



2001

ANZBMS

ABSTRACTS

Oral Abstracts follow in the order of their presentation

I	Invited Speaker
O	Oral
MO	Mini Oral

I1

INVESTIGATION OF POLYGENIC DISEASES: OSTEOPOROSIS

SH Ralston, Bone Research Group, Department of Medicine and Therapeutics, University of Aberdeen, Aberdeen, U.K.

Osteoporosis is a common disease with a strong genetic component. Twin and family studies have shown that genetic factors play an important role in regulating bone mineral density, ultrasound properties of bone, skeletal geometry, and bone turnover and well as contributing to the pathogenesis of osteoporotic fracture itself. Bone mineral density and other osteoporosis-related phenotypes are usually determined by the effects of several genes, but occasionally, osteoporosis or unusually high bone mass may occur as the result of mutations in a single gene. Linkage studies in man have so far defined loci on chromosomes 1p36, 1q21, 5q33-35, 6p11-12 and 11q12-13 which show definite or probable linkage to bone mineral density but the causative genes remain to be defined. Linkage studies in experimental animals have also identified several loci which regulate BMD and bone structure, and a future challenge will be to investigate the relevance of these in humans. Most work in the field has focussed on candidate gene studies in population studies, clinical cohort studies and case-controls. Amongst the most widely studied candidate genes are the vitamin D receptor (VDR) and Collagen type I alpha 1 genes (COL1A1). Polymorphisms of VDR have been associated with bone mass in several studies and there is evidence to suggest that these effects may be modified by dietary calcium and vitamin D intake. A Sp1 binding site polymorphism has also been identified in the COL1A1 gene which predicts osteoporotic fractures independently of bone mass, and there is functional evidence to suggest that this polymorphism influences collagen gene regulation and bone quality. From a clinical standpoint, advances in knowledge about the genetic basis of osteoporosis are important since they offer the prospect of developing new markers for assessment of fracture risk and the identification of novel molecular targets for design of new treatments for osteoporosis.

I2

VDR GENE POLYMORPHISMS AND BONE: THE CLINICAL SIGNIFICANCE OF GENETIC MARKERS.

JA Eisman, Bone and Mineral Research Program, Garvan Institute of Medical Research, St Vincent's Hospital, Sydney, NSW 2010, Australia.

Osteoporosis is recognised to be a major problem in relation to costs, quality of life and even mortality. Effective therapies now available have lesser effects in some individuals and are not widely used. The responses to prevention-related life-style changes have been particularly difficult to quantify. A major genetic component, independent of other medical and health factors, was shown in twin studies initially and subsequently in family based approaches. The difficulty of identifying specific genetic contributors was recognised given the likelihood of several modest genetic effects being summed in any individual.

Attempts to identify such loci were initiated based on understanding of bone and calcium homeostasis and candidate genes, e.g. the vitamin D receptor gene. Using several conservative (non-coding difference) polymorphisms in the vitamin D receptor gene, linkage and association were found with bone turnover and bone density. Although subsequent studies have shown weaker (or no) effects, this work opened the field to others seeking other causative genes; such as the collagen Ia1 gene and various other receptor genes. Several such genetic loci have been shown to have significant relationships with these parameters. Association studies have been most widely used with few linkage studies. Extended pedigree models and the data from the human genome project are certain to enhance progress.

However, while the number of publications in this area continues to double every 2-3 years, no single gene locus or cluster of loci has been shown to convey clinically useful information on bone density or fracture risk. The major value of such genetic loci may be to identify potential targets for new therapies and to help select individuals, who are more or less likely to respond to particular treatments. The issue of how and what genetic information could enhance clinical care deserves consideration.

O1

BONE CYP27B1 IS REGULATED BY DIETARY CALCIUM AND NOT PTH
PH Anderson, S Iida, AJ Moore, S Dunn, A Stiliano, PD O'Loughlin, M Cochran, BK May, HA Morris Institute of Medical and Veterinary Science, Adelaide, Australia. Depts of Physiology & Biochemistry, Adelaide University, Adelaide, Australia.

Regulation of renal 1,25dihydroxyvitamin D₃ (1,25D) activation by 25-hydroxyvitamin D-1a-hydroxylase (Cyp27b1) has been identified for 20 years, however, regulation of bone cell Cyp27b1 activity remains largely unknown. We have compared the effects of vitamin D status and dietary calcium (Ca) on Cyp27b1 mRNA expression levels in kidney and bone tissues. Vitamin D-deplete female Sprague-Dawley rats were raised on a vitamin D-deficient, 1% Ca diet and housed in a UV-free environment from birth. 6 month old vitamin D-replete and vitamin D-deplete rats were fed either the 1% Ca diet (high) or a 0.1% Ca diet (low) for 3 months. Total RNA was extracted from one femur and kidneys by phenol/chloroform methods. Real-Time RT-PCR quantified mRNA for Cyp27b1. The second femur was processed for histology and in situ hybridisation for Cyp27b1. Vitamin D-deplete rats exposed to low-Ca developed hypocalcaemia, hyperparathyroidism and significant increase in osteoid consistent with osteomalacia. In these rats, kidney Cyp27b1 mRNA levels increased 100-fold compared with kidneys levels in vitamin D-replete rats fed low Ca ($p < 0.001$) which was confirmed by ribonuclease protection assay. Additionally, kidney CYP27b1 levels correlated with serum PTH ($R^2 = 0.78$). In high Ca challenged rats, however, bone Cyp27b1 mRNA levels were increased 5-fold independent of vitamin D status and serum PTH ($p < 0.01$). Additionally, there was a correlation between bone mRNA levels for Cyp27b1 and the catabolic enzyme for 1,25D, 25-hydroxyvitamin D-24-hydroxylase (CYP24) ($R^2 = 0.89$). There was, however, no association between bone Cyp27b1 and kidney CYP24. Most interestingly, the high-Ca challenge in low-vitamin D rats normalised serum Ca and PTH levels as well as reduced osteoid and normalised mineralisation lag time. Preliminary identification of bone Cyp27b1 by in situ hybridisation indicates that the majority of the signal-positive cells are bone marrow derived normoblast-type, haematopoietic progenitor cells. It is evident from these rat studies that unlike kidney Cyp27b1 mRNA, bone levels are up regulated by dietary calcium and unaffected by vitamin D depletion or PTH and is associated with structural changes and normalisation of mineralisation lag time.

O2

SITE AND SURFACE SPECIFIC EFFECTS OF EXERCISE IN CORTICAL BONE DURING GROWTH: A LONGITUDINAL STUDY

L Saxon, SL Bass, RM Daly. Health & Behavioural Sciences, Deakin University, Burwood, Vic.

While BMD can account for much of the variance in bone strength, bone structural and geometric properties can affect bone strength with or without changes in BMD. We asked i) does exercise lead to periosteal expansion before puberty and endocortical contraction after puberty? ii) is the osteogenic response constant along the length of the bone? and, iii) does exercise result in an increase in the material density of the bone?

Cross-sectional data was collected in 47 female tennis players (17 pre-, 11 peri-, 19 post- pubertal). 12 mth follow-up was obtained in 31 players (5 pre to pre-, 9 peri to peri- & 17 post to post- puberty, baseline to 12 months respectively). Bone geometry was assessed by magnetic resonance imaging (MRI) at 30 to 40% (distal) and 50 to 60% (mid) of the humeral length from the distal end. BMC was assessed by DXA. Cortical BMD (material density of bone) = BMC/cortical volume (g/mm^3).

The cross sectional data showed that exercise during growth resulted in a bone that was 6 to 8% larger ($p < 0.001$). The cross sectional area of the cortical bone (ie cortex) was 8 to 12% greater due to periosteal expansion in the pre-pubertal years and endocortical contraction in the post-pubertal years ($p < 0.05$ to < 0.001). The effect of exercise (expansion at the periosteal surface) occurred in the pre-pubertal years and did not increase during the peri- or post- pubertal years despite more years training and advanced maturation. There was heterogeneity in the surface specific effect of growth and exercise at the mid compared with the distal humerus. Cortical BMD increased during growth ($p < 0.01$) and was greater in the playing arm of the pre- and peri- pubertal players ($p < 0.01$). The longitudinal data support the cross sectional findings. Over 12 mths cortical area of the non-playing arm at the distal and mid sites increased by 14-25%, 30-40% and 1-6% in the pre- peri- and post- pubertal players ($p < 0.001$). However, there was no additional exercise effect at the periosteal surface in any pubertal group, but an exercise effect at the endocortical surface (2.7% contraction) at the distal site in the post-pubertal players.

In conclusion, these cross-sectional and longitudinal data show that there was heterogeneity in the osteotrophic response to exercise that was site-specific and maturity-dependent. Furthermore, the increase in periosteal expansion due to exercise appeared to be achieved in the pre-pubertal years.

O3

OSTEOCLAST GENE ARRAY TOOL IDENTIFIES GENES REGULATED BY THE EXPRESSION OF RANKL IN OSTEOCLASTOGENESIS.

T Vaughan¹, Cathy Aitken², Jethro LeRoy¹, Geoff Nicholson² and Nigel Morrison¹ School of Health Science, Griffith University Gold Coast, Southport 4215, AUSTRALIA. 2 Department of Medicine, The University of Melbourne, Barwon Health, Geelong Hospital, Geelong 3216 AUSTRALIA

Macrophage colony stimulating factor (M-CSF) stimulates the differentiation of macrophage-like cells from monocytes. Addition of recombinant soluble receptor activator of NFkB ligand (RANKL) in the presence of M-CSF results in differentiation of osteoclast-like cells (OCLs). Total RNA from monocytes treated with M-CSF alone and treated with RANKL and M-CSF together was isolated at 8hours, 12 hours, 24hours, 8 days and 21 days after treatment. Differential display (DD) was performed on RNA (8hour, 12 hour, 24hour, 8days treatment with RANKL) the DD products were pooled to generate a probe for screening a gene array system derived from a human osteoclast cDNA library. cDNA hybridisation experiments against the array revealed additional regulated genes. Gene clones that showed significant regulation in M-CSF treated cells compared with M-CSF and RANKL treated cells represent genes that are targets for RANKL-specific regulation. Osteopontin, creatine kinase and cystatin B were up regulated by the treatment of RANKL. Thymosin beta-4, proteasome ATPase and adrenocorticotrophic hormone receptor were some of the genes down regulated by the treatment of RANKL. The regulation of cysteine protease cathepsin K and the cysteine protease inhibitors cystatin C and cystatin B were confirmed by real-time PCR. Cathepsin K relative expression peaked at one week treatment and subsequently decreased with increasing time of exposure to RANKL, while cystatin C expression peaked at two weeks RANKL treatment and cystatin B showed a steady increase in expression. Genes that are regulated by the action of RANKL during osteoclastogenesis may provide potential therapeutic targets for bone diseases like osteoporosis.

O4

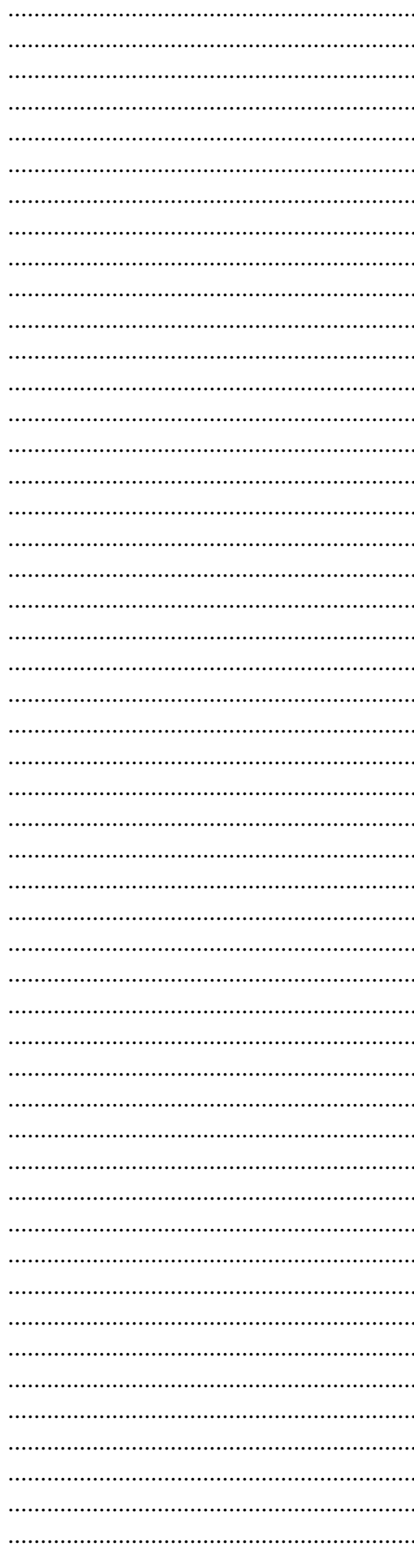
INVESTIGATION OF THE FUNCTIONAL DOMAIN BY TRUNCATION OF RAT RANKL

J W-Yin Tan, Jiake Xu, Ming-Hao Zheng

Department of Orthopaedic Surgery, QEII Medical Centre, University of Western Australia, Perth, WA, 6009

Receptor activator of NF-kB ligand (RANKL) has been identified as the 'master cytokine' in the differentiation and activation of osteoclasts. RANKL binds to the specific receptor, RANK, that expresses on the cell surface of osteoclasts and their progenitors. The interaction of RANKL with RANK is an important step leading to the activation of several signal transduction pathways and osteoclastogenesis. Previously we have expressed and purified soluble rat RANKL containing the TNF-like core domain (aa160-318) as a recombinant GST-rRANKL fusion protein and shown that it is capable of 1). Inducing the formation of bone resorbing osteoclast from RAW_{264.7} cells; 2). Stimulating mature rat osteoclast polarization and bone resorption ex vivo; 3). Inducing systemic hypercalcemia in vivo.

In the present study we have mutated the TNF-like core domain by truncation and investigated the effects of various regions on receptor binding and osteoclastogenesis. Our data suggests that full length TNF-like core binding domain is required for efficient binding to RANK and induction of osteoclastogenesis while the truncated binding domain aa160-300 (p3rRANKL2), aa160-268 (p3rRANKL3), aa239-318 (p3rRANKL4) and aa246-318 (p3rRANKL5) has decreased capabilities of binding to RANK and osteoclast inductivity. These findings suggest that full length TNF-like core is necessary for complete induction of osteoclastogenesis. Disruptions to the TNF-like core region may be able to down regulate osteoclastogenesis and thus, control diseases associated with abnormal osteoclasts activities.



O5

PAGET'S DISEASE OF BONE: LINKAGE TO A NOVEL REGION ON HUMAN CHROMOSOME 18Q23. D. Good,¹ F. Busfield,¹ B. Fletcher,¹ D. Duffy,² J. Kesting,¹ J. T. E. Shaw¹. ¹Dept of Diabetes and Endocrinology, Princess Alexandra Hospital, ²Queensland Institute of Medical Research, Brisbane, Australia.

Paget's disease of bone (PDB) is characterised by localised increased osteoclast activity and abnormal bone remodelling. PDB has a significant genetic component demonstrating linkage on chromosome 6q21.3 (PDB1), and 18q21.2-21.3 (PDB2) in some pedigrees. Mutations in exon 1 of TNFRSF11A on 18q21.2-21.3 have been identified in two PDB pedigrees. We have a large pedigree in which multiple family members are affected by PDB. Linkage analysis has excluded the PDB1 and PDB2 regions in this family, whilst sequencing has excluded mutations in exon 1 of TNFRSF11A. We proceeded to a 10cM genome-wide microsatellite scan, with 61 individuals typed for 474 markers. Initial linkage analysis suggested evidence for linkage to chromosome 7p and 18q23. Fine mapping, followed by two-point linkage analysis was performed in regions of interest on chromosomes 7p and 18q, including 88 subjects of whom 30 were affected. Peak LOD scores obtained were LOD = +2.75 at D7S507 and LOD = +1.76 at D18S70. Multipoint and haplotype analysis of markers flanking D7S507 did not support linkage to chromosome 7. Haplotype analysis of markers flanking D18S70 showed a consistent haplotype associated with PDB in a large subfamily within the pedigree. This subfamily consists of 54 genotyped subjects of whom 17 are affected. The age at diagnosis is significantly lower in this sub-family than in the rest of the pedigree (51.2 + 8.5 vs 64.2 + 9.7 years, p=0.0012). Multipoint linkage analysis of this subfamily gave a peak multipoint LOD score of +3.59, 0.79 cM telomeric of marker D18S1390. Our data are consistent with genetic heterogeneity within the pedigree, and indicate that 18q23 harbours a novel susceptibility gene for PDB in this pedigree.

O6

LOW-INTENSITY PULSED ULTRASOUND STIMULATES AN IN VITRO BONE-FORMING RESPONSE.

SJ Warden,¹ J Favaloro,² KL Bennell,¹ JM McMeeken,¹ KW Ng,² JD Zajac,² and JD Wark.² ¹Centre for Sports Medicine Research and Education, School of Physiotherapy, and ²Department of Medicine, The University of Melbourne, VIC 3010 Australia.

Low-intensity (<100 mW/cm²) pulsed ultrasound (US) is an established therapy for fracture repair. In animal and clinical trials, such US has been shown to facilitate fresh fracture repair and initiate healing in fractures with repair defects. However, the mechanism by which US achieves these outcomes is not clear. One possible mechanism is the direct stimulation of bone formation. To investigate this hypothesis, the current study investigated the mRNA response of isolated bone-forming cells (UMR-106 cells) to a single 20-minute dose of low-intensity pulsed US. Using a novel US-cell coupling method, cells were exposed to 200 is bursts of 1.0 MHz sine waves repeating at 1.0 kHz. The spatial-averaged temporal-averaged intensity was set at 30 mW/cm². The response of selected mRNA species was assessed at 0, 20, 40 mins, 1, 2, 4, 8 and 24 hrs post-US exposure. US stimulated expression of the immediate-early response genes c-fos and cyclooxygenase-2. Both genes showed initial transient responses peaking at 20 mins and returning to control levels by 1 hr post-US exposure. c-fos levels remained at control levels at each successive timepoint. In contrast, mRNA levels for COX-2 showed a secondary elevation at 4 and 8 hrs post-exposure. ALP and OC showed a delayed response to low-intensity pulsed US, with both genes being upregulated at 24 hrs post-US exposure but not at earlier timepoints. IGF-I, TGF-β or BSP expressions were not influenced by low-intensity pulsed US at any of the timepoints assessed. These findings suggest that low-intensity pulsed US has a direct effect on bone formation. This may contribute to the beneficial effect of low-intensity pulsed US on fracture repair.

MO1

THE INVOLVEMENT OF RANK LIGAND (RANKL) AND OSTEOPROTEGERIN (OPG) IN MULTIPLE MYELOMA.

GJ Atkins¹, A Farrugia², B Pan², P Liapis^{1,2}, P Bardy³, N Horvath¹, LB To², DM Findlay¹, ACW Zannettino². ¹Department of Orthopaedics and Trauma, Adelaide University, Adelaide, South Australia. ²Myeloma and Mesenchymal Research Laboratory, Hanson Centre for Cancer Research, Adelaide, South Australia. ³The Australian Red Cross Blood Service, Adelaide, South Australia

Multiple myeloma (MM) is an incurable B cell malignancy able to mediate massive destruction of the axial skeleton. It is characterised by the presence of a monoclonal population of end-stage plasma cells which localise to sites in the bone marrow in close proximity to the bone trabecular. The precise mechanisms responsible for the observed bone pathology remain unclear; nevertheless, it is generally accepted that it is due to MM cell-mediated disruption of the normal equilibrium between bone formation by osteoblasts and bone resorption by the multinucleated osteoclasts (OC). The aim of this study was to examine the involvement of the newly defined TNF-ligand family member, RANKL and its naturally occurring antagonist osteoprotegerin (OPG) in MM biology. We found that most human myeloma cell lines tested expressed RANKL and OPG at both the mRNA and protein level. MM patient derived BM mononuclear cells, including purified CD38⁺⁺ myeloma plasma cells, were also found to express RANKL mRNA. Flow cytometric analysis confirmed that CD38⁺⁺ cells in MM marrow express surface RANKL, implying that they could potentially participate in osteoclastogenesis directly. Indeed, co-culture of sorted patient-derived CD38⁺⁺/RANKL⁺ cells with murine splenocytes resulted in the formation of multi-nucleate, tartrate resistant acid phosphatase (TRAP) positive OC-like cells. Our data suggest that RANKL expression by MM cells confers on them the ability to participate directly in the formation of OC, and the resulting focal osteolytic lesions characteristic of multiple myeloma.

MO2

ACTIVATION OF PROTEASE-ACTIVATED RECEPTOR-2 LEADS TO INHIBITION OF OSTEOCLAST DIFFERENTIATION

R Smith¹, M Ransjö², RN Pike¹, EJ Mackie³. Department of Biochemistry and Molecular Biology, Monash University; Department of Oral Cell Biology, Umeå University, Sweden; School of Veterinary Science, University of Melbourne.

Protease-activated receptor-2 (PAR-2) is a G protein-coupled receptor that is activated specifically by a small number of tissue proteases. PAR-2 is also activated by peptides corresponding to the 'tethered ligand' created by cleavage of the extracellular N-terminal domain. We have demonstrated expression of PAR-2 by osteoblasts, but have not been able to identify any physiological responses to activation of PAR-2 in osteoblasts, other than elevation of [Ca²⁺]_i. The effect of a PAR-2-activating peptide (RAP) on osteoclast differentiation has been investigated in mouse bone marrow cultures. RAP alone had no effect on osteoclast differentiation, but when administered concomitantly with PTH or 1,25-dihydroxyvitamin D₃ (1,25D), RAP caused a significant inhibition (>50%) of osteoclast formation as assessed by tartrate-resistant acid phosphatase staining or calcitonin binding. In the presence of 1,25D, RAP was equally effective when administered at days 0-2, 2-5 or the entire 7-day culture period, but ineffective when administered only from days 5-7. In RT-PCR studies of RNA extracted from 3-day bone marrow cultures, RAP treatment reduced PTH- or 1,25D-stimulated cyclooxygenase-2 expression to below control levels. In PTH-, but not 1,25D-treated 3-day cultures, RAP reduced the ratio of RANKL:OPG below control levels. RAP also decreased levels of RANK expression in PTH-treated cultures at days 3 and 9, and in 1,25D-treated cultures at day 9. These observations indicate that activation of PAR-2 leads to inhibition of PTH- or 1,25D-stimulated osteoclast differentiation. The mechanisms of this effect appear to be different for the two hormones, and may involve direct effects on osteoclast precursors as well as on bone marrow stromal cells.

MO3

THE EFFECTS OF SIX-MONTH TREATMENT WITH DEXAMETHASONE AND/OR PROGESTERONE ON FEMORAL MID-SHAFT BONE IN SHEEP
SL O'Connell¹, LM Paton², B Fam², B Kaymakci², CDL Thomas¹, JG Clement¹ & JD Wark². ¹School of Dental Science & ²Department of Medicine, RMH, University of Melbourne, Victoria, Australia.

Glucocorticoid-induced osteoporosis appears to result from uncoupling of bone turnover, resulting in major suppression of formation activity but little change in resorption. Progesterone, a putative bone trophic hormone, may act as a glucocorticoid antagonist. We investigated this in sheep using dexamethasone, a potent glucocorticoid in humans.

Forty-eight Merino ewes (3-4 years) were divided into 4 groups and given a placebo (subcutaneous pellet), dexamethasone (D) (145mg/60 days, pellet), medroxyprogesterone (P) (300mg/6 weeks, injected I.M.) or both D and P (DP) for 28 weeks. Following previous measurement of end-point plasma osteocalcin (increased in P and decreased in D and DP), and areal BMD^a, photographs of femoral midshaft sections were computer analysed (Bioscan Optimas software) for total subperiosteal area (TSPA), medullary area (MA) and cortical area (CA).

TSPA decreased (mean, SEM: 263.76, 6.60 vs. 287.24, 7.48 mm², P<0.05) and MA marginally decreased (150.65, 5.29 vs. 165.91, 6.52 mm², P<0.10) in DP but not D or P. The resultant CA in DP was decreased compared to P (121.22, 3.45 vs. 124.04, 2.12 mm²). Earlier data showed that aBMD decreased with D and DP at the trabecular femoral head (9% each) and trochanter (15%, 13%), but not in midshaft bone. However, lumbar spine aBMD decreased in DP only (12%)^a.

Hence, P did not alleviate the loss of trabecular bone observed with D treatment. Rather, DP appeared to suppress both periosteal apposition and endosteal resorption, an effect that might over a longer period be reflected in a reduction in appendicular cortical bone but might already have contributed to the bone loss observed in the axial skeleton.

^aPaton LM, O'Connell SL, Fam BC, Kaymakci B, Reade PC, Ebeling PR, Wark JD, 1999, J Bone Miner Res. 14: S268

MO4

BONE DENSITY CHANGES IN PAMIDRONATE TREATMENT OF PAGET'S DISEASE: ENHANCED INCREASE IN THE NONPAGETIC SPINE WITH CALCIUM PLUS CALCITRIOL.

R.I. Price¹, L. Ward¹, D.H. Gutteridge¹, R.W. Retallack¹, G.O. Stewart², R.L. Prince¹, Charles Gairdner¹ & Fremantle² Hospitals, W. Australia.

Pamidronate (ivAPD) causes forearm bone loss, from 2^o HPT (1), that is abolished by Ca+calcitriol (1,25D) supplement (SUP) (2). We report effects on BMD of ivAPD, +/- SUP, in spine (LS), hip and whole body (WB).

49 patients were stratified into; (i) "moderate" (M, n=27) with Hyp_E = 5-10mmol L GF; (ii) "severe" (S, n=22) with Hyp_E >10. Patients randomised within-group to Ca+1,25D SUP, or UNSUP, ie total of 4 groups. S had 360 mg ivAPD as 6 doses of 60mg once-weekly; M had 240 mg as 4 weekly doses of 60mg. SUP had oral Ca 1.0-1.2 g/day for 6 months (mo) plus 1,25D 0.25mg twice daily for 4 weeks, starting at 1st ivAPD infusion. DXA BMD at 0, 3, 6, 12 & 24 mo in LS, hip and WB. Pagetic bone assessed separately in LS and hip. Only patients completing 24 mo included.

WB BMD of all groups rose significantly by 6 mo, notably in S-SUP (2.2-8.9% [(95%CI), p<0.005]. All groups elevated at 12 mo, but only M-SUP was still above baseline at 24 mo (1.1- 4.4%, p<0.005). SUP and UNSUP in S or M were not different at any time. LS Pagetic BMD rose markedly by 3 mo (Table), but SUP and UNSUP were not different at any time in S or M. Indeed, in combined M+S groups, Pagetic LS BMD of SUP & UNSUP elevated equally at 24 mo (Table). SUP BMD in nonpagetic LS rose mildly compared with Pagetic, but markedly compared with UNSUP, (Table; 95%CI, % rise in LS BMD. * = p<0.05, SUP vs UNSUP at same time).

SPINE	UNSUP(M+S) 3mo (n=24)	UNSUP(M+S) 24mo	SUP(M+S) 3mo (n=25)	SUP(M+S) 24mon
Pagetic	5.2-23.3	-1.4-18.9	10.5-25.2	1.7-16.6
Nonpag	-1.5-1.7	-0.3-4.4	0.4-13.6*	-0.1- 10.3

Hip Pagetic BMD responded similarly to the spine, with increases of about one half. Nonpagetic hip BMD showed neither treatment nor SUP effects.

We conclude (i) UNSUP bone loss limited to forearm; (ii) Ca+1,25D SUP does not enhance rise in Pagetic BMD, or its retention at 24 mo, relative to UNSUP; (iii) SUP markedly increases BMD in nonpagetic spine, relative to UNSUP, hinting at a role for SUP in osteoporosis treatment using PTH. (1) Price R *et al* (1993) J Bone Miner Res 8:210; (2) Stewart G *et al* (1999) Bone 24:139.

MO5

CLINICAL UTILITY OF ULTRASOUND AND GRIP STRENGTH TO PREDICT INCIDENT FRACTURE IN ELDERLY WOMEN.

SS Dhaliwal, A Devine, I Dick, A Marangou, R Naheed, RL Prince Department of Medicine, University of Western Australia, Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Western Australian Institute for Medical Research, Nedlands, Western Australia

In light of improved methods of fracture prevention it is important to improve future fracture prediction. Both skeletal integrity and neuromuscular function contribute to fracture propensity. In 1998 we recruited 1499 women over the age of 70 directly from the population. At two years 8% had sustained at least one incident fracture. We determined whether baseline skeletal strength and neuromuscular function measures predicted fracture.

Skeletal strength was measured by the Lunar Achilles heel ultrasound machine. Neuromuscular function was assessed by grip strength using a hand held dynamometer. Incident clinical fractures were verified from x-ray reports after the event. The mean age at baseline was 75 yrs, mean weight was 69kg, 34% had a prevalent fracture, and mean grip strength was 20.

In logistic regression analysis, incident fracture was predicted by prevalent baseline fracture, grip strength, the timed up and go test and the ultrasound parameters, bone ultrasound attenuation (BUA), speed of sound (SOS) and stiffness, but not age, SES or weight. In multivariate logistic regression only SOS (Relative Risk (RR) per SD 1.4, 1.1-1.7) and grip strength (RR per SD 1.3, 1.0 – 1.5) remained significant after adjustment for other demographic variables. There was no interaction between SOS and grip strength. The table shows the two-year RR (c.f. SD of 0) and the model predicted two-year actual fracture risk (AR (%)) by SD of grip strength or SOS.

Grip\SOS	SD 0	SD -1	SD -2
SD 0	RR 1.0, AR 7.4	RR 1.4, AR 10.1	RR 2.0, AR 13.5
SD -1	RR 1.2, AR 9.1	RR 1.8, AR 12.3	RR 2.4, AR 16.4
SD -2	RR 1.6, AR 11.2	RR 2.2, AR 15.0	RR 3.1, AR 19.7

The high baseline fracture risk in this population and the important role of simple measures of bone strength and neuromuscular function in defining a group of patients who have up to a 20% risk of fracture in just two years is illustrated. However, the proportion of potential fracture patients detected as a proportion of all fractures is small because of the small proportion of population in the high-risk groups.

MO6

VERTEBRAL BODY MORPHOMETRY IS RELATED TO INTERVERTEBRAL DISC DISORGANISATION

NL Fazzalari^{1,2}, E Simpson^{1,2}, IH Parkinson^{1,2}, B Manthey¹. ¹ Institute of Medical and Veterinary Science, Adelaide 5000. ² Adelaide University, Adelaide 5000.

