ANZBMS 2005 Annual Scientific Meeting

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ABSTRACTS

IP - INVITED SPEAKERS

IP1 7.9.05: 0830 – 0930 hours

NEW ROLES FOR OLD HORMONES AND THE GORDIAN KNOT OF HUMAN BIRTH

R Smith
The Mothers & Babies Research Centre, Hunter Medical Research Institute, John Hunter Hospital, Newcastle, NSW

While some aspects of endocrine function have been extraordinarily conserved across hundreds of millions of years of evolution the biology of pregnancy is extremely varied. As examples, parturition in goats is precipitated by luteolysis while in the sheep fetal mechanism predominate. This diversity is thought to be due to maternal-paternal genetic conflict as proposed by David Haig. From a medical perspective this diversity means that extrapolation from animal studies to the human are unwise. Experiments in pregnant women are necessarily restricted. For these reasons we remain substantially ignorant of the processes that regulate human birth and observational studies predominate. Surprisingly the hypothalamic stress hormone CRH is made in the placenta of primates and in the human increases in maternal blood in an exponential pattern peaking at delivery. In a prospective cohort study we have shown that the timing of birth is related to the rate of the exponential rise in this placental hormone. Effectively a biological clock exists in the placenta that determines the timing of birth. As labour approaches the uterus is transformed into an actively contracting pump for the expulsion of the fetus, in most mammals this event is signalled by a fall in circulating progesterone, but not in humans. In humans we have produced evidence for a functional progesterone withdrawal generated by changing expression of progesterone receptor isoforms. The stimulus to this change in receptors has been explored using novel mathematical approaches which allow cause and effect relationships between variables to be deduced without the need for experimental intervention. The approach uses Directed Graphs together with statistical testing of the likelihood that particular graphs are consistent with the data set on the variables. Using this approach strong evidence has been produced that inflammatory pathways are activated early in the pathway to human birth. Understanding the physiology of normal human birth provides a foundation upon which predictors of preterm birth can be developed and will also allow the identification of appropriate targets for novel therapeutics to arrest preterm labour.

IP2 7.9.05: 1000 – 1030 hours

MECHANISMS OF ACTION IN CURRENT AND EMERGING OSTEOPOROSIS THERAPIES

TJ Martin
St Vincent's Institute of Medical Research, Melbourne, VIC

Until recently osteoporosis treatment has relied upon drugs that inhibit bone resorption, with successful trials of bisphosphonates, SERM's and a strontium salt. Current novel treatments are aimed at interfering with osteoclast development (e.g. anti-RANKL), as well as inhibiting osteoclast activity (e.g. V_ATPase, cathepsin K and chlroide-7 channel inhibitors. The only proven anabolic therapy for bone is PTH. The anabolic effect of PTH is dependent upon intermittent administration, but when an elevated PTH level is maintained even for a few hours it initiates processes leading to new osteoclast formation, and the consequent resorption over-rides the effects of activating genes that direct bone formation. The observation that concurrent treatment with bisphosphonates impairs the anabolic response to PTH, adds to other clues that osteoclast activity is necessary to complement the direct effect that PTH has in promoting differentiation of committed osteoblast precursors. This might involve the generation of a coupling factor from osteoclasts that are transiently activated by RANKL in response to PTH. Both human and mouse genetics provide evidence supporting the view that osteoclasts, despite in some circumstances being unable to resorb bone, e.g. failure of acidification, can nevertheless be associated with normal, or even increased bone formation. An implication is that it may be possible to design resorption inhibitors that do not block PTH anabolic action when given simultaneously.

New approaches to anabolic therapies come from the discovery that an activating mutation in the LRP5 gene is responsible for an inherited high bone mass syndrome, and the fact that this can be recapitulated in transgenic mice, whereas inactivating mutations result in severe bone loss. This has focused attention on the Wnt/frizzled/β-catenin pathway as an important one in bone formation, and provides intriguing choices of any of a number of drug targets in this pathway.

IP3 7.9.05: 1030 – 1100 hours

CALCIUM SUPPLEMENTATION AND/OR VITAMIN D IN OSTEOPOROSIS - MYTH OR REALITY?

R Prince
University Of Western Australia, School Of Medicine & Pharmacology, Nedlands, WA

One of the central problems in nutrition science is to determine the optimal intake of nutrients to prevent disease in an individual taking into account their genetic, environmental and disease status. In the first part of the 20th century the principal nutritional focus was on the

prevention and treatment of rickets in children, still a substantial problem. With increasing longevity the focus now includes the other end of life where fragility fracture presents a major social, health and financial threat to millions of individuals who have survived other threats because of much improved public and personal health services. The aetiology of the threat includes the combined and independent effects of estrogen, calcium and vitamin D deficiency.

We have shown effects in humans of high endogenous estrogen to reduce fracture propensity, increase DXA bone density and reduce renal calcium and phosphate excretion, effects mediated in part by both aromatase and estrogen receptor gene polymorphisms. These data are supported by in-vitro and in-vivo animal and cell culture studies showing stimulation of calcium transport by estrogen. We and others have shown that this bone loss can be prevented by increased dietary calcium intake.

However the size of the treatment effect on fracture remains uncertain. We have recently completed a public health based, prospective, double blind, randomised trial of 1200 mg calcium compared to placebo in women mean age 75. After 5 years of treatment the intention to treat analysis showed a hazard ratio for clinical fracture of 0.86 95% CI 0.67-1.11. In those consuming 75% of tablets the HR was 0.48 95% CI 0.24-0.97. Thus calcium therapy may be effective if compliance is high, vitamin D and calcium remain indicated as a first step those with marginal vitamin D intake.

IP4 7.9.05: 1100 – 1130 hours

SEX HORMONE REPLACEMENT AND SERMS

JA Eisman
Bone and Mineral Research Program, Garvan Institute of Medical Research, Sydney, NSW

Postmenopausal bone loss is characterised by increased remodelling at different skeletal sites and in different bone compartments. Oestrogen sex hormone replacement, which can block this excessive 'turnover', had been the mainstay of postmenopausal osteoporosis prevention. The Women's Health Initiative demonstrated HRT efficacy in reducing fragility fractures but increased cardiovascular adverse events in a double blind RCT. The women were older and largely overweight women at relatively low risk for osteoporosis and high risk for vascular adverse events. Despite the limited relevance to younger postmenopausal woman, this reported adverse effect profile led to calls to restrict HRT to 1-2 years postmenopause and only for relief of menopausal symptoms.

Bone density improvements following HRT gradually disappear over about 5 years after cessation. Although oestrogen-only therapy appears to have a better safety profile, this could only be recommended post-hysterectomy.

Tibolone may prevent postmenopausal bone loss with less breast or uterine effects. However a large scale RCT of its safety and efficacy on fragility fractures is still in progress.

Raloxifene, the best-studied SERM, has been shown to reduce bone loss and reduce vertebral but perhaps not peripheral fragility fractures with a good safety profile with fewer breast cancer diagnoses. Studies are continuing on its cardiovascular safety profile and more potent SERMs are under evaluation.

Despite some concerns about their longer-term safety and efficacy, HRT and SERMs offer useful alternatives in the armamentarium for the prevention and treatment of the earlier stages of postmenopausal bone loss.

Conflicts of Interest (all 3 and/or 4):
Eli Lilly, Merck Sharp and Dohme, NPS Pharmaceuticals, Novartis, Organon, Roche, Sanofi-Aventis, Servier

IP5 7.9.05: 1130 – 1200 hours

OSTEOPOROSIS: WHOM TO TREAT? WHAT DRUG?

R Eastell
Professor of Bone Metabolism, University of Sheffield, Sheffield, UK

The drugs for osteoporosis are best used in those in whom they have been clearly shown to reduce fractures – patients with vertebral fractures or with a low spine or hip BMD (at or below a T-score of -2.5). The targeting of these treatments may also be done on health economic grounds. In the UK, such an approach has been applied by the National Institute of Clinical Excellence. The treatments for osteoporosis that are effective in reducing the risk of fracture have been recently renamed 'anti-catabolic' and 'anabolic'. The anti-catabolic treatments work by reducing the activation frequency and possibly restoring remodelling balance. This class includes potent agents such as alendronate, risedronate, and hormone replacement therapy, and weaker agents such as raloxifene and calcitonin. The anabolic treatments work by increasing the activation frequency and by causing a positive remodelling balance. This class includes parathyroid hormone (teriparatide). Some treatments, such as strontium ranelate, don't fit easily into this classification. The choice of treatment is based on the evidence for efficacy, its cost-effectiveness, and side effect profile. The choice of treatment is also dictated by the

patient's history and the efficacy of treatment in the individual may be monitored by serial measurements of bone mineral density or bone turnover markers.

IP6 7.9.05: 1300 – 1430 hours

PROTOS: A NEW WAY TO TREAT POST-MENOPAUSAL OSTEOPOROSIS

Chair: Prof Jack Martin
Presenters:
Prof Ego Seeman
Prof John Eisman
Prof Philip Sambrook

Lifestyle changes may reduce the risk of fracture in some cases but in general established osteoporosis requires drug therapy. Anti-resorptive agents inhibit bone resorption while bone-forming agents act by increasing bone formation but they may also increase bone resorption. In contrast, Protos, strontium ranelate, acts differently from currently available agents. It decreases bone resorption whilst allowing bone formation to continue.

To rigorously evaluate the efficacy of Protos, a comprehensive Phase 3 protocol was developed including a run-in study (FIRST) to normalise any vitamin D and calcium deficiencies before entry into two large well-designed studies to confirm that this drug reduces fracture risk at the vertebra, and non-vertebra levels.

The Spinal Osteoporosis Therapeutic Intervention trial (SOTI) involving 1649 post-menopausal women showed that treatment with Protos reduced the relative risk of vertebral fracture by 49% in the first year (RR=0.51; 95%Cl, 0.36 to 0.74; p<0.001) and by 41% at 3 years (RR=0.59; 95%Cl, 0.48 to 0.73; p<0.001) with no significant differences between the groups in the incidence of serious adverse events. The Treatment Of Peripheral OsteoporosiS (TROPOS) trial involved 5091 post-menopausal women and demonstrated Protos treatment for three years reduced the relative risk of non-vertebral fractures by 16% (RR=0.84; 95%Cl, 0.702 to 0.995; p=0.04). This included a reduction in the relative risk of hip fracture of 36% (RR=0.64; 95%Cl, 0.412 to 0.997; p=0.046) in those women aged ≥74 years with a femoral neck T-score ≤ -2.4(NHANES).

Pre-planned pooling of data from SOTI and TROPOS have also allowed analysis of patients aged over 80 years and those with osteopenia. In both of these subgroups Protos significantly reduce both vertebral and non-vertebral fractures.

The tolerability profile will also be presented.

IP7 7.9.05: 1430 – 1500 hours

GENETICS OF PARATHYROID GLAND DEVELOPMENT AND HYPOPARATHYROIDISM

RV Thakker
University of Oxford, Nuffield Department of Clinical Medicine, OCDEM, Churchill Hospital, Headington, Oxford, OX3 7LJ, UK

Molecular genetic studies have identified some of the genes (e.g. GATA3, Gcm2, Hoxa3 and SOX3) involved in the developmental pathways of the parathyroids. GATA3 haploinsufficiency causes the hypoparathyroidism, deafness and renal dysplasia (HDR) syndrome. GATA3 belongs to a family of zinc-finger transcriptional factors that are involved in vertebrate embryonic development, and the HDR phenotype is consistent with the expression pattern of GATA3 during human and mouse embryogenesis in the developing kidney, otic vesicle and parathyroids. The homeobox gene, Hoxa3, is also of importance in this pathway, as homozygous mutant knockout mice (-/-) lacking Hoxa3 have an absence of the thymus and parathyroids. Homozygous mutant mice (-/-) that are deleted for Gcm2 (glial cells missing 2), which is the mouse homologue of the Drosphilia gene, Gcm, also lack parathyroids. However, these Gcm2 deficient mice had PTH concentrations identical to those of normal mice, indicating an auxillary source of PTH, was found to be a cluster of PTH – expressing cells under the thymic capsule. Gcm2 has a role in human parathyroid development as a deletion in a patient has been reported to lead to hypoparathyroidism. Finally, investigations of kindreds with X-linked recessive hypoparathyroidism have identified a complex deletion-insertion involving chromosome Xq27 and 2p25. This deletion likely alters SOX3 expression, which occurs in the embryonic mouse parathyroid between 10.5 and 15.5 days post-coitum. These studies of hypoparathyroid patients and mouse models have helped to elucidate some of the genes involved in the embryological development of the parathyroids.

IP8 7.9.05: 1500 – 1530 hours

HYPERPARATHYROIDISM AND PARATHYROID CARCINOMA – INSIGHTS FROM GENE PROFILING

DJ Marsh
Kolling Institute of Medical Research, Royal North Shore Hospital and Department of Molecular Medicine, University of Sydney, NSW

Hyperparathyroidism is a common endocrinopathy characterised by calcium insensitive hypersecretion of parathyroid hormone and increased parathyroid cell proliferation. In primary hyperparathyroidism, the majority of tumours are sporadic, however approximately 5% occur as a component of one of the familial syndromes Hyperparathyroidism-Jaw Tumour Syndrome (HPT-JT), Multiple Endocrine Neoplasia Types 1 and 2A, or Familial Isolated Hyperparathyroidism (FIHP). Parathyroid tumours are classified as adenomas (80-85%), hyperplasia (15-20%) or carcinomas < 1%, however the histological differences between these tumours can be subtle. We undertook gene expression profiling for 53 parathyroid tumours and identified three distinct clusters – predominantly hyperplasia, predominantly adenoma, and a cluster characterised by mutation in the tumour suppressor gene *HRPT2*[1]. This "*HRPT2* Cluster" contained parathyroid tumours from patients with HPT-JT, FIHP and sporadic carcinoma. Expression of a number of genes that discriminated this cluster was confirmed by Quantitative Real Time RT-PCR and immunohistochemistry, including UCHL1 and VCAM1. *HRPT2* encodes parafibromin that is part of the Human Paf1 complex. The Paf1 complex is known to interact with RNA polymerase II and is important for expression of genes involved in cell cycle regulation and metabolism. We have identified a functional bipartite nuclear localisation signal in parafibromin and shown that a number of HPT-JT and parathyroid carcinoma-associated *HRPT2* mutations disrupt nuclear localisation[2].

[1]Haven *et al*. Gene expression of parathyroid tumours: molecular subclassification and identification of the potential malignant phenotype. *Cancer Research* 2004; 64: 7405-7411.

[2]Hahn & Marsh. Identification of a functional bipartite nuclear localisation signal in the tumour suppressor parafibromin. *Oncogene* (in press).

Disclosure:
No conflict of interest

IP9 7.9.05: 1530 – 1600 hours

THE CHANGING FACE OF PARATHYROID SURGERY

L Delbridge
University of Sydney Endocrine Surgical Unit, Sydney, NSW

Accurate 99mTc sestamibi parathyroid scanning has changed the face of parathyroid surgery, allowing the introduction of "focused" minimally invasive parathyroidectomy (MIP). Prior to this, four gland open neck exploration was standar, with the outcome largely determined by the surgeon's experience with the embryological vagaries of parathyroid descent. Whilst providing 95% cure, a cervical incision with often extensive dissection was required. Ectopic glands were either missed, or encountered only after lengthy exploration. Single gland disease should now be localised by sestamibi scan in the majority of cases. If in the neck, MIP will result in a 97% cure. If mediastinal, then a focused direct exploration can be undertaken as the initial procedure. Multiple gland disease is usually associated with a negative sestamibi scan, selecting out such patients for open exploration. With this approach, up to 70% of cases of primary HPT can now be performed as a day only, minimally invasive procedure. The availability of MIP, associated with an increasing awareness of the potential adverse outcomes of untreated hyperparathyroidism, has led to a marked increase in parathyroid surgery worldwide, with the recent AACE/AAES guidelines now recommending parathyroidectomy for all patients. There are however potential pitfalls with MIP. Multiple gland disease with enlarged but apparently non-functioning parathyroids is well documented, and these will be missed at MIP. While there has been no medium-term increase in persistent or recurrent hyperparathyroidism, this is likely to emerge as a potential problem long-term. Our own studies of perifusion of parathyroid cells have demonstrated considerable variability in PTH secretion by enlarged parathyroid glands, supporting the apparent discrepancy between size and function.

IP10 7.9.05: 1630 – 1700 hours

THERAPEUTIC APPROACHES TO METASTATIC BONE DISEASE

BF Boyce[1], L Xing[1], Y Wang[2], R Sundaramoorthi[2], T Keenan[2], W Shakespeare[2], C Metcalf[2], R Bohacek[2], MR vanSchravendijk[2], Z Yao[1], T Yamashita[1], D Dalgarno[2], J Iuliucci[2] & T Sawyer[2]
[1] *University of Rochester Medical Center, Rochester, NY, USA*
[2] *ARIAD Pharmaceuticals, Cambridge, MA, USA*

c-Src tyrosine kinase has been implicated in multiple signaling pathways that regulate cell growth, migration and survival and is over-expressed in a variety of human tumors. It is activated following integrin adhesion and downstream of tyrosine kinase growth factor and G-protein coupled receptors as well as RANK (Receptor Activator of NF-κB), whose expression is required in osteoclasts for osteoclast formation, activation and survival. Thus, src is a potential therapeutic target for several diseases, including osteoporosis, bone metastases and cancer. We generated a number of purine-based src inhibitors to investigate this possibility and targeted them to bone with a phosphonate group that lacked intrinsic anti-osteoclast activity to attempt to minimize the risk of unwanted side-effects. AP23451, a lead compound, inhibited src kinase activity with high specificity and prevented PTH-induced hypercalcemia and bone resorption as well as

ovariectomy-induced bone resorption and bone loss. It prevented the development of osteolytic metastases as effectively as Zometa when given daily to nude mice inoculated with MDA-231 human breast cancer cells by intracardiac injection, and also significantly reduced tumor cell volume in the fore- and hind-limbs of the animals, which Zometa did not do. Like Zometa, AP23451 inhibited the invasion of MDA-231 cells through Matrigel in vitro. Our findings suggest that src kinase inhibitors, such as AP23451, could have dual effects to prevent osteolysis and the growth of metastases in patients with cancers that metastasize to bone and inhibit bone resorption in conditions characterized by increased bone resorption.

IP11 7.9.05: 1700 – 1730 hours

TARGETING OF BONE CANCER WITH APO2L/TRAIL

A Evdokiou
Department of Orthopaedics and Trauma, The University of Adelaide, Royal Adelaide Hospital, Hanson Institute, Adelaide, SA

To examine the *in vivo* antitumour effects of Apo2L/TRAIL we used a mouse model in which MDA-MB-231-TXSA human breast cancer cells are transplanted orthotopically into the tibiae of athymic mice. Untreated animals transplanted with breast cancer cells all developed large lesions that invaded the marrow cavity and eroded the cortical bone, as assessed by radiography, micro computed tomography (mCT) and histology. In contrast, animals treated i.p with 30 mg/kg/dose of Apo2L/TRAIL for five consecutive days followed by once weekly for 4 weeks, showed dramatic conservation of the tibiae and tumour burden reduced by ten-fold. Although tumour cells persisted in the marrow cavity of Apo2l/TRAIL treated mice, the tumours were significantly smaller and were confined to the site of transplantation. The presence of tumour cells in the Apo2L/TRAIL treated animals may be an indication that the therapy and dosing is insufficient or that Apo2L/TRAIL treatment results in the selection of Apo2L/TRAIL resistant clones. We have tested this hypothesis by isolating tumour cells from the bones of Apo2L/TRAIL-treated animals to assess their resistance to Apo2L/TRAIL *in vitro*. Indeed, cancer cells explanted from Apo2L/TRAIL-treated animals were significantly more resistant to the effects of Apo2L/TRAIL when compared to cells explanted from the untreated animals. However, this resistance was readily reversed when Apo2L/TRAIL was used in combination with other agents, including chemotherapeutic drugs such as taxol and etoposide or the histone deacetylase inhibitor SAHA. Our data suggests that while Apo2L/TRAIL monotherapy may be an effective treatment for bone cancer, the acquisition of Apo2L/TRAIL resistance *in vivo* is of concern. Therefore, a combinatorial approach with other therapeutics may be required to ensure the complete eradication of cancer cells from the bone microenvironment.

IP12 7.9.05: 1730 – 1800 hours

PROSPECTIVE ISOLATION OF MESENCHYMAL STEM CELLS IN MAN AND MOUSE

P Simmons
Peter MacCallum Cancer Centre, Stem Cell Biology, East Melbourne, VIC

Mesenchymal stem cells (MSC) represent a second population of bone marrow (BM)-resident stem cells in adult mammalian bone marrow. There is considerable interest in the use of MSC as a cellular therapy for the treatment of not only of musculoskeletal defects and diseases but also in a range of other clinical applications such as cardiovascular repair. In contrast to the well-defined cell and molecular features of hematopoietic stem cells (HSC), the corresponding properties of MSC are much less well understood. Much of our current knowledge of MSC has been gained through in vitro assays and culture manipulations. In defining MSC by their *in vitro* properties much remains unknown about the cellular identity, ontogeny and anatomical location of MSC in the marrow in vivo. In addition, a physiological role for MSC has not been established. Seeking to address these issues we have sought to develop methodologies to prospectively isolate MSC in highly enriched form from primary hematopoietic tissues in order to explore the biological properties of these cells in an unmanipulated state, unaltered by culture epiphenomena. For the isolation of MSC from human BM antibody STRO-1 has proved to be a key reagent in purifying these rare cells. In addition, culture conditions have been developed to allow the *ex vivo* expansion of MSC under cGMP compliant conditions. Recent studies have culminated in the first successful prospective isolation of MSC from murine bone marrow. Notably, these studies indicate that bone tissue is the major reservoir of MSC in the mouse.

IP13 8.9.05: 0830 – 0910 hours

THE CALCIUM SENSING RECEPTOR (CaSR) AND ITS SEVEN CLINICAL DISORDERS

RV Thakker
University of Oxford, Nuffield Department of Clinical Medicine, OCDEM, Churchill Hospital, Headington, Oxford, OX3 7LJ, UK

The human calcium-sensing receptor (CaSR) is a 1,078 amino acid cell surface protein, which is predominantly expressed in the parathyroids and kidney, and is a member of the family of G protein-coupled receptors. The CaSR allows regulation of parathyroid hormone (PTH) secretion and renal tubular calcium reabsorption in response to alterations in extracellular calcium concentrations. The

human CaSR gene is located on chromosome 3q21.1 and loss-of-function CaSR mutations have been reported in the hypercalcaemic disorders of familial benign (hypocalciuric) hypercalcaemia (FBH or FHH) and neonatal severe primary hyperparathyroidism (NSHPT). However, some individuals with loss-of-function CaSR mutations remain normocalcaemic. In addition, there is genetic heterogeneity amongst the forms of FHH. Thus, the majority of FHH patients have loss-of-function CaSR mutations, and this is referred to as FHH type 1. However, in one family, the causative gene for FHH is located on 19p13, referred to as FHH type 2, and in another family it is located on 19q13, referred to as FHH type 3. Gain-of-function CaSR mutations have been shown to result in autosomal dominant hypocalcaemia with hypercalciuria (ADHH), Bartter's syndrome type V, and idiopathic hypercalciuria. CaSR auto-antibodies have been found in FHH patients who did not have loss-of-function CaSR mutations, and in patients with an acquired form (i.e. autoimmune) of hypoparathyroidism. Thus, abnormalities of the CaSR are associated with 3 hypercalcaemic, and 3 hypocalcaemic disorders, and one normocalcaemic disorder associated with hypercalciuria.

Reference:
Thakker RV (2004). Diseases associated with the extracellular calcium-sensing receptor. Cell Calcium 35: 275-282.

IP14 8.9.05: 0910 – 0940 hours

CLINICAL IMPLICATIONS OF THE CALCIUM-SENSING RECEPTOR'S MULTIPLE SENSING DOMAINS

AD Conigrave
School of Molecular and Microbial Biosciences, University of Sydney, NSW and Department of Endocrinology, Royal Prince Alfred Hospital, Camperdown, NSW

Structure-function analysis of the CaR indicates that it has multiple sensing domains and responds to multiple sensing modalities including Ca^{2+}, other divalent and polyvalent cations, L-amino acids, pH, ionic strength as well as calcimimetics and calicilytics. The locations of some of the sensing domains are now clear. Thus, L-amino acids bind in the Venus Fly Trap (VFT) domain of the CaR as previously described for metabotropic glutamate receptors. On the other hand, phenylalkylamine calcimimetics and calcilytics bind in the transmembrane domain region. Unlike amino acids and phenylalkylamines, Ca^{2+} appears to bind at several sites in both the VFT domain and transmembrane domain regions. A recent site-directed mutagenesis study indicates that the Ca^{2+} binding sites in the VFT domain can be clearly distinguished from those for L-amino acids.

What are the clinical implications of the CaR's multiple sensing domains? Firstly, because of its central role in the control of calcium homeostasis, the CaR is a target for drug therapy that has led in a short space of time to two novel classes of therapeutics: calcimimetics and calcilytics. The recently developed pharmacotherapeutics (e.g., cinacalcet) target the phenylalkylamine binding site in the receptor's seven transmembrane domain region. However, even newer classes of activators and inhibitors that target the receptor's amino acid binding site are feasible and have the potential to modify the effects of agents that bind in the seven transmembrane domain region. Secondly, depending on the location of a specific mutation, mutations of the CaR, which underlie various clinical disorders including familial hypocalciuric hypercalcemia (FHH) and autosomal dominant hypocalcemia (ADH), respond differently to different activators. Thus, the behaviours of some CaR mutations are modified, even rescued, by CaR-active amino acids or calcimimetics. Finally, the impact of compartment-specific variations in CaR modulators such as L-amino acids, pH and ionic strength may help to explain phenotypic variations between different kindreds with FHH. CaRs in the luminal membrane of the pancreatic duct, for example, are exposed to much lower amino acid concentrations than found in systemic blood. Thus CaR mutants may be rescued in some locations but seriously disabled in others leading to a severe tissue-specific phenotype (e.g., pancreatitis) on a background of mild hypercalcemia.

IP15 8.9.05: 1305 – 1335 hours

THE ROLE OF BONE STRENGTH IN OSTEOPOROSIS

P Sambrook
University of Sydney, Sydney, NSW

Bone mineral density (BMD), usually measured by dual energy x-ray absorptiometry, measures the amount of bone mineral in a projected area (or areal density) and has been shown to correlate well with whole bone strength. Although a good predictor of fracture risk, BMD does not distinguish specific attributes of three dimensional bone geometry such as the size and shape of bone, cortical versus trabecular components, microarchitecture or the intrinsic properties of the bone matrix. The importance of non-BMD bone related factors in fracture risk is also emphasised by the observation from epidemiological studies that the presence of a prior fracture increases the risk of further fracture independently of BMD. Similarly, only a relatively small component of the fracture risk reduction explained by anti-resorptives can be explained by the BMD gain.

Because of these limitations of BMD, there has been increasing interest in these other determinants of bone strength. These non-BMD factors are sometimes called 'bone quality'. Ultimately, bone strength is a function of the structural and material properties of bone. The structural properties include its geometry and microarchitecture whereas the material properties include its mineral and collagen components. Because bone undergoes continuous renewal by the process of coupled bone resportion and formation, so called bone remodelling, this process may also the influence the structural and material properties of bone and so affect bone strength. Indeed, there

may exist a threshold of reduction in bone resorption with bisphosphonates below which no further decrease in vertebral fracture risk was observed.

Markers of bone turnover are currently available and methods for measuring the structural and material properties of bone (such as MRI and uCT) will gradually become available to assess these non BMD factors that determine bone strength.

IP16 8.9.05: 1335 – 1425 hours

SURROGATE MARKERS: HOW MUCH DO THEY REALLY TELL US ABOUT FRACTURE RISK REDUCTION?

R Eastell
Academic Unit of Bone Metabolism, University of Sheffield, Sheffield, UK

We have excellent data from a number of large randomised controlled trials in osteoporosis that fractures are reduced by a number of therapies. It is appropriate the fracture is the endpoint for such trials. However, if we had surrogates for fracture risk reduction, this would help us in the management of the individual patient and it would help us to design either shorter trials, or at least trials of equivalent when testing new products. This would accelerate the development of new treatments. Bone mineral density has been used a surrogate marker for fracture. This test performs well in the prediction of fractures and is currently the basis for the definition of osteoporosis. However, the proportion of the fracture risk reduction explained by the change in bone mineral density is only about 10%, and fracture risk is reduced long before the increase in bone mineral density is complete [1]. Bone turnover markers have been proposed as a surrogate marker for fracture. This test hasn't performed consistently well in the prediction of fractures. However, the proportion of the fracture risk reduction explained by the change in bone turnover markers appears to be more than 50%, at least for anti-catabolic therapies such as the bisphosphonates risedronate [2] and alendronate [3] and the SERM, raloxifene [4]. It is likely that the change in bone turnover markers is indicating that there are fewer stress risers acting as weak points on the surface of bone, and that the probability of plate perforation is decreased, all this resulting in preservation of micro-architecture of trabecular bone. We still need to learn more about whether bone turnover markers are appropriate surrogates when stopping therapy – does the increase in bone resorption markers when stopping HRT indicate that the fracture benefit is lost? We still need to learn more about the use of bone turnover markers as surrogates for fracture risk reduction with anabolic therapy. In the meantime, bone turnover markers allow us to assess the individual's response to therapy, and may allow us to identify effective new treatments for osteoporosis.

References:

[1] Riggs BL, Parfitt AM. Drugs used to treat osteoporosis: the critical need for a uniform nomenclature based on their action on bone remodeling. J Bone Miner Res 2005; 20: 177-184.

[2] Eastell R, Barton I, Hannon RA, Chines A, Garnero P, Delmas PD. Relationship of early changes in bone resorption to the reduction in fracture risk with risedronate. J Bone Miner Res 2003; 18: 1051-1056.

[3] Bauer DC, Black DM, Garnero P, Hochberg M, Ott S, Orloff J, Thompson DE, Ewing SK, Delmas PD. Change in bone turnover and hip, non-spine, and vertebral fracture in alendronate-treated women: the fracture intervention trial. J Bone Miner Res 2004; 19: 1250-1258.

[4] Sarkar S, Reginster JY, Crans GG, ez-Perez A, Pinette KV, Delmas PD. Relationship between changes in biochemical markers of bone turnover and BMD to predict vertebral fracture risk. J Bone Miner Res 2004; 19: 394-401.

IP17 8.9.05: 1530 – 1600 hours

CHONDROCALCINOSIS AND ANKYLOSING SPONDYLITIS – TWO SIDES OF THE PYROPHOSPHATASE COIN

M Brown
University of Oxford, Botnar Research Centre, Oxford, UK

Inorganic pyrophosphate (PPi) PPi is a natural inhibitor of tissue mineralisation, the deposition of calcium hydroxyapatite crystals. Excess extracellular PPi (ePPi) leads to formation of calcium pyrophosphate dihydrate (CPPD) crystals as well as inhibition of calcium hydroxyapatite crystal formation, and conversely, reduced ePPi levels favour calcium hydroxyapatite formation, leading to ectopic mineralisation.

CPPD chondrocalcinosis is present in over 50% of joints in patients undergoing total hip replacement and is thought to accelerate the rate of joint degeneration in osteoarthritic cartilage. Genetic studies of this condition have demonstrated that different mutations in genes influencing PPi transport and metabolism are causally associated with the development of the condition. Functional studies of those variants suggest that these mutations may also influence chondrocyte maturation/differentiation, and cause CPPD deposition through influences on PPi levels and other mechanisms.

Ectopic mineralization is an important pathogenic factor in a variety of diseases including the common inflammatory arthritis, ankylosing spondylitis (AS). This condition is known to be highly heritable, but the key factors involved in the risk of developing AS are different from those involved in determining its severity and the rate of joint ankylosis which inevitably complicates the condition. Mouse studies and studies in a related disorder, ossification of the posterior longitudinal ligament of the spine, implicate disturbed ePPi levels in the

pathogenesis of spinal fusion in AS, and point to the PPi metabolism and transport pathways as potential therapeutic targets in the condition.

IP18 8.9.05: 1530 – 1600 hours

REGULATING PATHWAYS SUBSERVING OSTEOBLAST FUNCTION

J Cornish
Department of Medicine, University of Auckland, Auckland, New Zealand

Our knowledge in bone biology has exploded over the last decade with information on skeletal development derived from 'experiments of nature' in man and genetic manipulations in mice. Indeed, studies on bone development have revealed roles for peptides such as BMP / TGFβ family members, FGF, sclerostin and Wnt; obligatory transcription factors for osteoblastogenesis such as Runx2/Cbfa1, osterix, and LEF/TCF family; and signal transducing molecules such as tyrosine kinases, MAP kinases, β-catenin and Smads. All these factors, along with many others, are involved in bone cell fate determination, differentiation, and formation of mature bone. Dysfunction of these obligatory factors leads to pathology. In contrast many important cytokines and growth factors that modulate osteoblast function do not produce a phenotype, owing to the redundant or compensatory mechanisms.

Recent research involving BMP proteins has accentuated the importance of the regulatory network involving the interplay between the BMPs and the soluble BMP antagonists. SOST is a novel BMP antagonist and negative regulator of bone formation expressed in osteogenic cells. Sclerostin, the protein product of SOST, binds to BMPs and modulates the activity of the osteoblasts.

Cbfa1 is a key regulator of osteoblast differentiation, the genetic pathway of which is still incompletely elucidated, and it activates promoters of various genes specifically expressed in osteoblasts. Cbfa1 expression is regulated by products of at least three genes, including further transcription factors. Osterix is a novel zinc finger-containing transcription factor in osteoblast progenitors whose expression appears to be controlled by Cbfa1.

Evidence also exists that Cbfa1-independent pathways control osteoblast differentiation or proliferation. Recent studies demonstrate low-density lipoprotein receptor related-protein (LRP5) is a regulator of bone mass. Wnt proteins binding to frizzled-LRP5 receptor complex affect osteoblast proliferation. β-catenin is a downstream mediator of canonical Wnt signalling that forms a transcription-regulating complex with TCF/LEF transcription factors.

This smorgasbord of novel factors illustrates the complexity of bone mass regulation and provides new potential therapeutic avenues to treat osteopenia, or osteoporosis.

IP19 9.9.05: 0900 – 0945 hours

CYTOKINE REGULATION OF OSTEOCLAST FORMATION THROUGH NF-κB, C-FOS AND NFATS

T Yamashita[1], Z Yao[1], L Xing[1], F Li[1], Q Zhang[1], IR Badell[1], K Matsuo[2] & BF Boyce[1]
[1] *Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, NY, USA*
[2] *Keio University, Tokyo, Japan*

NF-κB, c-Fos and NFAT are transcription factors which are activated in immune cells and in most other cell types following stimulation by a variety of factors, including cytokines, growth factors and hormones. They regulate the expression of a large number of genes, including cytokines, and are activated in osteoclast precursors after RANKL, IL-1 or TNF bind to their respective receptors. However, of these cytokines, only RANKL is required for the induction of osteoclast formation in vivo during skeletal development. Nevertheless, it is likely that IL-1, TNF and other cytokines participate in the up-regulation of osteoclast formation seen in a variety of pathologic conditions that affect the skeleton in which cytokine production is increased, including estrogen deficiency and inflammatory bone diseases. Our studies indicate that following RANKL/RANK interaction in osteoclast precursors there is sequential activation of NF-κB, followed soon by c-Fos and later by NFAT1 and 2. Retroviral over-expression of NFAT2, but not NF-κB or c-Fos, in splenic osteoclast precursors induces osteoclast formation in the absence of cytokine treatment, while RANKL, IL-1 and TNF can augment this effect and induce osteoclast formation in the absence of NF-κB when c-Fos is over-expressed. IL-1 and TNF induce c-Fos expression in osteoclast precursors. Our findings suggest that in inflammatory conditions affecting bone, IL-1 and TNF could induce osteoclast formation without the requirement for increased RANKL signaling by inducing c-Fos and NF-κB signaling in osteoclast precursors and initiating self up-regulatory cycles in which NF-κB induces more cytokine expression and aggressive bone resorption.

IP20 9.9.05: 0945 – 1015 hours

PAGET'S DISEASE – GENES, HYPERPARATHYROIDISM, BISPHOSPHONATES, BMD AND FRACTURE RESPONSES

DH Gutteridge
Endocrinology & Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA

Sir James Paget's superb 1876 description of his disease remains valid in 2005; his title "chronic inflammation" less certain. **Mutations** in the SQSTM1 gene have been identified in up to 50% of patients with late-onset familial PD and in 10-30% of patients with sporadic PD. What is the role of genetic counselling?

In **assessment**, concurrent primary hyperparathyroidism, present in ~ 4% of PD, should be excluded. Though likely a chance association, the pagetic biochemical response to parathyroid surgery is predictable, linear, related to pre-operative plasma Ca ($p<0.01$); and may be profound.

In **management**, analgesics, rheumatology (splints, heel-raising, local steroids) and orthopaedics (joint replacement, wedge resection) have a potential role- as do potent bisphosphonates (BIS), to reduce bone turnover, and bone pain. Based on clinical trial results, approximate BIS equivalents are proposed – IV zoledronate 5mg (15 mins), IV pamidronate 120 mg (6 hrs), oral alendronate 40 mg/d 3 mths, oral risedronate 30 mg/d 3 mths. Assessment, and re-treatment based on biochemistry, 3 monthly, has proven useful. Febrile acute-phase responses are greater with IV drugs. Acquired resistance to one BIS does not limit the response to another

BMD responses– IV pamidronate produces profound increases in PD BMD at spine (20%) and hip (10%) at 6-12 months. Increases in non-PD bone at these sites are much less (4% and 1%). Post-BIS PTH-related forearm bone loss, cortical and trabecular, occurs in moderate and severe PD, and is preventable by calcitriol and calcium. Reliable data on the **fracture** (#) **responses** to BIS are limited, but suggest significant ($p = 0.003$) prevention in PD #s with a NS response in non PD, largely osteoporotic, #s – perhaps reflecting the BMD responses, and/or preferential uptake of BIS at PD sites.

Collaborators – the Paget's Disease Research Group of WA.

IP21 9.9.05: 1145 – 1215 hours

THE BURDEN OF FRACTURE

JA Pasco
The University of Melbourne, Department of Clinical and Biomedical Sciences: Barwon Health, Geelong, VIC

In excess of 100,000 fractures occur in Australia each year, contributing substantially to the $7.4b annual cost of osteoporosis. 30-50% of women and 15-20% of men suffer the consequences of fracture, which contribute significantly to mortality and morbidity in both sexes. The financial and human costs are set to escalate as the population ages.

Diagnosed fracture rates (persons/10,000/year) in Australian women and men are highest at the hip (28, 10), spine (21, 7), wrist (18, 4) and humerus (11, 3) and are 3-4 fold higher in women. Almost all hip and a third of non-hip fracture cases are hospitalized resulting in 64,500 admissions annually.

Survival is reduced after fragility fracture in all age-groups, more so in men. All fractures impact on well-being; most cause loss of confidence and independence. Nearly half of women need help with ADLs for several weeks. After 6mo, 3% of all, 20% of hip, 13% of humeral and 5% of spine fracture cases require assistance with personal care. After 12mo, 30% with any and 90% with hip fracture have not regained pre-fracture mobility. Half of hip fracture patients require long-term nursing.

BMD, age and prevalent fracture contribute independently to fracture risk. Although risk is highest for women with osteoporosis, only 27% of fractures arise from this group (osteopenia 56%, normal BMD 17%). Reducing the population burden of fractures requires attention to women with osteopenia, as well as osteoporosis, because over half the fractures arise in these individuals and osteopenia-plus-prevalent-fracture confers the same risk of fracture as osteoporosis.

IP22 9.9.05: 1145 – 1215 hours

INTRACELLULAR VESICLE TRANSPORT IS REQUIRED FOR BONE RESORPTION

NJ Pavlos
Unit of Orthopaedics, School of Surgery and Pathology, University of Western Australia, Perth, WA

Osteoclasts require strict ordering of intracellular membrane trafficking to sustain their unique structural and functional polarization during bone resorption. While it has become increasingly evident that multiple vesicular transport pathways regulate specific stages of the resorptive process, the molecular mechanisms driving these energetic events remain unclear. Accumulating evidence now hints that members of the small ras-related Rab GTPase family, established components of membrane trafficking machinery, play crucial roles in osteoclast function. Like other GTPases, Rabs act as molecular switches, cycling between GTP/GDP-bounds states, enabling them to recruit specific effector complexes through which they elicit their biological functions. Several Rabs have recently been identified in osteoclasts, each associating with a distinct vesicle trafficking step. Of these, Rab3D has been shown modulate a post-trans-Golgi pathway that is required for osteoclastic bone resorption. Mice lacking Rab3D manifest a phenotype of osteosclerosis due to dysfunctional osteoclasts. Consistently, overexpression of dominant-inhibitory Rab3D mutants in osteoclasts impairs bone resorption in vitro. In search of candidate Rab3D effector molecules, we have successfully identified a novel interaction between the N-terminus of Rab3D and Tctex-1, a light chain of the multimeric cytoplasmic dynein complex. Biochemical studies confirmed the specific binding of Rab3D with Tctex-1. In addition, our data indicate that this interaction is both GTP- and microtubule-dependent suggesting that Rab3D-mediated vesicle transport is regulated by the dynein motor complex thus controlling the microtubule-dependent targeting of post-Golgi vesicles to the osteoclastic ruffled border. Unraveling osteoclast vesicle transport pathways may uncover potential new drug targets for the treatment of bone diseases.

