs -13.2% with treatment). Furthermore, urinary level of deoxypyridinoline/ creatinin, the marker of bone resorption, was significantly reduced from 100.22 nM/mM in the OVX group to 63.92 nM/mM in the peptide-treated OVX group. These results indicate that the WP9QY peptide inhibited bone resorption induced by estrogen deficiency. The peptide inhibitor designed by using the technique of molecular modeling would be the promising therapeutic drug for inhibiting bone resorption.

P515 F

LASER BASED IMPLANTATION OF A NEW DESIGNED ANCHORAGE DEVICE FOR FRACTURE TREATMENT IN OSTEOPOROSIS

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Introduction: Common implants tend to fail in osteoporotic bone by cutting through .New developed implant geometries should diminish this problem .In a first step we optimized a star shaped anchorage device with respect to minimal stress in the adjacent bone. A second study was performed to investigate whether an Ee:YAG laser is suitable to open the cortical bone before insertion of these implants.

Materials and methods: The cross section of the implant was subjected to a two dimensional finite element analysis .Different loading directions were applied with 1000N.Outcome variables were number of flanges, radius of curvatures outside and thinning of the wings. For the laser test samples of cortical cal of bone were used (2 to 7 mm thick).The pulse energy of the Er: YAG laser was increased from 250 ml to 1.1 J and the pulse was calculated and the local bone reactions recorded. A handling test with a commercially available Er: yag laser was performed, where the complete profile was cut in a human cortical bone.

Results: A tri-flanged profile having a thickening outside of 5mm showed lowest amount of stresses within the adjacent bone .Perforations of the bone samples between 0.9 and 1.1 mm diameter were possible with the laser without side effects like melting or carbonisation. Extension of the pulse duration led to a higher increase of the oblation state than the increase of pulse energy. Preparation of the entry side with the laser and subsequent implant insertion was possible.

Conclusions: The new implant design reduces the stress concentration within the adjacent bone thus creating an improved interface especially for osteoporotic bone. Implant insertion after opening of the cortical bone with an Er: yag laser is possible and offers a great potential for further applications.

P516 S

CONCURRENT TREATMENT OF OVARIECTOMIZED RATS USING TWO OF ETIDRONATE, 1-ALPHA-OH VITAMIN D_3, AND VITAMIN K_2

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Although concurrent therapy of osteoporosis has been frequently employed in daily practice, there has been little data regarding what combination of agents improves the effect of each single regimen. Thus, we investigated the efficacy of concurrent treatments in ovariectomized rats. Eight-months-old female Wistar rats were divided into the following 11 groups (8 rats in each group): BC, S3, O3, S9, O9, D, K, D+K, E, E+D, and E+K groups. BC rats were sacrificed at the baseline (start) of the experiment. S3 and S9 rats were sham-operated and sacrificed 3 months and 9 months after operation, respectively. The other 8 groups of rats were ovariectomized. O3 and O9 rats were sacrificed 3 months and 9 months after ovariectomy, respectively. The remaining 6 groups of rats were treated for 6 months, from 3 months till 9 months after ovariectomy, with the following drugs: 1) 1-alpha-OH vitamin D_3 (0.1 micro-g/kg/d po, 5 d/w) (D); 2) vitamin K_2 (7 mg/kg/d po, daily) (K); 3) D+K; 4) etidronate (1 mg/kg sc 5 d/w for a week followed by a 3-week intermission) (E); 5) E+D; and 6) E+K. These rats were sacrificed at the end of treatment, 9 months after ovariectomy (at the age of 17 months). Bone mineral density (BMD) of lumbar spines (L2-4) was compared among the groups. Nine months after ovariectomy, the mean BMD was significantly lower in O9 rats than in S9 rats. Although the mean BMDs in K, E, and E+K rats were not significantly different from the mean value in O9 rats, the mean BMDs in the D, D+K, and E+D rats were significantly higher than the mean value in the O9 rats. The mean BMD in the E+D rats was significantly higher than that in the D rats. In this animal model of osteoporosis, with the drugs at the given doses, D with or without K or E was significantly effective in increasing BMD to the level of the sham-operated rats. The combination of D and E has been shown to be more effective in increasing the lumbar BMD than each single administration of these drugs.

P517 W

PREDICTIVE VALUE OF BIOCHEMICAL MARKERS OF BONE TURNOVER FOR BONE DENSITY IN POSTMENOPAUSAL WOMEN ON HORMONE REPLACEMENT THERAPY

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Osteoporosis is a common health problem in postmenopausal women. In this study we aimed to evaluate the predictive value of biochemical markers of bone turnover for bone density in postmenopausal Japanese women on hormone replacement therapy(HRT). Nineteen postmenopausal Japanese women (51-66 years old) were enrolled diagnosed as osteopenia or osteoporosis based on the diagnostic criteria of osteoporosis of the Japanese Society of Bone and Mineral Metabolism. Conjugated equine estrogen 0.625mg/day and medroxyprogesterone acetate 2.5mg/day were administered to womem with intact uterus and conjugated estrogen 0.625mg/day alone was given to those who underwent hysterectomy. Lumbar spine bone mineral density (BMD) was measured using dual-energy X-ray absorptiometry (DXA) (QDR-2000, Hologic) over 1 year. Bone formation markers, serum intact amino-terminal propeptide of type I procollagen (PINP) and bone alkaline phosphatase (BAP), and bone resorption markers, urinary cross-linked N-telopeptide of type I collagen (NTX) and free deoxypyridinoline (DPD) were measured before treatment and 1, 3, 6 and 12 months after treatment. The predictive value of biochemical markers of bone turnover for bone density was evaluated by calculating signal/noise ratios using the minimum significant change (MSC). Lumbar spine BMD was increased by 6.39±4.39% one year after treatment. The percent changes of each biochemical marker at 3 and 6 months after treatment were significant as compared with baseline values; PINP -31.9±4.6% and -51.7±4.9%, BAP -17.8±4.2% and -27.0±3.9%, NTX -20.1±11.3% and -35.6±6.6%, and DPD -28.6±7.6% and -29.2±6.1%. The percent changes of PINP and BAP at 3 and 6 months after treatment, NTX at 6 months and DPD at 1, 3 and 6 months were greater than MSC. The percent change of PINP at 6 months alone exceeded twice MSC value. In all women who gained bone mass with HRT the percent change of PINP at 6 months was greater than MSC. We could find significant inverse correlations only between the percent changes of BAP at 6 month and those of vertebral BMD at one year. The results suggest that biochemical markers, especially PINP and BAP, are useful for predicting the effect of HRT in postmenopausal Japanese women.

P518 F

GASTRIC PENTADECAPEPTIDE BPC 157 ATTENUATES OSTEOPOROSIS INDUCED BY OVARIECTOMY OR EXPERIMENTAL GASTRO-ESOPHAGEAL REFLUX DISEASE IN RAT

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Gastric pentadecapeptide BPC 157 is stable peptide currently in clinical phase II for inflammatory bowel disease (PL-10, PLD-116, PL-1473) that improves internal and external wounds heals (i.e., (i.e., Burns 27 817, 2001), and pseudoarthosis (Bone 24 195, 1999). Even sooner than ovariectomy, gastrointestinal tract dysfunction (i.e., gastrectomy, inflammatory bowel disease Dig Dis Sci 42 1029 1997) leads to osteoporosis. We investigate whether this peptide improves osteoporosis presented in Wistar rats, (i) ovariectomized female, or (ii) male with experimental gastro-esophageal reflux disease (GERD) induced by sphincters dysfunction (one tube secured by two sutures is placed in pylorus and other one in esophageal sphincter). Ovariectomized rats received agents topically, intravaginally, 1, or 0.01 microg/g neutral cream, or neutral cream (Belobaza, Belupo, Croatia, or nothing (controls)), while GERD-rats received BPC 157 10 microg/kg or 10 ng/kg or saline (5.0 ml) intraperitoneally, or 2microg or 2 ng/ml in drinking water), first application immediately following injury, last 24 h before sacrifice (at 4 weeks days (ovariectomy) or 3 and 5 weeks (GERD) post-injury). Densitometry analysis reveals significant osteoporosis in ovariectomized control rats, that was reversed in animals treated with gastric pentadecapeptide BPC 157. Also, a significant osteoporosis appeared in GERD-control rats, a finding probably important for further osteoporosis-research. This osteoporosis was also fully attenuated in BPC 157 rats (distal femur bone density in control rats was 54% that of BPC treated rats, $p<0.01$).

P519 S

IMPROVED LUMBAR BMD AND BIOMECHANICAL STRENGTH BY SURFACE INTERFERENTIAL STIMULATION DEVICES IN ESTABLISHED OVX OSTEOPENIA OF RATS. A PILOT STUDY

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This report describes a pilot study that was to determine the effect of Surface interferential stimulation (SIS) on established bone loss in an ovariectomized rat model of human osteoporosis. Twelve (12) weeks after the ovariectomy operation (age 28 weeks) on a total of 55 16-week old virgin female Sprague Dawley rats, the spine bone mineral density (BMD, g/cm2) was determined and used to re-randomize the OVX rats to 11 homogenous treatment groups. The SHAM (n=5) and one (1) OVX group did not receive treatment and served as controls. The remaining ten (10) OVX groups received various interferential electrical stimulation seven (7) days a week for eight (8) weeks. The devices stimulated at 1.8, or 4, or 7.3 mA rms with a base frequency (BaF) at 5,000 or 7,500 Hz, a beat frequency (BeF) sweep from 1 to 150 Hz or set at 150 Hz, and with continuous stimulation (S1) or with 5 seconds on 5 seconds off (S2) sine wave for 20 or 60 or 120 minutes per day. The 5th lumbar vertebrae (LV) and left femurs were subjected to ex vivo DEXA scans and biomechanical testing.

Increase in ex vivo BMD of the 5th LV was observed in Group 1 (0.1934 ± 0.0121 with BaF 5,000; Bef 1; S1 for 20 minutes), Group 5 (0.1892 ± 0.0100 with BaF 5,000; BeF 1; S2 for 120 minutes), Group 9 (0.1917 ± 0.0175 with BaF 5,000; BeF 150; S1 for 60 minutes), as compared to that of OVX control (0.1793 ± 0.0151) and SHAM (0.2154 ± 0.0159). An increase in MaxLoad (N) was observed in Group 1 (242.6 ± 66.4) and Group 5 (240.8 ± 83.0), as compared to that of OVX control (189.4 ± 83.4) and SHAM (255.4 ± 43.6). A similar trend was observed for these groups for the ex vivo BMD and the MaxLoad in the femurs.

The ex vivo DEXA scans for BMD and biomechanical testing on the 5th lumbar vertebrae and femurs indicated that the SIS has the potential to increase bone mass in ovariectomized female rats with an 8-week stimulation period.

P520 W

EFFECT OF BETA-CRYPTOXANTHINE ON BONE FORMATION AND BONE RESORPTION IN TISSUE CULTURE: PREVENTIVE ROLE IN OSTEOPOROSIS

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Bone loss with increasing age induces osteoporosis. Chemical factors in food can help to prevent bone loss with aging.

The effect of beta-cryptoxanthine (CX), which greatly is present in fruits (especially orange), on bone metabolism is not been clarified so far. The effect of CX on bone formation and bone resorption was investigated in tissue culture in vitro.

Rat femoral-diaphyseal (cortical bone) and -metaphyseal (trabecular bone) tissues were cultured for 48 hours in Dulbecco's modified Eagle's medium supplemented with antibiotics and bovine serum albumin. The experimental cultures contained 0.01 to 10 micromole CX. The presence of CX (1 or 10 micromole) caused a significant increase in calcium content, alkaline phosphatase activity and deoxyribonucleic acid content in the diaphyseal and metaphyseal tissues. These increases were completely prevented in the presence of cycloheximide, an inhibitor of protein synthesis. Beta-carotene or xanthine had no effect on diaphyseal and metaphyseal calcium contents.

The bone-resorbing factors parathyroid hormone (PTH; 0.1 micromole) or prostaglandin E2 (PGE; 10 micromole) caused a significant decrease in calcium content in the diaphyseal and metaphyseal tissues. The decrease in bone calcium content induced by PTH or PGE was completely inhibited by CX. In addition, CX completely inhibited the PTH- or PGE-induced increase in medium glucose consumption and lactic acid production by diaphyseal and metaphyseal tissues. The inhibitory effect of CX on PTH- or PGE-stimulated decrease in the diaphyseal calcium content was significantly prevented in the presence of vanadate, an inhibitor of protein tyrosine phosphatase. Vanadate alone did not have a significant effect on calcium content and lactic acid production in control bone tissues.

The present study demonstrates that beta-cryptoxanthine (CX) has a direct stimulatory effect on bone formation and an inhibitory effect on bone resorption in tissue culture in vitro. Presumably, the intake of dietary CX has a preventive effect on osteoporosis. This finding is the first time in our knowledge.

P521 F

EFFECTS OF RALOXIFENE ON BONE MINERAL DENSITY, BONE METABOLISM AND SERUM LIPIDS IN CHINESE POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS

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Aim: To determine the effect of raloxifene (RLX) on lumbar spine and total hip bone mineral density (BMD), bone metabolism and serum lipids in Chinese postmenopausal women with osteoporosis.

Methods: Postmenopausal women with osteoporosis, 50-80 years of age and with or without prevalent fractures, were eligible to participate in this multi-center, double-blind, placebo-controlled, randomised trial. 204 postmenopausal women (mean age 65.3 yr +6.0 sd) were randomised to receive RLX (60 mg) or placebo treatment daily for 12 months. BMD was measured by dual energy x-ray absorptiometry.

Results: There were no significant differences between the two treatment groups at baseline. The subjects receiving RLX showed significant increases in lumbar spine and total hip BMD compared with those receiving placebo (both between-group $p<0.001$). For lumbar spine BMD, the mean increase was 3.3% with RLX and 1.0% for placebo. For total hip BMD, the mean increase was 1.4% with RLX, with a mean decrease of 0.9% with placebo. No new vertebral fractures were observed in the RLX group while 5 subjects in the placebo group had one or more new fractures (Fisher's exact $p=0.059$). RLX treatment produced significant decreases in serum osteocalcin and C-telopeptide levels: the median decrease was 5.4 ng/ml (41.6%) for osteocalcin and 2458 pmol/L (61.5%) for C-telopeptide (both $p<0.001$). These decreases in serum osteocalcin and C-telopeptide were significantly greater than in placebo subjects (both between-group $p<0.001$). RLX had favourable effects on total and LDL cholesterol levels and no significant effect on HDL cholesterol and triglycerides compared with placebo treatment. Six subjects discontinued the study due to an adverse event (1 in the RLX group, 5 in the placebo group). There were no significant differences between the two groups in the incidence of hot flushes (3 subjects with RLX, 1 with placebo) or muscle cramps (9 subjects with RLX, 4 with placebo) and no venous thromboembolic events were reported.