The purpose of this study was to examine the influence of the morphological disorganisation of the intervertebral disc on vertebral cancellous bone architecture. Lumbar motion segments T12-L1, L2-L3 and L4-L5 were collected from 27 cadavers. There were 8 females aged 35-94 years and 19 males aged 20-90 years. An intervertebral disc macroscopic grade signifying the severity of disc disorganisation was assigned to each disc. Trabecular bone morphometric analyses were performed on the vertebral bodies. It was found that disorganisation of the intervertebral disc becomes more common with increasing age. Data were age adjusted and the relationship of morphological disc disorganisation on cancellous bone architecture analysed. A 10% increase in BV/TV was observed in the presence of advancing intervertebral disc disorganisation. Vertebral bodies adjacent to degenerate discs showed increased Tb.Th and decreased BS/BV. Significant bony changes were observed in the anterior regions of the vertebral body, while minimal alterations were found at posterior regions. Bone loss was observed in central regions of the vertebral body as intervertebral disc disorganisation increased, through a reduction in both Tb.N (1.5 ± 0.3 to 1 ± 0.2 [#/mm]) and Tb.Th (100 ± 38 to 75 ± 25 [microns]). About a 30% BV/TV increase in anterior areas of the centrum may be a response to a redistribution of load to the vertebral body periphery as a result of intervertebral disc disorganisation. It appears that trabecular morphology is related to the condition of the associated intervertebral disc, rather than being the sole consequence of a loss of bone with age. This relationship could influence the occurrence of vertebral body crush fracture.

MO7

IDENTIFICATION, EXPRESSION AND LOCALIZATION OF VACUOLAR H⁺ ATPASE ACCESSORY SUBUNIT 1, ATP6S1 IN MOUSE OSTEOCLASTS. J Xu, K Yip, N Pavlos, L Huang, A Carrello, MH Zheng. Department of Orthopaedic Surgery, University of Western Australia, Nedlands, WA 6009, Australia

Using PCR-selective subtraction hybridization of cDNAs from mouse osteoclasts and their precursor cells, we have identified a cDNA fragment encoding the mouse vacuolar H⁺ ATPase accessory subunit 1, ATP6S1 from osteoclasts. Sequence analysis revealed that the mouse ATP6S1 gene encoded a putative polypeptide of 463 amino acids displaying characteristics of a type I transmembrane glycoprotein. The amino acid sequence of the mouse ATP6S1 has a high degree of identity with that of rat ATP6S1 (98%), bovine (85%), and xenopus (58%). Furthermore, RT-PCR analysis showed that ATP6S1 transcripts were expressed highly in mouse osteoclasts and brain, and at various levels in other tissues including heart, kidney, muscle, spleen, liver and lung. The expression of ATP6S1 mRNA was slightly upregulated in osteoclasts compared with osteoclast precursor cells. An ATP6S1-EYFP fusion protein was generated and expressed in COS-7 cells. Confocal microscopy analysis revealed that ATP6S1 was localised to perinuclear region and vacuolar structures in the cytoplasm. Partial localisation was detected between ATP6S1 and microtubules in the perinuclear region but no apparent co-localisation was evident between ATP6S1 and F-actin filament. In osteoclasts, ATP6S1 was mainly distributed in the perinuclear region, and also expressed in vacuolar structures in cultured osteoclasts. A minor portion of ATP6S1 was co-localized with early endosomes by EEA-1 (an early endosome marker) antibody. In addition, ATP6S1 co-localised with pH-dependent lysotracker, suggesting that ATP6S1 is late endosome/lysosomal like. In all, the expression and localization of ATP6S1 suggests that it play a role in the acidification of osteoclast suborganells.

MO8

VARIATION IN CANCELLOUS BONE ARCHITECTURE BETWEEN SKELETAL REGIONS: A MORPHOMETRIC FRACTAL ANALYSIS.

IH Parkinson, NL Fazzalari. Division of Tissue Pathology, Institute of Medical and Veterinary Science, Adelaide, South Australia.

The complex morphology of cancellous bone architecture determines its' mechanical strength, therefore the susceptibility of bones to fracture is not solely determined by BMD. The fractal dimension, as a descriptor of complexity, enables differences in cancellous bone architecture, modulated by differences in mechanical loading, to be quantitated.

Fractal analysis was performed by a box counting method on histological sections of cancellous bone from three skeletal regions, subchondral bone from the proximal femur and the knee (n=106), vertebral bodies (n=58) and iliac crest (n=33). There were three fractal dimensions for each histological section.

For fractal 1, all three regions are the same. For fractal 2, the subchondral bone is greater than the iliac crest, which is greater than the vertebral bodies (1.38±0.06 versus 1.31±0.07 versus 1.19±0.07). For fractal 3, the subchondral bone is significantly greater than the iliac crest and vertebral bodies (1.74±0.06 versus 1.67±0.07 and 1.67±0.07).

These data show that cancellous bone architecture differs between skeletal regions. In particular, the fine surface detail as described by fractal 1 is the same in each region, while the shape of individual trabeculae as described by fractal 2 is more complex in the subchondral bone than the iliac crest and vertebral bodies. The overall spatial complexity of the cancellous structure as described by fractal 3 is the same for the iliac crest and vertebral bodies while in subchondral bone it is significantly greater. Fractal dimensions enable regional differences in cancellous bone architecture to be quantified so that pathology affecting these regions can be studied.

MO9

ARE ALL FRACTURES IN POSTMENOPAUSAL WOMEN DUE TO OSTEOPOROSIS? June Schroeder, R Burnet, G. Wittert, S.Fitzgerald and BEC Nordin. From the Endocrine Bone and Menopause Centre and the Endocrine and Metabolic Unit of the Royal Adelaide Hospital, Adelaide, SA.

There is a widespread assumption that all fractures from minor trauma in postmenopausal women are related to osteoporosis. We have tested this assumption by examining the bone mineral density (BMD) at spine, hip and forearm in a series of 514 postmenopausal women 234 of whom had suffered one or more fractures from minor trauma.

All measurements were made with the Norland XR 36 DEXA densitometer. We used logistic regression to calculate the increase in relative risk for each SD fall in BMD at 5 sites in 5 fracture groups after correction for menopausal age and weight with the results shown in the Table

RELATIVE RISKS OF FRACTURE (P VALUES:)

Site	Wrist(89) Other(73)	Foot/Ankle(63)	Vert(61)	Rib(54)
ForearmA	1.59(.004) 1.54(.012)	1.36(NS)	1.97(.001)	1.39(NS)
ForearmB	1.81(<.001) 1.40(NS)	1.18(NS)	1.88(.004)	1.71(.0036)
ForearmC	1.59(.004) 1.26(NS)	1.19(NS)	1.67(.010)	1.48(.015)
Fem.Neck	1.59(.005) 1.40(NS)	1.38(NS)	1.55(.036)	1.52(.040)
L2-4	1.62(.0039) 1.33(NS)	1.11(NS)	1.90(.0025)	1.83(.0044)

Note: ForearmA=ultradistal, B=distal, C=proximal

Wrist fractures are significantly related to BMD at all sites. Foot and ankle fractures are not related to BMD at any site. Vertebral fractures are related to BMD at all sites. Rib fractures are related to BMD at all but one site. 'Other fractures' are only related to BMD at one site. Thus wrist, rib and vertebral fractures are related to bone status – and presumably also hip fractures of which there were too few to analyse in this series. Other fractures (clavicle, humerus, tibia, nose etc.) will need to be studied in larger numbers to determine which of them can be regarded as 'osteoporotic'. In the meantime our data on foot and ankle agree with those of Greenfield and Eastell (2001,Osteoporosis Int. 12:97-103).

MO10

BMD RESPONSE TO HRT DEPENDENT ON BODY MASS INDEX.

S Korn, JA Pasco, MJ Henry, GC Nicholson, MA Kotowicz. The University of Melbourne, Department. of Clinical and Biomedical Sciences, Barwon Health, Victoria, Australia.

There is a wide range of BMD response to HRT when it is used to treat osteoporosis. In this study we investigated the relationship between the change in BMD after commencing HRT and body mass index (BMI). We assessed women (aged 60+ yr) referred for serial bone densitometry at The Geelong Hospital between 1991-8 and who used HRT+calcium (n=94) or calcium alone (n=40) for a minimum of 6 months (median duration (range): HRT 1.5(0.6-5.6) yr, calcium 1.3 (1.0-2.2)yr). BMD was measured at PA-spine and proximal femur (Lunar DPX-L) at baseline and at follow-up (time interval mean \pm SD, 1.7 \pm 0.9 yr). BMD for total hip was estimated (Mazess, 1999). Height and weight were measured at baseline and BMI calculated (kg/m²). Medication exposure was abstracted from patient records. Regression analysis was used to investigate the relationship between annual change in BMD (DBMD) with baseline BMI, before and after adjusting for baseline BMD and annual change in weight.

Change in BMD (g/cm²/yr) at the spine and hip were predicted by
DBMD_{SPINE}=0.0322+0.0018 BMI+0.00203 Dwt/yr-0.061 BMD_{BASELINE}
DBMD_{HIP}=0.0225+0.00452 BMI+0.00534 Dwt/yr -0.177 BMD_{BASELINE}
Mean change in BMD at the spine and hip was 2.0% and -2.2% at BMI=20, and 4.1% and 7.1% at BMI=30 (mean change in weight (0.213 kg/yr) and baseline BMD (spine=0.8497 g/cm²/yr and hip 0.7945 g/cm²/yr)).

Change in BMD at both the spine and hip was positively related to baseline BMI. This relationship existed before and after adjusting for weight change and baseline BMD (p<0.05). There was no corresponding association between calcium treatment and BMI. The mechanism of this effect is unclear. Nutritional, lifestyle, biomechanical and/or humeral factors associated with fat tissue may alter the bone remodelling process, resulting in a greater HRT response associated with increased adiposity.

MO11

EFFECTS OF VITAMIN D DEPLETION AND DIETARY CALCIUM ON OSTEOBLAST AND OSTEOCLAST VARIABLES IN THE RAT.

HA Morris, AJ Moore, S Iida, PH Anderson, PD O'Loughlin, Hanson Centre and Division of Clinical Biochemistry, Institute of Medical and Veterinary Science, Adelaide South Australia 5000.

1,25 dihydroxyvitamin D is an important hormone in calcium and bone mineral metabolism with both major bone cells, osteoblasts and osteoclasts, expressing vitamin D (VD) responsive genes. It is well established that vitamin D deficiency causes osteomalacia as a result of a delay in mineralisation. However when vitamin D is inactive, bone mineralisation defects can apparently be resolved by intravenous calcium and phosphate. We have developed a vitamin D deplete rat model and now report the interaction between vitamin D status and dietary calcium (Ca) on in vivo osteoblast and osteoclast variables. VD deplete rats (n=10) were bred in a UV free environment and fed a VD- diet containing 1 % Ca. VD+ rats were fed a diet containing 1% Ca and 1000 U/kg VD. At 6 months of age 5 rats from both the VD- and VD+ groups were changed to a 0.1 % Ca diet. At 9 months of age all rats were killed and femora were processed for histological examination. Fluorochrome labels were injected 6 and 2 days before death. Double label surface (dLS %) and mineral apposition rate were significantly elevated in VD- rats at baseline compared with VD+ and on 1% Ca (P<0.01). On 0.1% Ca, no change occurred in VD+ rats, with a significant increase in VD- (P<0.01). Osteoclast surface (OcS %) was decreased in VD- rats at baseline (P<0.01), remained unchanged on 1% Ca and increased on 0.1% Ca, in both groups. When bone cell activity was increased by ovariectomy, dLS increased in all groups but Oc.S only increased in VD+ groups. These data indicate that in vitamin D depletion, osteomalacia can develop by lowering dietary calcium levels. Osteoblast proliferation or activity is uncompromised by vitamin D depletion, however osteoclastogenesis appears to be compromised.

MO12

URINARY HYDROXYPROLINE AND CROSS-LINKS EXCRETION IN OSTEOPOROSIS CLINIC PATIENTS: EFFECTS OF VITAMIN D INSUFFICIENCY. DR Jesudason, AG Need, PD O'Loughlin, BEC Nordin. Institute of Medical and Veterinary Science, Adelaide, Australia.

It is generally accepted that hip fractures are associated with low serum 25-hydroxyvitamin D (25(OH)D) levels. The mechanism has not been fully elucidated, although there is a step wise increase in serum parathyroid hormone (PTH) when serum 25(OH)D falls below 50 nmol/L. Our objective was to study the relationship between the increasing serum PTH which occurs at low 25(OH)D levels and markers of bone breakdown (fasting urine hydroxyproline, deoxypyridinoline and pyridinoline corrected for creatinine (OHPr/Cr, Dpd/Cr and Pyd/Cr)) as well as serum osteocalcin (a marker of bone formation).

We obtained cross-sectional data on 291 subjects of mean age 61±10SD years who attended our osteoporosis clinic between 1994-2000 and who provided fasting blood and urine samples.

Serum PTH was significantly inversely related to serum 25(OH)D as levels fell below 50 nmol/L. At serum 25(OH)D levels below 50 nmol/L there were also stepwise increases in OHPr/Cr, Dpd/Cr and Pyd/Cr for each successive 10 nmol/L decrease in serum 25(OH)D. No relationship was demonstrated between serum osteocalcin and serum 25(OH)D. OHPr/Cr, Dpd/Cr and Pyd/CR were significantly higher in subjects with 25(OH)D levels below 30 nmol/L than in those with 25(OH)D levels above 30 nmol/L. In the whole set plasma ionised calcium was significantly positively related to serum 25(OH)D (r=0.15;P=0.02). The increase in bone resorption markers noted in subjects with 25(OH)D levels below 30nmol/L suggests that age-related bone loss will be greater in these subjects than in those with serum 25(OH)D above 30 nmol/L.

The data suggest that low serum 25(OH)D is associated with low plasma ionised calcium which stimulates increased PTH production, producing increased bone resorption.

MO13

DECLINING PREVALENCE OF PAGET'S DISEASE IN NEW ZEALAND

T Doyle, J Gunn, G Anderson, T Cundy Department of Radiology, Dunedin and Auckland Hospitals, and Department of Medicine, University of Auckland
Paget's disease of bone is reported to be common in New Zealand (NZ), but data suggest that the severity of Paget's disease has declined in recent decades. An epidemiological study from Britain, which has the world's highest prevalence of the disorder, has reported a decline in the prevalence between 1975 and 1995. In order to determine the current prevalence of Paget's disease in NZ, and to establish whether there has been any change in the prevalence, we have undertaken surveys in Dunedin and Auckland, and compared the results to an earlier survey from Dunedin*. Consecutive plain abdominal Xray films in subjects over the age of 55, who identified as of European or NZ European origin, were reviewed by two radiologists for the presence of Paget's disease. The films included the pelvis, sacrum, lumbar spine and upper femora. About 85% of patients with Paget's disease have at least one of these sites involved.

Age	Male			Female				
	No.	<70	71-80	>80	No.	<70	71-80	>80
Auckland 1999	482	1.9%	4.8%	5.1%	497	0%	1.4%	2.3%
Dunedin 2001	496	1.4% [†]	4.7% [†]	5.7% [†]	523	1.2% [†]	2.4% [†]	2.2% [†]
Dunedin 1983	500	3.1%	9.4%	9.5%	500	2.3%	3.1%	8.1%

[†] P<0.05 in comparison to 1983 figures

There was no significant difference in the prevalence between the Dunedin and Auckland surveys. Prevalence increased with age, and a significantly greater prevalence in men was shown (p<0.01), as most surveys have done in the past. Comparing the two Dunedin surveys, the prevalence rates across all age groups were significantly lower (25-85%) in the recent survey. From the 1996 census, we estimate that the number of patients with Paget's disease in NZ is about 16,000 (10,200 men and 5,800 women). However, as in Britain, the prevalence appears to be decreasing, emphasising the importance of environmental factors (as yet unidentified) in the aetiology of the disease.

* JC Reasbeck et al BMJ 1983 ; 286 : 1937. *Funding: National Association for the Relief of Paget's Disease (United Kingdom) and Paget's Disease Trust (Auckland).*

MO14

OPTIMAL FRACTURE PREDICTION FROM BMD MEASUREMENT AT MULTIPLE SITES: GEELONG OSTEOPOROSIS STUDY

MJ Henry, JA Pasco, KM Sanders, GC Nicholson, E Seeman, MA Kotowicz. The University of Melbourne, Dept of Clinical and Biomedical Sciences: Barwon Health, Geelong, Australia, 3220.

BMD is a strong predictor of fracture risk but it is unresolved which anatomical site(s) best predict fracture. We have investigated the ability of BMD at different sites and age (a), weight (w), height and body mass index (BMI) to predict fracture. Women sustaining low trauma fractures were recruited from an area in south-eastern Australia (n=470, aged 35-92yr) and included fractures of the hip (48), spine (108), humerus (30) and wrist (91). A non-fracture group (n=1120, aged 35-94yr) was established from an age-stratified population-based study. BMD was measured at the femoral neck (FN), Ward's triangle (WT), trochanter (T), PA spine (S), midradius (MR) and ultradistal radius (UR) sites using a Lunar DPX-L densitometer. Predictive discriminant analysis (a regression technique involving dichotomous response variables) was used to optimize fracture prediction. This technique generates a score that indicates an individual's fracture risk.

The risk score for fracture at the following sites are calculated as follows, with a positive score predicting fracture:
(%Sens,%Spec)

Hip = 1.69 -15.01 BMDT + 6.51 BMDFN +0.06a + 0.04w (88,78)

Spine = 6.23 - 4.98 BMDS - 2.09 BMDMR (79,71)

Humerus = 0.27 - 4.05 BMDS + 0.14 BMI (63,68)

Wrist = 0.93 - 3.75 BMDUR - 2.55 BMDWT + 0.03w (67,60)

All of the above sites =

4.25 - 2.27 BMDS - 2.23 BMDMR - 3.56 BMDT + 0.03w (71,66)

Discordance in BMD across sites has led to reliance on BMD measurements at a single site for fracture prediction. This cross-sectional data suggests that fracture prediction is optimized by a combination of multiple sites. These models require validation with prospective longitudinal data.

MO15

SEASONALITY OF FALLS AND FRACTURE.

JA Pasco¹, JR Pasco², MJ Henry¹, GC Nicholson¹, MA Kotowicz¹. ¹The University of Melbourne Dept Clinical and Biomedical Sciences, Barwon Health and ²Emergency Dept, Geelong Hospital, Australia.

Seasonal variations in hip and distal forearm fractures have usually been attributed to extreme wintertime weather conditions. Our study is set in Geelong (latitude 38°S) where the climate is temperate. We have previously demonstrated a higher fracture rate during winter (May-October) among women from the Barwon Statistical Division and postulated a causal association with both lower serum 25-hydroxyvitamin D and higher PTH levels. It is possible, however, that increased fracture rates result from increased frequency of falls during winter. We aimed to determine the seasonality of falls and falls resulting in fractures within our region. During 1995-7, we identified all resident women aged 35+ yr who presented with falls to the Emergency Department (ED) at the Geelong Hospital, the sole ED in the region. Consequent fractures were confirmed radiologically.

From a total of 1972 falls (from 1799 patients), there were 1092 fractures. Overall, 55.4% of falls resulted in fracture. Odds ratio (95% CI) for falls-related fracture in winter was 1.29 (1.08, 1.54).

	Summer (Nov-Apr)	Winter (May-Oct)	p
Falls presentations(n)	944	1028	0.1
Fractures (n)	492	600	0.003
Fractures/Falls (%)	52.1	58.4	0.005
Age (median (range), yr)			
Falls	73 (35-99)	73 (35-101)	0.8
Fractures	76 (35-98)	76 (35-100)	0.6

Our data indicate a trend towards more presentations from falls to ED in winter. During winter a greater proportion of falls resulted in fracture suggesting increased bone fragility during this period. This supports our hypothesis that, even at this latitude, low vitamin D levels and compensatory rises in PTH during winter impair bone mineralisation, stimulate bone resorption and diminish bone strength.

MO16

AUTOLOGOUS CHONDROCYTE IMPLANTATION: A REVIEW OF 26 CASE STUDIES

M. H. Zheng, R. Salleh, B. Robertson, D. J. Wood. Department of Surgery (Orthopaedics), University of Western Australia, QEII Medical Centre, 2nd Floor M Block, Nedlands WA 6009 Australia

Articular Cartilage defects of the knee occur commonly in sport injury and trauma. Increasing evidence suggests that the only technique that enables the regeneration of articular hyaline cartilage in chondral defects is autologous chondrocyte implantation (ACI). Here we have reported our clinical experience of autologous chondrocyte implantation using biodegradable type I/III collagen membrane (CACI). A total of 26 patients (aged range from 19 to 60 years, average 37 years) was conducted with CACI. Preoperative MRI scans were performed on all patients. Postoperative MRI scans were planned for approximately 3 and 12 months to determine the success of integration of implanted chondrocytes. The result demonstrated that the initial postoperative MRI scans at three months show the presence of oedematous tissue at the defect sites in 23 patients, contrasting with the fluid filled defects seen preoperatively and with and MRI signal differing from that of the surrounding normal hyaline articular cartilage. MRI scans in 9 patients at 12 months after operation showed maturation of cartilage graft in all patients. Apoptotic test of chondrocytes using Annexin IV before implantation showed that viability of chondrocytes was over 85% where apoptotic rate of chondrocytes was less than 2%. One patient with apoptotic rate of over 10% has a delayed repair in cartilage defects as evidenced by MRI. In conclusion, an early phase clinical studies showed that autologous chondrocyte implantation remains promising for the treatment of chondral defect with restoration of hyaline cartilage. Longer clinical follow-up of the patients and better assessment of cellular phenotype of chondrocyte before implantation are required.

MO17

EFFICACY OF A SHORT ORAL COURSE OF HIGH DOSE CALCIFEROL IN CORRECTING VITAMIN D DEFICIENCY.

F Wu, A Horne, J Clearwater, B Orr-Walker, IR Reid. Department of Medicine, University of Auckland, Auckland, New Zealand

Treatment of subclinical vitamin D deficiency is important, as it is common and it predisposes to osteoporosis and osteoporotic fractures. The use of daily low-dose calciferol replacement is slow to replenish vitamin D stores and requires long-term compliance. We examine the efficacy of a short course of oral high-dose calciferol in correcting vitamin D deficiency.

Thirty-two asymptomatic postmenopausal women (age 76 ± 4 yrs), with serum 25-hydroxyvitamin D (25OHD) ≤ 10 mg/L were given oral calciferol 50,000IU daily for 10 days. All were mobile and living independently, and none had diseases or were taking medications that influenced vitamin D or calcium metabolism.

The mean \pm SE pre- and post-treatment 25OHD levels were 8 ± 1 mg/L and 25 ± 5 mg/L respectively. All patients, except one, had 25OHD within the reference range following treatment (RR: 14 – 76 mg/L). The post-treatment levels were measured at 17 ± 7 wk; there was no correlation between post-treatment 25OHD level and time since treatment, suggesting that the levels peaked early and remained stable. There was no difference in the increase in 25OHD after treatment in those treated in the summer versus those treated in winter. Following treatment, serum parathyroid hormone levels decreased by 0.7 ± 1.7 pmol/L ($P < 0.05$), while serum calcium increased by 0.06 ± 0.08 mmol/L ($P < 0.001$). Serum alkaline phosphatase activity decreased ($P < 0.05$) indicating reduced bone turnover. There were no significant side effects, and no one had 25OHD or calcium levels above the reference range following treatment.

This simple regimen is a safe, effective, cheap, and well tolerated method of treating vitamin D deficiency and is convenient for both in- and out-patient settings.

MO18

LOCALIZATION OF THE L-AMINO ACID BINDING SITE ON THE HUMAN CALCIUM-SENSING RECEPTOR

R Mun and AD Conigrave Department of Biochemistry (G08), University of Sydney, NSW 2006, Australia.

The calcium-sensing receptor (CaR) is a seven transmembrane domain receptor that plays a key role in whole body calcium metabolism mediating feedback regulation of parathyroid hormone secretion as well as regulating renal calcium excretion (review: 1). It belongs to sub-group C of the seven transmembrane receptor family that also includes the metabotropic glutamate receptor, the GABA(B) receptor and a number of pheromone receptors. We have recently demonstrated that the CaR is stereo-selectively modulated by L-amino acids from several different classes (2). It is especially sensitive to amino acids that belong to the aromatic and aliphatic classes. In the presence of active L-amino acids, the set-point for Ca^{2+} ions is left-shifted. Furthermore, in the presence of constant submaximal Ca^{2+} concentrations, the receptor acts as a sensor for L-amino acids and is sensitive to changes in total amino acid concentration either side of the resting total L-amino acid level. The presence of an allosteric site for L-amino acids on the CaR would appear to explain how dietary protein intake modulates parathyroid hormone levels and renal Ca^{2+} excretion rates (review: 3)

In the current work we have used chimeric receptors constructed from domains of the homologous human CaR and rat metabotropic glutamate type-1 receptors to define the location of the amino acid binding site. The data indicate that the binding site localizes to the extracellular domain of the receptor rather than the seven transmembrane domain region.

1. E.M. Brown and R.J. MacLeod (2000) *Physiol. Rev.* 81, 239-297.
2. A.D. Conigrave, S.J. Quinn and E.M. Brown (2000a) *PNAS* 97, 4814-4819.
3. A.D. Conigrave et al. (2000b) *TIPS* 21, 401-407.

MO19

VERTEBRAL SIZE AND VOLUMETRIC DENSITY IN YOUNGER AND OLDER MEN WITH SPINE FRACTURES.

XF Wang, Y Duan, E Seeman. Department of Medicine, Austin & Repatriation Medical Centre, Melbourne, Australia.

Reduced peak bone accrual, excessive bone loss and impaired age-related periosteal bone formation may variably contribute to the smaller vertebral size and lower volumetric bone mineral density (vBMD) in men with spine fractures. We hypothesized that younger men with spine fractures would have smaller vertebral size due to reduced growth while older men would have normal bone size and reduced vBMD due to bone loss. We studied 44 men aged 54.3 yrs (45 – 65), 64 men aged 75.2 yrs (66 – 90) with one or more nontraumatic spine fractures, and 395 healthy men aged 17 to 91 yrs without fractures. Vertebral width, height, and vBMD of the third lumbar vertebra (L3) were measured by postero-anterior (PA) scanning using dual x-ray absorptiometry. Vertebral volume (V) was estimated as: $V = (\text{scan area of L3})^{3/2}$. Vertebral vBMD was estimated as BMC/V . The data were expressed as standardized deviation (SD) scores (Z score, Mean \pm SEM).

Advancing age was associated with increased vertebral width ($r = 0.17$, $p < 0.001$) but not vertebral body height in healthy men. Vertebral width was reduced in younger (-0.25 ± 0.15 SD, $p = 0.10$) and older patients with fractures (-0.57 ± 0.11 SD, $p < 0.001$). Vertebral height was similarly reduced in younger and older patients (-0.30 ± 0.16 vs -0.29 ± 0.12 SD, NS). After adjusting for standing height and body weight, vertebral width was reduced only in older patients (-0.33 ± 0.11 SD, $p < 0.01$). vBMD deficit was similar in younger and older patients before (-1.30 ± 0.13 vs -1.37 ± 0.10 SD, NS) and after adjusting for height and weight (-1.37 ± 0.13 SD vs -1.45 ± 0.10 SD, NS).

We infer that, contrary to our hypothesis, younger men with spine fractures do not have reduced bone size but only reduced vBMD, probably due to reduced accrual of bone. Older men have both smaller bone size and reduced vBMD, both may be the result of reduced periosteal apposition. The pathogenesis of spinal fragility may differ in younger and older men.

MO20

CHARACTERISATION OF CHONDROCYTE LINEAGE IN AUTOLOGOUS CHONDROCYTE TRANSPLANTATION.

RJ Wealhall, E King, D Liu, J Xu, MH Zheng. Department of Orthopaedic Surgery, University of Western Australia, Nedlands, WA 6009, Australia.

Autologous chondrocyte transplantation (ACT) has been shown to be a promising method for restoring hyaline cartilage defects. Since first reported in 1994, clinical follow up studies have indicated that ACT has provided an excellent outcome in the restoration of hyaline cartilage. As ACT relies on the use of cultured cells, and the biosynthetic profile of cultured chondrocytes has been shown to be altered during *in vitro* expansion, cultivation of chondrocytes for ACT has presented many technical and quality control challenges. An assessment of the cellular phenotype of cultured chondrocytes, consistent with differentiation of articular hyaline cartilage, is required to ensure the delivery of ACT. Using RT-PCR, flow cytometry, and scanning electron microscope (SEM) analyses, we have characterised the cellular phenotype of cultured chondrocytes used for ACT. We have examined several transcriptional factors, cytokines and matrix proteins necessary for the differentiation of chondrocytes in cases of ACT. These include Sox9, BMP-2, TGF β 3, PTHrP, CEP-68, type I and II collagen, aggrecan, and alkaline phosphatase. While there was variation in the expression of some of these genetic markers among patients, all patients expressed chondrocyte-specific genes. Furthermore, SEM and flow cytometry analyses demonstrated variation between patients in cell morphology and in the level of apoptosis of chondrocytes. Magnetic resonance imaging studies of two patients that were unable to restore hyaline cartilage show that these coincided with an apoptotic level greater than 8%. In short, given that there is a medical need for ACT in the treatment of articular cartilage injury, processes such as these for monitoring the quality of cultured chondrocytes prior to implantation may provide a better clinical outcome for ACT.

MO21

CALCIUM AND PROTEIN INTAKE PREDICT APPENDICULAR BUT NOT AXIAL BONE MASS IN CHILDREN AND ADOLESCENTS.

S. Iuliano-Burns, M. Bradney, L. Saxon*, S Bass*, E. Seeman

Dept. of Med., Austin & Repatriation Medical Centre, Melb, Aust.

*School of Health Sciences, Deakin University, Melb, Aust.

Protein and calcium (Ca) intakes may influence peak bone mass. Pre-pubertal growth is characterised by a greater rate of bone mass accrual at the appendicular than axial skeleton. Dietary changes during this time may be more likely to affect the appendicular skeleton. In animals and humans, the evidence for a beneficial effect of protein supplementation and deleterious effect of protein malnutrition is more compelling than the corresponding data concerning calcium deficiency and treatment. Although some investigators suggest calcium supplementation results in a higher peak bone mass in children, the data to support this notion is contradictory. We hypothesise that dietary protein and calcium will influence appendicular but not axial bone mass and a greater proportion of the variance in bone mass is accounted for by dietary protein than dietary calcium intake.

