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ABSTRACTS

'MEET THE PROFESSOR'

MEET THE PROFESSOR 1
Best practice for mouse skeletal analysis
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Genetically manipulated mice are a powerful tool for exploring the effects of gene products on bone structure and bone cell function, and their use is becoming more essential to prove the physiological relevance of cell culture studies or to explore mechanisms of human disease. Unfortunately, however, bone phenotypes can be subtle and easily missed or are often misinterpreted by inaccurate or incomplete analysis.
It need not be so! This talk will explore the use of genetically altered mice, including transgenics, knockouts and chemically-induced or naturally-occurring mutants. Best practice and common pitfalls of analysis of mouse skeletons by simple Xrays, histology and histomorphometry will be explored, including a discussion of appropriate ages and regions for analysis as well as appropriate software. Common physiological interventions such as anabolic PTH treatment and ovariectomy will be discussed. Finally, the use of ex vivo cell culture systems to determine changes in osteoblast and osteoclast differentiation, function and gene expression will be discussed.
Hopefully you will leave this session with not only a full stomach, but also a better understanding of how to analyse a mouse model, and how to interpret published papers (or papers under review) that feature skeletal analyses of mouse models.

MEET THE PROFESSOR 2
Management and diagnosis of osteoporosis
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Calcium and vitamin D are important for preventing non-vertebral and hip fractures in the institutionalised elderly. They also reduce falls and are important adjunctive treatments for osteoporosis.

Therapies for osteoporosis are classified as either anti-catabolic or anabolic. Potent anti-catabolic drugs are intravenous bisphosphonates and denosumab. Given parenterally once or twice a year, they avoid the important issue of non-compliance. However, they may have a different side effect profile to oral bisphosphonates or raloxifene. Annual infusions of 5 mg zoledronic acid for three years reduce spinal, non-spinal and hip fractures in women with postmenopausal osteoporosis. There is a small increase in absolute risk of atrial fibrillation.

Subcutaneous teriparatide injections are the first anabolic therapy and significantly increase BMD and reduce vertebral and non-vertebral fracture risk. The mode of action of PTH(1-34) differs from anti-catabolic drugs in that it restores trabecular microarchitecture and increases cortical and trabecular bone volume. Combining anabolic and anti-catabolic drugs diminishes beneficial effects; sequential therapy is preferred. Anti-catabolic therapy should be reinitiated after PTH(1-34) to further increase BMD.

Strontium ranelate, reduces vertebral; non-vertebral; and hip fracture risk. It increases osteoblastic, and reduces osteoclastic cellular activity *in vitro* and in animal studies. Rapid BMD increases occur and about half of the increase in spinal BMD is related to increased bone strontium content.

Bone mineral density measured by DXA remains the gold standard for diagnosing osteoporosis and monitoring therapy. Biochemical bone markers also have a role. Although therapeutic options for osteoporosis are rapidly increasing, translating evidence into effective treatment strategies is problematic - the majority of osteoporotic fractures remain untreated.

IS - INVITED SPEAKERS

IS1
Catabolic and anabolic actions of PTH
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Daily injections of PTH increase bone tissue mass and reduce the risk of fractures. The anabolic effect of PTH requires intermittent administration, but when an elevated PTH level is maintained even for a few hours it initiates signalling that leads to new osteoclast formation and bone resorption that predominates. The anabolic response likely requires the activation of new bone metabolic units (BMU's) throughout the skeleton, with PTH /PTHrP acting upon committed precursors in the osteoblast lineage to promote their differentiation and upon osteoblasts and osteocytes to reduce apoptosis. These effects could result from any of several local cell responses to PTH and PTHrP in the osteoblast lineage, important among which are increased runx2 activity, enhanced production of ephrin B2, reduced production of sclerostin by osteocytes, and co-operative effects with Wnt signalling. The discovery through mouse genetics that PTHrP is a crucial bone-derived paracrine regulator of remodelling implicates osteoblast-derived PTHrP as a physiological regulator, with PTH as its pharmacological surrogate. Furthermore, several lines of evidence point to the fact that osteoclasts might generate an activity that complements the direct effect that PTH has in promoting differentiation of committed osteoblast precursors. Such an activity might or might not require resorption by the osteoclast, and is analogous to the coupling factor considered to act upon the osteoblast to favour differentiation. Participation of the ephrin/eph family in such a process has been proposed.

IS2
Calcitonin: essential hormone or vestigial peptide
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The calcitonin (CT) hormonal system is elegant, extensive, and elusive. Apparently simple and straightforward, CT, a small 32 amino acid peptide turns out on investigation to be wonderfully complicated and contradictory. The small simple structure derives from a complex gene with mRNA splicing encoding two peptides, CT and calcitonin gene related peptide (CGRP) with multiple levels of regulation. CT is a very potent peptide in vivo and in vitro with effects at concentrations as little as 10-100 pM in vitro. It lowers serum calcium in states of high bone turnover and has very potent activity to inactivate osteoclast function. However its main physiological role remains to be clearly defined, with some arguing CT has no physiological role in regulating serum Ca or osteoclast function. The receptor for CT (CTR) has multiple ligand recognition sites and multiple receptor subtypes encoded in one gene.
Genetically modified animal models and study of human mutations are common approaches to understanding gene function. As yet, no human diseases have been described with mutations in either CT or CTR. No genetically determined CT deficiency state has been described and CT excess has no effect on mineral metabolism in adult life. This suggests either the CT and CTR genes are redundant or that any mutation is lethal.
Study of animal models has been more useful. The complex CT/CGRP knockout yielded live mice with interesting but apparently contradictory results. Surprisingly to some, global deletion of receptors often leads to different phenotypes from deletion of ligands, possibly because of multiple ligands or multiple receptors. Homozygous global deletion of CTR is lethal in utero but heterozygotes have suggested tantalising effects on bone formation.
In order to clarify these issues we have used the Cre/LOX system to develop a family of genetically modified CTR animal models. True to form this has led to interesting but complex results;

1) Histomorphometry demonstrates decreased trabecular bone volume in mice with OC specific deletion of the CTR consistent with CTR inhibiting bone resorption.
2) CT or the CTR inhibits bone formation.

These data suggest that there are major functions of CT and the CTR in regulating bone cell metabolism. They also indicate a role in regulating both bone resorption and more surprisingly bone formation. Experiments investigating protection of the skeleton at times of calcium stress are underway.
Physiological and pharmacological effects on brain, kidney and cartilage may expand the repertoire of CT.

IS3
The role of receptor activity-modifying proteins in receptor modulation
Naot, D.
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The closely related peptide hormones calcitonin, calcitonin gene-related peptide, amylin and adrenomedullin have diverse effects on bone metabolism. These peptides signal through two seven-transmembrane G protein-coupled receptors (GPCR): the calcitonin receptor (CTR) and calcitonin receptor-like receptor (CRLR), which form heterodimers with one of the three members of the receptor activity-modifying proteins (RAMPs) 1-3. CTR itself and the different combinations of RAMPs with either CTR or CRLR give rise to seven receptors with distinct affinities for the peptides of the calcitonin family. Recent studies have shown that associations with RAMPs are not restricted to CTR and CRLR, as RAMPs can interact with other class B GPCRs. RAMPs form

stable complexes with their receptor partners and participate in ligand binding and internalization. They have been shown to modify GPCR activity by altering their glycosylation and regulating receptor trafficking to the plasma membrane.
Although the effects of peptides of the calcitonin family on bone cells have been studied in many in vitro model systems and more recently in transgenic and knockout animals, some observations are still awaiting explanation. For example, amylin is known to signal through heterodimers of RAMP1-3 and CTR. We have shown that amylin stimulates osteoblast proliferation in vitro, even though CTR is not expressed in these cells, suggesting that another unrecognised amylin receptor may exist. It is evident that the calcitonin family of peptides and the receptor subunits are pieces in a puzzle that is yet to be completed.

IS4
Risk of Fracture and treatment of osteoporosis
Cummings, S.R.
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Ultimately, the goal of assessing risk of fracture is not to estimate the absolute risk of fracture, but to estimate the benefit of treatment. It is assumed that benefits of treatment are proportional to the risk, regardless of why the risk is high. This may not be true.

Areal bone mineral density (aBMD) of the hip and spine and age are such strong risk factors for fracture that it is difficult to improve on their ability to estimate risk and identify patients who will benefit from treatment by assessing risk factors or making additional measurements. In particular, it is not yet clear whether assessing risk factors improves the ability of aBMD to identify patients who will benefit most from treatment.

The ability of sophisticated and expensive imaging methods, such as QCT, to improve the prediction of fractures and treatment benefit based on aBMD remains to be proven. Some data suggest that measurement of markers of bone turnover might improve the estimation of benefit from alendronate. Trials have consistently found that treatment benefits people who have vertebral fractures, regardless of their aBMD. Vertebral fracture assessment (VFA) by DXA finds vertebral fractures in 10 to 20% of older women who would otherwise not be treated based on BMD or risk. VFA may have the greatest and cost-effective potential for identifying people who will benefit from treatment.