Conclusion: Daily RLX treatment for 12 months increased lumbar spine and total hip bone mineral density (BMD), decreased bone turnover and had favourable effects on serum lipids in Chinese postmenopausal women with osteoporosis.

P522 S

INCREASE IN BONE FORMATION INDUCED BY LOW-LEVEL MECHANICAL STIMULI IS FREQUENCY DEPENDENT IN OVARIECTOMIZED RATS

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Extremely low-magnitude mechanical vibrations producing strain magnitudes in the weight-bearing skeleton of less than 5ue can be anabolic to bone and prevent the decrease in anabolic activity related to mechanical disuse. Here, we used the ovariectomized (OVX) rat model to assess the efficacy and frequency-dependency of these mechanical signals in the hormonally challenged skeleton. Retired breeders (Sprague-Dawley) were OVX and subsequently subjected to low amplitude (0.3g) mechanical loading at either 45Hz (n=6) or 90Hz (n=6) for 10 min/day, or served as OVX controls (n=6). Following 28d, bone histomorphometry was used to monitor static and dynamic indices of bone formation in the metaphyseal proximal tibia while micro-computed tomography assessed micro-architectural trabecular changes in the metaphysis of the distal femur. Mechanical stimulation at 90Hz increased bone formation rates in OVX rats by 160% ($p<0.05$), mineral apposition rate by 60% ($p<0.05$), and the percentage of mineralizing surface by 50% when compared to age-matched control rats. Despite this increase in bone formation, tibial trabecular bone volume was not significantly affected. In stark contrast to the 90Hz mechanical vibrations, low-level mechanical stimulation at 45Hz was ineffective at increasing anabolic activity. In the distal femur, no differences in either bone quantity or architecture were detectable between OVX controls and the two stimulated groups. These data indicate, that even skeletons deprived of estrogen are capable of responding to low magnitude mechanical vibrations when these stimuli are applied at very high frequencies (90Hz). The ability of the skeleton to discriminate between the two frequencies used here is intriguing, particularly as both of them, at least in the context of habitual mechanical loading events, are considered to be physiologically high. While the complex mechanical environments induced by vibration at both the tissue and cellular level are not yet understood, preliminary strain gage recordings indicate that tissue-level strain magnitudes induced at 90Hz are significantly lower than vibrations induced at 45Hz, corresponding perhaps to the reduced displacement. Thus, it is entirely possible that factors other than matrix strain, per se, are regulating the anabolic response, such as intramedullary pressure, fluid flow, or neuromuscular feedback. The data do indicate, however, that these low-level stimuli could be used as an effective means of stimulating new bone formation, and perhaps in combination with antiresorptive therapies, could stimulate bone gain.

P523 W

EFFECT OF FOUR DIFFERENT THERAPY OPTIONS ON BONE DENSITY AND BONE METABOLISM PARAMETERS - A RETROSPECTIVE FIELD STUDY

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Are the therapeutic effects, observed in experimental studies, of calcium/vitaminD, bisphosphonates, fluorides or estrogens on bone density and -turnover reflected in everyday clinical practice?

This is an authentic field study since no publication had initially been planned. The parameters obtained between 1992 and 2001 (168 women, 67 men, average age 62.2±10.2 years, osteoporosis out-patient unit), have been evaluated retrospectively. Records were kept of height/weight, risk factors, laboratory data, BMD, Z-score and T-score (DXA). After filtering the data, the following were evaluated: bone mineral density (BMD), T-score and desoxypyridinoline (four treatment groups: calcium/vitaminD, bisphosphonates, fluorides and estrogens, observation period: 60 months subdivided into periods 1-4). Statistics: Exzess, Exel and SPSS for Windows.

For older, lighter and smaller patients bisphosphonates or estrogens were used more often than calcium/vitaminD or fluorides. Major changes in the parameters were observed up to period 3 (end of the treatment), followed by stable courses.

Bisphosphonate patients had the lowest T-score at the beginning (3.59±0.84, $p<0.05$), calcium/vitaminD patients the highest (-2.66±0.73). The most evident rise in the T-score occured with bisphosphonates (+0.89), the lowest with calcium/vitamionD (+0.29).

Before therapy the T-score was signifikant lower in the bisphosphonate group as compared to the estrogen group. This difference, however, disappears in the course of therapy with bisphosphonates.

The highest increase in BMD was achieved with bisphosphonates (+0.1g/cm2, $p<0.05$). There was less increase with calcium/vitaminD (+0.06), fluorides (+0.06) and estrogens (+0.05). Calcium/vitaminD led more rapidly to an increase in BMD than estrogen (+0.03g/cm2 vs. +0.01g/cm2, period 1 vs.2).

Desoypyridinolin in the urine was initially highest in bisphosphonate patients (7.11±3.65nmol/mmol Crea, $p<0.05$) and lowest in fluoride patients (4.55±2.18, $p<0.05$). The strongest decline in desoxypyridinoline was observed in the calcium/vitaminD group (-0.9nmol/mmol Crea), followed by the bisphosphonates (-0.8), and the estrogen group (-0.3). With fluorides desoyxpyridinoline rose (+0.2). Only estrogens continuously lower desoxypyridinoline.

Bisphosphonates have the strongest therapeutic effects.

Calcium/vitaminD or estrogens lower desoxypyridinoline and increase bone density.

Fluoride results in an increase in bone density, but the desoxypyridinoline increases.

P524 F

IMPROVED BONE MINERAL DENSITY IN POSTMENOPAUSAL WOMEN ON LONG-TERM TREATMENT WITH LOOP DIURETICS

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Background: in a previous study, we found an altered diurnal rhythm in plasma PTH levels in response to short-term treatment with loop diuretics, with a marked increase in PTH levels two hours after intake of tablets.

Aim: to study whether long-term treatment with loop-diuretics (LD) affects calcium homeostasis and bone metabolism.

Subjects and methods: in a cross-sectional design, 140 postmenopausal women treated with a loop diuretic (LD-group) for more than two years (median seven years) were compared to 140 age- and gender-matched population based controls. We measured bone mineral density (BMD), body composition and plasma levels of calcitropic hormones (PTH and 25-hydroxyvitamin D) and biochemical markers of bone turnover (osteocalcin, bone-specific alkaline phosphatase, and CTx). Study groups were compared by two-sample tests and associations between measured variables were assessed by multiple stepwise regression analysis.

Results: in the LD- compared to the control-group, bone mineral density (BMD) was increased at the spine (+7.5%, $p<0.001$), hip (+4.8%, $p<0.001$), and forearm (+3.7%, $p<0.001$). Plasma PTH was 37% higher ($p<0.001$), whereas 25-hydroxyvitamin levels were 20% lower ($p<0.001$) in the LD- than in the control-group. Levels of biochemical bone markers did not differ between groups. Body composition showed a significantly higher fat- and lean tissue-mass at all regions, and scale body weight was 17% higher ($p<0.001$) in the LD- compared to the control-group. The regression analyses showed that body weight was a consistent independent predictor of BMD at all measuring sites, whereas no association was found between treatment with loop diuretics and BMD at any measuring site. However, within the LD-group, BMD at the lumbar spine was positively associated with duration of LD-treatment ($p=0.01$), body weight ($p=0.001$), menopausal age ($p=0.002$), and PTH ($p<0.01$). Moreover, independent predictors of whole body BMD were duration of LD-treatment ($p=0.04$), body weight ($p=0.001$), and menopausal age ($p=0.001$). Finally, treatment with LD remained associated with increased PTH levels ($p=0.002$) even after correction for plasma 25-hydroxyvitamin D levels.

Conclusion: treatment with loop diuretics is not harmful to bone. Long-term treatment may improve BMD. This may suggest a potential bone anabolic effect of loop diuretics mediated through increased PTH levels.

P525 S

EFFECTS OF RALOXIFENE ON BONE MINERAL DENSITY, BONE METABOLISM AND SERUM LIPIDS IN HEALTHY CHINESE POSTMENOPAUSAL WOMEN

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Objective: To determine the effect of raloxifene (RLX), on bone mineral density (BMD), bone metabolism markers and lipids in healthy Chinese postmenopausal women.

Methods: This was a multicenter, double-blind, placebo-controlled, randomized study conducted in China with a total of 204 healthy postmenopausal women (mean age 59.5 yr ±5.0 sd and weight 62.8 kg ±8.7 sd) treated with either RLX 60 mg (n=102) or placebo (n=102) daily for 12 months. BMD was measured by dual-energy x-ray absorptiometry.

Results: There were no significant differences in baseline characteristics between the two treatment groups. Compared to placebo, RLX produced a significant increase in both total lumbar spine and total hip BMD. For the lumbar spine, percentage increase in total BMD was 2.3% with RLX compared to a decrease of 0.1% with placebo (between-group p less than 0.001). Corresponding values for total hip BMD were increases of 2.5% for RLX and 1.1% for placebo (between-group p=0.011). The biochemical markers of bone metabolism, serum osteocalcin and serum C-telopeptide, decreased significantly with RLX compared to placebo. For serum C-telopeptide, the median decrease was 894.5 pmol/L (24.0%) with RLX compared to a median increase of 603.0 pmol/L (15.8%) with placebo (between-group p less than 0.001). The median changes in serum osteocalcin were decreases of 4.0 ng/ml (27.7%) and 1.5 ng/ml (10.6%) with RLX and placebo, respectively (between-group p less than 0.001). Both total cholesterol and low-density lipoprotein cholesterol decreased significantly with RLX compared to placebo (both between-group p less than 0.001). There were no between-group differences for high-density lipoprotein cholesterol or triglyceride levels. Five patients reported serious adverse events, with endometrial carcinoma, cardiac neurosis, enlarged uterine fibroids and vascular disorder for RLX and angina pectoris for placebo (between-group p=0.174). There were no significant differences between groups for vasodilatation (13 patients with RLX and 8 patients with placebo) or leg cramps (13 patients with RLX and 8 patients with placebo), and deep venous thrombosis was not reported in either group.

Conclusions: This study confirms that RLX exerts positive effects on the skeleton, increasing BMD and decreasing biochemical markers of bone metabolism, and has a positive effect on the overall serum lipid profile in healthy Chinese postmenopausal women.

P526 W

HESPERIDIN, A CITRUS FLAVONOID, INHIBITS BONE LOSS AND DECREASES SERUM AND HEPATIC LIPIDS IN OVARIECTOMIZED MICE

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The purpose of this study was to examine whether or not hesperidin inhibits bone loss in ovariectomized mice (OVX), an animal model of postmenopausal osteoporosis. Forty 8-wk-old female ddY mice were assigned to five groups: sham-operated group (AIN93G), OVX group (AIN93G), OVX+HesA group (AIN93G+hesperidin diet), OVX+HesB group (AIN93G+alpha-glucosylhesperidin diet) and OVX+E_2 group (AIN93G+17beta-estradiol 0.03 microg/d with a mini-osmotic pump). After 4wk, the mice were killed and blood, femoral, uterine and liver was sampled immediately. Hesperidin administration did not affect the uterine weight at all. In OVX mice, BMD of the femur markedly decreased, and this bone loss was significantly prevented by the intake of diet containing hesperidin or alpha-glucosylhesperidin. The Ca, P and Zn contents in the femur were significantly higher in the hesperidin-administered groups than those in OVX group. Histomorphometry showed that the trabecular bone volume and trabecular thickness in the femoral distal metaphysis was markedly decreased by OVX, and alpha-glucosylhesperidin significantly prevented this bone loss. Furthermore, hesperidin decreased the osteoclast number of femoral metaphysis in OVX mice, as did E_2. Serum and hepatic lipids were decreased by intake of the

hesperidin-containing diet. These results suggest that the intake of citrus flavonoid hesperidin prevents bone loss as well as the decrease of cholesterol level in serum and liver.

P527 F

CONCURRENT TREATMENT OF OVARIECTOMIZED RATS USING TWO OF RISEDRONATE, 1-ALPHA-OH VITAMIN D_3, AND VITAMIN K_2

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Although concurrent therapy of osteoporosis has been frequently employed in daily practice, there has been little data regarding what combination of agents improves the effect of each single regimen. Thus, we investigated the efficacy of concurrent treatments in ovariectomized rats. Eight-months-old female Wistar rats were divided into the following 11 groups (8 rats in each group): BC, S3, O3, S9, O9, D, K, D+K, R, R+D, and R+K groups. BC rats were sacrificed at the baseline of the experiment. S3 and S9 rats were sham-operated and sacrificed 3 months and 9 months after operation, respectively. The other 8 groups of rats were ovariectomized. O3 and O9 rats were sacrificed 3 months and 9 months after ovariectomy, respectively. The remaining 6 groups of rats were treated for 6 months, from 3 months till 9 months after ovariectomy, with the following drugs: 1) 1-alpha-OH vitamin D_3 (0.1 micro-g/kg/d po, 5 d/w) (D); 2) vitamin K_2 (7 mg/kg/d po, daily) (K); 3) D+K; 4) risedronate (2 mg/kg sc, 2 d/w) (R); 5) R+D; and 6) R+K. These rats were sacrificed at the end of treatment, 9 months after ovariectomy. Bone mineral density (BMD) of lumbar spines (L2-4) was compared among the groups. Nine months after ovariectomy, the mean BMD was significantly lower in O9 rats than in S9 rats. Although the mean BMDs in K, R, and R+K rats were not significantly different from the mean value in O9 rats, the mean BMDs in D, D+K, and R+D rats were significantly higher than the mean BMD in the O9 rats. The mean BMD in the R+D rats was significantly higher than that in the D or D+K rats. In this animal model of osteoporosis, with the drugs at the given doses, D with or without K or R was significantly effective in increasing BMD to or over the level of the sham-operated rats. The combination of D and R has been shown to be more effective in increasing the lumbar BMD than each single administration of these drugs.

P528 S

CLINICAL ANALYSIS OF 156 OLD PATIENTS WITH HIP FRACTURE

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Objective: To report the treatment of hip bone fracture in the senile patients,to analyse its characteristics and key points in diagnosis and treatment .