Dietary data were collected from 140 healthy males and females aged 7-17 years using three-day weighed food diaries. Diets were analysed using FoodWorks nutrition program. Bone mineral content (BMC) was measured using DEXA. The proportion of the total variance accounted for by protein and Ca were determined using multiple regression controlling for age, height and weight.

Ca and protein intakes separately predicted leg BMC in each sex ($R^2 = 7-19\%$), arm BMC in males ($R^2 = 9-17\%$) but not spine BMC. When protein and Ca intakes were included in the regression equation, Ca but not protein, remained an independent predictor of arm BMC in each sex and leg BMC in males (Table 1.).

Table 1. Proportion of BMC variance accounted for by calcium intake

Variance (%)	Boys		Girls	
	Arms	Legs	Arms	Legs
* p < 0.01, ^p < 0.05	5.4*	4.3*	3.1^	NS

In summary, although the effect of colinearity cannot be excluded, these data indicate that calcium intake is a stronger independent predictor of appendicular bone mass than protein intake.

MO22

THE PRESENCE OF THE APOE4 ALLELE IS NOT ASSOCIATED WITH PREVALENT OR INCIDENT FRACTURE IN A WESTERN AUSTRALIAN POPULATION.

IDick, A Devine, E Jamieson, RL Prince. Dept of Medicine, University of Western Australia, Sir Charles Gairdner Hospital, Nedlands, WA.

A number of conflicting studies have been published indicating either an association, or no association, of the APOE4 allele with an increased risk of fracture and/or decreased bone mineral density (BMD). The aim of this study was to examine the association of the APOE4 allele with bone mineral density (BMD), quantitative ultrasound (QUS) and prevalent and incident fracture in a Western Australian population of women aged over 70 years who are participating in the The Calcium Intake Fracture Outcome Study (CAIFOS).

The study population consists of 1500 women who were randomised into the trial. This comprises 6.2% of 24800 women over the age of 70 available in the elderly population in Perth. The mean age of the population is 75.1 ± 2.7 . APOE genotyping was performed in 1136 subjects and comprises the subject group for this study. Of these, QUS was performed at baseline on 1118 subjects. At 12 months after randomization, Dual Energy X-ray Absorptiometry (DEXA) (Hologic) was performed at the hip on 950 of these subjects and at the spine in 224 subjects. Based on a T score cutoff for the total hip site of < 2 SDs below the premenopausal normal range, the subjects were classified as osteoporotic (n=322) or non-osteoporotic (n=628). Biochemical parameters of bone formation and resorption, measures of physical function (Barthel, Timed Up and Go) and balance (Romberg), grip strength, weight and lean body mass were measured. A total of 278 prevalent fractures resulting from minimal trauma, that had occurred prior to the commencement of the study, were reported by the subjects. A total of 85 minimal trauma incident fractures, up to 24 months after commencement of the study, were confirmed radiologically in this group.

APOE genotyping indicated that 24.2% of the subjects had an APOE4 allele, with 2% being homozygous. The presence of the APOE4 allele was not associated with an increase in prevalent fractures (OR=1.13, 96% CI 0.82-1.56) or an increase in the risk of incident fracture (RR=1.06, 95% CI 0.63-1.77). There was no significant difference in BMD at any site, nor was there a difference in any of the QUS parameters, biochemical parameters, age or functional tests in the APOE4 allele group. The APOE4 allele was not associated with an increased prevalence of DEXA defined osteoporosis at the hip (OR 1.37, 95% CI 0.99-1.89).

The results obtained from this study therefore do not support a role for APOE genotype in determining bone mineral density or fracture risk in a free living, elderly Caucasian female population.

MO23

LUMBAR SPINE AND HIP BMD IN HIV-INFECTED MEN.

N Pocock, J Gold, J Freund. Department of Nuclear Medicine and Bone Densitometry, St Vincent's Hospital, and Albion St Centre, Sydney.

Reduced bone density has been previously described in HIV affected men from studies of whole body BMD. The whole body site however is suboptimal for assessment of bone loss. There are no published studies of BMD at axial sites in HIV infected men.

BMD was measured in the lumbar spine and proximal femur using DXA (DPXL, Lunar Madison WI) in 110 HIV infected men. Additional parameters assessed included age, duration of antiretroviral NRTI therapy, testosterone and weight/lean body mass (measured by Bioelectrical Impedance Assessment - BIA). The BMD results were compared to the manufacturers normal range, which has been previously shown to not differ significantly from normative Australian data. The relationship of the additional parameters to BMD was assessed using multiple regression analysis.

Results: The patients ranged in age from 35 to 71 (median 48yrs). The median duration of known HIV infection was 10yrs (range 1 to 18). The median lumbar spine, total proximal femur (TPF) and femoral neck (FN) results are shown in the Table.

	Z Score (range)	T score (range)
Lumbar Spine	-0.3 (-4.8 to 2.7)	-0.7 (-4.1 to 1.8)
Total Proximal Femur	-0.3 (-3.9 to 1.6)	-0.7 (-3.5 to 1.6)
Femoral Neck	-0.4 (-3.0 to 1.6)	-1.0 (-3.9 to 1.6)

The mean Z scores were significantly reduced at the LS, TFF and the FN ($p < 0.001$ at all sites). The prevalence of osteopenia and osteoporosis at one or both sites was 44.5% and 10.9 % respectively. A total of 55.5% of patients were classified as osteopenic or osteoporotic at one or both sites. Multiple regression analysis indicated that the independent predictors of LS, TPF and FN BMD were age, duration of NRTI use and lean body mass.

Conclusion: There is significantly reduced axial BMD for age in HIV infected men. The mechanism is likely to be multifactorial. Antiretroviral therapy may be one of the underlying mechanisms.

MO24

IV PAMIDRONATE, BUT NOT ORAL ALENDRONATE, INCREASES HDL CHOLESTEROL AND DECREASES TRIGLYCERIDES IN PATIENTS WITH PAGET'S DISEASE. SD Vasikaran*, DH Gutteridge, LC Ward, S Dhaliwal, JR Burnett*, RL Prince, JP Walsh. Department of Core Clinical Pathology and Biochemistry*, Royal Perth Hospital; Department of Endocrinology, Sir Charles Gairdner Hospital; Perth, Australia.

The recent demonstration that the mechanism of action of aminobisphosphonates (N-BPs) was mediated via the mevalonate pathway prompted the measurements of lipids and lipoproteins in stored frozen serum from patients with Paget's disease enrolled in a randomised controlled trial of oral alendronate (40 mg daily), and intravenous (iv) pamidronate (APD, 60 mg 3 monthly).

Of 67 patients with active Paget's disease recruited for the study, 14 receiving lipid-lowering therapy were excluded. Of the remaining patients, 27 received alendronate (3 to 9 months) and 26 received APD (1 to 5 infusions at 3 monthly intervals) until biochemical normalisation of pagetic activity. The baseline sample as well as a post-treatment sample (end of treatment with alendronate, and 3 months after final infusion with APD) were analysed for total cholesterol, triglycerides, high density lipoprotein (HDL) cholesterol, apolipoprotein (apo) A-I and apoB.

There were no significant changes in serum lipids from baseline with alendronate treatment. In contrast, there was an 8% increase in HDL cholesterol ($p < 0.05$) and a 18% decrease in triglycerides ($p < 0.05$) concentrations with iv APD treatment. Total cholesterol, apoA-I and apoB levels were unchanged.

These results suggest iv N-BPs have a beneficial effect on serum lipid profile while oral N-BPs have no effect. More marked effects of iv APD may be observed if measurements were performed earlier (1 to 2 months) after the infusion. The effects of iv N-BPs on atherosclerosis and vascular outcomes need to be studied.

I3

MODULATION OF BONE CELL FUNCTION BY TENASCIN-C.

TM Campbell¹, EM Tudor¹, WT Wong¹, R Fässler² and EJ Mackie¹. ¹School of Veterinary Science, University of Melbourne, Australia. ²Lund University Hospital, Sweden.

Tenascin-C (TnC) is an extracellular matrix protein which is expressed by osteoblasts but is generally absent from mineralized bone matrix. We have used mice genetically incapable of expressing TnC ('knockout' mice) to investigate the role of TnC in bone cell function. A model of bone repair has been developed to investigate this process in TnC knockout mice. A hole was drilled through the tibial diaphysis, and mice were killed for histological examination at various times after drilling. In wildtype mice, osteogenic cells migrated into the defect then laid down trabecular bone, which was later remodelled into cortical bone, such that by 28 days after drilling normal architecture had almost been restored. In knockout mice, osteogenic cells invaded the drill site 2 days earlier than in wildtype mice, and the new bone formed in the early repair phase showed excessive mineralization and altered matrix composition in comparison with bones from wildtype mice. At day 28, exostoses were visible adjacent to the repair site on the surface of the tibia of knockout but not wildtype mice. Primary osteoblast cultures prepared from knockout and wildtype mice were used to investigate the ability of osteoblasts to undergo differentiation and proliferation in the absence of TnC. Alkaline phosphatase (AP) activity increased with time in culture (up to 19 days) in wildtype cells; in TnC knockout cells, AP activity per cell was lower than in wildtype cells at all time points examined, and was 4-fold lower than in wildtype cells by day 19. Unlike wildtype cells, knockout cells did not proliferate in response to transforming growth factor β or fibroblast growth factor-2. In contrast, knockout cells showed a normal proliferative response to platelet-derived growth factor. These results indicate that TnC, as part of the cellular environment in bone, modulates responses of bone cells to physiological and pathological stimuli.

I4

THE VITAMIN D RECEPTOR: MOLECULAR VARIATION AND FUNCTIONAL OUTCOMES. EM Gardiner, J Flanagan, KL Sunn, T-A Cock, AP Kouzmenko, JA Eisman. Bone and Mineral Program, Garvan Institute of Medical Research, Sydney 2010, Australia.

Active hormonal vitamin D acts in many cell types to regulate calcium homeostasis, cellular proliferation and differentiation. Only one human vitamin D receptor (VDR) was known, however, raising the question of how such functional diversity could arise. Evidence for other VDR protein isoforms was therefore sought, based on the earlier detection of alternatively spliced VDR transcripts encoding N-terminally variant proteins. Western blots of cell and tissue extracts using isoform-specific polyclonal antibodies detected one of the predicted protein isoforms, VDRB1, which had a 50 amino acid N-terminal extension and co-existed at slightly reduced or equivalent levels with the standard VDRA isoform. Transcriptional kinetics of VDRB1 and VDRA appeared to differ, with VDRB1 activity higher at earlier time points but lower at later times after ligand treatment in transient transfection assays. The N-terminal region of VDRB1 showed strong ligand-independent activation function-1 (AF-1) activity, with weaker but significant AF-1 activity also detected for the VDRA N-terminus in yeast and mammalian one-hybrid analyses. The N-terminal region of VDRB1 interacted strongly with the VDR AF-2 domain, the transcriptional co-activator CBP/p300 and co-repressors NCoR and SMRT in mammalian two hybrid experiments. Interaction of the VDRA N-terminus with the AF-2 region and these cofactors was weak or non-existent. Intracellular localisation was examined for other clues to a mechanism of the transcriptional difference. VDRB1 fused to green fluorescent protein accumulated in nuclear speckles that were AF-2 dependent and dispersed by 1,25-dihydroxyvitamin D₃, whereas VDRA exhibited diffuse nuclear fluorescence regardless of ligand. These clear differences in VDRB1 protein interactions and intranuclear localisation may contribute to its distinct transactivation characteristics and thus to the diversity of physiological vitamin D responses.

O7

EFFECTIVE TREATMENT OF POSTMENOPAUSAL OSTEOPOROSIS WITH INTERMITTENT (3-12 MONTHLY) TREATMENT WITH THE POTENT BISPHOSPHONATE, ZOLEDRONIC ACID.

MJ Hooper¹ for the Zoledronic Acid Study Group².¹The University of Sydney, Australia & ²25 centres in 10 countries

The potent oral bisphosphonates are effective in the prevention and treatment of osteoporosis but their poor bioavailability and gastrointestinal tolerability limits their usefulness and compliance. Intermittent intravenous bisphosphonates have the advantage of ensuring bioavailability and compliance and, in this study, we explored the use of zoledronic acid, which appears to be the most potent bisphosphonate to date.

In this one year, randomised controlled trial including 351 postmenopausal women with low bone mineral density (T-scores <-2.0), zoledronic acid was given intravenously in a total annual dose of between 1-4mg as 0.25mg, 0.5mg and 1mg at three-monthly intervals, 2mg at 6-monthly intervals and 4mg as a single initial dose. Bone mineral density results (intention to treat analysis) showed a mean increase from baseline (differences from placebo) of between 4.3-5.1% increase in the lumbar spine and 3.1-3.6% in the femoral neck (table below). After one year, there was a 40-50% decrease in markers (BSAP, serum CTX, urine NTX).

REGIMEN	SPINE BMD %	HIP BMD %
4x0.25mg (every 3 months)	+5.1	+3.1
4x0.5mg (every 3 months)	+4.9	+3.1
4x1.0mg (every 3 months)	+4.3	+3.2
2x2.0mg (at start and at 6 months)	+4.3	+3.6
1x4.0mg (at start only)	+4.6	+3.3

The increases in bone density and decreases in markers were significantly different from placebo in all groups.

The treatment was generally well tolerated but myalgia and pyrexia occurred more often in the active treatment arms.

This study demonstrates that intravenously administered zoledronic acid, given at intervals of up to one year between injections, produces effects on bone markers and bone density as great as those seen with the potent oral bisphosphonates of proven anti-fracture efficacy. This result indicates that a six-monthly or even twelve-monthly intravenous injection of zoledronic acid is likely to be an effective treatment for postmenopausal osteoporosis without the problems of bioavailability and compliance seen with oral bisphosphonates.

O8

INCIDENCE OF FRACTURES IN ELDERLY MEN AND WOMEN: DUBBO OSTEOPOROSIS EPIDEMIOLOGY STUDY.

KP Chang, JR Center, JA Eisman. Bone and Mineral Research Program, Garvan Institute of Medical Research, Sydney, Australia.

The Dubbo Osteoporosis Epidemiology Study is an ongoing longitudinal study of fracture incidence in men and women aged 60 and over since 1989. Fractures due to minimal trauma and non-pathological fractures were ascertained by reviewing all radiology reports from the two area radiology services.

From April 1989 to December 2000, 1055 symptomatic fractures occurred in 807 persons, over 39,357 person-years of observation. Fracture incidence was 3412 and 1632 per 100,000 person years for women and men respectively. The incidence of vertebral, proximal humerus, wrist, hip and pelvis fractures increased exponentially with age, typical of osteoporotic fractures. Ankle, hand and foot fractures did not show this exponential increase. However, 42% of men and 15% of women with ankle fractures had a subsequent osteoporotic fracture. Although fracture incidence was greater in women than men for all but rib fractures, the female:male ratio was less than 2 for most fracture types (eg hip 1.8:1, vertebral 1.4:1) in the 80+ age group. Absolute fracture numbers peaked in the 70-79 age group for men and in the 80-84 age group in women before declining, due to the smaller 'at risk' population.

This is the first study examining fracture incidence beyond 5 years. It highlights that 1 in 3 of all fractures occurred in men, and that with increasing age, the female:male ratio is less than 2 for most fracture types. Ankle fractures don't increase with age, however, in men at least, they appear to be forerunners for subsequent osteoporotic fractures. Although fracture incidence increases exponentially with age, absolute fracture numbers are higher, especially for men, in the younger age group. The data highlight the need for early preventative treatment as the fracture burden on society actually occurs younger than previously thought, especially in men.

O9

CALCIUM ABSORPTION AND SERUM VITAMIN D METABOLITES IN POSTMENOPAUSAL SMOKERS

AG Need, AJ Kemp, M Horowitz, HA Morris, BEC Nordin. Division of Clinical Biochemistry, Institute of Medical and Veterinary Science, and Department of Medicine, Royal Adelaide Hospital, Adelaide, SA

Smoking is associated with poor intestinal calcium absorption, low bone density and increased fracture risk but there are conflicting reports on its effects on the vitamin D endocrine system. We therefore took a fracture history and measured intestinal radiocalcium absorption (with ⁴⁵Ca using a 20 mg calcium carrier and a single blood sample taken one hour after the dose), serum vitamin D metabolites (25 hydroxy- vitamin D (25(OH)D) by competitive protein binding and calcitriol by HPLC and radioimmunoassay) and serum parathyroid hormone (PTH)(by immunoradiometric assay) in a group of 405 consecutive postmenopausal patients attending our osteoporosis clinics. We compared mean values in the 74 smokers and the 331 non-smokers and examined the relationships between them in each group. Vertebral compression fractures were more common in smokers than non-smokers (mean 1.5(2.5SD) vs 0.7(1.4);P=0.013) as were peripheral fractures (1.2(1.8) vs 0.6(1.0);P=0.016). The hourly fractional rate of calcium absorption was less in smokers than non-smokers (0.56(0.29) vs 0.68(0.27) fx/h;P=0.001) as were serum calcitriol (95(32 vs 122(37) pmol/L;P<0.001) and serum PTH (3.8(1.6) vs 4.9(2.6) pmol/L; P<0.001). Serum 25(OH)D was similar in each group (60(25) nmol/L in the smokers and 62(26) nmol/L in the non-smokers;P=0.57). Calcium absorption was related to serum calcitriol in both smokers and non-smokers (P=0.001 and <0.001 respectively). There was no difference in this relationship between smokers and non-smokers. In the smokers, serum calcitriol was significantly related to serum PTH (P=0.001) and serum 25 (OH)D (P=0.027). Hence the poor calcium absorption in the smokers was due to suppression of the PTH-calcitriol axis. This may aggravate their bone loss and also limit the effect of calcium therapy.

O10

BODY SEGMENT LENGTHS AND ARM SPAN IN NORMAL MEN AND WOMEN AND PATIENTS WITH SPINE FRACTURES.

Y Duan, XF Wang, J Edmonds, BT Kim, E Seeman. Department of Endocrinology, Austin & Repatriation Medical Centre, University of Melbourne, Melbourne, Australia.

Men and women with spine fractures are shorter and leaner and have smaller vertebral volume than controls. Whether the shorter stature in fracture cases is due to the fracture is not clear. We asked: (i) What are the age- and gender-specific differences in upper or lower body segment length in men and women? (ii) Do men and women with spine fractures have shorter body segment length compared to controls? We studied 101 healthy men and 233 healthy women aged 18 to 92 years, and 34 men and 67 postmenopausal women with spine fractures aged 46 to 90 years. Standing height, sitting height and leg length were measured by using a Holtain stadiometer. Arm span was measured by a tape which from the middle finger of the right hand to the middle finger of the left hand along the wall. All measurements were recording to the nearest 0.1 cm. The results were expressed in absolute term and standard deviation (SD) or Z scores (mean \pm sem). Arm span was greater by 1 and 5 cm than standing height in young men and young women, respectively (women, 164.6 \pm 0.6 vs 163.8 \pm 0.6 cm; men, 180.5 \pm 1.4 vs 175.8 \pm 1.6 cm, both p < 0.01). Advancing age was associated with decreased sitting height in healthy men and women (r = -0.29 to -0.36, both p < 0.01); leg length was independent of age in both genders (r = -0.03 to -0.12, NS). Sitting height and leg length were 6% and 9% higher in young men than young women, and 8% and 9% higher in elderly men than elderly women, respectively (all p < 0.01). Men with spine fractures had lower sitting height (Z = -1.02 \pm 0.13 SD, p < 0.01) not leg length (Z = 0.05 \pm 0.14 SD, NS) or arm span (Z = -0.22 \pm 0.14 SD, NS) relative to age-predicted mean. Women with spine fractures have lower sitting height (Z = -0.81 \pm 0.16 SD, p < 0.01), leg length (Z = -0.36 SD, p < 0.05) and arm span (Z = -1.46 \pm 0.53 SD, p < 0.01). We concluded that women, not men with spine fractures, come from a population with short stature.

O11

INTEGRATION OF AGE, PREVALENT FRACTURE STATUS AND DEXA BONE DENSITY TO PROVIDE PRECISE 5 YEAR RISKS OF FRACTURE

R Prince, D Doherty, S Dhaliwal Dept. of Medicine, Univ of Western Australia, Dept of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Western Australian Institute for Medical Research

Appropriate clinical care for osteoporosis is directed at management to prevent fractures occurring in the future. We have recently published precise 5-year risks of appendicular and axial fracture by age dichotomised by the presence or absence of previous fracture (Doherty et al Osteoporos. Int. 2001;12:16-23). We now include DEXA bone density measurement in the risk evaluation.

Using data from Marshall et al (BMJ. 1996;312:1254-59) we have selected the total hip site as representative of the approach. At this site the change in risk per SD change in BMD is 2.6. We apportioned the population risk of fracture by bone density SD (Z score) taking into account the proportion of the population in each SD category. We then calculated the proportion of fracture patients in each Z score category. Finally we have illustrated the effects in a population of 75 to 79 year old women without prevalent spine fracture who have a 5-year risk of first clinical spine fracture of 3.3%.

SD group (Z score) (2.6/SD)	Relative risk of Fracture population	Proportion of total population	5 year Z score group risk (%) for a total population risk of 1%	5 year Z score group risk (%) for a total population risk of 3.3%
3.5 - 2.5	0.057	0.006	0.03	0.1
2.5 - 1.5	0.148	0.061	0.09	0.3
1.5 - 0.5	0.385	0.242	0.24	0.8
0.5 - -0.5	1.000	0.383	0.61	2.0
-0.5 - -1.5	2.600	0.242	1.59	5.3
-1.5 - -2.5	6.760	0.061	4.14	13.7
-2.5 - -3.5	17.576	0.006	10.77	35.5

Calculation of age specific fracture rates which include DEXA bone density Z scores and previous history of fracture provide a simple clinical tool to make treatment decisions in consultation with the patient. Clinical trials have illustrated the treatment benefit of therapy in patients with a T score of -2.5 which corresponds to a Z score of -1 in 75 to 79 year old women. In these patients spine fracture rates are reduced by up to 50 % of baseline risk. Thus the absolute fracture risk reduction should be a saving of approximately 2.8 to 17.6 fractures per 100 women per 5 years depending on BMD category. Similar simple risk calculations can be made for other fracture sites at other ages. These calculations should aid clinical decision-making.

O12

AREAL AND VOLUMETRIC BONE MINERAL DENSITY (BMD) AND BONE VOLUME IN MEN WITH PRIMARY OSTEOPOROSIS AND FIRST-DEGREE MALE RELATIVES.

B. Erbas¹, S. Risteovski², S. Yeung², C. Poon², P.R. Ebeling². ¹Dept General Practice & Public Health, University of Melbourne, ²Dept Diabetes & Endocrinology, Royal Melbourne Hospital, Vic 3050

Osteoporosis in men is set to become an important public health problem. There are few data examining familial effects on areal and volumetric BMD, or bone volume, in relatives of men with primary osteoporosis. We assessed areal (aBMD) and volumetric BMD (vBMD), and bone volume in men with primary osteoporosis and their first-degree male relatives (FDMR) to determine if there was a familial effect on the development of primary osteoporosis in men. 65 normal, healthy men (Gp1); 113 men with primary osteoporosis (Gp2); and 66 of their FDMR were examined. FDMR were divided into 2 groups according to whether they had a t-score <-2 (Gp 3) or >-2 (Gp 4). BMDs at the spine (LS) and femoral neck (FN), and total body (BMC) were measured by DXA. Vertebral and FN volumes were calculated by standard formulae. Groups were age-corrected.

Osteoporosis (t< -2) occurred in 41% of FDMR. Age was negatively related to areal and vBMDs at the FN and to FN volume, while fat mass increased with age. All values were lower in OP men than in normal men, except fat mass and FN vBMD. While LS and FN aBMD were both lower in FDMR only LS, but not FN vBMD, was lower in FDMR.

Value	Gp 1 vs Gp 2	Gp1 vs Gp 3	Gp 1 vs Gp 4
LS aBMD	-0.249 (0.024) **	-0.211 (0.033) **	-0.0345 (0.031) NS
FN BMD	-0.241 (0.021) **	-0.210 (0.029) **	-0.015 (0.028) NS
TBBMC	-0.278 (0.102) **	-0.147 (0.134) NS	-0.139 (0.130) NS
% FAT	1.560 (1.119) NS	2.536 (1.471) NS	4.609 (1.438) **
VERT Vol	-0.057 (0.027) *	-0.040 (0.036) NS	0.006 (0.034) NS
LS vBMD	-0.238 (0.026) **	-0.197 (0.035) **	-0.034 (0.033) NS
FN Vol	-0.167 (0.073) *	-0.115 (0.097) NS	-0.028 (0.092) NS
FN vBMD	-0.122 (0.071) NS	-0.111 (0.095) NS	-0.022 (0.090) NS

Indicates Log to the base transform; *, p < 0.05; **, p < 0.001.

The high prevalence of low BMD in FDMR provides further evidence for a familial effect in primary osteoporosis in men. The familial effect may have a greater impact on spinal vBMD, while age-related effects may predominate at the proximal femur.

O13

A RANDOMISED TRIAL COMPARING SIMPLE VITAMIN D PLUS CALCIUM, CALCITRIOL AND ALENDRONATE IN PREVENTION & TREATMENT OF CORTICOSTEROID OSTEOPOROSIS

Sambrook PN, Kotowicz M, Nash P, Eisman JA, Nicholson GC. University of Sydney, Bone & Mineral Program, Garvan Institute of Medical Research, Sydney, South Queensland and University of Melbourne, Dept of Clinical and Biomedical Sciences, Barwon Health.

Bone loss leading to osteoporotic fractures is a well-recognised complication of corticosteroid therapy. A number of recent randomised trials in corticosteroid bone loss suggest treatment with vitamin D analogues or bisphosphonates can reduce bone loss due to corticosteroids. However whether active vitamin D metabolites have advantages over simple vitamin D and whether bisphosphonates are superior to active vitamin D metabolites is unclear.

We conducted a randomised, open label, parallel group study in 184 patients in 4 centres. Subjects were stratified for whether they were commencing corticosteroids for the first time or receiving chronic corticosteroids. They were allocated to one of three groups for two years treatment: (a) ergocalciferol (Ostelin) 0.25 mg (10,000 IU) 3 times per week plus calcium carbonate (Caltrate 600mg daily); (b) calcitriol (Rocaltrol 0.25mg) 0.5 mcg/day; (c) alendronate (Fosamax) 10mg/day. Efficacy was evaluated by bone mineral density (BMD) measurements of the lumbar spine and femoral neck at 0, 12 and 24 months.

For the total population, the mean % change in lumbar BMD after 24 months was -0.2% for ergocalciferol, -0.3% for calcitriol and +4.8% for alendronate. At the femoral neck, the mean % change after two years was -2.5% for ergocalciferol, -1.9% for calcitriol and +1.2% for alendronate. Analyses of the response according to recent commencement versus chronic use corticosteroids are in progress.

In summary, bone density outcomes were significantly better at both spine and hip in those receiving alendronate than either form of vitamin D (p= 0.02 for lumbar spine). The response was somewhat better for calcitriol than simple vitamin D at the hip, although no group differences were significant at this site. These data suggest that, although the active form of vitamin D may have a modest benefit over simple vitamin D, the potent bisphosphonate, alendronate, is a more effective treatment for corticosteroid-related bone loss.

O14

ANDROGEN THERAPY IN MEN ON LONGTERM GLUCOCORTICOIDS - A 12 MONTH RANDOMISED, PLACEBO-CONTROLLED TRIAL OF TESTOSTERONE OR NANDROLONE ON BONE DENSITY AND BODY COMPOSITION.

B Crawford¹, P Liu², M Kean¹, J Bleasel³, D Handelsman². ¹Department of Endocrinology, ²ANZAC Research Institute and Department of Andrology, ³Institute of Rheumatology and Orthopaedics, Royal Prince Alfred Hospital & University of Sydney

The aim of this study was to assess the effect of an aromatisable androgen, testosterone (Sustanon), and a non-aromatisable androgen, nandrolone (Deca-durabolin), on bone density and body composition in men requiring long-term glucocorticoid therapy for chronic illness such as lung or rheumatoid disease.

This was a 12 month randomised, double-blind, placebo controlled trial in 45 men (aged 61 ± 3 yrs). The dose of both androgens was 200 mg IMI q2 wk. All patients received a 600 mg calcium supplement daily. Bone density and body fat and lean mass was measured by DEXA, muscle strength by isokinetic dynamometry.

The main effects on BMD were seen in the lumbar spine which increased by 4.7% in the testosterone group (n=17) versus 0.6% with placebo (n=15) and a decrease of 0.7% with nandrolone (n=13). Both androgen treated groups gained lean mass (13.5%) by 12 months versus a decrease of 3.3% in the placebo group. Knee flexion and extension was also stronger with androgen treatment than placebo.

Testosterone treatment in steroid-dependent men prevents bone loss and improves muscle mass and strength. Aromatisation appears important for anabolic bone, but not muscle, effects.

O15

HYPOTHALAMIC Y2 NPY RECEPTORS ARE IMPORTANT FOR THE CENTRAL REGULATION OF BONE FORMATION.

PA Baldock, A Sainsbury#, GP Thomas, M Couzens#, RE Enriquez, H Herzog#, EM Gardiner. Bone Program, Neurobiology Program#, Garvan Institute of Medical Research, Sydney, Australia.

Neuropeptide Y (NPY) is a neuropeptide abundantly expressed in the arcuate nucleus of the hypothalamus. Acting through 5 receptor subtypes (Y1, Y2, Y4, Y5 and y6) in diverse tissues, it has effects on many physiological functions. NPY has been implicated in the central control of bone mass by leptin (Ducy et al., 2000). In order to assess the role of NPY further, bone volume and cell activity were assessed in mutant mouse lines with a germline knockout for the NPY Y2 receptor (Y2 KO) and a conditional, arcuate nucleus-specific knockout of Y2 (ARC Y2 KO).

Sagittal sections of the distal femoral metaphysis of 4 month old male mice were assessed by light microscopy. Bone formation was estimated by fluorescent labels, and resorption by TRAP staining.

Y2 KO mice had greater trabecular bone volume $12.4\% \pm 1.7$, compared to wildtype $5.3\% \pm 0.2$ ($P < 0.05$), with increased trabecular thickness $29.9\ \mu\text{m} \pm 2.0$ vs $18.9\ \mu\text{m} \pm 0.7$ ($P < 0.005$) and a non significant increase in trabecular number $4.0/\text{mm} \pm 0.3$ vs $2.8/\text{mm} \pm 0.002$ ($P < 0.06$). ARC Y2 KO mice were assessed one month after injection of recombinant adenovirus expressing CRE recombinase into the brains of mice with floxed Y2 receptor genes. Results five weeks after injection were similar to previous germline Y2 KO mice, with greater trabecular bone volume ($P < 0.01$) and thickness ($P < 0.0005$) compared to wild type. Bone formation in the conditional knockouts was elevated due to an increase in mineral apposition rate $1.4\ \mu\text{m}/\text{d} \pm 0.1$ compared to wildtype $0.96\ \mu\text{m}/\text{d} \pm 0.03$ ($P < 0.02$). Bone resorption was not altered in either germline or conditional Y2 knockout mice.

