



President's Poster Abstracts

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PP-TUE-02**Expression of the human CYP27B1-luciferase transgene in the mouse: tissue specific regulation and cellular localisation**

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The enzyme 25-hydroxyvitamin D 1 α -hydroxylase (CYP27B1) is the key enzyme in the synthesis of 1,25-dihydroxyvitamin D (1,25D). In recent times, a number of organs, other than the kidney, have been identified to express CYP27B1 activity and are targets for 1,25D activity. In order to investigate the localisation and transcriptional regulation of CYP27B1 expression in various tissues we have generated a transgenic mouse line that expresses the proximal -1501bp of 5' flanking region of the human CYP27B1 gene fused to the firefly luciferase reporter gene. Using bioluminescent imaging (BLI), luciferase expression was observed *in vivo* in a variety of tissues following an 2.5mg i.p. dose of D-luciferin. Discrete luciferase expression was located to organs such as the testes, jejunum, ileum, colon, anterior lobes of the prostate, growth plate of the femur, cerebellum, as well as in the kidney. No bioluminescence was observed in the liver or duodenum. Using confocal microscopy, both luciferase protein and the endogenous CYP27B1 protein were localised to proximal convoluted tubule cells of the kidney, neurons and Purkinje cells of the cerebellum, and novel sites such as epithelial cells of the choroid plexus. The testes also expressed a high level of the luciferase transgene which was co-located with CYP27B1 in Leydig and Sertoli cells. These findings demonstrate that the proximal -1501 bp 5' flanking region of the CYP27B1 gene is capable of directing the expression of CYP27B1 in the kidney, brain and testes, as well as in other tissues, such as bone, distal intestine and prostate in a tissue-specific manner.

PP-TUE-04**Analysis of physiological role of lactoferrin in bone using knockout mice**

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Lactoferrin is an iron-binding protein present in milk, epithelial secretions, and the secondary granules of neutrophils. The 80 kDa single chain glycoprotein binds iron tightly, circulates at 2 – 7 μ g/ml, and has multiple biological roles, including regulation of iron metabolism, immune function, and embryonic development.

We have established that lactoferrin is anabolic to bone. *In vitro*, it stimulates osteoblastic cell proliferation, differentiation, is anti-apoptotic to osteoblasts as well as inhibiting osteoclastogenesis; *in vivo*, it increases bone formation in a local injection model. However, whether lactoferrin has a physiological role in bone remains to be established. To directly address any essential physiological role in bone we have studied the bones, using microCT analyses, of lactoferrin knockout (LFKO^{-/-}) mice, generated by homologous gene targeting. The mice do not display any gross abnormalities.

The tibiae of 2, 4 and 12 week LFKO^{-/-} animals, compared to their wild-type litter mates, were studied using 2 different manufacturers' microCT machines. At all 3 time-points there was an indication of decreased bone area and width in the LFKO^{-/-} animals. Specifically there were significant decreases in the 4 week animals in

total bone area of 13.8% ($p < 0.05$). The periosteal and endosteal circumferences were also significantly reduced ($p < 0.05$).

The results of this study confirm the previous *in vitro* and *in vivo* data, indicating that lactoferrin promotes bone growth. In addition, these studies have provided insights into a physiological role of lactoferrin in bone, positively regulating bone growth.

PP-TUE-06

The complete inhibition of marginal bone loss by structure-base designed anti-TNF α peptide in murine collagen-induced arthritis. Comparison to anti-TNF α antibody

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Objective: The cyclic peptide, WP9QY (YCWSQYLCY) mimics the tumor necrosis factor α (TNF α) receptor ligand contact site and prevents the TNF α /its receptor interactions. In this study, we compared the effects of the WP9QY peptide on bone resorption in collagen- induced arthritis (CIA) mice with the anti-TNF α monoclonal antibody (anti-TNF).

Methods: CIA was induced by 2-time immunization method. The osmotic minipumps were implanted in the back of all mice on the day of the booster injection (day 21), and either vehicle or the anti-TNF (4 mg/kg/day) or the WP9QY (2 or 4 mg/kg/day) peptide was continuously infused until sacrifice (day 40), and thereafter, clinical, radiological, and histological assessments were performed.