Methods: All 156 cases over 60 years(age ranged from 60 to 93)with hip fracture were treated from January 1997 to December 1999.With male 55 cases, average 71.8 years, female 101 cases, average 73.1 years.Bone fracture types include the femoral neck fracture (94 cases), among those, male (20), female (74); The femoral intertrochanteric fracture (62 cases), male(35), female (27). 41 cases were treated with non-operational methods; 115 patients received operational treatment,among them 45 cases were treated with internal fixation, 68 cases were replaced with artificial femoral head, 2 cases were ablated femoral head and neck. Some were given drugs of osteoporosis.

Results: 110 cases were followed up from 0.5 to 3.5 years. Two cases were venous thrombosis in low limbs after operation, two cases had femoral head ischemia and necrosis,the moving and slipping of internal fixation were 2 cases, and the internal fixation break-up was one case, pain caused by artificial femoral head sinking were 4 cases, the death shortly after operation were 2 cases (died of cardiac infarction and respiratory infection), 97 cases got satisfactory result (88.2%).

Conclusion: 1.Hip bone fractures are mostly seen in senile woman (64.7%);while less in senile male (35.3%), which related to the osteoporosis after menopause in female. Femoral neck fractures are mostly seen in senile female, while femoral intertrochanteric fractures are mostly seen in senile male,when over 70 years, the hip bone fracture occurs more frequently than before, which is related to the osteoporosis,it shows that the bones fracture occurrence rate increases with the age, the danger of it increases too.

2.The hip bone fracture in the senile man belongs to that of osteoporosis, but the femoral intertrochanteric fracture has obvious injury history. The femoral neck fracture is often with slight force(torsion), so we should make a right diagnose and try to prevent omitting or mistaking, which will have a negative effect.

3.We should treat the osteoporosis together with fracture, which is of great significance in relieving bone pain,promoting bont healing and preventing hip bone from re-breaking up.

P529 W

DW1350, A NEWLY SYNTHETIC ANTI-OSTEOPOROTIC AGENT: 2. EFFECT ON OVARIECTOMIZED OSTEOPOROSIS MICE MODEL,A HISTOMORPHOMETRICAL ASPECT

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In the present study, the effect of DW-1350, a newly synthesized anti-osteoporotic agent, was evaluated in ovariectomized mice. Female ddY mice underwent bilateral ovariectomy for prevention study that test article was administered from 2 days after ovariectomy for one month, for therapeutic study it was conducted from 3 weeks after ovariectomy for three months. Body weight, bone weight and histological profiles of epiphyseal regions of tibia and femur such as cortical bone thickness, trabecular bone number, thickness and length with trabecular bone volume percentage (TBV), were observed respectively. In addition, formation of osteoclast cell was also monitored in *ex vivo* bone marrow cell culture and numbers of multinucleated TRAP positive cells were counted. Results were compared to that of alendronate, well-documented anti-osteoporotic agents. Histomorphometrical changes and TRAP positive cells were observed or calculated using image analyzer system, AnalySIS-auto (SIS Co., Germany). In prevention and therapeutic studies, DW-1350 showed favorable inhibitory effect to histomorphometrical changes induced by ovariectomy. We can also find that DW-1350 dose-dependently (10 or 50mg/kg, *p.o*) increased the TBV, trabecular bone length and width, and cortical bone width or decreased the osteoclast cell activity. Especially more favorable effects were observed in DW-1350 compared to that of alendronate on cortical bone thickness. Base on these results, DW-1350 may act as both a suppressor of bone resorption and an enhancer of bone formation *in vivo*. In addition, DW-1350 significantly decreased osteoclast cell formation in the *ex vivo* marrow cell culture dose dependently. In conclusion, it should be suggested that DW-1350 has enough and favorable effect to prevention and therapy of estrogen-deficient osteoporosis.

P530 F

THE EFFECT OF MENATETRENONE ON BIOCHEMICAL MARKERS IN BONE REMODELLING

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OBJECTIVE: To determine the influence of vitamin K2 on the blood levels of undercarboxylated osteocalcin (ucOC) and bone mineral density (BMD) among Thai postmenopausal women.

METHODS: 83 Thai postmenopausal women were recruited in the study and randomized into the control group (n=40) and the treatment group (n=43). Every woman received elemental calcium carbonate 800 mg / day and the treatment group received vitamin K2, 45 mg /day in addition. Serum NMID osteocalcin, betacrosslab (CTx), total alkaline phosphatase and ucOC were measured at baseline, 1, 3, 6 and 12 months after. BMD measurement was performed at baseline, 6 and 12 months after. At the end of 6 months, the calcium group was shifted to the vitamin K2 group for another 6 months in order to confirm the efficacy of vitamin K2 on BMD and ucOC level.

RESULTS: The serum level of ucOC in the vitamin K2 treated group were 10.47 ± 3.22, 9.57 ± 1.20, 5.31 ± 0.89, 4.17 ± 0.63 and 1.35 ± 0.39 ng/dl at baseline, 1, 3, 6 and 12 months after treatment respectively. The serum level of ucOC in the vitamin K2 treated group showed a significant decrease from the baseline at each point of observation (60.17%, p=0.0001 at 6 months and 87.11%, p=0.0001 at 12 months). At the end of 6 months, the calcium treated group was switched to the vitamin K2 treated group for another 6 months and showed a decrease in the level of ucOC and CTx to the same extent as the original vitamin K2 treated group. At the conclusion of the study, BMD of the hip did not increase in both groups, but BMD of the lumbar spine increased by 0.6% in vitamin K2 treated group and CTx was decreased by 58.84% (p=0.0001) compared to the baseline.

CONCLUSION: This study demonstrated an effect of vitamin K2 in decreasing level of ucOC which enhanced bone matrix formation and decreasing bone resorption. Vitamin K2 is suitable for normalize bone remodelling in postmenopausal osteoporosis patients. This is pilot study and the large scale study will be continued.

P531 S

DW1350, A NEWLY SYNTHETIC ANTI-OSTEOPOROTIC AGENT FOR TREATMENT OF OSTEOPOROSIS

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Most compounds currently available for the treatment of osteoporosis (eg. estrogen, calcitonin, Vitamin D, bisphosphonate, SERMs etc.) are bone resorption inhibitors.

The purpose of this research is to develop a new anti-osteoporotic agent which has both bone formation stimulating and bone resorption inhibiting effect without altering the mechanical properties of bone.

Our *in vitro* research for DW 1350, new synthetic anti-osteoporotic agent showed good inhibitory efficacy of osteoclastic bone resorptive action from osteoclatogenesis, fusion and fit formation assay, as well as favorable stimulating efficacy of osteoblastic bone formation from ALP and bone nodule assay. *In vitro* histomorphomertrical observation, we can find that the loss of trabecular bone of ovariectomized and neurectomized mice was suppressed after administration (10 or 50 mg/kg, *P.O*)of DW1350. In *ex vivo* of these two models, DW1350 significantly decreased osteoclast cell formation. In addition, DW1350 showed no abnormal signs on gastric ulcer healing on the indomethacin induced gastric ulcer rat model, quite differed from those of alendronate, which were induced more serve and numerous ulcerative lesions in that models.

In conclusion, it should be suggested that DW1350 has favorable effect to prevention and therapy of osteoporosis. In addition, DW1350 suppressed the loss of trabecular bone as well as cortical bone. Therefore, these results suggest that DW1350 might be a promising candidate, for treatment of osteoporosis.

P532 W

DW1350, A NEWLY SYNTHETIC ANTI-OSTEOPOROTIC AGENT: 1. DW-1350 INHIHITED BONE RESORPTION AND PROMOTED BONE FORMATION

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DW-1350, a synthetic benzamidine derivative compound, was selected on a new class of bone sparing candidates through the *in vitro* randomized screening studies on bone cells. We identified inhibitory activities of DW-1350 for each step of osteoclast differentiation, fusion and pit formation process in co-culture system with bone marrow of mice and primary osteoblasts ranging from 0.1nM to 1000nM. As a result of these studies, we could find that DW-1350 suppressed the formation of TRAP-positive osteoclasts induced by $1a,25(OH)_2D_3$, PTH, PGE_2 and IL-11 with 50% inhibitory range of 10nM~100nM. This study also showed that DW-1350 exhibited significant decrease of the fusion process to mature osteoclasts and the bone-resorbing activity measured by pit number formed on dentine slice in a dose-dependent manner. Treatments of DW-1350 in $1a,25(OH)_2D_3$-stimulated primary calvarial cells were showed highly increased level of osteoprotegerin(OPG) mRNA expression. On the other hand, DW-1350 affected cell proliferation and stimulated ALP activity and mRNA expression of differentiation marker genes using osteoblastic MC3T3-El cells and primary osteoblasts. And we identified the positive effects of DW-1350 on bone nodule formation determined by amount of minerals deposited on the formed bone matrix.

These results suggest that DW-1350 might be a promising agent for treatment of osteoporosis not only by inhibiting osteoclast formation and bone-resorbing action, but also by stimulating osteoblast differentiation

P533 F

DW-1350, A NEWLY SYNTHESIZED ANTI-OSTEOPOROTIC AGENT: 3. EFFECT ON SCIATIC NEURECTOMIZED IMMOBILIZATION OSTEOPOROSIS MICE MODEL, A HISTOMORPHOMETRICAL ASPECT

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In the present study, the effect of DW-1350, a newly synthesized anti-osteoporotic agent, was evaluated in sciatic neurectomized mice. Female ddY mice underwent bilateral ovariectomy for prevention study that test article was administered from 2 days after ovariectomy for one month, for therapeutic study it was conducted from 3 weeks after ovariectomy for three months. Body weight, thickness of hind limb, bone weight and histological profiles of epiphyseal regions of right tibia such as cortical bone thickness, trabecular bone number, thickness and length with trabecular bone volume percentage (TBV), were observed. In addition, formation of osteoclast cell was also conducted in *ex vivo* bone marrow cell culture and numbers of multinucleated TRAP positive cells were counted. The results were compared to that of alendronate, well-documented anti-osteoporotic agents. Histomorphometrical changes and TRAP positive cells were observed or calculated using image analyzer system, AnalySIS-auto (SIS Co., Germany). In prevention and therapeutic study, DW-1350 showed favorable inhibitory effect to histomorphometrical changes induced by neurectomy. We can also find that DW-1350 dose-dependently (10 or 50mg/kg, *p.o*) increased the TBV, trabecular bone length and width, and cortical bone width or decreased the osteoclast cell activity. Particularly, more favorable effects were demonstrated in DW-1350 compared to that of alendronate on cortical bone thickness. Base on these results, DW-1350 may act as both a suppressor of bone resorption and an enhancer of bone formation *in vivo*. In addition, DW-1350 significantly decreased osteoclast cell formation in the *ex vivo* marrow cell culture dose dependently. In conclusion, it should be suggested that DW-1350 has enough and favorable effect to prevention and therapy of immobilization osteoporosis.

P534 S

THE EFFECTS OF ALENDRONATE, AND ELCATONIN ON THE PROCESSES OF FRACTURE REPAIR IN CYNOMOLGUS MONKEYS (MACACA FASCICULARIS)

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We investigated the effects of antiresorptive agents-calcitonin and alendronate, on the processes of fracture repair in the femora of cynomolgus monkeys (Macaca fascicularis). Twelve monkeys were randomized into control group (CNT), elcatonin group (ECT), and alendronate group (ALN). Animals in ECT and ALN were subcutaneously injected respectively with elcatonin (5U/kg/day) and alendronate (100ug/kg/day), while animals in CNT were injected with saline vehicle. After 3 weeks pretreatment, transverse osteotomies were performed at the midshafts of right femora for all monkeys and fixed with AO plates. Injection was performed twice a week until euthanasia at 26 weeks post-fracture. Soft x-ray radiography, contact microradiograph, Micro-CT, biomechanical testing, and histomorphometry were performed. Both elcatonin and alendronate clearly delayed the fracture lines disappearance, while only alendronate induced a significantly larger endosteal callus than CNT group. Both elcatonin and alendronate significantly reduced N.Oc/BS in callus, however only alendronate significantly suppressed callus remodeling accompanied with much more woven bone in callus. Biomechanical properties of callus were not significantly different among the three groups, suggesting that the larger callus in ALN appeared to be a morphological adaptation to restore the mechanical strength of the fracture with inferior material

P535 W

COMPARATIVE EFFICACY OF HORMONE REPLACEMENT THERAPY, ETIDRONATE, CALCITONIN, VITAMIN D AND VITAMIN K IN POSTMENOPAUSAL OSTEOPOROSIS

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This study was conducted to assess the comparative efficacy of several pharmaceutical agents in postmenopausal women with osteoporosis. A total of 377 postmenopausal osteoporosis, aged 50 to 75 years, were randomly allocated into six groups: hormone replacement therapy (HRT); etidronate; calcitonin (CT); vitamin D3 (D3); vitamin K2 (K2); and control (no treatment). Thoracic and lumbar spine radiographs were taken every 3 months during the 2-year study period. Bone mineral density (BMD) at distal 1/3 radius was measured at baseline and at every 3 months, along with markers of bone turnover [serum bone specific alkaline phosphatase (BAP), serum osteocalcin, urinary N-telopeptide of type I collagen (NTX), and urinary deoxypyridinoline (DPD)]. Changes in BMD after the 2-year treatment in HRT, etidronate, CT, D3, K2 and control was 2.1%, -1.1%, 1.8%, -3.6%, -1.9% and -3.3%, respectively. The new vertebral fracture incidence in HRT, etidronate, CT, D3 and K2 was reduced by 58%, 42%, 50%, 25% and 33% versus control, respectively. Logistic regression analysis revealed that changes in BMD at month 3 significantly predicted changes in BMD after 2 years (odds ratio: 2.39 in HRT; 8.54 in etidronate; 9.80 in CT; and 5.38 in D3) and the new vertebral fracture risk (odds ratio: 2.17 in HRT; 2.54 in etidronate; and 3.53 in CT). Changes in NTX and DPD after 3 months were significant predictors of the incidence of new vertebral fractures (odds ratio: 1.83 and 2.02 in HRT, respectively; 2.04 and 1.87 in etidronate; 3.07 and 2.07 in CT). None of factors predicted BMD change or new vertebral fracture incidence in K2. In conclusion, antiresorptive medications, such as HRT, CT and etidronate are more potent to reduce the risk of vertebral fractures. An increase in BMD plays an important role in reducing the vertebral fracture risk, but other mechanisms, not measured by BMD, are also important particularly in D3 and K2. This study demonstrates the clinical utility of changes in BMD and markers of bone resorption at month 3 to predict both BMD change and fracture risk in response to antiresorptive pharmacologic treatments in postmenopausal osteoporosis.