NPY has therefore been strongly implicated in the control of bone formation, with a specific NPY receptor mediating an increase in osteoblast function. While a peripheral Y2 effect has not been ruled out, the arcuate-specific KO studies are consistent with regulation of bone mass by central action of NPY through the Y2 receptor.

O16

HAEMOPOIETIC CELLS SIGNAL TO OSTEOBLASTIC CELLS DURING HUMAN OSTEOCLAST FORMATION.

Atkins GJ¹, Zannettino ACW², Haynes DR³, and Findlay DM¹ Departments of ¹Orthopaedics, ²Pathology, University of Adelaide, and ³Myeloma and Mesenchymal Research Laboratory, Hanson Centre for Cancer Research, Adelaide, South Australia, 5000. We have previously used a culture system of human peripheral blood mononuclear cells (PBMC) as a source of osteoclast (OC) precursors and murine ST2 stromal cells to define and determine the separate contributions of the haemopoietic and stromal elements during human OC formation¹. ST2 cells expressed mRNA encoding many known osteoclastogenic factors, including RANKL and its inhibitor, OPG. Key findings from these experiments were that, firstly, the RANKL:OPG mRNA ratio increased in the cocultures over 21d, consistent with a key role for RANKL in the promotion of OC formation. Secondly, we found coordinate expression by PBMC of IL-1/IL-17, and IL-6/sIL-6R mRNA. Thirdly, PBMC secreted IL-1, IL-6 and TNF- α only in coculture with ST-2 cells early in OC development. Similarly, PGE₂, shown to upregulate RANKL mRNA expression and to synergise with RANKL during OC development, was secreted only in cocultures¹. To extend these studies to human osteoblasts, we cocultured monocytes and human osteoblast-like cells, derived from trabecular bone biopsies of normal donors, in media containing dexamethasone and PTH. Under our culture conditions, human OC form over a 21d period. Analysis after 72h by RT-PCR showed coculture dependent increases in the RANKL:OPG mRNA ratio. Similarly, we found coculture dependent secretion of PGE₂ and IL-6. Both PGE₂ release and RANKL transcription were inhibited by indomethacin. Together, these data show that monocytes, including pre-OC, provide signals to osteoblast-like cells during human OC formation. We propose that this bidirectional signalling is involved in a maintenance of the equilibrium between bone resorption and formation.

1. Atkins *et al* 2000 Bone 26:653.

O17

a-MELANOCYTE STIMULATING HORMONE (a-MSH) – A NOVEL FACTOR AFFECTING BONE TURNOVER

¹J. Cornish, ¹K.E. Callon, ²K.G. Mountjoy, ¹C.Lin, ¹U.Bava, ¹J-M Lin, ¹I.R. Reid. Departments of ¹Medicine and ²Paediatrics, University of Auckland, New Zealand.

Recent evidence suggests that various neuropeptides that influence energy expenditure and body weight, in particular fat mass, also regulate bone mass. Fat mass is one of the principal determinants of bone density but the mechanism underlying this relationship remains controversial. The importance of central melanocortin pathways has been highlighted recently in genetic studies in obese humans and mice. Melanocortin-4 receptor (MC4-R) deficient human subjects are obese and have a marked increase in bone mineral density. The endogenous ligand most probably involved in the control of body weight is a-MSH which binds with high affinity to MC4-R. There are data to suggest that peripheral actions of melanocortins, along with central pathways of action, are involved in controlling weight. Melanocortins circulate in the periphery and the receptors are found in peripheral tissues. We have recently isolated MC4-R in osteoblasts and in the current study, we have investigated the effects of a-MSH on bone cells *in vitro* and *in vivo*.

In primary cultures of fetal rat osteoblasts, a-MSH dose-dependently stimulated proliferation from nanomolar concentrations and greater. Similarly, a-MSH ($>10^{-10}$ M) increased proliferation of cultures of canine chondrocytes. In mouse bone marrow cultures, a-MSH ($>10^{-9}$ M) stimulated osteoclastogenesis, but it had no effect on bone resorption in 2 assays using mature osteoclasts. Systemic administration of a-MSH to male adult mice (20 injections of 4.5 mg/day over 4 weeks) decreased the trabecular bone volume in the proximal tibiae (from $19.5 \pm 1.8\%$ to $15.2 \pm 1.4\%$, $p=0.03$) and reduced trabecular number ($p=0.001$).

It is concluded that there are direct effects of a-MSH on bone that tend to increase bone turnover, and that when a-MSH is administered systemically this leads to decreased bone volume.

O18

NITRIC OXIDE SYNTHASES DURING FRACTURE HEALING.

W Zhu, AD Diwan, JH Lin, EM Gardiner, GAC Murrell. Orthopaedic Research Institute, St George Hospital / University of New South Wales, 4-10 South Street, Kogarah, NSW 2217, Australia.

We previously reported [1] that nitric oxide synthases (NOS) which are responsible for the generation of nitric oxide (NO) was induced during fracture healing. Inhibition of NOS inhibited healing process. Addition of NO reversed this impairment. The purpose of this study was to identify the temporal expression patterns of three NOS isoforms (iNOS, eNOS and bNOS), and localise their cellular distribution during the phases of fracture healing at both mRNA and protein levels.

In a rat right femoral fracture model, competitive PCR and *in situ* hybridisation were used to evaluate and localise NOS mRNA expression. Western blot and immunohistology were used for NOS protein evaluation and localisation. Healing samples were investigated at 4, 7, 14 and 21 days following fracture (n=7), with intact left femur as internal control.

There were no detectable iNOS and low expression of eNOS and bNOS in unfractured bone. iNOS mRNA and protein was maximally expressed at 4 days after fracture, in periosteum cells and osteoblasts, within the periosteal callus. eNOS mRNA and protein expression was found maximally at 7 and 14 days after fracture, in blood vessel endothelial cells and chondroid type cells during endochondral ossification. bNOS mRNA and protein was maximally expressed at 21 days after fracture, in chondroid cells and fibroblasts, at the junction of fibrous tissue and cartilaginous area of callus.

We demonstrate for the first time that the expression of NOS isoforms during fracture healing is isoform-specific, time-dependent and cellular distinctive. Different NOS isoform may subserve different functions at various stage of fracture repair.

[1] Diwan AD, Wang MX, Jang D, Zhu W, Murrell GAC 2000 Journal of Bone and Mineral Research 15(2): 342-351.

O19

EFFECTS OF RAT RANKL, MURINE RANKL AND HUMAN OPG ON MATURE OSTEOCLAST ACTIVITY

JM Lin, KE Callon, U Bava, C Lin, IR Reid, J Cornish. Department of Medicine, University of Auckland, New Zealand.

RANKL, a protein produced by osteoblasts, modulates osteoclast development by binding to the receptor RANK, which exists on the surface of pre-osteoclasts. Osteoprotegerin (OPG) is a decoy receptor interrupting the action of RANKL, thus inhibiting osteoclast development. Less is known about the action of RANKL and OPG on mature osteoclast activity. In this study we have investigated human OPG, rat RANKL (rRANKL) as well as two of its truncated fragments and compared their effects with murine RANKL (muRANKL) in a mature isolated osteoclast model.

Mature osteoclasts were isolated from the long bones of neonatal rats. The osteoclast-rich cell suspension was plated onto bone slices and cells were allowed to settle for 25 mins. After rinsing well the slices were incubated for 24 h in acidified media containing test substances or vehicle. The slices were then fixed and stained for tartrate resistant acid phosphatase (TRAP). TRAP-positive multinucleated cells on each bone slice were quantified and then cells were removed by gentle scrubbing. Bone slices were assessed for "pits" excavated by the osteoclasts using reflected light microscopy and metallurgical lenses. Osteoclast activity is expressed as a ratio of the number of pits: number of osteoclasts per bone slice.

rRANKL-(160-318) and muRANKL-(158-316) significantly increased while OPG significantly decreased osteoclast activity at concentrations of 10-100 ng/ml. There was no significant difference in osteoclast numbers with any of the treatments. These results demonstrate that RANKL and OPG directly affect mature osteoclast activity. These findings are consistent with our previous preliminary report of the peptides' action on bone resorption in a mouse calvarial organ culture system which predominantly reflects mature osteoclast activity. The effects of truncated fragments of RANKL are currently being investigated.

O20

OESTRADIOL MODULATES 1,25 DIHYDROXYVITAMIN D-INDUCIBLE GENE TRANSCRIPTION.

PD O'Loughlin, ZM Wurst, P Dwivedi, B May and HA Morris.

Division of Clinical Biochemistry, Institute of Medical & Veterinary Science and Departments of Physiology and Molecular Life Sciences University of Adelaide, Adelaide, SA, 5000.

Oestrogen replacement can correct the intestinal calcium malabsorption associated with menopause or oestrogen deficiency without altering circulating 1,25-dihydroxyvitamin D (1,25D). The mechanism for this oestrogen action is yet to be established. We used the intestinal cell line Caco-2 to investigate the combined effect of 1,25D and 17 β -oestradiol (E₂) on the induction of the 25-hydroxyvitamin D-24-hydroxylase (CYP24) promoter/luciferase reporter gene construct. Cells were treated with E₂ (10⁻⁸ to 10⁻¹²M) at 3 doses of 1,25D (10⁻⁸ to 10⁻¹²M). 1,25D (10⁻⁹M) treatment alone resulted in a 1.94-fold induction of luciferase activity compared to ethanol vehicle (p<0.001), while E₂ (10⁻⁹M) alone caused no induction above vehicle. E₂ (10⁻⁹M) in combination with 1,25D (10⁻⁹M) resulted in 3.45-fold induction (p<0.05 Vs 1,25D). When cells were treated with 1,25D 10⁻¹⁰M there was a positive response to increasing doses of E₂, however when treated with 1,25D 10⁻⁸M there was a negative dose response to E₂ treatment (1,25D/E₂ interaction p<0.001). The data indicate that the intestinal cell responsiveness to 1,25D at low doses is synergised by E₂, but at higher doses of 1,25D, E₂ has a repressive effect. These findings support the hypothesis that E₂ enhances the induction of vitamin D-responsive promoters, possibly via MAP kinase-dependent co-activation factors. However, at higher doses of 1,25D, E₂ actually interferes with induction, providing evidence for squelching. E₂ may only enhance intestinal vitamin D-responsiveness when circulating 1,25D is sub-optimal.

O21

CFU-GM- BUT NOT CFU-M-DERIVED CELLS FROM HUMAN UMBILICAL CORD BLOOD FORM OSTEOCLASTS AT HIGH EFFICIENCY.

JM Hodge, MA Kirkland*, GC Nicholson. The University of Melbourne, Department of Clinical and Biomedical Sciences: Barwon Health, Geelong, VIC. 3220. *Douglas Hocking Research Institute, Barwon Health, Geelong, VIC. 3220.

Osteoclasts (OC) are derived from hematopoietic cells, but the pathway of differentiation has not been clarified. Conflicting evidence suggests OC precursors may be derived from CFU-M, CFU-GM or a lineage distinct from either of these. To further investigate OC lineage we performed progenitor assays of human umbilical cord blood followed by *in vitro* studies of osteoclastogenesis.

Cord blood mononuclear cells (CBMC) were cultured in semi-solid media containing GM-CSF, IL-3 and stem cell factor. Individual colonies identified as CFU-GM or CFU-M on the basis of morphology were harvested following 14 days and transferred into 96-well plates in the presence of RANKL and human M-CSF for 6 days. Osteoclastogenic potential of these colonies was compared with pooled colonies or CBMC. Resorptive capacity of mature OC was assessed on bone substrate.

The efficiency of OC formation by pooled CFU was significantly increased compared with CBMC. When osteoclastogenesis by individual CFU colonies was compared to pooled CFU the number of osteoclasts formed on bone from CFU-GM was increased by 88% and resorption by 45%, whereas CFU-M were poorly osteoclastogenic. These results demonstrate that clonal expansion of CFU-GM colonies from human CBMC markedly increases osteoclastogenic potential. More committed CFU-M-derived colonies appear to have lost their capacity to form OC.

O22

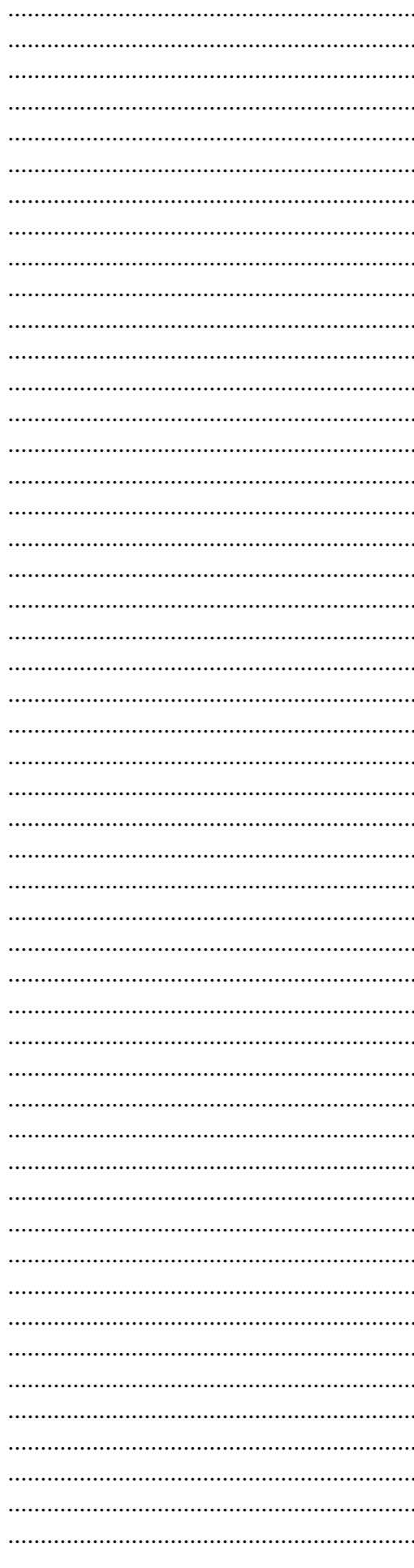
THE CHONDROCYTE IS A TARGET CELL FOR AMYLIN AND ADRENOMEDULLIN.

D Naot, J Cornish, KE Callon, CA Poole, CQX Lin, CL Xiao, U Bava, M Clatworthy*, GJS Cooper, IR Reid. University of Auckland and *Middlemore Hospital, Auckland, New Zealand.

Adrenomedullin and amylin are peptide hormones that show structural and functional similarities. We have previously shown that these peptides, as well as the amylin N-terminal fragment amylin-(1-8) are mitogenic to primary osteoblasts *in vitro* and promote bone growth *in vivo*. Systemic administration of amylin increased the tibial length and thickness of the epiphyseal growth plate, suggesting a direct effect of amylin on cartilage. The aim of the present study was to investigate whether the chondrocyte cell is a target for adrenomedullin and amylin.

Proliferation studies were performed on chondrocytes isolated from adult canine articular cartilage. The effects of amylin, amylin-(1-8) and adrenomedullin were compared to those of IGF-I, a known chondrocyte mitogen. Significant increases in cell numbers were observed for amylin, amylin-(1-8), adrenomedullin and IGF-I at concentrations of 3×10^{-10} M, 3×10^{-9} M, 3×10^{-9} M and 3×10^{-12} M, respectively. To further investigate amylin and adrenomedullin as potential regulators of chondrocyte growth, RNA was extracted from human articular chondrocytes and used to study the expression of genes for these peptides and their receptors in chondrocytes. RT-PCR analysis demonstrated the expression of adrenomedullin and its putative receptors in these cells.

Our results suggest that amylin and adrenomedullin regulate chondrocyte proliferation at periphysiological concentrations and that the chondrocyte is a likely target cell for these peptides *in vivo*. Thus amylin and adrenomedullin may influence bone length during development and might also induce chondrocyte proliferation during articular cartilage regeneration.



I5

NEW INSIGHTS INTO OSTEOCLAST REGULATION.

KW Ng, Department of Medicine, St Vincent's Hospital, Fitzroy 3065, Australia.

Osteoclast Inhibitory Lectin (OCIL) is a predicted type II membrane-bound molecule of 207aa containing a C-lectin domain in its extracellular domain. Recombinant protein to the extracellular domain of mOCIL inhibits osteoclast formation in murine osteoblast with spleen cell cocultures as well as monocyte cultures treated with M-CSF and RANKL, suggesting that OCIL acts upon hemopoietic cells to inhibit osteoclast formation (1).

In rodent tissues, Northern blotting, *in situ* hybridization, and immunohistochemistry demonstrated OCIL expression in osteoblasts, chondrocytes as well as in a variety of extraskelatal tissues, in parallel with RANKL and OPG. OCIL was expressed in synovial cells lining normal, osteoarthritic and rheumatoid joints in humans. Furthermore, OCIL protein and mRNA were highly expressed in lymphoid aggregates and in osteoclasts, but only those localised at sites of active inflammation where joint destruction occurred, co-localising with the expression of RANKL and OPG. In the rat collagen-induced arthritis model, a similar picture was obtained at sites of active joint erosion. In contrast, OCIL was not expressed in trabecular osteoclasts in control rats.

Two other novel C-lectins, designated OCILrp1 and OCILrp2, that share substantial identity with murine OCIL were identified. Their extracellular domains share 83% and 75% identity, respectively, with the extracellular domain of OCIL. Recombinant OCILrp1 and OCILrp2 inhibited osteoclast formation in murine spleen cultures with similar potencies to OCIL, implying redundancy for OCIL. However, while the intracellular domains of OCILrp1 and OCILrp2 share 83% identity, they showed no identity to the intracellular domain of OCIL.

- (1) Zhou H et al, 2001, JBC, 276:14916.

I6

THE FRACTURE BURDEN PERSPECTIVE FOR AUSTRALIA

Hearn TC, Wigg A, McCall K, Wells V, Chipchase L and Krishnan J
Dept. Orthopaedics, Repatriation General Hospital & Flinders Medical Centre, Flinders University, Adelaide, South Australia.

Hip fracture numbers are expected to increase by 66% over the next twenty years in South Australia. Fractures of the distal radius are also a common problem representing a growing public health concern. The purpose of this study was to determine incidence rates and management methods for distal radius fractures admitted to hospital in Australia and to calculate projections over the next 50 years.

Age and gender specific incidence rates and ICD9 procedures used for fractures of the distal radius for the last five years in Australia were obtained from the Australian Institute of Health and Welfare. Population projections from the Australian Bureau of Statistics 1997 census were combined with the age and gender specific fracture incidence rates. Estimated numbers of wrist fractures requiring hospital admission for 2021 and 2051 were then calculated.

In the over 50 age group, approximately 65% of distal radius fractures admitted to hospital in Australia were treated with closed reduction and no surgical fixation. The remaining 35% were treated with either internal or external fixation. The number of wrist fractures in the over 50 year olds in Australia was estimated to increase by approximately 60% in men and 55% in women by the year 2021 and by 75% in men and 100% in women by 2051.

As long as conditions contributing to wrist fractures in Australia remain unchanged, the number of fractures in the over 50 age group will increase in far greater proportion than the overall population in the next 50 years.

O23

GENDER DIFFERENCES IN BONE GEOMETRY DURING THE ADOLESCENT GROWTH SPURT. MR Forwood, DA Bailey, TJ Beck, RJ Mirwald, T Wallace, RA Faulkner, The Uni of Queensland, Uni of Saskatchewan, and Johns Hopkins University.

To investigate if gender differences occur in bone geometry of the proximal femur during adolescence, we used "Hip Strength Analysis" (Beck et al., Invest Radiol. 1990, 25:6-18.) to derive femoral neck geometry from DXA bone scans (Hologic 2000, array mode). We analysed longitudinal data from 70 boys and 68 girls over a 7 year period. Individual distance and velocity curves for height were fitted using a cubic spline, and the age of peak height velocity (PHV) was determined. To control for gender and maturational differences between children of the same chronological age, section modulus (Z), cross sectional area (CSA), sub-periosteal width (SPW), and BMD at the neck and shaft were determined for points on each individual's curve at PHV and 1 and 2 years either side of peak. To control for size, height and weight were used as co-variables in a 2-way ANOVA assessing gender at the maturational age points: -2, -1, age of PHV, +1, +2. To account for muscle mass, lean body mass was subsequently added as a covariate in the analysis

After maturity (PHV), independent of body size, BMD differed between genders at the shaft but not at the neck. At both sites, Z showed that male bones become stronger after PHV. Underlying these maturational changes, male bones became wider (SPW) and enclosed more material (CSA) at both the neck and shaft. Accounting for lean body mass, only sub-periosteal width remained greater in boys than girls after PHV. Thus, for the neck and shaft of the femur, bending strength during growth (indicated by Z) is dominated by lean mass and gives rise to the gender difference occurring at this time. For the shaft (with and without normalisation to shaft length) this only becomes evident as body size disperses, at and after maturity.

O24

THE EFFECT OF CALCIUM SUPPLEMENTATION ON BONE DENSITY IN PREMENARCHEAL FEMALES – A CO-TWIN APPROACH.

M. Cameron, C. Nowson, L. Paton, J. Alexander, JD Wark. University of Melbourne, Department of Medicine, Bone & Mineral Service, Royal Melbourne Hospital, Australia.

The adolescent years provide the best opportunity to maximise peak bone mass. Calcium is the major mineral in bone and increasing dietary calcium intake has been proposed to be an effective way of increasing bone mass. However the skeletal response to increased dietary calcium on bone is not clear. The aim was to assess the effect of calcium supplementation upon bone mineral density (BMD) in premenarcheal female twins, using the co-twin model.

Fifty-one pairs of premenarcheal female twins (26 monozygotic, 25 dizygotic) with a mean age (10.3 ± 0.1 yrs), height (1.4 ± 0.01 m) and weight (37.6 ± 1.4 kg), participated in a randomised, single blind, placebo-controlled study with one twin receiving a 1200mg calcium carbonate (Caltrate) supplement. BMD was measured using a Hologic QDR 1000W densitometer at baseline, 6 and 12 months. Dietary calcium intake was determined using a validated short food frequency questionnaire. The mean daily dietary calcium intake was 1382.3 ± 94.9 mg.

There was no difference in height, weight or dietary calcium intake between the placebo and calcium-supplemented twins at baseline. Calcium supplementation significantly increased the within-pair difference in BMD at the hip over 6 (1.5%, $p < 0.001$) and 12 months (1.6%, $p < 0.05$), and at the spine after 12 months (1.5%, $p < 0.05$). A trend was seen for a difference in total body bone mineral content (TB BMC) from baseline to 12 months (2.5%, $p = 0.07$). However, in the first 6 months there was no significant increase in spine BMD or TB BMC.

In this group of prepubertal females, there was no significant effect of calcium supplementation at the spine or total body after 6 months. However, there was a 1-2% increase in the within-pair difference in BMD at the hip at both 6 and 12 months and at the spine after 12 months. Subjects will be evaluated up to 18 months of calcium supplementation and the ultimate effect on peak bone mass will be established.

O25

PREPUBERTAL CHILDREN WHO HABITUALLY AVOID DRINKING COW'S MILK HAVE POOR BONES. A.Goulding, R Black, IE Jones, SM Williams. Dept. of Medical & Surgical Sciences, Medical School, Otago University, PO Box 913, Dunedin, New Zealand.

Although dairy products are the major dietary source of calcium in most western countries information concerning bone health in children who do not drink cow's milk is very limited. To evaluate the current calcium intakes and bone development of prepubertal children aged 3-10 years with a history of chronic avoidance of drinking cow's milk we recruited 50 subjects (30 girls, 20 boys) by advertisement. Dietary calcium intakes were estimated using a validated food frequency questionnaire. Body composition and areal bone mineral density (g/cm^2) were measured by dual energy x-ray absorptiometry (Lunar DPX-L). Subjects had low dietary calcium intakes, the mean (SD) value being only 443 (230) mg Ca /day and 24% of our sample had already suffered bone fractures. Most subjects (58%) did not consume either substitute calcium-rich drinks or calcium supplements. Although a high proportion of the study population (18%) were obese (BMI > 95th percentile), our milk-avoiders were significantly shorter, with smaller skeletons and lower total body bone mineral content than milk-drinking controls of similar gender and age ($P < 0.05$). In addition milk-avoiders had lower areal bone mineral density z scores ($P < 0.05$) at all regional skeletal sites (femoral neck, hip trochanter, lumbar spine, ultradistal radius and 33% radius) than reference populations of fracture-free controls drawn from the same community. The volumetric bone mineral density values (g/cm^3) were also severely reduced ($P < 0.001$) in children avoiding cow's milk: mean (SD) z scores being: L2-4 spine -0.72 (1.17), 33% radius -0.72 (1.35). We conclude that in growing children habitual avoidance of cow's milk is a major concern for bone health. (Grant support NZ HRC & NZ DAB).

O26

WHY DO FRACTURES RUN IN FAMILIES? A TWIN STUDY

N.El Haber [1,2], K.Hill [2], AM.Cassano [1,2], R.MacInnis [1], J.D.Wark [1] [1] Department of Medicine, Royal Melbourne Hospital, University of Melbourne, Parkville, Australia.

[2] National Ageing Research Institute, Parkville, Australia

Falls, osteoporosis and fractures in the older population, particularly women are a major global cause of injury, disability and death. An increase in postural sway, gait impairment and poor muscle strength are factors associated with falling and fractures, where hip fractures account for the most serious outcome of both osteoporosis and falls.

Theoretical modelling (1) indicated that the heritability of bone mineral density and hip axis length explain only a minority of familial aggregation of hip fractures. Therefore, a classic twin study was performed to investigate whether genetic or shared environmental effects on falls risk contributed to the familial aggregation of fractures.

A series of screening tests and validated clinical and laboratory tests of gait and balance were performed on 35 monozygotic (MZ) and 39 dizygotic (DZ) female twin pairs aged 46-82 years.

Results showed moderate to high (0.48-0.86) within-pair MZ correlations on several of the outcome measures (adjusted activity scores, muscle strength, Chattecx balance testing, stride velocity, double support duration, Lord's balance test, step test). Within-pair DZ correlations were generally lower, suggesting the presence of moderate to strong additive genetic influence.

Results presented in this study provide support for the presence of a heritable component in balance performance and gait function which contributes to the familial clustering of osteoporotic fractures.

- (1) Hopper J, Carlin J. 1992. American Journal of Epidemiology.136:1138-47.

O27

INTENSIVE EXERCISE DURING ADOLESCENCE: BENEFICIAL FOR BOYS BUT NOT FOR GIRLS?- A PILOT STUDY.

KM Sanders¹, MA Kotowicz¹, SL Bass², GC Nicholson¹. ¹Clinical and Biomedical Sciences, Barwon Health, The University of Melbourne. ²Health & Behavioural Sciences, Deakin University, Burwood, Vic.

Year 9 (14 to 15 year-olds) is an appropriate time for alternative school-based programs. This study compares changes in bone accrual between Year 9 adolescents participating in a school-based compulsory intensive activity regimen with normoactive controls from 2 other schools. Although boys are likely to have a greater osteogenic response than girls, 15% of the skeleton is still accrued in the two years after menarche so that there appears to be a window of opportunity to increase bone mass in both genders from such school-based programs.

Nine boys (Tanner stage 3-4) and 11 girls (1.4 years postmenarche) participating in the 10 month exercise program of cross-country hiking, skiing and running, were compared with the control group of 12 boys (Tanner stage 2-4) and 10 girls (1.4 years postmenarche). The annual changes were determined in BMD at the spine, hip and total body; calcaneal ultrasound, lean and fat mass, anaerobic threshold, quadriceps muscle strength, calcium intake, regularity of menses, pubertal staging and injury/illness.

Boys in the exercise group tended to have greater increases than controls (case vs control) in BMD at the femoral neck (FN, g/cm²; mean, {range} 0.079 {0.06, 0.11} vs 0.048 {-0.01, 0.14}, p=0.06), and quadriceps strength (mean \pm se: 0.03 \pm 0.03 vs -0.17 \pm 0.05, p=0.005). Girls from the exercise group had a lower increase in BMD than controls (FN, g/cm² {range} 0.01 {-0.13, 0.06} vs 0.07 {-0.12, 0.16}, p=0.02) and a higher proportion of irregular menses (8/11 vs 2/10, p=0.23) but had a higher increase in indices of heel ultrasound (Stiffness; 8.2 {-5.5, 27.9} vs 2.4 {-3.4, 13.6}, p=0.15). These preliminary findings suggest that intensive exercise in Year 9 girls may be deleterious for maximising peak bone mass although the exercise may have stimulated beneficial changes in bone geometry.

O28

TOTAL BODY MINERAL GAIN: A FOUR YEAR STUDY IN GIRLS WITH AND WITHOUT PAST FOREARM FRACTURES. IE Jones, RW Taylor, PJ Manning, SM Williams, A Goulding. Department of Medical and Surgical Sciences, Medical School, Otago University, PO Box 913, Dunedin, New Zealand.

We have previously shown that girls with a distal forearm fracture have poorer skeletons than girls who have never fractured. It is not yet known whether girls with a past fracture maintain lower bone mineral or enhance their relative bone gain compared with girls who have never fractured. Initially we recruited 100 girls with a distal forearm fracture aged 3-15 years and 100 age-matched fracture free controls. We assessed height, weight, Tanner stage of development, fracture history and total body bone mineral content (BMC) using dual energy x-ray absorptiometry at baseline. Four years later 170 girls were restudied (81 girls who remained fracture-free (group 1), 58 girls who had at least one fracture at baseline but no new fractures (group 2) and 31 girls (group 3) who sustained a fracture in the 4 years of follow-up). In data adjusted for age, height, weight, pubertal status and bone area, groups 2 and 3 had lower total body BMC than group 1 both at baseline (ratios (95% CI) 0.978(0.959-0.997) and 0.941(0.919-0.964) and at follow-up (0.983(0.968-0.999) and 0.960(0.941-0.980), respectively). Moreover, the relative gain in total body BMC in group 2 did not differ from that in group 1, 0.992(0.979-1.006). By contrast, group 3 showed a lower relative gain in total body BMC over 4 years than group 1, 0.969(0.952-0.986). We conclude from our four year study that girls suffering new fractures show the lowest gain in BMC, while girls with fractures at baseline continue to display lower BMC and show no catch-up improvement in mineral accrual versus girls remaining fracture-free lifelong. (Grant support: HRC of New Zealand).