O – ORAL PRESENTATIONS

O1 8.9.05: 0940 – 0950 hours

HIGH BONE TURNOVER PREDICTS MORTALITY IN THE FRAIL ELDERLY: A PROSPECTIVE COHORT STUDY

PN Sambrook[1], C Chen[1], L March[1], I Cameron[2], R Cumming[3], J Simpson[3] & MJ Seibel[4]
[1] Institute of Bone and Joint Research, Royal North Shore Hospital, Sydney, NSW
[2] Rehabilitation Studies Unit, [3] School of Public Health, University of Sydney, NSW
[4] ANZAC Research Institute, University of Sydney, NSW

Background:
Osteoporotic fractures are associated with accelerated bone turnover and excess mortality. In a prospective cohort study of 1112 elderly subjects, we assessed whether the rate of bone turnover is a direct predictor of mortality.

Methods:
Baseline data from elderly subjects (mean ± SD age: 86 ± 6.9 yrs; 21% male) living in residential care facilities were analysed. Parameters included: age, gender, co-morbidity, incident hip fractures, serum aminoterminal propeptide of type I collagen (PINP), carboxyterminal telopeptide of type I collagen (CTX-I), intact parathyroid hormone (PTH), serum 25 hydroxyvitamin D (25OHD). Serum calcium, phosphate and creatinine were measured in a randomly selected subgroup of 448 subjects (40%).

Results:
Over a mean ± SD follow-up of 782 ± 414 (range: 4 - 1883) days, a total of 517 (46.5%) subjects died. In univariate analyses, time to death was significantly ($p < 0.05$) associated with age (HR 1.64, per 10 yrs), gender (HR 1.32, male vs. female), comorbidity (HR 0.31, mild symptoms vs. severe illness), hip fracture (HR 2.36, yes vs no), log serum creatinine (HR 1.81), log PTH (HR 1.26), log CTX (HR 1.44) and log PINP (HR 1.33). These associations remained unchanged when adjusted for serum creatinine levels. Mortality rates/ person/ year continuously increased with increasing serum CTX or PINP concentrations (highest quintile vs. lowest quintile, CTX: 31.4 vs 17.6%, PINP: 30.1 vs. 19.5%). Similarly, the rate of hip fractures increased with increasing serum CTX-I, but not with PINP levels. In multivariate analyses adjusting for age, gender, comorbidity, 25OHD, PTH and hip fracture status, both bone turnover markers remained significantly associated with all cause time to death: log CTX, HR 1.21 (95%CI 1.05-1.40, $p = 0.01$), log PINP, HR 1.21 (95%CI 1.05-1.40, $p = 0.007$). For individual causes of death, bone turnover markers were associated with deaths from cardiac and cerebrovascular causes, but not infections.

We conclude that in the frail elderly, high bone turnover predicts death independent of age, gender, comorbidity, renal function, serum PTH levels and hip fracture status.

Disclosures:
All authors, 1

O2 8.9.05: 0950 – 1000 hours

IL-11 AND IL-6 STIMULATION OF OSTEOCLAST FORMATION DEPENDS ON STAT- BUT NOT ERK-MEDIATED PATHWAYS IN OSTEOBLASTS

JMW Quinn, A Nakamura, BJ Jenkins, NA Sims, M Ernst, TJ Martin & MT Gillespie
St. Vincent's Institute, Fitzroy, VIC, The Ludwig Institute of Cancer Research, Melbourne, VIC and Department of Medicine at St. Vincent's Hospital, The University of Melbourne, Fitzroy, VIC

The gp130 cytokines IL-11 and IL-6 play important roles in osteolysis. Both cytokines stimulate RANKL production by osteoblasts (OB), thereby triggering osteoclast formation. Several osteolytic factors also induce IL-11 in OB, resulting in an autocrine stimulus. As two gp130 signaling pathways are known, involving STAT1/3 and SHP2/ras/ERK, we aimed to determine their roles in osteoclast formation.

We employed cells from two knock-in mouse strains: $gp130^{deltaSTAT/deltaSTAT}$ (d-STAT), which express truncated gp130 incapable of signalling via STAT1/3, and $gp130^{Y757F/Y757F}$ (Y757F), which cannot signal via SHP2/ras/ERK. When bone marrow cells from these mice were stimulated by recombinant RANKL and M-CSF, d-STAT bone marrow formed similar numbers of osteoclasts to wild type (WT); Y757F bone marrow yielded two-fold more. IL-6 and IL-11 powerfully inhibited RANKL-stimulated osteoclast formation in Y757F bone marrow but had little effect on d-STAT and WT cultures. Coculture of WT bone marrow cells with OB from WT, Y757F or heterozygous $gp130^{deltaSTAT/+}$ mice resulted in formation of numerous osteoclasts in response to 1,25 dihydroxyvitamin-D3, IL-11 and IL-6, although for IL-6 the addition of IL-6 soluble receptor (IL-6sR) was required. In cocultures using d-STAT OB, IL-11 and IL-6+IL-6sR no osteoclast differentiation occurred, and the stimulus provided by 1,25 dihydroxyvitamin-D3 was greatly diminished, consistent with ablation of autocrine IL-11 action. ERK pathway blockade by PD98059 did not rescue d-STAT OB supported osteoclast formation.

We conclude that gp130 STAT1/3 signals in osteoclast progenitors reduce osteoclast differentiation, but gp130 STAT1/3 signals in OB are essential for IL-6 and IL-11 stimulation of osteoclast formation in co-cultures.

Disclosures:
1

O3 8.9.05: 1000 – 1010 hours

AC45, A V-ATPASE ACCESSORY SUBUNIT INTERACTS WITH VO DOMAIN SUBUNITS AND IS NECESSARY FOR OSTEOCLASTIC BONE RESORPTION

T Cheng[1], H Feng[1], KHM Yip[1], A Carrello[1], R Seeber[2], KM Kroeger[2], K Eidne[2], MH Zheng[1] & J Xu[1]
[1] *Molecular Orthopaedic Laboratory, Orthopaedics Unit, School of Surgery and Pathology,* [2] *Western Australia Institute for Medical Research, University of Western Australia, Nedlands, WA*

Solubilization of bone mineral by osteoclasts is dependent on the acidification of the extracellular resorption lacuna by means of a multimeric vacuolar type proton pump (V-ATPases). Besides such specialized function the V-ATPases is also essential for acidification of diverse intracellular compartments that includes the Golgi apparatus, endosomes, lysosomes and secretory granules. The core structure of V-ATPases comprises of two functionally and structurally distinct domains, V_1 and V_0. The peripheral cytoplasmically oriented V_1 domain is responsible for ATP hydrolysis which provides the energy for the translocation of protons across the integral membrane bound V_0 domain. Here, we have identified an accessory subunit, Ac45 that interacts with the V_0 domain and is involved in V-ATPase-mediated function in osteoclasts. Ac45 was localized to the ruffled border region of polarized resorbing osteoclasts and partially colocalized with pH-dependent transferrin receptor and lysotracker, marker of lysosomal structures. Furthermore using bioluminescence resonance energy transfer (BRET) assays, we showed that Ac45 interacts with subunits a_3, c, c" and the newly identified e, but not d of the V_0 domain. Deletion of the 26 residue-cytoplasmic tail (aa437-463) of Ac45 distorted the interaction of Ac45 with subunits of V_0 domain. Osteoclasts over-expressing the deletion mutant of Ac45 were smaller in size, exhibited an increase of intracellular pH and had impaired bone resorption capability. Currently, retroviral-based RNA silencing (siRNA/RNAi) is being developed to study the effect of Ac45 gene expression knockdown in osteoclasts. To conclude, our data suggest that Ac45 is involved in V_0 domain interactions, acidification and bone resorption.

O4 8.9.05: 1010 – 1020 hours

CALCIUM SUPPLEMENTATION FOR IMPROVING BONE MINERAL DENSITY IN CHILDREN: SYSTEMATIC REVIEW

T Winzenberg[1], K Shaw[2], J Fryer[1] & G Jones[1]
[1] *Menzies Research Institute, Hobart, TAS*
[2] *Dept of Health and Human Services, Hobart, TAS*

Trials of calcium supplementation in children have given inconsistent results particularly as to whether any benefit persists after supplementation is ceased. We performed a systematic review of randomised placebo-controlled trials of calcium supplementation in healthy children with measurement of bone mass at any site as an outcome. We searched multiple databases including Medline and Embase and used hand-searching to identify 232 potential studies. Assessment by 2 independent reviewers, yielded 34 references to 18 studies. Of these, 16 provided data which could be used in meta-analysis. Results are given below:

Site	Effect at end of trial[1]	Effect at longest point after cessation of supplementation
spine	0.12 (0.00, 0.25)[2] (n=948)	-0.01 (-0.18, 0.16) (n=531)
hip	0.12 (-0.01, 0.26)[3]	0.02 (-0.15, 0.19) (n=531)
total body	**0.20 (0.06, 0.34)** (n=802)	0.00 (-0.40, 0.40)[4] (n=96)
arm	**0.28 (0.17, 0.40)** (n=1372)	**0.19 (0.04, 0.35)** (n=754)

[1]standardised mean difference (SMD) (95% CI); an SMD of 0.3 is regarded as small. Bold de statistical significance.
[2]p=0.06 [3]p=0.07 [4]single study only

There was no significant heterogeneity at any site.

In conclusion, calcium supplementation has little effect on BMD at the hip or lumbar spine. Total body bone mass increased during supplementation but this effect does not persist. Upper limb bone mass increases with supplementation and two thirds of this effect persists after cessation. The differences between the sites are difficult to explain biologically. Taken as a whole, this overview suggests calcium supplementation in childhood as a measure for improving long-term bone density is of marginal benefit at best.

Disclosures:
1.

O5 8.9.05: 1020 – 1030 hours

GLOBAL KNOCK-DOWN OF THE CALCITONIN RECEPTOR (CTR) IN YOUNG FEMALE MICE RESULTS IN DECREASED TRABECULAR BONE VOLUME

RA Davey[1], JF McManus[1], AJ Moore[2], WSM Chiu[1], AM Axell[1], AJ Notini[1], HA Morris[2], DM Findlay[3] & JD Zajac[1]
[1] *Department of Medicine, University of Melbourne, Austin Health, Heidelberg, VIC*
[2] *Hanson Institute, IMVS, Adelaide, SA*
[3] *Orthopaedics and Trauma, University of Adelaide, SA*

To investigate the role of calcitonin (CT) in bone turnover, we have generated a global calcitonin receptor (CTR) knock-down (KD) mouse model using the Cre-loxP system. We estimate that the extent of CTR deletion in the CTR KDs is >90% at the mRNA level compared to controls.

Male and female heterozygous (het) CTR-lox controls, het CTR KDs and homozygous (hom) CTR KDs were studied at 6 and 12 weeks of age. Trabecular bone volume (BV/TV %) was decreased in female hom CTR KDs at 6 weeks of age ($P<0.05$). Trabecular number was decreased ($P<0.05$), while trabecular thickness and mineral apposition rate were unaffected, suggesting increased bone resorption. Osteoclast surface was unchanged in female hom CTR KDs, suggesting that the increased bone resorption may be due to increased osteoclast activity and/or lifespan with no change in the number of osteoclasts. Serum calcium, total protein and intact PTH levels were unchanged in female CTR KD mice. No changes in any of the bone histomorphometric variables were observed in hom CTR KD males at 6 weeks of age. However, serum calcium was decreased in male hom CTR KD mice compared to controls ($P<0.05$), while serum total protein and intact PTH levels were unaffected.

In conclusion, we have demonstrated that global knockdown of the CTR results in decreased trabecular bone volume in young female mice which is associated with increased bone resorption. These data suggest that the role calcitonin plays in regulating bone cell turnover is greater in young female mice than males.

Disclosures:
1.

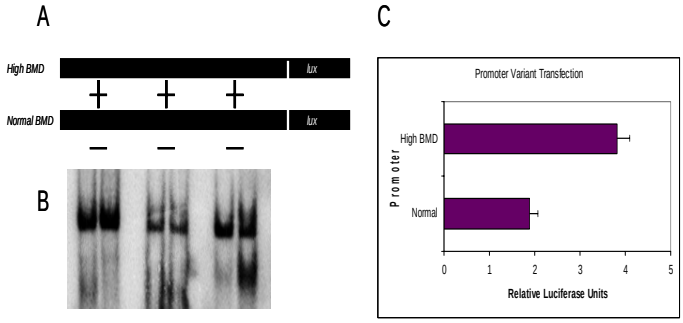
O6 8.9.05: 1100 – 1115 hours

DIFFERENTIAL ACTIVITY OF HUMAN RUNX2 P2 PROMOTER ALLELES ASSOCIATED WITH AN ALLELE-SPECIFIC DNA-PROTEIN INTERACTION

JD Doecke[1], CJ Day[1], AJ Stephens[1], G Nicholson[2] & N Morrison[1]
[1] *School of Medical Science, Griffith University, Gold Coast Campus, Gold Coast, QLD*
[2] *Clinical and Biomedical Health Sciences - Barwon Health, The University of Melbourne, The Geelong Hospital, Geelong, VIC*

We hypothesized that by taking subjects from the extremes of a population, we would specifically find alleles related to the bone mineral density trait. We tested this idea using RUNX2, the well known osteoblast transcription factor. From a population of 1300 subjects, the age-weight adjusted femoral neck bone mineral density (BMD) was ranked and the upper and lower deciles taken to represent the adjusted extremes. In these 260 subjects, we identified 19 allelic variations within the RUNX2 gene and promoter (P1 and P2), and characterized these novel variations with respect to BMD strata by genotype using dHPLC. Minor allele frequencies ranged between 0.36 to 23.91% for subjects with high BMD and 0.36 to 25.91% for subjects with low BMD. Concentrated within the P2 promoter were three polymorphic nucleotides for which the minor alleles were over represented in the upper decile of BMD. These alleles are in near complete LD with each other and represent a haplotype block that is significantly associated with increased BMD. Constructs made from the "normal" and the "high BMD" alleles were cloned upstream of otherwise identical luciferase constructs. Despite there being only 3 nucleotide differences between the two constructs, when transfected into osteoblast-like cells the high BMD allele construct showed significantly greater P2 promoter activity than the normal allele. An EMSA identified aberrant DNA-protein binding affinity between polymorphic and non-polymorphic variants, providing an allele-specific nuclear factor as a candidate for the regulation of the RUNX2 promoter.

Figure 1



Differential DNA-protein binding affinity caused by a single nucleotide polymorphism in the RUNX2 proximal promoter is involved in increased luciferase activity *in-vitro*.

Disclosure:
1

O7 8.9.05: 1115 – 1130 hours

MCP-1 INDUCES SUPER ABUNDANT NFATC1 EXPRESSION AND RESULTS IN TRAP+ MULTINUCLEAR CELLS WITH CALCITONIN RECEPTOR EXPRESSION, BUT THESE FAIL TO RESORB BONE AND ARE NOT OSTEOCLASTS
MS Kim, CJ Day, CL Magno, SRJ Stephens & NA Morrison
School of Medical Science, Griffith University, Gold Coast, QLD

Monocyte chemotactic protein 1 (MCP-1) is a CC chemokine that is induced by RANKL in human osteoclasts and enhances osteoclast formation in the presence of RANKL. In the absence of RANKL, treatment of peripheral blood mononuclear cells (PBMC) with MCP-1 and M-CSF results in TRAP+ multinuclear cells that are positive for a large range of osteoclast marker genes including NFATc1. Despite super abundant NFATc1 mRNA and nuclear NFATc1 protein, these TRAP+ multinuclear cells are negative for bone resorption and cannot be called osteoclasts. Therefore, despite NFATc1 expression levels 50fold greater than osteoclasts does not automatically result in a bone resorbing phenotype, rather NFATc1 is associated with multinucleation and TRAP+ status. The purpose of this study was to investigate the action of MCP-1 in osteoclast formation using PBMCs.

RANKL mediated osteoclast formation was inhibited by p38MAPK (SB203580) and ERK1/2 (U0126) antagonists. In contrast, MCP-1 mediated TRAP+ multinuclear cells were only suppressed by U0126, while SB203580 had no effect. In the presence of RANKL, MCP-1 rescued osteoclast formation from p38MAPK blockade (SB203580), but MCP-1 could not rescue the effects of U0126. These data show that formation of osteoclasts requires both the p38MAPK and ERK1/2 pathways, and that blockade of p38MAPK can be circumvented by MCP-1. Furthermore, formation of TRAP+ multinuclear cells induced by MCP-1 requires the ERK1/2 pathway only. We propose that the MCP-1 induced TRAP+ multinuclear cells represent an ERK1/2 dependent stage in osteoclast differentiation, after NFATc1 induction and cellular fusion, but prior to the development of bone resorption activity.

Disclosures:
1

O8 8.9.05: 1130 – 1145 hours

REMAINING LIFETIME RISK OF FRACTURES IN ELDERLY MEN AND WOMEN
ND Nguyen[1,2], H Alhborg[1], JR Center[1], JA Eisman[1,2] & TV Nguyen[1,2]
[1] *Bone and Mineral Research Program, Garvan Institute of Medical Research, St Vincent's Hospital, Sydney, NSW*
[2] *Faculty of Medicine, The University of New South les, Sydney, NSW*

Remaining lifetime risk of fracture (RLRF) is a preferred absolute-risk approach of assessment fracture risk in an individual. Although BMD is the best predictor of fracture risk, the RLRF for various BMD levels is not known. The present study was undertaken to estimate the RLRF in elderly men and women by age and BMD level.

A sample of 2216 (1358 women) subjects aged 60+ years as at 1989 of Caucasian background from the Dubbo Osteoporosis Epidemiology Study had been followed for 15 years. Incident fracture and all-cause mortality were recorded. BMD at the femoral neck was measured by DXA at baseline.

The death-adjusted RLRF (from the age of 50) was 54% (95% CI: 48-59) in women and 32% (23-36) in men. In women, the 10-year risk of fracture increased from 16% (12-21) among those aged 60-69 yr to 49% (40-61) among those aged 80+. In men, the 10-year risk also increased from 11% (5-17) among those aged 60-69 yr to 26% (13-38) among those aged 80+.

In women with BMD T-scores ≤–2.5, the 10-year and RLRF were 31% (19-44) and 71% (66-79), respectively; among men with the same BMD levels, these were 30% (2-56) and 47% (35-77).

The remaining lifetime risk of fracture is 1 in 3 men and 1 in 2 women. The presence of low BMD increased the risk to 1 in 2 for men and 7 in 10 for women. These estimates can be used to promote identification of high-risk individuals and target for treatment.

SOURCE OF FINANCIAL SUPPORT: NATIONAL HEALTH AND MEDICAL RESEARCH COUNCIL, AUSTRALIA

O9 8.9.05: 1145 – 1200 hours

PAGET'S DISEASE IN PEOPLE INHERITING MUTATIONS IN THE P62 GENE

M Bolland, K Callon, D Naot, PC Tong, D Wattie & T Cundy
Department of Medicine, University of Auckland, Auckland, New Zealand

Mutations in Sequestosome1 (P62) are present in 25-50% of families with familial Paget's disease (FPD). We sought to determine the prevalence and phenotype of PD in the offspring of affected family members known to carry P62 mutations. 63 offspring from 5 families (18 index patients) with mutations in P62 were approached, and 41 (12 men, 29 women; mean age 44 yrs, range 30-63) agreed to participate. The ubiquitin-binding domain region of the P62 gene was sequenced and the presence or absence of the known mutation established. Subjects inheriting a mutation then had bone scintigraphy, from which skeletal involvement was calculated.

14/41 subjects had inherited a familial mutation in P62. The mean ALP was 74u/L (normal 30-120) in subjects with mutations and 81u/L in those without mutations. To date, 13 of the 14 subjects inheriting P62 mutations have had bone scintigraphy. Scans from 4 subjects (mean age 46, mean ALP 139) showed PD, but were normal in the other 9 (mean age 46, mean ALP 58). In the 4 with PD scans, the skeletal involvement was less extensive in comparison to their parents. Thus only a third of subjects inheriting a P62 mutation had evidence of PD on scintigraphy, and their PD was less extensive than in their affected parents.

This genetic trait thus shows incomplete penetrance. The data are also consistent with the hypothesis that an environmental factor is important in the pathogenesis of PD, and that exposure to this factor may be falling.

Disclosure:
1

O10 8.9.05: 1200 – 1215 hours

GLUTAMINE REPEAT MUTATIONS DEFINE A NEW CBFA1/RUNX2 RELATED SYNDROME WITH DECREASED FEMORAL NECK BMD, DECREASED CALCANEAL BROADBAND ULTRASOUND AND INCREASED RISK OF OSTEOPOROTIC FRACTURE

ASJ Stephens[1], JD Doecke[1], SH Ralston[2], RL Prince[3], GC Nicholson[4] & NA Morrison[1]
[1] *School of Medical Sciences, Griffith University Gold Coast Campus, QLD*
[2] *The Bone Research Group, Department of Medicine and Therapeutics, Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK*
[3] *Western Australian Institute of Medical Research, and the Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA*
[4] *Department of Medicine, Geelong Hospital, Geelong, VIC*

RUNX2 is an essential transcription factor required for skeletal development and cartilage formation. Haploinsufficiency of RUNX2 leads to cleidocranial displaysia (CCD) a skeletal disorder characterised by gross dysgenesis of bones derived from intrachondral bone formation. A notable feature of the RUNX2 protein is the polyglutamine and polyalanine (23Q/17A) domain coded by a repeat sequence. Since none of the known mutations causing CCD characterised to date map in the glutamine repeat region, we hypothesised that Q-repeat mutations would exist and may be related to a more subtle bone phenotype. We screened subjects derived from multiple normal populations for Q-repeat mutations. A total of 28 subjects were identified who were heterozygous for a wild type allele and were carriers of Q variants: (deletions 15Q, 16Q, 17Q, and extension 30Q). Collectively, Q-repeat variants presented with significantly decreased femoral neck BMD (p=0.0006) with a reduction of 0.56SD in BMD. Broadband ultrasound attenuation (BUA) and speed of sound (SOS) measured in the calcaneus were available on 18 Q-variant subjects. Q-variants displayed significantly decreased BUA (p=0.006) with an effect of similar magnitude (0.65SD decrease). The transactivation function of the 16Q and 30Q alleles were analyzed using a RUNX2 reporter gene assay. These alleles displayed transactivation function but at levels significantly lower compared to wild type (23Q). Our analysis has identified novel Q-repeat mutations that occur at a collective frequency ~0.8%. These mutations significantly alter BMD and BUA and display impaired transactivation function introducing a new class of functionally relevant RUNX2 mutants.

Disclosure: 1

O11 8.9.05: 1215 – 1230 hours

VARIATIONS IN THE MODE OF LOADING ALONG THE BONE INFLUENCES ITS ARCHITECTURE RATHER THAN THE AMOUNT OF ITS MINERALISED MASS

RM Zebaze[1], A Jones[2], M Knackstedt[2] & E Seeman[1]
[1] *Endocrinology, Austin Health/Northern, The University of Melbourne, Melbourne, VIC*
[2] *Department of Applied Maths, RSPhysSE, Australian National University, Canberra, ACT*

It is unclear how a long bone adapts its structure to the varying type of biomechanical demands along its length. We sought to determine how the femoral neck (FN) adapts its structure to the varying mode of loading along its length. Cross-sectional slices were analysed every 50μm along the FN in 13 specimens from Caucasian women aged 29 to 85 using high resolution micro-CT. Near the trochanter, the total cross-sectional area (T-CSA) was large and ellipsoid with a supero-inferior long axis. Moving proximally, T-CSA decreased, then increased becoming more circular; the cortical mass diminished, while trabecular mass increased in a reciprocal fashion so that total bone mass (represented by bone area) from section to section was almost constant. From the FN-shaft junction (region mainly subjected to bending) to FN-femoral head junction (region subjected mainly to compressive and shear stresses), cortical bone mass decreased by ~ 60% (199.6 ± 12.7 vs. 78.5 ± 8.6 mm^2); this was associated with a similar ~ 60% increase in trabecular bone mass (72.8 ± 9.4 vs. 174.2 ± 16.3mm^2); the section modulus decreased by 67% (1491 ± 101.9 vs. 496 ± 66.1mm^3) while the coefficient of circularity (the degree to which the T-CSA approximates a circle) increased from 59 to 87.5%.

Along the FN, variations in the mode of loading is adapted for, by changes in architecture (T-CSA, size, shape and proportion cortical/trabecular bone), *not* mass; ellipticity and cortical bone favours resistance to bending, while circularity and trabecular bone favours resistance to compression and shear stresses. Loading conditions may influence bone architecture with little or no effect on the amount of its mineralised mass.

Disclosure:
1

O12 8.9.05: 1230 – 1245 hours

TARGETED SILENCING OF CATHEPSIN K WITH SIRNA IN PRIMARY HUMAN OSTEOCLAST CELLS

CI Selinger, CJ Day & NA Morrison
School of Health Science, Gold Coast Campus Griffith University, QLD

siRNA transfection results in knockdown of gene expression through gene silencing. Using transfected siRNAs these effects are short-lived, usually of the order of 72 hours, whereas osteoclast driven bone resorption assays have a larger time-frame. We developed a simple method to match the time frames of these two phenomena, by directly transfecting siRNA onto mature human osteoclasts seeded on dentine.

Our aim was to use siRNA to knockdown Cathepsin-K expression, significantly enough to inhibit the bone resorbing action of primary human osteoclasts. Cathepsin K is a major target of pharmaceutical development, and a prime target for siRNA knock-down.

Mature osteoclasts harvested from collagen-coated plates were seeded onto dentine. Cultures were exposed to siRNAs and allowed to resorb bone for 96 hours. All osteoclast cultures displayed the TRAP+ multinuclear phenotype. CTSK siRNA treatment resulted in inhibition of bone resorption with significant reduction in pit numbers and area of resorption compared with untreated osteoclasts (P=0.018, P=0.01) and GFP siRNA treated osteoclasts (P=0.004, P=0.032). Compared to untreated osteoclasts, GFP siRNA did not significantly alter pit number (P=0.93) or pit area (P=0.91). Reduced bone resorption observed in CTSK siRNA treated cells was not due to decreased cell number, with total cell count not significantly different between CTSK siRNA and controls (P=0.85). CTSK siRNA achieved knockdown of ≥60%, with 12.5nM siRNA, without producing off-target effects (cathepsin B, P=0.18, 2′,5′-oligoadenylate synthetase 1, P=0.39).

We provide evidence that siRNA can be used to specifically inhibit gene expression in mature primary human osteoclasts to alter osteoclast resorption.

Disclosures:
1.

O13 8.9.05: 1430 – 1440 hours

PAMIDRONATE THERAPY FOR ONE YEAR AFTER ALLOGENEIC STEM CELL TRANSPLANTATION (AlloSCT) REDUCES BONE LOSS FROM THE LUMBAR SPINE, FEMORAL NECK AND TOTAL HIP

PR Ebeling, P Shuttleworth, J Reynolds, J Szer, AP Schwarer, AW Roberts, KF Bradstock, C-H Hui, R Herrmann & AC Grigg
Bone Marrow Transplant Service, Depts. Diabetes and Endocrinology and Medicine, Royal Melbourne Hospital, Melbourne; Peter MacCallum Cancer Centre, Melbourne; The Alfred, Melbourne, VIC; Westmead Hospital, Westmead, NSW; Royal Adelaide Hospital, Adelaide, SA; Royal Perth Hospital, Perth, WA

Aims:
Bone loss is most severe at the proximal femur following allogeneic SCT (alloSCT). We studied effects of pamidronate therapy and interactions with glucocorticoid dose intensity on femoral neck, lumbar spine and total hip BMD post-alloSCT.

Methods:
We randomised 116 alloSCT recipients to receive pamidronate 90mg IV monthly, from day -7 to one-year post-alloSCT (n=63) or no pamidronate (n=53) in an open-label, prospective, controlled trial. All patients received oral calcitriol (0.25 mcg/day) and calcium carbonate (600 mg/day). All women received hormone therapy. Patients were classified into low (<12.5 mg), medium (12.5-25 mg) or high glucocorticoid (>25 mg) groups, based on mean daily prednisolone dose.

Results:
83 of 116 randomised patients were evaluable. Pamidronate therapy reduced bone loss at 12 months by 5.5% to 8.2%, but significant bone loss from the hip occurred.

Site	No pamidronate		**Pamidronate**		P value (Difference between groups)
	n	% Change *	n	% Change *	
Lumbar spine BMD	30	-3.97	49	2.70	<0.0001
Femoral neck BMD	30	-9.88	46	-2.64	<0.0001
Total hip BMD	26	-8.76	41	-3.25	0.0019

*Median percentage BMD change at 12 months

Pamidronate decreased bone loss at all sites in patients receiving medium or high mean daily glucocorticoid doses, but did not prevent hip bone loss in patients on low doses. Spinal bone loss did not occur in the in the control group on low glucocorticoid doses.

Conclusions:
Prophylactic monthly intravenous pamidronate infusions significantly reduce bone loss from both the spine and hip after alloSCT, however, hip bone loss is not prevented.

Disclosures:
4 Novartis

O14 8.9.05: 1440 – 1450 hours

ZOLEDRONIC ACID IN CHILDREN: EFFECT ON GROWTH, BONE DEVELOPMENT AND BONE AND MINERAL METABOLISM

CFJ Munns[1], JYH Hong[1], J Briody[2], M McQuade[1], DG Little[3] & CT Cowell[1]
Departments of Endocrinology and Diabetes[1], Nuclear Medicine[2] and Orthopaedic Surgery[3], The Children's Hospital at Westmead, Sydney, NSW

Zoledronic acid (ZA) is an intravenous bisphosphonate, 100 to 200 times more potent than pamidronate. Animal studies suggest ZA preserves bone architecture following avascular necrosis (AVN) and enhances fracture union, but impairs bone growth. Little is known of its effects in children. To address this, we evaluated 21 children (15 males; mean age 10.1±2.7 years), with focal bone lesions: 18 osteonecrosis (10 AVN, 8 Perthes disease), 2 fracture non-union and 1 congenital pseudarthrosis, treated for 1.1±0.5 years, at a dose of 0.207±0.05 mg/kg/year. All children received calcium 1000 mg twice daily and ergocalciferol 5000 IU daily, for one week before their initial infusion.

	Baseline$^{\Phi}$	12 months$^{\Phi}$	p$^{\psi}$
Height*	-0.10 (1.5)	-0.20 (1.7)	0.3
Lumbar spine aBMD for Age*	0.02 (1.5)	1.37 (1.7)	<0.001
Total body BMC for Age*	0.34 (1.8)	0.86 (2.0)	<0.001
Bone Area for Height*	0.24 (1.6)	0.76 (1.2)	<0.001
BMC for Lean Tissue Mass*	0.40 (1.4)	1.48 (1.6)	0.03
% femoral cortical area	67.85 (12.24)	72.43 (10.45)	0.075
Serum			
Calcium (mmol/l)	2.4 (0.09)	2.3 (0.12)	0.012
Phosphate (mmol/l)	1.4 (0.15)	1.3 (0.28)	0.237
Alkaline phosphatase (U/l)	208.5 (70.85)	207.4 (56.98)	0.949
Creatinine (umol/l)	54.4 (8.94)	51.9 (10.34)	0.289
25-hydroxyvitamin D (nmol/l)	71.1 (18.77)	59.2 (15.47)	0.006
Osteocalcin (nmol/l)	8.5 (4.25)	4.9 (2.98)	0.010
Urine			
Deoxypyridinoline/creatinine ratio (nM/mM)	26.2 (10.38)	20.2 (6.77)	0.084

* =Z-score; Φ =Mean (SD); ψ =Paired t-test for means; aBMD= areal bone mineral density; BMC= bone mineral content.

After ZA therapy, 11 children (50%) had a lumbar spine and/or total body aBMD z-score >2.0. None developed nephrocalcinosis.

These data suggest that 12 months of ZA is safe for growth and mineral metabolism. The reductions in serum calcium and 25-hydroxyvitamin D may reflect premedication with calcium and ergocalciferol. In association with a reduction in bone turnover, ZA led to significant gains in bone area and BMC for age and lean tissue mass. Of concern, 50% of children had an aBMD z-score >2.0 at 12 months, which may indicate the need for dose modification. No skeletal complications have been noted. Further studies are required to determine the minimum effective ZA dose.

Disclosures:
4. Novartis

O15 8.9.05: 1450 – 1500 hours

CLINICAL OUTCOMES, RISK OF NEW INCIDENT VERTEBRAL FRACTURES AND SURVIVAL OF PATIENTS PRESENTING WITH ACUTE OSTEOPOROTIC VERTEBRAL FRACTURES: *PERCUTANEOUS VERTEBROPLASTY IS SUPERIOR TO CONSERVATIVE THERAPY*

T Diamond, C Bryant, L Browne & WA Clark
Department of Endocrinology and Radiology, St George Hospital Campus, UNSW, Sydney, NSW

Aim:
To compare the acute and chronic benefits of percutaneous vertebroplasty (PV) to conservative therapy (CT) for acute osteoporotic vertebral compression fractures.

Methods:
During 2001/02, 126 consecutive osteoporotic patients presenting with acute vertebral fractures were enrolled in a prospective, nonrandomised, "intention-to-treat" 2-year study. There were 88 patients treated by PV and 38 by CT. Baseline osteoporotic risk factors and BMD were measured, while pain scores and level of function were recorded on presentation, at 24 hours, 6 weeks, 6-12 months and 24 months post therapy.

Results:
There were 39 men and 87 women, aged 51-95 years, followed for 629 days (range 42-730). Of these, 21 (17%) died and 7 (6%) were lost to follow up. Only 3 minor complications (fractured pedicle and psoas haemorrhage) occurred in the PV group in the first year of the study. PV-treated patients demonstrated better outcomes (reduced pain scores and improved functioning activities) at 24 hours and at 6 weeks post therapy, a quicker return to function (as assessed by Barthel Index) and lower rates of hospitalization than those treated

conservatively. Moreover, PV-treated patients did not demonstrate increases in new incident vertebral fractures (clinically and by radiographic assessment) or mortality as compared to those treated conservatively.

Conclusion:
If the benefits of PV are contrasted against the very low rates of complication, then this form of therapy appears to be much better than CT for treating acute osteoporotic vertebral compression fractures.

O16 8.9.05: 1500 – 1510 hours

HORMONE THERAPY USE AFTER THE RELEASE OF PRINCIPAL RESULTS FROM THE WHI TRIAL

S Korn, JA Pasco, MJ Henry, GC Nicholson & MA Kotowicz
The University of Melbourne, Department of Clinical and Biomedical Sciences: Barwon Health, Geelong, VIC

In July 2002 the initial results of the oestrogen plus progesterone arm of the Women's Health Initiative (WHI) were released[1]. The writing group reported increased rates of breast cancer and risk of adverse cardiovascular events that outweighed the benefits of decreased fracture risk.

We assessed 390 women (median age 70.5yr, range 50-97) recruited in the Geelong Osteoporosis Study with assessments spanning the WHI publication. Self-reported hormone therapy (HT) exposure was documented by questionnaire. Subjects received BMD results at each visit. The median time of scan pre-WHI 1.5yr (IQR 1.2-2.1) and post-WHI 0.3yr (0.1-0.6).

Age, yr (n)	HT use pre-WHI (%)	HT use post-WHI (%)
50-54 (21)	55	36
55-59 (84)	30	8
60-64 (77)	33	14
65-69 (75)	20	7
70-79 (169)	9	3
80+ (86)	8	4

After the release of WHI results, 67% of women with normal BMD (T-score>-1), 56% of women with osteopenia and 63% of women with osteoporosis stopped using HT. Of these, one with osteopenia, and three with osteoporosis commenced an alternative osteoporosis medication.

Extrapolated to the Australian population aged ≥50yr (2001), 772,231 women (27%) of were using HT prior to WHI, 389,487 (13.5%) stopped HT post-WHI. Although indications for use of HT were not documented, a relatively high proportion of younger women used HT, possibly for menopausal symptoms. Treatment cessation was not influenced by BMD and few women with reduced BMD commenced alternate therapies. These responses to the WHI results are likely to adversely affect future fracture rates.

[1] Rossouw JE et al (2002) JAMA 288: 321-33

O17 8.9.05: 1510 – 1520 hours

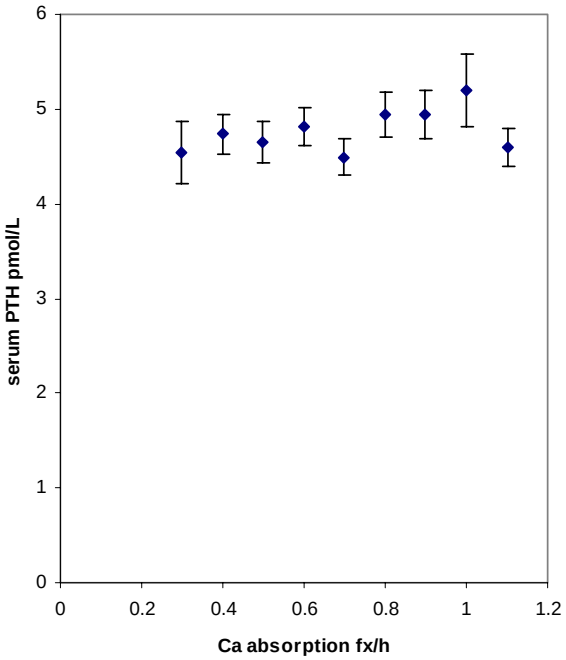
CALCIUM MALABSORPTION DOES NOT INCREASE SERUM PARATHYROID HORMONE IN POSTMENOPAUSAL WOMEN

A Need, M Horowitz, P O'Loughlin, P Coates & C Nordin
Clinical Biochemistry, Institute of Medical and Veterinary Science and Royal Adelaide Hospital, Adelaide, SA

It is commonly claimed that the rise in serum parathyroid hormone (PTH) with age is caused by malabsorption of calcium [1] but this, to our knowledge, has never been demonstrated. To test this hypothesis we have re-examined our data on serum ionised calcium (Ca^{2+}), radiocalcium absorption, PTH, calcitriol ($1,25(OH)_2D$) and 25-hydroxyvitamin D (25(OH)D), measured by previously described methods [2] in 1017 postmenopausal women attending our osteoporosis clinics.

PTH was not related to calcium absorption (figure) but was inversely related to ionised calcium and 25(OH)D (see correlation matrix). The positive correlation between PTH and calcitriol indicates that PTH drives calcitriol and not vice versa.

COEFFICIENTS OF CORRELATION



	Age	PTH	Ca^{2+}	Ca absorption	$1,25(OH)_2D$	
PTH	0.12***					
Ca^{2+}	0.09**	-0.14***				
Ca absorption	-0.20***	0.04	-0.01			
$1,25(OH)_2D$		-0.74*	0.12***	-0.05	0.34***	
25(OH)D		-0.15***	-0.22***	0.03	0.09**	0.21***

* P<0.05 ** P<0.01 *** P<0.001

The rise in PTH with age is not due to falling calcium absorption but to falling 25(OH)D levels and loss of the "calcaemic" effect of vitamin D [3].

1. Seeman E, Lancet 2002;359:1841-50
2. Need et al, J Clin Endocr Metab 2004;89:1646-49
3. Carlsson & Lindquist, Acta Physiol Scand 1955;35:53-55

Disclosure:
1

O18 8.9.05: 1520 – 1530 hours

CALCIUM-VITAMIN D_3 FORTIFIED MILK REDUCES BONE LOSS IN MIDDLE AGED AND OLDER MEN: A 2-YEAR RANDOMISED CONTROLLED TRIAL

RM Daly, M Brown, S Bass, S Kukuljan & C Nowson
Centre for Physical Activity and Nutrition Research, School of Exercise and Nutrition Sciences, Deakin University, Melbourne, VIC

The aim of this 2 year RCT was to examine the effects of calcium-vitamin D_3 fortified milk on BMD, bone geometry and strength in men aged over 50. A total of 167 community living men were randomised to receive either 400 mls of reduced fat UHT fortified milk providing 1000 mg/d of calcium and 800 IU/d of vitamin D_3 or to a control group. aBMD was assessed every 6 months by DXA; L_1-L_3 trabecular vBMD and mid-femur bone geometry and cortical vBMD were assessed by QCT. A total of 149 men completed the study [milk compliance averaged 88%]. At the FN, total hip and UD-radius, the mean loss in aBMD after 24 months was 0.9% to 1.6% less in the milk compared to control group [p<0.05 to <0.001]. No differences were detected for L_2-L_4 aBMD. Both groups experienced a similar and

significant reduction in L_1-L_3 trabecular vBMD [milk 2.5%; control 2.4%, $p<0.05$]. In contrast, the reduction in mid-femur cortical vBMD was less in the milk group [1.5% vs 2.5%, interaction $p=0.06$]. Medullary area remained unchanged in the milk group [-0.04%] but increased in controls [0.85%, $p=0.06$]. Serum 25(OH)D levels increased and PTH decreased in the milk relative to control group after 12 months [$p<0.001$]. These differences remained after 24 months. Body weight remained unchanged in both groups. In conclusion, supplementing the diet of men aged over 50 with calcium-vitamin D_3 enriched milk may represent an effective strategy to stop or slow age-related bone loss at clinically important skeletal sites.

Disclosure:
No conflict of interest

O19 8.9.05: 1430 – 1440 hours

ADULT DELETION OF HYPOTHALAMIC Y2 RECEPTORS IMPROVES BONE MASS FOLLOWING GONADECTOMY IN MICE

SJ Allison[1], PA Baldock[1], A Sainsbury[2], RF Enriquez[1], M During[4], JA Eisman[1], H Herzog[2] & EM Gardiner[3]
[1] *Bone and Mineral Research Program, Garvan Institute of Medical Research, Sydney NSW*
[2] *Neurobiology Program, Garvan Institute of Medical Research, Sydney, NSW*
[3] *School of Medicine, University of Queensland , Brisbane, QLD*
[4] *Department of Molecular Medicine and Pathology, University of Auckland, Auckland, New Zealand*

The neuropeptide (NPY) Y2 receptors regulate bone formation in the mouse, with increased cancellous bone volume (BV/TV) in Y2-receptor knockout models.