IS5
Toward the individualisation of fracture risk
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From the age of 60, one in two women and one in four men will sustain a fracture during their remaining lifetime. Several clinical risk factors are associated with fracture risk independent of BMD measurement. At present, a major priority in osteoporosis research is the translation of risk factors into a prognostic tool for assessing fracture risk and adverse outcomes. A common approach to developing prognostic models is to group individuals with similar characteristics by some arbitrary thresholds and to make a prediction for each group. While this risk-stratification is a logical approach, it is inefficient and usually has low predictive value.
Because the relation of a risk factor and fracture risk is continuous, reliance on a threshold value to determine therapeutic behaviour is unlikely to be valid. We have proposed an alternative approach in which an individual's multiple characteristics (e.g., BMD, age, weight, prior fracture, fall, etc) are simultaneously considered in a multivariable prognostic model and represented by a nomogram. This model recognises the fact that there are different ways two individuals can attain the same risk. For example, a 60 year-old woman with BMD T-score=-2.5 and a history of fracture is predicted to have the same 5-year risk of fracture as an 80 year-old woman with a T-score=-1 without a previous fracture.
Accurate estimates of fracture risk are critically important for informed decision-making and patient counselling. Since fracture risk is determined by multiple factors, any unidimensional risk assessment is unlikely to be helpful. A multivariable-based nomogram can be an effective tool for individualising short-term and long-term absolute risks of fracture.

IS6
Why should we think about calcium?
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Four physiological realities combine to make calcium a critical nutrient. The first is the imperative requirement to maintain the ionised calcium in the tissue fluids for optimal neuromuscular function. The second is that, regardless of calcium intake, there are always obligatory losses of calcium through the bowel, kidneys and skin, which is why the calcium requirement of adults is as high as 800 mg/day. The third reality is that 99% of the body's calcium is stored in the skeleton and can be mobilised by bone breakdown when required to maintain the ionised calcium. The fourth is that calcium is not widely distributed in our diet, being found mainly in dairy products. It is, therefore, not surprising that the rise in calcium requirement at the menopause (due to a fall in calcium absorption and rise in obligatory calcium excretion) increases bone resorption and leads to loss of bone. These changes can be reversed with estrogen therapy, particularly if supplemented with calcium, but calcium supplementation is also very effective

in its own right. With advancing age, the effects of estrogen deficiency are compounded by declining vitamin D status and secondary hyperparathyroidism which is due to loss of the calcaemic action of vitamin D on bone rather than to the independent decline in calcium absorption. This important but neglected calcaemic action on bone explains why the effect of vitamin D deficiency (rickets/osteomalacia) is different from the effect of calcium deficiency (osteoporosis) in adult experimental animals. Osteoporosis in men has a different origin from that in women but all forms of osteoporosis are aggravated by calcium deficiency.

IS7
Calcium and Vitamin D for whom, when and why
Richard Prince

IS8
Bone and non-bone effects of calcium
Reid, I.R
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The Auckland Calcium Study was a randomized controlled trial in 1471 normal postmenopausal women, assessing the effects of calcium on bone density over 5 years. The women received calcium 1g/day calcium or placebo.
Calcium reduced bone loss by two-thirds in both the hip and total body sites. Fracture data were inconclusive, but there were downward trends in hazard ratios for most fracture categories. Serum PINP levels were 22% lower in the calcium group at 5 years (P=.03).
Calcium reduced LDL cholesterol by almost 10%, increased HDL comparably, thus increasing the HDL/LDL ratio by almost 20%. In the calcium group, there were small transient decreases in blood pressure at six months, but thereafter the two groups were indistinguishable. There was no evidence of any between-groups difference in weight loss, lean mass or fat mass.
There were no between-groups differences in falls, tooth loss, or iron status. Constipation and discontinuation of trial medication were more common in the calcium group. Myocardial infarction was more common in those allocated to calcium, though this effect was of marginal significance following event adjudication.
It is concluded that calcium produces sustained benefits on BMD but these are likely to be overwhelmed from any deleterious effect on heart health.

IS9
Mechanisms of bone erosion in chronic tophaceous gout
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Gout is a prevalent form of inflammatory arthritis caused by intra-articular deposition of monosodium urate (MSU) crystals. Chronic tophaceous gout is characterized by bone erosion and associated joint damage. We have recently examined the mechanisms of bone erosion in gout, focusing on osteoclast development and function.
These experiments demonstrated that peripheral blood mononuclear cells (PBMC) from patients with severe erosive gout have preferential ability to form osteoclast-like cells in culture with RANKL and M-CSF. The number of PBMC derived TRAP positive multinucleated cells strongly correlated with the number of tophi. Furthermore, higher numbers of TRAP positive multinucleated cells were cultured from synovial fluid mononuclear cells from gouty knee effusions, compared with paired PBMC. Immunohistochemical analysis of tophi demonstrated numerous multinucleated cells localized to the areas surrounding uric acid crystal deposits. These cells expressed the osteoclast markers TRAP, vitronectin receptor and cathepsin K. MSU crystals did not directly promote osteoclast formation from RAW264.7 cells *in vitro*. However, MSU crystals inhibited OPG gene and protein expression in ST2 cells, without significantly altering RANKL gene expression. Conditioned medium from ST2 cells cultured with MSU crystals promoted osteoclast formation from RAW264.7 cells in the presence of RANKL.
In summary, chronic tophaceous gout is characterized by disordered osteoclast development. MSU crystals may promote osteoclastogenesis by alteration of the RANKL/OPG balance in stromal cells.

IS10
The role of bone in osteoarthritis
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It has been hypothesised for many years that bone plays a key role in osteoarthritis but evidence to support this has been lacking until recent years due to the lack of suitable animal models and the use of insensitive imaging techniques such as X-ray. Recent developments have primarily been due to the use of DXA and especially MRI in large scale epidemiologic studies. This talk will cover human studies on bone mineral density (both general and subchondral), bone marrow oedema and bone size. To date, there has been only one study suggesting higher bone density increases the risk of knee radiographic osteoarthritis. The study of subchondral bone density is expanding rapidly but there are methodological issues such as reproducibility and which area to measure prior to an increase in knowledge. Bone marrow oedema is common on MRI scans even in community living elderly. It is strongly associated with pain. The pathology remains uncertain but the change is likely to be cellular as BMD is increased in affected areas. Tibial bone size appears to have a key initiating role in the development of cartilage defects which is turn lead to

cartilage loss and eventually osteoarthritis. An osteophyte most likely represents substantial bone expansion based on comparative studies. Tibial bone size also predicts knee replacement in longitudinal studies. In conclusion, bone has a key role in osteoarthritis initiation and size matters.

IS11
Insights on arthritis from animal models
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The complex pathobiologic changes of human joint disease, particularly osteoarthritis (OA), normally take several decades to develop and may be influenced by a multitude of factors. The need to clarify the molecular events that occur in joint tissues at the onset and during the progression of OA has necessitated the use of models, which, although imperfect, can exhibit many of the pathologic features that characterize the human disease. In vitro studies have proven invaluable in defining specific molecular and cellular events in degradation of joint tissues such as cartilage. However, to fully understand the complex inter-relationship between the different disease mechanisms, joint tissues and body systems, studying OA in animal models is necessary. Models of inflammatory arthropathies have proven predictive of clinical efficacy, with therapies that are beneficial in animals having significant benefit in treatment of rheumatoid arthritis in humans (e.g. anti-TNF and anti-IL-1). While none of the available animal models of OA can truly be said to be "predictive" as anti-no OA therapies have yet proven to be disease modifying in human trials, this approach represents a cornerstone for discovery of new anti-OA therapeutic targets and drugs. Data will be presented from recent studies using genetically modified mice which have defined the degradative mechanisms in cartilage, in particular the enzymes responsible for breakdown of the principle structural proteoglycan, aggrecan. This work has shown for the first time that inhibition of a single proteolytic cleavage in aggrecan can abrogate cartilage erosion in OA, and may actually augment cartilage repair.

IS12
Role of αvβ3 integrin in osteoclast function: from attachment assays to clinical trials
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Adhesion of osteoclasts to bone matrix results in activation of these cells, leading to cytoskeleton reorganization, migration between resorption sites, and ultimately to their polarization during the resorption process. αvβ3 Integrin, a cell/matrix attachment protein, which recognizes the amino acid motif Arg-Gly-Asp (RGD) in vitronectin, osteopontin and bone sialoprotein, play a role in all these processes. αvβ3 integrins are abundantly (>10^6 receptors/cell) and selectively expressed in osteoclasts therefore can be effectively targeted for inhibition of bone resorption. Small-molecule, αvβ3 antagonists with a basic nitrogen separated some distance from a carboxylic acid to mimic RGD have been synthesized and the pharmacological properties of one such compound, MK-0429, are the following: *In vitro*, MK-0429 (i) inhibits αvβ3-mediated attachment to vitronectin and binding to purified human αvβ3 with IC50s of <1 nM; (ii) inhibits osteoclast formation and bone resorption with IC50s of ~10 nM. *In vivo,* administered at 10 and 30mg/kg, p.o., b.i.d. (i) increases bone mineral density (BMD) in growing male rats, (ii) and in ovariectomized (ovx) rats, increases BMD of distal femur and bone volume of proximal tibial cancellous bone and reduces bone turnover rate, when administered orally at 10 and 30 mg/kg, b.i.d. for 4 weeks; and (iii) suppresses urinary N-telopeptide (uNTx) in ovx rhesus monkeys, administered orally at 5, 15 and 40 mg/kg/day. In postmenopausal osteoporotic women, 200 mg MK-0429 b.i.d. increased lumbar spine and hip BMD and reduced NTx. In conclusion, αvβ3 is rate limiting for osteoclast function and provides a novel target for inhibition of bone resorption.