Results: The WP9QY treatment inhibited the CIA-induced increases in the arthritis score, but onset of the disease was not delayed by the peptide. The inhibitory effect of the WP9QY on inflammation was definitely weaker than that of the anti-TNF. Micro-CT and histological analyses, however, revealed that WP9QY blocked the CIA-induced bone destruction at the knee joints in a same extent as anti-TNF. Furthermore, WP9QY completely inhibited the CIA-induced marginal bone loss, while the inhibitory effect of the anti-TNF was not apparent.

Conclusion: WP9QY could be a useful template for developing a drug to prevent both inflammatory bone destruction and the marginal bone loss in rheumatoid arthritis.

PP-TUE-08

Deletion of the androgen receptor in mineralising osteoblasts results in trabecular bone loss in young male mice

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The aim of this study was to investigate the role of androgens acting directly via the androgen receptor (AR) on mineralising osteoblasts. To achieve this, we generated a genetically modified mouse line in which the DNA binding domain of the AR was deleted specifically in mineralising osteoblasts using the Cre/loxP system. The bone phenotype of mineralising osteoblast-specific AR knockout (mOb-ARKO) and control mice (wild-type, Cre and Lox) were assessed at 6, 12 and 24 weeks of age.

mOb-ARKO mice had decreased trabecular bone volume at 6 weeks of age ($P < 0.02$) which was associated with a decrease in trabecular number ($P < 0.01$) while trabecular thickness was unaffected compared to wild-type controls, suggesting increased bone resorption. In contrast, this effect on bone volume was not observed in adult mOb-ARKO mice at 12 or 24 weeks of age, although trabecular number was decreased ($P < 0.01$) in 24 week-old mOb-ARKO mice compared to wild-type controls. Despite the reduction in trabecular number, serum levels of C-telopeptide α -1-chain of type I collagen in 6 week-old mOb-ARKO mice did not differ from wild-type controls. This suggests that perhaps in the absence of AR action in mineralising osteoblasts, accrual

of trabeculae is impaired while thickening of existing trabeculae is maintained. Further characterisation of the bone phenotype of mOb-ARKO mice is ongoing.

In conclusion, deletion of the DNA-binding domain of the AR results in decreased trabecular bone volume in young male mice.

PP-TUE-10

Vitamin K2 activates the transcription of bone-related genes in osteoblastic cells by steroid and xenobiotic receptor and a novel pathway independent of gamma-carboxylation

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Vitamin K is known as a potent stimulator of the bone building process, and it is clinically used as a therapeutic agent for osteoporosis in Japan. We have recently shown that vitamin K₂ is a transcriptional regulator of bone marker genes in osteoblastic cells by activating the steroid and xenobiotic receptor, SXR (J Biol Chem 278: 43919, 2003). To explore the vitamin K₂ signaling network in bone homeostasis, we explored genes up-regulated by both vitamin K₂ and the prototypic SXR ligand rifampicin as SXR target genes in MG63 osteoblastic cells stably expressing SXR by using Affymetrix oligonucleotide microarray analysis. A small leucine-rich proteoglycan tsukushi, a collagen-associated protein matrillin-2, and CD14 antigen were identified as SXR-regulated genes. Vitamin K₂ enhanced collagen accumulation in MG63 cells, which could be modulated by the expression of tsukushi. We also identified genes up-regulated only by menaquinone-4 (MK4), one of the vitamin K₂ isoforms, in MG63 cells expressing either SXR or empty vector. These MK4 target genes were shown to be activated via a novel pathway, being independent of either γ -carboxylation or SXR signaling. Taken together, vitamin K₂ can potentiate bone homeostasis by using distinct pathways outside its activity as a cofactor of γ -carboxylase.

PP-TUE-11

Decreased osteogenic activity of mesenchymal stem cells in glucocorticoid-induced osteoporosis rats

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Aims: Colony-forming-units fibroblasts (CFU-Fs) and their differentiation into osteoblasts and adipocytes were evaluated using a glucocorticoid-induced osteoporosis animal model.

Methods: Twenty-four mature male SD rats were randomly assigned into experimental and control group. The rats in experimental group received 1.5mg/kg dexamethasone injection intramuscularly, twice a week, for up to 8 weeks. The control group received physiological saline. The bone marrow from bilateral femurs were aspirated and cultured for CFU-Fs. The CFU-Fs were stained with Giemsa and quantified. After two weeks' culture in osteogenic or adipogenic medium, the CFU-Fs were evaluated for alkaline phosphatase

(ALP) activity and lipid droplets formation stained by Oil Red O to analyze osteoblasts and adipocytes differentiation. At the same time, RT-PCR was used to analyze the expression of core binding factor {alpha}1 (Cbfa1) and peroxisome proliferator activated receptor {gamma} 2 (PPAR γ 2).