O29

CONTROL OF CYP27b1 EXPRESSION BY THE CYP27b1 PROMOTER

I Hendrix, BK May and HA Morris

Depts of Physiology and Biochemistry, University of Adelaide, Adelaide
Clinical Biochemistry, IMVS, Adelaide

The enzyme 25-hydroxyvitamin D₃-1 α -hydroxylase (CYP27b1) is essential for metabolising vitamin D₃ to 1 α ,25-dihydroxyvitamin D₃ (1,25D), its biologically active form, which is required for normal bone structure and the maintenance of plasma calcium levels. The kidney is considered to be the major site of expression of the CYP27b1 gene although expression in extra-renal cells and tissues has been reported. To investigate the control of the tissue-specific expression of CYP27b1 by its 1.5 kb proximal promoter, a transgenic mouse model, containing the 1.5 kb CYP27b1 promoter linked to the luciferase-reporter gene, has been established. To study the promoter-directed tissue-specific luciferase expression, three twelve week old transgenic mice were fed a 0.4%Ca, 0.34%P diet. After 4 weeks, mice were killed and 11 tissues were collected for analysis. The promoter-induced luciferase expression was determined in each of these tissues using a luciferase reporter assay. The luciferase expression was highest in the brain, testis, bone marrow and bone. Luciferase activity was also detected in the heart, skeletal muscle and ovaries. Minimal levels of luciferase were detected in the kidney, proximal and distal gut, liver and lung. It is surprising that only minimal levels of activity were detected in the kidney, suggesting that control of CYP27b1 expression in this tissue may reside outside the 1.5 kb promoter region. Tissues in which expression was strongest with this construct contain macrophage like-cells, suggesting that the 1.5 kb promoter is a strong regulator of CYP27b1 expression in these cells.

O30

PHOSPHOLIPIDS AS SURVIVAL FACTORS IN OSTEOBLASTIC CELLS – EVIDENCE FOR A CELL-TYPE SPECIFIC ROLE FOR PHOSPHATIDYLINOSITOL 3-KINASE.

Q Chen, K Callon, C Xu, I Reid, J Cornish, A Grey. Dept of Medicine, University of Auckland, Auckland, New Zealand.

The naturally occurring phospholipid growth factors lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) have been shown to influence fundamental cellular processes in a number of cell types *in vitro*. We have recently shown that both LPA and S1P are mitogenic to osteoblastic cells. In the current study, we examined the effect of these compounds on osteoblast survival *in vitro*. Using TUNEL and DNA fragmentation assays, we found that both LPA and S1P dose-dependently (0.01-10 μ M) inhibited the apoptosis induced by serum withdrawal in cultures of primary rat osteoblasts, and SaOS2 cells. We further examined the signaling pathways involved in these anti-apoptotic effects using a DNA fragmentation assay. In primary rat osteoblasts, the ability of both LPA and S1P to prevent apoptosis was completely blocked by pertussis toxin (PTx, 10ng/ml). LY294002, a specific inhibitor of phosphatidylinositol 3-kinase (PI 3-K), dose-dependently inhibited the survival-promoting actions of each phospholipid. Neither PD-98059 nor U-0126, specific inhibitors of MEK, the kinase responsible for activating p42/p44 MAP kinases, abrogated the anti-apoptotic effect of either phospholipid. In Swiss 3T3 fibroblastic cells, while PTx blocked the anti-apoptotic actions of both LPA and S1P, LY294002 inhibited the ability of S1P, but not LPA, to induce cell survival. PD-98059 and U-0126 modestly abrogated the pro-survival effects of LPA, and strongly inhibited the anti-apoptotic effects of S1P. We conclude that (a) the phospholipids LPA and S1P are potent survival factors in osteoblastic cells, (b) G_i proteins and PI 3-K, but not p42/44 MAP kinases, are involved in transducing the survival signal activated by LPA and S1P in osteoblasts and (c) the signaling pathways which mediate the pro-survival effects of LPA and S1P are cell-type specific.

O31

GENE EXPRESSION IN HUMAN CANCELLOUS BONE IS SIMILAR BETWEEN SKELETAL SITES

JS Kuliwaba^{1,2}, DM Findlay³, GJ Atkins³, MR Forwood⁴, NL Fazzalari^{1,2}. ¹Div of Tissue Pathology, IMVS, ²Dept of Pathology & ³Dept of Orthopaedics & Trauma, Uni of Adelaide, Adelaide, SA and ⁴Dept of Anatomical Sciences, Uni of Queensland, Brisbane, QLD.

The mechanisms that lead to the structure and turnover of cancellous bone are complex, involving both mechanical and chemical inputs. In this study we have examined the expression of mRNA encoding a number of bone cell markers and regulatory molecules in several skeletal sites: iliac crest (IC), femoral neck (FN) and intertrochanteric (IT) cancellous bone. These bone samples were obtained from 10 routine autopsy cases (6 women, 57-85 years; 4 men, 42-84 years; postmortem interval (PMI) 16-108 hours). Total RNA was isolated from each bone sample for semi-quantitative RT-PCR analysis of RANKL, osteoprotegerin (OPG), RANK, IL-6, TRAP, osteocalcin (OCN) and osteopontin (OPN) mRNA expression. The relative ratios of the amplified products, with respect to GAPDH, were determined.

We were interested to find no significant differences, either within individuals or in the mean data, in the gene expression pattern between the three skeletal sites (Anova single factor analysis):

Ratio	IC	FN	IT
RANKL/GAPDH	0.41±0.26	0.61±0.70	0.48±0.30
OPG/GAPDH	0.34±0.23	0.43±0.24	0.41±0.21
RANKL/OPG	1.24±0.29	1.31±0.87	1.49±1.33
RANK/GAPDH	0.16±0.06	0.14±0.04	0.17±0.11
IL-6/GAPDH	0.99±1.09	1.26±0.91	1.06±0.96
TRAP/GAPDH	0.35±0.10	0.39±0.13	0.40±0.14
OCN/GAPDH	0.95±0.49	1.18±1.01	0.88±0.44
OPN/GAPDH	0.36±0.11	0.38±0.26	0.37±0.13

Values are mean ± SD

Although differences in bone turnover have been reported between these skeletal sites, we did not observe significant differences in gene expression of the above skeletal factors between the IC, FN and IT sites. It will be informative to perform a similar analysis with osteoarthritic samples to determine whether the altered gene expression we have found at the proximal femur, compared to controls (1,2), reflects the local joint disease or suggests a wider skeletal difference in bone turnover.

1. Fazzalari et al. 2001 *JBMR* In Press; 2. Kuliwaba et al. 2000 *JBMR* 15:332-41.

O32

CHEMOTHERAPY SENSITISES CANCER CELLS BUT NOT NORMAL CELLS TO TRAIL-INDUCED APOPTOSIS

S Bouralexis, DM Findlay, S Hay, GJ Atkins, M Clayer, and A Evdokiou.

Department of Orthopaedics and Trauma¹, The University of Adelaide, Adelaide, South Australia.

Despite improvements in outcome, osteogenic sarcoma remains a difficult clinical challenge. The aim of this study was to investigate the cytotoxic activity of Apo2L/TRAIL by itself or in combination with clinically relevant anticancer drugs, on osteogenic sarcoma cell lines, and their non transformed counterpart, human osteoblast like cells (OB). TRAIL is a member of the tumour necrosis factor (TNF) family of cytokines, which induces apoptosis by interacting with its two death-domain, containing receptors. Expression of three "decoy" receptors, protect normal cells from apoptosis. When used alone TRAIL induced apoptosis in only 1/6 osteogenic sarcoma cell lines. However, treatment of resistant cell lines with TRAIL in combination with low doses of the chemotherapeutic agents, doxorubicin, cisplatin and etoposide, significantly augmented the effect of either agent alone. Our results indicate that chemotherapy and TRAIL act synergistically to kill cancer cells differentially compared to normal cells, which have exciting implications in the treatment of these sarcomas. Importantly, neither TRAIL alone, nor in combination with any of these drugs, affected human OB under the same conditions. We found a strong association between the expression of TRAIL death receptors (DR4 and DR5) and the TRAIL decoy receptors (DcR1 and DcR2) in some cells, suggesting a potential mechanism for protection of OB from TRAIL-induced apoptosis. In contrast osteogenic sarcoma cells usually lacked expression of either or both decoy receptors. Further work is designed to investigate the mechanisms for these observations. The augmentation of apoptosis by chemotherapy in osteogenic sarcoma cell lines was mediated by caspase activation as measured by caspase 8 and caspase 3 activity. The broad caspase inhibitor zVAD-fmk, or the caspase 3 specific inhibitor zDEVD-fmk, prevented apoptosis in response to TRAIL and to TRAIL/drug combinations.

O33

FGF-23 IN ONCOGENIC OSTEOMALACIA CELL LINES

^{1,2}M Mirams, ^{1,2}EA Martin, ¹BG Robinson, ^{1,2}RS Mason & ^{1,2}AE Nelson. ¹Cancer Genetics, Kolling Institute of Medical Research, Royal North Shore Hospital, Sydney, ²Physiology Department, Institute of Biomedical Research, University of Sydney.

Missense mutations in Fibroblast Growth Factor 23 (FGF-23) have been found to cause the condition of ADHR (1) (Autosomal Dominant Hypophosphataemic Rickets), an inherited form of hypophosphataemic rickets with features similar to oncogenic osteomalacia (OOM). This raises the possibility that FGF-23 may be a candidate for the factor causing OOM. OOM is characterised by impaired renal phosphate reabsorption associated with a tumour, leading to impaired bone mineralisation. The circulating factor secreted by the tumour has not yet been fully characterised. Mice implanted with FGF-23 secreting cells have recently been shown to develop hypophosphataemia (2). The purpose of this study is to investigate FGF-23 in OOM cell lines.

We are examining two fibroblastic oncogenic osteomalacia tumour cell lines, the R-cell and JH-cell lines. Conditioned media from the cell lines inhibited phosphate uptake by renal OK3B2 (opossum kidney) cells. Sequencing of genomic DNA revealed no mutations in FGF-23 in either cell line. Expression of FGF-23 is being studied. The activity from conditioned R-cell media passed through 10kDa cut-off Centricon filters, indicating that the factor is smaller than the 32kDa full-length FGF-23, as also reported from tumour extracts (3).

While FGF-23 could play a role in OOM, it appears from data in these cell lines that mutations in FGF-23 may not be the cause for the disorder as they are in ADHR. It is possible that altered levels of FGF-23 expression, an FGF-23 fragment, &/or alternate downstream phosphate regulating factors could be responsible for this condition.

(1) ADHR Consortium (2000). Nature Genetics 26: 345-348

(2) Shimada, T et al. (2001). PNAS 98(11): 6500-6505

(3) Jonsson, KB et al. (2001) J Endocrinol. 169(3):613-620.

O34

THE PHOSPHOLIPID GROWTH FACTOR SPHINGOSINE-1-PHOSPHATE STIMULATES OSTEObLAST PROLIFERATION IN A G_i PROTEIN- AND P42/44 MAP KINASE-DEPENDENT FASHION

C Xu, B Hill, K Callon, Q Chen, I Reid, J Cornish, A Grey. Dept of Medicine, University of Auckland, Auckland, New Zealand.

The related naturally occurring phospholipid compounds lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P), which circulate in low micromolar concentrations *in vivo*, are now recognized to be growth factors in many cell types. The cellular effects of these compounds are transduced by members of the *edg*/LP family of G protein-coupled receptors. We recently reported that LPA is a potent osteoblast mitogen *in vitro*, exerting its proliferative effects via a signaling pathway that includes G_i proteins and protein kinase C (PKC), but not p42/44 MAP kinases. In the current study, we have examined the effects of S1P on osteoblast mitogenesis. In cultures of both primary rat osteoblastic cells and SaOS2 cells, S1P dose-dependently stimulated cell proliferation, as judged by incorporation of [³H]-thymidine. At equimolar concentrations, S1P and LPA exerted comparable fold increases in cell proliferation. As with LPA, the proliferative response to S1P was strongly blocked by the G_i protein inhibitor, pertussis toxin (10 ng/ml). However, in contrast to LPA, the proliferative effects of S1P in osteoblastic cells were inhibited by the specific MAP kinase inhibitor, U-0126. S1P induces time- and concentration-dependent phosphorylation of p42/44 MAP kinases in primary osteoblastic cells and UMR 106-06 cells. These data demonstrate that (a) S1P is a potent osteoblast mitogen *in vitro*, and (b) the mitogenic signaling pathway used by S1P in osteoblastic cells differs significantly from that activated by LPA, despite the fact that the two compounds signal through structurally related cell-membrane bound receptors.

I7

ORGAN TRANSPLANTATION – THE ROLE OF LABORATORY SCIENCE

M.M. Müller, Institute of Laboratory Diagnostics, Kaiser Franz Joseph Hospital, Vienna, Austria

Introduction: In the last years the frequency of transplantation of organs like kidney, pancreas, liver, heart and lung has dramatically increased.

Immunology: Cellular and humoral mechanisms are involved in graft rejection and infections leading to release of cytokines, mediators, acute phase proteins, activation of the complement system and immune-competent cells. T-helper cells play the central role in cell-mediated immune response as they produce a variety of cytokines essential for activation and differentiation of other cells. It can occur within hours of transplantation (hyperacute graft rejection) in recipients pre-sensitised against donor antigens. The cellular mechanisms involved in the rejection of a graft are a combination of specific actions of cytotoxic T-cells, pro-inflammatory cytokines and unspecific proteolysis.

The Clinical Laboratory: Main diagnostic tasks are the diagnosis of transplant rejection, infection status, and the monitoring of immunosuppressive drug levels and of drug effects. The diagnosis of graft rejection is based on 2 principles: 1) Monitoring of organ dysfunction and tissue damage with so-called organ specific routine analytes for kidney, liver and pancreas function are used. 2) Monitoring of immunologic status. For monitoring immunosuppressive drugs (cyclosporine A, FK506, azathioprin, mycophenolic acid) mainly the measurement of blood target levels by means of HPLC or immunoassays is used. The interpretation of test results are method dependent and the therapeutic regimens are different for the various organs transplanted. In addition biological and toxic effects of the drugs can be monitored. Therapeutic drug monitoring in organ transplantation is a responsible and challenging task, since the clinician uses the blood drug levels and organ function tests reported for drug prescription and for estimation of the patient's compliance.

I8

THE PREDICTIVE VALUE OF BONE BIOCHEMICAL MARKERS.

R Eastell, Bone Metabolism Group, University of Sheffield, Sheffield, UK.

High rates of bone turnover may not only be associated with bone loss but also with a disruption of the trabecular network that gives bone its structural integrity. Loss of connectivity through disruption of the trabecular network is not necessarily reflected in a decrease of bone mass. In other words, biochemical markers may be independent predictors of fracture by providing insight into this qualitative feature of bone.

In two prospective studies, hip fracture has been associated with elevated levels of bone resorption. In both studies, markers of formation (i.e., serum osteocalcin and bone alkaline phosphatase) were not associated with an increased hip fracture risk. However, in two recent studies high levels of bone alkaline phosphatase were associated with increased fracture risk. Vertebral fractures have also been associated with high levels of bone resorption markers. Impaired gamma-carboxylation of osteocalcin may be associated with an increased risk of hip fracture.

Taken together, present evidence indicates that older individuals with increased bone resorption and perhaps increased bone formation are at increased risk of osteoporotic fracture and the information is complementary to that provided by bone mass measurements.

I9

THE BIOCHEMISTRY OF COLLAGEN BREAKDOWN PRODUCTS.

GN Kent, Division of Clinical Pathology, The Western Australian Centre for Pathology and Medical Research (PathCentre), Nedlands, Western Australia.

Type I collagen forms the major component of the organic matrix of bone. During bone formation there are specific biochemical modifications of this type I collagen (e.g. hydroxylation and glycosylation). The maturation process of bone collagen from monomer to polymer involves the formation of reducible and non-reducible crosslinks (pyridinium and pyrrolic) to form lamellar collagen fibrils. This process confers the properties required for the mechanical and structural role for collagen within bone.

The process of bone resorption involves osteoclast-mediated removal of mineral from the organic matrix followed by enzymatic degradation of the organic matrix. Collagenases, cathepsin K and other proteases produce a number of partially degraded collagen peptides from the mature collagen. These degradation products can be released into the circulation or further metabolized at their site of production. The nature and amount of these products of bone collagen breakdown found in blood and urine is dependent upon 1) the tissue source (e.g. cartilage release of pyridinoline); 2) biological rhythms (diurnal variation of bone resorption); 3) tissue-specific catabolism (e.g. bone, liver and kidney); 4) physiological changes (e.g. fasting, growth, age, gender and menopause) and, 5) pathological changes (e.g. rate of bone resorption, bone metastases, residence time in bone affecting extent of beta-isomerization of aspartic acid residues).

A number of these collagen degradation products have been used in the development of assays used as indices of bone turnover or bone resorption. These "specific" degradation products and their assays fall into two broad categories – amino acid derivatives (e.g. deoxypyridinoline, hydroxylysine glycosides) or peptides derived from the regions of the crosslinking sites between collagen monomers (e.g. N- and C-terminal telopeptides).

All the factors outlined above have to be considered, together with pre-analytical and analytical factors, in deciding upon the utility of any marker of collagen degradation as a valid indicator of the rate of bone resorption.

I10

MONITORING BONE DISEASES

IR Reid

Monitoring of bone diseases has been the subject of fewer investigations and cost-effectiveness analyses than have the use of diagnostic tests and therapeutic procedures. This is probably because the questions involved are more complex. However, this is an important issue since tests can be expensive, and they may involve discomfort (eg. blood sampling) or risk (eg. radiation). Furthermore if tests are used incautiously, inappropriate changes to therapies may result. Followup investigation may be necessary when the natural history of the condition is unpredictable, when an intervention will need to be introduced or modified at a certain point in the evolution of a condition, or when response to therapy is unpredictable. It is important that the test being monitored has the necessary precision to reliably detect the changes being sought. For instance, annual femoral neck bone density measurements will not detect a therapeutic response to most antiosteoporotic therapies, whereas serum alkaline phosphatase will almost always change substantially in response to potent bisphosphonate therapy in Paget's disease. However none of these tests are useful in the context of monitoring therapy, unless the clinician has a clear idea of what responses are required and of what further therapeutic interventions are appropriate, if the required responses are not found. It is not possible at this time to set out a fully justified, evidence based follow-up protocol for patients with osteoporosis but it is possible to outline the requirements that should be met by such a protocol.

I11

BONE TURNOVER MARKERS AND THE MENOPAUSE TRANSITION.

PR. Ebeling, Dept. Diabetes and Endocrinology, The Royal Melbourne Hospital, Parkville 3050, Victoria, Australia.

The menopause transition is a time of great variability in oestrogen levels, rates of bone turnover and bone loss. Women with osteopaenia at the onset of menopause may have a greater risk of osteoporosis and fracture if they are losing bone at a rapid rate.

Bone turnover markers may be useful in predicting rates of future bone loss, providing independent information about fracture risk beyond that available from BMD measurements alone. The diagnostic utility of a single bone turnover measurement is limited because individuals with low levels of bone turnover have rates of bone loss that range from 0 to 10 %/year; the test, therefore, has a low specificity. Nevertheless, a person with a high value of a bone turnover marker is generally at greater risk of bone loss than a person with a low value.

Most studies have shown a highly significant correlation between bone turnover markers and subsequent rates of bone loss. Postmenopausal women with high bone turnover have bone mass that is 8-14% lower compared with women with low bone turnover.

Bone markers are not usually adjusted for age, sex, height or weight, however the premenopausal mean is often used as a reference value. Postmenopausal women with a bone turnover rate 2 SDs above the premenopausal mean lose 2-6 times more bone from the distal forearm over a 4-year period, depending on the marker chosen. Taken together, it would seem reasonable to measure BMD during the menopause transition if preventive therapy is being contemplated for women at risk for osteoporosis. If the BMD is in the osteopaenic range, the finding of a urine or serum bone turnover marker elevated above the upper limit of normal for premenopausal women would add further impetus to the recommendation for preventative therapy.

I12

THE LABORATORY APPROACH IN THE DIAGNOSIS OF MENOPAUSE AND BONE METABOLISM.

L Vankrieken, DPC Benelux, Breda, The Netherlands.

Gerontology and associated age-related diseases are now high priorities within the medical community. In terms of women's health issues, menopause is a focal point. Determining the age of menopause is difficult due to individual and population variability as well as sampling bias (retrospective sampling, differences in study population). Physiological changes during the peri-/postmenopausal period include decreases in estradiol levels (<30 pg/mL), an increased estrone/estradiol ratio (>1) and increased FSH and LH serum levels. Osteoporosis is of particular concern and if the diagnosis of elevated bone resorption is made early in the course of decreasing estrogen production, then appropriate therapy can be instituted and the clinical complications of osteoporosis prevented. Bone turnover can be assessed by measuring products produced during bone resorption and bone formation. Deoxypyridinoline is a pyridinium crosslink found almost exclusively in bone collagen and is released during osteoclastic activity. Osteocalcin (OC) is specific for bone tissue. It provides a specific and sensitive marker for bone formation and the levels correlate with bone formation. The circadian variation of OC concentration has been reported to peak at night and drop in the morning. This should be taken into account when taking the blood sample; the recommended time of sampling is between 8.00-11.00 am.

Clinical applications of bone markers include the following: Paget's disease, menopause, osteoporosis, metastatic bone disease, chronic renal failure and early assessment of a loosening hip replacement. Evaluation of bone integrity should be based on both BMD measurement (although it provides a static assessment only, not current bone turnover) and measurement of bone markers for formation and resorption (Dpd and OC).



2001

ANZBMS

POSTERS

P1

GENERATION OF TRANSGENIC MICE THAT EXPRESS CRE RECOMBINASE SPECIFICALLY IN OSTEOCLASTS.

W.S.M. Chiu, R.A. Davey, A.J. Notini, J.F. McManus, J.D. Zajac. Department of Medicine, The University of Melbourne, Austin and Repatriation Centre, Studley Road, Heidelberg, Victoria 3084, Australia.

The *Cre-lox* system is a powerful technique, which allows tissue specific deletion of a target gene. The purpose of this study is to generate a transgenic mouse line that expresses Cre recombinase specifically in osteoclasts. We propose to utilise two promoters, tartrate-resistant acid phosphatase (TRAP) and cathepsin K (CK), to generate two osteoclast specific Cre transgenic mouse lines.

To test whether the TRAP and CK promoters are suitable to drive Cre expression specifically in osteoclasts, tissues were collected from 4 male and 4 female F1 CBA x C57Bl/6 mice and total RNA extracted. The mRNA levels of TRAP and CK were determined by Northern blot analysis. TRAP mRNA levels did not differ between males and females. TRAP mRNA is expressed in a number of tissues, however is significantly higher in bone compared with brain (14 fold), colon (4 fold), heart (25 fold), kidney (5 fold), liver (4 fold), lung (13 fold), muscle (15 fold) and stomach (11 fold) ($P < 0.05$). Preliminary results demonstrate CK mRNA is expressed highly in bone and at lower levels in lung and ovary.

In conclusion, TRAP (ranging from 4 fold to 25 fold) and CK are expressed at much higher levels in bone than other tissues. We are currently using these promoters to generate two Cre constructs. The resulting transgenic mice with the highest level of tissue specificity of Cre expression will be mated with *lox* mice to delete target genes of interest specifically in osteoclasts.

P2

HISTOMORPHOMETRY OF MALE HYPOGONADAL MOUSE BONE AND EFFECT OF 17 β -ESTRADIOL.

Dianne Hughes^{1,2}, Chris White², Michael Hooper², David Handelsman¹.

¹ ANZAC Research Institute, Concord Hospital, Concord NSW 2139

² Endocrinology and Metabolism, Concord Hospital, Concord NSW 2139

The *hpg* is a mutant mouse strain originally derived from C3H/HeHx101/H F1 hybrids. Lacking the ability to produce mature gonadotrophin-releasing hormone, *hpg* mice have a life-long deficiency of androgen and estrogen. The current study used this unique mouse model to investigate the consequences of bone development without sex steroids and effect of estrogen exposure. Wild type (wt) and *hpg* mice were raised to six weeks of age, then 0.2 cm 17 β -estradiol (E2) implants were inserted into some *hpg* mice (*hpg*+E2) for seven weeks. Histomorphometry of unmineralised cancellous bone was evaluated in 1st lumbar vertebrae of 4 wt, 7 *hpg* and 6 *hpg*+E2 mice at 13 weeks of age. There was no significant difference between final body weight (g) of *hpg* (20.7 \pm 1.4; mean \pm SEM) and *hpg*+E2 (21.9 \pm 2.0), both being significantly lighter than wt (25.3 \pm 2.4). Bone volume (BV/TV%) was over 200% greater in *hpg*+E2 (44.9 \pm 4.7) than in wt (12.0 \pm 1.9) and *hpg* (14.4 \pm 1.5). Trabecular thickness (Tb.Th mm) was 54% greater in *hpg*+E2 (54.2 \pm 5.2) than in *hpg* (35.1 \pm 4.5), and 86% greater in *hpg*+E2 than wt (29.2 \pm 3.1). Trabecular number (Tb.N /mm) was 100% greater in *hpg*+E2 (8.4 \pm 0.6) than *hpg* (4.2 \pm 0.3) or wt (4.1 \pm 0.4). Doubling trabecular number resulted in a 68% reduction of trabecular spacing (Tb.Sp mm) in *hpg*+E2, 68.6 \pm 8.9 compared to 213.3 \pm 21.6 in *hpg* and 226.0 \pm 29.1 in wt. Osteoid surface (OS/BS%) did not differ amongst wt (3.1 \pm 1.5), *hpg* 9.7 \pm 5.4 or *hpg*+E2 (1.13 \pm 0.5). Osteoclast surface (OcS/BS%) was increased in *hpg*+E2 (6.3 \pm 2.3) compared to *hpg* (1.0 \pm 0.3) and wt (1.2 \pm 0.7). These results indicate that steroid hormone deficiency has little effect on vertebral cancellous histomorphometry in 3 month *hpg* mice and that there is a marked response to a pharmacologic dose of estradiol.

P3

INFLUENCE OF SOY CONSTITUENTS ON OSTEOPOROTIC BONE.
U Wehr, P Hörmann, E Lochmüller#, M. Schlachter*, C Riegger*, P Weber*,
WA Rambeck, Institute for Animal Physiology, #Depart. of Medicine,
University of Munich, Germany, *F. Hoffmann-LaRoche Ltd., Basle,
Switzerland

Hormone replacement therapy (HRT) is often recommended for osteoporosis prevention and treatment. However, HRT may be associated with side effects. Thus, increased research into alternatives to estrogen for postmenopausal women is of clinical, scientific and health policy importance. Phytoestrogens including soybean isoflavones have structural similarity to estrogen and exhibit beneficial effects on bone tissue to protect against bone loss under estrogen-deficient conditions.

The aim of this study was to investigate the bone protective effect of soy and its main constituents genistein and daidzein. 60 female Fisher 344 rats were either sham-operated (SHAM) or ovariectomized (OVX). OVX rats (n = 10) were assigned to soy or to different doses of the soy constituents genistein and daidzein. After 4 weeks pyridinoline (Pyd in nmol/mmol Crea) and osteocalcin (Oc in ng/ml) were assessed, after 12 weeks the bone mineral density (BMD in g/cm²) of the right tibia was assessed.

	PYD	Oc	BMD
SHAM	361±23*	40±3	0.142±0.008*
OVX	538±19	65±4	0.129±0.007
Soy	407±36*	53±7	0.134±0.009
Genistein (60 mg/kg BW)	455±21*	49±6*	0.138±0.006*
Daidzein (60 mg/kg BW)	416±34	44±5	0.133±0.008
Gen/Daid (30+60mg/kg BW)	453±19*	59±11	0.137±0.003*

*p<0.05 in comparison to OVX without treatment

In conclusion, soy and the soy constituents genistein (most efficient) and daidzein suppressed the ovariectomy-induced increase in bone turnover and prevented ovariectomy-induced bone loss.

P4

THE EFFECTS OF CIRCULATING PTH LEVELS ON CORTICAL THICKNESS IN THE VITAMIN D DEplete RAT

AJ Moore, PH Anderson, S Iida, PD O'Loughlin, HA Morris
Institute of Medical and Veterinary Science, Adelaide, S.A. 5000.

Decreased Vitamin D status is strongly associated with hip fracture in the elderly. A placebo controlled trial of Vitamin D (VD) and dietary calcium (Ca) supplements reduced the incidence of hip fracture by 30 % in nursing home residents. The aim of this study was to investigate the structural changes to cortical bone that occur as a result of VD restriction in the rat. VD deplete rats (n=10) were bred in a UV free environment and fed a VD deficient diet containing 1 % Ca. VD replete rats were fed a diet containing 1000 U/kg VD and 1% Ca. At 6 months of age 5 rats from both the VD deplete and VD replete groups were changed to a 0.1 % Ca diet. At 9 months of age all rats were killed and femora were processed for histological examination using established resin embedding techniques. Cortical thicknesses were measured using a digital calliper. Plasma PTH levels were measured using an IRMA kit. An increase in PTH levels was observed with a reduction in dietary Ca or VD depletion (P<0.01). Cortical thickness was lower in VD deplete rats at BL compared to VD replete rats (P<0.01). Cortical thickness in VD replete rats decreased from BL in rats fed 0.1 % Ca, with no change in VD replete rats maintained on 1 % Ca (P<0.05). In VD deplete rats, no detectable changes were observed in cortical thickness from BL in either the 1 % Ca or the 0.1 % Ca. group. In VD deplete rats fed 0.1 % Ca, a significant increase in cortical thickness was positively correlated with increasing levels of PTH (r² = 0.58, P<0.05). In VD replete rats, a low Ca diet significantly reduced cortical thickness. With VD depletion, however, cortical thickness was reduced independently of dietary Ca. Of interest is the significant association between increased PTH levels and cortical thickness in the VD deplete, low Ca rats. These data suggest that suppression of PTH by dietary Ca in vitamin D deplete patients may exacerbate cortical bone loss and therefore increase risk of fracture.

P5

INFLUENCE OF CALCIUM SUPPLY IN OVARECTOMIZED RATS UNDER PHYTOESTROGEN THERAPY.