IS13
The effect of surface chemistry modification of implants
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Biomaterials and scaffolds used for skeletal tissue regeneration need to be biocompatible, osteo-inductive, osteoconductive and mechanically compatible with bone to meet the requirements for bone tissue engineering. The aim of our research is to deliver 1) a new generation of stable, life-long orthopedic/dental implants that offer strong bone–implant anchorage. 2) Novel smart scaffolds to permit greater control over the location and quality of bone regeneration, allowing faster healing.
Orthopaedic implants: Despite major advances in prosthetic technologies, implants have a finite life of 10-15 years, due to their premature failure. Novel micro-engineered surfaces are required to anchor prosthetic implants to the surrounding bony skeleton. Various surface chemical modifications have been applied to prosthetic devices to enhance osseointegration. To-date none have resulted in a stable interface strong enough to support functional loading for the lifetime of the implant. Our group demonstrated that surface chemisrty modification of biomaterials with bioactive molecules have the potential to provide a surface on a prosthesis that is conducive to normal bone metabolism.
Synthetic scaffolds: Currently available modalities for treating large bone defects, are limited in their success. Developing synthetic scaffolds that promote bone growth and adequate vascularization is vital in orthopaedic and maxillofacial surgeries. The current generation of synthetic scaffolds, does not combine the required posorsity, mechanical properties and bioactivity. This presentation will highlight some of our newly developed novel highly porous and mechanically strong silicate scaffolds that promote

the migration, proliferation and differentiation of bone and endothelial cells for effective skeletal tissue integration and vascularization.

IS14
Telling the effects of treatments: randomized trials vs observational studies
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Randomized trials have a major advantage: random assignment eliminates bias due to confounding. Trials also have disadvantages: 1) it is often not feasible or ethical to conduct a randomized trial of some drugs, 2) trials may be too small to find effects on clinical outcomes and 3) populations in trials may be different than those in practice.

Observational studies have several advantages. They can be very large, include unselected people, and study longer durations of treatment with fewer ethical issues. Their main disadvantage is potential confounding by differences in important characteristics between treatment and no-treatment populations.

Confounding can be reduced by measuring and 'adjusting' for differences. However, it may not be possible to have accurate measurement of all potential confounders. For studies of treatments it particulary difficult to control for: 1) confounding by indication, 2) healthy user effect and 3) confounding by compliance.

Features of an observational study that are important to its validity include 1) results have been adjusted for important and accurately measured confounders; 2) the association is strong; 3) the effect of the treatment is biologically plausible; and 4) the study shows an expected increase in effect with higher dose or longer duration of treatment.

There have been several prominent examples of major differences between the results of observational studies about a treatment and the corresponding trials such as hormone therapy for heart disease. On the other hand, surveys have found that results from observational studies and randomized trials agree most of the time.

IS15
Falls prevention – risk factor and RCTs
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About 30% of people aged 65 years and older fall each year. During the 1980s, several longitudinal epidemiological studies were conducted to identify risk factors for falls. Risk factors for falls include increased age, female gender, muscle weakness, poor balance, impaired gait, medical conditions like stroke and Parkinson's disease, cognitive impairment, psychotropic medications, poor vision, environmental hazards and inadequate footwear. Since the early 1990s more than 50 randomized trials of interventions to prevent falls have been conducted. Most of these trials have involved cognitively intact older people living in the community. Interventions proven to be effective in this population include multidisciplinary, multifactorial programs; exercise programs that emphasize balance improvement (such as Tai Chi); home modifications arranged by an occupational therapist; reduced use of psychotropic medications; and cataract surgery. Much more research is needed on the best way to prevent falls in hospital and residential aged care facilities, and among people with cognitive impairment.

IS16
Effectiveness of fracture prevention
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There are two broad approaches to fracture prevention: the 'population strategy' attempts to 'shift' the population mean of risk factors and 'high-risk identification' referring to 'case-finding' and targeted treatment.
Population differences in fracture rates are largely explained by environmental rather than genetic factors. Strategies to lower the population's absolute fracture risk could most optimistically prevent 30% of fractures. Vitamin D sufficiency could reduce hip and Colles fractures by 16% and 31%, respectively. High compliance of calcium supplementation may reduce fractures by up to 34% similar to exercise prescription in young people. Effective falls prevention programs may reduce hip fractures by 27%.
Pharmacological intervention offers up to 50% risk reduction but PBS eligibility of 'osteoporosis plus prior fracture' only relates to 17% of fractures. The recent amendment extends this to 24%. Australian data suggests <30% of those eligible are offered treatment. Incorporating compliance rates of ≤60% implies that drug therapy addresses <5% of fractures. This proportion of the fracture burden could be extended through case-finding algorithms incorporating risk factors such as glucocorticoid use. Such algorithms can have an acceptable positive predictive value without sacrificing specificity.
Of the 50,000 fractures p.a, 9% occur at sites unrelated to fragility. Extending eligibility for pharmacological intervention and improved compliance could improve 'case-finding' to 25% of fractures. Economic modelling suggests a population-based approach without screening is cost-effective from age 80 onwards. In younger age groups the biggest challenge will always be the 50% of the fracture cases that arise from the large number of people at lower fracture risk. To this group intervention programs offering multi-disease protection may offer the most cost-effective fracture prevention.

IS17

Cathepsin K, a new molecular target for treatment of osteoporosis

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Cathepsin K (Cat K), is a member of the papain family of cysteine proteases of which 11 human members have been identified. It is highly and selectively expressed in osteoclasts, and has the unique ability to cleave both the helical and telopeptide regions of type I collagen over a pH range of 4-7. In addition, loss of function of Cat K in humans results in reduced bone turnover and dense bones and knock out of Cat K in mice causes osteopetrosis. Co-crystal structures of the active site of Cat K have revealed opportunities for obtaining Cat K inhibitors with favorable selectivity for Cat K compared to other cathepsins. L-006235, is a reversible, potent inhibitor of human Cat K with a Ki of 0.2 nM, with >5000-fold selectivity for Cat K vs. cathepsins B, L and S. L-006235 inhibits rabbit Cat K with a Ki of 0.5 nM and rabbit osteoclast mediated bone resorption in vitro with an IC_{50} of 5 nM. When given orally once daily for 27 weeks to newly-ovx'd rabbits, L-006235 partially (2 mg/kg/d) and fully (10mg/kg/d) prevented bone loss. However, unlike other bone resorption inhibitors, Haversian remodeling of cortical bone or remodeling of cancellous bone were not suppressed. Consistent with its inhibitory effects on breakdown of collagen, L-006235 inhibited urine N-telopeptides dose (0.6, 3 and 15 mg/kg/q.d.) dependently (30-76%) after 7 days of administration, in a reversible fashion. These results suggest that inhibition of Cat K may offer a novel therapeutic approach for treatment and/or prevention of post-menopausal osteoporosis.

IS18

Protease-activated receptors and thrombin

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Protease-activated receptors (PARs) are seven transmembrane domain G protein-coupled receptors that mediate responses of cells to extracellular proteases including thrombin. Murine osteoblasts express two of the thrombin-responsive PARS, PARs-1 and -4. Thrombin stimulates proliferation and inhibits differentiation of osteoblasts through the mediation of PAR-1. Thrombin also exerts a number of effects on bone cells for which the receptors have not been characterized, including stimulation of prostaglandin E_2 (PGE_2) and interleukin-6 (IL-6) release by osteoblasts, and osteoclastic bone resorption in organ culture. Using a variety of culture systems and cells from PAR-1-null and wildtype mice, we have investigated mechanisms of these responses. Stimulation by thrombin of IL-6 or PGE_2 release from primary mouse osteoblast cultures could be mimicked by treatment with a PAR-1- but not a PAR-4-activating peptide. These effects were found to be dependent on thrombin's proteolytic activity and the presence of PAR-1. Thrombin's induction of IL-6 was inhibited by selective inhibitors of cyclooxygenase (cox)-1 or –2, and PGE_2 treatment induced IL-6 transcript expression and release into the medium. Cox-2 expression was also stimulated by thrombin treatment. Thrombin stimulated an increase in the ratio of RANKL to osteoprotegerin transcripts in primary mouse osteoblast cultures. Osteoclast differentiation induced in mouse osteoblast-bone marrow co-cultures by PGE_2, but not by 1,25-dihydroxyvitamin D_3, was inhibited by thrombin treatment. These observations indicate that PAR-1 mediates thrombin-induced IL-6 and PGE_2 release by osteoblasts. Moreover, despite these pro-osteoclastogenic effects, thrombin inhibits osteoclast differentiation.

IS19

PPARγ – an osteoblast and adipocyte switch

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Mesenchymal stem cells (MSC) are multipotential cells able to differentiate into osteoblasts, adipocytes, chondrocytes, myoblasts, fibroblasts and stromal cells. A reciprocal relationship exists between differentiation of MSC into adipocytes and osteoblasts, the former regulated by activation of the lineage-specific transcription factors/nuclear receptors, peroxisome proliferator activated receptor-γ2 (PPARγ2) and C/EBP, and the latter by Runx2, Dlx5 and Osterix.

Peroxisome proliferators are chemicals that induce dramatic proliferation of rodent hepatic peroxisomes. The receptor for these ligands, PPARα, a nuclear hormone receptor, was cloned in 1990, followed by identification of PPARγ and PPARδ. All are activated by dietary fats and derivatives and thus function as lipid sensors. The isoforms share a common domain structure and molecular mechanism of action, binding to cognate DNA elements as obligate heterodimers by partnering with a retinoid X receptor. The PPARγ1 isoform is widely expressed at low levels but PPARγ2 is restricted to adipocytes and is necessary for their differentiation. Reduced expression decreases adipocytic and increases osteoblastic differentiation of MSC. Decreased PPARγ activity in mice increases osteoblastogenesis and BMD, and prevents age-related osteopenia. In addition to natural ligands, the antidiabetic thiazolidinedione (TZD) drugs activate PPARγ2. Reduced bone formation, reduced BMD, and increased fractures associated with TZD therapy is an emerging clinical issue.

TAZ (transcriptional coactivator with PDZ-binding motif) may be a molecular switch in MSC differentiation. TAZ enhances Runx2 transcription activity, but suppresses that of PPARγ, and inhibits TZD-induced adipogenesis.