The aims of this study were to investigate whether Y2-receptor knockout mice are resistant to gonadectomy-induced bone loss, and whether conditional deletion of hypothalamic Y2-receptors can improve bone following gonadectomy.

Male, 8 week old wild type and Y2KO mice underwent orchidectomy (ORX) or sham-operation. Parameters of bone formation were determined in the distal femora at 16 weeks. Values: mean ± SEM.

ORX significantly reduced BV/TV [%] in both genotypes to comparable levels, associated with significantly increased osteoclast surface. Interestingly, the greater mineral apposition rate (MAR) of the Y2KO model was maintained following ORX.

The demonstration that Y2KO mice maintained anabolic activity suggested that selected hypothalamic Y2-deletion might improve bone formation following gonadectomy. Y2-floxed mice were bilaterally injected into the hypothalamus with either cre-recombinase (CRE) or empty expressing adeno-associated virus 8 weeks after ORX, and were collected 6 weeks later.

Despite bone loss following ORX, BV/TV was 2-fold greater in conditional Y2KO mice compared with controls (CRE, 4.0±0.8 vs empty, 2.1±0.4 $p<0.05$). Importantly, this was associated with significantly greater MAR (CRE, 2.5±0.2µm/d vs empty, 1.4±0.1µm/d).

Together these data demonstrate that the Y2KO anabolic pathway is active following sex hormone deficiency. Further, that adult-induced disruption of hypothalamic Y2-receptor activity is able to improve bone mass following gonadectomy.

Disclosures:
4 (Pfizer)

O20 8.9.05: 1440 – 1450 hours

CENTRAL REGULATION OF CORTICAL BONE MASS AND OSTEOBLAST ACTIVITY BY Y2 RECEPTORS: COMPARISON TO LEPTIN PATHWAY

PA Baldock[1], S Allison[1], A Sainsbury[2], RF Enriquez[1], H Herzog[2],EM Gardiner[3] & JA Eisman[1]
[1] *Bone and Mineral Research Program,* [2] *Neurobiology Program, Garvan Institute of Medical Research, St Vincent's Hospital, Sydney, NSW*
[3] *School of Medicine, University of Queensland, Brisbane, QLD*

Y2 receptor knockout [Y2KO] and leptin-deficient [ob/ob] mice display increased bone volume and osteoblast activity in cancellous bone, via anabolic signals from the hypothalamus. The effects of these central pathways on cortical bone, critical to bone strength, were examined.

Femora from male, 16 week old wildtype [wt], Y2KO, ob/ob and Y2/ob mice and hypothalamic Y2KO mice were examined by DXA and histology.

In Y2KO, shaft and distal BMC were greater than wt, with greater cortical area and osteoblast activity [mineral apposition rate, MAR, µm/d]. In ob/ob, despite greater weight, shaft and distal BMC was reduced compared to Y2KO. In the Y2/ob cross, despite shaft BMC

being reduced compared to Y2KO, shaft cortical area and thickness and distal BMC were similar to wt. BMD did not differ throughout. Periosteal and endocortical MAR was similar in Y2KO and Y2/ob and greater than wt, and elevated from controls after adult hypothalamic Y2KO (0.23 ±0.01 vs 0.17 ±0.02, p<0.05).

	wt	Y2KO	ob/ob	Y2/ob
Body weight (g)	26.7 ±4	29.8 ±1	52.4 ±4 ab	47.3 ±5 ab
Shaft BMC (mg)	7.8 ±1	8.8 ±1a	6.7 ±1 b	6.6 ±1 b
Shaft Cortical Area (mm^2)	0.9 ± 0.03	1.1 ±0.04 a	0.7 ±0.06 ab	0.9 ±0.02 b
Distal BMC (mg)	10 ±1	12 ±1a	9 ±1 b	11 ±1
Distal Endocortical MAR	0.21 ±0.03	0.31 ±0.03 a	0.24 ±0.01b	0.32 ±0.01ac
Shaft Periosteal MAR	0.16 ±0.2	1.1 ±0.2 a	1.0 ±0.3 a	1.8 ±0.4 a

a p< 0.05 vs wt, b p< 0.05 vs Y2R KO, c p<0.05 ob/ob vs Y2R/ob.

The Y2 receptor pathway represents an adult-inducible stimulator of cortical and cancellous bone formation. Y2 deficiency increased BMC and osteoblast activity in Y2R/ob mice, in part counteracting the leptin-deficient reduction in cortical bone. These data delineate opposing effects of Y2KO and leptin pathways on cortical bone.

O21 8.9.05: 1450 – 1500 hours

INHIBITION OF GLUCOCORTICOID SIGNALING BY OVEREXPRESSION OF 11BETA HSD2 IN MATURE OSTEOBLASTS INHIBITS NODULE FORMATION AND INCREASES ADIPOGENESIS IN VITRO

H Zhou, K Brennan, Y Zheng, CR Dunstan & MJ Seibel
Bone Research Program, ANZAC Research Institute, Sydney, NSW

Transgenic (tg) expression of 11beta-hydroxysteroid dehydrogenase type 2 (HSD2), a glucocorticoid (GC) inactivating enzyme, under the control of a 2.3Kb collagen type I promotor (Col2.3-HSD2) abrogates intracellular GC signalling in mature osteoblasts (1). To characterise osteoblasts overexpressing Col2.3-HSD2, primary osteoblast cultures were generated from the calvaria of 1-day-old tg mice and WT littermates.

Col2.3-HSD2 tg cultures developed 50% fewer nodules than WT after 2 weeks culture. Alkaline phosphatase (ALP) activity was decreased relative to WT from day 3 in Col2.3-HSD2 tg cultures and a significant increase in adipocyte numbers was observed, suggesting a shift in lineage commitment from osteoblast to adipocyte.

mRNA for HSD2 was only detected in Col2.3-HSD2 tg mice with increased levels observed from day 3, indicating number of mature osteoblasts was increasing. At day 1, mRNA for Runx2, ALP, and osteocalcin were expressed at low but similar levels in cells from Col2.3-HSD2 tg and WT. These mRNA levels remained low in cells cultured from Col2.3-HSD2 tg mice, while they increased with osteoblast differentiation in the WT culture. Conversely, mRNA expression of adipogenic transcription factors C/EBPα and PPARγ increased at day 3 in Col2.3-HSD2 tg cultures but not in WT cultures.

These results indicate that disrupting GC signalling in mature osteoblasts impairs early osteoblast differentiation and promotes adipogenic differentiation. This suggests the existence of a GC regulated paracrine signal between mature osteoblasts and their mesenchymal precursors that influences lineage commitment.

(1) Sher et al Endocrinology 145:922-9, 2004. The authors thank Prof B Kream for providing the transgenic animals.

Disclosures:
1. (no conflict of interest)

O22 8.9.05: 1500 – 1510 hours

OSTEOBLAST TARGETED OVEREXPRESSION OF HYDROXYSTEROID DEHYDROGENASE TYPE 2 INDUCES DELAYED CALVARIAL DEVELOPMENT IN TRANSGENIC MICE

CR Dunstan, H Zhou, K Brennan, Y Zheng & MJ Seibel
Bone Research Program, ANZAC Research Institute, Sydney, NSW

Transgenic (tg) expression of 11beta hydroxysteroid dehydrogenase type 2 (HSD2), a glucocorticoid inactivating enzyme, under the control of a 2.3Kb collagen type I promotor abrogates intracellular glucocorticoid signalling in mature osteoblasts (1). We evaluated calvaria and tibiae of wild-type (WT) and tg mice aged 1, 7 and 42 days. HSD2 mRNA and protein expression was present in the calvaria and long bones of tg mice but absent in WT. Skeletal expression of HSD1 was similar in WT and tg mice.

Day 1 HSD2 tg mice had incompletely formed calvaria. The amount of bone was reduced by 33% relative to WT. Cartilage was present in all tg mice but almost absent in WT mice. In 7-day old mice, increased cartilage was present, but the amount of bone was similar to WT. Calvaria appeared normal at 6 weeks of age indicating recovery. The tibiae were similar in transgenic and wild type mice at all ages evaluated.

These results indicate that, in mice, glucocorticoid signalling in osteoblasts is required for normal development of the calvarial bone. Calvarial bones form by intramembranous bone formation above a cartilaginous template that is removed without undergoing hypertrophy or mineralisation. Blocking of glucocorticoid signalling through the overexpression of HSD2 in mature osteoblasts results in a delay in bone formation and in cartilage removal in the calvaria. This may reflect a specific requirement for glucocorticoid signalling in the formation of intramembranous bone structures.

(1) Endocrinology 145:922-9, 2004. The authors thank Prof B Kream for kindly providing the transgenic mice.

Disclosures:
1. (no conflict of interest)

O23 8.9.05: 1510 – 1520 hours

DIFFERENTIAL ACTIVATION OF INTRACELLULAR CA^{2+} MOBILIZATION AND ERK1/2 PHOSPHORYLATION BY THE EXTRACELLULAR CA^{2+}-SENSING RECEPTOR STIMULATED BY Ca^{2+} IONS OR L-AMINO ACIDS

HJ Lee[1], H-C Mun[1], NC Lewis[1], EL Culverston[1], RS Mason[2] & AD Conigrave[1]
[1] *School of Molecular and Microbial Biosciences, University of Sydney, NSW*
[2] *Department of Physiology, School of Medical Sciences, University of Sydney, NSW*

The calcium-sensing receptor (CaR) mediates feedback control of extracellular ionized Ca^{2+} concentration (Ca^{2+}_o) by regulating parathyroid hormone (PTH) secretion and renal calcium excretion. The CaR couples to several important intracellular signalling enzymes including PI-PLC, leading to intracellular Ca^{2+} (Ca^{2+}_i) mobilization, and ERK1/2. In addition to Ca^{2+}_o, the CaR is activated, allosterically, by several sub-classes of L-amino acids including the aromatics, L-Phe and L-Trp which markedly enhance the Ca^{2+}_o sensitivity of Ca^{2+}_i mobilization in CaR-expressing HEK293 cells and normal human parathyroid cells. They also induce a small but physiologically significant enhancement of Ca^{2+}_o-dependent suppression of PTH secretion. In the current study, we report that L-amino acids induce a small but significant enhancement of Ca^{2+}_o-stimulated ERK1/2 phosphorylation by Western blotting. In CaR-expressing HEK293 cells, 10 mM L-Phe lowered the EC_{50} for Ca^{2+}_o by around 0.3 mM from control values of 2.0-2.8 mM in three distinct series. The effect was stereoselective (L-Phe > D-Phe) and another aromatic amino acid L-Trp, but not the branch chain amino acid L-Leu, also enhanced Ca^{2+}_o-stimulated ERK1/2 activation. The effect of amino acids was further investigated in HEK293 cells that stably expressed a CaR mutant S169T that, in assays of Ca^{2+}_i mobilization, was found to exhibit markedly reduced sensitivity to Ca^{2+}_o that was restored to normal by L-Phe. In these experiments, L-Phe lowered the EC_{50} for Ca^{2+}_o-stimulated ERK1/2 phosphorylation from around 2.2 mM to 1.6 mM. The data indicate that L-Phe enhances the Ca^{2+}_o sensitivity of CaR-stimulated ERK1/2. The effect is comparatively small and appears to be a fine-tuning mechanism. The results indicate that two distinct activators of the CaR, extracellular Ca^{2+} and amino acids, have differential effects on intracellular signalling pathways that are coupled to CaR activation. The relative impacts of extracellular Ca^{2+} and amino acids on CaR-dependent biological responses are likely to depend critically on the signalling pathways involved.

O24 8.9.05: 1520 – 1530 hours

MUTATIONAL ANALYSIS OF EXOLOOP-3 RESIDUES OF THE CALCIUM-SENSING RECEPTOR POINTS TO A CONSTRAINING EFFECT OF CORE RESIDUES ON WILD-TYPE CALCIUM-SENSING RECEPTOR ACTIVATION

AJ Rea, BK Ward, AL Magno & T Ratajczak
Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital and Centre for Medical Research, University of Western Australia, Nedlands, WA

The calcium-sensing receptor (CaR), a G-protein-coupled receptor responsible for the regulation of calcium homeostasis, is comprised of a large extracellular ligand (Ca^{++}) binding domain, from which a signal must be transmitted to the transmembrane domain for receptor activation. The mechanism of signal transmission is not fully understood but a number of domains, including extracellular loop (exoloop) -3 in the transmembrane domain, have been implicated. In order to examine the contribution of exoloop-3 to receptor activation, exoloop-3 residues (^{828}TYGKFVSAVE^{837}), were subjected to alanine scanning mutagenesis. Wild-type and mutant receptors transfected into HEK293 cells were first assessed for cell surface expression using a biotin-streptavidin immunoprecipitation assay. Dose response of mutant receptors to Ca^{++} stimulation, in the presence or absence of the allosteric CaR modulator, NPS-R-467, was then assessed using an inositol phosphate assay. Mutant residues at either end of exoloop-3, T828A, Y829A and E837A, caused a decrease in sensitivity to calcium. By contrast, the other mutants (G830A, K831A, F832A, V833A, S834A and V836A) demonstrated an increase in sensitivity to calcium. Native core residues of exoloop-3 would therefore be predicted to have a constraining effect on CaR activation. Cell-surface expression of some activating mutants was greater than that of wild-type receptor, suggesting that this may contribute to their increased

sensitivity. E837A prevented potentiation of CaR activation by NPS-R-467, whereas F832A appeared to enhance potentiation beyond that seen with wild-type CaR in the presence of NPS-R-467; other mutants failed to influence potentiation. We speculate on the mechanisms whereby exoloop-3 residues might influence CaR activation.

Disclosures:
1 for all authors

O25 8.9.05: 1630 – 1640 hours

MATERNAL VITAMIN D STATUS AND OFFSPRING BIRTH SIZE: A PROSPECTIVE STUDY

R Morley[1], JD Wark[2], J Carlin[1] & J Pasco[3]
[1] Clinical Epidemiology and Biostatistics Unit, University of Melbourne Department of Paediatrics, Murdoch Children's Research Institute, Royal Children's Hospital, [2] University of Melbourne Department of Medicine and Bone and Mineral Service, Royal Melbourne Hospital, Parkville and [3] University of Melbourne Department of Clinical and Biomedical Sciences, Barwon Health, Geelong, VIC

Maternal vitamin D deficiency has been linked with reduced birth size of the offspring, an indicator of fetal growth. However, trials of vitamin D supplementation in pregnancy have yielded inconsistent results. Therefore we conducted a prospective study of 475 healthy Australian women with singleton pregnancies, measuring maternal circulating 25–hydroxyvitamin D (25-OHD) and parathyroid hormone (PTH) levels, and infant size at birth. Maternal blood was collected before 16 weeks (w) and at 28-32 w gestation. The main hypothesis was that offspring of mothers with 25-OHD levels <28 nmol/L at 28-32 w would be smaller at birth than offspring whose mothers had higher 25-OHD levels. Data from 374 pregnancies were suitable for analysis. 25-OHD was <28 nmol/L in 27 women (7.2 %) at 28-32 w. After adjusting for potential confounders (including gestation length), and for log PTH, log serum calcium and log serum albumin, infants in this group differed as follows from infants of mothers with higher 25-OHD levels: knee-heel length –3.2 mm (95% CI – 5.9, - 0.6), mid-upper arm and calf circumference – 0.2 mm (-0.6, 0.1) and – 0.3 (-0.7, 0.06) respectively. The knee-heel difference was greater unadjusted for gestation length. Maternal 25-OHD at 28-32 w was weakly positively associated with gestation length (doubling the 25-OHD equated to a 0.3 w increase in gestation), which partly explained the association between 25-OHD and birth size. Maternal PTH at 28-32 w was negatively associated with 25-OHD. After adjustment, log2 PTH was related positively to infant knee-heel length, birth weight, and mid-upper arm and calf skinfold thickness. These associations were independent of 25-OHD.

Low maternal 25-OHD in late pregnancy is associated with reduced intra-uterine long bone growth. The longterm consequences for linear growth require follow-up. There is also a strong positive relationship between maternal PTH in late pregnancy and infant size, which warrants investigation.

Disclosures:
1.

O26 8.9.05: 1640 – 1650 hours

FACTORS ASSOCIATED WITH SKELETAL AGE DEVIATION IN CHILDREN

M Gutteridge, D Ma & G Jones
Menzies Research Institute, Hobart, TAS

Aims:
We recently reported an independent, dose-response relationship between skeletal age deviation (SAD, the difference between bone age and chronological age) and both bone density and fracture risk in children. In addition, there was wide biological variation in SAD from -3.38 to +4.65 years but little is known about factors responsible for this variation. The aim of this study, therefore, was to describe factors associated with SAD.

Methods:
A convenience sample of 642 children aged 7-17 years was studied. All were assessed for body composition (DXA), diet, strength, dexterity, habitual physical activity, sunlight exposure, smoking and medication use. Skeletal age was assigned using the TW2 method.

Results:
In multivariate analysis, SAD was significantly associated with:

VARIABLE	β-coefficient (yrs)	95% CI
Inhaled corticosteroids (Y v N)	-0.10	-0.77, -0.13
Ever smoking (Y v N)	-0.14	-0.68, -0.19

Grip strength (kPa)	+0.12	+0.00, +0.03
Milk (serve/day)	+0.13	+0.05, +0.25

No association was observed with handedness, dexterity, TV/video watching, physical activity, sun exposure and consumption of cola/carbonated drinks. Both lean and fat mass were independently and positively associated with SAD. Approximately 20% of variability in SAD was explained by these factors.

Conclusion:
This study suggests body composition, diet, ever smoking and inhaled corticosteroids may modify bone maturity relative to age and thus impact on fracture risk in children. However, further studies are necessary to explore other determinants of SAD such as genetic and perinatal factors and whether SAD influences peak bone mass

Disclosures:
1

O27 8.9.05: 1650 – 1700 hours

A COMPARISON OF BMD AND DEMOGRAPHIC DATA FOR PREDICTING CLINICAL FRACTURES OVER 5 YEARS
I Dick[123], A Devine[1234] & RL Prince[123]
[1] University of Western Australia, [2] Sir Charles Gairdner Hospital, [3] Western Australian Institute of Medical Research, [4] Edith Cowan University, Perth, WA

Some have suggested that BMD is not required to predict fracture risk if demographic data for the individual is known.

Demographic variables, total hip BMD (Hologic 4500A), and 5-y incident fracture data were collected on 1125 women (75±3y). Two models were developed for fracture prediction Model 1 - using BMD SD (Z) score and Model 2 using demographics - Age, BMI, TUAG, smoking and prevalent fracture. Beta coefficients were used to calculate 5-y fracture hazards for each person and plotted as ROC's compared to actual incident fractures.

185 individuals (16.4%) sustained ≥1 fractures over 5-y. Areas under the curves for each ROC were identical for each model (0.62±0.02). In both models fewer fractures occurred in the lower compared to the upper quartiles (Model 1: 9.5% vs. 23.8%; Model 2: 9.4% vs. 23.6%). However, only 39% of patients in the lowest risk quartile using demographics had high BMD in Model 1, only 42% in the highest risk quartile using demographics had low BMD in Model 1.

Thus BMD and demographic data have similar predictive power to determine fracture risk but in many patients predicted to be at high or low risk of fracture using demographic data the risk is not related to bone structure and therefore may not respond to therapies designed to improve bone structure.

Disclosures:
All authors 1

O28 8.9.05: 1700 – 1710 hours

GENETIC EFFECTS ON BONE LOSS IN PERI- AND POST-MENOPAUSAL WOMEN: A LONGITUDINAL TWIN STUDY
J Makovey[1], V Naganathan[1,2], TV Nguyen[3] & P Sambrook[1]
[1] *Institute of Bone and Joint Research, Royal North Shore Hospital, University of Sydney, Sydney, NSW*
[2] *Centre for Education and Research on Ageing, Concord Hospital, University of Sydney, Sydney, NSW*
[3] *Bone and Mineral Research Program, Garvan Institute of Medical Research, Sydney, NSW*

Cross-sectional twin and family studies have shown that bone density variance is mainly under genetic influences. The relative magnitude of genetic and environmental components on bone loss variance however is not clear. The aim of this study was to assess the heritability of bone loss in peri- and post-menopausal women.

A sample of 176 pairs of twins (88 MZ and 88 DZ), (mean age 57 years (range: 45 – 57)) were seen. Each individual had base line BMD measurements at lumbar spine and the femoral neck measured by a HOLOGIC QDR-4500W bone densitometer and a repeat measure, on average 3.9 years (range: 1 – 7.5 years) later. Change in BMD (dBMD) was expressed as percent gain or loss per year. Intraclass correlation coefficients in dBMD were calculated for MZ and DZ pairs. Genetic model-fitting analysis was used to partition the total variance of dBMD into three components: genetic component (G), common environmental component shared by the twins

(C), and specific environmental component, including measurement error (E). The index of heritability was estimated as the ratio of genetic variance over total variance.

The mean (± SD) of annual change in BMD was –0.42±1.4 at the lumbar spine and -0.31±1.28 at the femoral neck. There was no significant difference in rate of bone loss between MZ and DZ twins. At the lumbar spine the intraclass correlation in MZ twins (0.39) was significantly higher than in DZ twins (0.19). However, at the femoral neck the intraclass correlation was low in both MZ twins (0.09) and DZ twins (0.00). Results of genetic model-fitting analysis indicated that the index of heritability for dBMD at the lumbar spine was 0.4. There was, however, no significant genetic effect on dBMD at the femoral neck.

These data suggest that although genetic effects on bone loss with ageing are less pronounced than those on peak bone mass, they still account for approximately 40% of the between-individuals variation in bone loss at the lumbar spine in pre- and post-menopausal women.

O29 8.9.05: 1710 – 1720 hours

GENETICS AND REMAINING LIFETIME RISK OF FRACTURE: THE COLLAGEN I ALPHA 1 GENE

TV Nguyen, ND Nguyen, JR Center & JA Eisman
Bone and Mineral Research Program, Garvan Institute of Medical Research, St Vincent's Hospital, Sydney, NSW

The association between genetic polymorphisms and fracture risk is commonly examined in a relative risk-based approach. However, a lifetime risk approach is more meaningful and appropriate. This study examined the association between polymorphisms of the collagen type I alpha 1 (COLIA1) and remaining lifetime risk of fracture (RLRF) in elderly men and women.

COLIA1 genotypes (*SS, Ss* and *ss*) were determined in 698 women and 378 men aged 60+ years as at 1989 of Caucasian background, who have been followed for up to 15 years, during which, fractures were ascertained. Femoral neck BMD was measured by DXA (GE-LUNAR) at baseline. RLRF was estimated by using the survival analysis, with adjustment for the competing risk of death.

The death-adjusted RLRF in women with *ss* genotype was 66% (95%CI: 45-92), significantly higher than those with *SS* (51%; 44- 58) or *Ss* genotype (47%; 38-56). The RLRF for women with osteoporosis (BMD T-score ≤-2.5) was 65% (58-74), which was equivalent to those with *ss* genotype. Moreover, the RLRF for osteoporotic women with *ss* genotype was 94% (95 CI: 82-100). In men, there was no significant difference in RLRF between those with *S* allele (32%; 22-39) and *ss genotype* (27%; 10-41).

These data suggest that women with the COLIA1 *ss* genotype had equivalent remaining lifetime risk of fracture to that with osteoporotic BMD and additive to it. Although there appeared to be no genotype effect for men, the combination of COLIA1 genotypes and BMD measurement could potentially increase the accuracy of identifying high-risk women.

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O30 8.9.05: 1720 – 1730 hours

ESTIMATING THE PREVALENCE OF COELIAC DISEASE IN OSTEODEFICIENT PATIENTS

L Wienholt[1,2], MJ Hooper[2,3,4], AM Kean[3], A Conigrave[2,3], R Loblay[1,2] & AJ Williams[1,2]
[1] *Department of Clinical Immunology, Royal Prince Alfred Hospital, Sydney, NSW*
[2] *University of Sydney, Sydney, NSW*
[3] *Department of Endocrinology, Royal Prince Alfred Hospital, Sydney, NSW*
[4] *Department of Endocrinology, Concord Hospital, Sydney, NSW*

Introduction:
Coeliac disease (CD) is a life-long intolerance to dietary gluten resulting in an inflammation of the small intestine. Research has shown that CD is a highly prevalent, largely undiagnosed illness affecting approximately 0.3% of the general Australian population.

Aim:
To determine the prevalence of CD in patients presenting with osteopaenia or osteoporosis and patients presenting to hospital with non-traumatic fractures.

Methods:
Subjects
All patients attending endocrinology clinics at Royal Prince Alfred Hospital and Concord Hospital, Sydney from April 03 onwards, who were found to have a T-score of -1 or lower on bone mineral density and all patients admitted to Royal Prince Alfred Hospital from April 2003 onwards with the diagnosis of a non-traumatic fracture were serologically screened.

Coeliac Serology
Anti transglutaminase antibodies, anti endomysial antibodies and/or anti gliadin antibodies were measured. Serologically positive patients were asked to undergo a small bowel biopsy for definitive diagnosis of CD.

Results:
Three hundred and fifty patients with presenting osteopaenia or osteoporosis and 450 patients with non-traumatic fractures have been screened for CD. Results confirm that CD is at 7 times more prevalent in patients with osteopaenia or osteoporosis, and 4 times more prevalent in non-traumatic fracture cases than the expected population mean.

Conclusion:
These findings suggest that the routine screening of CD is warranted in all patients with osteopaenia or osteoporosis.

Disclosures:
1

O31 8.9.05: 1630 – 1640 hours

OSTEOCLAST FORMATION IS STRONGLY REDUCED IN THE ABSENCE OF CD47/SIRPα-INTERACTION
P Lundberg[1,3], C Koskinen[1], H Löthgren[1], Å Stenberg[2], UH Lerner[1] & P-A Oldenborg[2]
[1] Department of Odontology, Section for Oral Cell Biology and [2] Department of Integrative Medical Biology, Section for Histology and Cell Biology, Umeå University, Sweden
[3] Neurobiology Program, Garvan Institute of Medical Research, Sydney, NSW

Interaction between the leukocyte receptors CD47 and signal regulatory protein alpha (SIRPα has been found to regulate cell migration, phagocytosis, and macrophage fusion. Since macrophages and osteoclasts have the same haematopoietic origin we investigated if the CD47/SIRPα-interaction could also regulate formation of osteoclasts.

We cultured spleen cells from wild type mice and bone marrow macrophages (BMM) from wild type and CD47 knockout mice. After incubation with macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor (NF)-κB ligand (RANKL), with and without antibodies to either CD47 (mAb miap 301) or SIRPα (mAb P84) the cells were fixed and stained for tartrate-resistant acid phosphatase (TRAP). In parallel wells the TRAP activity was determined and RNA was collected for quantitative real time PCR analyzes.

Functional blocking of SIRPα or CD47, but not isotype controls, inhibited osteoclast formation by 60±4% and 70±2%, respectively, in M-CSF/RANKL-stimulated spleen cell cultures. Similarly, osteoclast formation in BMM cultures was strongly inhibited. In BMM cultures from CD47-deficient mice, the number of osteoclasts was significantly reduced (79±3%), as compared to that in wild-type cultures. Neither P84 nor miap301 affected the enzymatic TRAP activity at the end of spleen cell cultures. Addition of mAb P84 significantly decreased M-CSF/RANKL induced mRNA expression of the osteoclast-specific genes for *calcitonin receptor* and *cathepsin K*, but not that for *TRAP* indicating that the decreased osteoclast formation is not exclusively due to inhibition of osteoclast progenitor cell fusion but also involves effects on RANKL induced differentiation.

We conclude that the CD47/SIRPα interaction is important for M-CSF/RANKL-stimulated osteoclast formation.

Disclosures:
1. No conflict of interest

O32 8.9.05: 1640 – 1650 hours

CARDIOTROPHIN-1 REGULATES OSTEOCLAST FORMATION AND FUNCTION IN A MANNER DISTINCT FROM OTHER GP130 CYTOKINES
NA Sims, JMW Quinn, H Saleh & TJ Martin
Department of Medicine at St. Vincent's Hospital, The University of Melbourne, St. Vincent's Institute, Fitzroy, VIC

The gp130 family of cytokines signal by complexing with the gp130 receptor subunit. In gp130 null mice there is a dramatic increase in osteoclast generation *in vivo* and *in vitro*, in contrast to the stimulatory effects of gp130 family members on cultured osteoclasts. Knockout (KO) mice for some members of this family have distinct bone phenotypes, suggesting distinct signaling pathways induced by the cytokine-specific receptor complex formed. We examined the skeletons of cardiotrophin-1 (CT-1) null mice to determine the unique roles of CT-1 in bone.

Compared with wild type (WT), adult male CT-1 KO mice demonstrated a significant increase in trabecular bone volume (+ 229%) and trabecular BMD (+ 31%). Bone formation was unchanged in CT-1 KOs. The increase in bone mass was associated with an increased number of abnormally large osteoclasts; osteoclast surface and number were approximately doubled and the amount of bone surface covered by each osteoclast was significantly elevated. These osteoclasts appeared to be functionally impaired, suggested by the increased bone mass, and by an increase in cartilage remnants within the trabecular bone. Stimulation of CT-1 KO bone marrow cultures with RANKL and M-CSF led to a significant elevation in osteoclast number in comparison with WT marrow. The osteoclasts generated in vitro were also abnormally large, with more nuclei than those from WT, suggesting that the osteoclast phenotype was cell-lineage autonomous.

CT-1 is therefore unique in its actions on gp130 signaling and essential for normal osteoclast function. This phenotype suggests that CT-1 inhibition may provide a therapeutic target for inhibiting bone resorption.

O33 8.9.05: 1650 – 1700 hours

SCAFFOLD PROTEIN P62 MODULATES OSTEOCLASTOGENESIS THROUGH THE REGULATION OF RANKL SIGNALING CASCADES AND TRAF6 IN THE UBIQUITIN-MEDIATED PROTEASOMAL PATHWAYS

KHM Yip, MH Zheng & J Xu
Department of Orthopaedic Surgery, QEII Medical Centre, University of Western Australia, Perth, WA

p62 is a scaffold protein that mediates diverse cellular signalling cascades including NF-κB and ubiquitin proteasomal pathways. Mutations in p62 gene (also known as SQSTM1) have been found in patients with Paget's disease of bone (PDB), a common bone disorder that is characterized by increased bone resorption and irregular bone formation. Interestingly, all these mutations appear to be clustered at the ubiquitin-associated (UBA) domain of the p62 protein. Up to date, relationship between mutation of p62 UBA domain and pathogenesis of PDB is not yet defined. Moreover, it is still not clear how p62 plays a role in osteoclastogenesis and RANKL cascades during pathological bone resorption. In present study, by using *in-vitro* osteoclastogenesis assays, luciferase gene reporter assays and western blotting we have shown that overexpression of a p62 UBA domain deletion mutant potentiates osteoclastogenesis and RANKL-induced NF-κB and NFAT activation, ERK as well as whole cell tyrosine kinase phosphorylation in comparison with wild type p62. Furthermore, preliminary results from confocal analysis suggest that the p62 UBA domain mediates TRAF6 localization to proteasomal compartments, indicating that p62 might target TRAF6 for ubiquitin-mediated proteasomal degradation. Based on these we propose that the p62 UBA domain is involved in the modulation of RANKL induced osteoclastogenesis, which correlate with the pathogenesis of PDB. More importantly, investigation of the role of p62 and its UBA domain in osteoclastogenesis and RANKL-induced signaling pathways will undoubtedly bring significant insight into the role of this scaffold protein in osteoclast related pathological bone conditions.

O34 8.9.05: 1700 – 1710 hours

THIOREDOXIN BINDING PROTEIN-2 IS A KEY INHIBITOR OF OSTEOCLASTIC BONE RESORPTION

JM Hodge, GC Nicholson & CJ Aitken
The University of Melbourne, Department of Clinical and Biomedical Sciences: Barwon Health, Geelong, VIC

Thioredoxin binding protein-2 (TBP-2) inactivates thioredoxin-1 (Trx-1) by binding to its redox-active site. We have previously shown that adenoviral overexpression of TBP-2 inhibits human osteoclast differentiation, down-regulates Trx-1 expression and inhibits AP-1 activation. This study aimed to test our hypothesis that downregulation of TBP-2 will stimulate osteoclastogenesis.

TBP-2 gene expression was partially silenced using a retrovirus expressing shRNA targeting TBP-2. The control was a nonsense shRNA. CFU-GM osteoclast precursors were infected with the constructs and co-treated with GM-CSF (10 ng/mL) for 24h to stimulate proliferation. The cells were then incubated with hM-CSF (25 ng/mL) and sRANKL (125 ng/mL) for 14d with dentine slices.

TBP-2 shRNA down-regulated TBP-2 mRNA 33-54%. The nonsense shRNA did not affect osteoclastogenesis. TBP-2 shRNA caused moderate increases in osteoclast formation (+24%, p=0.038), size (+15%, p=0.009) and total area (+31%, p=0.027). Increases in total resorption (+152%, p=0.005) and resorption/osteoclast (+137%, p=0.014) were more marked. Nuclei/osteoclast was increased (+22%, p=0.0001) suggesting that increase in cell size was due to fusion.

We have shown that TBP-2 is a key inhibitor of osteoclastic bone resorbing activity and, to a lesser extent, differentiation. The mechanism of the effect remains to be determined although we propose that it is mediated by modulation of nuclear Trx-1 and RANKL-induced AP-1 and NF-κB activation.

Disclosures:
1

O35 8.9.05: 1710 – 1720 hours

HUMAN OSTEOCLAST DIFFERENTIATION AND FUNCTION ARE STIMULATED BY EXOGENOUS ROS AND RANKL-INDUCED INTRACELLULAR ROS PRODUCTION

LAB Hodgson, CJ Aitken, JM Hodge, MA Chacksfield & GC Nicholson
The University of Melbourne, Department of Clinical and Biomedical Sciences: Barwon Health, Geelong, VIC

It has been shown in animal models that reactive oxygen species (ROS) activate osteoclasts and act as second messengers in RANKL signaling. Our aim was to investigate the role of ROS in human osteoclast differentiation and function.

The Amplex® Red Hydrogen Peroxide/Peroxidase assay was used to measure intracellular H_2O_2 in mature osteoclasts generated by culture of human CFU-GM for 14d in hM-CSF (25ng/ml) and sRANKL (125ng/ml). Cells were serum-starved for 20h prior and then treated with sRANKL (125ng/mL). Cells were lysed, Amplex Red reagent added and incubated for 30min before reading on a fluorescence microplate reader. There were significant ($p=0.001$) increases in H_2O_2 levels at 10, 15, 30 and 45min post-RANKL treatment, with peak effect (+35%) at 10min. Similarly, using fluorescence microscopy and the ROS-sensitive dye CM-H_2DCFDA, we found that treatment of serum-starved mature osteoclasts with sRANKL for 30min increased intracellular ROS levels.

Co-treatment of osteoclastogenesis cultures with H_2O_2 (50μM), increased resorption and osteoclast size even when added as late as day 13. Co-treatment with H_2O_2 caused a left-shift in the RANKL dose-response curve, particularly enhancing the effect of sRANKL at 12.5 ng/mL. H_2O_2 increased basal (+53%) and sRANKL-stimulated (+184%) NF-κB activation, as assessed by EMSA.

We have shown sRANKL signals via ROS (ligand-mediated ROS production) and that ROS promote osteoclast formation and resorptive activity. It is likely that the effects of ROS are mediated by NF-κB and other downstream RANKL signaling pathways.

No conflicts of interest exist for any of the authors

O36 8.9.05: 1720 – 1730 hours

CAFFEIC ACID PHENETHYL ESTER, A NATURAL COMPONENT OF HONEYBEE PROPOLIS INDUCES OSTEOCLAST APOPTOSIS AND ATTENUATES OSTEOCLASTOGENESIS VIA THE SUPPRESSION OF RANKL-INDUCED NF-κB AND NFAT ACTIVITY

LY Chai[1], ESM Ang[1], N Pavlos[1], KHM Yip[1], JH Steer[2], DA Joyce[2], MH Zheng[1] & J Xu[1]
[1] Molecular Orthopaedic Laboratory, School of Surgery and Pathology, [2] Pharmacology Unit, School of Medicine and Pharmacology, University of Western Australia, Nedlands, WA

NF-κB is a key regulator of osteoclast differentiation, activation and survival. Caffeic acid phenethyl ester (CAPE), a natural NF-κB inhibitor from honeybee propolis has been shown to have anti-tumor and anti-inflammatory properties. However, the effect of CAPE on osteoclast formation and RANKL signaling is unknown. In this study, we investigated its effect on the regulation of RANKL-induced osteoclastogenesis and osteoclast survival. Both RAW264.7 cells and primary bone marrow cells were used to examine the effect of CAPE on RANKL-induced osteoclastogenesis. In order to determine the action of CAPE on signaling pathways, we used reporter gene assays for NF-κB and NFAT activity, and Western blotting for phospho-IKBα. To assess rates of apoptosis we measured changes in annexin staining, caspase-3 activity, chromatin and microtubule structure. Our results showed that low concentrations of CAPE (<0.5 uM) dose dependently inhibited RANKL-induced osteoclastogenesis in RAW 264.7 cells and in bone marrow cell cultures. At higher concentrations, CAPE induced apoptosis of RAW 264.7 cells and RAW 264.7 cell-derived osteoclast like cells (OLCs). Consistently, we found that CAPE increases caspase-3 activity and disrupts the microtubule network in OLCs. Furthermore, CAPE inhibited both basal and RANKL-induced NF-κB and NFAT activation in a dose dependent manner. This study implies that attenuation of osteoclastogenesis and induction of osteoclast apoptosis through the inhibition of NF-κB and NFAT activation by this natural compound might be useful for the treatment of osteolysis attended with enhanced osteoclast formation and activation.

O37 9.9.05: 1045 – 1055 hours

A NOVEL MUTATION (K378X) IN THE SEQUESTOSOME 1 GENE: ASSOCIATION WITH A SEVERE PAGETS DISEASE OF BONE PHENOTYPE AND FUNCTIONAL IMPLICATIONS ON NF-κB ACTIVATION

SL Morgan[1,2], L Ward[1], BK Ward[1,2], J Walsh[1], J Xu[3], B Stuckey[2], N Kent[4] & T Ratajczak[1,2]
[1] Laboratory for Molecular Endocrinology, Western Australian Institution for Medical Research and UWA Centre for Medical Research, The University of Western Australia, Nedlands, WA
[2] Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA
[3] Department of Orthopaedics, School of Surgery, The University of Western Australia, Nedlands, WA
[4] Department of Clinical Pathology, PathCentre, The Queen Elizabeth II Medical Centre, Nedlands, WA

The second most common bone disease in Caucasian populations is Paget's disease of bone (PDB), a disorder generally affecting persons over 55. A significant number of PDB patients have recently been reported to harbour mutations within the Sequestosome 1 (SQSTM1/p62) gene. Our study aimed to screen patients with either sporadic or familial PDB for p62 mutations. The significance of mutations on the activation of transcription factor NF-κB, important for osteoclastogenesis, was also investigated.

We report the prevalence of P392L to be 7.69% (8 of 104) for sporadic and 14% (2 of 14) for familial patients. In addition, a novel mutation was identified, a transversion mutation (A to T) at position +1132, resulting in a premature stop codon at amino acid 378 (K378X). This mutation was identified in a familial patient displaying a severe PDB phenotype.

To determine the affect of p62 (WT, K378X, P392L, and E396X) on NF-κB activation, Cos1 cells were transfected with a luciferase reporter gene and one of the p62 expression plasmids or an empty vector control. Our results have consistently shown that WT p62 has an inhibitory effect on NF-κB activation ($p = 0.0068$). In addition, all mutants activate NF-κB to a significantly greater extent than WT p62: K378X ($p = 0.0228$), P392L ($p = 0.04$) and E396X ($p = 0.00046$). Similar results were observed in HEK293 cells.

Our study therefore supports continued investigation of the p62 gene, as well as the effects of identified mutations, on osteoclast signalling pathways, in relation to PDB.

O38 9.9.05: 1055 – 1105 hours

HIGH IGFBP-2 LEVELS ARE ASSOCIATED WITH AN INCREASED RISK OF OSTEOPOROTIC FRACTURES IN ELDERLY MEN

MJ Seibel[1], TV Nguyen[2], JR Center[2], C Meier[1] & JA Eisman[2]
[1] *Bone Research Program, ANZAC Research Institute, The University of Sydney, NSW*
[2] *Bone and Mineral Research Program, Garvan Institute of Medical Research, St Vincent's Hospital and UNSW, Sydney, NSW*

Serum IGFBP-2 levels increase with advancing age, are inversely associated with bone mineral density (BMD) and are positively correlated with bone resorption rates. We examined the relationship between serum IGFBP2 and fracture risk in community-dwelling men.

This case-cohort control study included 139 men, aged 71 ± 5.3 yrs (mean±SD) who had been prospectively followed in the Dubbo Osteoporosis Epidemiology Study for a median of 6.3 yrs (range, 2-13 yrs). During this period, 38 men had incident low-trauma non-vertebral fractures (cases), while 101 men had no fractures (controls). Femoral neck BMD, markers of bone resorption (ICTP) and formation (PINP), and serum levels of IGF-I, IGFBP-2, IGFBP-3 were measured at baseline.

In the entire sample, IGFBP-2 was negatively correlated with femoral neck BMD ($r= -0.21$; $p= 0.01$) and positively correlated with age ($r= 0.34$; $p<0.001$). Men with a subsequent fracture had lower baseline IGFBP-2 levels and higher baseline ICTP levels than men without a fracture. There were no significant differences in IGF-I, IGFBP-3 between cases and controls. In univariate logistic regression analysis, increased fracture risk was associated with higher IGFBP-2 (odds ratio [OR] 1.9; 95%CI 1.3-2.9) or lower ICTP levels (OR 2.3; 95% CI 1.5-3.5). After adjusting for age and femoral neck BMD, the effect of IGFBP-2 remained statistically significant (OR 1.73; 95%CI: 1.1–2.7). However, in the presence of ICTP, age and femoral neck BMD, the magnitude of effect of IGFBP-2 on fracture risk reduced to OR of 1.4 (95% CI: 0.8-2.3).