Results: The number of CFU-Fs in osteoporosis group is only 22.8% of control group. In osteoporosis group, ALP activity accounts for 43.2% of control group, but lipid droplets are 3.1 times higher than control group. Cbfa1 mRNA expression decreases by 33.5%, while PPAR γ 2 increases by 2.5 times in osteoporosis group, as compared with control group.

Conclusions: Decrease in the number of CFU-Fs, and priority differentiation into adipocytes at the cost of osteoblasts differentiation may be one of the important mechanisms of the glucocorticoid-induced osteoporosis.

PP-TUE-13

The vitamin E analogue, γ -tocotrienol blocks osteoclastogenesis and promotes osteoblast activityS. Hay¹, V. Liapis¹, T. Vincent¹, G.J. Atkins¹, D.M. Findlay¹, A.D. Odysseos³, A.D. Keramidas² and A. Evdokiou¹¹*Discipline of Orthopaedics, University of Adelaide, Hanson Institute, Adelaide, South Australia.*²*Department of Chemistry, University of Cyprus, Nicosia, Cyprus*³*EPOS-IASIS, R&D, Nicosia, Cyprus*

There is now compelling evidence to suggest that elevated levels of reactive oxygen species are involved in the process of osteoclastic bone resorption and that antioxidant therapy may be useful in the prevention and treatment of bone loss in osteoporosis and other bone disorders. We investigated the effects of γ -tocotrienol, a potent anti-oxidant vitamin E analogue, on osteoclast differentiation and bone resorbing activity using three independent *in-vitro* model systems of osteoclastogenesis. When human peripheral blood mononuclear cells (PBMCs) and the RAW264.7 murine monocytic cell line were cultured with the receptor activator of nuclear factor kappa B-ligand (RANKL), γ -tocotrienol dose-dependently inhibited the formation of tartrate-resistant acid phosphatase positive multinucleated cells and bone resorption when these cells were plated on bone slices. Similarly, γ tocotrienol dose-dependently inhibited the bone resorbing activity of mature osteoclasts that were isolated from human Giant Cell Tumours of bone when cultured on bone slices. The effect of γ -tocotrienol on osteoblast function was also investigated by using mineralized bone forming primary human osteoblast cultures. Osteoblasts harvested from normal human bone donors were cultured up to 21 days in serum-containing medium, with ascorbate, potassium phosphate and dexamethasone. γ -tocotrienol progressively increased mineralization in osteoblast cultures when compared to untreated cells. Taken together these results demonstrate for the first time that γ -tocotrienol has an inhibitory effect on the differentiation and activity of osteoclast, whereas it promotes osteoblast activity.

PP-TUE-15

Pharmacological disruption of the proteasome pathway inhibits osteoclastogenesis and bone resorption via modulation of inhibitor of NF κ B ($I\kappa$ B α) and TRAF6/p62N.J. Pavlos¹, E. Ang¹, T.L. Chai¹, K. Yip¹, S. Lea², T. Ratajczak², M.H. Zheng¹ and J. Xu¹⁽¹⁾ *Molecular Orthopaedic Laboratory, School of Surgery and Pathology, (2) Laboratory for Molecular Endocrinology, The University of Western Australia, Nedlands WA 6009.*