Greater understanding of the molecular mechanisms controlling MSC differentiation may allow development of therapies to prevent TZD-related bone loss.

IS20
The skeletal effects of thiazolidinediones in humans
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Thiazolidinediones (TZDs) are agonists of the peroxisome proliferator-activated receptor gamma (PPARγ) nuclear transcription factor. Two members of this drug class, rosiglitazone and pioglitazone, are commonly used in the management of type II diabetes mellitus, a disease associated with increased skeletal fragility, and have emerging roles in the treatment of other clinical conditions characterized by insulin resistance. Over the past decade, a consistent body of preclinical data has demonstrated that PPARγ activation regulates the fate of pluripotent mesenchymal cells, favoring adipogenesis over osteoblastogenesis, and promoting bone loss in rodents. Until recently, there were no bone-related data available from studies of TZDs in humans. In the past year, however, several clinical studies have reported adverse skeletal actions of TZDs in humans. Collectively, these investigations have demonstrated that the TZDs currently in clinical use decrease bone formation and accelerate bone loss in healthy and insulin resistant subjects, and increase the risk of fractures in the appendicular skeleton in women with type II diabetes mellitus. These observations should prompt clinicians to evaluate fracture risk in patients for whom TZD therapy is being considered, and initiate skeletal protection in at-risk individuals.

IS21
Multipotential mesenchymal stem/ precursor cells
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Bone marrow derived mesenchymal stem/precursor cells (MPC) give rise to different cellular lineages following ex vivo expansion such as myeloid supportive stroma, smooth muscle cells, osteoblasts, adipocytes, chondrocytes and myoblasts. We have recently developed an immunoselection protocol to purify human MSC populations directly from bone marrow aspirates and other tissues, based on their high expression of the pre-osteoprogenitor marker, STRO-1 and co-expression of the vascular/smooth muscle antigens, CD106 and CD146. Clonal analysis has identified populations of MPC with high proliferation potential and the capacity for multi-differentiation. Preliminary studies have shown that those MPC that continue to express high levels of the STRO-1 antigen in vitro, exhibit a more immature phenotype. Furthermore, high STRO-1 expressing human MPC demonstrated an enhanced capacity to facilitate angiogenesis and new bone formation when implanted into small animal models. We have recently identified a bone marrow derived multipotential ovine MPC population that has been used to facilitate bone repair in both an autologous and allogeneic models of non-union segmental bone defects in sheep. Our studies demonstrate that the use of purified populations of MPC may be an effective cell therapy strategy for a range of tissue engineering orthopaedic applications.

IS22
Chemokines and cytokines involved in PTH's actions
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PTH is an essential regulator of calcium homeostasis but also has a role as an anabolic hormone for bone. We hypothesized that the anabolic effects of PTH, in contrast to its catabolic effects, are due to different kinetics of gene regulation as well as different genes being expressed. Microarray analyses show clear differences in the profile of genes between intermittent and continuous infusion. Many of the genes identified from the intermittent microarrays were regulated extremely rapidly both in vitro and in vivo, with maximal mRNA expression 1-2 h after PTH (1-34) treatment. Even after 14 daily intermittent injections of PTH (1-34) in rats, many genes subsided to low levels within 24 h and prior to the next injection but some of the genes showed dramatic amplification with repeated injection. With infusion of PTH, most genes changed very slowly and only showed 2-5-fold changes. Notable novel genes which were identified as highly regulated by PTH and involved in its anabolic effects were interleukin-18, Jagged1 and monocyte chemoattractant protein-1 (MCP-1). It appears that PTH acts on osteoblastic cells, inducing abundant and transient expression by these cells of many growth factors, cytokines and chemokines which are then involved in signaling to all the cells in the surrounding bone marrow. We conclude that these are rapidly and transiently induced in the osteoblast by intermittent PTH injection and are either not stimulated by continuous presence of the hormone or are stimulated to a constant low presence leading to continuous bone resorption.

IS23
Osteocytes and bone health
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Osteocytes derive from cells of the osteoblast lineage, which become encased in bone matrix during bone formation, where they form a dense network of cell bodies and cell processes. Osteocyte viability depends on interstitial fluid flow along the osteocyte canaliculi. The appropriate density and viability of osteocytes are essential for bone health because osteocytes perform many important functions in bone. Osteocytes appear to initiate bone repair in response to microcracks, as well as new bone formation

in response to increased loading of bone. Osteocytes are able to regulate the amount of new bone formation in bone remodelling cycles, at least in part by the production of a molecule called sclerostin. Production of a mutant form of sclerostin has been associated with particularly dense, fracture resistant bones, and this information has led to development of anti-sclerostin antibodies as a potential anabolic therapy for bones. Deletion of the osteocytic gene encoding DMP-1 has identified a role also for osteocytes in bone apposition and in systemic mineral metabolism. The latter appears to be due to their production of FGF-23, which controls the renal production of 1,25 $(OH)_2$ vitamin D. Reduced osteocyte density has been reported in association with osteoporotic fracture, which is thought to relate to reduced ability to detect microdamage of the bone matrix, and is consistent with the reported accumulation of microdamage with age. Reduced osteocyte formation and/or survival could be the result of skeletal disuse, reduced blood flow to bone and therefore relative hypoxia, and pharmacobiology, such as chronic exposure to glucocorticoids. Because of the important roles of osteocytes in bone, new approaches to bone health may well involve the identification of agents to protect these cells from harmful influences in disease and ageing.

IS24
Osteoclast – osteoblast communication
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Osteoblasts play an important role in controlling osteolysis through their regulated expression of RANKL, a TNF-related molecule that stimulates osteoclast (OC) formation, survival and activation. However there is now good evidence that OCs also stimulate osteoblast action.
In bone remodelling, bone resorption is followed in a spatially and temporally coordinated fashion by osteogenesis, with OCs and neighbouring osteoblasts forming recognisable structures termed basic multicellular units. This suggests some uncharacterised OC to osteoblast communication occurs. Products of bone matrix released by OCs may stimulate osteoblasts but it is likely activation causes OCs to osteogenesis-stimulation factors. Evidence for this comes from two research areas. Firstly, anti-osteolytic agents (e.g. bisphosphonates) frequently cause reduced osteogenesis. Furthermore such agents, including calcitonin (which acts specifically on OCs to reduce activity) can also reduce the pro-osteogenic actions of PTH. However, there are anti-osteolytic factors that do not reduce osteogenesis, including inhibitors of the resorption process that do not block OC formation or activity. Secondly, studies of murine models where OC-osteoblast coupling may have broken down suggest the identities of possible coupling factors. For example, mutant gp130 Y757F mice have high bone turnover osteopenia with high OC and osteoblast numbers, while gp130 Y757F mice crossed with IL-6 knockouts have high OC numbers but low osteoblast numbers, suggesting a role for IL-6. Other molecules have also been proposed to participate in OC-osteoblast coupling, notably ephrin B2 and EphB4.
Thus, OC-osteoblast communication is critical to osteogenesis in bone remodelling and understanding the process could lead to improved bone anabolic therapies.

OR - ORAL PRESENTATIONS

OR1

The calcitonin receptor plays a physiological role in maintaining trabecular bone volume in young female and adult male mice

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To investigate the physiological role of the calcitonin receptor (CTR) in calcium homeostasis and bone turnover, we have generated a mouse model in which the CTR is deleted specifically in osteoclasts (OCL-CTR KO).
The phenotypes of OCL-CTR KO and control littermates were assessed at 6 and 12 weeks of age (n=8-10/group). Serum calcitonin, PTH and calcium levels were unchanged in female and male OCL-CTR KOs at 6 and 12 weeks of age. Osteoclast-specific deletion of the CTR in males resulted in a 37% decrease in trabecular bone (BV/TV) at 12 weeks of age (P<0.01). This was associated with a 23% decrease in trabecular thickness (TbTh) (P<0.05), a 14% decrease in mineralised surface (P<0.05), and a 24% decrease in bone formation rate (P=0.06). Although trabecular number was lower in OCL-CTR KOs, this was not significant (P=0.08). This bone loss may be attributed to an increase in bone turnover prior to 12 weeks of age, as evidenced by preliminary gene expression data demonstrating elevated RANK (P<0.01), TRAP, collagen 1a1 (P<0.05) and alkaline phosphatase (P<0.05) mRNA levels in OCL-CTR KO males at 6 weeks of age.
In contrast, OCL-CTR KO females had a 17% reduction in BV/TV (P<0.05) at 6 weeks of age due to an 8% decrease in TbTh (P=0.05).
We are further assessing the physiological role of the CTR in protecting against hypercalcemia in OCL-CTR KO and control mice.
In conclusion, we have demonstrated a physiological role of the CTR in trabecular bone maintenance in young female and adult male mice.

OR2

Can bone density assessed by DXA at age 8 predict fracture risk in males and females during puberty?

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Introduction: The aim of this study was to determine if pre-pubertal dual energy x-ray absorptiometry (DXA) can predict fracture risk during puberty.
Methods: We studied 183 children who were followed for 8 years (1460 person years). Bone densitometry was measured at the total body, hip and spine by DXA and reported as bone mineral content (BMC), bone mineral density (BMD) and bone mineral apparent density (BMAD). Fractures were self-reported at age 16 with x-ray confirmation,
Results: There were a total of 63 fractures (43 upper limb). In unadjusted analysis, only total body BMD showed an inverse relationship with upper limb fracture risk (p=0.03). However, after adjustment for height, weight, age (all at age 8) and sex, total body BMC (HR/SD 2.47 95% CI 1.52 - 4.02), spine BMC (HR/SD 1.97 95%CI 1.30 - 2.98), total body BMD (HR/SD 1.67 95% CI 1.18 - 2.36), total body BMAD (HR/SD 1.54 95% CI 1.01, 2.37) and spine BMD (HR/SD 1.53 95%CI 1.10, 2.22) were all significantly associated with upper limb fracture risk. Similar, but weaker associations were present for total fractures. There was a trend for overweight/obesity to be associated with increased upper limb fracture risk (HR 1.53/category, p=0.08)
Conclusions: Measurement of bone mass by DXA is a good predictor of upper limb fracture risk during puberty. Although we did not measure true bone density, the constancy of fracture prediction following a single measure suggests bone strength remains relatively constant during puberty despite the large changes in bone size.