S Krammer, U Wehr, E Lochmüller[#], C Riegger^{*}, P Weber^{*}, M Schlachter^{*}, WA Rambeck, Institute for Animal Physiology, [#]Depart. of Medicine, University of Munich, Germany, ^{*}F. Hoffmann-LaRoche Ltd., Basle, Switzerland

Phytoestrogens, such as the soy isoflavones genistein and daidzein, are natural compounds, with a biological activity similar to estrogen. They are currently extensively investigated in molecular, preclinical and clinical studies to determine their potential health benefits. Phytoestrogens may protect against chronic diseases such as hormone-dependent cancer (e.g., breast and prostate cancer), cardiovascular disease and osteoporosis.

The aim of our study was to investigate the relationship between calcium supply and the effect of genistein on bone density in ovariectomized rats, as a model for postmenopausal osteoporosis.

60 female Fisher 344 rats were either sham-operated (SHAM, n = 10 per group) or ovariectomized (OVX, n = 10 per group). Half of the animals received adequate, the other half inadequate supply of calcium. Two groups were assigned to 30 mg genistein/kg BW. After 12 weeks the bone mineral density (BMD in g/cm²) of the right tibia was assessed. As a control the weight of the uterus was also determined.

Results	Ca supply in feed	Weight of uterus (g)	BMD
SHAM	0,5%	1,0±0,2	0,142±0,008*
SHAM	0,25%	1,0±0,1	0,125±0,008
OVX	0,5%	0,1±0,0*	0,131±0,007
OVX	0,25%	0,1±0,0*	0,124±0,011
Genistein	0,5%	0,1±0,0*	0,138±0,008*
Genistein	0,25%	0,1±0,0*	0,123±0,008

*p<0.05 in comparison to OVX (0.25% Ca)

Conclusion: A sufficient supply of Ca is an absolute requirement for preventing OVX induced bone loss in rats by genistein.

P6

CALCIUM BIOAVAILABILITY: EFFECTS OF FRUCTO-OLIGOSACCHARIDES

MC Kruger, *LM Schollum. Milk and Health Research Centre, IFNHH, Massey University, Private Bag 11222, Palmerston North; *New Zealand Dairy Research Institute, Palmerston North, New Zealand.

Fructo-oligosaccharides (FOS) increase intestinal calcium absorption and enhance bone calcium stores in rats. Mechanisms may include an effect on intestinal calcium binding protein or an increase in fermentation of FOS in the large intestine to produce short-chain fatty acids, which in turn reduce luminal pH. However a direct comparison of FOS with different degrees of polymerisation (DP) has not been done simultaneously in the same experimental model.

The purpose of the study was to compare the effects of FOS with different DP on calcium bioavailability. Forty nine day old male Sprague Dawley rats were randomised into 4 groups of 10 each. The control animals were fed the base diet, which contained 5% sucrose. The remaining three groups were fed the base diet in which the sucrose was replaced with 5% Raftilose[®] P95[#] (DP 2-8), or Raftiline[®] HP[#] (inulin DP >23) or Raftilose[®] Synergy1[#] (DP 90% inulin and 5-6% 2-8). Animals were fed the test diets for 4 weeks. At weeks 0 and 4 serum calcium and parathyroid hormone levels were measured. At week 4 the animals were sacrificed and bone density, bone calcium content and urinary bone resorption markers measured.

Preliminary results indicate that both *in vivo* and *ex vivo* femur bone density were significantly higher in the group fed Raftiline[®] HP compared to control. In addition, the excretion of collagen cross-links decreased most significantly in the Raftiline[®] HP group compared to control. Serum PTH and calcium did not change significantly. We conclude that not all fructans are equally efficient in stimulating calcium absorption and bone mineralisation. The inulin from which small molecular weight oligomers have been eliminated, increased calcium bioavailability significantly compared to sucrose.

Orafiti Active Food Ingredients.

P7

CHANGES IN CONDYLAR CANCELLOUS BONE VOLUME FRACTION (BV/TV) AND TURNOVER IN SHEEP FOLLOWING FORWARD MANDIBULAR DISPLACEMENT.

B Ma, N Fazzalari, WJ Sampson, DF Wilson and O Wiebkin. (Orthodontic Unit, Dental School, Adelaide University)

Forward displacement of the mandible in animal models is associated with faster and/or redirected condylar growth. In this study we investigated the effect of mandibular forward displacement on modelling and remodelling of the mandibular condyle. Eight male sheep (mean age 4m) were randomly allocated to experimental and control groups with four animals in each group. Forward mandibular displacement was induced with an intraoral appliance. The study period was 15 weeks during which time, calcein (day-1) tetracycline (day-91) and alizarin red S (day-102) labels were administered to all animals. Mid-sagittal sections of the temporomandibular joints were selected for analysis. BV/TV, trabecular architecture, bone formation and resorption were estimated using histomorphometry. The sampling site was divided into two regions for analysis: (1) a “subchondral region,” (labelled by 2nd and 3rd labels), believed to comprise bone newly formed during the experimental period; and (2) a “central region,” (labelled by calcein label), believed to comprise bone which existed before the experiment. The condylar growth was found to be faster in the experimental group. In the experimental group, BV/TV of the subchondral regions decreased, although the specific bone surface and bone turnover increased. This low BV/TV was due to decreased trabecular thickness and increased trabecular separation. In the central region of the experimental group’s condyle, BV/TV was unchanged, but revealed a significantly higher bone turnover. These results indicated rapid condylar growth following forward mandibular displacement was achieved by forming thinner trabeculae and larger trabecular spacing.

P8

CHARACTERISATION OF CHONDROCYTE LINEAGE IN AUTOLOGOUS CHONDROCYTE TRANSPLANTATION.

RJ Wealthall, E King, D Liu, J Xu, MH Zheng. Department of Orthopaedic Surgery, University of Western Australia, Nedlands, WA 6009, Australia.

Autologous chondrocyte transplantation (ACT) has been shown to be a promising method for restoring hyaline cartilage defects. Since first reported in 1994, clinical follow up studies have indicated that ACT has provided an excellent outcome in the restoration of hyaline cartilage. As ACT relies on the use of cultured cells, and the biosynthetic profile of cultured chondrocytes has been shown to be altered during *in vitro* expansion, cultivation of chondrocytes for ACT has presented many technical and quality control challenges. An assessment of the cellular phenotype of cultured chondrocytes, consistent with differentiation of articular hyaline cartilage, is required to ensure the delivery of ACT. Using RT-PCR, flow cytometry, and scanning electron microscope (SEM) analyses, we have characterised the cellular phenotype of cultured chondrocytes used for ACT. We have examined several transcriptional factors, cytokines and matrix proteins necessary for the differentiation of chondrocytes in cases of ACT. These include Sox9, BMP-2, TGFβ3, PTHrP, CEP-68, type I and II collagen, aggrecan, and alkaline phosphatase. While there was variation in the expression of some of these genetic markers among patients, all patients expressed chondrocyte-specific genes. Furthermore, SEM and flow cytometry analyses demonstrated variation between patients in cell morphology and in the level of apoptosis of chondrocytes. Magnetic resonance imaging studies of two patients that were unable to restore hyaline cartilage show that these coincided with an apoptotic level greater than 8%. In short, given that there is a medical need for ACT in the treatment of articular cartilage injury, processes such as these for monitoring the quality of cultured chondrocytes prior to implantation may provide a better clinical outcome for ACT.

P9

EFFECT OF LOW-INTENSITY ULTRASOUND STIMULATION IN A RABBIT MODEL OF DISTRACTION OSTEOGENESIS.

MG Uglow, J Briody, M Hile, L Bilston, EJ Smith, RA Peat, DG Little. Orthopaedic Research Unit, Children's Hospital at Westmead, NSW 2145, Australia.

Low-intensity pulsed ultrasound has been shown to accelerate fracture healing in animals and humans^{1,2}. Low-intensity pulsed ultrasound therapy (SAFHS: Sonic Accelerated Fracture Healing System, Exogen Inc.) was used to treat 21 immature New Zealand white rabbits following a mid diaphyseal tibial osteotomy and 1cm bone lengthening using an Orthofix M-100 device, 20 minutes/day. Treatment consisted of pressure wave signals with a frequency of 1.5Mhz producing an intensity of 30mW/cm². Twenty-one matched controls underwent an identical procedure with the ultrasound transducer switched off. At 4 and 6 weeks postoperatively, tibiae from 15 rabbits were analysed using DXA, QCT and 4-point mechanical testing. No significant differences were identified between active and control groups with respect to bone mineral content or cross-sectional area of the regenerate, nor the bone proximal and distal to it. No improvement in strength of the regenerate was identified in either group, nor was there a reduction in stress-shielding related osteopenia occurring in bone adjacent to the regenerate. Tibiae from the remaining rabbits at 2, 4 and 6 weeks postoperatively, were used for histomorphometry. Preliminary observations on limited sample number indicated that total regenerate bone volume did not change although the active group had fewer trabeculae, with increased thickness, which may correlate with an apparent reduction in osteoclast number. Previous studies have shown differences at earlier time points, but in these studies differences were not significant at later time points³. Although proven to be effective in unconstrained systems (plaster), this study does not support the role of SAFHS as a useful adjunct for patients undergoing distraction and other rigid bone fixation procedures. Further research is needed to definitively support the use of SAFHS in such constrained situations.

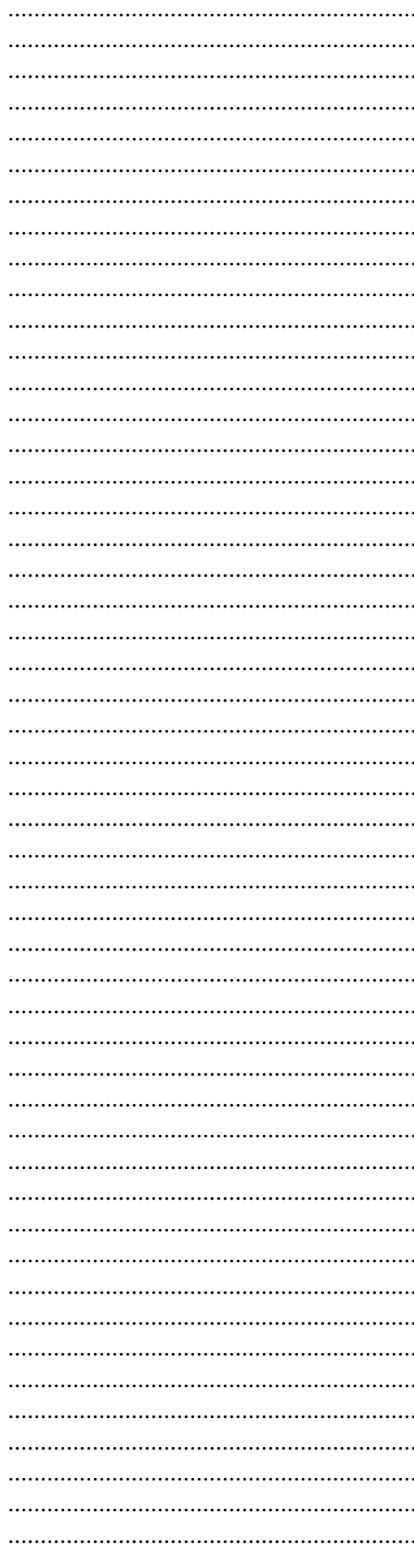
1. Heckman J, et al. 1994. *J Bone Joint Surg (Am)* 76-A:26-34.
2. Kristiansen TK, et al. 1997. *J Bone Joint Surg (Am)* 79-A:961-973.
3. Shimazaki A, et al. 2000. *J Bone Joint Surg (Br)* 82 (7):1077-1082

P10

EXPRESSION OF PROTEASE-ACTIVATED RECEPTOR-1 DURING BONE HEALING

S-J Song, TM Campbell, EJ Mackie. School of Veterinary Science, University of Melbourne, Parkville, Victoria 3010, Australia

Protease-activated receptor-1 (PAR-1) is a member of the family of seven trans-membrane-spanning, G-protein-coupled receptors. It can be activated by thrombin or synthetic peptides corresponding to the PAR-1 tethered ligand sequence that arises through thrombin's cleavage of the extracellular amino-terminus. PAR-1 is expressed by a number of cell types, including osteoblasts. Many cellular responses to thrombin are mediated by PAR-1, such as inflammation, wound repair following tissue injury, and osteoblast proliferation. The expression of PAR-1 in healing bone has not however been described. Male mice (12 weeks old) were used in a model of bone repair recently described in our laboratory. A single hole was drilled through both cortices of the tibia of anaesthetized mice, then the animals were killed at various times after drilling. In this model, osteoblast precursors migrate from the bone marrow into the drill site by 5 days after injury, and subsequently deposit woven trabecular bone. A polyclonal antibody raised against a peptide corresponding to the post-cleavage amino terminal sequence of PAR-1 was used to localize PAR-1 expression in the repairing bone at different time points after injury. Elevated PAR-1 expression at the edge of the drill site and adjacent periosteal surface was detectable 3 days after bone injury. The intensity of PAR-1 staining within the drill site and periosteal surface increased over days 5-9. Osteocytes within new bone and cartilage cells in periosteum present at days 7 and 9 expressed PAR-1. By day 14 the PAR-1 expression was much weaker within the drill site. The pattern of PAR-1 expression observed suggests that PAR-1-mediated responses to thrombin plays an important role in bone repair during the early response to injury.



P11

THE XANTHINE DERIVATIVE, PROPENTOFYLLINE, INHIBITS EARLY FRACTURE REPAIR IN RATS.

MG Brown, JA Schuijers and BL Grills. School of Human Biosciences, Department of Human Physiology and Anatomy, La Trobe University 3086, Victoria, Australia.

Propentofylline (PPF) is a drug that stimulates nerve growth factor (NGF) production in neural tissue *in vitro* and *in vivo*. Previous research in our laboratory has shown that NGF is formed by osteoblasts, is present in fracture callus and its application to fractures improves repair. Therefore, it was proposed that if PPF administration stimulated callus NGF concentrations in a rat rib fracture model, then PPF might improve the repair process.

Treated rats (n = 16) received a subcutaneous injection of 25 mg of PPF/kg of body weight/day for 14 days post-fracture and control rats (n = 16) received isotonic saline. NGF concentrations were measured by ELISA in 2 week-old fracture calluses and unfractured ribs (treated = 8, control = 8) and calluses were assessed histologically at 3 weeks post-fracture (treated = 8, control = 8).

At 2 weeks post-fracture, NGF concentrations (per wet weight of tissue) in fracture calluses from controls were approximately two-fold higher than unfractured control ribs (p = 0.002), possibly signifying that NGF production increased after fracture. Osseous NGF concentrations were almost two-fold higher in unfractured PPF-treated ribs compared to unfractured control ribs (p = 0.04), suggesting that PPF treatment stimulated NGF synthesis in intact bone. Paradoxically, callus NGF concentrations in PPF-treated fractures were nearly 50% lower than in control calluses (p = 0.03). At 3 weeks post-fracture, all 8 fractures in control animals had been fully united by bone and cartilage, however, PPF-treatment interfered with fracture repair resulting in 3 total non-unions and 5 partial non-unions (out of 8 fractures).

In conclusion, PPF treatment for the first 2 weeks of fracture repair resulted in reduced callus NGF concentrations and impaired fracture repair up until 3 weeks post-fracture.

P12

NERVE GROWTH FACTOR STIMULATES UMR 106.01 OSTEOBLASTIC PROLIFERATION BUT DOES NOT AFFECT OSTEOCLASTOGENESIS.

TN Uebergang, RJ Thomas, MT Gillespie and BL Grills. School of Human Biosciences, Department of Human Physiology and Anatomy, La Trobe University 3086, Victoria, Australia and St Vincent's Institute of Medical Research, Victoria Parade, Fitzroy 3065, Victoria, Australia.

Nerve growth factor (NGF) has an important role in developing and maintaining sensory and sympathetic nerves. There is evidence that NGF also influences non-neuronal tissue such as bone. Research from our laboratory has shown that both NGF mRNA and NGF receptor mRNA are expressed by clonal osteoblastic cells UMR 106.01 cells (1). These findings suggest that NGF may directly influence both bone metabolism and osseous nerves. The present study investigated possible mechanisms by which NGF may regulate bone cell function.

Firstly, the effects of NGF on osteoblastic cell proliferation were investigated by exposing recombinant NGF to clonal UMR 106.01 cells for 7 days. Secondly, since the low-affinity NGF receptor is a member of the Tumour Necrosis Factor (TNF) superfamily, NGF was administered to a murine osteoblastic and haematopoietic coculture system in order to establish possible roles for NGF in osteoclastogenesis.

NGF treatment caused an increase in cell number by approximately 13.4 fold by day 7 compared to 11.8 fold increase in control cell numbers (p < 0.0001). NGF had no effect alone on either supporting (p > 0.05) or inhibiting (p > 0.05) osteoclastic formation.

These findings suggest that the action of NGF may be more important in stimulating osteoblastic proliferation rather than having any obvious role in osteoclastogenesis.

P13

APOPTOSIS OF TENDON FIBROBLASTS IN ROTATOR CUFF TEARS.

TL Dela Rosa, A Wang, MH Zheng. Dept. of Orthopaedic Surgery, Univ. of Western Australia, Nedlands 6009, WA, Australia.

Rotator cuff tears are a common injury which affects both the young athlete and the sedentary alike. This condition is commonly treated with glucocorticoid injections as part of initial management. The effects however of these injections on the histology of collagen and the metabolism of tendon fibroblasts are still controversial. In this study, samples from patients with rotator cuff tears, with or without prior steroid injections, were taken during surgery. Examinations done include histopathology, in situ hybridization to detect glucocorticoid receptor mRNA and TUNEL assay for apoptosis. Tenocytes were also cultured and then exposed to different doses of steroid in culture. H & E stains showed marked cellularity although there were no signs of inflammation. The nuclei were noted to be rounded and a significant number showed pyknosis. Angiogenesis was also noted in the sections, consistent with previous findings of angiofibroblastic hyperplasia as a characteristic of tendinosis. Collagen structure was abnormal, with longitudinal clefts and focal areas of marked disorganization of fibers. In situ hybridization showed strong signal for glucocorticoid receptor mRNA in all of the samples. TUNEL assay showed strong signals for apoptosis. There was a dose-dependent relationship between tenocyte numbers and exposure to steroids in culture. Our results suggest that although an overall picture of hypercellularity is seen in cases of tendinosis and tendon tears, a high percentage of these cells are undergoing apoptosis. This may reflect a natural high rate of turnover of cells during the process of repair of may be due to exogenous factors. Glucocorticoids almost certainly affect metabolism of tendon fibroblasts as seen by the effect on cell numbers in culture.

P14

A MOLECULAR STUDY OF CHONDROCYTE PROLIFERATION AND MATURATION USING DIFFERENTIAL DISPLAY IN THE CHICKEN MODEL

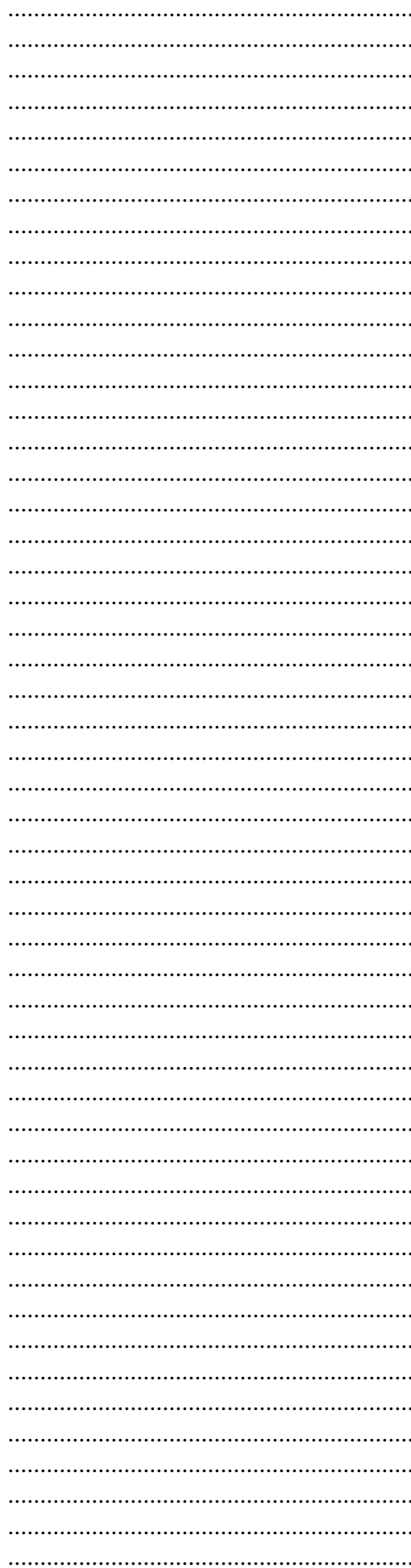
Simcock, W.E., Day, C.J., and Morrison, N.A. School of Health Sciences, Griffith University, Gold Coast Campus, Queensland

Chondrocytes play a pivotal role in skeletal development and juvenile growth. Aberrant chondrocyte behaviour results in growth deformities such as dwarfism and gigantism.

The chondrocytes at the end of the long bones do not undergo apoptosis after mineralization of the embryonic skeleton. These chondrocytes form the growth plate, and by proliferating and maturing in a linear fashion drive the elongation of bones during childhood and puberty.

The molecular mechanisms regulating the chondrocyte life-cycle are not yet fully understood. In this study, we have isolated the distal ends of chicken tibiae, separated the chondrocytes of the growth plates out into their constituent populations, and isolated mRNA from each population. The mRNA was then used as the starting material for differential display, a technique which uses non-specific primers to amplify gene transcripts.¹ Products specific and common to different populations of cells from the growth plates were then sequenced to provide data on the molecular mechanisms involved in regulating this process.

¹Liang, P., and Pardee, A.B. (1998) *Mol Biotechnol* 10:261-267



P15

FLUID SHEAR STRESS AS A MEDIATOR OF FIBROBLAST GROWTH FACTOR RECEPTOR (FGFR) EXPRESSION AND OSTEOBLAST PHENOTYPE.

RA Jackson, V Nurcombe, SM Cool. School of Biomedical Sciences, Department of Anatomical Sciences, University of Queensland, Brisbane, QLD 4072, Australia.

Osteoblasts rely on the ability to receive and interpret external signals to regulate their growth, differentiation and death. Fluid shear has been implicated as an important mediator of these events, having a profound influence on cell phenotype *in vivo* and *in vitro*.

Statement of Purpose: This study aimed to develop a better understanding of the relationship between osteoblast phenotype and FGFR expression in response to mechanical stimulation.

Statement of Methods: To examine the effects of fluid shear on osteoblast phenotype, MC3T3-E1 osteoblasts were cultured for up to 20 days. At 3 day intervals, triplicates for both loaded and control groups were assayed for proliferation (BrdU incorporation), differentiation (alkaline phosphatase expression) and maturation (Von Kossa positive) status. FGFR expression was simultaneously assessed by Western blotting and quantified by ELISA.

Summary of Results and Conclusions: This study demonstrated that fluid shear increases the proliferation, differentiation and maturation of MC3T3-E1 osteoblasts. FGFR expression was also examined in order to establish a role for these receptors in the control of osteoblast phenotype. All four FGFR isoforms (FGFR1-4) were expressed in MC3T3-E1 cultures; however their expression mirrored each other, and was not significantly different for loaded versus control groups. In addition, cultures were observed to progress through a proliferation phase that lasted up to 10 days, followed by a differentiation phase, and a maturation phase. As culture time increased, FGFR expression also increased, peaking at day 10, and plateauing thereafter. However, this expression was unaffected by fluid shear. These results indicate a correlation between FGFR expression and osteoblast differentiation and maturation that is independent of fluid shear loading.

P16

PERITONEAL FLUID: A POTENTIAL SOURCE OF STEM CELLS WITH OSTEOGENIC POTENTIAL?

SM Cool, RA Jackson, JH Campbell, V Nurcombe. School of Biomedical Sciences, Department of Anatomical Sciences, University of Queensland, Brisbane, QLD 4072, Australia.

Among the approximately 90 million fractures suffered in Australia every year, about 15% are difficult to heal. For such cases, orthopaedic surgery is currently in the midst of a transformation from conventional bone grafting to bone tissue engineering. A suitable and readily expandable source of stem cells capable of differentiation toward the osteoblast lineage might represent a useful tool for tissue engineering and repair.

Statement of Purpose: This ongoing study aims to show that peritoneal fluid contains a ready source of adult stem cells, not unlike bone marrow stroma, that when given the right stimuli is capable of differentiation towards the osteoblast lineage.

Statement of Methods: To examine the osteogenic potential, cells obtained by peritoneal lavage are being maintained *in vitro* for extended periods of time in medium containing osteogenic supplements. Cultures of bone marrow stromal cells are being used as controls. Immunofluorescence and flow cytometry is used to determine the origin of the peritoneal and marrow cells whilst immunohistochemistry and ELISA is used to examine osteogenic markers.

Summary of Results and Conclusions: Expanded bone marrow cultures contain a pool of mesenchymal stem cells that is capable of differentiation into cells of the osteoblast lineage. However, given the problem of access to marrow cells we have been seeking alternate sources of cells with comparable plasticity. Recent work by our colleagues (1) has shown cells that coat foreign bodies in the peritoneum can be induced to become myofibroblasts and to further differentiate into blood vessels when transplanted into an arterial bed. We are keen to test whether this plasticity will extend to the differentiation of osteoblastic cells. A variety of osteogenic markers have been used to characterise these cells after their exposure to osteogenic supplements, with promising results.

1. Campbell, J. H., Efendy, J. L., and Campbell, G. R. (1999) *Circ Res* 85(12), 1173-8.

P17

DOES HAVING BONE MASS IN THE LOWER TERTILE OF A HEALTHY POPULATION OF FEMALE TWINS EFFECT THE WITHIN-PAIR DIFFERENCE IN BONE MASS ASSOCIATED WITH PREGNANCY.

L. Paton¹, C. Nowson², J. Alexander¹, C. Margerison¹, M. Frame¹, B. Kaymakci¹, and J.D. Wark¹. ¹University of Melbourne, Bone and Mineral Service Royal Melbourne Hospital, Melbourne, Australia, ² School of Health Science, Deakin University, Melbourne

There is uncertainty about the long term effects of pregnancy and lactation on measures of bone mineral in healthy women. Women with low bone mineral density (BMD) may be at even greater risk of bone loss in this situation. The aim of this investigation was to examine twin pairs with average BMD in the lowest tertile from a population of healthy Australian female twins, age greater than 18 years who were 1) discordant for ever being pregnant or 2) differed in their absolute number of pregnancies.

Study 1 consisted of 83 female twin pairs mean age, 42 years (range 18-88 years); study 2 consisted of 498 female twin pairs, mean age 42 years (range: 18-89 years). BMD at the lumbar spine (LS) and total hip (HP) and total body (TB) bone mineral content (BMC) were measured (QDR 1000W). The number of pregnancies, breast feeding history and calcium intake were obtained by questionnaire. The mean LS BMD was calculated for each pair and the population divided into tertiles based on this mean value. Within-pair differences (WPD) were calculated for each tertile.

Study 1: In the tertile with the lowest mean LS BMD there was a significant within-pair difference in TB BMC (8.5%, $p = 0.03$) and total body fat (18.3%, $p = 0.03$) with the ever pregnant individual having high TB BMC and fat mass. There was no WPD in height but a marginal difference in weight (6.9%, $p = 0.05$). After adjustment for age, lean and fat mass, there was no WPD in TB BMC. Study 2: Similarly, there was a significant WPD in TB BMC (2.8%, $p = 0.02$) and total body fat (6.8%, $p = 0.04$). After adjustment for age and lean the WPD in TB BMC (1.0%, $p = 0.04$) remained. After adjustment for age, lean and fat mass, no WPD was seen. There was no WPD in height or weight. No within-pair differences were observed in the 2nd or 3rd tertiles for either population for any measures of regional BMD, BMC or body composition.

In this investigation of BMD in the lower tertile of a healthy population of females, history of pregnancy was associated with higher unadjusted TB BMC and total body fat mass. After adjustment for fat the difference in TB BMC was no longer evident.

P18

PREGNANCY AND LACTATION HAVE NO LONG TERM EFFECTS UPON BONE DENSITY IN A HEALTHY POPULATION: A TWIN STUDY.

L. Paton¹, C. Nowson², J. Alexander¹, C. Margerison¹, M. Frame¹, B. Kaymakci¹, and J.D. Wark¹. ¹University of Melbourne, Bone and Mineral Service Royal Melbourne Hospital, Melbourne, Australia, ² School of Health Science, Deakin University, Melbourne

Pregnancy and lactation impose a high calcium demand upon the body. Do these extra stresses have long term effects upon bone density? To investigate this question we investigated whether there was a difference in bone mineral density (BMD) between female twin pairs, age greater than 18 years who were 1) discordant for ever being pregnant or 2) differed in their absolute number of pregnancies.

Study 1 consisted of eighty-three female twin pairs (21 MZ and 62 DZ) mean age, 42 years (range 18-88 years); study 2 consisted of 498 female twin pairs, mean age 42 years (range: 18-89 years). All subjects were at least 6 months post-partum. BMD at the lumbar spine (LS) and total hip (HP), and total body (TB) bone mineral content (BMC) were measured (Hologic QDR 1000W). The number of pregnancies beyond 20 weeks, breast feeding history, calcium intake, and calcium supplementation during pregnancy and/or breast feeding were obtained by questionnaire. Analysis of the within-pair differences and between group differences were performed. (SPSS). Study 1: No significant within-pair differences in BMD at the LS, TH or in TB BMC (paired t-tests) in twins discordant for ever being pregnant. When pairs were divided according to the number of pregnancies of the ever pregnant twin (1, 2, or 3 or more), there was no difference in BMD or BMC (ANOVA). There was no difference in BMD with the total number of months spent breast feeding. Study 2: No within-pair difference in HP BMD, LS BMD or TB BMC was observed in 498 twin pairs related to the absolute difference in number of pregnancies. Pairs were grouped according to the absolute difference in the number of pregnancies (range: 0 – 6). Subjects were also divided into three groups: equal number of pregnancies, different by 1 or 2 pregnancies, or different by 3 or more. No differences were observed in BMD/BMC, adjusted for age and lean mass.

No long term effect of either pregnancy or breast feeding on bone density was observed in healthy female twins.

P19

HRT, BMD AND ABDOMINAL FAT: A CO-TWIN ANALYSIS

C Margerison, L Paton, B Kaymakci, L Lan, C Nowson, and JD Wark. University of Melbourne, Dept of Medicine, Royal Melbourne Hospital.

Hormone replacement therapy (HRT) has been shown to improve bone density but its affect on body composition is not well defined.