P536 F

EFFICACY OF FOSAMAX VS. EVISTA COMPARISON TRIAL (EFFECT-INTERNATIONAL): RESULTS AT 6 MONTHS

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This multinational study was designed to compare the efficacy of alendronate and raloxifene when used for treatment of osteoporosis in postmenopausal women. We presented here the 6 month results from this 12 month study.

This study population comprised 487 postmenopausal women at 50 centers in 15 countries representative of Eastern and Western Europe, Asia-Pacific, and South America. Patients were randomly assigned in a 1:1 ratio to receive alendronate 70 mg once weekly (Fosamax, Merck & Co., Inc.) and raloxifene placebo daily or raloxifene

60 mg daily (Eli Lilly) and alendronate placebo once weekly. This interim analysis assessed the response in markers of bone turnover at 6 months. The markers measured were urinary NTx and serum BSAP.

The mean age of women enrolled was 62 years (range 42 to 90 years). 79% were Caucasian. They were on average 15 years post menopause. At 6 months, the decrease in urinary NTx from baseline in the alendronate group was 68.1%, compared to a decrease of 28.6% in the raloxifene group (P<0.001). The decrease in serum BSAP from baseline in the alendronate group was 43.8%, compared to a decrease of 11.1% in the raloxifene group (P<0.001). For both urinary NTx and serum BSAP, changes from baseline within treatment group were significant for both treatments (P<0.001 for both alendronate and raloxifene).

This study is planned to continue through a 12 month treatment duration. The 6 month results presented here indicate that weekly alendronate provides larger decreases in bone turnover than does daily raloxifene, and is therefore a more potent antiresorptive agent when used for treatment of osteoporosis in postmenopausal women.

P537 S

COMPARISON OF THE TISSUE PROFILES OF PROSTAGLANDIN EP RECEPTORS IN RAT, CYNOMOLGUS MONKEY AND HUMAN

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The evidence that PGE2 increases bone mass in many different species of animals has been well documented. Recently, sufficient evidence has supported the hypothesis that the bone anabolic effect of PGE2 is mediated mainly through EP4, a subtype receptor of PGE2. EP4 receptors are expressed in many tissues other than bone, which may complicate the development of an EP4 agonist as a therapeutic bone anabolic agent. Since physiological functions may be regulated by more than one type of prostanoid receptor, it is necessary to understand the tissue distribution of EP receptor subtypes. Additionally, the receptor profile may vary from species to species. In order to determine an appropriate animal species to predict human response to PGE2 agonists, we have compared the tissue profile of EP receptors in rat, cynomolgus monkey, and human. Total RNA from multiple tissues (brain, heart, liver, lung and kidney) of animals and humans were obtained. The expression of EP1, EP2, EP3 and EP4 was analyzed by quantitative PCR (Taqman) after RT reactions. Primers and probes were designed to ensure amplifications of equal efficiency by PCR. We found EP3 and EP4 to be more highly expressed than EP1or EP2 in all three species. The level of EP4 expressed in rat is lower than in monkey or human, and varies less from tissue to tissue. The level of EP4 expression in monkey and human are nearly identical in four tissues: brain, heart, liver and lung. However, EP4 is much more highly expressed in monkey than human in the kidney. Similar analyses were performed for the other EP receptor subtypes. Our data support the concept that the cynomolgus monkey is a better predictor of human response than the rat, and may be advantageous for evaluating the therapeutic window of EP4 agonist and other prostaglandin E analogs.

P538 W

ALBIZZIAE CORTEX PREVENTS BONE LOSS IN OVARIECTOMIZED (OVX) RATS

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Estrogen deficiency after menopause induces bone loss and results in osteoporosis. To find estrogenic efficacy from herbal extract, effect of Albizziae Cortex (AC) extract was investigated on osteoporosis *in vitro* and *in vivo* models. AC is the stem bark of *Albizziae julibrissin* Durazzini (Leguminosae). AC is used in Korea as tonics, to ease the mind and calm the nerves, antibacterial and antiparasitic drug. Proliferation of osteoblast (Saos-2) was tested with MTT and alkaline phosphatase (ALP) assays. Adult OVX SD rats (10 weeks old) were divided into four groups; Sham, control, 17beta-estradiol at 1 microg/kg/day (E2), and AC at 5 g/kg/day. Animals were given an oral administration everyday for 9 weeks (E2 was given an i.p. injection instead). Trabecular bone area (TBA) of tibia and lumbar were measured by bone histomorphometry. In results, AC increased cell proliferation and ALP activity of Saos-2 (113% and 135% of control). The TBAs of tibia and lumbar in control group was reduced 35% and 70% compared to sham (P<0.01). The TBAs of tibia and lumbar in AC group was increased upto 165% and 133% of control (P<0.01), which were better than that in E2 group (147% and 124% of control, P<0.05). In conclusions, extract of Albizziae Cortex prevents OVX-induced cancellous bone loss for 9 weeks in OVX rats. (Supported partially by a grant, #01-PJ2-PG6-01NA01-0002, from HPEB, Korea)

P539 F

EFFECT OF ALENDRONATE AND MENATETRENONE ADMINISTRATION ON BONE METABOLISM DURING GN-RH AGONIST TREATMENT FOR ENDOMETRIOSIS

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Objective: Present study was performed to elucidate whether bisphosphonate (alendronate) and menatetrenone administration could prevent bone loss more effectively than estrogen progestogen (EP) preparations add back therapy during Gn-RH agonist treatment for endometriosis.

Materials & Methods: Nine patients of endometriosis who were undertaken Gn-RH agonist treatment (Leuprorelin, 1.88mg/month) were subjected (Group A). They were administered alendronate (5mg/day) and menatetrenone (45mg/day) during Gn-RH agonist treatment for the prevention of bone loss. The bone mineral density (BMD) of the lumber spine was examined by DXA method before and 6, 12 months after the treatment. The biomarkers of the bone metabolism (urinary deoxy-pyridinoline (d-Pyr), for bone resorption marker, and plasma intact osteocalcin (OC), for bone formation marker) were also measured in the same time to evaluate the effects of these medications. The same examinations were also done in 14 cases of endometriosis who were undertaken Gn-RH agonist treatment with EP add back and menatetrenone administration (group B).

Results: There was a significant increase in BMD values of the group A during the treatment compared to those in the group B (A vs. B, 6 months: 1.22±0.25 vs. 1.17±0.18, 12 months: 1.26±0.21 vs. 1.19±0,36, p<0.01). Urinary d-Pyr levels in the both groups decreased significantly on 6 and 12 months after the treatment compared to the previous values, and there was also significant differences between group A and B after the treatment (6 months: 3.3±1.3 vs. 4.2±2.0, 12 months: 2.5±1.5 vs. 3.6±1.4 micro mol/mol.cre., p<0.02). There was no significant change in plasma OC levels between group A and B (6months: 8.2±2.5 vs. 8.4±3.0, 12 months: 8.9±2.9 vs. 9.1±3.3 ng/dl).

Conclusion: From these results, it was concluded that there was a beneficial effect for the BMD values during Gn-RH agonist treatment using alendronate and menatetrenone administration. And it was suggested that this method might be a standard regimen during Gn-RH agonist therapy for the patients who could not take EP preparations.

P540 S

ZOLEDRONIC ACID ADMINISTERED AS A SINGLE INTRAVENOUS DOSE PRESERVES CANCELLOUS BONE IN OVARIECTOMIZED RATS WITHOUT CAUSING 'FROZEN BONE'

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Bisphosphonates are potent suppressors of bone remodeling and exert a long-term bone protective effect in postmenopausal women. One of the main concerns with agents which strongly reduce activation frequency is that this may result in the accumulation of microdamage and eventually increased bone fragility. The aim of our study was to investigate the duration of action of a single intravenous dose of zoledronic acid (ZA), and to investigate its effect on bone formation parameters. Seven-month-old virgin Wistar rats were sham-operated or ovariectomized and treated with a single intravenous injection of ZA at doses of 0.8, 4, 20, 100 or 500 microg/kg. Changes in cancellous bone were measured in the proximal tibia metaphysis at 4-weekly intervals for 32 weeks by peripheral quantitative computed tomography. Osteocalcin levels were measured in serum at selected time-points. Dynamic histomorphometric parameters were evaluated at necropsy by prior administration of double fluorochrome labels at weeks 31 and 32 after i.v.-administration of ZA. OVX-rats showed rapid cancellous bone loss which equilibrated at 55% (p<0.01). Full protection of cancellous bone parameters was achieved at 20 microg/kg for up to 32 weeks, while higher doses even resulted in a 5% increase compared to sham-operated controls. At 20 microg/kg, all dynamic bone formation parameters in cancellous bone were at the level of sham-operated animals, while the two higher doses significantly suppressed the double-labeled surface, mineral apposition rate and bone formation rate to levels below those of sham-operated rats. Plasma osteocalcin in rats treated with 20 microg/kg ZA remained at the level of sham-controls throughout the study except at week 32 when it started to rise. At the two higher doses, plasma osteocalcin decreased significantly but the inhibition did not exceed 50% of the baseline value. These data clearly argue against a total suppression of bone turnover at any point in time, even for the highest dose of ZA. The results indicate that a single intravenous dose of 20 microg/kg ZA exerts a significant, long-term protection against cancellous bone loss for up to 32 weeks without suppressing bone formation below a critical level. Based on the different length of the bone remodeling cycle in rats and humans, these data support the use of an infrequent dosing regimen for ZA for the treatment of osteoporosis (Reid et al. NEJM 2002 346:653-661).

P541 W

METABOLIC BONE MARKERS AS TOOLS FOR MONITORING EFFICACY OF ALENDRONATE IN JAPANESE POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS

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Alendronate (ALN) is the third-generation bisphosphonates established as first-line agent for the treatment of osteoporosis. However, utilities of metabolic bone markers in Japanese individuals receiving ALN at a dose of 5 mg/day have not been fully investigated. Enrolled were 65 osteoporotic postmenopausal women who were informed to participate in the present prospective study with ALN and aged 67.5 years. More than 50 % of the patients showed high bone turnover as judged by the levels of resorption markers more than 2 SD above the level of premenopausal women. Lumbar spine BMD significantly increased by 4.8% from the baseline at 24 Weeks following the initiation of receiving ALN. Urinary free-DPD, total-DPD, and NTX were evaluated and significantly decreased to 19%, 23%, and 38%, respectively, from the baseline at 4 Weeks following the initiation of receiving ALN, although the significant changes in serum bone specific ALP (BAP) were observed later (-16% at 12 Weeks, and -29% at 24 Week, $p<0.05$). The magnitude of decrease of U-NTX was larger compared to that of f-DPD ($p<0.01$) and total-DPD ($p<0.05$). By the 4 Weeks, U-NTX in 80% of the patients went down within reference range (mean ±2 SD of premenopausal women). Intraindividual coefficient of variation (CVi) of each marker was estimated as double long term CV in calcium supplemented subjects. The proportion of responders who showed signal/noise ratio of over 1.0 was much higher in U-NTX than free/total DPD. These findings suggest that most of Japanese osteoporotic women respond metabolically to the treatment with smaller dose of ALN, and that correction of bone metabolism can be achieved as early as 4 Weeks if evaluated by U-NTX. The advantage of measurement of serum NTX over U-NTX will also be discussed. (Working Group on Bisphosphonate and Bone Markers in Japan)

P542 F

EARLY EFFECT OF STRONTIUM RANELATE ON CLINICAL VERTEBRAL FRACTURES IN WOMEN WITH POSTMENOPAUSAL OSTEOPOROSIS

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Strontium ranelate (SR) has been reported to increase bone formation and decrease bone resorption, an effect found in preclinical and clinical studies. Spinal Osteoporosis Therapeutic Intervention (SOTI), a phase III randomised, double blind, placebo controlled study, enrolled 1649 patients [age: 69.7 ±7.3; Lumbar BMD: 0.73 g/cm^2 ±0.12, mean (SD)]; 87.5% of patients had at least one prevalent vertebral fracture (2.2 prevalent VF per patient). Treatment using 2g/d orally during 3 years significantly ($p<0.001$) reduced the risk of new vertebral fracture (VF) by 41% in postmenopausal women with osteoporosis.

To assess the efficacy of SR on the incidence of clinical fractures (fractures with at least 1 cm body height loss and/or acute back pain), body height was measured with a Harpenden stadiometer by 2 successive measurements after repositioning.

The incidence of patients experiencing a new clinical VF was reduced by 38% over 3 years of treatment (RR=0.62, 95% CI [0.46;0.83], $p<0.001$) with an early reduction by 53% after the first year (RR=0.47, 95% CI [0.28;0.79], $p=0.003$).

The decrease in body height from baseline to last value was less pronounced in the SR group as compared to placebo ($p=0.006$). There was also a lower percentage of patients with a body height loss of at least 1 cm in the SR group as compared to placebo (30.1% versus 37.5% respectively, $p=0.003$).

The quality of life of the patients was assessed by a general scale, SF-36 and a specific scale, QUALIOST. Quality of life analysis showed a significant difference between treatment groups in favour of the SR group for all QUALIOST scores (physical and mental scores) as well as for the General Health perception score of the SF-36.

SR was well tolerated. The results suggest that SR reduces the risk of vertebral fractures early and that these effects are sustained, and are associated with improvement in the quality of life for specific issues related to osteoporosis.

P543 S

KATSURAGI CALCIUM STUDY: A RANDOMIZED, PROSPECTIVE DOUBLE-BLIND PLACEBO-CONTROLLED COMPARISON OF ACTIVE ABSORBABLE ALGAL CALCIUM WITH $CACO_3$ ON LUMBAR MINERAL DENSITY, SPINAL FRACTURE, SPONDYLOTIC DEFORMITY AND BODY COMPOSITION

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A prospective double-blind, placebo-controlled study on the effect of supplementation with 900 mg/day of calcium as active absorbable algal calcium (AAA Ca) or calcium carbonate on lumbar bone mineral density (BMD) carried out in elderly in-patients with osteoporosis at Katsuragi Hospital was re-evaluated as to the effect on vertebral fracture and spondylotic deformity. In addition to the already reported significant increase in lumbar bone mineral density after 2 years on supplementation with AAACa but not $CaCO_3$, AAA Ca but not $CaCO_3$ was found to inhibit new occurrence of vertebral fracture. Increase of intra-individual variation of L_1-L_4 BMD expressed by coefficient of variation indicating an augmentation of the degree of spondylotic deformity was also inhibited significantly by AAACa but not by $CaCO_3$. According to the whole body DXA study in the first and second year of the study, increase of fat content was significantly inhibited by AAACa but not by $CaCO_3$. As to the regional distribution of bone mineral content, AAA Ca but not $CaCO_3$ decreased head mineral content, reversing the general trend accompanying aging. In addition to increasing BMD and reducing facture, AAACa was found to inhibit spondylotic deformity and obesity.