OR3

Endogenous sex hormones and incident fracture risk in older men: the Dubbo Osteoporosis Epidemiology Study

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One third of osteoporotic fractures occur in men. The present study examined the relationship between serum testosterone (T) and estradiol (E2) levels and fracture risk in men 60+ years participating in the DOES. The analysis included men who had serum samples for baseline measurements (n=609) with follow-up of up to 13 yrs. Clinical risk and lifestyle factors and BMD were assessed at baseline. Serum T and E2 were measured by tandem mass spectrometry. Low-trauma symptomatic fractures were ascertained by X-ray record.
During follow-up, 113 men suffered at least one low-trauma fracture. Fracture risk was increased in men with reduced serum T (HR 1.33; 95%CI: 1.09, 1.62). After adjustment for SHBG, both low serum T (HR 1.48; 95%CI: 1.22, 1.78) and serum E2 (HR 1.21: 95%CI: 1.00, 1.47) were associated with increased overall fracture risk. After adjustment for major fracture risk factors (age, weight

or BMD, fracture history, smoking status, calcium intake, SHBG), lower T was still associated with increased risk of hip (HR 1.88; 95%CI: 1.24, 2.82) and non-vertebral (HR 1.32; 95%CI: 1.03, 1.68) fracture. By contrast, lower E2 was only associated with increased fracture risk in the presence of body weight (HR 1.25; 95% CI: 1.02, 1.54), but not at any site after adjustment for BMD (HR 1.19; 95% CI: 0.69, 1.03).
In community-dwelling men over 60 years of age, serum T, but not E2, is an independent predictor of osteoporotic fracture and its measurement may provide additional clinical information for the assessment of fracture risk in elderly men.

OR4

Bone turnover is higher and bone density lower in adolescents with subclinical vitamin D deficiency

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Aims: It is important to identify modifiable influences on childhood bone development as suboptimal development is important in the pathogenesis of osteoporosis. This study aimed to examine the relationship between serum vitamin D and bone density (BMD) and bone turnover in 16 year old children.
Methods: From a birth cohort of 1435 children born in 1988-89, 888 were studied in 1996-7 and 415 were studied in 2004-05. We measured serum 25-hydroxy vitamin D ((25-OH)D), bone density at the hip, spine, radius and total body and bone turnover markers (bone-specific alkaline phosphatase (BAP) and deoxypyridinoline (DPD).
Results: The prevalence of vitamin D deficiency (<50nmol/l) increased from 10% at age 8 years, to 36% at age 16, and was as high as 50% in children with low summer sun exposure. After adjusting for weight, height and age, log-transformed serum (25-OH)D at age 16 was positively correlated with lumbar spine BMD in males ($r=0.25$, $p< 0.001$) but not females ($r=0.08$, $p =0.39$) and hip and ultradistal radius BMD in males ($r=0.29$, $p< 0.001$ and $r=0.16$ $p=0.015$) and females ($r=0.22$, $p= 0.015$ and $r=0.21$ $p=0.025$), and negatively correlated with urinary DPD/creatinine ($r=-0.17$, $p=0.002$) and BAP ($r=-0.18$, $p=0.001$). There was no evidence of any threshold effect.
Conclusion: Vitamin D deficiency appears detrimental to both bone density and turnover and is common in teenagers but not prepubertal children, possibly due to decreasing physical activity associated sun exposure with age. Randomised controlled trials are needed to determine whether vitamin D supplementation can improve peak bone mass.

OR5

Low vitamin D status increases osteoclastogenesis and bone loss in the rat

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The vitamin D status required to maintain bone strength is controversial. We generated 6 groups of Sprague-Dawley rats with stable, mean 25D ranging from 11 nmol/L to 118 nmol/L. D-depleted animals fed a diet with 0.4% Ca and varying vitamin D (0ng-500ng/d) for 4 months were killed at 7 months. Serum 25D, PTH, Ca, P, crosslaps and ALP and bone tissue expression of RANKL and OPG were determined. Proximal femoral structure was analysed by μCT. Distal femoral histomorphometry was analysed in 5μm undecalcified sections. Fasting serum Ca, PTH and phosphate were normal in animals with low 25D. Osteomalacia was seen only in animals fed zero vitamin D. OMT was constant in animals fed between 50 and 500ng/d vitamin D and trabecular bone volume (BV/TV) was positively related to circulating 25D ($R^2=0.51$, $P<0.001$), but not to $1,25(OH)_2D_3$ or PTH levels. Animals fed 50 to 100ng/d vitamin D (mean 25D <80nmol/L) had lower BV/TV ($P<0.05$) and increased osteoid surface ($P<0.05$) and osteoclast surface ($P<0.05$) compared to animals fed higher vitamin D. Bone RANKL:OPG was negatively related to 25D (R:O = -0.001*25D + 0.3, R^2 0.19, $P<0.01$) and positively related to osteoclast surface (OS = 15*R:O + 2.87, R^2 0.22, $P<0.005$). By multiple linear regression 25D was inversely related to osteoclast surface ($P<0.002$), but circulating 1,25D was not. In conclusion, animals fed adequate dietary Ca, required >80nmol/L to prevent bone loss. Our data support the hypothesis that bone cells utilise serum 25D, presumably to synthesise 1,25D for paracrine action rather than responding to circulating 1,25D.

OR6

Accelerated bone resorption, due to dietary calcium deficiency, promotes tumour growth in a murine model of breast cancer bone metastasis

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The skeleton is a major site of breast cancer metastases. High bone turnover increases risk of disease progression and death. However, there is no direct evidence that high bone turnover is causally associated with the establishment and progression of metastases. In this study, we investigate the effects of high bone turnover on tumour growth in a rodent model of bone metastasis.
Female nude mice commenced a diet containing normal (0.6%) ('Normal-Ca') or low (0.1%) ('Low-Ca') calcium content. Mice were concurrently treated with vehicle or recombinant osteoprotegerin (OPG; 1mg/kg/day sc; n = 16/group). Three days later (day 0), 50,000 MDA-MB-231-TXSA cells were implanted by intra-tibial injection and mice were followed until day 17.
PTH and TRAcP5b levels, indicating secondary hyperparathyroidism and high bone turnover, which was maintained until day 17. Treatment with OPG increased serum PTH but profoundly reduced bone resorption. On day 17, in mice receiving 'Low-Ca' alone, lytic lesion and tumour area and cancer cell proliferation increased by 43%, 24% and 24%, respectively compared to mice receiving 'normal Ca' ($p<0.01$). In contrast, OPG treatment completely inhibited lytic lesions, reduced tumour area, decreased cancer cell proliferation and increased cancer cell apoptosis.

We conclude that increased bone turnover, due to dietary calcium deficiency, promotes tumour growth in bone, independent of the action of PTH. These findings have clinical implications as breast cancer patients, much like the older population in general, frequently have a low dietary calcium intake and high bone turnover.

OR7

Osteoclastogenesis inhibitors modulate experimental osteoarthritis

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Subchondral bone remodelling in osteoarthritis (OA) predicts progression of disease and the need for joint replacement surgery. Monoiodoacetate (MIA)-induced arthritis initiated by intra-articular injection of the glycolytic inhibitor MIA is a model of joint injury. MIA-induced arthritis leads to chondrocyte loss, cartilage matrix degradation, subchondral bone remodelling and osteophyte formation.

AIMS: The aims of this study were to investigate the effect of osteoclast inhibitors on evolution of MIA-induced arthritis.

METHODS: Wistar rats were injected into the left knee joint with MIA while the right knee was injected with saline vehicle. At the same time, the rats were treated either with saline (control), osteoprotegerin (10 mg/kg S.C. twice weekly) or zoledronate (0.5 μg/kg S.C. once every other week). The rats were sacrificed at 14 or 28 days. Control and target knee joints were evaluated by plain x-rays and histology using a modified Mankin score. CTX-II serum levels were measured by ELISA.

RESULTS: At 14 days, zoledronate but not OPG, reduced damage to cartilage structure, chondrocyte integrity and proteoglycan content by 20 – 30%. Zoledronate also prevented the increase in CTX-II after joint injury (135 pg/mL vs 80 pg/mL, p=0.027). Zoledronate and OPG reduced osteoclast numbers and subchondral bone remodelling by 60 and 90% respectively. OPG dramatically reduced tidemark invasion at 14 and 28 days and prevented bone marrow fibrosis and bone remodelling. Both OPG and zoledronate alleviated radiological progression.

CONCLUSION: Modulating bone remodelling with osteoclastogenesis inhibitors attenuates radiological and histological progression of experimental OA. Zoledronate appears to exhibit additional chondroprotective properties.