Proteasome-mediated pathways regulate diverse signaling cascades which play fundamental roles in cell differentiation and apoptosis. The mechanism(s) by which the proteasome pathway regulates osteoclast formation and pathological bone destruction is presently unclear. In this study, we investigated the effects of several pharmacological inhibitors of the proteasome pathway (MG-132, MG-115 and Epoxomicin) on osteoclast formation and bone resorption as well as their effects on key RANKL-mediated signaling molecules inhibitor of NF-kappaB ($I\kappa$ B α) and sequestosome 1/p62, both associated with the proteasome pathway. Using a combination of primary bone marrow monocytes (BMMs) and RAW 264.7 cell osteoclastogenic culture systems, we demonstrate all proteasome inhibitors dose-dependently attenuate RANKL-induced osteoclastogenesis. At higher concentrations, proteasome inhibitors were found to trigger apoptotic cell-death in osteoclasts as evidenced by nuclear fragmentation and disorganization of the cytoskeleton. Under pathological conditions, epoxomicin also blocked bone resorption by multinucleated osteoclast-like giant cells derived from giant cell tumors of bone. The same proteasome inhibitors were also found to suppress RANKL-induced NF- κ B activation as well as prolong the proteasome-targeted degradation of $I\kappa$ B α . Interestingly, treatment of proteasome inhibitors also altered the cellular localisation, but not the physical association of p62 and TRAF6 *in vivo*. Collectively, our findings demonstrate that proteasome inhibitors perturb osteoclast formation and function by influencing key RANKL-mediated signaling cascades ($I\kappa$ B α and TRAF6/p62). We propose that selective proteasome inhibitors might offer potential therapeutic value for the treatment of osteoclast-mediated bone diseases.

PP-TUE-17

SMYD1, a histone methyltransferase, is expressed in osteoblasts and chondrocytes

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The SET and MYND domain containing protein-1 (SmyD1), also known as CD8b opposite (BOP), is a novel histone methyltransferase that has been shown to play a key role in cardiac and skeletal muscle development. In microarray transcript profiling of OSVDR transgenic mice, which exhibit increased periosteal bone formation due to over-expression of the vitamin D receptor in mature osteoblasts and osteocytes, there was a decrease in SmyD1 transcript levels in marrow-flushed long bone diaphyses, suggesting an involvement of SmyD1 in osteoblast development or function. To confirm and extend this finding, SmyD1 mRNA expression was examined in adult bone tissue and in differentiating cultures of the mouse MC3T3-E1 osteoprogenitor cell line, using in situ hybridization and quantitative real-time PCR (qRT-PCR).

Smyd1 transcripts were detected in mature osteoblasts and osteocytes and in articular and growth plate chondrocytes of adult mouse bone by in situ hybridization. In qRT-PCR analysis of marrow-flushed diaphyseal bone samples, Smyd1 transcripts were 50% lower in OSVDR bone compared to wildtype ($p=0.001$). This transgene-associated reduction was maintained after treatment with 1,25-dihydroxyvitamin D-3. Interestingly, SmyD1 expression was significantly lower in differentiated MC3T3-E1 cultures than in mature long bone tissue. The reason for this difference between expression in tissue and the osteogenic cell line is not yet known. Taken together, these results are consistent with a role for Smyd1 in adult bone tissue and suggest a potential vitamin D-regulated role for this histone methyltransferase in mature cells of the osteoblast lineage.

PP-TUE-19

Alkylamines prevent osteoclast formation and activity by inhibiting the mevalonate pathway

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Alkylamines (and their precursors) are found in a variety of edible plants and plant products such as tea, mushrooms, apples and grapes. Alkylamines such as *sec*-butylamine (SBA), *n*-butylamine (BA) and *iso*-butylamine (IBA) contain a primary amino group and exhibit marked structural homology with the R² side-chain of nitrogen-containing bisphosphonates (N-BPs). Due to this structural similarity, we sought to determine whether alkylamines could inhibit the mevalonate pathway *in vitro* and thus disrupt osteoclast function.

Preliminary studies with J774 macrophage cells revealed that $\geq 5\text{mM}$ SBA, IBA, or BA induced accumulation of the unprenylated form of the small GTPase Rap1A, indicative of mevalonate pathway inhibition. When J774 cells were metabolically labelled with [¹⁴C]-mevalonate, 1-10mM alkylamine dose-dependently inhibited the incorporation of [¹⁴C]-mevalonate into prenylated proteins. Furthermore, like N-BPs, this inhibitory effect of alkylamines on Rap1A prenylation was overcome by replenishing cells with geranylgeraniol. Since N-BPs are potent inhibitors of FPP synthase, we examined the effect of alkylamines on recombinant human FPP synthase. All alkylamines tested were found to be weak inhibitors of rhFPP synthase (IC₅₀ values 1-10mM), with potencies corresponding to that observed for inhibition of protein prenylation.