In conclusion, these results suggest that IGFBP-2 predicts fracture risk in men independent of BMD and age, possibly mediated through an association between IGFBP-2 and bone resorption.

Disclosures:
All authors, 1

O39 9.9.05: 1105 – 1115 hours

ESTRADIOL PREDICTS 5 YEAR FRACTURE RISK AND FOREARM CORTICAL DENSITY, VOLUME AND STRENGTH

A Devine[1235], RL Prince[123], SS Dhaliwal[34] & IM Dick[123]
[1] *University of Western Australia,* [2] *Sir Charles Gairdner Hospital,* [3] *Western Australian Institute of Medical Research,* [4] *Curtin University,* [5] *Edith Cowan University, Perth, WA*

In women 25 years post-menopause we examined the effects of endogenous estrogen status on incident fracture risk and peripheral quantitative computerized tomography (pQCT) 3D measured cortical structure.

Study design: In 1150 women mean (SD) age 75(2.6) baseline serum estradiol and SHBG were measured and the free estradiol index (FEI) calculated; radiologically verified fractures were recorded over five years; pQCT was measured at the radius 4% site (Stratec XCT 2000) to

evaluate bone structure in 3D and bone strength (Stress Strain Index SSI) in the three directions. Analyses were adjusted for BMI, age and calcium supplementation; hazard ratios (HR) for time to fracture were calculated by Cox regression.

16% of patients fractured over five years. Fracture risk was related additively to baseline FEI (HR per SD 0.75, 0.58-0.97) and the total pQCT bone density (HR per SD 0.67, 0.55-0.82), fracture rates in the bottom and top quartile of FEI and pQCT bone density were 21.7% and 11.8% and 26.3% and 10.5% respectively.

FEI was positively associated with cortical bone volume and density (β coefficients 0.16, $p<0.001$ and 0.21, $p<0.001$) and strength (SSI) in the x, y and polar directions (0.16, $p<0.001$; 0.19, $p<0.001$ and 0.19, $p<0.001$ respectively).

In elderly women bioactive oestrogen is an important determinant of both cortical bone structure and strength and fracture risk.

Disclosures:
All authors 1

O40 9.9.05: 1115 – 1125 hours

HAVING A NON-MELANOMA SKIN CANCER DECREASES THE RISK OF SUBSEQUENT FRACTURE: A RECORD LINKAGE STUDY

V Srikanth, J Fryer, L Blizzard, A Venn, L Newman, H Cooley, T Albion & G Jones
Menzies Research Institute, Hobart, TAS

Aims and objectives:
It is uncertain whether low levels of sun exposure are sufficient for vitamin D homeostasis or whether higher levels of exposure (which increase the risk of non-melanoma skin cancer (NMSC) may also have benefit. We tested the hypothesis that, if the latter is true, people ≥ 50 years of age with a non-melanoma skin cancer will have lower rates of fracture.

Methods:
A record-linkage study was performed between the Tasmanian Cancer Registry (TCR) which monitors incident skin cancer from 1991 to the present and the Southern Tasmanian Fracture Registry which monitored incident fracture, based on X-ray reports, from 1997-2002. The expected number of people with prior NMSC in the fracture cohort was calculated based on NMSC incidence data from the Southern Tasmanian residents of the TCR using indirect standardization. Relative risks were calculated as the ratio between observed and expected number of NMSC cases.

Results:
All fractures were more common in those without NMSC.

Fracture Site	Observed cases	Expected cases	RR (95% CI)
All Fractures	268	388	**0.69 (0.61, 0.78)**
Hip	73	130	**0.54 (0.44, 0.71)**
Wrist	96	117	**0.80 (0.66, 1.00)**
Spine	60	78	**0.77 (0.59, 1.00)**

Conclusion:
A diagnosis of NMSC is associated with a substantial decrease in subsequent fracture risk most likely reflecting that the outdoor lifestyle that increases skin cancer risk also decreases fracture risk. Thus, it may not be possible to balance sun exposure for both bone and skin health but only to achieve a compromise position based on individual risk profiles.

Disclosure:
1

O41 9.9.05: 1125 – 1135 hours

OLDER WOMEN WITH HIGHER LEVELS OF PHYSICAL ACTIVITY AND DIETARY CALCIUM INTAKE HAVE STRONGER BONES

MK Nurzenski[1], NK Briffa[1], RI Price[2], B Khoo[2], A Devine[3,4,5], TJ Beck[6] & RL Prince[3,4]
[1] *School of Physiotherapy Curtin University of Technology, WA*
[2] *Medical Technology and Physics, Sir Charles Gairdner Hospital, WA*

[3] *School of Medicine and Pharmacology, University of Western Australia, WA*
[4] *Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, WA*
[5] *School of Exercise, Biomedical and Health Science, Edith Cowan University, WA*
[6] *Department of Radiology, Johns Hopkins University School of Medicine, Baltimore, USA*

Introduction:
In a population-based study of elderly women it has been shown that those in the highest tertile of physical activity (PA) and the highest two tertiles of dietary calcium intake (CI) had 4.4 to 6.4% higher hip BMD. The effects of these two lifestyle factors on geometric indices of bone strength have now been examined in 1010 women from the same cohort.

Materials and Methods:
Baseline PA and CI were assessed by validated questionnaires, and one year DXA scans (Hologic 4500A) were analysed using hip structural analysis software (v3). Section modulus (Z), an index of bending resistance, and cross-sectional area (CSA), an index of axial bone strength were measured at the femoral neck (NN), intertrochanter (IT) and femoral shaft (S) sites. These data were divided into tertiles of PA and CI and the results compared using ANCOVA (SPSS v11.5) with correction for age, BMI and treatment (calcium/placebo).

Results and Conclusions:
PA showed a significant dose response effect on CSA and Z at all hip sites ($p<0.004$). For CI, there was a dose response effect for CSA and Z at the IT and S regions ($p<0.05$). These effects were additive, women (n=241) with PA levels in the upper two tertiles and CI in the upper tertile had greater femoral axial strength (NN 6.5%, IT 6.1%, S 5.3%) and bending resistance (NN 6.6%, IT 3.8%, S 4.9%). This supports the use of population-based lifestyle interventions to improve femoral strength to levels already achieved by almost one quarter of the population.

O42 9.9.05: 1135 – 1145 hours

NON-INVASIVE, NONIONIZING DISCRIMINATORS OF PREVALENT VERTEBRAL FRACTURE CASES: A QUS-BASED MODEL

B-K Tan[1], RI Price[2], S Song[3], SS Dhaliwal[4] & KP Singer[5]
[1] *Centre for Musculoskeletal Studies, School of Surgery and Pathology, The University of Western Australia; School of Physiotherapy, Curtin University of Technology, Perth, WA*
[2] *Department of Medical Technology and Physics, Sir Charles Gairdner Hospital, Perth, WA*
[3] *Division of Imaging Services, Royal Perth Hospital, Perth, WA*
[4] *School of Public Health, Curtin University of Technology, Perth, WA*
[5] *Centre for Musculoskeletal Studies, School of Surgery and Pathology, The University of Western Australia, Perth, WA*

Aim:
This study aimed to investigate if a composite model, comprising calcaneal quantitative ultrasonometry and assessments of thoracic spinal curvature and risk factors associated to falls, can differentiate a group of elderly women with prevalent vertebral fractures from those without.

Methods:
104 community-dwelling women (mean age 71.3 ±5.8) underwent proximal femur and lumbar spine DXA BMD and calcaneal quantitative ultrasound (QUS) evaluations; thoracic spinal curvature measurements and; falls risk assessments. These women were dichotomised into a group with radiological evidence of prevalent vertebral fractures (VF, n = 24; combined vertebral morphology & radiologist) or a group without vertebral fractures (NVF, n = 80).

Results:
The VF group had lower QUS measurements ($p<0.05$) and greater thoracic curvature ($p = 0.04$) independent of age. The final composite discriminant model, identified BUA, thoracic curvature and postural sway when standing with eyes closed (EC), with a final Wilk's Lambda of 0.79 ($p<0.0001$). A composite model comprising of BUA and thoracic curvature had higher area under the ROC curve (AUC = 0.75), compared to DXA total hip BMD alone (AUC = 0.60).

Conclusions:
Low calcaneal QUS, increased thoracic curvature and increased postural sway significantly discriminated those at high risk of future osteoporotic fractures. The equipment required for these assessments is non-ionising, easy to administer, reliable and can be set up in community and rural settings. This model may be useful as a pre-screening strategy to identify individuals at high risk of osteoporotic fracture and to refer them for further assessment.

O43 9.9.05: 1045 – 1055 hours

EVIDENCE FOR LRP1-INDEPENDENT ACTIONS OF LACTOFERRIN IN OSTEOBLASTS

Q Zhu, J Cornish, M Watson, K Callon, IR Reid & A Grey
Department of Medicine, University of Auckland, Auckland, New Zealand

Lactoferrin (Lf) is anabolic to bone. *In vitro*, it stimulates osteoblastic cell proliferation and differentiation, and inhibits osteoclastogenesis; *in vivo*, it increases bone formation. We recently reported that the low density lipoprotein receptor-related protein1 (LRP1) mediates Lf-induced mitogenic signalling in osteoblastic cells, thereby contributing to the anabolic skeletal actions of lactoferrin.

In the present study, we demonstrate that Lf dose-dependently inhibits osteoblast apoptosis induced by serum withdrawal. This action is not sensitive to receptor associated protein (RAP), a specific LRP1 and LRP2 inhibitor. Furthermore, the ability of Lf to prevent apoptosis in LRP1-expressing fibroblastic cells is not sensitive to RAP. Lf prevents apoptosis in fibroblastic cells to a similar degree in the presence and absence of functional LRP1, suggesting that LRP1 is not necessary for the anti-apoptotic actions of Lf. Lf activates PI3-kinase-dependent phosphorylation of Akt in osteoblastic cells. The activation of Akt signalling by Lf is not sensitive to RAP. Inhibition of Lf-induced Akt activation did not abrogate the anti-apoptotic actions of Lf. We previously demonstrated that Lf activates p42/44 MAPK signalling in osteoblastic cells. The MAPK inhibitor U-0126, however, did not inhibit the pro-survival actions of Lf.

Taken together, our data demonstrate that Lf promotes osteoblast survival, but that, unlike the mitogenic actions of Lf, this effect is not mediated by LRP1. Neither does it involve the PI3K or p42/44 MAPK signalling pathways. These data suggest that Lf signals through more than 1 membrane-bound receptor to produce its anabolic skeletal effects.

Disclosures:
1. No conflict of interest
2. No shares in company
3. No paid consultancy
4. No company financial support
5. No further information

O44 9.9.05: 1055 – 1105 hours

DICKKOPF'S ARE EXPRESSED BY BREAST CANCER CELL LINES AND REGULATED BY PTHRP

K Häusler, M Ciccomancini, N Ilievski, , TJ Martin & M Gillespie
St. Vincent's Institute, Melbourne, VIC

Dickkopf (Dkk) proteins have been shown to bind to LRP-5 and prevent Wnt signalling, a pathway involved in bone formation. We have investigated the role of the Wnt pathway in breast cancer growth in bone using a candidate gene approach.

We determined mRNA expression levels of secreted frizzled-related proteins (sFRP's), Dkk's and other Wnt pathway molecules using MCF-7 human breast cancer cell lines. MCF-7, vector control cell lines and several clonal cell lines overexpressing PTHrP, which induce greater osteolytic damage *in vivo* than parental MCF-7 cells, were found to express components of the Wnt pathway. Notably, the cell lines overexpressing PTHrP had increased Dkk-2 mRNA expression relative to vector control and parental MCF-7 cells.We investigated the association between Dkk-2 and PTHrP mRNA in MCF-7 cells using recombinant and synthetic peptides corresponding to various portions of PTHrP.C-terminal region of PTHrP (107-139aa), but not N-terminal sequences (1-34 and 1-108aa), were found to upregulate both Dkk-2 and Dkk-3 mRNA's 4-fold in MCF-7 and MCF-10A (mammary epithelial) cell lines. There was no effect of any fragments of PTHrP on Dkk-1 mRNA.

We established that there is an active canonical Wnt signalling pathway present in MCF-7 cells by measuring translocation of β-catenin to the nucleus (immunocytochemistry) and activation of Tcf/Lef gene transcription (luciferase reporter) in the presence of either LiCl (GSK3β inhibitor) or recombinant Wnt3a.

To determine the effect of Dkks upon osteoclast formation, we used recombinant Dkk-1 and found that this inhibits osteoclast formation *in vitro*. sFRP-1, another inhibitor of the Wnt pathway, also inhibited osteoclast formation. Both sFRP-1 or Dkk-1 was able to dose dependently inhibit osteoclast formation in either osteoblast/bone marrow co-cultures, or RANKL+M-CSF-treated bone marrow cultures. The action of Dkk-1 and sFRP-1 to limit osteoclast formation may be explained by their ability to inhibit Wnt signaling (canonical, canonical and non-canonical respectively), suggesting that Wnt signaling is involved in osteoclastogenesis.

Combined these data suggest that Dkk's produced by breast cancers may influence the proposed vicious cycle of destruction characteristic of breast cancer growth in bone in an autocrine manner, particularly since PTHrP, a driver of osteolysis, regulates expression of Dkk's. In addition, there may be paracrine actions of Dkk's on resident osteoclast and osteoblast cells.

O45 9.9.05: 1105 – 1115 hours

CYCLOOXYGENASE-2 MEDIATES THROMBIN'S INHIBITION OF OSTEOBLAST APOPTOSIS BY A PAR-1-INDEPENDENT MECHANISM

L Loh[1], CN Pagel[1], S Merlin[1], E Tudor[2], RN Pike[3] & EJ Mackie[1]
[1]School of Veterinary Science, [2]Department of Pharmacology, University of Melbourne, [3]Department of Biochemistry and Molecular Biology, Monash University, Melbourne, VIC

We have previously shown that treatment of serum-deprived or dexamethasone-treated primary mouse calvarial osteoblasts with 100 nM thrombin significantly reduces apoptosis in these cultures, and that this effect is not mediated by any of the known thrombin-responsive protease-activated receptors (PAR-1, -3 and -4), but is mediated by a secreted inhibitor of apoptosis. In the current study we have used inhibitors of various intracellular signaling pathways to identify the pathways involved in thrombin's inhibition of osteoblast apoptosis. As only inhibitors of cyclooxygenases prevented thrombin's effect on osteoblast apoptosis, the expression of cyclooxygenase-2 (COX-2) and synthesis of prostaglandin E_2 (PGE_2), following thrombin treatment of PAR-1 null primary osteoblasts, and the ability of PGE_2 to inhibit serum deprivation-induced osteoblast apoptosis, were studied. Six hours after thrombin treatment of PAR-1 null osteoblasts, expression of COX-2 mRNA and secretion of PGE_2 were significantly elevated. To study the potential role of prostaglandin E_2 in the inhibition of osteoblast apoptosis, serum-deprived primary mouse osteoblasts were treated with either PGE_2 (1 □M) or the cAMP analogue 8-bromo cAMP (1pM-1□M). Both PGE_2 and between 1 pM and 1 nM 8-bromo cAMP significantly inhibited serum deprivation-induced osteoblast apoptosis as judged by staining for DNA strand breaks and nuclear morphology. Taken together these results suggest that thrombin stimulates COX-2-mediated PGE_2 production by a mechanism that does not require PAR-1 and that this rise in PGE_2 signals through cAMP to inhibit serum-deprivation-induced osteoblast apoptosis.

Disclosures:
No conflict of interest

O46 9.9.05: 1115 – 1125 hours

ANABOLIC AND CATABOLIC BONE DEFECTS IN A MOUSE MODEL OF TYPE 1 NEUROFIBROMATOSIS (NF1)

A Schindeler & DG Little
The Department of Orthopaedic Research & Biotechnology and The CHW Clinical School, The Children's Hospital at Westmead, Sydney, NSW

NF1 is a common autosomal dominant disorder that affects 1 in 3500 individuals. The orthopaedic manifestations of NF1 can be severe and have a significant negative impact on quality of life. Congenital pseudarthrosis of the tibia (CPT) is a feared condition that occurs in 2-4% of children with NF1. These children make up 55% of all cases of CPT. Despite continuing advances in orthopaedics, the treatment of CPT remains problematic with residual disability or amputation remaining common. NF1 patients also show higher instances of osteopenia and their bone healing following a fracture is often deficient, thus implying fundamental defects in bone homeostasis and repair. We are studying these processes in a mouse model of NF1.

Based on the human disease, we hypothesised that NF1+/- mice may exhibit defects in both osteoblast-driven bone formation (anabolism) and osteoclast-driven bone resorption (catabolism). Consistent with an anabolic deficiency, cultured calvarial osteoblasts from NF1+/- mice showed decreased ALP+ cells and decreased mineralisation. In contrast, NF1-derived bone marrow lymphocytes stimulated with RANKL/M-CSF demonstrated increased osteoclastic differentiation and resorption of a calcified substrate, congruous with exuberant catabolism in the NF1+/- state. Pilot co-culture experiments have suggested crosstalk between NF1+/- osteoblasts and osteoclasts may compensate and attenuate these defects in vivo. In conclusion, our initial findings using cultured NF1+/- bone cells have indicated dual defects in anabolism and catabolism. These data may support the combination of pro-anabolic and anti-catabolic therapies for the treatment of CPT.

O47 9.9.05: 1125 – 1135 hours

PTHrP SENSITISES BREAST CANCER CELLS TO Apo2L/TRAIL-MEDIATED CELL DEATH

S Bouralexis, TJ Martin & MT Gillespie
Bone, Joint and Cancer Unit, St. Vincent's Institute, Fitzroy, VIC

Parathyroid hormone-related protein (PTHrP) is a multifunctional protein that is produced by approximately 80% of breast cancers results. PTHrP positive primary breast cancers are correlated with an improved patient prognosis, with fewer metastases at all sites, including bone. We have established that PTHrP produced by tumors locally in the bone contributes to bone metastasis formation, yet its production in primary cancers is associated with a more favourable phenotype. The latter clinical finding led us to discover that PTHrP

over-expression in human breast cancer cells, or treatment of those cells with PTHrP, resulted in enhanced production of several DNA repair enzymes, including, BRCA-1, BRCA-2, ATM, ATR, BARD1, Rad51 and Mdm-2. Structure-function studies demonstrated that the domain within PTHrP corresponding to residues 107-111 (or TRSAW), a portion of PTHrP that has been shown in other studies to have biological activities distinct from those mediated by the amino-terminal region of the molecule responsible for mediating bone destruction. Whilst the interplay between DNA repair and apoptotic genes has been well documented, the interaction of PTHrP with other molecules known to link into cell death pathways has not been investigated. One such pathway, which has received considerable interest is the Apo2L/TRAIL apoptotic pathway. Apo2L/TRAIL is a member of the tumour necrosis factor (TNF) family of cytokines and induces death of tumour cells, but not normal cells. Its potent apoptotic activity is mediated through cell surface death domain-containing receptors, DR4/TRAIL-R1 and DR5/TRAIL-R2. Whilst, there is some evidence in the literature for the ability of PTHrP to sensitise cells to apoptosis, the interaction has not been investigated intensely. We have over expressed PTHrP in a number of breast cancer cell lines and determined their responsiveness to Apo2L/TRAIL. Compared with parental and vector transfected cells, PTHrP overexpression in either MCF-7, MDA-MB-231 renders these cells more susceptible to Apo2L/TRAIL-mediated apoptosis: apopotosis was increased 2 fold. This highlights a new action for PTHrP as well as a possible mechanism to treat breast cancers.

O48 9.9.05: 1135 – 1145 hours

ZOLEDRONIC ACID TREATMENT ENHANCES HARD CALLUS FORMATION WITHOUT DELAYING ENDOCHONDRAL REPAIR IN A RAT FRACTURE

M McDonald, S Dulai, C Godfrey & DG Little
The Children's Hospital at Westmead, Sydney, NSW

Premature catabolism of callus may adversely affect fracture repair. We used zoledronic acid (ZA) in a closed rat fracture model, to examine endochondral ossification, callus remodelling and strength using different, clinically relevant dosing regimes.

Rats were treated with saline or 0.1 mg/kg ZA [bolus or 5 divided, weekly doses], commencing 1 week post-fracture and collected at 4, 6, 12 and 26 weeks.

Importantly, cartilaginous callus content was unaltered by ZA, indicating osteoclasts are not essential to soft callus removal. Hard callus BMC and volume, however, were increased in ZA groups over saline at all times ($p<0.01$), with mechanical strength significantly increased at 6 weeks (23-34% $p<0.05$).

Important differences between treatment regimes were apparent. In the bolus group callus, volume decreased by 8% between 4-6 weeks and 25% between 6-12 weeks, suggesting continued callus remodelling and resorption.

In contrast, the weekly dosed group callus volume increased by 24% between 4-6 weeks suggesting a lack of remodelling. From 6-12 weeks, a reduction similar to that of the bolus group occurred but callus volume remained 26-27% larger in the weekly group compared to bolus at 12 and 26 weeks ($p<0.01$). Differences in lamellar bone content were also noted.

ZA did not delay endochondral ossification, indicating that osteoclast function is not essential for soft callus removal. Bolus treatment was superior to weekly dosing; hard callus remodelling commenced, reducing volume whilst increasing strength. This highlights the likely safety of ZA treatment during fracture repair. Bolus ZA provides a larger, stronger callus without delaying endochondral repair.

P - POSTERS

P1

SERUM CATHEPSIN K IS A MARKER OF BONE TURNOVER IN OSTEOPOROSIS AND PAGET'S DISEASE OF BONE

C Meier[1], TV Nguyen[2], CR Dunstan[1] & MJ Seibel[1]
[1]*ANZAC Research Institute, University of Sydney, NSW*
[2] *Garvan Institute of Medical Research, University of NSW, Sydney, NSW*

Cathepsin K, a cysteine protease, plays an essential role in osteoclast-mediated collagen degradation. Recently, an immunoassay to quantify cathepsin K in serum has been developed. We assessed the usefulness of serum cathepsin K as a marker of bone turnover in cross-sectional and longitudinal studies of patients with metabolic bone disease.

The study cohort consisted of 40 healthy controls (13 premenopausal women [age, 30.6±6.6 yrs (mean±SD)]; 11 postmenopausal women [64.1±8.3 yrs]; 16 men [41.6±12.4 yrs]), 21 women with postmenopausal osteoporosis (66.1±7.9 yrs) and 10 patients with Paget's disease of bone (67.1±11.6 yrs). Patients were started on oral or intravenous bisphosphonate treatment and were followed prospectively over 6 months. Circulating cathepsin K levels were determined by a specific sandwich enzyme immunoassay (Biomedica, Austria). In addition, serum carboxyterminal cross-linked telopeptide of type I collagen (βCTX-I) and bone-specific alkaline phosphatase (BALP) were measured for comparison.

Serum cathepsin K levels were similar in the three healthy control groups with a mean (±SD) level of 3.1 (±1.7) pmol/L. When compared to healthy controls, mean cathepsin K levels were significantly elevated in women with postmenopausal osteoporosis (11.3 ± 13.1 pmol/L, p=0.01) and in patients with Paget's disease of bone (6.2 ± 4.4 pmol/L, p=0.04). In postmenopausal osteoporotic women, both oral and intravenous bisphosphonate treatment resulted in a significant reduction in serum cathepsin K levels with a nadir at one month (-33.9 % vs. baseline; p=0.01) and stable suppression thereafter. The magnitude of these changes was intermediate to those seen with βCTX-I and BALP. In patients with mild Paget's disease (baseline BALP, 73.3±50.4 U/L), cathepsin K tended to decrease during bisphosphonate treatment (mean % change after one month: -30.3%).
Serum cathepsin K levels appear to reflect osteoclastic activity in patients with postmenopausal osteoporosis and Paget's disease and may hold promise as a direct cellular marker of osteoclast activity.

Disclosures:
All auhors, 1

P2

BONE TURNOVER IS REDUCED AT 18 MONTHS AFTER A SINGLE INTRAVENOUS DOSE OF ZOLEDRONIC ACID

CAM Kulak[1], GJ Paz-Filho[1], JP Bilezikian[2], VZC Borba[1] & MJ Seibel[3]
[1] *Serviço de Endocrinologia e Metabologia da UFPR, Curitiba, Brazil*
[2] *Columbia University, New York, USA*
[3] *ANZAC Research Institute, The University of Sydney, Sydney, NSW*

Zoledronic acid (ZOL) is a highly potent bisphosphonate. A single intravenous dose of 4mg ZOL has been reported to inhibit bone resorption in women with low bone mineral density (BMD) for at least 12 months (1). The duration of this effect remains unknown. We evaluated changes in bone turnover at 12 (T12) and 18 (T18) months after a single injection of 4 mg of ZOL (T0) in 29 patients (24 female; age 61.2 ± 15 years) with low BMD. Serum CTX-I, a marker of bone resorption, serum BALP, a marker of bone formation and lumbar spine, femoral neck and total hip BMD were measured concurrently in all patients.

Results:
Median CTX levels were 0.183 ng/ml at T0 (range: 0.01 - 0.69), 0.039 ng/ml (0.01 - 0.37) at T12 ($p<0.001$ vs. T0) and 0.108 ng/ml (0.012 - 0.42) at T18 (p= 0.063 vs T0, p= 0.068 vs T12). There was no significant change in median BALP levels at any time point. The median percent increase in BMD (vs. T0) at the lumbar spine was 3.3% (-9.5 to +26.6) at T12 and 5.1% (-5.9 to +21.93) at T18. At the femoral neck, the corresponding changes were 2.1% (-4.7 to +83.3) and 0.13% (-6.8 to +116.2), and at the total hip 2.5% (-5.2 to +68.3) and 1.14% (-6.2 to +7.5). Changes in hip BMD and serum CTX correlated inversely at T12 (r= -0.509, p=0.01), but no correlations were found between other skeletal sites and bone markers at T12 and T18.

Conclusion:
A single administration of 4mg of ZOL inhibits bone resorption for 12 months, with substantial reductions still being observed at 18 months. Therefore, a dosing interval of 12-18 months may be appropriate in patients with low BMD to inhibit bone resorption.

(1) Reid IR et al. New Engl J Med 28; 346: 653-61, 2002.

P3

FALL-RELATED FACTORS PREDICT FRACTURE RISK IN ELDERLY MEN AND WOMEN WITHOUT OSTEOPOROSIS

TV Nguyen, ND Nguyen, JR Center & JA Eisman
Bone and Mineral Research Program, Garvan Institute of Medical Research, St Vincent's Hospital, Sydney, NSW

About 50% of women with fragility fracture do not have osteoporosis by BMD, yet risk factors for fracture in the non-osteoporosis group have not been well-documented. The aim of this study was to examine the contribution of non-BMD factors to low trauma fracture risk in elderly men and women.

Among individuals aged 60+ years who participated in the Dubbo Osteoporosis Epidemiology Study, 939 women and 719 men were found to have femoral neck BMD T-scores>-2.5 (non-osteoporosis) and were followed for up to 15 years (1989-2004). During this period, the incidence fracture was ascertained. At baseline, femoral neck BMD was measured by DXA (GE-LUNAR), and postural sway was measured by a swaymeter. During the follow-up period, 227 women and 103 men had sustained a fracture. The majority of symptomatic fractures were vertebrae (30%), hip (11%) and forearm and or wrist (20%). The incidence of fracture (per 1000 person-years) was 23.5 for women and 14.3 for men. In the multivariate Cox's proportional hazards model, the following risk factors were significantly related to fracture risk: postural sway (HR: 1.2; 95% CI: 1.1-1.3 in women and 1.3; 1.1-1.4 in men); fall in the previous 12 months (HR: 2.0, 1.1-3.5 in women and 2.3; 1.5-3.5 in men), and baseline femoral neck BMD (HR: 1.6, 1.5-2.6 in women and 1.3; 1.1-1.4 in men).
Thus, in non-osteoporotic women and men, the combination of low but non-osteoporotic BMD, a history of fall, and postural instability could identify a subgroup of individuals with higher-risk of fracture.

SOURCE OF FINANCIAL SUPPORT: NATIONAL HEALTH AND MEDICAL RESEARCH COUNCIL, AUSTRALIA

P4

INCIDENCE AND RISK FACTORS FOR LOW TRAUMA FRACTURES IN MEN WITH PROSTATE CANCER

HG Ahlborg[1,2], ND Nguyen[1], JR Center[1] , JA Eisman[1] & TV Nguyen[1]
[1] *Bone and Mineral Research Program, Garvan Institute of Medical Research, Sydney, NSW*
[2] *Department of Orthopaedics, Malmö University Hospital, Malmö, Sweden*

The relative importance of BMD and non-skeleton risk factors for fragility fracture among men with prostate cancer (PC) has so far not been examined. This study was aimed at determining the risk of low trauma fracture in men with prostate cancer, and to characterize the association between potentially risk factors and fracture risk.

From the Dubbo Osteoporosis Epidemiology Study, 43 men were diagnosed to have PC; among whom, 20 received androgen deprivation therapy (ADT). Low trauma fractures were ascertained and compared with expected numbers in the study population and expressed as standard incidence ratio (SIR). BMD at the femoral neck (FNBMD) and postural instability were measured.

During the 313 person-years of follow-up, 15 (35%) men sustained at least one fracture after the diagnosis of PC. Overall, fracture risk was increased (SIR 3.1; 95%CI, 1.8-5.2) predominately among men with ADT (SIR 5.8; 2.6-11.0). FNBMD in men with PC were 0.4 SD higher than in the underlying population ($P<0.05$). Additionally, each SD lower FNBMD and each SD higher rate of FNBMD loss were significantly associated with 1.7- (1.0-2.8) and 1.6- (1.0-2.2) fold increase of fracture risk, respectively.

These results suggest that men with prostate cancer, particularly those treated with ADT have an increased risk of sustaining a low trauma fracture. Although the average BMD in men with prostate cancer prior to treatment seems to be higher than expected, a low BMD prior to treatment or increased rate of bone loss after initiation of treatment is each a significant predictor of fracture.

SOURCE OF FINANCIAL SUPPORT: NATIONAL HEALTH AND MEDICAL RESEARCH COUNCIL, AUSTRALIA

P5

BONE LOSS AND WEIGHT FLUCTUATION PREDICT MORTALITY RISK IN ELDERLY MEN *AND WOMEN*

ND Nguyen, JR Center, JA Eisman & TV Nguyen
Bone and Mineral Research Program, Garvan Institute of Medical Research, St Vincent's Hospital, Sydney, NSW

Low bone mineral density (BMD) has been suggested as a risk factor for mortality in women. The present study examined the contribution of bone loss, weight loss and baseline BMD to the risk of all-cause mortality risk in both elderly men and women.

Data from 2153 (1059 women) subjects aged 60+ years as at 1989 of Caucasian background from the Dubbo Osteoporosis Epidemiology Study were analyzed. All-cause mortality was recorded annually between 1989 and 2004. Information of concomitant diseases was also recorded. Baseline femoral neck BMD (GE-LUNAR DXA) was measured at baseline and every two years afterwards. The rate of change in BMD was derived for each individual by linear regression.

During the follow-up period, 254 and 295 women died, yielding mortality incidence (per 1000 person-years) of 41.2 in men and 20.4 in women. In both sexes, the following factors were independently significant predictors of mortality: bone loss (HR: 1.6; 95%CI, 1.0-2.4 in men and 1.6; 1.2-2.2 in women) and weight fluctuation measured by the coefficient of variation (HR: 1.2; 1.0-1.3 in men, and 1.1; 1.0-1.2 in women). Moreover, in women baseline femoral neck BMD (HR: 1.3; 1.1-1.5) and weight loss (HR: 1.2, 1.1-1.4) were additional independent predictors of mortality risk. Omitting the last weight measurement (before death) did not alter the results.

These data suggest that in addition to low BMD, BMD loss and weight change are also significant predictors of all-cause mortality in elderly men and women, independent of age and concomitant diseases.

SOURCE OF FINANCIAL SUPPORT: NATIONAL HEALTH AND MEDICAL RESEARCH COUNCIL, AUSTRALIA

P6

THE ANTIOXIDANT N-ACETYL CYSTEINE HAS A BIPHASIC EFFECT ON OSTEOCLASTOGENESIS AND INHIBITS RESORBING ACTIVITY AND SURVIVAL OF MATURE OSTEOCLASTS

N Zimmerman, CJ Aitken, JM Hodge, MJ Constabl & GC Nicholson
The University of Melbourne, Department of Clinical and Biomedical Sciences: Barwon Health, Geelong, VIC

We have previously demonstrated that N-acetyl cysteine (NAC) dose-dependently inhibits the formation of osteoclasts from CFU-GM precursors, and their ability to resorb. This study further examined the effects of NAC on precursors and mature osteoclasts.

Human CFU-GM were cultured on dentine for 14d with sRANKL and hM-CSF. When co-treatment with NAC (10mM) was commenced on d1, 2, 3, 4 or 7 and continued until d14, osteoclast formation was markedly inhibited and resorption abolished. Treatment from d10-14 or 12-14 inhibited resorption but had only minor effects on formation. When NAC treatment was commenced at the start of the cultures and removed on d1-14, a biphasic effect was observed; early, short-term exposure (removal on d1 or 2) enhanced formation and resorption whereas exposure for the first 7d or more caused marked inhibition of both. In cultures of mature osteoclasts transferred to fresh dentine slices and cultured for a further 48hr, NAC (1-10 mM) concentration-dependently inhibited resorption (complete inhibition at 10mM) and, to a lesser extent, survival (50% inhibition at 10mM).

We have shown that NAC inhibits the later phases of osteoclast differentiation but paradoxically stimulates during the early proliferative phase. NAC also inhibits survival and resorptive activity of mature osteoclasts. The molecular mechanisms of these effects remain to be determined.

No conflicts of interest exist for any of the authors

P7

TWO INACTIVATING MUTATIONS OF THE CALCIUM-SENSING RECEPTOR ASSOCIATED WITH FAMILIAL HYPOCALCIURIC HYPERCALCAEMIA DEMONSTRATE A DOMINANT NEGATIVE EFFECT ON WILD-TYPE RECEPTOR ACTIVITY

AL Magno[1,2], BK Ward[1,2], BGA Stuckey[1] & T Ratajczak[1,2]
[1] *Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA*
[2] *Western Australian Institute for Medical Research and UWA Centre For Medical Research, University of Western Australia, Nedlands, WA*

The calcium-sensing receptor (CaR) is a plasma membrane G-protein coupled receptor that plays an integral role in regulating extracellular calcium concentrations. The activation of the human CaR induces a signalling pathway that results in a decrease in parathyroid hormone secretion in parathyroid cells and inhibits reabsorption of Ca^{2+} in the kidneys. Heterozygous loss-of-function mutations are associated with familial hypocalciuric hypercalcaemia (FHH), a benign condition characterized by lifelong hypercalcaemia.

A key aim of our research program is to identify and characterize CaR mutations associated with FHH. We report here two inactivating CaR mutations, L174R and G778D, identified in families diagnosed with FHH. In order to confirm the biochemical phenotype, these mutations were introduced by site-directed mutagenesis into the pcDNA3.1 expression vector for human CaR. Cell surface expression of

the mutant and wild-type receptors in transfected HEK293 cells was determined using a biotinylation and immunoprecipitation technique. The mutant receptors expressed in HEK293 cells were functionally characterized using an inositol phosphate assay. At the cell surface the wild-type and G778D mutant receptors exhibited equivalent expression. The cell surface expression of the L174R mutant receptor was noticeably lower than that of wild-type. The inositol phosphate assay revealed that both mutants completely disrupted the CaR's ability to respond to extracellular calcium. Experiments in which each mutant was cotransfected with wild-type receptor, to emulate the heterozygous state found in FHH, showed that both mutants impaired the function of the wild-type receptor, revealing a dominant negative effect.

P8

VITAMIN D, BONE HEALTH AND FRACTURES IN PEOPLE WITH INTELLECTUAL DISABILITY

S Vanlint[1] & M Nugent[2]
[1] *Department of General Practice, University of Adelaide, Adelaide, SA*
[2] *Strathmont Centre, Oakden, SA*

Background:
Osteoporosis is believed to be more common in people with intellectual disability who are in institutional care. The most likely causes of this are hypovitaminosis D, lack of weight-bearing exercise and drugs interfering with vitamin D metabolism. The extent of this problem and its precise impact on fracture rates are not well-documented in the literature. Our hypotheses are that hypovitaminosis D is common in this population, and that this is principally due to a lack of exposure to UV light due to poor mobility. This and a reduction in weight-bearing result in poor bone health, which when combined with an increased rate of falls leads to an increase in the incidence of fractures. We also intend to demonstrate that thrice-yearly vitamin D supplementation is effective in correcting hypovitaminosis D in this population.

Methods:
We audited the records of 300 adults at a residential centre for the intellectually disabled. Data collected included age, sex, history of fractures, whether or epilepsy had been diagnosed, vitamin D and albumin levels (if known), medication use, ambulatory ability and dietary status. Those found to be deficient were commenced on vitamin D_3 100,000 IU every 4 months, orally.

Results:
Full data will be available at the meeting. Preliminary analysis indicates a high incidence of vitamin D deficiency (over 60% of individuals tested), and an association between vitamin D levels and ambulatory ability. It is anticipated that conclusions regarding the efficacy of this form and dose vitamin D supplementation will also be made.

No conflict of interest is declared.

P9

OSTEOPOROSIS IN PATIENTS WITH LOW TRAUMA FRACTURES: THE BONE PROTECTION PROJECT

CA Inderjeeth, D Glennon, J Bates & A Petta
Dept of Rehab & Aged Care, Sir Charles Gairdner Hospital, Nedlands, WA

Purpose:
The purpose of this project was to identify and increase awareness of osteoporosis and prevent future osteoporosis related fractures.

Methods:
All patients over 50 years of age identified in a tertiary hospital database as presenting to an emergency department with a low trauma fracture were included in a postal/telephone survey. Two hundred random participants were chosen. Data collected included fracture site, co-morbidities, risk factor profile, falls risk and medications for osteoporosis.

Results:
Mean age was 74 years with 89% being female and 91% of Caucasian origin. It included 35% of patients who were discharged from the Emergency Department. Of the 200 subjects the main fracture types were wrist (103), shoulder (46), hip (20) and (70) reported multiple fractures. 98% reported a fall as a precipitating event. One third reported a prior fracture. Only a minority had anti osteoporotic treatments initiated on discharge from hospital. The majority were either prescribed calcium (27%), vitamin D (20%) or a bisphosphonate (20%). Only a minority of patients had any of the relevant investigations including BMD.

Conclusion:
Patients with fractures are often not investigated or treated for osteoporosis putting them at risk of further fracture.

P10

OSTEOPOROTIC FRACTURE: RISK FACTORS, AWARENESS, INVESTIGATION AND TREATMENT

CA Inderjeeth, D Glennon & A Petta
Dept of Rehab & Aged Care, Sir Charles Gairdner Hospital, Nedlands, WA

Purpose:
Osteoporosis is often under recognised and under treated. We looked at the level of awareness, investigations and treatment of patients with fracture discharged from a teaching hospital.

Methods:
Female patients identified in the hospital database as having DRG codes for fracture between June 1997 and June 2002 were selected. Of 1,584 surveys sent 366 valid questionnaires were returned. There were specific questions on history of fractures, awareness of osteoporosis, bone mineral density testing and treatment for osteoporosis.

Results:
The mean age of respondents was 80+/- 8 years with a range of 60 to 99 years. 59% reported one fracture, 22% two fractures and 20% three or more fractures. 64% reported a prior hip fracture. Only 48% were aware that they had osteoporosis and 35% reported having a BMD. The majority reported at least one risk factor for osteoporotic fracture. Only 37% reported being on any treatment for osteoporosis on discharge with the majority being on calcium (34%).

Conclusion:
This relatively large study of women discharged from a tertiary hospital confirms the findings from other studies that a significant proportion of patients at risk of further fracture are not offered specific treatment for osteoporosis.

P11

BISPHOSPHONATE THERAPY IN PREGNANCY AND LACTATION-ASSOCIATED OSTEOPOROSIS

S O'Sullivan, A Grey & I Reid
University of Auckland, Auckland, New Zealand

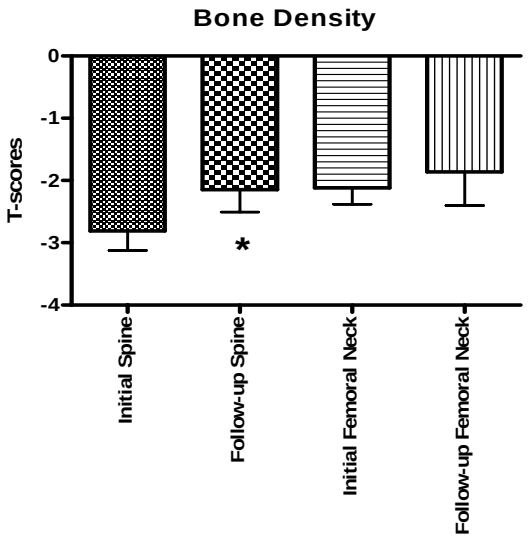
Aims:
Pregnancy and lactation-associated osteoporosis is a rare condition, characterised by presentation with fracture(s), most commonly vertebral, in the early post-partum period. Cessation of lactation and anti-resorptive therapy are commonly recommended, but the response of this disorder to treatment is uncertain.

Methods:
We performed a retrospective chart review of cases of pregnancy and lactation-associated osteoporosis seen at our osteoporosis clinic between 1986 and 2004.