Our objective was to determine if HRT use affects body composition, in particular the distribution of fat in the abdominal area.

Forty female twin pairs were identified, where one twin had more than 3 months of current continuous HRT use and the other had no exposure to HRT. The mean duration of HRT use was 53 months. Bone mineral density (BMD) was measured (Hologic QDR 1000W) at the lumbar spine (LS) and 1/3 distal radius (FA). Fat mass and lean mass were calculated using Hologic software for the total body, limb and trunk regions. Central abdominal fat (AF) was measured in the region from the upper edge of L2 to the lower edge of L4, and laterally to the inner aspect of the rib cage.

Within-pair t-tests were performed using SPSS. There were no significant weight, height or BMI differences between the HRT users and non-users. LS BMD and FA BMD were significantly greater (7.2% and 4.7% respectively, $p < 0.05$) in HRT users compared to non-users. There were no significant within-pair differences in fat or lean mass at any of the regions measured (as described above).

When the within-pair absolute difference in total body fat was plotted against the within-pair absolute differences in LS BMD and FA BMD, marginal associations were observed (LS $p = 0.06$; FA $p = 0.05$). When total fat was broken down into specific regions, marginal associations were still present between the within-pair absolute difference in trunk fat and the within-pair absolute difference in LS BMD ($p = 0.03$) but not FA BMD ($p = 0.11$). Strong associations were observed between the within-pair absolute differences in LS BMD and FA BMD and the within-pair absolute differences in both measures of AF (total AF: LS $p = 0.001$ FA $p = 0.02$; percentage AF: LS $p = 0.008$, FA $p = 0.03$). The within-pair absolute difference in limb fat was associated with the within-pair absolute difference in FA BMD ($p = 0.03$) but not LS BMD ($p = 0.15$).

The strong association between the within-pair difference in abdominal fat and the within-pair difference in LS BMD may help to explain the within-pair difference in LS BMD in HRT discordant twins. Further investigations are needed to increase understanding of the mechanisms of associations between body fat and bone mineral density.

P20

HORMONE REPLACEMENT THERAPY AND BONE MINERAL DENSITY: A CO-TWIN ANALYSIS.

C Margerison, L Paton, B Kaymakci, C Nowson, and JD Wark. University of Melbourne, Dept of Medicine, Royal Melbourne Hospital, Australia.

Hormone replacement therapy (HRT) has been shown to improve bone density in clinical trials but its effect in routine clinical practice is less well defined. Our objective was to estimate the difference in measures of bone mass at clinically relevant sites within female twin pairs discordant for HRT use, using a co-twin model.

Forty female twin pairs were identified, where one twin had more than 6 months of current continuous HRT use and the other had no exposure to HRT. The mean duration of HRT use was 53 months (range 6 to 276 months). Bone mineral density (BMD) was measured (Hologic QDR 1000W) at the lumbar spine (LS), total hip (TH), femoral neck (FN), 1/3 distal radius (FA). Total body (TB) bone mineral content (BMC) was also measured and total fat and lean mass calculated using Hologic software. HRT use, calcium intake, physical activity and lifetime smoking were determined by questionnaire.

Within-pair differences were calculated and expressed as absolute differences and percentage differences. Adjustments were made for age, lean mass and fat mass. TB BMC was also adjusted for height. Within-pair t-tests were performed using SPSS.

There were no significant within-pair differences in weight, height BMI, fat mass or lean mass with HRT use. LS BMD and FA BMD were significantly greater (7.2%, $p < 0.02$ and 4.7%, $p < 0.05$ respectively) in HRT users compared to non-users. After regional BMD was adjusted for age, lean and fat mass, the significant within-pair differences remain (LS BMD 7.1%, $p < 0.02$; FA BMD 2.9%, $p < 0.05$). The within-pair difference in TB BMC approached significance (3.5%, $p < 0.1$) when adjustments were made for height (a known univariate predictor of TB BMC). There was no significant effect of HRT duration on any of the sites measured.

These results suggest that HRT in routine clinical use significantly protects against menopausal bone loss at the lumbar spine and forearm in keeping with randomised clinical findings.

P21

VARIABILITY IN BONE MINERAL ACCRUAL OVER TWO YEARS IN 3 TO 6 YEAR OLD GIRLS: ASSOCIATIONS WITH BODY COMPOSITION AND LIFESTYLE. RW Taylor, IE Jones, A Goulding. Departments of Human Nutrition and Medical and Surgical Sciences, University of Otago, PO Box 56, Dunedin, New Zealand.

Maximising bone mineral accrual during growth may help prevent osteoporosis in later life. Although the acquisition of bone mass is maximal during puberty, variation in bone gain in younger children may also be important. The dietary calcium intake (validated food frequency questionnaire), physical activity level (questionnaire) and bone development (dual energy x-ray absorptiometry, Lunar DPX-L) of 39 girls aged 3 to 6 years was measured annually for two years. Two-year gain in total-body bone mineral content (BMC, g) ranged from 135-325g (9-26% of baseline values). The median gain in BMC once adjusted for age, height, weight and bone area (multiple regression) was 204g. Girls who gained more BMC (> median) had similar age, height and weight as girls who gained less BMC (\leq median), but had greater BMC (mean \pm SD, 618 ± 83 vs 555 ± 91 , $P < 0.05$), bone area (800 ± 83 vs 734 ± 97 , $P < 0.05$) and lean tissue mass (15.0 ± 1.6 vs 13.7 ± 1.8 , $P < 0.05$) at baseline. In addition, these girls gained more body weight (5.4 ± 1.8 vs 4.5 ± 0.8 , $P < 0.05$) and more lean tissue mass (4.0 ± 0.6 vs 3.5 ± 0.6 , $P < 0.05$) over the two year study, and their parents rated them as more physically active (5-point Likert scale; 3.7 ± 0.6 vs 3.2 ± 0.6 , $P < 0.05$). Girls with consistently high calcium intakes did not appear to gain more bone mineral (212 ± 43 vs 212 ± 35 g, $P > 0.05$) than girls with lower calcium intakes. In conclusion, considerable variability in bone mineral accrual was observed in young prepubertal girls. Girls who gained more bone tended to have more bone at baseline, gain more lean tissue mass and were rated as more physically active by their parents (Grant support: Otago Medical Research Foundation).

P22

IS THE MUSCLE-BONE INTERACTION DEPENDENT ON STAGE OF PUBERTY? *RM Daly, *L Saxon, †C Turner, †A Robling, *S Bass *School of Health Sciences, Deakin University, Melbourne. †Biomechanics and Biomaterials Research Center, Indiana University, USA.

Growth is associated with large increases in bone mass, size, strength, muscle mass and muscle strength. Since muscles exert the largest voluntary loads to bones, muscle mass should be a primary determinant of BMC, bone size and bone strength. The aim of this study was to examine the relationship between muscle mass and BMC, bone size and bone strength during different stages of puberty. We studied the non-playing arm of 17 pre-, 11 peri- and 19 post- pubertal (age menarche 12.5 ± 0.3 yrs) female tennis players (age 10.5 ± 0.3 , 12.2 ± 0.3 and 14.3 ± 0.4 yrs respectively). Average total and cortical bone areas (TotAr and CortAr), muscle area (MuAr), and bone strength [polar second moment of area I_p (mm^4)] were determined at a site 30-60% of the humeral length from the distal end by MRI; BMC was assessed by DXA. Cortical BMD (g/mm^3) = BMC/total volume. All muscle and bone indices increased with advancing age ($r=0.58$ to 0.77 , $p < 0.001$). BMC, TotAr, CortAr, cortical BMD and I_p were linearly associated with MuAr ($r=0.59$ to 0.91 , $p < 0.001$). These muscle-bone relationships were similar (no slope differences) for pre-, peri- and post- pubertal girls. To assess the maturity related changes in bone length, BMC, bone size, strength and muscle mass, data was expressed relative to predicted peak young adult values. As shown in the Table, bone and muscle traits matured at different rates and followed the temporal sequence: length, TotAr, MuAr, bone strength and BMC.

% from predicted peak	Pre-pubertal	Peri-pubertal	Post-pubertal
Length	84 ± 1.5^a	91 ± 1.6^a	99 ± 1.4^a
Total area	74 ± 2.1^b	83 ± 3.4^b	90 ± 2.5^e
Muscle area	68 ± 1.7^c	77 ± 3.3^d	92 ± 3.3^e
Bone strength (I_p)	52 ± 2.9	65 ± 5.0	83 ± 4.0
BMC	49 ± 2.8	62 ± 3.7	90 ± 3.6

^a $p < 0.07$ to < 0.001 vs TotAr, MuAr, I_p , BMC; ^b $p < 0.05$ to < 0.001 vs MuAr, I_p , BMC; ^c $p < 0.001$ vs I_p , BMC; ^d $p < 0.001$ vs BMC ^e $p < 0.01$ vs I_p

Despite differences in the pattern of growth for bone mass, size, strength and muscle mass, the intrinsic relationship between these parameters was maintained during different stages of maturation.

P23

PHYSICAL ACTIVITY AND BONE STRENGTH AT THE PROXIMAL FEMUR DURING THE ADOLESCENT GROWTH SPURT. MR Forwood, DA Bailey, TJ Beck, RL Mirwald, T Wallace, TL Oreskovic. School of Biomedical Science, The Univ of Queensland, University of Saskatchewan, and John Hopkins University.

To investigate the influence of physical activity on bone strength during adolescence, we analysed 7 years of longitudinal data from 70 boys and 68 girls who were part of a longitudinal study monitoring bone mineral and bone geometry changes in healthy, growing children. Physical activity data and anthropometric measurements were taken every 6 months and DXA bone scans were measured annually (Hologic QDR2000, array mode). On hip DXA scans, we used "Hip Strength Analysis (Beck et al., Invest Radiol. 1990, 25:6-18) to estimate structural determinants of bone strength including the section modulus at the femoral neck. Distance and velocity curves for height were fitted for each child using a cubic spline, and the age of peak height velocity (PHV) was determined. To control for maturational differences between children of the same chronological age, section modulus values were determined for points on each individual's curve at the age of PHV and one and two years on either side of peak. Subjects were categorised into activity groupings using all their physical activity assessments as measured by the (PAC-Q) physical activity inventory score. This was transformed into an age specific mean Z-score. On the basis of mean Z-score ranking, subjects were compared between the most and least active activity quartiles. There were no statistical differences between these activity groups in terms of attained height and weight at the age of PHV.

Two way analyses of variance revealed significant ($P < 0.05$) activity and maturational age effects in both boys and girls. These results suggest that a modifiable lifestyle factor like physical activity may play an important role in the optimisation of bone strength at the proximal femur during the adolescent years.

P24

EVIDENCE OF ASSOCIATION AND LINKAGE DISEQUILIBRIUM BETWEEN ESTROGEN RECEPTOR α GENE POLYMORPHISM AND BONE MINERAL DENSITY IN FEMALES

R Sapir-Koren, G Livshits, S. Trofimov E Koblyansky. Anatomy and Anthropology Department, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978, Israel.

This study investigated existence of association and linkage disequilibrium between radiographic hand BMD and ER α gene polymorphism, in human pedigrees of a European population, Chuvasha, living in Russia. Similar environmental conditions and minimal genetic flow characterize this ethnically Caucasian population. The study sample included 463 members of 113 pedigrees, mostly nuclear families. We performed association and linkage analysis of the combination of PvuII and XbaI RFLPs (haplotype) of ER α gene and BMD Z score values of trabecular or cortical bone at the hand 3rd finger. The analyses have been performed separately for both genders and for both generations, forming 4 groups (n; mean age): "fathers" (114; 64.2y), "mothers" (112; 62.7y), "sons" (125; 33.0y) and "daughters" (102; 33.4y). The Px haplotype was associated significantly with lower BMD Z scores in "mothers" only. The difference between subjects who carry one or two copies of the Px haplotype (n=24) and those lacking it (n=98), is 0.68 Z scores, p= 0.003 and 0.51 Z scores, p= 0.025 for trabecular and cortical bone, respectively. Multiple linear regression model with age, height weight, and Px haplotype status as predictors, explains 26.7% and 28.3% of the total observed variance in BMD, with Px haplotype as independent predictor explaining 5.9%; p= 0.002 and 3%; p= 0.028 (trabecular and cortical bone, respectively). Using t-TDT for trios of two parents and just one of their female offspring (but not male offspring), suggested the existence of linkage disequilibrium between the two loci of Px haplotype and of BMD trait (p= 0.047). We conclude that the Px haplotype of the ER α gene is associated and in linkage disequilibrium with low BMD values in females, as the phenotype is gender dependent (not observed in males).

P25

PREVALENT APPENDICULAR FRACTURES IN ELDERLY WOMEN WITH NORMAL DEXA ARE ASSOCIATED WITH LOW ULTRASOUND MEASUREMENTS

A Devine, A Marangou, SS Dhaliwal, RL Prince Dept. Medicine, University of Western Australia and Dept. Endocrinology & Diabetes, Sir Charles Gairdner Hospital, Perth, Western Australia.

The pathophysiology and aetiology of multiple fractures in people with normal DEXA bone density (BMD) has not been well investigated. The aims of this study were to examine the factors that may be associated with multiple appendicular fractures in women with normal DEXA BMD.

The study group consisted of 204 women aged 75 ± 2 y (mean \pm SD) who had been assigned a Total Hip BMD T score -1.0 and classified according to prevalent fracture status: 0/1 fracture (n=179) and ≥ 2 fractures (n=25).

Calcaneum QUS (Lunar Achilles) and DEXA (Hologic 4500A) BMD at the hip and bone turnover markers (osteocalcin, alkaline phosphatase and urinary deoxypyridinoline/creatinine ratio) were measured. Age at menopause, average daily calcium and alcohol intake, smoking history and body mass index (BMI) were ascertained. Significant differences between the fracture groups were determined by Student's t test.

BMD at Total Hip and Femoral Neck and bone turnover markers were not different between the two groups. There was no difference between the two groups for age at menopause, calcium and alcohol intake, years smoked and BMI. QUS measures were significantly lower in the multiple fracture group, by approx 1 SD, compared to those in the none or one fracture group (SOS 1510 ± 24 versus 1529 ± 24 m/s, $P < 0.01$; BUA 102 ± 8 versus 106 ± 7 db/MHz, $P < 0.05$; stiffness 71 ± 11 versus 79 ± 10 %, $P < 0.05$).

We conclude that patients with prevalent appendicular fractures with normal DEXA BMD may have qualitative differences in bone that can be detected by QUS.

P26

CAUSATION OF AGE RELATED INCIDENT FRACTURE: THE ROLE OF ESTROGEN.

A Devine, SS Dhaliwal, IM Dick, A Marangou, R Naheed, RL Prince. Dept. Medicine, University of Western Australia and Dept. Endocrinology & Diabetes, Sir Charles Gairdner Hospital, Perth, Western Australia.

Bone loss is a natural consequence of aging and is accelerated in women after menopause as a result of a decline in circulating estrogen (E2) levels. The aim of this study was to examine the effect of endogenous E2 levels in elderly women and its relation to incident fracture and bone parameters as determined by DEXA.

The study group consisted of 1499 women enrolled in a 5-year trial of the effects of calcium on fracture outcome, who were randomly recruited from the population over the age of 70 using the electoral roll. They were aged 75y and weighed 69kg. At two years 8% had sustained one incident fracture. Serum E2 27 ± 14 pmol/L was measured using sensitive RIA with a detection limit 5 pmol/L (Orion Diagnostica). One-year hip BMD was measured using DEXA (Hologic 4500A). Z scores were calculated for BMD and E2. Treatment was coded as placebo or calcium. Incident clinical fractures were determined from x-ray reports after the event.

In univariate logistic regression analysis incident fracture after adjustment for age, weight and treatment, was predicted by E2 (Relative Risk per SD below the mean, 1.37: 95% CI 1.08-1.76), BMD at the hip (1.47: 1.11-1.19), neck (1.55: 1.17-2.05), trochanter (1.37: 1.05-1.80), and intertrochanter (1.39: 1.05-1.83). In multivariate logistic regression, using incident fracture as the response variable and BMD and E2 as independent variables, E2 (1.43: 1.04-1.97) was still predictive of incident fracture after hip BMD (1.43: 1.05-1.94) and adjustment for BMD at all other sites, age, weight and treatment.

It appears that E2 predicts incident fracture after adjustment for hip BMD. These data confirm the importance of postmenopausal E2 concentration in the pathogenesis of osteoporosis in elderly women.

P27

THE DETERMINANTS OF BONE DENSITY IN NORMAL POSTMENOPAUSAL WOMEN- A 10-YEAR PROSPECTIVE STUDY. F Wu, R Ames, J Clearwater, MC Evans, G Gamble, IR Reid. Department of Medicine, University of Auckland, Auckland.

Osteoporosis is common in postmenopausal women, and as the population ages, it becomes increasingly so. We prospectively assessed bone mineral density (BMD), fracture rates and the factors determining the rate of bone loss over a 10-year period of postmenopausal life.

104 normal white postmenopausal women (baseline age 59 ± 5 yrs) completed the study (follow-up 10.2 ± 0.2 yrs). None had diseases or were on medications affecting bone metabolism at entry. Their medical, fracture, and lifestyle history were recorded. Body composition and BMD was measured by dual-energy x-ray absorptometry at baseline and 10-years. Biochemical, haematological, and hormonal analyses were performed.

24% of women started hormone replacement therapy (HRT) during the follow-up period, with most remaining on therapy at follow-up. The mean duration of use was 6.6 ± 1.7 yrs. The use of HRT was associated with significant gains in BMD and reduction in fracture risk (standardised risk ratio for vertebral fractures 0.42 [CI: 0.18 – 0.83]). The baseline and 10-year BMD in the non-HRT users were highly correlated ($0.82 < r < 0.91$, $P < 0.0001$), accounting for the majority of the variance of the follow-up BMD. Multivariate analyses showed that independent correlates of changes in BMD were weight and fat mass (baseline values and changes during follow-up), time since menopause, sex hormones, urinary calcium loss, parathyroid hormone levels and haemoglobin concentrations.

We conclude that BMD is highly predictable over an extended period in normal postmenopausal women, so does not require frequent measurement. Maintenance of body weight and good health reduces bone loss. HRT is an effective treatment and long-term compliance is achievable.

P28

The effects of exercise detraining on postmenopausal bone loss, two years after completing a strength training intervention.

DA Kerr¹, I Dick², TR Ackland², S Cummings², RL Prince². ¹Curtin University, Perth, ²The University of Western Australia, Perth AUS.

It is a commonly held view that the benefits of exercise on the skeleton can only be maintained while the person remains physically active. The aim was to investigate the effects of exercise detraining on BMD two years after postmenopausal women completed a randomised exercise intervention in which subjects were assigned to one of three groups: a strength (S), fitness (F) or non-exercise control (C) for the 2-year intervention. On completion of the study, all subjects had been advised to take at least 1 g of calcium per day and undertake either weight-bearing exercise or strength training three times per week. Eighty of the 90 women who completed the two-year exercise intervention study, returned for follow-up bone density testing after two years. The % change from baseline at 4 years and from 2 to 4 years were as follows:

% change BMD	Strength		Fitness		Control	
	at 4y	2-4y	at 4y	2-4y	at 4y	2-4y
Total hip	0.2±4.5*	-0.9±4.6	-0.4±3.9	0.2±4.0	-1.6±2.7	-0.7±2.8
Intertroch.	-1.2±4.8	-0.8±5.0	-0.5±4.9	0.9±4.5	-2.9±2.9	0.0±4.0
Troch.	-0.1±5.4	-0.2±5.7	0.1±4.3	-0.1±4.9	-0.8±3.6	-0.9±4.0
Neck	-1.2±4.8	-2.7±4.8	-0.5±4.9	-1.0±4.4	-2.9±2.9	-2.8±2.8

* $p < 0.05$

Using the baseline BMD as a covariate the data was analysed by repeated measures ANOVA. A significant exercise effect was observed at completion of the study at the total hip site in the strength group compared to control group ($p = 0.038$). The data was also analysed by whether subjects were taking a calcium supplement (>600 mg) and undertaken either weight-bearing or strength training. There was a significant interaction ($p < 0.05$) observed in subjects who were taking calcium and exercising but no effect observed separately for either calcium or exercise. These results suggest there was only a small residual benefit from the 2 year intervention for the strength group at the total hip site. There was an interaction between calcium and exercise in slowing postmenopausal bone loss, postmenopausal bone loss, reinforcing the importance of this public health message.

P29

THE EFFECTS OF EXERCISE INTERVENTION ON MARKERS OF BONE TURNOVER IN POSTMENOPAUSAL WOMEN.

A Randall¹, DA Kerr², TR Ackland³, N Kent¹, RL Prince³. ¹Path Centre, Perth, Western Australia. ²Curtin University, Perth, Western Australia. ³Dept of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Perth, Western Australia.

A two year strength training regime has previously been shown to increase hip BMD in postmenopausal women(1). We now evaluate the changes in bone turnover markers. Subjects were randomized into a strength (S), fitness (F) or non-exercise control (C) groups and were all calcium supplemented (600 mg). Fasting morning urine and serum samples were taken at 0 (enrollment) 6, 12, 18 and 24 months. Serum N-Mid Osteocalcin (OC) and beta Crosslaps (CTX) were analysed on the Roche Elecsys 2010 immunoassay analyser. Serum Bone Alkaline Phosphatase (BALP) was analysed with Metra Biosystems Alkphase-B kits. Urine Total Deoxypridinoline was analysed by our HPLC method (2) with results expressed as a ratio to creatinine (Dpd/Cr).

Initial analysis showed that the pattern of change for each marker over the trial period was almost identical for each subject group and that group differences at each time point for each analyte were minor. Group data was then pooled for further evaluation. OC and Dpd/Cr decreased at 6 and 24 months, BALP was increased at 24 months and CTX decreased at 6 months and then increased at 24 months.

For each marker mean (sem) at 0, 6 and 24 months is shown. For difference from 0 months; not significant = ns; $p < 0.01 = **$; $p < 0.05 = *$.

OC (ng/mL) : 30.5 (1.1); 26.0 (0.9)**; 27 (1.0) ns.

BALP (U/L) 15.8 (0.5); 15.3 (0.4) ns; 19.6 (0.6) **.

DPD/Cr (mmol/mol) 37.2 (2.5), 28.7 (1.1)**; 24.3 (0.8) **.

CTX (ng/mL) 0.33 (0.02); 0.25 (0.01)*; 0.42 (0.02)**.

Different exercise regimens did not cause notable differences in the bone markers. The changes over time seen in the markers may be due to calcium supplementation. The discordance between CTX and Dpd/Cr requires further investigation.

(1) Kerr et al. 2001. JBMR, 16:175-181

(2) Randall et al 1996 JBMR 8:175 181

P30

EARLY RETENTION OF INFUSED PAMIDRONATE IN PAGET'S DISEASE CORRELATES WITH BONE REMODELLING ACTIVITY.

SD Vasikaran*, DH Gutteridge, LC Ward, S Dhaliwal, PHR Barrett, ED Horgan*, RW Retallack, RL Prince, C Bhagat, J Walsh. Department of Core Clinical Pathology and Biochemistry*, Royal Perth Hospital; Department of Endocrinology, Sir Charles Gairdner Hospital; Department of Medicine, UWA; Perth, Australia.

This study was undertaken to examine retention of infused pamidronate (APD) in Paget's disease, and its association with: height, weight, BMI, serum creatinine, alkaline phosphatase (ALP), osteocalcin (OC) and fasting urine deoxypridinoline (DPD/Cr). Urine is the only route of excretion of infused bisphosphonates with most of the drug excretion occurring in the first 24 hours. The retained drug is mainly deposited at the resorption surfaces of bone.

We studied 11 patients with active Paget's disease who were treated with APD 60mg infusion over 4 hours. 24-hour urine was collected from the start of the infusion. APD was measured in urine by HPLC and fluorescent detection. APD excretion in the 24 hours varied from 3.2 mg-26.8mg in the 11 patients.

In univariate analysis (Pearson Correlation), APD retention was significantly correlated with DPD/Cr ($r = .67$, $p = 0.02$), and OC ($r = .68$, $p = 0.02$). There was no correlation with creatinine or ALP. In multivariate analysis OC accounted for 46% of the variation in APD retention and DPD/Cr accounted for 15% of the variation. Based on available models, a significant amount of APD is estimated to be retained in the body for many years.

The correlation of APD retention with DPD/Cr suggests that more drug is retained with increased bone resorption. Although OC is not generally considered to be a clinically useful marker in Paget's disease, its correlation with APD retention suggests that OC may possibly reflect a hitherto unrecognised aspect of pagetic activity.

P31

THE PARADOX OF INCREASED FEMORAL NECK SIZE IN WOMEN WITH HIP FRACTURE.

Y Duan, X-F Wang, J Edmonds, E Seeman. Department of Endocrinology, Austin and Repatriation Medical Centre, University of Melbourne, Melbourne, 3084, Australia

Longer hip axis length and reduced volumetric bone mineral density (vBMD) are reported to be independent determinants of hip fracture risk. As smaller bone size is also likely to be associated with increased fracture risk we hypothesized that women with hip fracture have reduced femoral neck size and reduced vBMD. We studied 200 women with hip fracture aged 74.4 years and compared them with 191 women with spine fractures aged 69.5 years, 45 women with both spine and hip fractures aged 74.7 years, and 801 healthy women aged 18 to 92 years. Bone width, volume and vBMD of the third lumbar vertebra and femoral neck were measured by using dual-energy x-ray absorptiometry. The results were expressed as absolute values and standard deviation (SD) or Z scores (mean \pm sem). Vertebral and femoral neck width increased with age in healthy women and being 0.3 SD higher in elderly than young women. Women with hip fracture had larger femoral neck width (0.74 ± 0.09 SD, $p < 0.001$) but normal vertebral width (-0.10 ± 0.10 SD, NS) relative to age-matched healthy controls. Patients with spine fractures also had increased femoral neck width (0.29 ± 0.08 SD, $p < 0.01$) but reduced vertebral width (-0.24 ± 0.07 SD, $p < 0.01$), while patients with both spine and hip fractures had increased femoral neck width (0.67 ± 0.21 SD, $p < 0.01$) and non-significantly reduced vertebral width (-0.21 ± 0.19 SD, $p = 0.26$). vBMD was more greatly reduced at the femoral neck than at the vertebra (-0.90 ± 0.06 vs -0.28 ± 0.08 SD, $p < 0.001$) in patients with hip fractures. Patients with spine fractures had greater vBMD deficits at the vertebra than at the femoral neck (-0.91 ± 0.06 vs -0.53 ± 0.06 SD, $p < 0.001$). Despite patients with hip fracture being 5 years older, spine fracture cases had greater deficit in vertebral vBMD than hip fracture cases (-0.91 ± 0.06 vs -0.28 ± 0.08 SD, $p < 0.01$). We infer that patients with fractures have bone fragility with a heterogeneous structural basis.

P32

STATIC HISTOMORPHOMETRY OF FEMORAL NECK BONE IN ELDERLY MEN WITH HIP FRACTURE OR OSTEOARTHRITIS.

DN Edis, PR Ebeling, BL Grills. Departments of Surgery (Orthopaedics), and Diabetes and Endocrinology, University of Melbourne, Royal Melbourne Hospital, Parkville and School of Human Biosciences, Department of Human Physiology and Anatomy, La Trobe University 3086, Victoria, Australia.

By 2010, 30% of all hip fractures will occur in men. Local factors may be important in the pathogenesis of fractures. Few studies have examined the microscopic changes occurring at the fracture site. We biopsied bone adjacent to the fracture site in 11 men with acute hip fracture and in 8 men with osteoarthritis having elective hip arthroplasty. Bone biopsies from the femoral neck were fixed and embedded in LR Gold resin as described previously by our group (1).

We used standard sectioning, staining and measurement techniques to estimate trabecular volume, mean wall width (MWT), osteoid volume, osteoid thickness and osteoid surface. Two patients with evidence of osteomalacia were excluded from further analyses. The mean data for each group were compared and analysed by Student t-tests. Men with hip fracture had reduced trabecular bone volume (mean) 16.39% vs 20.3% ($p = 0.04$) and lower MWT, 41 μ m vs 48 μ m ($p = 0.058$). The reduced MWT suggests that the imbalance in bone remodelling that underlies this condition could in part be accounted for by reduced bone formation. Osteoid volume, osteoid thickness and osteoid surface were similar in each group.

In conclusion, men with osteoporosis and hip fracture have evidence for lower bone formation at the fracture site.

1. Edis *et al.*, 1999. Proc. ANZBMS 9:83.

P33

VITAMIN D DEFICIENCY IS COMMON AND UNRECOGNISED AMONG RECENTLY- ARRIVED ADULT IMMIGRANTS FROM THE HORN OF AFRICA.

JYY Ngeow¹, SA Skull¹, BA Biggs¹, A Street¹, PR Ebeling², Victorian Infectious Diseases Service¹ Dept Diabetes & Endocrinology², The Royal Melbourne Hospital, Parkville, Victoria.

Persons with dark skin and reduced sun exposure are at particular risk of osteomalacia. We therefore examined the prevalence of Vitamin D deficiency in this new Melbourne community from the Horn of Africa.

A cross-sectional community survey was conducted from two Melbourne community health centres during 2000. Quantitative determination of 25-hydroxyvitamin D (25(OH)D) was performed using the DiaSorin[®] (Minnesota, USA) 25-OHD 125 I radioimmunoassay kit. Religion and skin coverage were used as indicators of sun exposure.

25(OH)D serology was performed in 116 participants. More than half (n= 61, 53%) had suboptimal levels (< 25 nmol/L), representing 74% of participating women and 20% of participating men. Two thirds of affected women were of childbearing age. All 23 (20%) participants with levels less than 15 nmol/L were women. Lower 25(OH)D levels were more likely in those who reported being mostly covered when outdoors (RR=3.4; 95%CI 2.0, 5.9); Muslims (RR=6.2, 95%CI 1.6, 23.1) and those with a longer duration of residence in Australia (RR=2.0; 95%CI 1.4,2.8).

Adult immigrants from the Horn of Africa have a high prevalence of Vitamin D deficiency. This is likely to be related to skin pigmentation, the cultural practice of largely covering the skin with clothing, and lifestyle changes associated with reduced sun exposure in Melbourne. Women are at particular risk, but men are also affected. These findings also have implications for the children of these families. Routine screening for Vitamin D deficiency should be considered for all immigrants from the Horn of Africa, particularly those who are predominantly shielded from sunlight by clothing.

P34

A NOVEL APPROACH TO ESTIMATING THE PREVALENCE OF OSTEOPOROSIS IN THE FRAIL ELDERLY.