Treatment of mature osteoclasts (isolated from neonatal rabbit long bones) with 1mM SBA, IBA or BA for 48hrs markedly decreased the number of TRAP positive multinucleated cells, F-actin rings and resorption area. Furthermore, 1mM SBA, IBA or BA decreased the RANKL-induced formation of murine osteoclasts from M-CSF-dependent bone marrow macrophage precursors by $\geq 50\%$.

These studies suggest that dietary alkylamines may have a bone-protective property *in vivo*.

PP-TUE-21

Macrophages: the forgotten bone-lining cell

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The presence of a population of bone-associated macrophages (BAMs) has not been fully appreciated. The aim of this study was to characterize the phenotype, distribution and cellular environment of BAMs on normal bone surfaces. We have demonstrated a network of BAMs on both osteal surfaces by *ex vivo* confocal imaging of bones from the transgenic MacGreen mouse (macrophages express GFP). The GFP⁺ BAMs co-expressed F4/80 and were observed across the entire osteal surface. Immunohistochemical examination of F4/80 and Mac-3 expression confirmed the presence of this BAM network on resting long bones. BAMs were also demonstrated to be associated with areas of bone remodelling and formed a canopy over cuboidal osteoblast-like cells, suggesting cellular interaction between osteoblasts and BAMs. Investigation of BAM regulation of osteoblast function is underway (see M. Chang abstract). Given that macrophages and osteoclasts develop from hematopoietic precursors and macrophages can differentiate into osteoclasts *in vitro*, it is possible that BAMs are *in vivo* osteoclast precursors. We demonstrated that BAMs have an enhanced capacity to form TRAP⁺ bone-resorbing osteoclasts *in vitro* compared to bone marrow macrophages. However, overlap of F4/80 and osteoclast marker expression *in situ* was minimal, implying that the trans-differentiation of BAMs to osteoclasts is uncommon or rapid and that BAMs have other functional roles *in vivo*, in addition to serving as potential osteoclast precursors. These data provide compelling evidence that there is an extensive cellular network of BAMs on osteal surfaces and suggest that these cells influence and participate in bone remodelling.

PP-TUE-23

Both phenylephrine and sodium nitroprusside cause contraction of early, soft tissue fracture callus *ex vivo*

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Early soft tissue bone fracture callus contracts in solutions that contract smooth muscles¹. Such contraction may promote bony apposition and facilitate fracture repair. Concentrations of noradrenaline (NA) increase in plasma and callus tissue after fracture. NA contracts smooth muscle by binding to α adrenergic receptors. Nitric oxide (NO) concentrations also increase in callus after fracture. Yet NO relaxes vascular smooth muscle, which is a characteristic that may impair fracture repair. The aim of the present experiment was to investigate the contractility of early soft tissue fracture callus in response to (i) the α_1 adrenergic agonist, phenylephrine (PE), an analogue of NA and (ii) sodium nitroprusside (SNP), a generator of NO formation in tissue.

The 6th rib of adult male rats was fractured under anaesthesia and the ensuing callus was resected 8 days later. The prep (approx 100 mg) was mounted on a sensitive force transducer, which enabled the force generated by calluses to be measured accurately to within 0.01 mN. Test solutions were normal Krebs-Henseleit (KH) (pH 7.4) solution and small variations of it. Experiments were performed at room temperature.

(i) In normal KH calluses neither increased nor decreased basal tone, but force transients were still evident. (ii) In zero-Ca²⁺ KH, calluses contracted producing an increase in forces in the range of 1.2-2.3 mN. (iii) PE (10⁻⁹-10⁻⁶ M) contracted calluses producing forces of 1-2.5 mN. (iv) SNP (10⁻⁶ M) potently contracted calluses producing forces in the range of 2.5-5 mN. Results for PE are consistent with the presence of α_1 adrenergic receptors in the contractile cells, presumably osteoprogenitor cells, of early callus. The seemingly paradoxical result of SNP causing contraction in this smooth muscle-like tissue is not unique as SNP has previously induced contraction in animal urinary bladder and small intestine preparations.

The contraction of soft fracture callus by NA and NO, should such occur *in vivo*, may contribute to static tension within the callus and thereby promote osteoprogenitor cell differentiation towards an osteoblastic phenotype and thus accelerate osteogenesis in the callus.

Dooley, PC Howgate, MA Schuijers, JA and Grills, BL. J. Orthop. Res. 22: 1063-1071 (2004), 23:499-500 (2005).