Results:
We identified 10 cases of pregnancy and lactation-associated osteoporosis. Nine women presented with painful low-trauma vertebral fractures, at a median of 1 month post-partum (range 0-10 months) and 8/10 women sustained multiple vertebral fractures (median 4). Recognised risk factors for osteoporosis (low BMI, smoking history, family history of osteoporosis/fracture, vitamin D deficiency) were variably present. The median duration of lactation was 5 months (range 0-18). The mean initial lumbar spine BMD T-score was -2.81; that at the femoral neck was -2.12. All patients were advised to cease lactation, and 9 received bisphosphonates (5 pamidronate, 5 alendronate, 2 zoledronate), for a median of 19 (1-48) months. At first follow-up (1-2 years) the mean LS BMD T score was -2.15 ($p=0.03$ vs baseline, $n=8$). Follow-up BMD data were limited at the proximal femur. Of the four women who had subsequent pregnancies, none sustained a fracture during or following the subsequent pregnancy.

Conclusion:
Pregnancy and lactation-associated osteoporosis causes considerable morbidity in the form of pain and disability to an otherwise young, healthy population. Patients tend to present with multiple vertebral fractures and significantly reduced bone density. Bisphosphonate therapy is associated with significant improvement in BMD.

Disclosures:
1.



P12

SERUM CATHEPSIN K MEASUREMENTS: REPEATABILITY, INTRA-SUBJECT AND POSTPRANDIAL VARIABILITY

U Meinhardt[1], C Meier[2], JR Greenfield[1], TV Nguyen[1], J De Winter[2] & MJ Seibel[2]
[1] *Garvan Institute of Medical Research, Sydney, NSW*
[2] *Bone Research Program, ANZAC Research Institute, Sydney, NSW*

Cathepsin K, a cysteine protease, plays a major role in bone matrix degradation. Recently, an enzyme-linked immunosorbant assay for cathepsin K measurement in serum and cell culture (Cathepsin K ELISA BI-20432, Biomedica, Austria) was developed using a polyclonal sheep antibody. In this study we assessed the repeatability and intra-subject and postprandial variability of cathepsin K measurements in human sera.

Serum samples from nine healthy postmenopausal women aged 65.9 ± 6.5 years (mean ± SD) were collected a) in the fasting state on two occasions separated by 1 - 4 weeks, and b) 120, 240 and 360 minutes after a standard breakfast (1385 Kcal, 37g protein, 88g fat, 104g carbohydrate). Samples were kept frozen at – 20 °C up to 6 months and at – 80°C up to 12 months before being analysed in batch duplicates by a single investigator.

Measurement of sera obtained on two separate occasions yielded mean (±SD) cathepsin K values of 3.20 ± 1.41 pmol/L and 2.65 ± 1.29 pmol/L, respectively, with no statistical difference between the two sets of data (p = 0.39). Mixed-effect analysis of variance suggested that the intra-subject variance of cathepsin K was 1.81 ± 0.90 pmol/L^2 of which biological and inter-assay variance accounted for 68 % (1.28 ± 0.87 pmol/L^2) and analytical variance accounted for 32 % (0.58 ± 0.28 pmol/L^2). The postprandial cathepsin K levels at baseline, 120, 240 and 360 minutes were 2.65 ± 1.29, 2.79 ± 1.07, 2.37 ± 1.00 and 2.75 ± 0.96 pmol/L, respectively; no statistically significant difference was observed between these results (p = 0.79). Cathepsin K levels did not appear to be affected by haemolysis of the blood sample (2.73 ± 1.15 vs. 2.28 ± 1.24 pmol/L, respectively, p= 0.12, n = 5).

We conclude that serum cathepsin K levels as measured by the Biomedica assay show good intra-subject repeatability and low postprandial variability.

P13

A 5 YEARS PROSPECTIVE STUDY ON VITAMIN D STATUS IN HEALTHY CHINESE ADOLESCENT GIRLS

LH Foo[1], A Trube[1], Q Zhang[1,2], X Du[1], K Zhu[1], H Greenfield[1] & DR Fraser[1]
[1] *Faculty of Veterinary Science, University of Sydney, Sydney NSW*
[2] *Institute for Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, Beijing, China*

The beneficial effect of an adequate vitamin D status for maximising bone mass accrual in childhood and adolescence is well documented. The aim of this longitudinal prospective study was to determine the vitamin D status of a group of 101 apparently healthy Chinese adolescent girls (10 years of age in 1999) at 3 intervals from 1999 to 2004 in Beijing, China (40°N). Blood was collected on school days at the end of winter from March to April. Vitamin D status was assessed from the 25-hydroxyvitamin D [25(OH)D] concentration in serum, measured by a competitive protein binding assay (CPBA) in 1999 and 2001 and by a commercial Diasorin radioimmunoassay (RIA) in

2004. Because cross-calibration comparison of 25(OH)D levels by both methods revealed that RIA consistently gave 23% higher values than CPBA, this factor was used to adjust the values of the RIA to those of the CPBA. Mean (SD) levels of serum 25(OH)D were 19.0 (7.8), 19.6 (11.0) and 29.9 (17.5)nmol/L in 1999, 2001 and 2004 respectively. The log transformed values, used to compare differences over the 3 time points, indicated that the mean level of 25(OH)D was significantly higher in 2004 compared to 1999 and 2001 ($P<0.0001$). However, these mean 25(OH)D concentrations are all lower than those of comparable subjects in other countries at a similar latitude and the higher value in 2004 confirms a continuing low vitamin D status in this population. There is debate about the 25(OH)D concentration that defines a state of vitamin D deficiency. If vitamin D deficiency is defined as a 25(OH)D concentration equal to or below 20.0nmol/L, 37.5nmol/L or 50 nmol/L, then the prevalence of deficiency in these subjects in 2004 was 23.0%, 80.3% or 94.1% respectively. Regardless of which cutoff point for deficiency is finally accepted, it is clear that vitamin D deficiency in this population is common. As in other regions where vitamin D deficiency is reported, a limited exposure of skin to sunlight in summer and the very low intake of dietary vitamin D are major factors, which contribute to vitamin D deficiency in these subjects. Other potential causative factors and the impact of low vitamin D status on health of adolescent children warrant further investigation.

P14

RPAH FIRST FRACTURE CLINIC

L Sullivan, C Bennett, J Vaile & J Bleasel
Royal Prince Alfred Hospital. Sydney, NSW

Previous work at many centres has shown that patients who suffer their first osteoporotic fracture are inadequately managed in terms of osteoporosis care. Currently available therapies have been shown to greatly reduce the risk of subsequent fracture in patients who have already fractured. We have set up a clinic to assess patients presenting to the Orthopaedic Fracture Clinic, to assess BMD, detect underlying contributors to osteoporosis, and to instigate therapy where appropriate. A dedicated Osteoporosis Nurse attends Orthopaedic Fracture Clinics and identifies patients with low trauma fractures. A standardised history is taken and BMD and appropriate blood testing is performed, with the results entered into a custom-built computer database. Patients are then assessed by a medical officer and treatment commenced if appropriate. The Osteoporosis Nurse then telephones the patient after one month to check compliance and assess problems, and arrangements are made for BMD and review in one year. Of the first 250 patients completing assessment, 40% (29%) were osteoporotic at the lumbar spine (femur) with the majority of the remainder osteopenic. Inadequate calcium intake and suboptimal vitamin D were present in most patients. Therapy was commenced in most patients (calcium 56%, vitamin D 61% and oral or intravenous bisphosphonate therapy in 72%). We expect that this intervention will reduce incidence of future fracture and thereby minimise associated morbidity, mortality and economic cost. At the 12 month follow up patient satisfaction and improved outcomes have been demonstrated.

Disclosure:
Start-up funding for our Osteoporosis Nurse has been provided by Merck Sharpe & Dohme. This is now a hospital funded position.

P15

NON-INVASIVE ASSESSMENTS FOR OSTEOPOROTIC FRACTURE RISK

B-K Tan[1], RI Price[2], R Day[3], SS Dhaliwal[4] & KP Singer[5]
[1] *Centre for Musculoskeletal Studies, School of Surgery and Pathology, The University of Western Australia; School of Physiotherapy, Curtin University of Technology, Perth, WA*
[2] *Department of Medical Technology and Physics, Sir Charles Gairdner Hospital, Perth, WA*
[3] *Department of Medical Engineering and Physics, Royal Perth Hospital, Perth, WA*
[4] *School of Public Health, Curtin University of Technology, Perth, WA*
[5] *Centre for Musculoskeletal Studies, School of Surgery and Pathology, The University of Western Australia, Perth, WA*

Aim:
Bone loss and the propensity to fall increase the risk of fragility fractures and this risk increases with aging. The aim of the study was to investigate if calcaneal quantitative ultrasonometry and falls risk assessments can identify a group of osteoporotic elderly women from a non-osteoporotic group.

Methods:
104 community-dwelling women (mean age 71.3 ±5.8), recruited prospectively, underwent DXA BMD and calcaneal QUS measurements; rasterstereographic thoracic curvature examination and performance-based assessment of strength, mobility and balance. The women were classified into a 'High Risk' or a 'Low Risk' of osteoporotic fracture based on the WHO recommended BMD T-score of ≤-2.5 and/or a history of fragility fracture.

Results:

Speed of Sound (SOS) and poor mobility (timed "Up & Go" [TUG]) were significant discriminators of the osteoporotic group, independent of age (SOS: age adjusted odds ratio (OR) = 3.08, 95% CI = 1.69 – 5.62, p<0.0001; TUG: age adjusted OR = 1.63, 95% CI = 1.02 – 2.63, p = 0.045). Composite risk OR showed that for an individual who had a decrease of one SD in SOS and a concomitant increase in one SD in TUG, her risk of osteoporosis increased by 5-fold compared to those individuals who were not at risk (SD '0' for both SOS and TUG).

Conclusion:
Calcaneal QUS, in combination with falls-risk assessments, can discriminate elderly women at risk of future osteoporotic fractures. Case finding for at-risk individuals may allow early intervention to be implemented, and consequently curb the spiralling cost of managing osteoporotic fractures.

P16

ELEVATED SERUM FGF23 CONCENTRATIONS IN PLASMA CELL DYSCRASIAS

I Stewart, C Roddy, A Gill, A Clarkson, M Mirims, AE Nelson, L Coyle, C Ward, P Clifton-Bligh, BG Robinson, RS Mason & RJ Clifton-Bligh
Kolling Institute, University of Sydney and Royal North Shore Hospital, NSW

Fibroblast growth factor 23 (FGF23) is now recognized as a key regulator of phosphate metabolism. The syndrome of tumour-induced osteomalacia (TIO) is typically associated with mesenchymal tumours and elevated serum FGF23 concentrations. TIO is less commonly associated with non-mesenchymal tumours and serum FGF23 has not yet been studied in these cases. We identified an elevated serum FGF23 concentration (161 RU/mL) in one patient with small B-cell lymphoma and hypophosphatemia, prompting us to examine serum FGF23 in other patients with B-cell neoplasms. Serum FGF23 concentrations measured using the FGF23 C-terminal ELISA (Immunotopics, CA) in 26 normal controls was 23 ± 11 RU/mL (mean ± SD). Serum FGF23 levels were elevated in 5/16 patients with myeloma (57, 83, 83, 89, 1111 RU/mL), 3/18 patients with monoclonal gammopathy of uncertain significance (MGUS)(81, 123, 177 RU/mL), and 1/12 patients with chronic lymphocytic leukaemia (249 RU/mL). In myeloma and MGUS patients, serum FGF23 concentrations were significantly and positively associated with serum paraprotein and beta-2 microglobulin concentrations (p = 0.0006). Hypophosphatemia was not observed even in those patients with elevated FGF23, and a weak positive correlation was noted between serum FGF23 and phosphate concentrations (p = 0.03). Dysplastic plasma cells in bone marrow biopsy samples showed intense FGF23 expression in a perinuclear distribution, a pattern also typical for FGF23 localisation in mesenchymal tumours. Our findings contribute to an expanding literature regarding abnormal FGF/FGF receptor-signalling in myeloma. The absence of hypophosphatemia in these cases suggests either that FGF23 produced by dysplastic B-cells lacks systemic bioactivity or that other factors contribute to maintain serum phosphate.

P17

THE DISPROPORTIONATELY SHORTER FEMORAL NECK LENGTH IN CHINESE CONTRIBUTES TO HIGHER HIP STRENGTH THAN IN CAUCASIANS

XF Wang, Y Duan, M J Henry* & E Seeman
*Department of Endocrinology, Austin Health, the University of Melbourne, *Department of Clinical and Biomedical Sciences: Barwon Health, the University of Melbourne, Geelong, VIC*

Racial differences in femoral neck (FN) traits are mainly due to differences in structure than volumetric BMD. We hypothesized that the lower hip fracture rate in Chinese than Caucasians may be partly due to Chinese having shorter FN length. We used DXA hip strength analysis program, to calculate safety factor (SF, estimated failure stress in tension/applied tensile stress in walking) in 821 healthy Chinese (540 females) and 1052 Caucasians (692 females) aged 18-93 years in Melbourne. Trunk and leg lengths were measured by anthropometry.

In both sexes, 85% of the shorter height in Chinese than Caucasians was due to shorter leg length. FN length was 6% shorter in Chinese than Caucasians after adjusting for their shorter stature. SF decreased with age but was 5-10% higher in Chinese than in Caucasians. About 13% of the racial difference in SF was independently attributed to the racial difference in FN length. Comparing sexes, shorter stature in women was equally due to shorter leg and trunk lengths in both races while FN length was 4-6% shorter in women than men after adjustment. There was no sex difference in SF in elderly Caucasians, but SF was 7.8% lower in elderly Chinese women than men ($p < 0.01$). Disproportionately shorter leg and FN length may partly account for the lower hip fracture risk in Chinese. Whether deleterious structural abnormalities in women overwhelm any protective effect of the shorter FN length is unknown.

Disclosures:
1.

P18

THE SKELETAL RESPONSE TO PROLONGED SUN DEPRIVATION IN ANTARCTIC CONDITIONS

S Iuliano-Burns, X-F Wang, J Ayton*, J Karpavicius & E Seeman
Austin Health / University of Melbourne, Heidelberg, VIC
**Australian Antarctic Division, Kingston, TAS*

Lack of exposure to solar ultra violet radiation (UVR) results in decreased synthesis of vitamin D, with low serum levels associated with increased bone turnover and bone loss. We investigated the skeletal response to solar UVR deprivation on healthy adults (n = 57) aged 38.6 yrs (range 21.0 – 61.2 yrs) during their wintering employment in Australia's Antarctic program. Anthropometry was measured at baseline and dietary intake at 6 months. Blood samples were taken at baseline and quarterly there on, and analysed for vitamin D and markers of bone turnover (OC), formation (P1NP) and resorption (Beta XL). Mean (± SE) height; 180.2 ± 1.4 cm, weight 85.7 ± 4.5 kg and calcium intake 755 ± 52 mg/day were recorded.

	Baseline	3 mths	6 mths	9 mths	12 mths
Vit. D (nmol/l)	**55.2**	**39.8***	**37.9***	**39.6***	**44.2***
OC (ng/ml)	**23.0**	**24.5**	**23.4**	**22.6**	**25.3**
P1NP (ng/ml)	**49.8**	**49.2**	**52.3**	**52.7**	**56.1**
Beta XL (ng/ml)	**0.312**	**0.344**	**0.320**	**0.332**	**0.339***

*Different to Baseline

Serum Vitamin D levels at 3 months were significantly lower than baseline, and remained lower for the year ($p < 0.01$). Bone resorption was higher at 12 months compared to baseline ($p < 0.06$). Time since arrival (TSA - duration of sun deprivation) was negatively correlated with vitamin D levels ($r = -.41$, $p < .001$), and positively correlated with bone resorption ($r = 0.18$, $p < 0.07$). After accounting for calcium intake TSA was positively correlated with OC ($p < 0.01$) and P1NP ($p < 0.05$). Short-term sun deprivation negatively impacts on bone, especially in extreme environments. The long-term detriment to bone is still to be determined.

Disclosures:
1

P19

BALANCE CHARACTERISTICS IN INDIVIDUALS WITH OSTEOPOROTIC VERTEBRAL FRACTURES

AM Greig[1], AM Briggs[1], PW Hodges[2], TW Wrigley[1], JD Wark[3] & KL Bennell[1]
[1] *Centre for Health, Exercise and Sports Medicine, School of Physiotherapy, University of Melbourne, VIC*
[2] *School of Physiotherapy, University of Queensland, QLD*
[3] *Department of Medicine, Royal Melbourne Hospital, University of Melbourne, VIC*

Vertebral fractures are recognized as one of the hallmarks of osteoporosis (OP). Vertebral fractures may affect balance due to pain, altered muscle function and postural changes. Balance impairments may increase falls risk and further fracture. The aim of this study was to compare balance characteristics between individuals with OP and vertebral fracture(s), individuals with OP and no fracture, and comparable controls with normal bone mineral density (BMD).

Thirty-one participants were recruited and stratified into three groups: OP and vertebral fracture(s) (n=10); OP and no fracture (n=12); and controls (n=9). Participants performed quiet standing balance tasks under different conditions (eyes open, eyes closed, shortened base). A force plate provided centre of pressure (COP) data and measurement of shear forces.

A 2 way mixed ANCOVA identified greater mean shear forces in all three conditions, in the medio-lateral (M-L) and antero-posterior (A-P) directions in the control group, compared with OP fracture (M-L: $p=0.006$) (A-P: $p<0.001$) and no fracture (M-L: $p<0.05$) (A-P: $p<0.001$) groups. The mean resultant COP velocity was also significantly reduced in the OP fracture group compared with controls ($p=0.013$), but not different to the OP only group.

Differences exist in balance characteristics between individuals with OP and individuals with normal BMD. The findings suggest that in quiet standing, individuals with OP are less likely to use balance strategies that result in the development of shear forces. As well, the decreased mean resultant COP velocity in the OP fracture group may indicate a reduced ability to respond and adjust to perturbations which could result in an increased falls risk.

Disclosures:
No conflict of interests exists for any of the authors

P20

SUBREGIONAL BONE MINERAL DENSITY CHARACTERISTICS IN THE LUMBAR SPINE: AN *IN VIVO* PILOT STUDY USING DXA

A Briggs[1,2], J Wark[2], B Phillips[3], A Greig[1,2], S Kantor[2], N Fazzalari[4] & K Bennell[1]
[1] *Centre for Health, Exercise and Sports Medicine, School of Physiotherapy, University of Melbourne, VIC*
[2] *Department of Medicine, Royal Melbourne Hospital, University of Melbourne, VIC*
[3] *Rehabilitation Sciences Research Centre, School of Physiotherapy, University of Melbourne, VIC*
[4] *Institute of Medical and Veterinary Science, SA*

Vertebral fractures are a hallmark of osteoporosis but their aetiology remains unclear. Previous research has demonstrated heterogeneity in vertebral bone properties. This pilot study was conducted to compare intra-vertebral BMD profiles between individuals with and without osteoporotic vertebral fracture.

Three cohorts were recruited – primary osteoporosis with (n=9) and without (n=15) a vertebral fracture, and comparable controls (n=7). AP and lateral-projection DXA scans were performed on the lumbar spine. Lateral scans were used to calculate areal BMD for the whole vertebral body and in 6 subregions. Significant differences in areal BMD were found between subregions (p<0.001), but independent of group. Post hoc tests revealed significantly lower areal BMD in the central and anterior zones (p<0.05). To account for the confounding effect of vertebral geometry when comparing subregional BMD (srBMD), ratios of srBMD were compared between groups. However no group differences were found. The inability to detect between-group differences was likely due to low power. Percent differences in mean BMD between fracture and non-fracture cases were calculated. Larger differences were observed comparing srBMD between groups (13.1-16.3%) compared to standard DXA variables (0.7-5.4%), suggesting that srBMD may be a more sensitive tool to discriminate between fracture and non-fracture cases. The greatest mean difference in areal srBMD between fracture and non-fracture cases was observed in the central subregion. Consistent with histological data this area contained minimal cortical bone and maximal trabecular bone. It therefore may be of significant clinical importance given that trabecular bone is known to account for the majority of vertebral strength [1].

1. Silva et al. *Spine*, 22: 140-50, 1997.

Disclosures:
No conflict of interest exists for any of the authors
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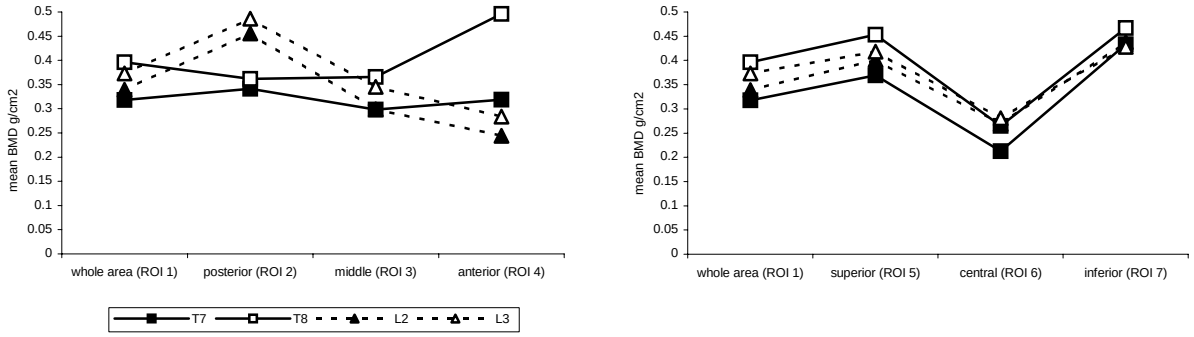
P21

DISTRIBUTION OF BONE MINERAL DENSITY IN THORACIC AND LUMBAR VERTEBRAE: AN *EX VIVO* STUDY USING DXA

A Briggs[1,2], J Wark[2], S Kantor[2], N Fazzalari[3], A Greig[1,2] & K Bennell[1]
[1] *Centre for Health, Exercise and Sports Medicine, School of Physiotherapy, University of Melbourne, VIC*
[2] *Department of Medicine, Royal Melbourne Hospital, University of Melbourne, VIC*
[3] *Institute of Medical and Veterinary Science, SA*

Heterogeneity exists in bone properties throughout the vertebral body. Patterns of distribution of BMD within a vertebral body may yield important clinical information and provide greater insight into fracture mechanics and bone loss. Compared to the lumbar spine, heterogeneity of BMD in the thoracic spine remains poorly understood. Knowledge of BMD distributions in the thoracic spine is important given the high prevalence of osteoporotic vertebral deformities in this area. However, DXA cannot be used to measure thoracic BMD *in vivo*. The aim of this study was to determine whether BMD profiles in thoracic vertebrae were consistent with lumbar profiles when measured by DXA. Six embalmed cadaver spines (mean age 81 years, SD 3.6 years) were scanned using antero-posterior and lateral projection DXA. Areal BMD was measured from the lateral scan in the whole vertebral body area and within six smaller subregions. A repeated-measures ANOVA demonstrated a significant difference in areal BMD within the vertebral bodies of T7, T8, L2 and L3 (p<0.05). Post hoc tests revealed significantly lower BMD in the central zone of thoracic and lumbar vertebrae (p<0.05) and in the anterior zone of lumbar vertebrae (p<0.05) (see Figs). These results, consistent with histological data, demonstrate heterogeneity in BMD within thoracic vertebrae when measured with DXA. Furthermore, similarities in BMD distributions exist between thoracic and lumbar levels. Therefore, ultimately measurement of lateral projection subregional areal BMD of the lumbar spine *in vivo* using DXA could be used to provide insight into thoracic areal BMD characteristics and ultimately vertebral fragility.



Disclosures:
No conflict of interest exists for any of the authors

P22

VERTEBRAL CENTROID AND COBB ANGLE MEASURES OF THORACIC KYPHOSIS

A Briggs, E Tully, P Adams, A Greig, T Wrigley & K Bennell
Centre for Health, Exercise and Sports Medicine, School of Physiotherapy, University of Melbourne, VIC

Several radiographic measures of thoracic kyphosis exist, though controversy surrounds their validity. This study examined validity and reliability of 2 measures. 35 participants (mean age = 63.1yrs, sd = 7.8) with normal and low BMD had lateral spinal radiographs taken to measure kyphosis and anterior vertebral height (AVH) loss. A traditional Cobb angle and a vertebral centroid angle were measured between T1-T12 (global measure) and T4-T9 (regional measure). Intra-rater reliability was tested with a random set of 15 films measured one week later.

Excellent reliability was achieved for each measure (SEM 0.86-1.17, ICC 0.97-0.99). High correlation was found between Cobb and centroid methods, globally (r = 0.82) and regionally (r = 0.80), providing evidence of concurrent validity. Only moderate correlation existed between global and regional measures for the Cobb (r = 0.61) and centroid (r = 0.75) methods. This suggests that for both measures, particularly the Cobb angle, a regional measure may better estimate kyphosis since curvature is maximal in this zone for pathological kyphosis. Overall, the centroid approach may better describe thoracic kyphosis since it encompasses more vertebrae and vertebral morphology.

Linear regression models demonstrated that only 7-16% of observed variance in maximal AVH loss could be explained using the 4 measures. However, 23-31% could be explained using summed multi-level vertebral height loss. No measure of kyphosis could sufficiently predict AVH loss. Kyphosis is more related to multi-level AVH loss than a single level. Results suggest that other factors such as disc, ligament and muscle status may influence kyphosis more than vertebral structural integrity.

Disclosures:
No conflict of interest exists for any of the authors
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P24

HIV INFECTION IS NOT ASSOCIATED WITH REDUCED BONE DENSITY IN CAUCASIAN MEN

MJ Bolland[1], AB Grey[1], AM Horne[1], S Briggs[2], MG Thomas[2], RB Ellis-Pegler[2], AF Woodhouse[2], GD Gamble[1] & IR Reid[1]
[1] *Department of Medicine, University of Auckland, Auckland, New Zealand*
[2] *Department of Infectious Diseases, Auckland Hospital, Auckland, New Zealand*

Recent studies have reported low bone mineral density (BMD) in HIV-infected patients, and higher than expected rates of osteopenia and osteoporosis. Frequently these findings have been attributed to treatment with HAART (highly active antiretroviral therapy). We sought to determine whether BMD in HIV-infected men treated with HAART is different from that in healthy control subjects, and if so what HIV-related factors might explain this finding.

We studied 59 HIV-infected Caucasian men treated with HAART for at least 3 months, and 118 healthy community-dwelling male volunteers as controls. Each HIV-infected man was age-matched (within 5 years) to 2 controls. All participants had measurements of BMD, and bone-related laboratory parameters. Adjustment for potential confounders was performed by analysis of covariance.

The mean duration of known HIV infection was 8.5 years, and of HAART treatment was 4.3 years. There were no significant differences in mean BMD between groups at the lumbar spine [HIV group (H) 1.23g/cm^2, controls (C) 1.25g/cm^2; P=0.53] or total body (H:1.18g/cm^2, C:1.20g/cm^2, P=0.09). At the total hip the HIV group had significantly lower BMD than controls (H:1.03g/cm^2, C:1.09g/cm^2, P=0.01). The HIV-infected men were on average 6kg lighter than the controls. After adjusting for the weight difference, HIV status was not an independent predictor of BMD at any site (lumbar spine P=0.87; Total hip P=0.15; Total body P=0.67)

We conclude that HAART-treated HIV-infected men are lighter than healthy controls. This weight difference is responsible for a small decrement in hip BMD. Overall, skeletal health is not significantly compromised in HIV-infected Caucasian men receiving HAART.

Disclosures:
1

P25

OSTEOCLASTS, BUT NOT OSTEOBLASTS, ARE NEGATIVELY AFFECTED WHEN GROWN ON A BISPHOSPHONATE-TREATED CALCIFIED SUBSTRATE

A Schindeler & DG Little
The Department of Orthopaedic Research & Biotechnology and The CHW Clinical School, The Children's Hospital at Westmead, Sydney, NSW

Bisphosphonates are potent inhibitors of osteoclast-mediated bone resorption. Chronic dosing of osteoblasts with solubilised bisphosphonates has been reported to enhance osteogenesis and mineralisation. This poorly reflects the in vivo situation, where free bisphosphonate becomes rapidly bound to bone surfaces. To establish a more clinically relevant cell culture model, we grew bone cells on calcium phosphate coated quartz discs pre-treated with the potent nitrogen-containing bisphosphonate, zoledronic acid (ZA). Binding studies utilising [^{14}C]-labelled ZA confirmed that the bisphosphonate bound in a concentration-dependent manner over the 1-50μM dose range. When grown on ZA-treated discs, the viability of bone-marrow derived osteoclasts was greatly reduced, while the viability and mineralisation of the osteoblastic MC3T3-E1 cell line was unaffected. This suggests that only bone resorbing cells are affected by bound bisphosphonate.

However, this system does not account for transient exposure to unbound bisphosphonate in the hours following a clinical dosing. To model this event, we transitorily treated osteoblasts with ZA in the absence of a calcified surface. Osteoblasts proved highly resistant to all transitory treatment regimes, even when utilising ZA concentrations that prevented mineralisation and/or induced cell death when dosed chronically. This study presents a pharmacologically relevant model of bisphosphonate treatment on cultured bone cells and implies that bisphosphonate therapies may not strongly affect osteoblasts at bone surfaces.

P26

POPULATION-BASED REFERENCE DATA ON MEASURES OF BALANCE, STRENGTH, GAIT AND ACTIVITY IN WOMEN

N El Haber[1,2], K Hill[1,2], A-M Cassano[1,2] & JD Wark[1]
[1] *Department of Medicine, Royal Melbourne Hospital, University of Melbourne, Parkville, VIC*
[2] *National Ageing Research Institute, Parkville, VIC*

To date, population–based reference data of postural sway, lower-extremity strength, gait and physical activity is limited in the literature. The value of the few reference data previously published is reduced by factors such as age range, sample size, type and conditions of testing.

The purpose of this study was 1) to present population-based reference values using validated measures of balance, strength, gait and physical activity in a healthy cohort of three hundred and eighty-nine community dwelling females aged 21.0-82.5 years (mean age ± SD; 51.1 ± 14.1 years), and 2) to determine age related decline in performance.

Testing included clinical [Lord's Balance Test and Step Test] and laboratory (static & dynamic) with and without a distractor task [using the Chattecx Balance System] balance measures. Physical activity was assessed using the Human Activity Profile questionnaire, and gait was monitored using the Clinical Stride Analyser.

Reference data were obtained for balance and related measures; a subset of the measures is presented in Table 1. The sample was divided into 6 groups, each representing an age decade. Reduced overall physical performance was confirmed in association with increased age, with significant differences in performance occurring between the younger and older groups (Figure 1).

Outcome Measure	**Mean (SD)** Group1	**Mean (SD)** Group 2	**Mean (SD)** Group 3	**Mean (SD)** Group 4	**Mean (SD)** Group 5	**Mean (SD)** Group 6
Chattecx Balance System† **-A-P, distraction**	**3.3 (1.0)**	**3.2 (1.1)**	**3.4 (1.1)**	**3.6 (1.0)**	**4.0 (1.3)**	**4.9 (1.5)***
Lord's Balance Test (mm) **-Eyes closed, foam** Step Test (no of steps/15 sec) **-Right leg support**	**69.3 (26.0)** **20.3 (2.8)**	**80.0 (58.9)** **20.3 (3.3)**	**95.8 (56.5)** **20.0 (3.6)**	**104.7 (70.7)** **18.3 (3.9)**	**107.0 (60.1)** **18.0 (3.7)**	**136.3 (57.3)*** **15.8 (3.1)***
Nicholas Manual Muscle Tester‡ -Average hip abductors	**11.7 (2.1)**	**12.7 (2.1)**	**12.4 (2.1)**	**10.7 (2.9)**	**9.8 (1.8)**	**8.6 (2.0)***
Human Activity Profile **-Adjusted Activity Score**	**89.5 (6.0)**	**85.4 (7.9)**	**82.0 (8.2)**	**76.4 (7.9)**	**73.8 (10.0)**	**64.7 (13.0)***
Clinical Stride Analyser **-Velocity (m/min)**	**77.3 (10.2)**	**81.2 (13.1)**	**81.2 (12.7)**	**79.0 (12.3)**	**75.0 (11.8)**	**63.0 (11.2)***

Table 1: Population-based reference values on measures of balance, strength, gait and activity
†Medio-lateral amplitude (cm) / height (m) ‡kg force (dynamometer)/weight (kg) *, P<0.001 significant differences between groups exist
A-P, Anterior-Posterior perturbations
Group 1 =21-30 years old; N=36 Group 2 =31-40 years old; N=58 Group 3 =41-50 years old; N=97 Group 4 =51-60 years old; N=96
Group 5 =61-70 years old; N=59 Group 6 =71-82 years old; N=43

Determination of balance and other related problems is supported by the application of population-based reference data. This may be of great clinical interest, supporting medical diagnoses and can be used to monitor performance in a variety of conditions, enabling implementation of preventative strategies for people at risk of falls.

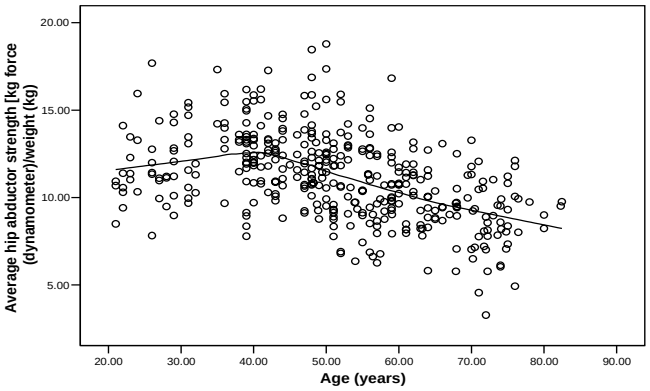


Figure 1: Relationship between age (years) and weight (kg) adjusted average [right and left] hip abductor strength on the Nicholas Manual Muscle Tester [kg force (dynamometer)/ weight (kg)].

P27

QUANTITATIVE BACKSCATTERED ELECTRON IMAGING OF NORMAL AND OSTEOPOROTIC BONE

P Sutton-Smith, H Beard & NL Fazzalari
Bone and Joint Research Laboratory, Division of Tissue Pathology, Institute of Medical and Veterinary Science and Hanson Institute, Adelaide, SA
Department of Pathology, University of Adelaide, Adelaide, SA

Bone strength is determined by a number of factors including the amount of bone, bone architecture and bone material properties. The first two of these have been investigated extensively, but investigations into material properties have been restricted due to technical limitations. Quantitative backscattered electron imaging is a technique, which allows the degree of mineralisation of trabeculae to be assessed. We have developed a modification of the technique of Roschger et al 1998 (Bone 23: 319-326) and applied this to inter-trochanteric biopsies from normal and osteoporotic individuals.

Biopsy cores from 19 normal post mortem controls and 17 osteoporotic individuals undergoing total hip replacement were processed into methyl methacrylate. The blocks were polished and analysed in a Philips XL20 scanning electron microscope. The microscope was calibrated using a carbon/aluminium standard and a mean and distribution of percent calcium were produced for each individual, and for the normal and osteoporotic groups.

Each individual showed a normal distribution of percent calcium. Each of the normal and osteoporotic groups also showed normally distributed data with the osteoporotic group being under mineralised relative to the normal control group. There was a significant correlation to increase mineralisation with age in the normal group except for three women who were markedly under mineralised similar to the osteoporotic individuals.

These data suggest there is an age-related increase in the degree of mineralisation in normal individuals and that osteoporosis results in under mineralisation of trabeculae. Three women from the normal group were markedly under mineralised suggesting they had increased risk of osteoporotic fracture.

Disclosure:
4. Eli Lilly

P28

AUDIT OF RURAL BMD SERVICE IN SOUTH AUSTRALIA

JL Wormald & KG Hughes
Department of Nuclear Medicine, PET and Bone Densitometry, Royal Adelaide Hospital, Adelaide, SA

The Mobile Bone Densitometry Service in South Australia was established in 1994. It provides a service to the people of rural South Australia and also the residents of Alice Springs. To ensure the service is provided fairly amongst areas with greater populations, an audit was performed comparing the annual schedule with data from the 2001 Census (Australian Bureau of Statistics).

Census data was obtained for each statistical subdivision where the service has been over the last five years. This showed the total population and the breakdown via age and gender. The service is mainly utilised by females over the age of 45. The Census figures for females 45-64 years and over 65 years were totalled giving an estimated number of potential users. From experience, the male attendance is low; therefore this data was not included.

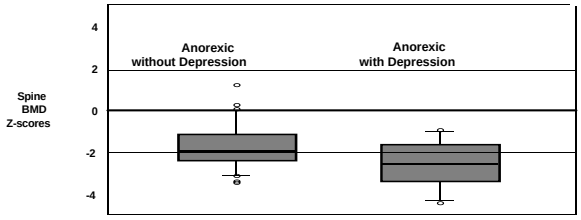
The proportion of weeks spent in the varying regions over the last five years matches the over 45 year old female population in these regions. There are four main sites where the number of weeks spent is low compared to the population; Mount Gambier, Clare, Victor Harbor and Alice Springs. This audit has enabled us to better plan the service schedule to ensure that the areas of greater need are taken into account. This should minimise occasions where the service is over or underbooked, ensuring full utilisation of the service. This information will be used in the planning of the schedule for 2006 and 2007. A further audit is planned after the 2006 Census.

P29

DEPRESSION IN ADOLESCENT GIRLS WITH ANOREXIA NERVOSA IS A RISK FACTOR FOR OSTEOPOROSIS

J Konstantynowicz[1,3], H Kadziela-Olech[1], M Kaczmarski[2], RMD Zebaze[3], S Iuliano-Burns[3], J Piotrowska-Jastrzebska[1] & E Seeman[3]
[1] *Department of Pediatrics and Auxology, Medical University of Bialystok, Poland*
[2] *3rd Department of Pediatrics, Medical University of Bialystok, University Children's Hospital 'Dr. Ludwik Zamenhof', Bialystok, Poland*
[3] *Department of Endocrinology & Medicine, University of Melbourne, Austin Campus, Heidelberg, VIC*

Both Anorexia nervosa (AN) and depression are associated with osteoporosis. To investigate whether depression is an independent risk factor for osteoporosis in adolescent girls with AN we compared 14 girls (mean age 16.9 years) with AN and depression with 31 anorexic girls without depression, matched by age, Tanner stage, weight, height, calcium intake and duration of AN. The diagnosis of depression was based on Hamilton Depression Rating Scale (HAM-D) and the Montgomery-Asberg Depression Rating Scale (MADRS). Dual energy X-ray absorptiometry (DXA) was used to determine total body and lumbar spine bone mineral density (TB BMD, LS BMD), fat mass (FM) and lean mass (LM). BMD was reduced in both groups but girls with AN and depression had lower BMD than those with AN alone (lumbar spine Z-scores -2.6 ± 0.3 *vs* -1.7 ± 0.3 SD; $p = 0.02$) (mean ± SEM). Quantitative assessment of depression correlated independently with TB BMD ($r = -0.4$; $p < 0.05$) and LS BMD ($r = -0.6$; $p < 0.001$).



We conclude that anorexic girls with depression are at higher risk of osteoporosis than those without depression. The mechanisms responsible for decreased BMD in depression are not known. Independent treatment of the depressive disorder in AN may partly alleviate the bone fragility.

P30

POTENTIAL MAGNIFICATION OF BMC USING FAN BEAM DXA IN CHILDREN DUE TO CHANGES IN SOFT TISSUE THICKNESS

K Fitzgerald[1], J Brody[2] & N Pocock[1]
[1] *Department of Nuclear Medicine and Bone Densitometry, St Vincent's Hospital, Sydney, NSW*
[2] *Department of Nuclear Medicine, Children's Hospital at Westmead, NSW*

Introduction:
Many paediatric centres use DXA Bone Mineral Content (BMC) to assess bone status. Recently a number of published paediatric studies utilised fan beam DXA. Fan beam DXA causes a magnification of BMC with decreasing distance from the X-ray source. Use of BMC has potential problems due to the soft tissue increase with growth varying the skeletal height above the scanning table

Aim:
Estimate the change in soft tissue thickness in children with growth and the potential magnification of BMC if measured using fan beam DXA.

Methods:
The variation in lumbar and pelvic soft tissue thickness with age was assessed in children undergoing radionuclide bone scans. Soft tissue thickness was measured from the lumbar spine and greater trochanter to the skin surface using the gamma camera ruler. Anthropomorphic measurements in children undergoing DXA were also used to measure pelvic height above the scanning table. Magnification of BMC using fan beam DXA (Hologic QDR 4500/A) was assessed in-vitro using a spine phantom scanned five times at multiple heights above the table.

Results:
There was a 40% linear decrease in BMC with increasing distance from the X-ray source over 0-24cm. Mean lumbar and pelvic soft tissue thickness in children between 5 and 16 years increased 3.5cm and 5cm respectively. The resultant error in lumbar spine and proximal femur BMC would be 5.8% and 8.3% respectively.

Conclusion:
Changes in soft tissue thickness in children with growth will result in significant errors in BMC when measured using fan beam DXA. Serial fan beam BMC in children should be interpreted with caution.

P31

MAGNIFICATION ERROR OF BMD IN PENCIL BEAM, FAN BEAM AND NARROW ANGLE FAN BEAM DXA
K Fitzgerald, J Freund & N Pocock
Department of Nuclear Medicine and Bone Densitometry, St Vincent's Hospital, Sydney, NSW

Introduction:
In bone densitometry there is an intrinsic magnification with decreasing skeletal distance from the X-ray source. This is most marked with fan beam DXA but theoretically may also occur with narrow angle fan beam and 'pencil beam' DXA. Previous studies using various scanners have reported significant errors with BMC and area, as soft tissue thickness changes, but generally indicate small changes in BMD. Comparison of these errors between the different machines is difficult due to the absence of a standard protocol.