P544 W

WITHDRAWN

P545 F

MAINTENANCE OF BONE PROPERTIES WITH ELECTRICAL STIMULATION IN IMMOBILIZED MODEL RATS

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This study investigated that electrical stimulation could prevent bone loss in sixty-four male Wistar rats aged 8 weeks. Rats were transected the sciatic nerve of both hind limbs (group B, C, D) or sham-operated (A). Group C and D were given 15 and 60 Hz electrical stimulation at the lower limbs, respectively. All the rats were sacrificed at week 1, 2, 3 and 4 weeks after operation. Three-point bending strength, dry bone weight, and ash content of tibia were measured. Two-way ANOVA and Fisher's PLSD were used to determine the effect of electrical stimulation on bone properties. p less than 0.05 was considered significant. Three-point bending strength, dry bone weight, and ash content of stimulated rats were significantly higher than those of denerved rats. Moreover, group D was kept bone properties compared with group C. Electrical stimulation inhibited bone loss of rat with the sciatic denervation. Electrical stimulation was considered to prevent bone loss with immobilization.

P546 S

DIAGNOSTIC AND PRESCRIPTION ATTITUDES IN OSTEOPOROSIS MANAGEMENT AMONG PHYSICIANS IN GREECE

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Aim: Attitudes and prescription practices differ considerably worldwide among specialists eligible to deal with osteoporosis .In order to evaluate this problem in Greece two hundred fifty osteoporotic women by WHO criteria, were randomly selected from an Osteoporosis Center in Greece, where the patients referred for evaluation.

Results: Mean age of the patients was 60±0.5 years (range: 43 to 83 years old) and mean age of diagnosis was 55±0.5 (range:39 to 73 years old). Of these patients 119 (46.85%) were originally diagnosed by an orthopedist, 59 (23.23%) by a rheumatologist, 20 (7.87%) by an endocrinologist, 7 (6.69%) by a general practitioner and 39 patients(15,35%) by a gynecologist. In case of severe osteoporosis, with or without a clinically apparent fracture, 63.0% of the patients were followed by an orthopedist and 20.55% by a rheumatologist. The original diagnosis of osteoporosis was based on DXA in 238 patients (93.33%), on U/S in 10 cases (3.92%) and on Xray of lumbar spine in 7 patients (2,75%). As a complementary diagnostic means an Xray was used by 61.86% of orthopedists, 31.67% of rheumatologists, 10% of endocrinologists, 41.48% of general practitioner and 10.26% of gynecologists. Chi-square analysis between specialty and Xray use revealed statistically significant differences ($p<0.0001$). The follow up of 39 patients (15,29%) was based on bone markers.

Analysis of 503 prescriptions concerning the attitudes among the various specialties showed that orthopedists prescribed mainly calcitonin (in 57.8% of their patients), rheumatologists mainly prescribed biphosphonates (53.42%), endocrinologists - HRT (50.85%), general practitioners - calcium or/and Vitamin D (29.79%), and gynecologists HRT (68.33%). Analysis of medication itself revealed similar results. Specialty and kind of treatment were correlated according to Chi-Square analysis ($p<0.0001$).

Conclusions: The main differences among specialists were noted in the therapeutic management of osteoporotics. Further studies are needed to confirm this problem and to explain the different attitudes among physicians that treat osteoporotic patients.

P547 W

AKG A POTENTIAL THERAPEUTIC DRUG AGENT IN THE TREATMENT OF OSTEPOROSE LIKE SCELETEAL SYSTEM DISORDERS.

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Trabecular bone losses are the primary type of bone compromise in postmenopausal osteoporosis. The greatest losses of bone density is seen in the femoral neck, lumbar vertebrae, and enriched sites of trabecular bone.

Recently it was proven that alpha ketoglutarate in the gut is partly converted to proline. Proline is an imported substrate for pro collagen syntheses where is converted to hydroxyproline. Collagen determines trabecular bone formation and thus, is the factor of bone elasticity and strenght.

The purpose of the present study was to investigate the effect of alpha ketoglutarate (AKG), as an anabolic agent for the treatment of osteoporosis on the rat model with established osteopenia.

Material and methods: The experiment was carried out on 40 females Wistar rats b.wt 200 g ± 10 at the starting age of 2 months with free access to food and tap water. The rats were divided into 2 groups; shame operated SHAM and ovariectomized OVX. The duration of the study was 7 months after bilateral ovariectomy/sham operation and 2 months administration of AKG and placebo drinks.

Blood was collected at the end of the study. Both femurs were removed from each rat, cleaned from soft tissue and frozen for the future analysis.

Results: AKG supplementation increase BMD in femur in ovariectomized rats in comparison to the AKG free groups. ovx/akg group had higher value (0.335 g/cm^2) than ovx/placebo one (0.299 g/cm^2). Similar tendency was observed in sham/akg and sham/placebo groups (0.349 g/cm^2 vs. 0.327g/cm^2). Analogous situation to the femur value was observed in the value of BMD in lumbar vertebrae, but changes were less pronounces (ovx/akg 0.239 g/cm^2 vs. ovx/placebo 0.233 g/cm^2 and sham/akg 0,257 g/cm^2 vs. sham/placebo 0,252 g/cm^2). Additionally, analysis of bone mechanical parameter of femora shown decreased Bone Robusticity Index in ovx/akg vs. ovx/placebo one. If more lower is this index bone is denser or more robust.

Conclusions: The obtained results documented positive effect of AKG on body weight gain and BMD of the bones in ovariectomized rats. Thus, presented data show the potential usefulness of AKG as a possible therapeutic drug- agent for the osteporose treatment.

P548 F

DOES ALPHA-KETOGLUTARATE PROTECT SKELETON IN CONDITIONS OF ESTROGEN DEFICIENCY IN RATS?

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The purpose of the study was to establish the possibilities of the alpha-ketoglutarate usage as a factor which protects bone losses in the rats during developing of osteopenia.

Material and Methods: The experiments were carried out on 40 female Wistar rats. The animals were maintained in controlled condition with free access to food and drink. The rats were divided into shame-operated (SHAM) and ovariectomized (OVX) groups. Seven day after surgery the animals were divided into groups received placebo and experimental drink including AKG. Sixty days after the animals were euthanised with CO_2. DEXA measurements of the femora and lumbar vertebrae (L 2-4) were done for determination of cortical and trabecular bone mineral density (BMD) respectively. The ultimate strength was estimated by three point bending test. Bone robusticity index (BRI) and the weight-length index (WLI) as whole bone morhometric parameters were measured. The following geometric parameters, such as the second moment of inertia in relation to the horizontal axis (Ix), the cross-sectional area (A) were assessed. Serum osteocalcin and Ctx were measured.

Results: The obtained results indicate that AKG supplementation decrease the body weight of OVX rats in comparison to the OVX rats received placebo (338,6 ±8,7 g vs. 345,6 ±7,5g). AKG treatment increased bone mineral density of cortical bone both in SHAM and OVX rats by 2,7 % (0,259 ±0,011 g/cm^2 vs. 0,266 ±0,018 g/cm^2) and 20,5 % (0,234 ±0,019 g/cm^2 vs. 0,282 ±0,012 g/cm^2) respectively. The OVX rats demonstrated the higher values of BMD in femora than SHAM rats by 6,02% after 60 days of AKG treatment. AKG supplementation increased bone mineral density of cortical bone both in SHAM and OVX rats by 2,7 % and 20,5% respectively. BMD of lumbar vertebrae of OVX rats treated with AKG was identical than shame-operated rats and 4,02 % higher than in SHAM rats treated with placebo.

Conclusion: The obtained results suggest the potential usefulness of alpha-ketoglutarate in the prophylaxis of the skeletal system osteoporoses-like disorders. This concept requires further research.

P549 S

EFFECTS OF ALENDRONATE AND HORMONE REPLACEMENT THERAPY, ALONE OR IN COMBINATION, ON BONE MASS AND MARKERS OF BONE TURNOVER IN ELDERLY WOMEN WITH OSTEOPOROSIS

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To compare alendronate, hormone replacement therapy (HRT), and their combination in treatment of elderly postmenopausal women with osteoporosis, 90 patients, aged 65-80 (mean 71) years, and with a T-score of bone mineral density (BMD) <2.5 at either the lumbar spine or the femoral neck were randomised to receive 10 mg of alendronate(n=30), 2 mg of estradiol plus 1 mg norethisterone acetate (n=30)(HRT), or their combination (n=30) for 2 years. BMD of the lumbar spine and the upper femur was measured at baseline and after 1 and 2 years of treatment. Urinary excretion of type I collagen aminoterminal telopeptide as related to creatinine (U-NTX) and serum type I procollagen aminoterminal propeptide (S-PINP) were assayed at baseline and with 6 months' intervals thereafter. Increases of 9.1-11.2 % in lumbar spine BMD at 2 years were similar in the study groups. Only HRT increased femoral neck BMD statistically significantly ($p<0.0001$) at both 1 (+4.9%) and 2 years (+5.8%; $p<0.05$ for differences to the other groups). The alendronate group exhibited the biggest increases in trochanter BMD both at 1 (+5.8% $p<0.01$ for differences to the other groups) and 2 years (+8.5% $p<0.01$ for a difference to the combination treatment group). Total hip BMD increased similarly in all study groups. Percent reductions in U-NTX in the HRT group (60.2-62.7%) were significantly less ($p<0.05$) than in the combination group (78.1-80.4%) and in the alendronate only group (72.4-76.1%). Also S-PINP decreased less ($p<0.05$) in the HRT group (-53.6% to -59.8%) than in the other groups (-73.0% to -75.0% in the alendronate group; -67.0% to -71.5% in the combination group). Six patients discontinued the study due to gastrointestinal complaints (2 in each group), and 5 receiving HRT due to breast tenderness. We conclude that in elderly postmenopausal women with osteoporosis the combination of hormone replacement therapy and alendronate did not offer an extra gain of bone mass over either treatment alone. In terms of BMD changes the single treatments were equally effective, but the reductions in bone markers were less on HRT than on alendronate.

P550 W

AWARENESS AND PATTERN OF MANAGEMENT OF OSTEOPOROSIS AMONG MEDICAL PRACTITIONERS IN HONG KONG

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Introduction: Epidemiology studies projected a vast increase in osteoporotic fractures in Asia by 2050. Awareness of osteoporosis among medical professionals and the pattern of management in Asia have not been explored.

Objectives: To evaluate the awareness of osteoporosis and the pattern of management in two groups of medical practitioners in Hong Kong: (1) hospital-based practitioners in the public sector (PH); (2) clinic-based private practitioners (PC).

Methods: 501 medical practitioners in Hong Kong were invited to complete a self-administered questionnaire. The questionnaire consisted of 35 questions in relation to the background characteristics of the practitioners as well as the diagnosis and management of their patients.

Results: Overall response rate was 41% (PH33%, PC51%). 76% of the whole cohort reported treating osteoporosis patients in their practice. 91% agreed that osteoporosis was under-diagnosed. Most practitioners (66%) believed that asymptomatic nature of the disease accounted for the under-diagnosis. Inaccessibility (45%) and high cost (54%) of diagnostic tools were believed to be the other major reasons for under-diagnosis. DEXA was employed as the diagnostic tool in only 51% of PH group and 55% of PC group. Similarly USG machines were used by only 31% of the practitioners, being more in the clinic setting (PC45% vs. PH17%, P<0.001). Concerning treatment goals, majority considered prevention of future fracture as critical or highly important (PH85%, PC80%; P=NS) whereas increase in BMD (PH46%, PC57%; P=NS), presence of analgesic effect (PH33%, PC40%; P=NS), low side effect of therapy (PH52%, PC60%; P=NS) and convenient dosing schedule (PH27%, PC38%; P=NS) were considered less important. On the contrary, the cost of therapy (PH61%, PC60%; P=NS) and improvement in the quality of life of patients (PH64%, PC70%; P=NS) were considered critical or highly important.

Conclusions: This survey showed that doctors in Hong Kong were aware of osteoporosis though they believed that the problem was still under-diagnosed due to inaccessibility and high cost of diagnostic tools. Prevention of future fracture was considered the most important element in osteoporosis therapy. This epidemiological study provides future directions for the development of health care towards prevention and treatment of osteoporosis in Hong Kong.

P551 F

INTERMITTENT ADMINISTRATION OF HUMAN PARATHYROID HORMONE(PTH) INCREASES BONE FORMATION ON THE INTERFACE OF THE HYDROXYAPATITE COATED TITANIUM RODS IMPLANTED INTO OVARIECTOMIZED RAT FEMORA

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Hydroxyapatite(HA) have good osteoconductive activity, however the presence of osteoporosis may limit the magnitude of this benefit. Intermittent administered PTH has an anabolic effect on the skeletal metabolism.The purpose of this study is to evaluate the effect of PTH on new bone formation on the interface of the HA-coated implants in experimental osteoporosis.