PP-TUE-25

Fracture healing delayed by impaired inflammatory responses in Fra1 transgenic mice

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Transgenic mice (Fra1-tg) overexpressing a subunit of transcription factor AP-1, Fra1, show enhanced bone formation leading to increased trabecular bone in the marrow cavities of long bones. To determine whether fracture healing is affected by increased bone formation, we created a transverse fracture of the tibial diaphysis in Fra1-tg mice. Unexpectedly, we found that fracture union was delayed in transgenics compared to wild-type controls, as evidenced by radiography, examination of tissue sections, and observation of altered expression of markers in cells at the fracture site. Specifically, while formation of bony callus by membranous ossification was intact, formation of chondrogenic callus during fracture healing was impaired with reduced expression of *Bmp2*, *Sox9*, and type II collagen (*Col2*) mRNA. Since chondrogenesis and growth plate formation were apparently normal in Fra1-tg mice, we searched for pathological cues at early stages of fracture healing such as changes in inflammatory mediators that could affect chondrogenic callus formation. Induction of proinflammatory cytokines IL-6 and TNF- α after fracture was suppressed in Fra1-tg mice, accompanied by reduced expression of cyclo-oxygenase2 (*Cox2*) and synthesis of prostaglandin E2 (PGE2). Currently, we are testing the hypothesis that PGE2 is a local bone anabolic activator and is required for fracture healing. These data reveal that Fra1/AP-1 suppresses initiation of chondrogenesis during fracture healing.

PP-TUE-27

Low bone mineral density in patients with inflammatory bowel disease and interleukin 6 and 10

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Background: Low bone mineral density could be a serious complication of inflammatory bowel disease (IBD).

Objectives: We assessed low bone mineral density by patients with Crohn's disease (CD) and ulcerative colitis (UC) in relation with disease activity and blood plasma levels of interleukin 6 and 10.

Methods: The retrospective study includes 71 patients with CD and 57 with UC. Bone mineral density was assessed by dual X-rays absorptiometry DXA at lumbar spine and total hip. We assessed disease activity at CD by CDAI (Crohn's disease activity index) and at UC by Truel. We also assessed the blood plasma levels of interleukin 6 and 10.

Results: By patients with CD we confirm statistic importance of blood plasma levels of interleukin 6 in relation with low bone mineral density on total hip ($p < 0,04$) and on lumbar spine ($p < 0,0003$). The statistic importance of blood plasma levels of interleukin 10 in relation with low bone mineral density on total hip ($p < 0,003$) and on lumbar spine ($p < 0,001$). Statistically important was also dependence of low bone mineral density on total hip ($p < 0,004$) and on lumbar spine ($p < 0,003$) on CD disease activity.

By patients with UC we confirm statistic importance of blood plasma levels of interleukin 6 in relation with low bone mineral density on total hip ($p < 0,0001$) and on lumbar spine ($p < 0,00001$). The statistic importance of blood plasma levels of interleukin 10 in relation with low bone mineral density on total hip ($p < 0,00001$) and on lumbar spine ($p < 0,00002$). Statistically important was also dependence of low bone mineral density on total hip ($p < 0,01$) and on lumbar spine ($p < 0,01$) on UC disease activity.

Conclusion: By patients we confirmed statistic importance of low mineral density in relation with blood plasma levels of IL6, IL10 and disease activity.

PP-TUE-29

The influence of endogenous excess of glucocorticoid on cortical bone geometry in Cushing's syndrome: comparison between menstrual and postmenopausal women

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Endogenous excess of Glucocorticoid (GC) in Cushing's syndrome (CS) leads to progressive bone loss and the development of fragility fractures. GC-induced osteoporosis (GIO) is associated with a preferential deterioration of trabecular and weight-bearing bone, but it remains little known how GC excess affects cortical and weight-unbearing bone. On the other hand, it is now recognized that cortical bone geometry is one of important factors in bone quality and estrogen deficiency markedly affects it. To evaluate the contribution of GC excess to changes in cortical bone geometry, we analyzed cortical geometric parameters of radius using peripheral quantitative computed tomography in women with CS and normal controls (Cont) matched to age, body size and menstrual state. Forty-four postmenopausal (CS; n=6, mean age 67yr, Cont; 38, 67) and 34 menstrual women (CS; 4, 34, Cont; 30, 33), were enrolled in this study. Plasma cortisol and urinary-free cortisol levels in CS were 17.5 ± 6.2 g/L and 243 ± 172 g/day, respectively. In menstrual women, periosteal circumference, cortical thickness, cortical area and strength strain index (SSI), a cortical bone strength index, were significantly lower in CS group than in Cont group. Menstrual women with CS showed slender and thin cortical bone, indicating structurally weak bone. These differences were not observed in postmenopausal women with CS, which suggested that GC action on cortical bone was masked by estrogen deficiency. In conclusion, the present findings indicate that GC excess markedly affects cortical bone geometry, which plays an important role in bone fragility of GIO.