Zochling J¹, March L¹, Cumming RG², Cameron ID³, Sambrook PN¹.

¹Institute of Bone and Joint Research, University of Sydney. ²Department of Public Health, University of Sydney. ³Rehabilitation Studies Unit, University of Sydney.

Osteoporosis in the institutionalised elderly has not been well studied despite a high fracture incidence. The 'gold standard' (DEXA) measurement is impractical, but calcaneal ultrasound measures using the CUBA (McCue) portable ultrasound machine is promising. The aim was to examine the use of a standardised ultrasound t-score using an approximation from population DEXA t-scores.

The CUBA was used to measure broadband ultrasound attenuation (BUA) and velocity of sound (VOS) at the calcaneus in 634 female residents of hostels and nursing homes over 65 years of age. Normal population values were obtained from 936 normal volunteers aged 20-80 for BUA and VOS and for BMD at the neck of femur (measured by DEXA). These values were converted to t-scores, and regression equations calculated for each modality. Equations were then determined for the conversion of BUA and VOS t-scores to their equivalent DEXA bone mineral density score at the neck of femur. A t-score at the hip of -2.50 was found to be equivalent to a BUA t-score of -1.77, and a VOS t-score of -2.59.

Using these levels as our definition of osteoporosis we found that 72% of our elderly population were classed as osteoporotic by BUA, and 81% by VOS. Different modalities of measuring BMD have different thresholds for defining osteoporosis, but in this population, ultrasound seems to be a reasonable approximation to DEXA at the hip. The predictive value of these t-scores will be tested during follow-up of this cohort.

P35

CLINICAL CORRELATES OF *RUNX2* ALLELES IN OSTEOARTHRITIS PATIENTS.

T Vaughan¹, PA Cheras² and NA Morrison^{1,1} Genomics Research Centre, Griffith University Gold Coast. ² Greenslopes Private Hospital, 4120, Australia.

Runt-related gene 2 (*RUNX2*)/core binding factor A1 (*CBFA1*) is a transcription factor that regulates osteoblast and chondrocyte differentiation. Over expression of *RUNX2* in chondrocytes of mice leads to accelerated chondrocyte maturation and endochondral ossification. *RUNX2* deficient mice show a complete lack of both endochondral and intramembranous ossification and display a retardation of chondrocyte differentiation and an absence of osteoblast differentiation. The aim of this study was to determine if DNA polymorphism within *RUNX2* was a determinant of serum measures relevant to osteoarthritis. *RUNX2* contains a glutamine-alanine repeat motif that acts as one of three transactivation domains in the protein. Within the repeat unit, we detected two common variants: an 18 base pair deletion and single nucleotide polymorphism (SNP) with alleles GCA and GCG.

Genotypes were determined on 84 osteoarthritis patients who had multiple repeated measures of serum variables. Statistically significant differences in osteocalcin (OC) and C-reactive protein (CRP) and TNF-alpha serum levels were observed between different genotype groups. *RUNX2* is known to bind in the osteocalcin promoter, suggesting a direct effect of genetic polymorphism on the serum levels of osteocalcin.

P36

ACUTE EFFECT OF MILK ON BIOCHEMICAL MARKERS OF BONE RESORPTION.

JH Green, CL Booth, RLW Bunning, P Pearce, J Yeo and T Walmsley Milk and Health Research Centre, Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand

The purpose of this study was to assess the responsiveness of 4 biochemical markers of bone resorption to high calcium skim milk. Ten women, age 65.5±3y, body mass index 29±2, and all =5y postmenopausal took part. They consumed

400ml of high calcium skim milk (Ca 1200mg, Mg 172 mg). Serum parathyroid hormone (PTH), C-telopeptides (CTx) and N-telopeptides (NTx) were measured at baseline, and again 1, 3 and 5 hours after milk. Urine free deoxypyridinoline (DPD) and creatinine (Cr) were measured at baseline, and between 0-2, 2-4 and 4-6 hours after milk. Data are expressed as mean ± SEM, and the impact of time on each variable was assessed by one-way ANOVA.

Variable	Baseline	1h	3h	5h	p
PTH					
pmol/l	2.78±0.27	2.84±0.22	2.57±0.23	2.57±0.24	NS
NTx					
nmol/l	17.95±0.90	16.48±0.91	15.52±0.69	15.66±0.52	NS
CTx					
ng/ml	0.34±0.04	0.23±0.03	0.17±0.02	0.12±0.02	<0.001
	Baseline	0-2h	2-4h	4-6h	p
DPD					
nmol/ mmol cr	8.14±0.78	7.07±0.36	6.77±0.68	4.93±0.64	=0.01

With respect to the postprandial response to high calcium milk, serum CTx was the most sensitive of the markers of bone resorption that we measured. NEW ZEALAND MILK funded this work.

P37

NO EVIDENCE OF GENE-ENVIRONMENT INTERACTION ON BONE DENSITY IN FEMALE TWINS

V.Naganathan, T.Nguyen and P. Sambrook. Institute of Bone and Joint Research, Royal North Shore Hospital, Sydney, Australia.

Twin and family studies have shown that genetic factors have the largest influence on bone density. It is unclear however if this genetic influence is modified by environmental influences known to influence bone density such as smoking. The aims of this twin study were to determine if an environmental influence such as smoking had a significant effect on the heritability of BMD.

Bone density of the hip was measured by DEXA scan on 475 adult female twin pairs. A smoking history of > 5 pack/year was regarded as a significant exposure (categorised as “Yes”). Twins pairs grouped by zygosity (identical-MZ and non-identical-DZ) and smoking status (Yes or No). This results in 4 groups of concordant pairs and 2 groups of discordant pairs. The following correlations for hip BMD were found for the twin pairs: MZ (No,No) = 0.80, DZ (No,No) = 0.44, MZ (Yes,Yes) = 0.80, DZ (Yes,Yes) = 0.15, MZ = (No,Yes) = 0.75 and DZ (No, Yes) = 0.32. The differences in heritability calculated from these correlations were not significantly. Using path-analytic genetic model fitting analysis the models fitted the data best when it was assumed that there was no differences in the heritability of hip BMD between smokers and non-smokers.

Two conclusions can be drawn from this study with regards to the heritability of BMD, either there is no significant gene-smoking interaction or that larger numbers of twins are necessary to show a significant gene-environment interaction. The data will be analyzed further to look for other possible gene-environment interactions by looking at the influence exposure to HRT, exercise and alcohol has on the heritability of BMD.

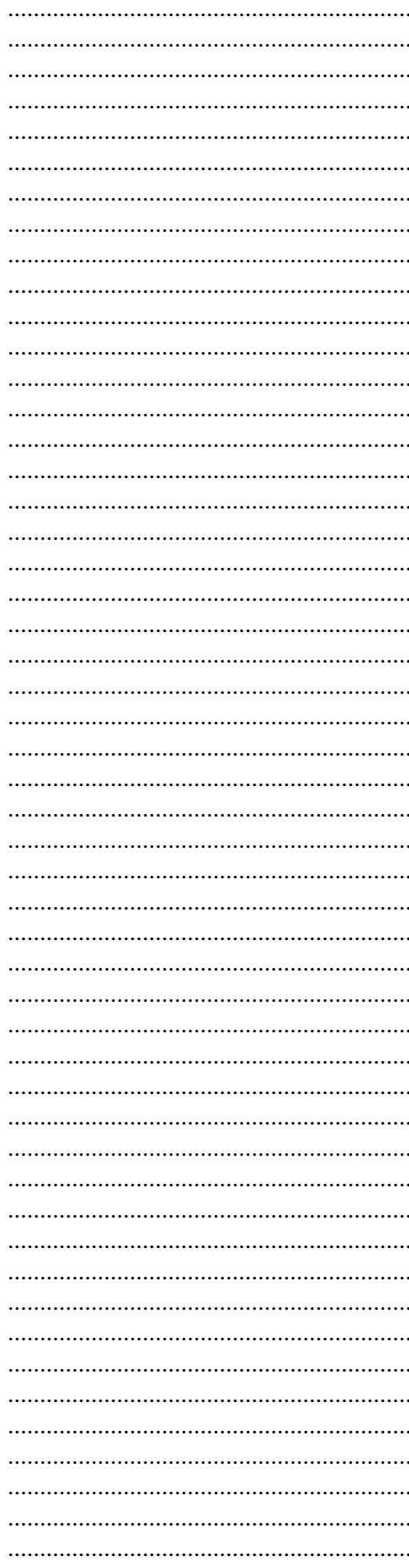
P38

DUAL FEMUR BONE DENSITY MEASUREMENT IN LOW IMPACT FRACTURES. JCH Wong, L McEwan. Department of Nuclear Medicine & Bone Densitometry, Royal Brisbane Hospital, QLD 4029.

The high correlation documented between the left and right femoral bone mineral densities in the normal population suggests that dual femur measurements are not justified in clinical practice. This study evaluates whether this premise holds for subjects who have sustained fractures with minimal trauma.

25 women aged 50-81 years (mean=76 years) with previous low impact fractures in the last 12 months had both proximal femora measured using a LunarÒ DPX-IQ densitometer. There was significant correlation between values in the left and right total hip (TH) ($r=0.92$; $p<0.05$) and left and right femoral neck (FN) ($r=0.78$; $p<0.05$). The mean differences between the left and right TH and FN densities were not significant. There was also no significant effect of side dominance on lower limb TH or FN values. However, the range of the limits of agreement for the TH (-0.079 to 0.103g/cm²) and FN (-0.135 to 0.149g/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 10% of subjects with a T-score discordance greater than 0.5 and 0.5% greater than 1 for the TH. For the FN, 20% have a T-score discordance greater than 0.5 and 5% greater than 1.

The utility of dual femur measurements in altering diagnosis and management is therefore limited for TH measurements. The FN, however, shows greater variability and dual measurements may be considered in clinical practice.



P39

A COMPARISON OF BONE MINERAL DENSITY IN CHINESE AUSTRALIAN, CAUCASIAN AUSTRALIAN AND CHINESE WOMEN. EL Bond, R Wong, L Lan, C Margerison, L Paton, JD Wark. Department of Medicine, University of Melbourne, Bone & Mineral Service, Royal Melbourne Hospital, Parkville, Australia.

There is marked heterogeneity in bone mineral density (BMD) and fracture epidemiology between Asian and Caucasian populations. Moreover, there is a lack of appropriate reference data to allow interpretation of BMD values in Asian Australians (AA), who comprise 8% of our population. To improve understanding of bone health in AA, we compared areal BMD of Asian Australian women (AA), Caucasian Australian women (CA) and Chinese women (Ch) from China. All women involved were healthy volunteers.

20 AA of Chinese ethnicity had lumbar spine (LS) BMD measured (Hologic QDR1000W). Cross-sectional data on 1300 CA were obtained on the same instrument. Results of LS BMD using a Lunar DPX from 1034 Ch were obtained. AA BMD were compared with CA BMD. The 20 AA were matched hierarchically for age, height and weight with the CA. Comparisons were made using paired t-tests for LS BMD, BMC and area. Mean CA BMD per decade was compared with Ch using a universal scanner calibration ¹ to enable comparison of results obtained on machines from two manufacturers.

Cross-sectionally, the BMD of the AA tended to fall below the CA mean, with Z scores of 0.56 to -1.75 (mean -0.54), and T scores of 0.51 to -3.88 (mean -1.24). No significant difference was found within the AA and CA matched pairs for BMD, BMC, and area. The Ch mean BMD were all lower than CA BMD, but within 1.96 C.I.

This sample of AA showed a trend towards low BMD when compared cross-sectionally with CA. However, there was no significant difference in BMD, BMC and area within the matched pairs. Future research will be aimed at establishing normative BMD and fracture risk data for AA. These data can assist in the understanding and management of bone health in Asian Australians.

1. Genant et al, 1994. J. Bone Min. Res. 9 (10):1503-14.

P40

RISK ASSESSMENT AND USE OF DXA TO ESTABLISH NORMATIVE DATA USING HEALTHY VOLUNTEERS.

EL Bond and JD Wark. Department of Medicine, University of Melbourne, Bone & Mineral Service, Royal Melbourne Hospital, Parkville, Australia, 3050.

Establishing specific bone mineral density (BMD) and fracture risk reference data for age, gender and ethnic populations is an important area of osteoporosis research in Australia. The current best method of measuring BMD is dual energy X-ray absorptiometry (DXA). This type of research necessarily involves exposing healthy volunteers to very low levels of ionizing radiation (LLR) comparable to the dose received on two Australia-Europe return airline flights, or less than 10% of average annual background radiation exposure in Melbourne. We have reviewed the literature to investigate what level of hazard may be involved with LLR. The health effect of foremost concern is cancer induction at the somatic level.

Difficulty arises when estimating cancer risk at LLR due the enormous population sizes required. Risk of exposure to LLR is currently inferentially and mathematically extrapolated from effects of large radiation doses including studies of survivors of the 1945 atomic bombings of Hiroshima. The estimated cancer risk for use of DXA from this extrapolation is in the order of 1:1,000,000. Yet there is a lack of clear evidence to support this linear extrapolation to LLR.

A body of evidence exists which supports the threshold hypothesis below which there is no increased risk of cancer, but above which the risk increases. Evidence for "radiation hormesis" which proposes that there is actually a reduced risk at LLR also exists.

Guidelines used by regulatory authorities for informed consent for research involving DXA can present problems for the conduct of this research if they rely on unsubstantiated evidence of risk and give rise to apparently unjustified concern among prospective research subjects. These issues require careful consideration to ensure that subjects' safety is protected, that consent is adequately informed and that appropriate research can be conducted.

P41

TEMPERATURE DEPENDENCY OF BONE MINERAL DENSITY USING THE LUNAR EXPERT.

N Culton, N Pocock. Department of Nuclear Medicine and Bone Densitometry, St. Vincent's Hospital, Sydney.

A recent problem with the air-conditioning unit within our department provided an opportunity to examine the effect of room temperature on the bone mineral density (BMD) measured by the Lunar Expert (Madison WI). The Bone Fide spine phantom (Madison, WI) consists of a bone equivalent material, L1-L4, surrounded by a tissue equivalent resin and is used for daily quality control procedures with an established mean of $1.145 \pm 0.006 \text{ g/cm}^2$. The phantom was scanned using the same imaging parameters at various intervals during several days, including before and after repeated calibration procedure (Lunar 'Quality Assurance' (QA)) which 'recalibrates' the expected BMD values at any given temperature to within an acceptable range. The temperature of the room was recorded at the time of the phantom and QA measurements. The change in phantom BMD measurements, obtained at two successive time points within a single calibration period was compared to the observed corresponding temperature change.

The range in temperature over several days was 19 to 23.6°C, however the greatest observed change in temperature between any two time points was 2°C. There was a moderate negative correlation between the change in room temperature and the change in phantom BMD ($r=-0.58$). The regression equation suggests that a temperature change of 10°C, could change the BMD result by approximately 5% from the expected mean. After a QA scan was performed, the bone density result for the phantom returned to a value within the expected range. Operators of densitometry machines should be aware of the need for constant room temperature throughout the day. Calibration procedures should be performed for a specific temperature and repeated if the temperature fluctuates significantly throughout the day.

P42

A QC ASSESSMENT OF MOBILE VERSUS FIXED DEXA UNITS.

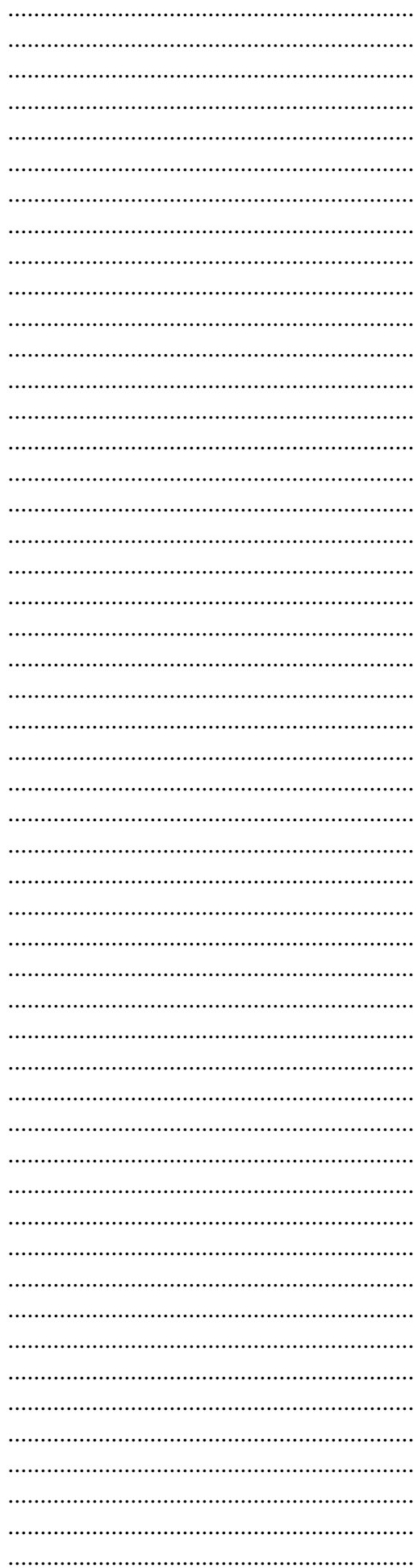
R Will, M Minchin, A Minchin, S Lenny, G Mastaglia. Bone Densitometry Australia, PO Box 673, Victoria Park W.A.

We have provided a DEXA service to rural and regional Western Australia since January 1999. The DEXA unit is housed in a purpose built caravan and is pulled by a 4 wheel drive vehicle. A daily check on the machine operating characteristics is undertaken as per the manufacturers requirements. A voltage regulator is installed in all mobile units to adjust for local voltage fluctuations. This mobile service has now been extended to NSW (2 units), Victoria, Queensland and Tasmania progressively from September 2000. Three fixed Lunar DEXA's are also operated in WA; 1 DPXL and 2 DPX IQ long table. Our DEXA operators undergo a training program on both fixed and mobile units.

We have undertaken a comparison of the QC results obtained with the Lunar Phantoms (Aluminium/water bath). Measurements are taken 3 times a week. The operators have also undertaken duplicate DEXA measurements at the lumbar spine and femoral neck on patients to assess their own internal QC's.

A running mean summary for the phantoms was undertaken and the CV's as a % for the fixed sites varied from 0.41-0.76% and on the mobile sites varied from 0.74% to 1.25%. At the lumbar spine, the precision (2 SD's) for detecting a significant change in BMD for 7 operators varied from 13-31mg or 1.0-2.6%. At the femoral neck for 6 operators this varied from 13-57 mg or 1.5-6.6%.

Conclusions: 1. There may be greater instability of a mobile compared with a fixed DEXA unit. For the phantom assessed, a maximum variation of 1.25% is acceptable but is likely to improve with further staff training. 2. There is considerable variation in the precision results obtained by different operators with better precision at the lumbar spine than the femoral neck. Further training is likely to lead to an improvement in these results.



P43

A COMPARISON OF DOCTOR-REFERRED VERSUS SELF-REFERRED PATIENTS FOR DEXA

R Will¹, M Minchin¹, A Minchin¹, S Lenny¹ and G Mastaglia¹. ¹Bone Densitometry Australia, PO Box 673, Victoria Park, W.A.

Bone Densitometry Australia has provided a mobile DEXA service (Lunar IQ short table scanner) to Darwin (population 84,000) in July 1999 and August 2000. Scans were undertaken by the same operator with acceptable QA data.

There were 301 patients who self-referred (SR) and 261 were doctor-referred. Patients who were DR were more likely to have sustained fractures (58 vs 32; $C^2 = 13.96; P < 0.001$). When pts were divided into 6 fracture groups (vertebral; hip; other major upper and lower limb; multiple; minor- toes, fingers, scaphoid etc and ribs) there was no difference in the distribution of the fractures between DR and SR groups; $C^2 = 8.9; DF = 5; NS$. In those patients with fractures there was no difference in the distribution of the T scores between those with a T score ≥ -1.4 vs ≤ -1.5 (lowest T score at the L1-L4 spine or femoral neck; $C^2 = 6.54; DF = 5; NS$). There were more DR patients with a history of fractures and a T score at the spine or hip ≤ -2.5 ($C^2 = 23.4; DF = 4, P < 0.001$), however if these patients are excluded the distribution in T scores at the spine or hip was no different between DR and SR ($C^2 = 3.4; DF = 3; NS$). There were more osteoporotic patients in the DR group and more osteopenic patients in the SR group (DR pts; 19.5% osteoporotic and 34.9% osteopenic and SR pts; 7.6% osteoporotic and 47.8% osteopenic; $C^2 = 14.8; DF = 1; p < 0.001$).

Conclusions: 1. Doctors identify patients who have more significant degrees of osteoporosis than SF patients with a greater concentration of spine and hip fractures. 2. "Minor" fractures may identify patients with osteoporosis. 3. There is no difference in the distribution of minimal trauma non-hip and non-spine fractures between low T score (≤ -1.5) and high T score (≥ -1.4) patients. 4. To identify 1 osteoporotic patient 13 patients need to be screened in this population and to identify 1 osteopenic patient 2.5 patients need to be screened.

P44

ULTRASOUND OF THE CALCANEUS IN A PRIMARY CARE SETTING: CORRELATION WITH TWO RISK FACTOR ASSESSMENT QUESTIONNAIRES.

R March RN, The Canterbury Geriatric Medical Research Trust; S Carson, Christchurch South Health Centre; N Gilchrist, The Canterbury Geriatric Medical Research Trust; C Frampton, Christchurch School of Medicine; et al

1. To evaluate the use of two osteoporosis risk questionnaires (SCORETM and DECO) in General Practice.
2. To evaluate the usefulness of mobile technology provided by the Achilles Ultrasound Bone Densitometer (Lunar Madison WI) in postmenopausal women aged 50-70 years.

Letters were sent to all women aged 50-70 who are registered at the Christchurch South Health Centre. The first 250 to reply were entered into the study. Ultrasound measurements of the calcaneus were made using an Achilles Ultrasound Bone Densitometer.

The osteoporosis risk questionnaire results were compared to T score using Pearson Product Moment Correlation. There was a correlation between both the questionnaires and T score (SCORE $r = .295^{***}$ and DECO $r = .385^{***}$). Using a T score of -2 and a SCORE of 6 as a cut off point as Lydick et al did during the validation and development of the SCORE questionnaire¹ we obtained a sensitivity of 81% and a specificity of 36.4%. The mean T score was -1.38 with a range of 3.3 to -4.8 . 23.6% (57/241) had a T score in the osteoporotic range, 38.2% (92/241) had a T score in the osteopenic range.

The SCORE and DECO osteoporosis risk assessment questionnaires may not be sensitive or specific enough to be a screening tool in this group. The mobile Achilles Ultrasound machine in a general practice setting is a useful and convenient tool, allowing patients to attend for a bone density measurement as part of a general health screening.

¹ Lydick E, Cook K, Turpin J, Melton M, Stine R, Byrnes C. Development and Validation of a Simple Questionnaire to Facilitate Identification of Women Likely to Have Low Bone Density. American Journal of Managed Care 1998;4:37-43

P45

MOBILE ULTRASOUND SERVICE AUDIT

M Merrilees, R March, CGM Research Trust, P Maguire, CGM Research Trust, P Reilly, CGM Research Trust, M Caesar, CGM Research Trust, N Gilchrist, J Palmer, Bone Health Services, W Gilchrist, Bone Health Services

The aim of this audit was to see how well the Ultrasound service was being used and if referrals were made appropriately.

All scans from January 1994 to December 1999 were identified from the patient database. The referral letter, patient questionnaire, scan and follow-up letter were read to determine; where the scan occurred, the reasons for referral, the T score value, the date of scan and recommendations from the specialist.

Data was collected on 3745 patients from a number of locations throughout the South Island of New Zealand. There were 3648 females and 97 males who had been scanned. Of the females 2621 were postmenopausal and 958 had had a hysterectomy at some stage.

From the fractures identified from the patient questionnaire there were 350 wrist fractures, 120 vertebral fractures, 28 hip fractures and 1232 other fractures. These were both traumatic and osteoporotic fractures. 1533 people had normal bone density, 1393 had osteopenia, 760 had osteoporosis and for 59 patients there were no scan details available.

In 2462 patients, no medication or change in medication was suggested. In 1283 patient's additional medication was suggested. Further investigation was recommended in over 100 patients and DEXA scans in approximately 250. Those with fractures had an average T score of -3.5. Average T score was -1.8 for follow-up scan recommendation.

This is a well-utilised service.

Fractures and risk factors are important reasons for referral.

57% of those scanned have Osteopenia or Osteoporosis.

10% are recommended for further investigations.

34% are recommended for therapy.

P46

EVALUATION OF THE SUNLIGHT OMNISENSE.

N Culton, S Kalnins, J Freund, N Pocock. Department of Nuclear Medicine and Bone Densitometry, St Vincent's Hospital, Sydney.

Sunlight Omnisense (SO) is a new ultrasound device which measures the speed of sound (SOS m/sec) through cortical bone. SOS can be obtained at various skeletal sites, including the distal 1/3 radius, proximal phalanx (III), mid-shaft tibia and metatarsal (V). We assessed precision of the SO in-vitro using perspex phantoms, and also in-vivo at the radius and phalanx. SO SOS measurements were compared to DXA lumbar spine (LS), total proximal femur (TPF) and femoral neck (FN) BMD (Lunar Expert) in 31 women aged 45 to 84. Three operators each performed 10 measurements on the radius of individual subjects. In addition, 2 of the operators performed 10 measurements on the phalanx of a single volunteer. The coefficient of variation (CV%) and standardised CV (sCV%) was calculated for each operator at both sites. Reproducibility was also assessed by performing paired measurements on 42 patients at the phalanx and radius. The SO SOS results were compared to the LS, TPF and FN BMD results using simple regression analysis. The SOS and BMD T scores were compared by paired T test.

Site	Rad	Rad	Rad	Phal	Phal	<i>Correl</i>	Rad	Phal
Operator	1	2	3	2	3	LS	0.43	0.46
CV (%)	0.5	0.6	0.4	1.4	0.8	TPF	0.38	0.39
sCV (%)	3.1	3.3	2.4	5.4	3.2	FN	0.32	0.27

The in-vitro CV = 0.11% for the Phalanx probe and 0.18% for the Radius probe. The SO T scores did not differ significantly from the DXA T scores at any site.

The SO precision obtained is comparable to published results. Intra-operator precision improved with experience. The precision results of this study suggest that the precision of Omnisense is similar to other QUS techniques but does not equal the reported precision of DXA. SO SOS showed moderate correlations to LS, TPF and FN BMD.

P47

ULTRASOUND BONE MINERAL DENSITY – NEED FOR SEPRATE NORMOGRAM FOR INDIANS.

CV Harinarayan, Department of Endocrinology & Metabolism, Sri Venkateswara Institute of Medical Sciences, Tirupathi – 517 507, AP, INDIA.

To study the ultrasound bone mineral Density of Indian Population and to correlate the same with the Serum calcium, 25 OH Vitamin D3, PTH-intact and Deoxy pyridinoline crosslinks of collagen.

Seventy-six postmenopausal women in the age group of 47 ± 10 years underwent ultrasound bone mineral density by LUNAR Achilles Ultrasound bone Densitometer of the right calcaneum (USBMD). Simultaneously fasting serum samples were analysed for Serum calcium, 25 OH Vitamin D3, PTH-intact and Deoxy pyridinoline crosslinks of collagen (DPD).

The mean + SD of their serum calcium (N= 8.5-10.5 mg%), 25 OH vitamin D3 (N=23-113 nmol/l), Intact PTH (N=0.8-5.2 pmol/l), DPD (nM/mM of creatinine) were 9.7+ 0.67; 49.4 + 17; 2.2 +1.6; 9.63 + 10 respectively. The USBMD measured using the German normogram as reference showed a stiffness index of 83 + 18; T score of -1.32 +1.38 and a Z score of -0.09 + 1.32. There was a positive correlation between the 25 OH vitamin D status and the Z scores measured (P <0.015). There was no significant correlation between the USBMD measurements and the serum parameters measured. Even among the group with negative Z and T scores, there was no correlation between the USBMD measurements and the serum parameters measured.

The study brings out the fact that there is an urgent need for a separate normogram of Indian population, of USBMD measurements, calcium and vitamin D status of the population and their correlation.

P48

STUDY OF BONE MINERAL MARKERES IN POSTMENOPAUSAL WOMEN WITH AND WITHOUT DIABETES.

CV Harinarayan, M Suresh #, M Dhanajayanaidu #, G Agarwal, Endocrinology & Metabolism Department, Sri Venkateswara Institute of Medical sciences, and S.V.University# Tirupathi – 517 507, AP, INDIA

Purpose: This study was aimed to understand the Vitamin D status, bone mineral markers (biochemical and hormonal) in postmenopausal women with and without diabetes as there are scant reports on this subjects.

Methods: Thirty-nine postmenopausal women with diabetes were studied for the vitamin D status, bone mineral markers to compare with diabetic women and regular cycles. Urinary calcium, phosphorous, creatinine along with serum calcium, phosphorus, alkaline were collected for analysis along with S.Intact PTH, 25OH Vitamin D3and urine for deoxypyridonoline cross links of collagen.

Results: The mean ± SD of age (yr.), S.Ca (mg%), S.Phos.(mg%), SAP (IU/l), S.Cr. (mg%), U.Cr. (mg/day), U.Ca. (mg/day), U.Phos. (mg/day), T.vol. (ml/day), S.PTH(Intact) (pmol/l), 25 OH Vit D3 (nmol/l), Urinary Deoxy pyridinoline Cross links (nM/mM of creatinine) of the post menopausal diabetics(n=39) were 53± 9; 9.7 ± 0.4; 3.37 ± 0.51; 163 ± 47; 0.9 ± 0.37; 886 ± 287; 162 ± 76; 454 ± 191; 2532 ± 894; 2.42 ± 1.55; 67± 40; 7.68 ± 4.7 respectively. When compared with postmenopausal women of similar age groups without diabetes (n=9) there was no significant difference between any of the parameters between the two groups. The 25 OH Vit D3 status was significantly higher in postmenopausal women with diabetes (P < 0.1). Postmenopausal women with diabetes were categorized into those with natural and surgical menopause. There was no significant difference in any of the parameters between both the groups and a similarly with postmenopausal women (surgical) with diabetes Vs postmenopausal women without diabetes. The 25 OH Vit D3 was significantly different (P<0.2) in postmenopausal women (natural) with diabetes were compared with postmenopausal women without diabetes.

Conclusions: There is no difference in the bone mineral parameters in postmenopausal women with and with out diabetes. Polypharmacy as a part of diabetes could be the cause of higher vitamin D levels.