Fifty-four 11-week-old female Sprague-Dawley rats were divided into six groups (n=9 each group). The baseline control group was killed at the beginning of the experiment. An ovariectomy(OVX) was performed in twentyseven rats and eighteen rats were subjected to a sham surgery. Four weeks after operation, HA-coated titanium implants (1.4mm in diameter and 15mm in length) were inserted into the medullary canal of the left femur. These animals were then subjected to treatment with PTH (30microg/kg/day, 5 days/week) for a period of 4 weeks. After sacrifice, transverse sections from the diaphysis of the femur at the level of middle one-third of the implant were prepared for histomorphometric measurements. The bone volume on the interface of the implant (BV.Im) in OVX group (7.03±1.01%) was smaller than that of sham group (28.84±2.07%), and OVX rats treated with PTH (27.48±1.90%) was greater than that in OVX group. The bone formation rate on the implant (BFR/Im) in OVX+PTH group (6.37±0.35mcm^3/mcm/day) was significantly higher than that in sham group (2.63±0.46mcm^3/mcm/day) and OVX group (2.07±0.29mcm^3/mcm/day). In order to evaluate connection between the implant and trabecula, node-strut analysis was performed. A strut that connects the implant with node was defined as ImNd. In the same way, connects the implant with terminus as ImTm. ImNd/total strut length (ImNd/TSL) in OVX+PTH group (33.64±8.91%) was higher than that in OVX group (17.37±6.53%), conversely ImTm/TSL in OVX+PTH group (17.42±7.23%) showed significantly decrease to OVX group (59.93±11.87%). These results indicate that PTH increase new bone formation on the interface of the HA-coated implant and maintain existing trabecula in osteoporotic condition. It suggests that PTH is useful to enhance initial fixation of orthopedic implants even in osteoporotic status.

P552 S

MECHANISMS FOR THE ENHANCEMENT OF FRACTURE HEALING IN RATS TREATED WITH INTERMITTENT LOW-DOSE HUMAN PARATHYROID HORMONE (1-34)

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Recent reports have demonstrated that intermittent treatment with parathyroid hormone (1-34) [PTH(1-34)] increases callus formation and mechanical strength in experimental fracture healing. However, little is known about the optimal dose required for enhancement of fracture repair or the molecular mechanisms by which PTH regulates the healing process. In this study, we analyzed the underlying molecular mechanisms by which PTH affects fracture healing and tested the hypothesis that intermittent low-dose treatment with human PTH(1-34) can increase callus formation and mechanical strength. Unilateral femoral fractures were produced and a daily subcutaneous injection of 10 microg/kg of PTH(1-34) was administered during the entire healing period. Control animals were injected with vehicle solution alone. The results showed that on day 28 and day 42 after fracture, bone mineral content (BMC), bone mineral density (BMD), and ultimate load to failure of the calluses were significantly increased in the PTH-treated group compared with controls (day 28: 61, 46, and 32%; day 42: 119, 74, and 55%, respectively). The number of proliferating cell nuclear antigen (PCNA)-positive subperiosteal osteoprogenitor cells was significantly increased in the calluses of the PTH-treated group on day 2, and TRAP+ multinucleated cells were significantly increased in areas of callus cancellous bone on day 7. The levels of expression of type I collagen (COLlA1), osteonectin (ON), ALP, and osteocalcin (OC) mRNA were increased markedly in the PTH-treated group and accompanied by enhanced expression of insulin-like growth factor (IGF)-I mRNA during the early stages of healing (days 4-7). The increased expression of COL1A1, ON, ALP, and OC mRNA continued during the later stages of healing (days 14-21) despite a lack of up-regulation of IGF-I mRNA. These results suggest that treatment of fractures with intermittent low dose PTH(1-34) enhances callus formation by the early stimulation of proliferation and differentiation of osteoprogenitor cells, increases production of bone matrix proteins, and enhances osteoclastogenesis during the phase of callus remodeling. The resultant effect to increase callus mechanical strength supports the concept that clinical investigations on the ability of injectable low-dose PTH(1-34) to enhance fracture healing are indicated.

P553 W

COLLAGEN CROSS-LINK MATURITY AND CRYSTALLINITY INDICES IN POSTMENOPAUSAL WOMEN AFTER TREATMENT WITH TERIPARATIDE

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Bone quality, a major component of bone strength (NIH Consensus Conference 2001), can be partitioned into architectural and material components. Factors including the structure and composition of the underlying matrix (mainly collagen) and mineral contribute significantly to bone strength. Collagen cross-link ratios and mineral crystallinity are typically higher in osteoporotic bone samples compared with normal bone. Previous studies found that antiresorptive agents either increased or had no effect on mineral crystallinity, consistent with a suppression of osteoclasts. Teriparatide [rhPTH(1-34), TPTD], a new treatment for osteoporosis, is a bone formation agent with a mechanism of action distinct from antiresorptive treatments for osteoporosis. This study examined the effects of teriparatide (TPTD) on bone quality as reflected in bone matrix mineralization and type 1 collagen cross-linking. Biopsies were obtained from women enrolled in the Fracture Prevention Trial (Neer NEJM 2001) [placebo, 20 microg and 40 microg TPTD] after 12-24 months of treatment. Specifically, matrix mineralization (mineral:matrix), mineral crystallinity and the ratio of non-reducible (pyridinoline, (pyr)) and reducible (dehydro-dihydroxy-lysinonorleucine (deH-DHLNL)) cross-links in the collagen of non-decalcified thin sections were determined using Fourier transform infrared microscopic imaging (FTIR).

Overall, matrix mineralization and mineral crystallinity decreased after teriparatide treatment in a dose-dependent fashion, compared with placebo. Similarly the collagen cross-link ratios at the different depths also exhibited a dose-dependent decrease in the teriparatide-treated groups compared with placebo. The decrease in these parameters related to bone matrix and mineral properties exemplifies the bone-forming action of teriparatide, which causes deposition of new, not yet fully mineralized, bone with a cross-linking pattern consistent with that encountered in younger tissue. Apart from the pronounced improvements in bone architecture previously demonstrated after teriparatide treatment, the current study demonstrates that the new bone formed during treatment with teriparatide has the normal mineral and collagen quality characteristics routinely encountered in younger bone.

	Placebo (n=10)	**TPTD20 (n=10)**	**TPTD40 (n=10)**
Periosteal mineral:matrix	6.78 ± 0.93	4.28 ± 0.98*	3.46 ± 0.97*
Periosteal mineral crystallinity	1.1 ± 0.01	0.95 ± 0.01‡	0.92 ± 0.02‡
Periosteal pyr/deH-DHLNL	3.5 ± 0.01	3.1 ± 0.2†	3.1 ± 0.1†
Endosteal mineral:matrix	6.71± 0.86	4.12 ± 1.04*	3.11 ± 0.99*
Endosteal mineral crystallinity	1.15 ± 0.12†	0.97 ± 0.03*	0.95 ± 0.04*
Endosteal pyr/deH-DHLNL	3.4 ± 0.1	3.1 ± 0.02*	3.1 ± 0.1*
Trabecular mineral:matrix	6.11 ± 0.95	4.20 ± 1.11*	3.22 ± 0.98*
Trabecular mineral crystallinity	0.94 ± 0.02	0.88 ± 0.02‡	0.85 ± 0.03†
Trabecular pyr/deH-DHLNL	2.5 ± 0.15	2.34 ± 0.16†	2.26 ± 0.15‡
*P<0.05 vs. Placebo; †P<0.02 vs. Placebo ; ‡P<0.001 vs. Placebo Data are mean ± SD			

P554 F

CHARACTERISTICS OF MODELING AND REMODELING OSTEONS AFTER TERIPARATIDE TREATMENT

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Teriparatide [rhPTH(1-34), TPTD], a new bone formation treatment for osteoporosis, is able to reverse osteoporotic changes in bone structure and decrease fracture rate (Neer et al. N. Eng. J. Med. 2001). A significant proportion of new bone formed during teriparatide treatment is thought to be formed via modeling, i.e. formation of new bone on quiescent bone surfaces without previous resorption. Indeed, Dempster et al. recently reported the presence of trabecular osteons with smooth cement lines, indicating modeling, in biopsies obtained from a small number of patients treated with teriparatide (ASBMR 2001 abstract 1171). The purpose of this study was to investigate the occurrence and characteristics of modeling osteons in biopsies obtained from patients treated with placebo or teriparatide (20 and 40 microg/day s.c., duration 12-24 months) in a large randomized, placebo-controlled trial. A total of 49 biopsies (placebo (n=19), TPTD20 (n=17), TPTD40 (n=13)) were studied. Active bone forming, tetracycline-labeled osteons on trabecular and endocortical surfaces were studied using polarized light for collagen orientation and cement line stains. The osteons were classified according to the presence of smooth or scalloped cement lines (i.e. modeling and remodeling osteons, respectively). No modeling osteons were found in placebo treated patients. In patients treated with teriparatide, however, a dose-dependent increase in modeling osteons (active bone forming units) was seen for TPTD20 and TPTD40 ($P<0.0001$). A significant proportion of osteons studied were, however, classified as mixed remodeling/modeling osteons in the TPTD20 and TPTD40 groups ($P<0.0001$). They were characterized by partly scalloped, partly smooth cement lines, suggesting the transition from remodeling to modeling during the bone formation period. This would suggest that teriparatide causes extension of new trabecular osteons beyond the boundaries of the original remodeling site.

In conclusion, our studies suggest that teriparatide not only induces pure modeling bone formation at quiescent surfaces, but also increases bone formation at remodeling sites, causing increased extension of trabecular bone packets. These mechanisms may all contribute to the increase in bone volume and change in trabecular morphology previously demonstrated after treatment with teriparatide.

P555 S

MICROARRAY ANALYSIS OF GENE EXPRESSION INDUCED BY PARATHYROID HORMONE AND PROSTAGLANDIN E2 IN VIVO IN BONE TISSUE AND IN VITRO IN OSTEOBLASTS

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Parathyroid hormone (PTH) and prostaglandin E2 (PGE2) are most powerful stimulaters of bone formation in animals and humans. Bone formation can be induced by local delivery of PGE2 or PTH, implicating the cell autonomous action of PGE2 and PTH via G protein-coupled receptors for these ligands expressed in osteoblasts. Accordingly, studies using in vitro cell culture systems showed the stimulatory effects of PTH and PGE2 on proliferation, differentiation and survival of osteoblastic cells. However, the molecular mechanisms leading to powerful osteoanabolic action of PTH or PGE2 are still not known largely due to the difficulty of understanding the molecular events induced by these osteoanabolic stimuli in bone tissue. To address this question, in the present study, we employed DNA microarray analysis to evaluate the pattern of gene expression induced by PTH and PGE2 in vivo in bone tissue. Ten-month old female rats were treated with 80 ug/kg/day rhPTH (1-34) or 3 mg/kg/day PGE2 for 1 or 4 days. Total RNA, isolated from tibial diaphysis of these animals, was transcribed to labeled probes, which were then hybridized to Affymetrix chips containing 8740 rat genes. Microarray analysis was also conducted in primary rat calvarial osteoblasts, treated with rhPTH(1-34) or PGE2 for 6, 24 and 72 hours, for in vitro and in vivo comparison. Cluster analysis of gene expression documented the significant homology between the pattern of gene expression induced by PTH and PGE2 in vivo in bone tissue with -150 genes regulated by both PTH and PGE2, pointing to the possibility of common mechanism leading to osteogenesis. Among these genes, including 120 genes with known function, 20 genes are markers of osteoblasts markedly regulated by PTH and PGE2. Interestingly, transcriptional profile induced by PTH and PGE2 in vitro in cultured osteoblasts showed very little homology to the profile induced in vivo in bone tissue. These results suggest the possible role for in vivo environment, such as cell-matrix, cell-cell communication and mechanical stress, in bone formation induced by PTH and PGE2.

P556 W

THE EFFECTS OF HPTH(1-34) ON FRACTURE HEALING IN RATS FEMUR

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The aim of this study was to investigate the effect of lower dose hPTH(1-34) on fracture healing in rat. Female SD rats (5 weeks old) were injected subcutaneously with either two doses of hPTH(1-34) (10 microg/kg and 30 microg/kg) or vehicle three times a week for 3 weeks, then bilateral femora was fractured and fixed with intramedullary wires. PTH treatment was stopped in pretreatment groups (P10, P30 groups); while the treatment was continued in continious treatment groups (C10 and C30 groups). Animals were sacrificed at 3, 6 and 12 weeks after surgery. Soft X-ray of all fracture was taken to evaluate fracture line. Bone mineral density in fracture callus was analyzed using DXA and pQCT. After densitometrical analysis, three-point bending mechanical test was performed. To derive the data of intrinsic material properties, such as ultimate stress, elastic modulus, toughness, we normalized structural mechanical properties by cross-sectional moment of inertia calculated by pQCT. In soft X-ray findings, there was no significant difference in fracture line disappearance ratio among the groups. Bone mineral density in fracture callus increased in C10 and C30 compared with other groups, which were not significantly different among CNT, P10 and P30 at each sacrifice. Ultimate load increased in C30 compared with CNT, P10 and P30 at 12 weeks after surgery. There were no significant differences in stiffness and work to failure among each group. Intrinsic material properties were not significant different among each group at each sacrifice. These results suggests that low dose hPTH(1-34) treatment could lead to accelerate mineralization in callus, and then increase the mechanical strength of fracture site.

P557 F

QUALITY OF LIFE AND INSTITUTIONALIZED SHELTERED ELDERLY PATIENTS: A SF-36 AND HAQ COMPARISON OF SOCIAL, CLINICAL AND DEMOGRAPHIC FACTORS

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The instruments SF-36(Medical Outcomes Study 36 item short-form Health Survey) and HAQ (Stanford Health Assessment Questionnaire) were applied to 127 elderly patients, sheltered in a geriatric institution, in order to study the quality of life related to variables as: social, demographic and clinical factors.

The following domains were evaluated: general health status, functional capacity, emotional aspects, pain, mental health and vitality.

It was applied the reliability Alpha Cronbach coefficients.

HAQ and SF-36 quality life domains showed a negative correlation, but a very close correlation could be seen between HAQ and SF-36 functional capacity.

This study shows that quality of life of sheltered elderly patients suffer influences from several variables expressed by them individually.

Better quality of life was related to gender, education, mental health, vitality, and functional capacity. Amazing is that patients between 70 and 80 years old showed sometimes better conditions than younger subjects (60 to 70).

P558 S

HEALTH RELATED QUALITY OF LIFE IN MEN AND WOMEN WITH VERTEBRAL FRACTURE IN A JAPANESE POPULATION

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To study the relationship between vertebral fracture and subjective health outcome indicators, a cross-sectional survey with health examination including spinal radiographs and questionnaire survey was conducted among the 756 men and 1414 women aged 55-99 years in a population-based study (Adult Heath Study (AHS)) in Japan. The AHS recruited a cohort of about 20,000 people in Hiroshima and Nagasaki

based on the 1950 Japanese national census; this cohort has received biennial health examinations since 1958. EQ5D was used as health outcome indicators, which consists with items of mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. The EQ5D score in the AHS population was 0.79 in average. EQ5D score decreased with increasing age, and was lower in women than in men. After adjusting age, sex, stroke, dementia, ischemic heart disease, rheumatoid arthritis, and osteoarthritis, EQ5D score was significantly lower in vertebral fracture patients. Comparing EQ5D score between patients with vertebral fracture and those with osteoarthritis, patients with osteoarthritis showed slightly lower EQ5D score than patients with vertebral fracture. Persons whose body height shortened more than 2cm during the past 10 years had significantly lower EQ5D score compared the persons whose change was less than 2cm. In conclusions, vertebral fracture and body height shortening were associated with decrease in health outcomes assessed by EQ5D in both men and women, after adjusting for the several potential diseases affecting QOL in the elderly.