PP-TUE-31

Vitamin D assay requests in a major metropolitan health service of 4 hospitals - audit of clinical usage

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Aim: To determine the practise of ordering Vitamin D in clinical practise. To review possible clinical and public health significance of the survey results.

Method: The results of all Vitamin D assays ordered on patients in Eastern Health over a 17 month period (January 2005 to May 2006) were audited. LIASON[®] 25-OH-Vitamin D assay (DiaSorin) a chemiluminescence immunoassay to detect Vitamin D was performed in one laboratory servicing the whole health service. Reference range of 60-200 nmol/L was used. The 4 hospitals involved were Box Hill Hospital (BHH), Maroondah Hospital (MH), Angliss Hospital (AH) – all predominantly acute hospitals and Peter James Centre (PJC) an Aged Care & Rehabilitation Hospital.

Results: A total of 5,287 Vitamin D assays were performed during the period, the majority of which were single patient assays. 4,038 (76.4%) were performed on admitted patients in medical, orthopaedic and geriatric wards in an acute or subacute hospital setting. 1,249 (23.6%) were performed on "out – patients" (Emergency, Outpatients, G.P's etc).

Overall only 723 (13.6%) assays were above the preferred level of 70 nmol/L. Only 1,541(29.2%) were above 50 nmol/L. Thus 3,746 (70.8%) were less than 50 nmol/L i.e. sufficiently low to be in the range associated with increased risk of fracture and falls.

Mean ages at the 4 hospitals across the Eastern metropolitan area was 49, 78, 87 and 93 respectively. Median (mean) Vitamin D levels at these hospitals were 44(49.3), 37(41.9), 33(37.2) and 30(36.3) respectively.

Conclusion: Vitamin D requests are popular among clinicians and results would suggest that majority are targeting appropriate patients. Vitamin D deficiency was so commonly found in a predominantly elderly patient population in general medical, orthopaedic and geriatric wards as to warrant questioning the justification of continuing to perform routine assays on such patients.

PP-TUE-33

Glucocorticoid suppresses the differentiation of osteoblasts by enhancing the expression of follistatin, Dan and sFRP, and suppressing both BMP and Wnt signal pathways.

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Although glucocorticoid is known to induce osteoporosis mainly by suppressing osteoblast-mediated osteogenesis, the mechanisms by which this agent affects osteoblast functions are still unclear. On the other hand, both BMP-Runx2 and Wnt signal pathways play crucial roles in the osteogenesis and are known to stimulate osteoblast differentiation. In the present study, we used osteoblastic MC3T3-E1 cells and examined whether or not dexamethasone (Dex) would modulate these two pathways and thereby suppress osteoblast functions. Dex (10^{-7} M) significantly inhibited the proliferation of the cells through Day 7 by cell counting and BrdU incorporation. Dex (10^{-9} – 10^{-7} M) also strongly and dose-dependently suppressed the mineralization of the cells at Days 21 and 28 by Alizarin red stain and von Kossa stain, while Dex (10^{-7} M) did not significantly affect either alkaline phosphatase staining or activity at Day 10. Real-time PCR revealed that Dex (10^{-7} M) reduced the mRNA expressions of type I collagen, osteocalcin, and Runx2 at Days 3, 5, and 7. In contrast, mRNA expressions of BMP antagonists, Follistatin and Dan, as well as a Wnt antagonist, sFRP, were increased by Dex (10^{-7} M) at Days 3, 5, and 7. Thus, the present study suggests that Dex may inhibit the differentiation of osteoblastic MC3T3-E1 cells by suppressing both BMP and Wnt signal pathways through enhanced expression of follistatin, Dan, and sFRP.