Aim:
Assess the change in BMD, BMC and area with varying height above the X-ray source, using a standard protocol, in pencil beam, fan beam and narrow angle fan beam DXA.

Methods:
Using pencil beam (Hologic QDR 4000, Lunar DPX-IQ and Norland X-36) fan beam (Hologic QDR 4500/A) and narrow angle fan beam (GE-Lunar Prodigy) BMD, BMC and area were assessed using a spine phantom (Lunar bar) scanned five times at multiple heights (0–28cm) above the table.

Results:
The change in BMD, BMC and area per cm with the different densitometers is shown in the table below

	Prodigy	DPX-IQ	QDR 4500/A	QDR 4000	Norland
BMD /cm^{-1}	-0.26	-0.22	-0.35	-0.34	-0.13
BMC /cm^{-1}	-0.28	-0.34	-1.66	-0.16	-0.15
Area /cm^{-1}	-0.04	0.00	-1.49	0.16	-0.15
Δ BMD 5-15cm	-2.62	-2.25	-3.54	-3.40	-1.31

Conclusion:
BMC, area and BMD change with distance from the X-ray source with all densitometers. The change in BMD is small but from previous studies of soft tissue variation could result in variations of BMD exceeding 3.5%.

P32

VALPROATE-INDUCED BONE MINERAL DEFICITS IN MICE
S Senn, S Kantor, S Andrikopoulos, TJ O'Brien, MJ Morris* & JD Wark
Department of Medicine and Bone and Mineral Service, The Royal Melbourne Hospital and Austin and Repatriation Hospital, and Department of Pharmacology, The University of Melbourne, Parkville, VIC*

Aims:
In order to determine the factors that place an individual at risk of AED-induced bone disease, our aim was to develop a mouse model of AED-induced bone loss, and to identify different genetic strains that are sensitive and resistant to this effect. The ultimate aim of this project is to identify the mechanisms including any genetic factors underlying this effect.

Method:
Seven different inbred mouse strains (n=40 per strain, n=10 per diet) were placed on a diet mixed with 0, 2, 4 or 6 g/kg valproate (VPA) for 8 weeks. Total BMC, fat mass and lean mass were assessed using dual energy x-ray absorptiometry (DXA). BMC was corrected for total body weight and total lean mass to account for differences in animal size.

Results:
Strain 1 was identified as being sensitive to VPA-induced bone disease, showing significant differences (95% CI) of 10.4% (2.7%-18.2%) and 8.4% (0.1%-16.2%), respectively, in weight-adjusted BMC compared with control mice whilst on the 2 and 6 g/kg VPA diets ($p<0.05$). Strain 2 was identified as a strain resistant to the effects of VPA on BMC at all doses. Other VPA-sensitive and resistant strains have also been identified.

Conclusion:
This study has successfully identified a unique animal model that may resemble AED-induced bone deterioration in humans. Genetic background was found to influence susceptibility to decreased bone mass in mice. These models can be used in future studies of the metabolic and genetic factors that are important in determining individual susceptibility to the development of AED-induced bone disease. This approach will facilitate the design of strategies for the prevention and treatment of AED-associated bone disease.

P33

IMPACT OF THE WOMEN'S HEALTH INITIATIVE (WHI) TRIAL ON OSTEOPOROSIS PREVENTION

T Yong, G Phillipov & P Phillips
Endocrinology, The Queen Elizabeth Hospital & Health Service, Woodville, SA

In July 2002, extensive media coverage of the WHI trial findings created the public perception that hormone therapy (HT) was associated with high absolute breast cancer risk, and precipitated a rapid decrease in HT use. We have evaluated the potential ramifications of this decrease for osteoporosis prevention. During the period July–November 2002 our monthly DXA scan rate increased 99% compared to the preceding year (239 vs 120; $P<0.01$). Subsequently (December 2002–June 2003) the scan rate decreased to within 4% (125; NS) of pre-WHI levels. Our results parallel a 54% increase, in national Medicare DXA reimbursements over the same period. In Aug 2002 the highest relative percentage (62.3%), for 14 months, of women's first ever DXA scan occurred, associated with a significant age increase (60.1 vs 59.0; $P<0.01$). Reviewing monthly Medicare data during 2003-2004 did not identify any surge in DXA services. The Pharmaceutical Benefits Scheme (PBS) statistics showed that post-WHI prescription rates for the two most commonly prescribed oestrogen and oestrogen-progestin medications, decreased 53 and 33% respectively, with no "rebound" as of Oct 2004. Our findings suggest that many women on HT and their physicians, concerned by the WHI findings, arranged a DXA scan to assess BMD before stopping HT. Other evidence from PBS and Medicare data suggests that HT discontinuation was not accompanied by a change to alternative osteo-protective medication. The publication of the WHI was associated with a substantial decrease in the number of post-menopausal women taking osteo-protective medication.

Disclosure:
1

P34

STRUCTURAL AND REMODELLING INDICES IN THE CANCELLOUS BONE OF THE PROXIMAL FEMUR ACROSS ADULTHOOD

H Tsangari[1], DM Findlay[2,3], & NL Fazzalari[1,2,3]
[1] *Bone and Joint Research Laboratory, Division of Tissue Pathology, Institute of Medical and Veterinary Science and* [2] *Hanson Institute, Adelaide, SA*
[3] *Department of Orthopaedics and Trauma, The University of Adelaide and Hanson Institute, Adelaide, SA*
[4] *Department of Pathology, The University of Adelaide, Adelaide, SA*

Pathologies involving the proximal femur, specifically proximal femoral fractures and osteoarthritis are widely researched, yet a reference range of normal hip morphology is lacking. The aim of this study was to map changes in the structure and architecture of cancellous bone, as a function of age (18 to 88 years of age) and sex, in bone samples taken from the intertrochanteric region (IT) of the proximal femur at routine autopsy. An age-dependent decrease in bone volume was observed in both females and males, as expected ($r = 0.75$ and $r = 0.63$, $p<0.001$, respectively). The static indices of bone remodeling, resorption (ES/BV; $p<0.03$) and formation (OS/BV; $p<0.001$) were found to correlate positively with age in the female group, and only ES/BV ($p<0.001$) correlated with age in the male group. Interestingly,

the underlying mechanisms for changing bone architecture appeared to be different between males and females. Consequently, the OS/ES ratio is increased for the older female group. The greater OS per unit of ES in females over 50 years of age highlights this difference and importantly implies that the formation and resorption surface balance is different between males and females. Surprisingly, resorption indices increased in older males compared with their younger counterparts, while bone formation indices increased in the older female cohort. The IT region in the proximal femur is adjacent to the site commonly involved in fragility fracture and distal to the subchondral changes associated with hip osteoarthritis. Therefore, these site-specific data represent an histomorphometric reference range for future studies of bone diseases that manifest in the proximal femur.

P35

GENERATION AND CHARACTERISATION OF OSTEOCLAST-SPECIFIC CALCITONIN RECEPTOR KNOCKOUT MICE

WSM Chiu[1], JF McManus[1], AJ Moore[2], AM Axell[1], AJ Notini[1], HA Morris[2], DM Findlay[3], JD Zajac[1] & RA Davey[1]
[1] *Department of Medicine, University of Melbourne, Austin Health, Heidelberg, VIC*
[2] *Hanson Institute, IMVS, Adelaide, SA*
[3] *Orthopaedics and Trauma, University of Adelaide, SA*

The aim of this study is to investigate the role of calcitonin in calcium homeostasis and to clarify its physiological role in regulating bone turnover. To achieve this we have deleted the calcitonin receptor (CTR) specifically in osteoclasts using the Cre/*loxP* system.

CTRloxP mice, in which exons 13 and 14 of CTR are flanked by loxP sites, were initially bred with TRAP-Cre mice, in which the expression of Cre is under the control of the TRAP promoter (1). From 5 breeding pairs, 46 and 43 female and male offspring were generated of which none were homozygous knockouts (KO), while the other expected genotypes were represented. Since global deletion of the CTR early in embryogenesis is lethal and TRAP is expressed in the placenta, the inability to generate homozygous KOs clearly demonstrates that deletion of exons 13 and 14 of the CTR inactivates the receptor.

We successfully generated Osteoclast-specific CTR KO mice (OC-CTR KO) by breeding the CTR-*loxP* and cathepsin K-Cre (1) transgenic mouse lines. Preliminary data indicates that fasting serum levels of intact parathyroid hormone, calcium and total protein are unaffected in male and female OC-CTR KO mice at 4 weeks of age. We are currently investigating the bone phenotypes of OC-CTR KO mice using static and dynamic bone histomorphometric analyses.

In conclusion, we have successfully generated a genetically modified mouse line in which the CTR is specifically deleted in osteoclasts. This mouse model will provide valuable insight into the regulation of bone resorption and formation by calcitonin.

(1) WSM Chiu, JF McManus, AJ Notini, AI Cassady, JD Zajac, RA Davey. 2004 Transgenic mice that express Cre recombinase in osteoclasts. Genesis 39(3):178-85.

P36

OPTIMISING THE DOSE OF FUGU PARATHYROID HORMONE PEPTIDE (1-34) FOR MAXIMAL BONE FORMATION IN YOUNG MALE RATS

JF McManus[1], JD Zajac[1, 4], NA Sims[2], A Axell[1], WSM Chiu[1], AJ Notini[1], RA Davey[1], HE MacLean[1] & JA Danks[3, 4]
[1] *University of Melbourne, Department of Medicine, Austin Health, Heidelberg, VIC*
[2] *University of Melbourne, Department of Medicine, St Vincent's Hospital, Fitzroy, VIC*
[3] *St Vincent's Institute of Medical Research, Fitzroy, VIC*
[4] Teeleostin Pty Ltd

Intermittent human parathyroid hormone (hPTH) is a proven bone anabolic agent in animals and humans. Previously we have shown that PTH peptide (1-34) derived from the Japanese puffer fish *Fugu rubripes* (fPTH) is capable of increasing bone formation *in vivo*. Our current experiment was designed to identify the dose of fPTH at which maximal bone formation could be achieved in young male rats.

Weanling male Sprague Dawley rats were injected subcutaneously with either 50ug or 100ug/100g body weight of fPTH peptide, 10 ug/100g body weight of hPTH peptide (1-34) or vehicle, daily for 30 days.

On completion of the treatment, tibiae were harvested for bone histomorphometry. In rats administered fPTH, histomorphometric markers including metaphyseal trabecular volume ($p<0.001$), trabecular thickness ($p<0.001$), trabecular number ($p<0.001$), bone formation rate ($p<0.01$) and mineral apposition rate ($p<0.005$) were significantly higher than in control animals. Conversely osteoclast surface ($p<0.001$) was significantly decreased compared with controls. These parameters were also significantly different in rats given hPTH (1-34) compared with controls with the exception of mineral apposition rate. There was no significant difference in histomorphometric markers between rats given 50 and 100ug/100g body weight of fPTH peptide.

The results indicate an increase in bone formation which is likely due to an increase in osteoblast number and a decrease in osteoclast number. These data support our previous findings that fPTH is an effective bone anabolic agent and demonstrate that maximal bone formation can be realized in 3-4 week old male rats.

Disclosures:
2, 4 Teeleostin Pty Ltd

P37

GENE EXPRESSION OF PASSAGED HUMAN ARTICULAR CHONDROCYTES

Z Lin*, J Xu*, J Fitzgerald§, D Wood* & MH Zheng*
** Department of Orthopaedic Surgery, QEII Medical Centre, University of Western Australia, Perth, WA*
§ Biological Engineering Division, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA

Autologous chondrocyte implantation (ACI) is considered to be one of the best cellular engineering approaches for the treatment of articular cartilage injury. ACI is able to repair cartilage defect with hyaline-like histology and improved function which conventional surgical treatments could not be achieved. As ACI employs in vitro cultured autologous chondrocytes to repair articular cartilage injury, the biosynthetic profile of cultured chondrocytes has been shown to be altered during in vitro monolayer cultivation. Thus, this study investigated the expression profile of several chondrocyte associated gene clusters in serially passaged human articular chondrocytes by quantitative Real-time PCR. The gene clusters include extracellular matrix proteins (aggrecan, type I collagen, type II collagen, type X collagen, fibromodulin, fibronectin, and link protein), matrix proteinases (MMP-1, MMP-3, MMP-9, MMP-13, ADAMTS-4 and ADAMTS-5), proteinase inhibitors (TIMP-1, TIMP-2 and TIMP-3), cytokines (IL-1β, TGFβ, TNFα, and IGF-1), transcription factors (Sox-9, c-fos and c-jun), and intercellular signaling (COX-2, MAPk1, and NOS2). Results obtained by clustering analysis (Euclidean distance) showed that with increasing passage number, the gene expression of the matrix proteins aggrecan, type II collagen, and fibromodulin decreased, while fibronectin and link protein increased. Matrix proteinases, MMP3, 9, 13 and ADAMTS-4, 5, decreased expression especially to passage 6, whilst the proteinase inhibitors, TIMP1, 2, 3, remained constant. Cytokine IL-1 showed increased expression with serial chondrocyte culture. No significant alternation in TNF-α, TGF-β, IGF-1, or transcriptional factors, Sox-9, c-fos, or c-jun expression were observed. These results suggest that the chondrocytic gene expression profile is altered at various degrees with increasing passage number, though the gene expression levels of transcriptional factors which contribute to hyaline cartilage regeneration remain unchanged. This data may prove important for the future development of more specific and efficacious cultivation technique for human articular chondrocyte-based therapies.

P38

FULVESTRANT, AN OESTROGEN RECEPTOR ANTAGONIST, BLOCKS PROLIFERATION AND DIFFERENTIATION OF CULTURED PRIMARY HUMAN OSTEOBLASTS

L Filgueira[1], J Meagher[1], M Al-Mushaiqri[1], AP Skirving[2] & R Zellweger[2]
[1] School of Anatomy and Human Biology, University of Western Australia, WA
[2] Department of Orthopaedic Surgery, Royal Perth Hostpital, Perth, WA

Osteoblasts have been cultured from cancellous bone biopsies of patients undergoing orthopaedic surgery after bone fractures of the lower limb in elderly patients (n=21; age: 74-97, median=87) and younger patients (n=13; age: 15-68, median=28). Primary osteoblast cultures were successfully established in 24% (5/16) of elderly and 92% (12/13) of young patients. The cells were tested for osteoblast markers using light and immunofluorescence microscopy and real time reverse transcription polymerase chain reaction (rtRT-PCR). All cultures formed bone-like nodules of calcified extracellular matrix. The cells stained positive for vimentin (all cells positive) and STRO-1 (stromal stem cell marker; 1-5% positive cells) and expressed mRNA for Cbfa1, BMP-2, BMP-4, BMP-6, BMP-7, AP, collagen type 1, RANK-L, oestrogen receptor alpha and beta.

The cells were cultured in the presence or absence of fulvestrant (1nM, 100nM, 10uM) for up to 1 week before tested for changes to the cells using light and electron microscopy, rtRT-PCR and proliferation assays. Fulvestrant decreased proliferation of the cells in concentration-dependent way and induced differentiation of cells towards lipocytes.

In conclusion:
1. The efficiency of osteoblast culture from cancellous bone derived from young individuals is much better than from elderly individuals.
2. However, there is no difference between the cultured cells of either group.
3. Blocking of oestrogen receptors impairs proliferation of osteoblasts at an early stage of differentiation.
4. Blocking of oestrogen receptors induces differentiation of lipocytes, indicating that deficiency diverts differentiation of osteoblasts towards lipocytes, resulting in an increase of yellow bone marrow and decrease of bone formation.

P39

MONOCYTE CHEMOTACTIC PROTEIN 1 EXPRESSION CORRELATES WITH MULTINUCLEATION OF HUMAN OSTEOCLASTS

CJ Day, SRJ Stephens, MS Kim & NA Morrison
School of Medical Science, Griffith University, Gold Coast, QLD

Osteoclasts are multinuclear TRAP+ cells that also express calcitonin receptor (CTR), cathepsin K (CTSK) and NFATc1. MCP-1 is a CC chemokine that is expressed by M-CSF and RANKL derived human osteoclasts. In the absence of RANKL, addition of exogenous MCP-1 results in the formation of multinucleated cells that are TRAP+ and express a range of osteoclast markers but do not resorb bone (multinuclear polykaryons).

In this study we examine the correlation between MCP-1 expression and multinucleation, using quantitative PCR to measure gene expression. The effects of various signaling pathways involved in human osteoclast differentiation were tested using specific antagonists: SB203580 (p38MAPK), U0126 (ERK1/2), rapamycin (mTOR) and cyclosporin A, CsA (NFAT inhibitor).

In the murine cell line RAW264.7, SB203580 and CSA blocked osteoclast formation, while U0126 and rapamycin excited osteoclast formation. In marked contrast, only rapamycin permitted human osteoclast formation, while U0126 inhibited the formation of osteoclasts from human precursors as did SB203580, and CSA. Furthermore, RANKL induction of MCP-1 was abolished by those agents that blocked osteoclast formation in the human model. Expression of TRAP and NFATc1 were not significantly altered by the inhibitors but expression of CTSK and CTR was significantly altered. CTSK was down-regulated by SB203580, while CTR expression was reduced by all inhibitors. The consistent outcome of the antagonist experiments is that MCP-1 expression is highly correlated with multinucleation. This correlation is consistent with the observation that adding exogenous MCP-1 in the absence of RANKL results in the formation of multinuclear polykaryons.

Disclosures:
1

P40

GRANULOCYTE COLONY STIMULATING FACTOR RECEPTOR UPREGULATED BY RANKL – DIRECT EFFECTS OF G-CSF ON HUMAN OSTEOCLAST ACTIVITY

RMS Granfar, CJ Day & NA Morrison
School of Medical Science, Gold Coast Campus Griffith University, Southport, QLD

Granulocyte Colony Stimulating Factor receptor (CSF3R/GCSF-R) has been found to be 11.8-fold up-regulated in human osteoclasts derived from adherent Peripheral Blood Mononuclear Cells (PBMC) using M-CSF and RANKL [1]. This regulation was investigated and supported by Real-time PCR (Q-PCR). In order to further clarify the physiological significance of G-CSF, an *in vitro* approach of generating human osteoclasts from adherent PBMCs was employed [1] with additions of recombinant G-CSF in dose- and time-dependant manner.

Selected markers of osteoclast maturity such as multi-nucleation, tartrate resistant acid phosphatase (TRAP) content, cathepsin K (CTSK), calcitonin receptor (CTR), vitronectin receptor (VNR), actin ring, cell size and ability to resorb bone were analysed. Where applicable molecular (mRNA template) [2], cellular (protein activity or presence) and functional (bone resorption) assays were utilised. The G-CSF treated cells were compared with osteoclasts, which in turn were compared with macrophage controls.

Preliminary results include increased bone resorption following G-CSF treatment of osteoclasts, where treatment with 25ng/mL G-CSF, 25ng/mL M-CSF and 20ng/mL RANKL enhanced bone resorption by ~2 fold when compared to M-CSF-RANKL treated cells. Increased cell size and multi-nucleation have also been observed.

G-CSF appears to have a significant effect of increasing human *in vitro* osteoclast function and as such warrants further investigation.

[1] Day CJ, Kim MS, Stephens SR, Simcock WE, Aitken CJ, Nicholson GC, Morrison NA. J Cell Biochem. 2004 Feb 1;91(2):303-15.
[2] Granfar RM, Day CJ, Kim MS, Morrison NA. Mol Cell Probes. 2005 Apr;19(2):119-26.

Disclosures:
1

P41

CIRCULATING DONOR DNA IN RECIPIENTS OF BONE ALLOGRAFT

T Partsalis, M Hurworth, MH Zheng, S Kumta, D Lo & D Wood
Department of Orthopaedic Research, University of Western Australia, Nedlands, Perth, WA
Perth Bone and Tissue Bank, Perth Orthopaedic Institute, Nedlands, Perth, WA
Prince of Wales Hospital, Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR

Aims:
Our aim was to investigate the possible transfer of cellular material with the use of bone allograft, by detecting the presence of donor DNA in recipient plasma.

Methods:
Fifty one female patients who received bone allograft from male donors were included in the study. Forty patients who received allograft from the Perth Bone and Tissue Bank were identified, with a blood sample analysed between 6 weeks and 18 months of surgery. In addition, 11 patients were prospectively recruited from the Prince of Wales Hospital, Hong Kong SAR. These patients had blood samples analysed both preoperatively and at 1 day, 1, 2, and 3 months postoperatively.

The blood samples were analysed via Polymerase Chain Reaction, (PCR), for the presence of the SRY gene on the Y Chromosome.

Results:
Of the total 51 female patients receiving bone allograft from a male donor, 6 tested positive for the SRY sequence. Of the 6 positive patients, 5 were positive at day 1 postoperatively and negative thereafter, with the remaining patient positive at 3 months postoperatively, having initially tested negative. No preoperative specimens were positive.

Conclusions:
Our results document, for the first time, the presence of donor DNA in recipient circulation after bone allograft use. This occurs despite thorough preparation of the bone allograft designed to render the graft free of antigenic material. The significance of this finding is unclear. The positive findings at day 1 may be a consequence of operative handling of the graft, while the positive result at 3 months may reflect bone allograft physiology, possibly incorporation.

Disclosures:
1

P42

RAT ULNA LOADING IS AN EXCELLENT MODEL FOR INITIATING STRESS FRACTURES, BUT NOT MICRODAMAGE

LJ Kidd & MR Forwood
School of Biomedical Sciences, University of Queensland, QLD

Repair of microdamage by bone remodelling is vital to maintain micro architecture and mechanical properties. The loading model of rat ulnae has been central for investigating "targeted" remodelling of microdamage. Cyclic ulna-loading was performed *in vivo* on both ulnae of 40 female rats in a custom designed loading device. Loading was stopped at a predetermined loss of stiffness ranging from 5% to 30%. Ulnae were harvested on the day of loading or following 10 to 16 days. Ulnae were examined histologically following bulk staining with basic fuchsin. A displaced fracture occurred in 10 bones. Fifteen bones failed to fatigue or showed no evidence of microdamage or remodelling. The remaining 55 bones all had incomplete, non-displaced fractures of a consistent configuration and position more typical of "stress" fractures than "microcracks". Extensive new bone and numerous resorption spaces were seen adjacent to the fracture lines. These fracture lines occurred consistently, even with stiffness loss as low as 5-8%. Previous reports with this model have examined a small number of sections from the midshaft, alone. As these fractures occurred exclusively in the distal 1/3rd of the shaft, increased detection of fracture lines in this study was likely to be due to greater sectioning of the distal ½ of the ulna. These results indicate that rat ulna loading creates an excellent model of "stress" fractures, offering an ideal method to investigate stress fracture healing, but is not a reliable model for investigating microdamage.

P43

GHRELIN IS A WEAK OSTEOBLAST MITOGEN, AND ACTIVATES OSTEOCLASTIC BONE RESORPTION *IN VITRO*

M Watson, A Grey, J-M Lin, V Chan, K Callon, IR Reid & J Cornish
Department of Medicine, University of Auckland, Auckland, New Zealand

Ghrelin is a recently discovered hormone of nutrition, primarily synthesized in the stomach and released in response to fasting, such that circulating levels are maximal prior to meals. *In vivo*, ghrelin is thought to act in part as an orexin. Since bone turnover is acutely responsive to the fed state, such that bone resorption is increased during fasting, we have examined the effects of ghrelin on bone cell

function *in vitro*. The ghrelin receptor (GHS-R) is expressed in osteoblastic cells, and in cultures of primary rat and human osteoblasts, ghrelin weakly activates cell proliferation (fold-stimulation 1.1-1.2) at concentrations ranging from 10pM-10nM. Preliminary evidence suggests that ghrelin does not influence osteoblast differentiation or apoptosis induced by serum withdrawal. Ghrelin did not alter osteoclastogenesis in a murine bone marrow assay, but it did increase the bone-resorbing activity of isolated mature osteoclasts by 15-30% at concentrations of 1-10nM. No consistent effects of ghrelin were observed in *ex vivo* murine calvarial cultures. These data suggest that the elevated levels of ghrelin may contribute to the higher levels of bone resorption that occur in the fasted state.

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P44

GENETICS OF FRACTURE RISK: A RE-EVALUATION OF EVIDENCE OF ASSOCIATION

CYT Wang, ND Nguyen, JA Eisman & TV Nguyen
Bone and Mineral Research Program, Garvan Institute of Medical Research, St Vincent's Hospital, Sydney, NSW

The risk of osteoporotic fracture is partially determined by genetic factors. Several candidate genes have been shown to be associated with fracture risk. However, these associations have been highly inconsistent due to lack of replication. This study was undertaken to assess the posterior probability of true associations and propose a new statistical measure of association.

A Medline search was conducted to identify all studies that have reported a "positive" association ($p<0.05$) between a candidate gene polymorphism and fracture risk. For each study the Bayes Factor (BF), which is similar to the LOD score in linkage study, was calculated. Higher BF suggests a higher posterior probability of a true association.

The literature search resulted in 22 "positive" studies which involved 9 genes, including the collagen type 1α1 gene, vitamin D receptor gene and estrogen receptor gene. The average odds ratio in these studies was 1.95 (range: 1.23-4.1), with an overall standard error being 0.3312, and the observed p-value ranged between 0.0001 and 0.0463. For a given p-value <0.01, studies with larger sample size provides lower level of evidence of an association than studies with smaller sample size. The average posterior probability of a true association was 0.52. To achieve a posterior probability of >0.9, a p-value <0.0003 is required, and only 2 studies met this criterion.

Thus, the majority of positive association studies had low posterior probability of a true association, and that the Bayes Factor can be used as a more relevant measure of evidence for genetic association studies.

P45

A META-ANALYSIS OF RELATIONSHIPS AMONG LEPTIN, FAT MASS, LEAN MASS, AND BONE MINERAL DENSITY

C Pongchaiyakul[1], ND Nguyen[2], V Pisprasert[3] & TV Nguyen[2]
[1] *Division of Endocrinology and Metabolism;* [2] *Bone and Mineral Research Program, Garvan Institute of Medical Research, St Vincent's Hospital, Sydney, NSW*
[3] *Division of Nutrition, Department of Medicine, Khon Kaen University, Thailand*

The association between leptin and BMD is not clear due to conflicting findings from observational studies. The present study was aimed at examining the inter-relationships among leptin, FM, lean mass (LM) and BMD by a meta-analysis.

A systematic PubMed search of literature was conducted to identify all published studies in English on the association among the variables. Only studies on post-menopausal women were included in the analysis. The random-effects meta-analysis model was used to estimate the overall correlation coefficient and its 95% confidence interval.

Twenty-five studies were identified from the literature, which involved 5803 post-menopausal women aged between 50-90 years. Leptin was significantly correlated with FM (r=0.61; 95%CI: 0.55-0.67) and whole body BMD (r=0.13; 0.10-0.16). There was no significant correlation between leptin and femoral neck (FN) BMD (r=-0.04) or lumbar spine (LS) BMD (r=-0.03). Moreover, the correlation between LM and BMD (rLM) was consistently and significantly higher than that between FM and BMD (rFM): for whole body BMD: rLM=0.30 vs. rFM=0.19; for LSBMD: rLM=0.34 vs. rFM=0.19; and for FNBMD: rLM=0.37 vs. rFM=0.24. At the FN, there were 99.4% chances that rLM>0.3, but there were only 0.9% chances for rFM>0.3. In each correlation analysis, there was a significant heterogeneity among studies.

These results suggest that although leptin was highly correlated with FM, the effect of leptin on BMD was modest, accounting for around 1% of BMD variance. The modest leptin-BMD association maybe due to the fact that BMD being more strongly be correlated with LM rather than FM.

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P46

EVALUATION OF A PATIENT SUPPORT PROGRAM (ACTNOW) IN THE TREATMENT OF OSTEOPOROSIS

L Day[1], R Osborne[2], E Hristov[1] & JD Wark[1]
[1] *University of Melbourne Department of Medicine and Bone & Mineral Service, Royal Melbourne Hospital, VIC*
[2] *University of Melbourne and Centre for Rheumatic Diseases, Royal Melbourne Hospital, Parkville, VIC*

Obstacles to effective treatment of osteoporosis need to be identified and removed. ActNow is a patient information and support service designed to assist treatment effectiveness. It provides an information/education kit, support nurse and helpline access. Although very good long-term treatment compliance has been demonstrated among enrolled patients, the participation rate in ActNow has been low.

Therefore we evaluated ActNow to identify obstacles to program effectiveness. Structured interviews were conducted with: 25 osteoporosis patients enrolled in ActNow; 25 participants prescribed risedronate, but not enrolled in ActNow; and 25 medical practitioners (15 general practitioners, 10 specialists).

86 % of participants enrolled in ActNow assessed it as "beneficial" to "extremely beneficial." Once participants who were not enrolled in ActNow had an understanding of the program, 76 % felt that it would be " beneficial" to "extremely beneficial". In 72 % of this group, the reason for not enrolling was their lack of knowledge of ActNow. Of this 72 %, 89 % said that the likelihood of their enrolling when prescribed Actonel would be 'very high' to "high". 52 % of the medical practitioners treated 1-5 osteoporosis patients/week. 71 % sometimes encouraged their patients to utilize support services; 58 % did so believing that they increased patient education, compliance rates and reinforced positive messages. 63 % believed that ActNow had a positive impact on patient-doctor relationships.
It is concluded that ActNow is regarded positively by most participating patients. Lack of awareness about the program appears to be a major and remediable cause of non-participation in ActNow. Approaches to promoting such programs warrant further exploration.

Disclosures:
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P47

TAMOXIFEN-INDUCED BONE REMODELLING PROVIDES INCREASED PRECURSORS FOR *IN VITRO* TRANSGENIC OSTEOCLAST STUDIES

B Fletcher[1], D Ovchinnikov[1], A Pettit[1], M Chang[1] & I Cassady[1]
[1] *CRC for Chronic Inflammatory Diseases, Institute for Molecular Bioscience, University of Queensland, Brisbane, QLD*

Inducible, conditional knockout mice are commonly used to provide insight into organ specific effects in transgenic animals with an embryonic-lethal phenotype. We were interested in using such a system (tamoxifen-inducible Cre-ER^T recombinase) to examine osteoclast-specific gene knockout in transgenic mice. Because of the known effect of tamoxifen in increasing bone mineral density in post menopausal women treated for breast cancer, we first examined the effect of a short term, high dose, tamoxifen treatment regime (1 mg/day for 5 days, peritoneal injection) in male C57BL/6 mice.

In mice treated with tamoxifen at 7 weeks of age, we observed a significant increase in the number of RANKL-induced osteoclasts able to be derived from bone marrow *in vitro* . This effect persisted up to three weeks post treatment, with the peak difference in osteoclast generation seen one week post treatment. We also examined the *in vivo* effects of this drug regime on bone architecture and osteoclastogenesis. Histomorphometry on paraffin sections of the proximal tibia showed an initial increase in osteoclast number and activity in treated animals, closely followed by increased bone formation. An apparent increase in TRAP deposition throughout the bone matrix was also noted in the tamoxifen treated animals. At three weeks post treatment, extensive trabecular bone volume changes were obvious in the treated animals.

We conclude that whilst tamoxifen-inducible Cre-ER^T recombinase may be a very effective tool for *in vitro* transgenic osteoclast studies, it is important to take into account direct side effects on bone biology *in vivo* in male mice.

P48

25-HYDROXYVITAMIN D_3-1α-HYDROXYLASE (CYP27B1) PROMOTER ACTIVITY IN THE RAT OSTEOBLAST-LIKE CELL LINE ROS 17/2.8

AG Turner[1,2], PP Dwivedi[2], PH Anderson[2], BK May[1,2] & HA Morris[1,2]
[1] *School of Molecular and Biomedical Sciences, University of Adelaide, Adelaide, SA*
[2] *Endocrine Bone Research Laboratory, Hanson Institute, Adelaide, SA*

Biologically active vitamin D, 1,25 dihydroxyvitamin D_3 (1,25D), regulates both osteoblast proliferation and differentiation. Production of 1,25D is catalysed by the enzyme 25-hydroxyvitamin D_3-1α-hydroxylase (CYP27B1). Expression of CYP27B1 has previously been identified in non-renal cells including osteoblasts and it is hypothesised that local production of 1,25D in osteoblasts has an autocrine or paracrine role. The aim of this study was to investigate the regulation of CYP27B1 gene expression in the osteoblast-like cell line ROS 17/2.8. Full-length CYP27B1 promoter (1.5kb) activity was analysed by using the CYP27B1 promoter-luciferase construct transiently transfected into ROS 17/2.8 and then directly measuring luciferase activity. Luciferase activity using truncations of the CYP27B1 promoter was then tested. Three-fold higher CYP27B1 promoter activity was detected in the -997 bp construct when compared to the full-length promoter activity. In the –531 and –1100 bp constructs there was a reduction of 35% and 55% respectively when compared to activity in the –997 bp construct. This suggests that an enhancer region lies beyond the basal promoter between –531 and –997 bp with a repressive region between –997 and –1100 bp. The enhancer region contains two putative Ets-1 protein-binding sites, site-directed mutagenesis of which decreased luciferase activity by 60% and 40% when compared to wild-type. Two Smad binding elements were identified within the –997 to –1100 bp repressive region. Mutagenesis of these elements reversed the repression of CYP27B1 promoter activity. These results demonstrate the CYP27B1 gene in ROS 17/2.8 is stringently regulated by a complex interaction of enhancer and repressor elements.

P49

ANALYSIS OF CELL HYPERTROPHY IN TRIIODOTHYRONINE-TREATED THREE-DIMENSIONAL CULTURE OF MOUSE CHONDROCYTES

KS Chen, L Tatarczuch, Y Ahmed, M Mirams & EJ Mackie
School of Veterinary Science, University of Melbourne, Parkville, VIC

Hypertrophic chondrocyte death during endochondral ossification has been generally considered to be a process of apoptosis. However, classical apoptotic chondrocytes are rarely seen in growth cartilage (Roach and Clarke, 2000; J. Bone Joint Surg. 82: 601-13). Hypertrophic chondrocytes exist in two forms, "dark" and "light" chondrocytes, and we have recently observed in horses that these cells undergo two distinct non-apoptotic forms of physiological death. The aim of this study was to set up a culture system in which chondrocytes can be induced to hypertrophy so that molecular biological methods can be used to clarify the process of chondrocyte death in the future. Chondrocytes were isolated from the epiphyseal cartilages of the limb long bones in 5-day-old mice and cultured as three-dimensional pellets. These pellets were cultured in triiodothyronine (T3) containing or control medium. The pellets were collected at day 14 and examined by light and electron microscopy. Distinct hypertrophic dark and light chondrocytes were recognised in both treatment groups under electron microscopy. Chondrocytes treated with T3 showed a more active secretory phenotype than control cells, with abundant mitochondria, Golgi and rough endoplasmic reticulum. They also showed organization into columnar architecture which was not seen in control pellets. A few apoptotic cells were noted in control but not T3-treated cultures. In this culture system, chondrocytes treated with T3 resemble the hypertrophic chondrocytes in growth cartilage in vivo. This culture system will be extremely useful for studying mechanisms of physiological death of hypertrophic chondrocytes.

P50

EFFECTS OF DIETARY SUPPLEMENTATION OF OMEGA-3 FATTY ACIDS ON SKELETAL STRUCTURE IN GROWING RATS

AJ Moore, A Mulaibrahimovic, L Cleland[1], M James[1] & HA Morris
Endocrine Bone Research, Hanson Institute, Adelaide, SA
[1] *Department of Rheumatology, Royal Adelaide Hospital, Adelaide, SA*

Dietary lipids have been demonstrated to have an important role in skeletal biology and bone health. Polyunsaturated fatty acids (PUFA) have been shown to affect bone formation and resorption via prostaglandin biosynthesis. Alterations in histomorphometric parameters have also been observed with dietary PUFA supplementation. However, the effect of dietary n-3 and n-6 PUFA on bone histomorphometric variables during growth has not been investigated. The aim of this study was to determine the effects of dietary n-3 PUFA on skeletal architecture in growing rats. One month old female rats were fed diets containing either n-3 or n-6 PUFA and 1% calcium, and killed at 2, 3, 4, and 6 months of age. Femora were removed and prepared for histomorphometry. 5µm thick undecalcified sections of distal femora, stained with Von Kossa, were used to measure trabecular bone volume (BV/TV), trabecular thickness (Tb.Th) and trabecular number (Tb.N) using Quantimet imaging. BV/TV in the femoral metaphysis of rats fed n-3 PUFA increased by 15% by 6 months of age ($P<0.01$) when compared with animals fed n-6 PUFA. This increase was due to an increase in Tb.Th with no change in

Tb.N. Thickening of trabeculae from dietary n-3 PUFA is likely to be due to increased formation and possibly reduced resorption. An analysis of dynamic parameters will clearly identify the mechanism by which n-3 fatty acids can alter bone turnover in the metaphysis. A diet rich in n-3 PUFA may promote the accrual of bone mineral during growth resulting in higher bone density in adulthood.

P51

A SINGLE SYSTEMIC PERI-OPERATIVE DOSE OF ZOLEDRONIC ACID INCREASES CALLUS VOLUME AND STRENGTH IN RAT FRACTURE HEALING; DELAYED ADMINISTRATION AT ONE OR TWO WEEKS POST-FRACTURE FURTHER INCREASES THESE PROPERTIES

N Amanat[1,2], M McDonald[1], C Godfrey[1], L Bilston[3] & D Little[1]
[1] *Orthopaedic Research and Biotechnology, The Children's Hospital at Westmead, Sydney, NSW*
[2] *School of Aeromech, Mechanical and Mechatronic Engineering, The University of Sydney, Sydney, NSW*
[3] *Prince of Wales Medical Research Institute, Sydney, NSW*

Bisphosphonates (BPs) have high affinity for bone mineral and inhibit bone resorption, properties that may provide advantages for augmenting fracture repair. This study investigated the possibility of improving fracture repair by transiently interfering with bone catabolism.

Carbon-14 labelled zoledronic acid (ZA) was used in a closed rat fracture model. Five treatment groups were investigated (n=15 per group): saline control, local ZA injection peri-operatively (0.01mg/kg), single IV ZA dose peri-operatively (0.01mg/kg), single IV ZA dose one week after fracture (0.01mg/kg) and a single IV ZA dose two weeks after fracture. Rats were sacrificed six weeks after surgery.

Single dose IV ZA administration significantly increased callus BMC, volume and mechanical strength. Peri-operative IV treatment increased mechanical strength by 30% compared to controls ($p<0.05$). Administering the IV dose at one week or two weeks after fracture further increased mechanical strength compared to controls by 44% and 50% respectively ($p<0.05$). No significant differences were seen with local injection. Autoradiographic analysis indicated that ZA binds to bone that is present at the time of administration and that new bone formed after administration contains little ZA. Quantification of the concentration of ZA in fractured and non-fractured femoral diaphyses indicated that administration post-fracture significantly increased the uptake efficiency in the callus compared to peri-operative administration.

The results of this study showed that the timing of a single systemic dose of ZA plays an important role in improving callus properties in this rat fracture model. The results also show the potential for reducing systemic exposure with delayed IV administration due to increased ZA uptake in the callus.

P52

KLOTHO GENE POLYMORPHISMS ARE ASSOCIATED WITH OSTEOCALCIN LEVELS IN AGED POSTMENOPAUSAL WOMEN

RL Prince[1,2,3], BH Mullin[2], SG Wilson[2,3], FMA Islam[1,3], M Calautti[2], A Devine[1,2 3,4] & IM Dick[1,2,3]
[1] *University of Western Australia,* [2] *Sir Charles Gairdner Hospital,* [3] *Western Australian Institute of Medical Research,* [4] *Edith Cowan University, Perth, WA*

Osteoporosis is known to have a strong genetic basis. Polymorphisms within the *KL* (*klotho)* gene have a significant effect on the osteoblast defect of ageing. The association between polymorphisms within this gene and bone turnover markers, bone structure and fracture rates was studied in 1190 postmenopausal women with a mean age of 75 years.

Polymorphic sites were genotyped using Matrix-Assisted Laser Desorption Ionisation – Time of Flight (MALDI-ToF) mass spectrometry. Serum osteocalcin, urinary creatinine and DPD was determined. Clinical osteoporotic fractures were verified by x-ray. Hip BMD was measured using Hologic 4500A; femoral neck CV was 1.4%.

The *G* allele of SNP c.1775G>A (homozygous population prevalence 41%) was associated with a lower osteocalcin level than the *A* allele ($P = 0.004$) in a co-dominant model. SNPs C-387T and IVS1+8262c>t both showed non-significant associations with osteocalcin (P values of 0.063 and 0.068 respectively). A haplotype analysis of 2 of 5 haplotypes of the 3 SNPs with a frequency greater than 4% revealed a significant association with osteocalcin ($P = 0.036$). None of the individual polymorphisms or haplotypes analysed showed any associations with a DPD/creatinine ratio, bone structure or fracture.

The *G* polymorphism within the c.1775G>A SNP site, and a haplotype including this are associated with a reduced osteoblast product osteocalcin. These data suggest that variation in the *KL* gene product affects osteoblast activity independent of osteoclast activity but this defect does not result in an effect on bone structure in this population, perhaps because of "rescue" by other genetic or environmental factors.

Disclosures:
All authors 1.

P54

PATIENT PREFERENCES IN OSTEOPOROSIS THERAPY: EVALUATION USING TIME TRADE-OFF

E Hristov[1], R Osborne[2], A Dalton[3] & JD Wark[1]
University of Melbourne, Department of Medicine and Bone and Mineral Service, Royal Melbourne Hospital[1], University of Melbourne and Centre for Rheumatic Diseases, Royal Melbourne Hospital, Parkville, VIC[2], University of Melbourne, School of Population Health[3]

Uptake of interventions, compliance and adherence to therapy are key determinants of treatment effectiveness. Increasingly, patients seek to participate in informed decision-making about their treatment. Their preference is likely to influence their reliability in taking medication. Therefore, it is important to understand patients' treatment preferences and their determinants.