OR8

Sexual dimorphism in radial and longitudinal bone growth differ by tempo and magnitude: a study in male-female co-twins pairs

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An individual's bone trait position on the sample distribution influences fracture risk. Fracture risk is lower in males than females, in part due to sex differences in bone achieved during growth. We studied within pair differences in bone structure and mass in 66 dizygotic (DZ) boy-girl twin pairs aged 7 – 18 years to test the hypothesis that sexual dimorphism in bone traits emerge at puberty and are negligible prior to puberty. Total body and lumbar spine (LS) BMC and mid-femoral shaft (FS) dimensions were measured using DEXA. Regional BMC was acquired from the total body scan. Height (Holtain Stadiometer), and limb lengths (Harpenden anthropometer) we measured. 45 twin pairs were pre- and 21 pairs were or peri/post-pubertal based on Tanner staging (pubic hair, genital development). Within pair comparisons were performed using unpaired t-tests.

Contrary to the hypothesis, sexual dimorphism in bone structure and TB BMC was evident before puberty. LS width (not height) was greater in boys than girls before puberty (3.3 ± 0.03 v 3.0 ± 0.03 mm, $p < 0.0001$). Similarly, mid-FS periosteal widths (not femoral length) were higher in boys than girls (15.1 ± 0.3 v 14.5 ± 0.3 mm, $p < 0.1$). TB BMC was higher in boys than girls (1138 ± 28 v 1027 ± 29, $p < 0.01$). These existing differences remained during and after puberty.

Sexual dimorphism in bone structure and BMC are detectable prior to puberty challenging the notion that the dimorphism is driven only by differences in sex hormones.

OR9

Bone mass tracks strongly from childhood to adolescence

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It has been hypothesised that bone mass tracks but long term studies in children have not been done. The aim of this eight year longitudinal study was to describe tracking of bone mass from age eight to age 16 years and whether this was independent of change in body size.

183 subjects had anthropometric and DXA measures at age eight and age16-yrs. Bone mineral content (BMC), areal bone mineral density (aBMD) and bone mineral apparent density (BMAD) was assessed at the spine, hip, and total body by a Hologic QDR2000 densitometer.

Over the eight year period, BMC increased 192-285% in males and 168-189% in females, whereas aBMD increased similarly for both males and females (36-61% versus 35-61% respectively). Spine and hip BMAD increased 17% and 4% for males, and 22% and 21% respectively for females ($p<0.05$ for sex difference), whereas total body BMAD declined in both sexes (males: −6%, females: −4%). All DXA measures, except total body BMAD, tracked significantly from childhood to adolescence in both sexes after adjustment for change in height and weight (males: R^2: BMC = 55-72%; aBMD = 37-48%; BMAD = 31-52%, females: R^2: BMC = 41-71%; aBMD = 21-59%; BMAD = 25-50%) (all $p<0.01$).

In conclusion, DXA measures tracked moderately to strongly from childhood to adolescence with measures at age eight explaining up to 72% of the variation in adolescent bone mass. This was independent of change in body size. These results suggest a propensity to osteoporosis may be detectable in early childhood.

OR10

Clinical nomogram for individualizing 5-year and 10-year risk of fracture

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The translation of risk factors for fracture into a prognostic model that can be used in primary care setting has not been well realized. The present study sought to develop a nomogram that incorporates non-invasive risk factors to predict 5-year and 10-year absolute risks of fracture for an individual woman and man.

A sample of 2216 (1358 women) participants aged 60+ years, part of Dubbo Osteoporosis Epidemiology Study, were analyzed. Baseline measurements included age, femoral neck BMD, prior fracture, a history of falls and body weight. Between 1989 and 2004, 426 women and 149 men had sustained a low-trauma fracture. Two prognostic models based on the Cox's proportional hazards analysis were considered: model 1 included age, BMD, prior fracture and falls; and model 2 included age, weight, prior fracture and falls.

Model I (AUC=0.75 for both sexes) performed better than model II (AUC = 0.72 for women and 0.74 for men). Using the models' estimates, various nomograms were constructed for individualizing the risk of fracture. If the 5-year risk of 10% or greater is considered "high risk", then virtually all 80 years old men with BMD T-scores < -1.0 or 80 years old women with T-scores < -2.0 were predicted to be in the high risk group. A 60 years old woman's risk was considered high risk only if her BMD T-scores ≤-2.5 and with a prior fracture.

In conclusion, assessment of fracture risk for an individual can not be based on BMD alone, because the combination of clinical factors could substantially elevate an individual's risk of fracture. The nomograms presented here can be useful for individualizing the short- and intermediate-term risk of fracture and identifying high-risk individuals.

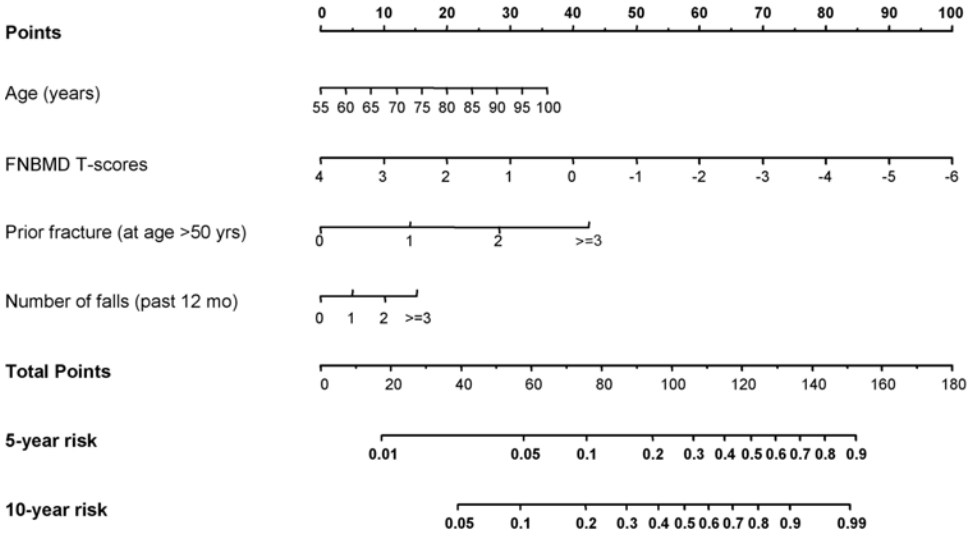

Figure: Nomogram for predicting 5-y and 10-y probability of any osteoporotic fracture for individual women

Source of financial support: National Health and Medical Research Council, Australia

OR11

Bone varies its spatial distribution rather than its mass to optimise strength and minimize bulk

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We hypothesized that (i) variability in cortical thickness (CTh) around the cross sectional (CS) perimeter, *NOT* the average C.Th determines cortical area (CA, indicator of compressive) and section modulus (Z, indicator of bending strength). (ii) The amount of material needed to achieve a given CA is minimized by varying CTh rather than having a constant CTh. Slices were made every 300 µm along the FN in post-mortem specimens from 13 females (mean age 69 years) using micro-CT. CTh was measured at every degree around the CS. For the entire FN, average CTh, SD of CTh distribution (CTh SD), CA, total CS area (TCSA) and Z were computed. The FN CS perimeter was directly measured and an estimated cortical area in the hypothetical situation where the CA was uniformly distributed was calculated. CTh SD *not* the average CTh was a determinant of CA and Z, respectively r = 0.76 and 0.65 ($p<0.01$). The estimated CA derived assuming a uniformly distributed CTh around the CS (as commonly done) was 26 ± 6% ($p < 0.05$) larger than the true CA. The greater the CTh SD, the greater the difference between the calculated and the true CA; that is, the greater the minimization of CA. We inferred that varying CTh minimizes the amount of material needed to optimise strength while avoiding the energy cost incurred by bulk. The CTh SD is a preferred measure over average CTh.

OR12

Hypovitaminosis D and parathyroid hormone response in the elderly: effects on bone turnover and mortality

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Background: Vitamin D deficiency is common in the elderly and usually leads to secondary hyperparathyroidism. However, not all elderly subjects respond in this way and therefore may have altered bone turnover, fracture risk and mortality.
Methods: We measured baseline serum 25 hydroxyvitamin D (25OHD), serum intact parathyroid hormone (PTH), serum aminoterminal propeptide of type I collagen (PINP) and serum carboxyterminal telopeptide of type I collagen (CTX-I) in 1054 institutionalized older people. Deaths and fractures were recorded prospectively.
Results: In the presence of hypovitaminosis D (25OHD <39 nmol/L), subjects with serum PTH levels ≤66.2 pmol/L (termed 'functional hypoparathyroidism', n=487) had significantly higher serum calcium, albumin and 25OHD levels and lower serum creatinine and bone turnover markers than individuals showing a hyperparathyroid response (PTH >66.2 pmol/L; n=425). Mortality was lower in vitamin D-deficient subjects with 'functional hypoparathyroidism' than in those with secondary hyperparathyroidism (HR=0.73, 95% CI: 0.60-0.88; P=0.001) after adjusting for age, sex, immobility, cognitive function, co-morbidities, number of medications and weight. All subjects with serum PTH levels ≤ 66.2 pg/L (n=629) were similar with regard to both bone turnover and mortality, independent of their actual vitamin D status. No differences in fracture incidence were observed between the two groups after adjusting for immobility, cognitive function, weight, residence and history of fracture (HR=0.92, 95% CI: 0.66-1.28; P=0.62).
Conclusion: Absence of secondary hyperparathyroidism in the presence of hypovitaminosis D appears to be common in the frail elderly and is associated with longer survival, similar to that observed in vitamin D replete elderly subjects.