P559 W

IMPROVEMENT EFFECTS OF ALFACALCIDOL ON BONE PROPERTY IN RAT OSTEOPOROSIS MODEL INDUCED BY IRRADIATION OF BOTH KIDNEYS

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Objective: To observe the effects of alfacalcidol on bone mineral density, bone histomorphometry and bone biomechanics in rat osteoporosis model induced by irradiation of both kidneys.

Material and Methods: Six-month male SD rats were randomly divided into three groups: sham (Group A), model (Group B), Onealfa (Group C), each with 8 rats. Under anesthesia, both kidneys of each rat in Group A were carefully pushed out of the body for 30 minutes and then sent back to abdominal cavity. Kidneys of rats in Group B were exposed as Group A and irradiated with 15 Gy gamar rays(non-renal tissues were protected by lead planks). Rats in Group C were operated as Group B. One week later, Onealfa, a kind of alfacalcidol, was given to rats in Group C (0.1μg/kgBW/d,po) for 90 days. All rats were raised for 3 months. BMD of L1-4 was measured by DEXA. L4 were fixed with 10% formaldehyde, decalcified, embed with paraffin, stained with hematoxylin and eosin for histomorphometry. Left tibia were fixed with acetone and embed with cold methyl methacrylate, then 15 micrometer undecalcified sections were made for fluorescence microscope observation, based on which mineral deposition rate was measured. The biomechanical property of L3 and right femurs was measured.

Results Compared with that of Group A, BMD of L1-4 in Group B decreased 7.4%(P<0.01), TV/BV decreased 20.7%(P<0.01), Tb.Th decreased 21.0%(P<0.05), Tb.Sp increased 37.8%(P<0.01), MAR decreased 35.5%(P<0.05), Maximum Load decreased 20.9%(P<0.01)in L3 and 15.7%(P<0.01)in femur, Maximum Stress decreased 20.8%(P<0.01)in L3 and 12.3%(P<0.05)in femur. Compared with that of Group B, BMD in Group C increased 17.5%(P<0.01), TV/BV increased 45.7%(P<0.01), Tb.Th increased 22.8%(P<0.01), Tb.Sp decreased 39.6%(P<0.01), MAR increased 80%(P<0.01), Maximum Load increased 30.5%(P<0.01)in L3 and 24.3%(P<0.01)in femur, Maximum Stress increased 30.4%(P<0.01)in L3 and 19.5%(P<0.05)in femur.

Conclusion We conclude that alfacalcidol (Onealfa) can improve bone property in rat model of osteoporosis induced by irradiation of both kidneys.

P560 F

LOW INCIDENCE OF HYPERCALCEMIA AND HYPERCALCIURIA IN GREEK FEMALE PATIENTS FOLLOWING DAILY ADMINISTRATION OF 1 MCG ALFACALCIDOL

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Aim: A number of studies, support that the administration of high alfacalcidol dosages results in osteoproductive effects in bones. In these studies, the incidence of hypercalcemia and hypercalciuria appears to be negligent. In the present study, we aimed to investigate the effect of daily 1 mcg alfacalcidol administration (Alpha D3, Gerolymatos, TEVA) to postmenopausal osteoporotic and osteopenic Greek women, on calciemia and calciuria.

Materials and Methods: 709 women participated in the study. All the patients were postmenopausal, aged from 40 to 79 years old (av. 61,93, sd 8,04), with a T score lower than (-1,5), had had basic education and received no other treatment for osteoporosis. Exclusion criteria were thyroid disease, GI surgery, corticosteroid p.o. administration, antiepileptics and diuretics, chronic diseases, mobility disturbances and all other diseases affecting bone turnover. All patients were subject to laboratory examinations for Ca, P, alkaline phosphatase, T3, T4, TSH, and 24-hour urine for Ca, P and creatinine. Patients presenting pathological levels were excluded from the study. A question on basic educational skills was also included. All patients received 1 mcg alfacalcidol daily in the morning for a period of 6 months. All examinations were repeated at 3 and 6 months, and recorded on patient sheets. Adverse effects and discontinuation of treatment were also recorded.

Results: Out of the 709 women initially included, 574 concluded the study. Out of those women 2.4% presented hypercalciuria (Ca 24-hours > 240 mg) at 3 months and 1.9% at 6 months, while hypercalcemia (serum Ca>10,2 mg/ml) was observed at 3 and 6 months at percentages of 2% and 1.7%. An increase of total alkaline phosphatase by 1,94% was also observed, which is not statistically significant, but is an indication of osteoproductive activity.

Conclusions: The administration of 1 mcg alfacalcidol (Alpha D3, Gerolymatos, TEVA) in Greek postmenopausal osteoporotic and osteopenic women is not associated with danger of hypercalcemia or hypercalcuria, and probably has osteoproductive activity, an effect which must be further confirmed by future studies.

P561 S

RANDOMIZED DOUBLE BLIND MONTHS STUDY OF 1 μG/DAY OF ALPHACALCIDOL VS PLACEBO IN HIGH BONE TURNOVER POSTMENOPAUSAL WOMEN

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Vitamin D metabolites have been used for osteoporosis treatment. Main actions on bone are: mineralization, stimulation of osteoblast and disminished resorption, however available information about action on resorption markers in a prospective double-blind, placebo control trial are started. 85 postmenopausal patients with low BMD (t-score between –1 and –3 at L2-L4 or femoral neck) and high turnover (N-telopeptide, NTx, Tartrate Resistant Acid Phosphatase, TRAP, and Alcaline Phosphatase, AP 1 SD or more above the premenopausal mean) were randomized in a double-blind, placebo control, 12 month trial of 1 μg/day of alfacalcidol vs placebo, according to the principles of the Helsinski Declaration. Exlusion criteria were: secondary osteoporosis, fracture 6 months prior to the study, medication such as HRT, insulin, sterids, diuretics, biphosphnates, calcitonin, raloxifene, antitubeculosis drugs and thyroid hormones, allergy to vitamin D.

Group A: afaclcidol 1μg/day + calcium citrate 500 mg/day, Group B: placebo+ calcium citrate 500 mg/day. Mean age was 61.58 ± 6.69 and 62.4 ± 6.30. Age of menopase was 45.6 ± 5.14 and 46.4 ± 5.5, there were no differences on risk factor between groups. Modest hipercalciuria occurs in 48% (Group A) and 18% (Group B), Calcium supplement was stopped to control this side effect. We did no have hypercalcemia. Droup out rate was 3.5% in the placebo group and 2.4% in the alfacalcidol group. BMD remained stable in both group. In conclusion the use of alfacalcidol 1 μg/day durin 12 months in postmenopausal women with low BMD and hight turnover was very well tolerated and decresed NTX by 27.7%, TRAP by 23.6% and AP by 16%.

	Placebo				Alphacalcidol			
Marker	**Initial**	**3 mo**	**6 mo**	**12 mo**	**Initial**	**3 mo**	**6 mo**	**12 mo**
AP (UI/L)	47 ±14	43.2 ±15	43.3 ±13	43.2 ±12	48.6 ±15	43.1 ±15	39.2 ±10**	40.8 ±10**
TRAP (UI/L)	10.2 ±3.3	9 ±2.3	8.4 ±1.9***	8.1 ±2.2***	9.9 ±2.9	8.67 ±12.6	7.4 ±1.8***	7.5 ±2.3***
U-NTx (nmBCE/mmcreat)	133.5 ±58.3	103.6 ±64*	118.5 ±68	114.5 ±85*	128.7 ±67	115.2 ±28*	102 ±63	92.9 ±56*
* p<0.05, **p<0.01, ***p<0.001								

Systemic and Local Regulation of Skeletal Metabolism

P562 W

THE HISTORICAL VIEW OF CHANGE OF CONTENTS OF CATIONIC EQUILIBRIUM FOR OSSEOUS TISSUES

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Results of our investigation concerning cationic equilibrium are compared to results calculated on the basis of elements concentration for ribs originating from Japanese excavations. Constant value for bone from excavations equals to 1,09, moreover the factor of equilibrium for given elements were established. For comparison the constants values of cationic equilibrium were following: for surface of femur capitulum-A, for cortical part-B, for spongiose part-C - tab.1, which concern women-F and men-M and total population-P. These values for femur capitulum of habitants of industrial region were equal: women 1,076; men 1,067; total population 1,060. The constants for teeth, no smoking and smoking peoples are equals 9,842 and 9,849. The samples were collected during operative procedures in period 1995-2001 year. It seems to be possible, that constant value of cationic equilibrium could be used as reference value in retrospective and prospective research works.

Table 1. The constants values of cationic equilibrium for given part of femur capitulum.

	A	**B**	**C**
F	1,166	1,038	1,039
M	1,112	1,029	1,067
P	1,134	1,030	1,044

P563 F

EARLY CLOSURE OF GROWTH PLATE CAUSES POOR GROWTH OF LONG BONES IN COLLAGEN-INDUCED ARTHRITIS RATS

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Juvenile rheumatoid arthritis (JRA), juvenile chronic arthritis and hemophilic arthropathy are sometimes associated with premature closure of juxta-articular epiphysis, resulting in joint malalignment, extremity length discrepancy or short stature. Although inflammatory changes may contribute to the pathogenesis of early epiphyseal closure in such clinical cases, the causality of this relationship has not proven. Experimental animal models, such as (CIA) and adjuvant arthritis.

The purpose of this study is to determine if growth retardation occurs at the epiphyseal plates in collagen-induced arthritis (CIA) rat, which are widely used to examine pathologies of arthritis with immunological abnormalities, and to describe the histopathology of the growth plate in this model.

Methods: CIA was induced in rats by immunization with type 2 collagen. The longitudinal growth of femur and tibia were measured radiographically. Rats were sacrificed periodically and tissue specimens were obtained for histological evaluation using hematoxilin-and-eosin (HE) and safranin-O staining. Expression of matrix metalloproteinase (MMP)-3 and vascular endothelial growth factor (VEGF) was evaluated by immnohistochemistry using serial sections.

Results: Early closure of epiphyseal growth plate was detected radiographically and histologically in the CIA rats. Growth retardation of femur and tibia was observed in CIA rats. The HE sections showed significant reduction of growth plate width in the proximal tibia along with morphological changes from 3 weeks after immunization, and growth plate width was greatly diminished at 6 weeks after immunization. Intensity of safranin-O staining in extracellular matrix (ECM) of growth plate in the proximal tibia was reduced from early stage after immunization. Chondrocytes expressing MMP-3 and VEGF increased in number in growth plate of CIA rats at 3-week after immunization compared with control rats.

Conclusions: The present study shows that disturbances of long bone growth with early closure of epiphyseal growth plate occur in CIA rats. It seems that up-regulation of MMP-3 and VEGF play an important role in this event. Further clarification of the mechanism of this phenomenon could yield clinical benefits, especially in prevention of the premature closure of growth plate that is seen in JRA and other diseases, and CIA rats could be a useful model for this research.

P564 S

A RAPID 35% FLUX IN BONE MASS OCCURS DURING PREGNANCY AND LACTATION CYCLES IN MICE

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The maternal skeleton provides mineral to the fetus and neonate during pregnancy and lactation, respectively, but little is known about the magnitude or regulation of this process. We have begun systematically studying skeletal metabolism during reproductive cycles of normal mice in order to establish a normal baseline. Subsequent comparative studies of normal mice versus mice that lack specific calcitropic hormones or receptors may identify the responsible regulatory factors.

Serial measurements of bone mineral content (BMC) and density (BMD) were made in normal Black Swiss mice by PIXIMUS (Lunar), a DXA machine scaled for mice. After calibrating to a standard phantom, both total and regional (forelimb, pelvis, spine, hindlimb) BMC and BMD were measured daily during prepregnancy (3-10 days), pregnancy (19 days), lactation (20-30 days), and post-lactation. In parallel studies, ionized calcium was measured on tail blood of normal mice every other day during reproductive cycles.

Compared to pre-pregnancy, total BMC rose steadily to peak at 117.7 ± 0.58% before delivery, rose further during the first day postpartum to 125.4 ± 0.36%, and dropped to 90.3 ± 4.0% during the second week of lactation. The BMC returned to baseline during the third week of lactation. From peak to nadir, the total BMC dropped 35.1%. Similar BMC changes were seen at all regional sites. All quoted values were significantly different from baseline and each other time point ($p < 0.002$). In several mice, the fetal contribution to the total BMC was determined by obtaining readings before and after removal of fetuses by C-section. The entire litter accounted for 1.9 ± 0.4% of the total BMC. Serum ionized calcium levels were unaltered across the entire reproductive cycle.

In summary, BMC of the pregnant mouse increases rapidly while she is simultaneously losing calcium to her litter through placental calcium transport. Mineral accretion in pregnancy prepares the maternal skeleton for a 35% fall in BMC induced by the calcium demands of lactation, followed by a recovery to baseline within one week. Despite these fluxes in BMC, normocalcemia is always maintained. It remains to be determined how these cyclic changes in BMC are achieved and regulated.

P565 W

POSTTRAUMATIC DISTURBANCES OF HUMORAL BIOCHEMICAL MARKERS OF BONE METABOLISM

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Traumatic Brain Injury (TBI) isolated or combined with fractures and Spinal Cord Injury (SCI) are often associated with enhanced osteogenesis, especially around the large joints. Heterotopic Ossification (HO) is a process in which new bone with bone marrow is formed in tissues, which normally do not ossify. Although a large number of locally bioactive substances in bone have been identified over the past decade, the complex mechanisms regulating the bone induction process still remains essentially unknown. Despite of many theories regarding the pathogenesis of heterotopic ossification, none of them has been proven. The main causes of enhanced ossification in trauma patients are brain or spinal cord Injuries and long term ventilation under neuromuscular blockade.

The aim of this study was to reveal whether post-traumatic changes of humoral factors levels in trauma patients could be associated with different injuries.