Using the time trade-off (TTO) technique, we evaluated treatment-related health state preferences in 25 unselected, treatment-naïve patients [23 female, 2 male; age (mean ± SD) 65.1 ± 8.3 years] attending a community-based osteoporosis clinic. Standardized vignettes were developed representing good health for an older person, the untreated health state with an osteoporotic vertebral fracture and health states with once-weekly oral medication (OM) or annual intravenous medication (IVM), with no, mild-moderate or severe side-effects. Utility (strength of patient preference) was calculated for each. The untreated health state was the poorest [median utility 0.50, inter-quartile range (IQR) 0.30 – 0.75]; i.e., patients would "trade" 10 years living in the morbid state for 5 years in the healthy state. Utilities were compared (Wilcoxon signed rank test with Bonferroni correction). Both medications with no side-effects were strongly preferred over the untreated state ($p<0.0001$). For each treatment, increasing severity of side-effects was associated with decreasing utility. The least preferred state was OM with severe side-effects (median, IQR utility 0.50, 0.30-0.60), equivalent to no treatment. OM with mild-moderate side-effects was preferred marginally over no treatment ($p<0.09$). IVM with no, mild-moderate or severe side-effects was preferred strongly over no treatment (median utility 1.00, 0.90, 0.80, $p <0.0001$).

This novel use of standard TTO techniques elicited osteoporosis therapy preferences among treatment-naive patients. When side-effects are considered, these patients strongly preferred IVM over OM. This tool will be applied in prospective studies of "real world" outcomes in osteoporosis.

Disclosures:
3,4 Novartis Pharma

P55

CHRONIC ANTI-EPILEPTIC DRUG TREATMENT IS ASSOCIATED WITH CLINICALLY SIGNIFICAN IMPAIRMENT IN BALANCE FUNCTION – A TWIN AND MATCHED SIBLING PAIR STUDY

SJ Petty[1], LM Paton[1], N El Haber[1], TJ O'Brien[1], B Metcalf[2], K Bennell[2], SF Berkovic[3] & JD Wark[1]
[1] *Department of Medicine, The Royal Melbourne Hospital, University of Melbourne, Parkville, VIC*
[2] *Department of Physiotherapy, The University of Melbourne, Parkville, VIC*
[3] *Epilepsy Research Centre, Austin Health, The University of Melbourne, Melbourne, VIC*

Rationale:
Anti-epileptic medication(AED) users have increased incidence of bony fractures. Potential causes for this association include bone disease and increased falls both during seizure and at other times.

Aim:
To assess the effect of chronic AED-use on muscle strength and balance function utilizing a twin and matched sibling AED-discordant pair approach.

Methods:
7 pairs (6 female, 1 male) 3 monozygous, 1 dizygous and 3 sibling pairs (within 3-years of age) were assessed. All pairs were discordant for AED-use (AED-user duration>12 months). Mean(SD) age 55.6(17.6) years, height 161(6)cm, weight 67.9(13.6)kg. Previously validated tests of muscle strength, static and dynamic balance, predictive of falls risk were performed: (i) Nicholas Manual Muscle Tester(NMMT), (ii) Kincom, (iii) Chattecx Balance System(CBS): Sway index(SI), anterior-posterior(AP) and lateral(LR) sway measured in 3 platform positions and (iv) Lord's Balance test(LBT). Human Activity Profile (HAP) utilized to measure physical activity. Paired t-tests utilized to assess mean within-pair differences (AED-user vs. non-user).

Results:

No significant within-pair difference in age, height, weight, muscle strength (NMMT, Kincom) or activity level (HAP). Significant within-pair difference (AED-user vs. non-user) seen in multiple balance measures: LBT: eyes closed (+87mm, p=0.026); foam mat eyes open (+46mm, p=0.043); CBS: Stable platform +distraction (SI+0.36cm, p=0.013, LR+1.28cm, p=0.00, AP+1.68cm, p=0.02); Anterior-posterior moving platform (SI+0.66cm, p=0.045, AP+2.66cm, p=0.022); Medial-lateral moving platform +distraction (AP +1.16cm, p=0.024).

Conclusion:
There is significant within-pair difference in balance function in chronic AED users compared to matched sibling/twin control. This may have important implications for AED-users indicating increased falls risk and therefore fracture risk.

Disclosure:
No conflict of interest.

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MORPHOLOGICAL DIVERSITY OF HYPERTROPHIC CHONDROCYTES IN GROWTH CARTILAGE

L Tatarczuch, YA Ahmed, HMS Davies & EJ Mackie
School of Veterinary Science, University of Melbourne, Parkville, VIC

During endochondral ossification chondrocytes undergo hypertrophic differentiation contributing to cartilage growth and bone formation. It is usually assumed that hypertrophic chondrocytes are a homogenous cell population, though some previous studies have indicated their morphological heterogeneity. The aim of this study was to determine the ultrastructural morphology of hypertrophic chondrocytes in equine articular–epiphyseal and physeal growth cartilage (AEGC and PGC, respectively). Specimens of AEGC and PGC collected from the head of humerus during foetal and postnatal growth were examined by transmission electron microscopy. Despite morphological variations in ultrastructural appearance "light" and "dark" hypertrophic chondrocytes were present at both locations. The light chondrocytes were characterised by electron lucent cytoplasm containing sparse cisterns of rough endoplasmic reticulum and an inconspicuous Golgi region. This is in contrast to more electron dense cytoplasm of dark chondrocytes that contained abundant, often dilated, rough endoplasmic reticulum and a prominent Golgi region. Both light and dark chondrocytes were observed in the proliferation zone and their subsequent maturation and death followed two distinctive patterns. The light chondrocytes appeared to disintegrate within a preserved cellular membrane, whereas dark chondrocytes undwent gradual condensation with the cytoplasm extruded into the extracellular space. The proportion of light chondrocytes was higher in foetal than postnatal specimens and greater in AEGC than PGC. The presence of morphologically distinct populations of hypertrophic chondrocytes in growth cartilage suggests they play specific roles during skeletal development and growth.

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IS REDUCED VERTEBRAL BONE STRENGTH ASSOCIATED WITH LOWER ERECTOR SPINAE AND PSOAS MUSCLE SIZE AND DENSITY IN OLDER MEN?

S Kukuljan, RM Daly & S Bass
Centre for Physical Activity and Nutrition Research, School of Exercise and Nutrition Sciences, Deakin University, Melbourne, VIC

Muscles place the largest loads on bones, and thus changes in muscle size should affect bone predictably and correspondingly. A reduction in the biomechanical competence of the spine predisposing to an increased risk of fracture can result from changes in both the material (mass) and geometric properties (shape, structure, distribution) of bone, often in parallel with changes in muscle. We used QCT to examine whether the relationship between psoas and erector spinae muscle area (cm^2) and density (HU) and lumbar spine (L_3) total area (TotAr, cm^2), total and trabecular BMC (g) and volumetric BMD (g/cm^3) and compressive strength (g^2/cm^4) differed in men (n=181) aged 50-79 years with normal (T-score >-1.0 SD) or low (-1.0 to -2.5 SD) BMD. Each of the bone traits were positively correlated with muscle area (r=0.21–0.36) and density (r=0.17–0.28, except TotAr) (all $p<0.05$-<0.001). No differences were detected for the slope of the muscle-bone relationships in men with normal and low BMD, but the intercepts for muscle area and density with total and trabecular BMC and BMD, and compressive strength were reduced in men with low BMD (all $p<0.001$). That is, these men had less bone for a given muscle area compared to those with normal BMD. Since the muscle to height relationship was similar in both groups, the lower vertebral BMD and compressive strength relative to muscle in men with low BMD indicates a primary bone defect rather than an indirect effect via a reduction in muscle size or density.

Disclosure:
No conflict of interest

P58

REGULATION OF GALANIN AND GALANIN RECEPTOR mRNA EXPRESSION BY LEUKEMIA INHIBITORY FACTOR AND TRI-IODOTHYRONINE IN UMR 106-01 OSTEOSARCOMA CELLS

M Yao[1], JA Schuijers[1], AL Gundlach[2] & BL Grills[1]
[1] *Department of Human Physiology and Anatomy, School of Human Biosciences, La Trobe University, VIC*
[2] *Howard Florey Institute of Experiment Physiology and Medicine, University of Melbourne, VIC*

Galanin (GAL) is a neuropeptide that acts via 3 G-protein receptors and its effects include the stimulation of nerve regeneration and the inhibition of both inflammation and mitosis. We have located GAL and its receptors in skeletal cells of fracture callus and in UMR 106-01 osteosarcoma cells and have shown that GAL aids bone formation in inflammatory situations and induces UMR 106-01 cell apoptosis.

Both leukemia inhibitory factor (LIF) and tri-iodothyronine (T3) regulate GAL in non-osseous tissues to affect physiologic/pathophysiologic processes. In bone, LIF and T3 act via osteoblasts to increase bone remodeling, whilst LIF stimulates UMR 106-01 cell division and T3 can decrease their proliferation. GAL is an inhibitor of mitosis seemingly via GALR1 activation and an instigator of apoptosis possibly via GALR2.

Thus the aims of the current experiments were to investigate the effects of LIF and T3 on GAL, GAL-R1 and -R2 receptor mRNA expression in UMR 106-01 cells.

Cells were incubated with either LIF (2.5, 5, 10 and 50 ng/ml) or T3 (10, 100 and 1000 nM) for either 2 h or 24 h. LIF treatment caused a downregulation in mRNA expression of GAL (2.5 and 5 ng/ml, $p < 0.05$) and GALR1 (5 ng/ml, $p < 0.01$) and an upregulation of GALR2 (2.5 - 50 ng/ml; $p < 0.01$).

After 2h, all T3 treatments resulted in no change to either GAL or GALR1 mRNA expression however, after 24 h 10 nM T3 caused an upregulation in both of these genes ($p < 0.05$). At 2h, only 1000 nM T3 downregulated GAL2 mRNA expression ($p < 0.05$) but by 24 h it had no effect on its expression.

The downregulation of GAL and GALR1 mRNA expression in LIF treated UMR 106-01 cells may explain in part the documented increase in cell division of these cells by LIF. Conversely, the upregulation of mRNA expression of these two genes in T3 treated cells may to some extent account for their previously reported decrease in proliferation due to T3 treatment. The influence on GALR2 mRNA expression by LIF and T3 treatment may also implicate different effects on apoptosis in these cells.

Disclosures:
1

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DAILY SUBCUTANEOUS INJECTION OF VEHICLE ONTO MOUSE CALVARIA INHIBITS MINERAL APPOSITION RATE AND UPREGULATES OSSEOUS mRNA EXPRESSION OF TNFα, IL-1β, COLLAGEN TYPE I AND MMP-2

AC McDonald[1], JA Schuijers[1], AL Gundlach[2] & BL Grills[1]
[1] *Department of Human Physiology and Anatomy, School of Human Biosciences, La Trobe University, VIC*
[2] *Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne, VIC*

Soft tissue inflammation can influence either adjacent or systemic bone remodeling by inhibiting bone formation. The rodent calvarial injection model is widely used to study the effects of substances on bone, yet no osseous changes have been documented in vehicle-injected, control animals even though adjacent soft-tissue inflammation is an inevitable scenario after repeated subcutaneous needle injections over an extended period.

Therefore, a 2 week regimen of the calvarial injection model was used to investigate the effects of local soft tissue injury on 1. mineral apposition rate (MAR), 2. osteoclastic surface and 3. osseous gene expression of the inflammatory cytokines TNFα and IL-1β along with collagen type I and two matrix metalloproteinases, MMP-2 and MMP-13. Two groups of 4 week-old mice were used: non-injected controls and vehicle-injected animals. Injected mice received 10 µl of a solution containing PBS, BSA and glycerol (pH 7.4) subcutaneously onto calvaria each day for 2 weeks.

Dynamic histomorphometric analysis showed that injected mice had a lower mineral apposition rate (MAR) at both 1 ($p = 0.024$) and 2 weeks ($p = 0.004$) compared with controls. No osteoclasts were found on calvarial parietal bone in either group. RT-PCR analysis demonstrated an increased expression of both TNFα ($p = 0.005$) and IL-1β ($p = 0.07$) in injected mice compared with controls. An increase in both TNFα and IL-1β is likely to inhibit bone formation. Injected mice also had higher osseous mRNA expressions of collagen type I ($p < 0.05$) and MMP-2 ($p = 0.014$) but not MMP-13 compared with controls. These data may signify that in injected mice, bone collagen lysis (by proteases such as MMP-2) is greater than its synthesis and thus results in the observed reduction in MAR compared with non-injected controls. Future studies using the calvaria injection model should take these above effects into account when undertaking similar procedures.

Disclosures:
1

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CHARACTERISATION OF BONE QUALITY IN THE FEMUR AND VERTEBRA IN A SHEEP MODEL OF STEROID-INDUCED OSTEOPOROSIS

MR Zarrinkalam[1], H Beard[1], GJ Atkins[2], DM Findlay[2,5], KJ Welldon[2], CG Schultz[3] & RJ Moore[1,4]
[1] *The Adelaide Centre for Spinal Research, Institute of Medical and Veterinary Science, Adelaide, SA*
[2] *Department of Orthopaedics and Trauma, University of Adelaide, Adelaide, SA*
[3] *Department of Nuclear Medicine and Bone Densitometry, Royal Adelaide Hospital, Adelaide, SA*
[4] *Department of Pathology, University of Adelaide, Adelaide, SA*
[5] *Hanson Institute, Institute of Medical and Veterinary Science, Adelaide, SA*

Aim:
The ovine model has been identified as one of the most suitable for pharmacological trials and *in vivo* assessment of vertebral augmentation procedures and orthopaedic devices (Beard et al., 2005). The aim of this study was further characterise the glucocorticoid-treated sheep as a model of steroid-induced osteoporosis.

Methods:
Osteopaenia was induced in ten 8-year-old lactating ewes and their lumbar spine (LS) and proximal femora (PF) were processed and analysed by quantitative histomorphometry (Beard et al., 2005). Osteoblast cultures were established from vertebral and femoral trabecular bone fragments (Atkins et al., 2003). The expression of bone-related genes in cultured cells and bone samples was determined using real-time RT-PCR analysis.

Results:
In treated sheep, PF and LS trabecular bone volume (BV/TV) decreased, by 42.5% and 29.5%, respectively, compared to controls ($P<0.05$). The decrease of BV/TV in PF region was greater than the LS region ($p<0.05$). Preliminary results suggest that there are differences in gene expression (i.e. Collagen type-I) in the PF and LS regions, which may account for the histomorphometric observations. Furthermore, the cells isolated from the two regions display differences in growth and gene expression patterns which may also contribute to the *in vivo* phenotype.

Conclusion:
These data support our sheep model as a valuable large animal model for osteoporosis studies. Ongoing investigations to characterise bone quality and gene expression in this animal model could highlight potential differences in bone characteristics between the femoral and vertebral regions. Such information could have significant value in pharmacological drug design and trials.

Reference:
Atkins, G. J., Kostakis, P., Pan, B., Farrugia, A., Gronthos, S., Evdokiou, A., Harrison, K., Findlay, D. M. and Zannettino, A. C. (2003) *J Bone Miner Res*, 18, 1088-98.
Beard, H., Schultz, C. G. and Moore, R. J. (2005) In *Spine Society of Australia* (Ed, McPhee, A. P. B.) Auckland, New Zealand, pp. 123.

P61

EXPRESSION OF RANKL, OPG, PTHRP AND OCIL IN MULTIPLE MYELOMA ARE INFLUENCED BY THE TUMOR CELLS' PROXIMITY TO BONE

V Kartsogiannis[1], C Ly[1], H Zhou[2], O Gallagher[3], C Buckle[3], E De Leenheer[3], K Vanderkerken[4], KW Ng[5], TJ Martin[1], MT Gillespie[1] & PI Croucher[3]
[1] *Bone, Joint and Cancer Unit, St. Vincent's Institute, Fitzroy, VIC*
[2] *Bone Biology Unit, ANZAC Research Institute, Concord, NSW*
[3] *Academic Unit of Bone Biology, Division of Clinical Sciences, University of Sheffield Medical School, Sheffield, United Kingdom*
[4] *Department of Hematology and Immunology, Free University Brussels, Brussels, Belgium*
[5] *Department of Endocrinology, St. Vincent's Hospital, Fitzroy, VIC*

Multiple myeloma is characterized by the development of lytic bone lesions mediated by increased osteoclastic bone resorption. A number of molecules have been implicated in promoting the increase in osteoclastic resorption including RANKL and macrophage protein-1 alpha. While the RANKL/RANK/OPG system may indeed play a critical role in the osteolysis in myeloma, other molecules may also contribute. One novel family of osteoclast inhibitors, named OCILs (Osteoclast Inhibitory Lectins) may also be effective in altering the progression of multiple myeloma since they are effective in blocking RANKL-, TNFα-induced and TGFβ-primed osteoclast formation. Using in situ hybridization and immunohistochemistry, mRNA and protein expression of OCIL was examined in the 5T2MM murine model of myeloma. OCIL was shown to be expressed by 5T2MM myeloma cells residing in bone. Approximately 80% of myeloma cells within the marrow cavity expressed OCIL. Myeloma cells residing closer to the growth plate, or those contained well within the bone

environment, exhibited higher levels of OCIL mRNA and protein than the population of myeloma cells outside the bone. We therefore examined the mRNA and protein expression for other key factors in myeloma including RANKL, OPG and PTHrP. Similar to OCIL, the expression of each of these was elevated when myeloma cells were in proximity to bone but not outside of bone. OPG mRNA and protein were expressed in only a limited number of myeloma cells. Furthermore, quantitative RT-PCR analysis, also revealed a consistent expression of OCIL in the 5T2MM and 5T33MM myeloma cell lines as well as the murine bone marrow endothelial cell lines, STR10 and STR12. These results demonstrate that murine myeloma cells express OCIL and raise questions about the potential role of OCIL in the progression of myeloma as well as the role of the bone microenvironment to influence the expression of other candidate molecules (RANKL, OPG and PTHrP) in the development of osteolytic bone disease.

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GLUCOCORTICOID-INDUCED MYOPATHY AND BONE LOSS: A PILOT STUDY

CL Lu[1], H Hadimulia[1], G McColl[2], K Bennell[3], T Wrigley[3], S Kantor[1] & J D Wark[1]
[1] *Department of Medicine, University of Melbourne, Bone and Mineral Service, Royal Melbourne Hospital, VIC*
[2] *Clinical School, Department of Rheumatology, University of Melbourne, Royal Melbourne Hospital, Melbourne, VIC*
[3] *Centre for Health, Exercise and Sports Medicine, University of Melbourne, VIC*

Background:
Steroid myopathy is likely to contribute to glucocorticoid-induced osteoporosis by reducing skeletal loading and increasing falls risk, thereby predisposing to fractures.

Hypotheses:
(i) Patients receiving long-term glucocorticoids will have lower levels of lean mass, muscle strength and bone mineral (BMD) measures than patients who are not on glucocorticoid or newly commencing treatment. (ii) There will be a relationship between bone mineral measures and lean mass and muscle strength.

Subjects and Methods:
To date, we have studied 18 patients receiving more than 6 months glucocorticoid therapy for musculoskeletal or other inflammatory diseases (mean age 38, range 21-50 years) and 3 control (46, 37-54 years) who were newly commencing glucocorticoid treatment within 2 weeks or were on methotrexate treatment. We measured BMD and soft tissue composition (Hologic QDR 4500A densitometer), calcaneal quantitative ultrasound (QUS) indices and lower limb muscle strength.

Interim Results:
There was a pattern of decreasing muscle strength based on cumulative steroid dose. Proximal lower limb lean mass, however, did not show this pattern. Knee flexion strength correlated with femoral neck BMD (r=0.499) in the steroid group. Other muscle strength components had similar correlation coefficients with femoral neck and total hip BMD, though were not statistically significant.

Conclusion:
This ongoing study will help us understand the relationship between muscle and bone with long-term glucocorticoid therapy, and may lead to improved prediction of glucocorticoid-induced bone loss.

Disclosures:
No conflict of interests (all authors)

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TRUNCATION MUTANTS OF RANKL INHIBIT RANKL-INDUCED OSTEOCLAST DIFFERENTIATION AND ACTIVATION

C Wang*, W-Y Tan*, N Pavlos, Jamie, J-M Li*[1], J Cornish[1], M-H Zheng & J Xu*
* *Department of Orthopaedic Surgery, QEII Medical Centre, University of Western Australia, Perth, WA*
[1] *Department of Medicine, University of Auckland, New Zealand*

Receptor activator of NF-kB ligand (RANKL) is a crucial factor necessary for osteoclast differentiation and activation. In this study we have examined the role of the TNF like core domain of RANKL in osteoclast differentiation and activation. To this end, a series of truncation mutants of the TNF-like core domain of RANKL were expressed as GST-fusion proteins, and their biological activities assessed using a number of pro-osteoclastogenic systems. Osteoclastogenesis assays revealed that while GST-rRANKL (aa160-318) containing the full TNF-like core region strongly induced osteoclast formation. RANKL truncation mutants GST-rRANKL (aa239-318), (aa160-268), (aa160-291), (aa246-318) display significantly decreased osteoclastogenic activity. Consistently, the decrease in osteoclast number correlates with decreased TRACP activity and reduced calcitonin receptor and cathapsin K gene expression. Furthermore, competition studies revealed that all RANKL truncation mutants were capable of inhibiting RANKL (aa160-318)-induced osteoclast formation but with different efficacy, RANKL mutant (aa246-318) been the most potent one. Bone resorption analysis showed that RANKL mutant

(aa246-318) competitively inhibits RANKL (aa160-318)-induced osteoclastic bone resorption *in vitro*. Interestingly, GST pull down studies revealed that all RANKL mutants have reduced binding affinity to RANK. In addition, all RANKL mutants display significant reduction in the activation of crucial osteoclastic signalling pathways includes NF-kB, ERK, JNKs and have decreased IKBα degradation compared to the full-length protein. Together, our data indicate that RANKL mutants may act as competitive inhibitors of RANKL-induced osteoclast differentiation and activation and thus may offer potented therapeutic effects to combat bone lytic disorders.

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EFFECT OF AN INFORMATION-BASED INTERVENTION ON THE MANAGEMENT OF MINIMAL AND MODERATE TRAUMA FRACTURES: A RANDOMISED STUDY

D Bliuc, JA Eisman & JR Center
Garvan Institute of Medical Research, St Vincent's Hospital, University of New South Wales, Sydney, NSW

Osteoporosis is sub-optimally managed even in high-risk people with prior fracture. This study aimed to: 1) determine whether an information-based intervention could change post-fracture management of osteoporosis, and 2) identify participant- and doctor-related barriers to osteoporosis management. Consecutive fracture patients (n=198) from outpatient fracture clinics at St Vincent's Hospital, Sydney were interviewed. Fracture risk factors, prior investigation and treatment for osteoporosis were collected at baseline. Participants were contacted after 3 months. Those not investigated or treated were randomised to either a personalised letter or the same letter plus a "free" BMD offer. Follow-up was obtained after another 6 months.

Of the 198 participants, less than 20% (39) had a primary care physician follow-up 3 months after fracture, leaving 159 who were randomized to the personalised letter (n=79) and personalised letter plus BMD (n=80). Significantly more people were investigated for osteoporosis in the letter plus BMD group (38% vs. 7%; p=0.001), but treatment rates were very low in both groups (6%). Of those tested, 66% had low bone density. Of those with low BMD who saw their primary care physician (n=18) only 33% were recommended treatment. Belief that fracture was osteoporotic was the strongest predictor of better osteoporosis management. Other factors were age >50, female gender and having a BMD scan.

Thus an information-based intervention did not improve post-fracture treatment. Low uptake of either BMD or visit to primary care physician together with low treatment recommendation among people who contacted their physician, reflect significant participant- and doctor-related barriers to osteoporosis management.

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NOVEL GENES REGULATED BY MICROPHTHALMIA TRANSCRIPTION FACTOR IN MACROPHAGES AND OSTEOCLASTS

N Meadows[1], G Faulkner[1], C Wells[1], T Ravasi[1], MC Ostrowski[2], DA Hume[1] & AI Cassady[1]
[1] *CRC for Chronic Inflammatory Diseases, Institute for Molecular Bioscience, University of Queensland, Brisbane, QLD*
[2] *Department of Molecular Genetics, Ohio State University, Columbus, OH, USA*

Microphthalmia transcription factor (Mitf) regulates osteoclast function by activating genes like TRAP and Cathepsin K. Mitf binds as a homo- or heterodimer to a modified E-box, termed the M-Box (TCANNTG). We have modulated the level of Mitf activity in the pre-osteoclastic, macrophage cell line, RAW264.7, to identify novel Mitf regulated genes. Stable transfectant cell lines expressing the Mitf-A isoform (RAW/Mitf-A) and the dominant negative isoform, *mi* (RAW/*mi*), under control of the EF1a promoter, were prepared. The expression and function of the exogenous proteins have been confirmed.

Microarray expression profiling using the 22,000 element murine Compugen cDNA array of RAW/Mitf-A and RAW/*mi* cell lines was performed before and after osteoclast differentiation. A Blast technique was developed in parallel, to download 5kb of DNA sequence 5' of the translation start site for all genes on the Compugen array. Promoters were searched for M-boxes and potential Mitf target genes were compared with targets generated from the microarray analysis. This approach identified 5 novel genes that were indicated to be Mitf regulated.

These genes were validated using Q-PCR and their promoters have been assayed for Mitf binding and transcription activation using gel shift and promoter-reporter assays. We have identified Mitf-regulated genes that have documented roles in osteoclast function but unknown regulation pathways, and genes with a novel role in osteoclast biology.

We propose that Mitf is a "master" regulator of genes involved in osteoclast function and delineation of Mitf regulated genes may provide novel therapeutic treatments of bone diseases.

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IMAGE RESOLUTION OF DXA SCANNERS IS IMPORTANT IN HIP STRUCTURAL ANALYSIS

BCC Khoo[1], Qiao QH[2], TJ Beck[2] & RI Price[1]
[1] *Sir Charles Gairdner Hospital, Perth, WA,*
[2] *The Johns Hopkins University, Baltimore, MD, USA*

Hip Structural Analysis (HSA) uses spatial distribution of pixel masses from DXA images to calculate structural geometrical outcomes predicting bone strength. HSA computations are thus influenced by spatial resolution properties of DXA scanners.

We measured the spatial resolution of 3 DXA scanners in terms of modulation transfer function, MTF. This technique employed Fourier transform of the first derivative of a sigmoid fit to pixel distributions traversing the image of an L-shaped copper piece. An oversampling method was used to eliminate aliasing. Spatial resolution was evaluated in the vertical and horizontal directions.

Resolution limit was defined as spatial frequency (cycles/mm) where MTF fell to 1%, as shown in Table 1, presented at 10 cm above couchtop, typical height for an adult hip DXA scan. All scanner models investigated had better vertical resolution (along scanner axis) compared to horizontal resolution. All scanners had comparable horizontal resolution, the main difference was with vertical resolution of QDR4500A.

The asymmetry in resolution implies that bone edges (as in bone width in HSA) are more smeared in the horizontal than vertical direction. Corrections for blurring effect on detection of bone margins would be different for either direction and is scanner model dependent.

Table 1: Limiting resolution for Hologic DXA scanners investigated

	Horizontal		**Vertical**	
	(cycles/mm)	***(mm)***	***(cycles/mm)***	***(mm)***
QDR1000W	0.18	2.8	0.29	1.7
QDR4500W	0.18	2.8	0.30	1.7
QDR4500A	0.18	2.8	0.40	1.3

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OMEGA 3 FATTY ACIDS AND BMD IN A COMMUNITY SAMPLE: GEELONG OSTEOPOROSIS STUDY

FN Jacka, MJ Henry, MA Kotowicz, GC Nicholson & JA Pasco
The University of Melbourne, Department of Clinical and Biomedical Sciences: Barwon Health, Geelong, VIC

Recent evidence suggests a role for omega-3 polyunsaturated fatty acids (n-3 PUFAs) in bone metabolism, however data is limited. This study investigated the association between dietary intakes of seafood-derived n-3 PUFAs and BMD in a random sample of postmenopausal women.

Self-reported seafood and fish oil consumption data were obtained from women aged >55yr enrolled in the Geelong Osteoporosis Study (n=723). N-3 intakes were divided into High (top quartile) and Low (lowest three quartiles) as intakes were highly skewed. Oral glucocorticoids and/or hormone therapy users were excluded, as were women with BMI≥25 to avoid interaction terms in ANCOVA analyses (n=410). Different associations between n-3 and BMD were observed for smokers (n=107) and non-smokers (n=206) and these groups were analysed separately.

Among smokers, High n-3 had significantly lower BMD than Low n-3 at the PA-spine (-0.52SD, p=0.02) and mid-forearm (-0.49SD, p=0.02), with a trend at the total hip (-0.41SD, p=0.06). There was no relationship between n-3 and age- and BMI-adjusted BMD at the PA-spine (p=0.2), total hip (p=0.4) or mid-forearm (p=0.1) in non-smokers. However the inverse relationship at the mid-forearm became significant after adjustment for calcium intake (-0.33SD, p=0.04).

These findings of an inverse relationship between n-3 and BMD do not support existing evidence regarding a positive effect of n-3 on bone metabolism in animal models [1,2]. However high ratios of n-6 to n-3 PUFAs are known to be associated with lower BMD. Further research that takes into account other dietary sources of n-3 PUFAs in conjunction with n-6 PUFAs is warranted.

[1]Li Y. et al. J Bone Miner Res. 1999; 14:1153-62
[2]Sakaguchi K .et al. Prostaglandins Leukot Essent Fatty Acids. 1994; 50:81-4

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MICROARCHITECTURAL BONE STRUCTURE DETERMINED USING MRI

AT Hattam[1], JA Pasco[1], PR Brotchie[2], J Umbers[2], KE Negus[2], MJ Henry[1], GC Nicholson[1] & MA Kotowicz[1]
[1] *The University of Melbourne, Department of Clinical and Biomedical Sciences, Barwon Health, Geelong, VIC*
[2] *Barwon Medical Imaging, The Geelong Hospital, Geelong, VIC*

The use of MRI in the assessment of trabecular bone is becoming recognised as a methodology for identification of patients at risk of fragility fracture. Microarchitectural parameters of bone, such as trabecular thickness, number, connectivity and alignment, have been used to complement the measurement of BMD, allowing a more accurate prediction of fracture risk.

MRI of trabecular bone microarchitecture is advantageous as the technique is non-invasive and does not require the use of ionising radiation. However, image acquisition times are lengthy and require subjects to lie motionless for up to 20 minutes.

Our laboratory has modified previously reported[1] scan sequences using a Picker Edge (Phillips) 1.5T scanner equipped with 27mT/m gradients to achieve suitable images of the calcaneum for microarchitectural analysis, with reduced scan time and subject discomfort.

The scan technique involves 3D gradient echo-fast imaging with steady state precession (3D turbofield echo Phillips) and a spatial resolution of 175x175x700µm. Imaging time has been reduced to less than 15 minutes.

Examination of calcaneal trabecular orientation in a group of 5 men aged 20-39yr indicates trabecular alignment angled at approximately 90° to the plane between the calcaneal spur and the posterior tuberosity. This orientation indicates the bone's grain and direction of maximal strength. This is consistent with heel-strike during gait, as it is between these two points where maximum forces are applied to the calcaneum.

Comparisons with trabecular alignment in elderly men and those with fracture may contribute to the understanding of the microarchitectural aspects of bone fragility.

[1]Boutry *et al* (2003) *Radiology* 227: 708-17

P70

THE ASSOCIATION BETWEEN FRACTURE AND DEPRESSION IN WOMEN

LJ Williams, MJ Henry, FN Jacka, M Berk, MA Kotowicz, GC Nicholson, S Dodd & JA Pasco
The University of Melbourne, Department of Clinical and Biomedical Sciences: Barwon Health, Geelong, VIC

High levels of disability, functional impairment and mortality are associated with both fracture and depression. The aim of this study was to investigate the relationship between fracture and depression in a community sample of women.

Two samples of women aged ≥ 35yr were drawn from the Geelong Osteoporosis Study. Eligible women with fracture (n=519) identified prospectively from radiology reports and non-fracture controls (n=970) were randomly selected from the electoral roll 1994-6. Symptoms of depression for both groups during the 12-month period 2000-1 were identified by self-report questionnaire based on the DSM-IV. Response rates were 57% and 61%, for women with fracture (n=296, median age 63.0yr, range 35-87) and women without fracture (n=590, median age 59.3yr, range 35-91), respectively.

Depression was identified in 29 women with and 67 women without fracture. Associations between fracture and depression differed for women <70yr and those ≥ 70yr. Unadjusted OR (95% CI) for depression following fracture were 0.60 (0.35-1.02, p=0.06) and 4.0 (1.32-12.11, p=0.01), respectively. Adjusting for age, weight, height and smoking status did not affect the OR significantly.

We acknowledge that both selection and response biases may have excluded frail women with concurrent comorbidities. These results indicate that among elderly women, risk of depression is increased after fracture. By contrast, among younger women, risk of depression is not increased and may be reduced. This possibly reflects a different response to fracture but could also indicate differences in pre-morbid state among younger women.

P71

FAMILIAL AND BEHAVIORAL FACTORS INFLUENCE THE DECISION TO UNDERGO A BONE MINERAL DENSITY SCAN

A Baheiraei[1], JA Eisman[1], NA Pocock[2] & TV Nguyen[1]
[1] *Bone and Mineral Research Program, Garvan Institute of Medical Research, St Vincent's Hospital, University of New South Wales, Sydney, NSW*

[2] *Nuclear Medicine, St Vincent's Hospital, University of New South Wales, Sydney, NSW*

Bone mineral density (BMD) is a primary predictor of fracture risk and a measurement of BMD plays a key role in the diagnosis and management of osteoporosis. However, several recent studies suggest that high-risk individuals are not being diagnosed or treated. The aim of the study was to investigate factors that could influence the decision to undergo a BMD measurement in a non-English speaking group within an English-speaking community.

The study was designed as a cross-sectional investigation, in which 131 Iranian- Australian women aged 35 years or older participated. Each woman completed a set of questionnaires from which data on socio-demographic, clinical characteristics and lifestyle factors were obtained. All women were offered a free BMD scan at the lumbar spine and femoral neck by DXA.

Only 90 women (or 69%) took up the offer to have a BMD measurement. Among these women, 13.3% were found to have osteoporosis. The following factors were found to be independent predictors of accepting a BMD scan: a familial history of osteoporosis (odds ratio [OR] 2.7; 95% CI 1.1-8.0), higher knowledge of osteoporosis (OR 1.3; 1.1-1.5), perception of susceptibility to osteoporosis (OR 1.1; 1.0-1.2), and surprisingly those with lower calcium intake (OR 0.4; 0.2-0.7) were less likely to accept the offer.

These findings suggest that familial history of osteoporosis was the strongest predictor of BMD scan decision. However, other behavioral factors such as knowledge and perception of osteoporosis also influence a woman's decision to undergo a BMD measurement. These findings should help the development of multilinguinal educational programs designed to overcome the underdiagnosis and undertreatment in osteoporosis.

P72

PARADIGM OF CARTILAGE REGENERATION BY MATRIX-INDUCED AUTOLOGOUS CHONDROCYTE IMPLANTATION (MACI): A HISTOLOGICAL ASSESSMENT

C Willers[1], M-H Zheng[1] & L Kirilak[1]
[1] *School of Surgery and Pathology (Orthopaedics), University of Western Australia, WA*

Conventional treatment regimes for articular cartilage injury only manage biomechanically inferior tissue comprised mainly of fibrocartilage. The development of autologous chondrocyte implantation (periosteal ACI) has seen improvements in patients outcomes over conventional therapy, but complications associated with periosteum has led to the search for alternative scaffolds for the seeding of autologous chondrocytes. Matrix-induced autologous chondrocyte implantation (MACI) presents arguably the best alternative to conventional ACI by obviating many of PACI's inherent limitations. We have conducted an objective assessment of MACI patients by histological examination, and assessed the integration and phenotypic maintenance of chondrocytes seeded onto the type I/III collagen membrane used in MACI. Ten biopsies were analysed at 48 hours, 21 days, 6, 8, 12, and 24 months postoperatively. Scanning electron microscopy and RT-PCR confirmed ACI-Maix type I/III collagen membrane efficiently integrates chondrocytes into its matrix and maintains the chondrolineage phenotype (aggrecan and collagen II expression). Results of sequential histology and collagen II staining at the six time points showed that MACI induces the regeneration of cartilage-like tissue as early as 21 days, with hyaline-like cartilage formed at 6 months. In summary, we have shown that MACI is a reliable cell-based treatment for the regeneration of articular cartilage defects of the knee.

P73

RELATIONSHIP BETWEEN PROGRESSION OF OSTEOLYSIS ADJACENT TO HIP PROSTHESES AND POLYETHYLENE WEAR

R Stamenkov, S Neale, M McGee, D Findlay[1], J Taylor[2], G Kourlis[2], S Pannach & D Howie[1]
Department of Orthopaedics and Trauma, Royal Adelaide Hospital and University of Adelaide, [1] Hanson Institute, and [2] Department of Radiology, Royal Adelaide Hospital, SA

Introduction:
Knowledge of the extent and progression of peri-prosthetic osteolysis (PPO) is important in monitoring and surgical management of total joint replacement patients, and to determine effects of medical treatments. The aim of this study was to use quantitative computed tomography (CT), with wear and migration analyses, to determine the rate of progression of PPO and its relationship to wear of the polyethylene (PE) cup.

Methods:
PPO adjacent to 19 well-fixed Harris-Galante acetabular components was measured at 12-month intervals. Migration analyses were used to exclude cases with migrated components. Volumetric PE wear was determined from digitised X-rays using the Polyware software program. Patient-related factors including age, gender, BMI, activity levels, comorbidities, and joint pain and function were recorded from our Joint Replacement Database.

Results:
Lesions in many of these patients were relatively quiescent, while others progressed markedly over a one year period. There was a significant association between progression of osteolysis and total PE wear, PE wear rate and volumetric PE wear in the 12 month period between CT scans (p=0.026, p=0.025 and p=0.035, respectively). None of the other covariates examined was significantly associated with the progression of osteolysis.

Conclusions:
CT measurement of PPO progression and accurate estimates of cup wear and stability provide important information to guide clinical management of total hip replacement patients. The data support the involvement of PE particles in bone destruction in these individuals.

P74

CHARACTERISATION OF RANK EXPRESSING CELLS IN MARROW AND PERIPHERAL BLOOD: A MONOCLONAL ANTIBODY STUDY

GJ Atkins[1], C Vincent*[1], P Kostakis*[2], AN Farrugia*[2], DM Findlay[1], A Evdokiou*[1], ACW Zannettino*[2]
[1] *Orthopaedics, University of Adelaide, Adelaide, SA*
[2] *Division of Haematology, Institute of Medical and Veterinary Science, Adelaide, SA*

The expression of RANK has been shown to be a prerequisite in order for osteoclast precursors (preOC) to differentiate into functional osteoclasts (OC). Although attributed to the monocytoid lineage, the true nature of the preOC *in vivo* has remained enigmatic. We tested a panel of murine monoclonal antibodies raised against full-length recombinant human RANK, for reactivity with normal human peripheral blood and bone marrow mononuclear cells (PBMC and BMMC, respectively) and Giant Cell Tumour cells (GCT). RANK immunoreactivity was observed with $CD19^+$ (B-cells), $CD56^+CD3^+$ (NK cells) and $CD14^+$ (monocytes) subpopulations of BMMC. Similar reactivity was observed in PBMC. Positive selection by magnet activated cell sorting (MACS) yielded a distinctive population of viable cells. However, exposure of $RANK^+$ cells to recombinant RANKL/M-CSF did not yield functional OC. In contrast, positive selection of $CD14^+$ cells yielded a population of monocytes that readily differentiated into OC. A possible explanation for the failure of $RANK^+CD14^+$ cells to differentiate into OC may be antagonism between the RANK MAb used and recombinant RANKL, for RANK receptors on these cells. Consistent with this, we observed differential effects of RANK MAbs when added to OC-forming assays from $CD14^+$ PBMC precursors; one MAb, designated R24, profoundly inhibited OC formation, whereas others did not. Furthermore, R24 inhibited the resorbing activity of GCT cells in a dose-dependent manner. Our results indicate that RANK+ preOC may be represented in the marrow and circulate in the periphery. However, positive selection of preOC using RANK MAbs may developmentally and functionally inhibit these cells.

Disclosures:
1

P75

ROLE OF RHO GTPASE EFFECTOR PROTEIN, RHOTEKIN-2, IN OSTEOCLASTOGENESIS

E Clark, FM Collier, JM Hodge, MA Kirkland & GC Nicholson
The University of Melbourne, Department of Clinical and Biomedical Sciences: Barwon Health, Geelong, VIC

Rhotekin-2 (RTKN-2) is a recently described Rho GTPase effector protein of unknown function that is highly expressed in haemopoietic cells. Rho is involved in the regulation of the osteoclast cytoskeleton. We wished to determine whether RTKN-2 is regulated during osteoclastogenesis and whether activation of Rho pathways modulates osteoclast differentiation.

Human osteoclasts were generated by incubating CFU-GM with hM-CSF (25 ng/mL) and sRANKL (125 ng/mL) for 14 days; while macrophages were generated in the presence of M-CSF alone. RTKN-2 mRNA expression (normalised to GAPDH) was quantified using real-time PCR.

Expression of RTKN-2 mRNA was abundant in CFU-GM and declined during both macrophage and osteoclast differentiation. However, at 2, 4 and 7d expression tended to be less in the osteoclastogenesis cultures. At 14d RTKN-2 mRNA expression was 73% less in mature osteoclasts than in macrophages (p=0.05).