OR13

The effect of a one-year randomized resistance exercise and walking intervention on the prevention of bone loss in older men

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In two previous studies[1,2], we have demonstrated a positive effect of resistance training on bone mineral density (BMD) in postmenopausal women. The aim was to examine the effect of a one-year resistance-training program on bone mass in 143 men aged 55 to 80 years, who were randomised to either a supervised resistance-training (S) or unsupervised walking program (W), conducted three times per week. In addition the S group were randomised so one arm exercised while the alternate arm was the non-exercise control. Resistance exercises were selected to strengthen the ipsilateral forearm, hip and spinal regions. BMD was measured at the whole body, hip, spine and both forearm sites at 0, 6 and 12 months. At baseline there was no significant difference in BMD between the two exercise groups. Retention was 86% and exercise compliance 70% for the S group. The S or W intervention had no effect on BMD at any site measured at 6 or 12 months. In addition no site-specific effects were observed on the forearm. The S group did however increase their lean body mass (1.5±2.7% $p<0.05$) compared with the W group (-0.3± 2.1% $p<0.05$) at 12 months. Muscle strength also increased significantly ($p<0.05$) in the S group but not in the W group. Both exercise interventions may have been sufficient to maintain bone mass. There was a clear treatment effect for muscle but not for bone mass. Therefore the stress/strain relationship may not be the same for men as it is in women.

[1]Kerr, D., et al. (2001). J Bone Miner Res **16**(1): 175-81.

[2]Kerr, D., et al. (1996). J Bone Miner Res **11**(2): 218-25.

OR14

Depression and falls: Geelong Osteoporosis Study

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Psychotropic agents known to cause sedation have been shown to be associated with falling. However, it is not clear whether depression is an independent risk factor. This study investigated the association between depression and falls risk in a population-based sample of women living in the community.
In this observational study, 101 women with depression and 775 healthy controls were drawn from an age-stratified, randomly-selected sample of women (aged 20-92 yr). Using a semi-structured interview (SCID-I/NP), depression was documented for the 12-month period prior to study visit; current psychotropic use, regular alcohol consumption and falling history were self-reported. Participants were classified as fallers if they had fallen to the ground at least twice during the same 12-month period.
Forty-five women (5%) were classified as fallers. Fallers were older [62 (IQR 50-74) *vs* 52 (38-67) yr, p=0.009] and more likely to have depression (27% *vs* 11%, p=0.001) and use psychotropic medication (33% *vs* 15%, p=0.001) than non-fallers. No difference in depression severity or alcohol consumption was detected. Those with depression were younger [48 (36-60) *vs* 54 (39-69) yr, p=0.02]. Psychotropic agents were associated with falls (age-adjusted OR 2, 95% CI 1-5, p=0.008). Age-adjusted odds for falling were 3.6-fold greater for women with depression (OR=3.6, 95% CI 1.7-7.3, p<0.001) and this relationship remained significant after adjustment for psychotropic drug use (OR=2.8, 95%CI 1.3-6.2, p=0.009).
Depression was associated with an increased risk of falling, independent of psychotropic drug use. Further research into the reasons for this association is warranted and planned.

OR15

Hypercalcaemia and undetectable serum PTH: a case of primary hyperparathyroidism due to secretion of a mutant PTH

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We report a case of primary hyperparathyroidism in whom pre-operative serum PTH was consistently undetectable because of an acquired truncating mutation that was restricted to the tumour-associated PTH gene.
A 59 year-old female presented with a 2 year history of musculoskeletal pain and polyuria. Serum calcium was elevated at 3.0 mmol/L (normal range 2.20-2.55 mmol/L) and serum intact PTH was consistently undetectable <3 ng/L (normal 3-50 ng/L, Immulite). Serum 1,25-dihydroxyvitamin D was normal on one occasion 82 pmol/L and elevated on another 208 pmol/L (normal 38-162 pmol/L). Urinary calcium excretion was elevated at 11.41 mmol/d (normal 1.25-7.5 mmol/d). Serum PTHrP, ACE, and IEPG were normal. A CT scan revealed a mass inferior to the right thyroid lobe and sestaMibi uptake co-localized to this area. At surgery a 4.0 g right inferior parathyroid adenoma was removed, and three normal glands were identified. The patient became normocalcemic within 24h of surgery and PTH was subsequently detected in serum at a normal level. Histopathology was consistent with a parathyroid adenoma albeit negative for PTH staining; a surrounding rim of normal parathyroid did stain strongly for PTH. Sequencing of the tumour PTH gene revealed a missense mutation CGA to TGA at codon 83 (R83X) that predicts a premature truncation after the 52nd amino acid in the secreted PTH peptide (ie "PTH1-52").
To our knowledge, this is the first case described of a parathyroid adenoma producing a mutant PTH that causes hypercalcaemia, but is not detected by two-site PTH assays in routine use.

OR16

A phase 1 genomewide association study in osteoporosis

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Background: Genomewide association studies (GWAS) are a powerful method for identifying genes of small to moderate effect involved in common heritable diseases. Apart from some genes of large effect, such as *LRP5*, most genes involved in BMD variation have yet to be identified. We sought to identify those genes, performing a GWAS study in individuals selected with extreme BMD values.

Method: A phase 1 GWAS was completed in 69 high (+1.5<Z-score<+4) and 66 low (-4< Z-score<-1.5) BMD cases genotyped for 317,000 SNPs using the Illumina HumanHap300 chip. All cases were women >50 years, >5 years postmenopausal. A phase 2 study is underway.
Results: For a range of different genetic models this study of 135 subjects has equivalent power to a cohort study of 850-920 unselected individuals. The overall genotyping success rate was 99.6%; a duplicate sample was identical at all 315953 successful genotypes. Many genes previously associated with osteoporosis were identified in this screen at significance levels that would carry them forward into phase 2, including *Alox12*, *ANKH*, *COL1A1*, *ESR1*, *IL-6*, *Klotho*, *LRP5*, *LRP6*. Nine SNPs in *NELL-1* achieved P<0.024, strongly suggesting that *NELL-1* is associated with BMD. Transgenic mice over-expressing *NELL-1* develop craniosynostosis, and have accelerated osteoblast differentiation and mineralizing activity. All three SNPs in *Sp7* encoding osterix were BMD-associated (p=0.02-0.0009). No difference could be identified comparing British and Australian samples; thus British historic controls can be used for comparison with Australian cases.
Conclusion: GWAS using efficient cohort selection designs are a powerful, cost-effective method for identifying BMD-associated genes.

OR17

RANKL/RANK/OPG in breast cancer metastasis to bone

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Receptor activator of NFκB ligand (RANKL) has recently been shown to stimulate the migration of epithelial cancer cells via its receptor, receptor activator of NFκB (RANK) and play an important role in the tissue-specific metastatic behaviour of breast cancer cells (Jones et al., Nature 2006). Here, we hypothesized that RANKL facilitates tumour metastasis to bone and that expression levels of RANKL:RANK:OPG (osteoprotegrin) correlate with disease burden and outcome in the clinical setting. To determine whether the expression of RANKL, RANK and OPG directly correlates with breast cancer metastases to bone we performed immunohistochemistry on tissue microarrays of breast, lymph node and bone biopsies from 100 cases of breast cancer both with and without bone and lymph node metastases. Expression levels of RANKL, RANK and OPG were assessed semi-quantitatively and scored according to their relative staining intensities (0=no staining; 1=weak staining; 2=strong staining). Preliminary results confirm that primary breast tumours, with and without bone metastases, abundantly express RANK. Interestingly, bone lesions exhibited markedly higher RANK expression as compared to the corresponding primary tumours (50% of bone lesions had a score=2 versus 7.7% and 18.5% of primary tumours). Furthermore, a significant proportion of bone lesions (91.7%) showed higher RANKL expression levels as compared to their respective primary breast tumours (61.5% and 74.1%). Taken together our data add strength to the notion that RANKL/RANK signaling plays a crucial role in the tissue-specific metastasis of breast cancer to bone.

OR18

Imatinib mesylate inhibits bone formation and decreases bone mass in vivo by inhibiting PDGFR-mediated osteoblast mitogenesis

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Imatinib mesylate, an orally active inhibitor of the c-abl (including bcr/abl), c-kit and platelet-derived growth factor receptor (PDGFR) tyrosine kinases, is in clinical use for the treatment of chronic myeloid leukaemia (CML) and gastrointestinal stromal cell tumours (GIST). Genetic interruption of both c-kit and c-abl signaling in mice induces osteopenia, and treatment with PDGF increases bone mass in rodents, suggesting that imatinib might have adverse effects on the skeleton. In vitro, imatinib exerts complex effects in bone cells. It inhibits the proliferation of osteoblast-like cells induced by both low-dose serum and PDGF. Neither addition of SCF, the c-kit ligand, nor siRNA-mediated knockdown of c-abl, alters osteoblast mitogenesis. Imatinib dose-dependently increases osteoblast differentiation, also by inhibiting PDGFR signaling, an observation that might explain the early increase in bone formation markers observed in humans with CML starting therapy with imatinib. In murine bone marrow cultures, imatinib inhibits osteoclastogenesis stimulated by 1,25 dihydroxyvitamin D_3, and partially inhibits osteoclastogenesis induced by treatment with RANKL and M-CSF. Consistent with these findings, imatinib partially inhibits osteoclastogenesis in RANKL-stimulated RAW-264.7 cells. Treatment with imatinib increases the expression of OPG in bone marrow from CML patients, and osteoblastic cells in vitro. In order to elucidate the skeletal effects of imatinib in vivo, we treated 6 month old Wistar rats with vehicle, imatinib 40mg/kg/day or imatinib 70mg/kg/day, for 5 weeks. Trabecular bone volume, assessed at the proximal tibia by micro-CT, declined by 20% in the imatinib-treated rats (p<0.05 vs vehicle). Serum osteocalcin (Rat-Mid) fell by 40% in response to imatinib therapy (p<0.01 vs baseline). There was no evidence for an anti-resorptive effect of imatinib in vivo (CTX-I). Collectively, these results suggest that the dominant skeletal action of imatinib in vivo is inhibition of osteoblast mitogenesis by interruption of PDGFR signaling, leading to decreased bone formation and a decline in bone mass. Clinical studies should be undertaken to assess the effect of long-term imatinib therapy on bone mass in humans. These results also suggest that PDGFR signaling in osteoblasts may regulate bone mass in vivo.