In 25 patients with fractures only (GCS=14±0.4, ISS=10.5±1.7), 25 patients with TBI and fractures (GCS=10±0.8 ISS=27.5±2.4) and 25 patients with TBI (GCS=6.9±0.85, ISS=26±0.9) we measured the following Parameters in serum during the first week after admission: Calcium (Ca), Inorganic phosphorus (Phos), Intact parathyroidhormone (iPTH), Osteocalcin (OC), Calcitonin (CT), Carboxyterminal propeptide of Type1 procollagen (PICP), Carboxyterminal piridinolin cross linked telopeptide of type 1 collagen (ICTP). The blood samples were taken on the day of trauma after admission (d0), and 1, 3, 5, and 7th day after trauma. OC level was TBI with Fracture group and in TBI group significantly decreased, iPTH and ICTP were increased compared to fracture only (Table).

	OC (ng/ml)			**iPTH (pg/ml)**			**ICTP (ug/ml)**		
	Frx	TBI+Frx	TBI	Frx	TBI+Frx	TBI	Frx	TBI+Frx	TBI
Day 0	4.5 ±0.4	4.10±0.35	3.20±0.29	64±10	110±12	84±11	5.5±1.0	4.6±0.5	4.1±0.5
Day 1	3.6 ±0.40	1.98±0.23	1.40±0.20	57±11	85±18	62±9	8.0±1.5	6.2±0.8	5.8±1.0
Day 3	3.2 ±0.44	1.96±0.18	1.70±0.22	52+10	87±28	49±8	12.1±2.6	9.4±1.1	6.4±0.9
Day 5	3.3 ±0.37	1.99±0.24	1.80±0.46	38±8	73±21	36±7	12.9±2.9	10.0±1.5	7.0±1.0
Day 7	3.4 ±0.59	2.40±0.39	2.20±0.52	22±6	37±9	39±11	15.0±3.7	13.0±1.5	9.0±1.3

Osteocalcin (OC), intact parathyroid hormone (iPTH) and Carboxyterminal piridinolin cross linked telopeptide of type1 collagen (ICTP) seems to be humoral factors which might influence fracture healing and HO.

P566 F

THE CHANGE OF CONSTANTS OF CATIONIC EQUILIBRIUM IN RELATION TO PROFILE OF FEMUR CAPITULUM

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Little is know about cationic equilibrium in femur capitulum for habitants living on the industrial region in comparison to recreational regions cationic equilibrium in caput of femur was analyzed on the basis of the following geometric means of elements contents (microgram/g): 0,26 Mn < 0,28 Cd, < 1,2 Ni < 2,0 Ag < 3,0 Sb < 4,0 Mo < 4,0 Sn < 4,05 Pb < 4,41 Cr < 5,08 V < 6,0 As < 12,72 Cu < 13,0 Ba < 124,75 Rb < 162,46 Fe < 200,04 Zn < 281,58 Sr < 395,0 Al/ < 720,0 Ti < 1590,0 Si < 1719,86 Mg < 2189,94 K < 5850,0 Cl < 6490,0 S < 17057,65 Na < 36525,67 Ca < 42400,0 P.

The constant values of cationic equilibrium are highest for surface of femur capitulum and equal to 1,166 for females; 1,112 for males and 1,134 for the whole population moreover for cortical part of femur capitulum: 1,038 for females; 1,029 for males and 1,030 for the whole population. Disregarding particular of femur capitulum the highest constant value is obtained for females 1,076; for males 1,066 and the whole population 1,060. The change of cationic equilibrium in relation to profile of femur capitulum are presented in table,1 surface of femur capitulum, 2 for cortical part, 3 for spongiose part.

The change of constants of cationic equilibrium			
	1	**2**	**3**
A	1,040	0,983	0,985
B	1,030	0,995	1,010
C	1,040	1,010	0,990
D	1,030	1 ,030	0,991
E	1,070	1,010	0,981
F	1,030	0,994	1,110

P567 S

ADVANCED GLYCATION END-PRODUCTS INDUCE MCP-1-MEDIATED ADHESION OF MONOCYTES TO SMOOTH MUSCLE CELLS AND VASCULAR CALCIFICATION THROUGH PPAR gamma

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Although calcification of blood vessel walls is usually seen in advanced stages of diabetes mellitus (DM), its mechanism has not been known. We studied effects of high glucose and advanced glycation end-products (AGE) on vascular calcification using coronary artery smooth muscle cells (SMC) and obtained the following results. 1) High concentration of glucose, beta-glycerophosphate and AGE increased both proliferation and calcification of SMC in a dose-dependent manner. 2) Calcification of SMC cultured with human peripheral monocytes was greater than in that of either cell type alone. 3) Glucose and AGE augmented expression of LFA-1 and CC-chemokine receptor 2 (CCR2) on monocytes and ICAM-1 and a chemokine MCP-1 mRNA on SMC in a dose-dependent manner. 4) High glucose and AGE induced LFA-1-mediated adhesion of momocytes to SMC. 5) Anti-LFA-1 antibody reduced calcification of SMC cocultured with monocytes. 6) PPARgamma ligands, 15d-PGJ2 and pioglitazone, decreased LFA-1 and CCR2 expression on monocytes and ICAM-1 and MCP-1 mRNA expression on SMC in the presence of high glucose or AGE. 7) These PPARgamma ligands decreased the calcification of SMC cocultured with monocytes. Taken together, high levels of glucose and AGE induce MCP-1-mediated adhesion of monocytes to SMC, resulting in proliferation and calcification of SMC, in which induction of both MCP-1/CCR2 and LFA-1/ICAM-1 pathway is mediated by PPARgamma. We thereby propose that PPARgamma ligands could be potentially useful for the prevention of vascular calcification seen in advanced stages of DM.

Vitamin D

P568 W

RAPID EFFECTS OF SOLUBLE FORM OF RECOMBINANT RECEPTOR ASSOCIATED PROTEIN (RAP)ON MEGALIN FUNCTION

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Megalin is a multi-ligand endocytic receptor present in renal proximal tubules and involved in the uptake of proteins. Although it has been reported that megalin-knockout mice exhibit impaired renal uptake of 25-hydroxyvitamin D, the function of megalin is not fully understood, partially because most of the knockout mice die perinatally from holoprosencephaly. In this study, to elucidate the function of megalin in kidney, we administered soluble form of recombinant receptor associated protein (RAP) to mice. RAP is a 39-kD protein binding to members of the LDL receptor family including megalin, and inhibits the binding of all other ligands to these receptors. Although native RAP contains an endoplasmic reticulum (ER) retention signal and is mainly located in relation to the ER, here we utilized His-tagged soluble form of recombinant murine RAP [a.a.39-365] which lacked N-terminal signal peptide and the C-terminal ER retention signal. Ligand blot analysis revealed the interaction between soluble RAP (sRAP) expressed in E. Coli and megalin. Male ICR mice (7-8 wk old) were given i.p. administration of sRAP (3.5 mg/dose, 3 times with 4-hr intervals), and urine samples were collected before and after the administration. Urinary excretion of low-molecular-weight proteins was increased by administration of sRAP, which was consistent with the previous reports where RAP was infused directly to the renal artery. Western blot analysis using antibody against vitamin D binding protein (DBP) confirmed the increased urinary loss of DBP by sRAP administration. Immunostaining using anti-His antibody demonstrated the apical localization of sRAP in the proximal tubules, suggesting that systemically administered sRAP targeted the proximal tubules and was internalized after binding to megalin. There was no obvious change in the amount of megalin expressed in the brush border by administration of sRAP. These results indicate that administration of recombinant sRAP might provide a useful in vivo model to investigate the roles of renal uptake of proteins in mineral metabolism.

P569 F

PROPERTIES OF THE ENZYME RESPONSIBLE FOR THE C-3 EPIMERIZATION OF VITAMIN D_3 METABOLITES

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1alpha,25-Dihydroxyvitamin D_3 [1alpha,25$(OH)_2D_3$] is metabolized into its epimer of hydroxyl group at C-3 of the A-ring beside side-chain cleaved products through the C-24 and C-23 oxidation pathways. We also have demonstrated that natural metabolites, 25-hydroxyvitamin D_3 [25$(OH)D_3$], 24,25-dihydroxyvitamin D_3 [24,25$(OH)_2D_3$] and synthetic analog, 22-oxa-1alpha,25$(OH)_2D_3$ [OCT] are metabolized into their respective C-3 epimers. In the present study, we examined the basic properties of the enzyme responsible for the C-3 epimerization of the above vitamin D_3 compounds using microsome fraction prepared from rat osteoblast like UMR-106 cells and liver of vitamin D receptor knockout (VDR KO) mice.

The microsome fraction of UMR-106 cells was incubated with 0-300 microM of either 1alpha,25$(OH)_2D_3$, 25$(OH)D_3$ or 24,25$(OH)_2D_3$ for 60 min in the presence of NADPH-generating substrates. Maximum velocity (Vmax) values for 1alpha,25$(OH)_2D_3$, 25$(OH)D_3$ and 24,25$(OH)_2D_3$ were 1.52, 2.34 and 0.51 pmol/min/mg protein, respectively and Michaelis constant (Km) values for 1alpha,25$(OH)_2D_3$, 25$(OH)D_3$ and 24,25$(OH)_2D_3$ were 98.9, 73.7 and 200.1 microM, respectively. These results indicate that 25$(OH)D_3$ has the highest specificity for C-3 epimerization among the three compounds. The same results were observed in various cell culture systems. The reverse C-3 (from alpha to beta) epimerization activity for 3-epi-25$(OH)D_3$ in microsome fraction prepared from UMR-106 cells was 6-fold lower than that of the C-3 (from beta to alpha) epimerization. These results suggest that the enzyme responsible for C-3 epimerization recognizes not only the side-chain structure but also the stereochemistry of the hydroxyl group at C-3 of the A-ring.

In addition, we examined the effect of cytochrome P450 inhibitor and anti-NADPH cytochrome P450 reductase on C-3 epimerization activity. C-3 epimerization activity was not inhibited by various cytochrome P450 inhibitor or anti serum of NADPH cytochrome P450 reductase. C-3 epimerization activity was not induced by 1alpha,25$(OH)_2D_3$ in UMR-106 cells. Furthermore, C-3 epimerization activity in microsome fraction prepared from liver of VDR KO mice was not reduced in comparison with wild type mice. Based on these results, the enzyme responsible for

the C-3 epimerization of vitamin D_3 compounds are thought to be differ from already-known cytochrome P450 related vitamin D metabolic enzymes and not induced by 1alpha,25$(OH)_2D_3$ through VDR.

P570 S

WINAC (WSTF INCLUDING NUCLEOSOME ASSEMBLY COMPLEX) IS A NOVEL CHROMATIN REMODELING COMPLEX MODULATING VITAMIN D RECEPTOR (VDR) TRANSACTIVATION

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Many complexes interact with nuclear receptors for their ligand-dependent transactivation through modulating chromatin structure in the promoters of a set of target genes. Distinct classes of co-regulator complexes like DRIP/TRAP complex, p160 family complex, and TRRAP/GCN5 complex are shown to interact with nuclear receptors in a ligand-dependent way. But still other unknown complexes are assumed to support VDR transactivation in different ways.

Using GST fused VDR ligand binding domain (LBD) as a bait, we purified nuclear complexes associated with liganded VDR-LBD from HeLa nuclear extracts, and identified WSTF(Williams syndrome transcription factor) as one of the interactants. WSTF has been reported to be one of the candidate genes causing Williams syndrome, which often accompanies infantile hypercalcemia. To examine WSTF function in VDR transactivation, we purified a VDR interacting WSTF complex using Flag-tagged WSTF stably expressing cells. Using MALDI TOF-MS and Western blotting, we found that the complex designated 'WINAC' is a novel SWI/SNF related ATP dependent chromatin remodeling complex composed of at least 13 components. In vitro chromatin reconstitution and disruption assay showed that this complex modifies chromatin configuration through recognizing VDR in a ATP-dependent way. A ChIP analysis showed that this complex is recruited on some VDR target gene promoters in a ligand- independent way and regulates vitamin D metabolism through VDR-mediated target genes expression by facilitating the recruitment of other co-activators and co-repressors to the promoters. As some components of the WINAC are shared with those of other complexes involved in DNA replication, we confirmed in vitro and in vivo that WINAC mediates DNA replication by reconstituting chromatin on newly replicated DNA. These results suggest that this complex has multiple functions by bridging multiple complexes..

Finally we confirmed the relation between the symptoms of Williams syndrome and the function of WINAC. First we confirmed that VDR transactivation is abnormal due to low expression of WSTF in primary culture cells of the patients' skin fibroblasts. Next we exmamined the expression pattern of the components of WINAC during the mouse embriogenesis. These results support our idea that WINAC plays an important role in the pathogenesis of Williams syndrome.

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RAR AND VDR COOPERATIVELY FUNCTION IN THE BONE TISSUE

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In the bone tissues, vitamin A and D are known to control physiological events, such as mineral metabolism and skeletal formation. These actions are mediated by the specific nuclear receptors VDR and RAR (alpha, beta, gamma) through ligand-dependent transcriptional regulation of the target genes. Actually, VDR-/-mouse developed the typical features of typeII ricket, like impaired bone metabolism and inadequate mineralization of cartilage. However, many of these abnormalities appear to be indirectly caused by hypocalcaemia, and hence direct function of VDR in the bone tissues still remains uncertain, suggesting presence of a functionally redundant factor VDR. From the similarity in the vitamin A and D actions in bone tissues, we hypothesized that RAR compensate VDR function. To test this hypothesis, we generated VDR-/-RAR-/- WKO mice and analyzed their bone tissues.

Although both VDR-/-/RARalpha-/- WKO mice (9 out of 161 pups) and VDR-/-/RARbeta-/- WKO mice (9 out of 102 pups) were born under the expected ratio, they showed growth retardation as compared with the VDR-/-mice. Surprisingly, soft-X-ray picture and bone mineral density (BMD) showed that bone loss of these mice tibia was more severe than that of VDR-/-mice. However, the abnormalities of growth plate and the serum level of mineral concentrations of these receptor WKO mice were indistinguishable from that of the VDR-/-mice. Accordingly, another reason other than decrease of serum mineral levels was supposed.

In this research, further bone loss, found out by generating VDR-/-RARalpha-/- WKO mice and VDR-/-RARbeta-/- WKO mice, is an unexpected phenotype from the single receptor KO mice, suggesting that both of vitamin A and D actions are essential for normal bone metabolism.