Co-treatment of CFU-GM osteoclastogenesis cultures with lysophosphatidic acid (LPA, 6.25 nM - 4 μM), a Rho signaling pathway activator, caused potent inhibition of osteoclastogensis at the lowest concentration used. Dentine resorption was also inhibited.

Our results indicate that RTKN-2 is differentially regulated during osteoclast versus macrophage differentiation and that low expression of this Rho effector protein might be required for the osteoclast phenotype. Inhibition of osteoclastogenesis by LPA, a Rho activator, is consistent with the Rho signaling pathway being inhibitory of osteoclast differentiation.

P76

MORPHOMETRIC ASSESSMENT OF MICRODAMAGE ACCUMULATION AND BONE RESORPTION IN FRAGILITY HIP FRACTURE PATIENTS

JS Kuliwaba[1,2], MR Forwood[3] & NL Fazzalari[1,2]
[1] *Bone & Joint Research Lab, Div of Tissue Pathology, IMVS & Hanson Institute,* [2] *Dept of Pathology, Uni of Adelaide, Adelaide, SA*
[3] *Dept of Anat & Developmental Biology, Uni of Queensland, Brisbane, QLD*

Unrepaired microdamage (Mdx) accumulates, resulting in decreased stiffness, strength and toughness of bone; however, the role of Mdx in fracture risk remains unclear. This study assessed Mdx accumulation and repair (bone resorption) in femoral trabecular bone from patients with a fragility hip fracture (Fx) compared to age- and sex-matched controls (C). Intertrochanteric bone cores were obtained from patients undergoing hip arthroplasty surgery for a fractured neck of femur (6f, 2m, aged 82±5 yrs [mean±SD]), and from controls at autopsy (6f, 2m, aged 79±7 yrs). Samples were *en bloc*-stained in basic fuchsin, resin-embedded, and *in vivo* Mdx identified in 70□m sections. Morphometric parameters were measured using a semi-automated digitising system. Trabecular bone volume, architecture, and indices of bone resorption were not different between the fracture and control groups (BV/TV[%]: Fx:4.7±2.3, C:5.8±2.3, p=NS). Linear microcrack density and crack length were similar between groups. However, diffuse damage density was increased in bone from fracture patients compared to controls (Df.Dn[#/mm^2]: Fx:1.61(0.18-2.81), C:0(0-0.58), p<0.05 [median(quartiles)]). Although the ratio of Mdx (cracks and diffuse) density to resorption site density was not statistically different between groups (Mdx.Dn/Rs.Dn: Fx:0.27(0.07-0.79), C:0(0-0.35), p=0.08), the data are suggestive of an unrepaired Mdx burden in the fracture group. The increased diffuse Mdx burden, with no bone architectural change, suggests that bone from hip fracture patients may be mechanically compromised due to defective damage repair mechanisms. Better understanding of the mechanisms by which bones are less likely to fracture will enable better targeting of drug therapy to individuals at risk of fragility fracture.

Disclosures:
1

P77

DIFFERENTIAL GENE EXPRESSION OF BONE ANABOLIC FACTORS AND TRABECULAR BONE ARCHITECTURAL CHANGES AT A DISTAL SKELETAL SITE IN PRIMARY HIP OSTEOARTHRITIS

JS Kuliwaba[1,2], L Truong[1,2], H Tsangari[1], DM Findlay[3] & NL Fazzalari[1,2]
[1] *Bone & Joint Research Lab, Div of Tissue Pathology, IMVS & Hanson Institute, Depts of* [2] *Pathology and* [3] *Orthopaedics & Trauma, Uni of Adelaide, Adelaide, SA*

Osteoarthritic (OA) subchondral bone is sclerotic, yet mechanically weak due to hypomineralisation, increased collagen metabolism, and the presence of collagen type-I homotrimer. This study examined whether gene expression of bone anabolic factors and trabecular bone architecture and turnover are altered at a distal femoral site in primary hip OA patients compared to age- and sex-matched controls. Intertrochanteric (IT) bone cores were obtained from 16 primary OA patients at hip arthroplasty surgery (8f, 8m, mean age 65 [48-85] yrs) and 16 non-OA controls at autopsy (8f, 8m, 64 [44-85] yrs). Samples were processed for RNA isolation and undecalcified bone histology. Semi-quantitative RT-PCR analysis revealed elevated mRNA expression levels of alkaline phosphatase (p<0.004), osteocalcin (p<0.004), and the collagen type-I genes COL1A1 (p<0.0001) and COL1A2 (p<0.002) in OA bone compared to control, suggesting increased osteoblastic activity and/or bone turnover at the IT region in OA. However, histomorphometric indices of bone turnover did not differ between groups. Interestingly, the ratio of COL1A1:COL1A2 mRNA was 2-fold greater in OA bone compared to control (p<0.0001), suggesting a possible presence of collagen type-I homotrimer at the distal site. Osteopontin, IGF-I, IGF-II, and TGF-β1 mRNA levels were similar between groups. OA and control IT bone was architecturally distinct; OA bone had increased surface density of bone (p<0.004), decreased trabecular separation (p<0.004), and increased trabecular number (p<0.004). The differential gene expression and trabecular architectural changes observed at a distal skeletal site in OA implicate the generalised involvement of bone in the pathogenesis of OA.

Disclosures:
1

P78

ALENDRONATE ALONE OR IN COMBINATION WITH RALOXIFENE PRODUCES A GREATER EFFECT THAN RALOXIFENE ALONE ON BONE TURNOVER MARKERS IN POSTMENOPAUSAL WOMEN WITH LOW BONE DENSITY AND PAST FRACTURE

H Wilson[1], H Zhou[1], R Yao[1], S Vasikaran[2], GN Kent[3] & RK Will[1]
[1] *Metabolic Bone and Musculoskeletal Diseases Research Unit, and* [2] *Clinical Biochemistry Royal Perth Hospital and* [3] *PathCentre Perth, WA*

Aims:
Alendronate (ALN) and Raloxifene (RLX) are both used in the therapy of osteoporosis in postmenopausal women. A recent study has shown that ALN and RXL in combination decreased bone turnover more than either drug alone[1]. Our study compares the efficacy of AXL and RXL alone or in combination on biochemical markers of bone turnover in postmenopausal women with osteoporosis.

Method:
A population of 166 calcium and vitamin D replete postmenopausal women with osteoporosis based on femoral neck or lumbar spine bone mineral density) was recruited. The women were at least 6 months beyond the menopause with a history of at least one past minimally traumatic fracture. Patients were randomised to ALN 70mg/week, RXL 60mg/day or both, and bone turnover was measured at 6 months. ^{45}Ca intestinal absorption was measured as previously described[2] .

Results:
Data is complete for 100 of the 166 patients. The ALN and ALN+RXL groups showed a significant decrease in urinary N-telopeptide (NTx) and serum ionised calcium at 6-months ($p<0.001$), however there was no change in the RXL group. All three groups experienced a significant decrease in Alkaline Phosphatase ($p<0.0001$) however there was no difference between ALN and ALN+RXL groups.

Urinary calcium/creatinine ratio significantly decreased from baseline to 6 month in ALN+RXL group ($p<0.001$), however ALN and RXL did not change. No significant changes in ^{45}Ca absorption were observed any group over time but we found a significant correlation between ^{45}Ca and urinary calcium/creatinine ratio at baseline ($r=0.365$, $p<0.0001$).

Conclusion:
Treatment with ALN, alone or in combination with RXL produced significant decrease in bone turnover markers in postmenopausal women with low bone density and a prevalent fracture in contrast to RXL alone. We found no significant difference between the ALN and ALN+RXL groups on bone turnover markers.

1. O. Johnnell, et al. 2002. J Clin Endocrinol Metab. 87(3): 985-992
2. Nordin BEC, et al. 1998. J Nucl Med. 39:108-13.

P79

BONE DENSITY IS INCREASED IN POSTMENOPAUSAL WOMEN WITH LOW BONE DENSITY AND PAST FRACTURE BY ALENDRONATE ALONE OR IN COMBINATION WITH RALOXIFENE, BUT NOT WITH RALOXIFENE ALONE

R Yao[1], H Wilson[1], H Zhou[1], GN Kent[2], G Roff[3] & RK Will[1]
[1] *Metabolic Bone and Musculoskeletal Disease Research Unit, RPH, Perth, WA*
[2] *PathCentre, Nedlands, WA*
[3] *Nuclear Medicine Department, RPH, Perth, WA*

Aims:
Antiresorptive agents Alendronate (ALN) and Raloxifene (RLX) can both be used in the treatment of osteoporosis in postmenopausal women. A recent study has shown that ALN and RXL in combination increased bone mineral density (BMD) more than either drug alone (1). Our study compares the efficacy of ALN and RXL alone or in combination on BMD change in postmenopausal women with osteoporosis with a prior prevalent minimal trauma fracture.

Method:
A population of 164 postmenopausal women with low bone density based on hip or lumbar spine BMD (T-score $\leq$-2.5) was recruited for the study. The women were at least 6 months beyond menopause with a history of at least one past minimally traumatic fracture. Patients were randomised to ALN 70mg/week, RXL 60mg/day or both. BMD was measured at baseline and at 12-month by dual x-ray absorptiometry (DXA).

Results:
In the 61 patients who have had 12 month DXA assessments, both the ALN and ALN+RLX groups demonstrated statistically significant increases in BMD, whilst the BMD increment in the RLX group was not significant at any site (Table). BMD changes were not significantly different between the ALN and ALN+RLX groups at all sites.

Conclusions:

Antiresorptive therapy including ALN increased BMD in postmenopausal women with low bone density and past fracture. ALN in combination with RLX was no more effective than ALN alone. RLX did not significantly increase BMD in this 12-month study.

Table. Percentage changes in BMD from baseline to 12 months [a]

	ALN (n=19)	RLX (n=20)	ALN+RLX (n=22)
Lumbar spine BMD	3.1±0.7[b]	1.2±1.0	4.2±0.6[b,c]
Femoral trochanter BMD	1.8±0.7[b]	1.1±0.8	3.8±0.8[b]
Total hip BMD	1.4±0.7[b]	1.0±0.8	2.6±0.7[b]

[a]BMD values are mean percentage change ± SEM, [b]Statistically significantly different from baseline ($p<0.05$), [c]Statistically significantly different from RLX ($p<0.001$).

1. O. Johnnell, et al. 2002. J Clin Endocrinol Metab. 87: 985-92

P80

FGF-8 IS ANABOLIC TO BONE

J-M Lin[1], M Watson[1], K Callon[1], J-S Lin[2], A Grey[1], D Naot[1], IR Reid[1] & J Cornish[1]
[1] *Department of Medicine, University of Auckland, Auckland, New Zealand*
[2] *AgResearch, Wallaceville, Upper Hutt, New Zealand*

Fibroblast growth factor (FGF) is a group of structurally-related peptides, involved in bone metabolism. FGF-8 was reported to affect fetal skeletal development and we have investigated its effects on bone cells. Recombinant murine FGF-8 at concentrations ranging from 0.05 - 500 ng/mL had diverse effects on the bone cells: potently stimulating primary rat and human osteoblast proliferation (2-fold), inhibiting osteoblast differentiation by 12% in bone nodule assays and moderately inhibiting osteoclast formation in mouse bone marrow cultures by 15%. FGF-8 does not act directly on osteoclasts as indicated by results from mature isolated osteoclast assays and bone organ culture.

In addition, we have elucidated mechanisms by which FGF-8 exerts its effects on these cells. In osteoblasts, FGF-8 appears to activate MAP kinase signalling as FGF8-induced osteoblast proliferation is blocked by a specific inhibitor of MAP kinase, U-0126. We determined if FGF-8 regulation of osteoclastogenesis was dependent on the RANKL/OPG pathway. The expression levels of RANKL and OPG from mouse bone marrow cultures, using real-time PCR, showed that FGF-8 at concentrations of 5 and 25 ng/mL did not significantly change OPG levels, however there was a significant increase in RANKL at 25 ng/mL and ratios of RANKL/OPG at both 5 and 25 ng/mL were increased. These data suggest that FGF-8's inhibitory effect on osteoclast formation is independent of the RANKL/OPG pathway.

In conclusion, our studies thus far indicate that FGF-8 has effects on bone cells and is indeed another member of the FGF family that is involved in regulating bone metabolism.

Disclosures:
1. No conflict of interest
2. No shares in company
3. No paid consultancy
4. No company financial support
5. No further information

P81

FASLODEX AND TAMOXIFEN INHIBIT 1,25D-INDUCED CYP24 EXPRESSION IN UMR-106 OSTEOBLASTS

M Leong, P Dwivedi, B May, H Morris & P O'Loughlin
Division of Clinical Biochemistry and Hanson Institute, Institute of Medical & Veterinary Science, Adelaide, SA and School of Molecular and Biomedical Science, University of Adelaide, Adelaide, SA

In vitamin D responsive tissues, 1,25-dihydroxyvitamin D (1,25D) regulates its intracellular concentration by inducing its catabolic enzyme, cytochrome P450-24 (CYP24). Maximal induction of the CYP24 gene involves both genomic and non-genomic signalling pathways. Similar non-genomic signals are illicited by estrogens. We have demonstrated in bone cells that 17β-estradiol (E_2) inhibits 1,25D induction of CYP24 by a mechanism that involves the Ets binding site (EBS) within the CYP24 promoter. We investigated the possible estrogen-like action of 2 selective estrogen receptor modulators (SERMs) on 1,25D-induced CYP24 expression in UMR106 osteoblast cells. UMR106 cells were transfected with a 298 base pair CYP24 promoter/luciferase reporter and treated with 1,25D, E_2, faslodex (ICI182780)

or tamoxifen alone or the combined treatment of 1,25D with either E_2 or SERM. The construct was induced by 1,25D treatment 37.4 ± 5.8 fold above the control ($p<0.05$). E2, faslodex and tamoxifen alone had no effect on the level of expression of construct. Co-treatment with 1,25D and either E_2, Faslodex or Tamoxifen suppressed 1,25D induction of the construct by 21 % ($p<0.05$), 53 % ($p<0.05$), 100 % ($p<0.05$) respectively. We conclude that the SERMs faslodex and tamoxifen have the ability to inhibit 1,25D induction of CYP24 in UMR106 osteoblast cells.

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FRACTURE RISK AND BMD: THE ODDS RATIO FALLACY

C Nordin[1,2], R Prince[3], I Dick [3], C Pearce[4] & P Baghurst[5]
[1] *Institute of Medical and Veterinary Science, Adelaide, SA*
[2] *Royal Adelaide Hospital, Adelaide, SA*
[3] *Sir Charles Gairdner Hospital, Perth, WA*
[4] *School of Mathematical Sciences, University of Adelaide, Adelaide, SA*
[5] *Public Health Research Unit, Women's and Children's Hospital, Adelaide, SA*

It is standard practice in the bone literature to use Logistic Regression (LR) to calculate the relation between fracture risk and bone density (BMD) and to describe the resultant Odds Ratio (OR) as a Relative Risk (RR). The OR usually lies in the range 1.5 to 2.5 per SD of BMD [1,2] and when equated with RR appears to take the Fracture Probability over 1 at low bone densities which is clearly absurd. This anomaly is due to a failure to appreciate that, even at low values, OR is only an approximation to RR, just as Odds are only an approximation to Risk; the Odds must be divided by 1+Odds to yield the true Probability or Risk. The difference between Odds and Probability is shown in the Table which relates clinical fractures to total hip BMD in 1,128 normal women of mean age 75 followed for up to 5 years as part of a calcium trial. The best fit of the Odds of any clinical fracture to BMD by LR was a multiplicative factor of 1.41 per unit fall in T score with an intercept of 0.084 at a T score of zero, yielding the Odds and Probability values shown below:

T score	-4	-3	-2	-1	0	1	2	
Fracture Odds	0.338	0.240	0.170	0.121	0.085	0.061	0.043	
Fracture Probability		0.254	0.194	0.145	0.107	0.078	0.057	0.041

When going from a T score of 2 to a T score of -4, the Fracture Odds rise by a factor of 7.9 but the Fracture Probability rises by a factor of only 6.2. This may seem a small difference but at higher ORs or lower T scores, the Fracture Odds soon exceed unity whereas the Probabilities always remains below 1, as they have to do; Odds are not the same as Risk.

We conclude that the current mode of relating fracture risk to BMD by equating OR with RR is seriously misleading.

1. Kanis JA, et al. (2001) Osteoporosis Int 12:989-95.
2. Stone KI, et al. (2003) J Bone Miner Res 18:1947-54.

Disclosure:
1

P83

REGIONAL AND TOTAL BODY BONE MINERAL DENSITY IN PATIENTS WITH NEUROFIBROMATOSIS-1

S Dulai, J Briody, DG Little, K North & R Howman-Giles
The Children's Hospital at Westmead, Sydney, NSW

Aim:
To evaluate regional and total body bone mineral density (BMD) in paediatric patients with Neurofibromatosis-1 (NF1).

Methods:
A cross-sectional cohort was recruited from children presenting at our institution's NF1 clinic. Subjects without a history of orthopaedic intervention, or treatment with medications known to alter BMD, had BMD of the total body, lumbar spine and both femoral necks measured using Dual energy X-ray Absorptiometry (Lunar Prodigy).

Results:

There were 23 patients (10 Males), which gives a power of >90% to detect a difference of 1 Z-score. Mean age was 10.8 years (5.8-17.2), height SDS was -0.8 (-4.8-1.8, p=0.01), weight SDS was -0.3 (-2.6-1.7) and BMI SDS was 0 (-4.1-1.7)

All regions had Z-scores significantly below zero (Table 1). There was also a significant within-patient difference in femoral necks (0.4±0.3, p<0.001), and a reduction in total body Z-score with age (p=0.03).

Table 1: Age and sex matched BMD Z-scores

Region	Mean	Std. Deviation	p
Total body	-0.83	1.08	0.001
Arms	-0.83	1.22	0.004
Legs	-0.73	0.81	<0.001
Lumbar spine	-0.64	1.09	0.009
Right femoral neck	-0.62	0.90	0.005
Left femoral neck	-0.72	1.10	0.007

Conclusions:
Results suggest that children with NF1 may have a tendency towards an osteopenic bone phenotype. Results also suggest that osteopenia in NF1 may become more severe with age, which we plan to test in a longitudinal study.

P84

EVALUATION OF NATURAL MARINE SPONGES AS POTENTIAL BIOSCAFFOLDS FOR THE ATTACHMENT, PROLIFERATION AND DIFFERENTIATION OF OSTEOBLASTIC CELLS

KL Solomon[1,2], C Willers[1], MA Borowitzka[2], J Xu, MH Zheng[1] & NJ Pavlos[1]
[1] *Unit of Orthopaedics, School of Surgery and Pathology, University of Western Australia, Nedlands, Perth, WA*
[2] *Biological Science Division, School of Biological Sciences and Biotechnology, Murdoch University, Perth, WA*

The use of bioscaffolds and cell constructs to support cell growth and tissue regeneration is becoming an ever increasing practice in orthopaedic surgery. However, the donor availability and the number of scaffolds suitable for the treatment of osseous injury remains limited. In search of potential bioscaffolds for cell-based bone tissue regeneration we have evaluated the use of natural marine sponges to support the growth and differentiation of osteoblastic cells *in vitro*. For this purpose, 5 unidentified sponge species of genus *Hippospongia* (n=1), *Callyspongia* (n=3) and family Chalinidae (n=1) were selected as candidate scaffolds based on i) hydration potential, ii) fiber matrix architecture, and iii) collagenous composition of spongin fibers. Primary osteoblastic cells seeded onto devitalised sponge matrices were assessed for their ability to attach to, invade, and proliferate in each sponge type using a combination of light, confocal and scanning electron microscopy. In short term cultures (7-days), cellular attachment was observed on all 5 species with cells often aligning along the longitudinal axis of sponge fibers. At 14-days, increased cellular invasion and proliferation was apparent, with osteoblastic cells displaying signs of early-phase matrix deposition. By 21-days culture, osteoblastic cells were found to completely bridge interconnecting spongin-fiber pores, with total encapsulation of sponge skeletons observed in some species. Importantly, the osteoblastic phenotype of these cells was confirmed by staining for alkaline phosphatase. Together with preliminary biocompatibility studies our data indicate that natural marine sponge skeletons may offer a potential new source of bioscaffold for the repair of bone injury.

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DETERMINANTS OF FOREARM BONE MASS IN CHINESE CHILDREN WITH DIFFERENT NATIONALITIES

Q Zhang[1,2], X Hu[1], B Zhang[1], B Cui[1], J Zhang[1] & G Ma[1]
[1]*Institute for Nutrition and Food Safety, Chinese Centre for Disease Control and Prevention, Beijing, China*
[2]*The Faculty of Veterinary Science, University of Sydney, Sydney, NSW*

Objective:
To evaluate the relationship between forearm bone mass and physical development and pubertal development in Chinese children with Han, Tibet and Qiang nationalities.

Methods:
Total 486 Han (243 boys, 243 girls), 662 Tibetan (332 boys, 340 girls), and 748 Qiang (379 boys, 375 girls) nationality children aged 7 to 17 yrs were studied in two counties of Sichuan Province, China. Puberty development was determined by Tanner stage. The forearm bone mass were measured using Norland peripheral dual energy x-ray bone densitometer (pDEXA).

Result:
The variability in nationality, bone area, age, height, weight, Tanner stage of pubic hair development and emission history for boys (or Tanner stage of breast development for girls) accounted for 61.5%-86.8% of the variation of distal forearm bone mineral density(DFBMD) and proximal forearm bone mineral density (PFBMD) in corresponding gender. Among that, nationality explained 0.8% - 4.0% the variability of DFBMD and PFBMD in each gender. Controlled for the bone area, age, physical and puberty development, DFBMD in Tibetan boys, DFBMD and PFBMD in Tibetan girls were 5.7%, 5.6% and 1.9%, respectively, higher than that of their Han counterparts (p<0.01).

Conclusion:
Besides age, physical development, puberty development and bone area, nationality is one of determinations of forearm bone mineral density in Chinese children.

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THE BONE MASS DEVELOPMENT OF CHILDREN WITH DIFFERENT NATIONALITIES IN CHINA

X Hu, Q Zhang, B Zhang, B Cui, J Zhang & G Ma
Institute for Nutrition and Food Safety, Chinese Centre for Disease Control and Prevention, Beijing, China

Objective:
To investigate the bone mass development of children with Han, Tibet and Qiang nationalities in China.

Methods:
Total 486 Han (243 boys, 243 girls), 662 Tibetan (332 boys, 340 girls), and 748 Qiang (379 boys, 375 girls) nationality children aged 7 to 17 yrs were studied in two counties of Sichuan Province, China. Their forearm bone mass were measured using Norland peripheral dual energy x-ray bone densitometer (pDEXA).

Result:
Their average age is about 11.7yr, 12.5 yr and 12.1 yr for Han Majority, Tibetans, and Qiang nationality. Bone mineral content (BMC) and bone area (BA) in proximal forearm and distal forearm of Tibetan was significantly higher than those of Han counterparts after adjusted for age, gender, height and weight.

Conclusion:
The bone mass development of children varies in different nationality.

Table Physical development and bone mass in different minority of China (Mean±SD)

	Han Majority		Tibetans		Qiang Minority		*P*
	Mean± SD	adj.	Mean ±SD	adj.	Mean± SD	adj.	
Proximal radius							
BMC(g)	0.613±0.215	0.647[a]	0.697±0.256	0.665[b]	0.648±0.236	0.653[a]	0.001
BA(cm^2)	1.128±0.148	1.146[a]	1.219±0.147	1.201[b]	1.164±0.150	1.168[c]	<0.001
BMD(g/cm^2)	0.532±0.129	0.552	0.559±0.156	0.541	0.544±0.143	0.547	0.103
Distal forearm							
BMC(g)	0.876±0.357	0.928[a]	1.047±0.434	1.001[b]	0.942±0.404	0.949[a]	<0.001
BA(cm^2)	3.082±0.569	3.165[a]	3.286±0.627	3.211[b]	3.200±0.633	3.212[b]	0.008
BMD(g/cm^2)	0.275±0.064	0.283[a]	0.308±0.075	0.300[b]	0.284±0.069	0.285[a]	<0.001

Adj: control for age, gender, height and weight.

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LRP5 GENE POLYMORPHISMS ARE ASSOCIATED WITH OSTEOPOROSIS IN MEN

A Koay[1], S Yeung[2], MA Brown[1] & PR Ebeling[2,3]
[1] Botnar Research Centre, Institute of Musculoskeletal Sciences, Oxford University, Headington UK
Departments of [2] Diabetes and Endocrinology, and [3] Medicine, The University of Melbourne, The Royal Melbourne Hospital, Parkville, VIC

Aim:
Genetic factors underlying BMD operate in a sex-specific manner. We proposed that *LRP5* gene polymorphisms modulate BMD and fracture risk in men.

Methods:
Two cohorts were recruited. The first cohort comprised men with spinal fragility fractures (n = 78, age 22-88 years) and healthy, fracture-naïve men (n=65, age 21-80 years). The second cohort comprised 56 families of men with fragility fractures. Lumbar spine, proximal femur and total body BMD and total body bone mineral content (TBBMC) were measured by DXA. *LRP5* allele, genotype and haplotype frequencies in control and fracture populations were compared using contingency table testing and the χ^2 statistic. Within- and between-family association between *LRP5* gene polymorphisms and bone mass indices were assessed by QTDT.

Results:
LRP5 allele, genotype or haplotypes were expressed at similar frequencies in men with and without fragility fractures. QTDT analysis of families of osteoporotic men with fractures identified association between the coding C135242T (F549F) polymorphism in exon 8 and FN BMD Z-score (p = 0.026). Restricting analysis to men did not abolish association (p = 0.048). Height was associated with the C165215T (A1330V) *LRP5* polymorphism in exon 18 (p = 0.062 in the entire family cohort, p = 0.092 men-only analysis). There was no linkage between *LRP5* polymorphisms and bone mass or height.

Conclusions:
Positive associations between the F549F polymorphism and femoral neck BMD, and the A1330V polymorphism and height, were noted in families of men with fragility fractures. No single *LRP5* polymorphism was predictive of fracture risk. This study confirms *LRP5* gene polymorphisms modulate BMD in men.

Disclosures:
None

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ARE THE WHO CLASSIFICATIONS FOR OSTEOPOROSIS IN WOMEN APPROPRIATE FOR MEN?

MJ Henry, JA Pasco, GC Nicholson, MA Kotowicz
The University of Melbourne, Dept Clinical & Biomedical Sciences: Barwon Health, Geelong, VIC

The WHO has defined osteoporosis in postmenopausal women as BMD more than 2.5SD below the young adult reference mean. Osteopenia has been classified between a T-score of -1.0 and -2.5 and normal above -1.0. Whether these thresholds are appropriate for men remains unclear.

An age-stratified random sample of men within the Barwon Statistical Division is currently being recruited for the Geelong Osteoporosis Study with interim data reported here (n = 1158, age 20-96yr). BMD was assessed at the femoral neck using a Lunar densitometer and T-scores were calculated using the mean and SD of men aged 20-29yr. Self-reported low trauma adult fractures have been recorded excluding those from falls greater than standing height and motor vehicle accidents (FRAC). The proportion (%) of the male population that fall into the WHO classifications for females and the pattern of FRAC across various age-groups are tabulated.

		Normal T >-1.0 (%)		Osteopenia -2.5<T≤-1.0 (%)		Osteoporosis T ≤-2.5 (%)	
Age(yr)	n	All	FRAC	All	FRAC	All	FRAC
60-64	83	46	16	50	14	4	1
65-69	72	33	8	59	10	8	4
70-79	195	30	4	58	17	13	5
80+	131	23	6	57	10	20	6

Most prevalent fractures are found in men with T >-2.5. Few men have 'osteoporosis' and fracture. This may in part be related to a survival effect. Nevertheless, the proportions of fractures do increase with lower BMD amongst the elderly. Large population-based studies will help address the appropriateness of the WHO classification for defining 'osteoporosis' in men.

P91

CALCIUM SENSING RECEPTOR PROPERTIES IN OSTEOBLASTIC CELLS

S Atwa[1], N Lewis[2], TC Brennan[1], AD Conigrave[2] & RS Mason[1]
School Of Medical Sciences and Institute for Biomedical Research[1], School of Molecular and Microbial Biosciences [2], University of Sydney, NSW

We previously demonstrated the calcium-sensing receptor (CaR) on osteoblastic cells and showed that Ca^{2+} (0.5–3 mM) and other CaR activators Gd^{3+}, Mg^{2+} and Al^{3+} significantly stimulated proliferation of MG63 cells and that Ca^{2+} and Gd^{3+} significantly suppressed the RANK-L:OPG expression ratio. The current study focuses on the effects of selective CaR activators, and activators and inhibitors of the related metabotropic glutamate receptors (mGluR). The specific CaR calcimimetic R-467 stereoselectively enhanced Ca^{2+}-dependent suppression of RANK-L: OPG but paradoxically suppressed the Ca^{2+}-dependent increase in cell proliferation. Further, Ca^{2+} (0.5–3 mM) dose-dependently increased alkaline phosphatase activity up to 6 fold, but this was stereoselectively suppressed by R467. This may suggest that Ca^{2+} and R467 both act through the CaR but activate distinct signalling pathways. Gd^{3+} and R467 enhanced Ca^{2+} -induced activation of ERK1/2 mitogen-activated protein kinase within 5 mins in CaR-expressing HEK293 cells, while no consistent response to Ca^{2+} was seen in osteoblastic MG-63 cells over 5min-24h. None of three mGluR antagonists tested, AIDA, (group I), LY341495, (group II and III) CGGP, (group III), nor ACPD, agonist for groups II and I, had any effect on Ca^{2+}-dependent proliferation. In contrast, DCG IV (100 μM), agonist of mGluR group II, significantly inhibited Ca^{2+}-dependent increase in cell proliferation ($P< 0.01$). Overall, the results demonstrate a role for the CaR in the regulation of RANKL and OPG and may indicate a role for proliferation and differentiation if Ca^{2+} and R467 activated signaling pathways are distinct. There is no evidence of a role for mGluRs in mediating Ca^{2+}-dependent effects on osteoblastic cells but indicate instead that mGluRs may suppress osteoblastic cell proliferation.

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CONTRIBUTION OF MYELOMA CELL PRODUCED RANKL TO BONE LOSS IN MULTIPLE MYELOMA

R Barnett, Q Lam, JW Sentry & H-G Schneider
Alfred Pathology Service, Alfred Hospital, Melbourne, VIC

Multiple myeloma (MM), a common haematological malignancy, is currently incurable with patient median survival of about 3 years, and accounts for approximately 2% of cancer deaths. MM increases bone loss by disrupting the checks that normally control signalling by receptor activator of nuclear factor kappaB ligand (RANKL, also known as, TNFSF11). RANKL is required for osteoclast differentiation and activation. RANKL binds to its functional receptor RANK (also known as TNFRSF11A) to stimulate osteoclastogenesis. Osteotropic cytokines regulate this process by controlling bone marrow stromal expression of RANKL. Further control over osteoclastogenesis is maintained by regulated expression of osteoprotegerin (OPG, TNFRSF11B), a soluble decoy receptor for RANKL. In normal bone marrow, abundant stores of OPG in stroma, megakaryocytes, and myeloid cells provide a natural buffer against increased RANKL. In multiple myeloma the disruption of the balance between RANKL and OPG is reported to occur as a consequence of the interaction of myeloma cells with stroma and other cell types triggering increased osteoclastogenesis. More recently we and others have provided clear evidence that myeloma cells can directly contribute to the pool of RANKL in bone. To investigate the contribution of myeloma cell production of RANKL to bone loss in MM we have constructed siRNAs to knock down mRNAs encoding RANKL in myeloma cells. We are validating the efficiency of these constructs in myeloma cell lines and will test these constructs following transfection into primary human myeloma cell isolates for use in *in vitro* culture systems, and also in the 5TMM myeloma mouse model.

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CLINICAL EXPERIENCE WITH 2 YEARS OF ZOLEDRONIC ACID IN OSTEOPOROSIS

D Hughes[1], B White[1], B Crawford[123] & M Hooper[123]
Endocrinology[1], Concord and RPA Hospitals[2] and The University of Sydney[3], NSW

Intravenous (IV) zoledronic acid (ZA:Zometa, Novartis Pharmaceutical) therapy is used in the management of osteoporosis (Reid et al. 2002). In this study we report our experience with ZA in routine clinical practice in regard to effect on bone mineral density (BMD) in osteoporosis. We reviewed the records of patients who had been given IV ZA (4mg) as a single annual dose. BMD was routinely measured on the day of the first, second and third annual infusion. Results were available at 0 and 1 years (Group 1) in 55 patients (36 women and 14 men), with a mean age of 68 years (range, 19-92 years) and 0, 1 and 2 years in a subset of 39 patients (all postmenopausal women) (Group 2). The analyses used paired t tests. Results are mean $\pm$ SEM. In group 1, lumbar spine BMD was increased significantly (3.8 $\pm$ 0.7%) at 12 months ($P<0.0001$), femoral neck also increased significantly (2.0 $\pm$ 0.5%) at 12 months ($P=0.0001$). In group 2, who were followed for a further 12 months, the change in BMD compared to baseline were at the lumbar spine +3.1 $\pm$ 0.7% and +4.3 $\pm$ 0.9% respectively at 1 and 2 years, and at the femoral neck 2.5 $\pm$ 0.6% and 2.5 $\pm$ 0.8%. No new incident fractures were observed.

Reid, IR et al. NEJM 2002; 346:653-661

Disclosures:
3,4 (Novartis, Mercke, Aventis, Sanofi, Wyeth, Pfizer, Lilly, Roche)

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IS STRONTIUM ANABOLIC?: MECHANISM OF ACTION IN HUMAN OSTEOBLASTS

TC Brennan[1], MM Muir[1], AD Conigrave[2] & RS Mason[1]
[1]*School of Medical Sciences and Institute of Biomedical Research, University of Sydney, NSW*
[2]*School of Molecular and Microbial Sciences, University of Sydney, NSW*

Strontium (Sr^{2+}) reduces vertebral and non-vertebral fractures in post-menopausal women. The mechanisms of action of Sr^{2+} at a cellular level are not well understood. We previously showed that Sr^{2+} induced significant increases in osteoblastic cell proliferation and more than halved RANKL:OPG mRNA ratio, with an EC_{50} of $\leq$ 0.1 mM. The current study evaluated whether Sr^{2+} increased alkaline phosphatase activity, a marker of osteoblastic differentiation, or affected the lifespan of osteoblastic cells. Primary human osteoblasts and MG63 cells were cultured in DMEM with 10% FBS then adapted to serum-free medium for 24h before experimental treatments were added. The tested concentrations of Sr^{2+} were 0.01 - 1 mM in the presence of 1 mM Ca^{2+}. Alkaline phosphatase activity in MG63 cells was dose-dependently increased ($p<0.001$, 1mM Sr^{2+}) by 2-3 fold after treatment for 72h. After treatment with Sr^{2+}, 0.01 -1 mM in 1 mM Ca^{2+}, for 48 h and then exposure to doxorubicin (5 □M) for a further 24 h, cell loss in MG63 cells was 74 ± 20 % with vehicle and was dose-dependently decreased by $Sr2^{+}$ from 0.01 – 1 mM, ($p< 0.05$ at 1 mM Sr^{2+}). Cell loss was reduced by more than 66 % at the highest tested dose of Sr^{2+}. Similar results were also observed in primary human fetal osteoblasts. These data indicate that in addition to proliferative and anti-resorptive effects, Sr^{2+} induces osteoblastic differentiation and may also contribute to enhanced osteoblastic lifespan, a characteristic also noted upon exposure to sex steroids or, intermittently, parathyroid hormone.

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GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR INDUCES OSTEOCLASTOGENESIS POTENTIALITY

R Lari[a], PD Kitchener[b] & JA Hamilton[a]
[a] *Department of Medicine, University of Melbourne, Royal Melbourne Hospital, VIC*
[b] *Department of Anatomy and Cell Biology, University of Melbourne, VIC*

Introduction:
Osteoclast-mediated bone loss is one of the major pathological processes of rheumatoid arthritis (RA). Granulocyte-macrophage-CSF (GM-CSF) is a potent pro-inflammatory cytokine that is normally difficult to detect in the circulation, but is present in the synovial fluid of the inflamed joint where it may influence osteoclast activity.

Method:
Bone marrow cells were cultured in high doses of GM-CSF and/or M-CSF for 7 days. The GM-CSF bone marrow derive cells (GM-BMM) or M-CSF derive macrophages (M-BMM) were then cultured with soluble RANKL and M-CSF.

Results:
Bone marrow cells cultured in GM-CSF (GM-BMM) before exposure to RANKL and M-CSF have high osteoclastogenic potential. All cells in this population were capable to differentiating to TRAP positive osteoclasts. In comparison to the M-BMM, the GM-BMM precursors generated more multinuclear osteoclasts ($p>0.01$) and expressed higher levels of osteoclast markers CTR, Cath k, *c-fos* and β- integrin (as measured by Q-PCR). Furthermore, when bone marrow cells were cultured in both M-CSF and GM-CSF, GM-CSF had a dominant effect on the cells potential to differentiate into an osteoclast.

Conclusions:
We found that GM-BMM precursors represent an easily obtainable and enriched source of osteoclast progenitors, with strong osteoclastogenic potential. GM-BMM are suitable progenitors to investigate the effect of pro-inflammatory cytokines on osteoclast differentiation and the mechanisms involved bone resorption in diseases like RA. Also, according to Hart et al the properties of RA synovial macrophages resemble those of GM-CSF-induced macrophages *in vitro(1)* and our results support the hypotheses that synovial precursors may have a higher potential to differentiate to osteoclasts.

1.Hart, P. H., Jones, C. A., and Finlay-Jones, J. J. Monocytes cultured in cytokine-defined environments differ from freshly isolated monocytes in their responses to IL-4 and IL-10. J Leukoc Biol 57:909-18; 1995.

Disclosures:
1

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COMPARISONS BETWEEN Ca2+-REGULATED PTH SECRETION FROM PERIFUSED NORMAL AND ADENOMATOUS HUMAN PARATHYROID CELLS

H-C Mun[1], L Delbridge[2], M Wilkinson[3] & AD Conigrave[1]

[1]School of Molecular and Microbial Science, University of Sydney, NSW, [2]Departments of Surgery and Endocrinology, [3]Clinical Endocrine Laboratory, Royal North Shore Hospital, St Leonards, NSW

We recently described a new method for the functional analysis of parathyroid hormone (PTH) secretion using perifusion of normal human parathyroid cells (Conigrave et al., 2004). We have now compared PTH secretion from normal and adenomatous human parathyroid cells. All tissue was obtained with patient consent under institutional guidelines. Human parathyroid cells were prepared by collagenase digestion and perifused as described previously (Conigrave et al., 2004). Compared to normal parathyroid cells, adenomatous cells exhibited significantly lower cellular PTH secretion rates and, as previously reported, a reduced sensitivity to extracellular Ca^{2+}. Maximum secretion rates for normal and adenomatous cells were, respectively, 3.9 ± 0.4 fg min^{-1} $cell^{-1}$ (mean ± SEM; n = 12) and 2.0 ± 0.4 fg min^{-1} $cell^{-1}$ (n = 14; unpaired Student's t-test: p = 0.002) and minimum PTH secretion rates for normal and adenomatous cells were, respectively, 0.7 ± 0.1 fg min^{-1} $cell^{-1}$ and 0.4 ± 0.1 fg min^{-1} $cell^{-1}$ (p = 0.008). Furthermore, the IC_{50} for Ca^{2+} was elevated from 1.1 ± 0.02 mM for normal cells to 1.2 ± 0.02 mM for adenomatous cells (p = 0.027). In 5/7 cases of patients with double adenomas functional analysis of PTH secretion by perifusion was consistent with the observed changes in serum PTH level in vivo upon sequential excision of the adenomas. Decreased cellular PTH secretion rates and reduced sensitivity to extracellular Ca^{2+} appear to be key functional characteristics of adenomatous parathyroid cells. Differences in cellular PTH secretion rate appear to be a major contributor to functional differences between adenomas *in vivo*.

A.D. Conigrave *et al.,* 2004 *J. Biol. Chem*. 279, 38151-38159

P97

LOCALIZATION OF ANGIOTENSIN RECEPTORS AND ANGIOTENSINOGEN IN THE EPIPHYSEAL PLATE OF RAT TIBIA

C Sernia, L Li & H Huang

The University of Queensland, Department of Physiology and Pharmacology, Brisbane, QLD

The Renin-Angiotensin System (RAS) is best known for its role in the regulation of cardiovascular functions. Clinical and experimental evidence from the use of inhibitors of RAS during pregnancy suggests that it is involved in normal bone growth. However there has been no attempt at identifying components of RAS in bone that would provide direct evidence to support its involvement in bone development. The aim of this study was to localize two components of RAS; the AT1 receptor (AT1-R) and angiotensinogen (AGT) -the obligatory substrate in the formation of angiotensin II, in the tibial epiphyseal plate of 14-day old rats. Immunohistochemistry was used to localize AT1-R and AGT in sections of paraformaldehyde-fixed, decalcified bone. AT1-R was present mainly in the chondrocytes of the proliferation and maturation zones, with lesser but still detectable staining in chondrocytes of the other epiphyseal zones. In contrast, AGT was mostly present in the resting zone, with lower intensity stain the maturation, hypertrophy and degeneration zone. No stain was detectable in chondrocytes of the proliferation zone. These results show that chondrocytes at the tibial epiphyseal plates may be targets of angiotensin action via AT1 receptors. Furthermore, the presence of AGT suggests that angiotensin II could be generated locally in the epiphyseal plate. In conclusion, our study supports a role for the RAS in normal bone growth. Further studies are needed to define the precise role of RAS in chondrocyte function.

Disclosures:
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