OR19

Androgens act directly via the androgen receptor in mineralising osteoblasts to maintain trabecular bone in male mice by regulating bone turnover

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The aim of this study was to define the target cells of androgen action via the androgen receptor (AR) in bone using mice in which the AR is deleted specifically in terminally differentiated, mineralising osteoblasts (mOBL-ARKOs).
mOBL-ARKO mice were generated by breeding floxed AR mice with osteocalcin-Cre mice. Male mOBL-ARKOs and littermate controls were assessed by histomorphometry in the distal femoral metaphysis at 6, 12 and 24 weeks of age (n=10/group).
Deletion of the AR specifically in mineralising osteoblasts resulted in 22-38% decreases in trabecular number (TbN) at 6, 12 and 24 weeks of age ($P<0.05$) in the secondary spongiosa. At 6 weeks of age, this loss of TbN resulted in a 36% decrease in trabecular bone volume (BV/TV) ($P<0.01$). In contrast, BV/TV in mOBL-ARKOs did not differ from controls at 12 and 24 weeks of age, as trabecular thickness was maintained or increased ($P<0.05$). The loss of TbN observed at all ages is likely to be attributed to increased bone resorption, evident from 12 weeks of age as measured by serum X-laps ($P<0.05$). Although X-laps was higher in 6 week old mOBL-ARKOs, this was not significant ($P=0.07$). BV/TV in the primary spongiosa was unaffected in mOBL-ARKOs indicating that new bone accrual is not dependent on the AR in mineralising osteoblasts.
Our findings conclusively demonstrate that in addition to the well-established actions of androgens via aromatisation to estrogen, androgens also act directly via the AR in mineralising osteoblasts to maintain trabecular bone mass by regulating bone turnover.

OR20

Oncostatin M is an essential stimulus of bone formation and osteoclastogenesis

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Murine oncostatin M (OsM) signals through gp130 and a specific OsMR expressed by osteoblasts. OsM is reported to increase osteoclastogenesis by stimulating RANKL production by osteoblasts, but the effects of OsM on bone formation are not defined.
To determine whether OsM is essential in vivo, we studied OsMR deficient mice. Male and female OsMR null mice demonstrated a 75% increase in trabecular bone volume in distal tibia and lumbar vertebrae. Femoral trabecular BMD was also elevated by 40% in males and females. This was associated with a significant reduction in osteoclast surface and reduced resorption. Bone formation was also reduced, with significant reductions in osteoid thickness, osteoblast surface and bone formation rate. In contrast, marrow adipocyte number was elevated 4 fold.
Consistent with this phenotype, OsM treatment dose-dependently increased alkaline phosphatase activity and mineralization by Kusa4b10 stromal cells compared to untreated controls, while adipocyte formation was more than halved. Real time PCR analysis demonstrated a 20-fold increase in expression of the transcription factor C/EBPd within 1 hour of OsM administration. C/EBPd synergises with runx2 to enhance osteocalcin transcription; consistent with this we observed a dose-dependent increase in activity of a 6xOSE-luciferase reporter construct. Furthermore, local injection of OsM over the calvariae of young wild type mice significantly increased calvarial thickness and bone formation rate indicating an anabolic effect in vivo.
This reveals an inhibitory role for OsM in adipocyte differentiation and a critical role in stimulating bone formation in vivo.

OR21

Neuropeptide Y protects the skeleton from bone loss induced by stress

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Neuropeptide Y has anxiolytic actions, attenuating the psychological effects of stress. The Y2-receptor, which mediates these anti-stress actions, also regulates bone. We investigated whether NPY could modulate skeletal responses to stress.
Stress responses in $NPY^{-/-}$ mice were examined using established behavioral models and showed anxiety-related parameters were much greater in $NPY^{-/-}$ mice. Skeletal responses to stress were examined at 14 weeks in male $NPY^{-/-}$ and wildtype mice after 4 week low and high stress protocols. The "stress" protocol involved regular handling, including rectal temperature, glucose tolerance test, 24h fasting and metabolic cage studies.
In low stress groups whole body, femoral (53 ±2 mg/mm^2 vs 65 ±2, $p<0.05$) and tibial BMD was greater in $NPY^{-/-}$ than wild type mice, with greater cortical volume and thickness. Femoral cancellous bone volume (16.7 ±1.5% vs 11.6 ±0.9, $p<0.01$) and mineral apposition rate (2.8 ±0. μm/d vs11.9 ±0.1, $p<0.0005$) were greater in $NPY^{-/-}$ mice.
In the "stressed" groups, body weight was reduced in $NPY^{-/-}$ mice (30.1 ±1g vs 25.5 ±1, $p<0.0001$) with no decrease in wildtype. Trabecular number reduced in wildtype without significant loss of cancellous bone volume. Stress reduced bone volume in $NPY^{-/-}$ mice (16.7 ±1.5% vs 13.2 ±0.7, $p<0.01$) and mineral apposition rate (1.6 ±0.1 μm/d vs 2.4 ±0.1, $p<0.0001$), both remained greater than wildtype.
NPY plays a powerful role in increasing bone during times of plenty and may inhibit loss due to stress in times of famine. This may explain a mechanism for bone loss associated with stress in otherwise healthy individuals.

OR22

Osteoclast formation and osteolysis are promoted by the AMP-activated protein kinase (AMPK) activator aminoimidazole-4-carboxamide ribonucleoside (AICAR) *in vivo*

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AMPK serves to maintain whole body and cellular energy balance in response to hormonal and metabolic stress signals. We investigated the effects of pharmacological activation of AMPK by AICAR on osteoclasts (OCs) *in vitro* and *in vivo*.

We studied AICAR effects on osteoclastogenic cultures of murine bone marrow and spleen cells stimulated by RANKL plus M-CSF or co-cultured with osteoblasts stimulated with dihydroxyvitamin D3, and RANKL-stimulated RAW264.7 cells. AICAR effects *in vivo* were studied using daily AICAR (500mg/kg) or saline (controls) intraperitoneal injections in male 12 week old C57Bl/6 mice, and their tibia examined by histomorphometry after 28 days.

AICAR (200uM) increased OC formation 40% in bone marrow cultures maximally stimulated by RANKL (100ng/ml) and M-CSF (30ng/ml); when 20ng/ml RANKL was employed AICAR increased OC formation up to 50-fold. AICAR also increased osteoclastogenesis in RANKL-stimulated spleen cells, bone marrow macrophages (BMM), RAW264.7 cells and osteoblast/bone marrow co-cultures. AICAR did not alter OC survival or dentine resorption per OC. AICAR stimulated phosphorylation of AMPK substrate acetyl-CoA carboxylase in BMM. *In vivo*, trabecular bone mass (BV/TV) was reduced (49.5%) in AICAR treated mice, with 2.2-fold greater OC numbers (OcS/BS). Trabecular number (Tb.N) was decreased but trabecular thickness (Tb.Th) was unaltered. Osteoblast numbers (ObS/BS) were also 2.8-fold higher with AICAR treatment, with increased osteoid surface.

Thus, AICAR promotes OC formation and causes high turnover osteopenia *in vivo* with increased OC numbers and a coupled increase in osteogenesis. These results are consistent with a role for AMPK in bone physiology by modulating OC differentiation.

OR23

Adiponectin knock-out mice have increased trabecular number and bone volume at 14 weeks of age

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Adiponectin, a hormone secreted by adipocytes, regulates energy homeostasis and glucose and lipid metabolism. Plasma levels of adiponectin are negatively correlated with body fat mass. Adiponectin inhibits the formation and activity of osteoclasts and increases the proliferation and differentiation of osteoblasts *in vitro*. The aim of our study was to determine the bone phenotype of adiponectin knockout mice.

Male adiponectin-deficient (Ad-KO) and wild-type (WT) C57BL/6J mice were sacrificed at 8, 14 and 22 weeks of age. Body weights did not differ between Ad-KO and WT mice. We scanned the left proximal tibia using micro-CT at 5μm resolution and analysed bone microarchitecture by 3D analysis.

We found significant increases in trabecular bone volume (BV/TV) (15.9±1.63 vs 12.2±0.72%, p=0.02) and trabecular number (3.20±0.18mm^{-1} vs 2.32±0.12mm^{-1}, p=0.0009) in 14-week old Ad-KO mice compared to controls. Similar differences between WT and Ad-KO were present in 8 and 22-week old animals but these did not reach statistical significance. Trabecular thickness was significantly greater (0.053±0.001mm vs 0.048±0.002mm, p=0.04) in 22-week old Ad-KO mice compared to WT.

Ad-KO mice have increased number and volume of trabeculae at 14 weeks of age indicating that the net effect of adiponectin on bone accrual *in vivo* is inhibitory. These effects are age-dependent. Our data concur with the observations from epidemiological studies in humans that adiponectin negatively correlates with both fat mass and bone mass. Therefore, adiponectin may be a contributor to the link between fat and bone mass.

OR24

Adverse effects of valproate on bone: defining a model to investigate the pathophysiology

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Background: Over the past four decades anti-epileptic drug (AED) therapy has been shown to significantly decrease bone mass and increase fracture rates. A multi-factorial mechanism of bone loss is likely but the exact mechanism through which AEDs exert their effects on bone is controversial, partly due to the lack of a well-established animal model for the disorder.

Aim: To develop a mouse model of AED-induced bone disease to investigate the effects of AEDs on total bone mineral content (BMC) and to characterize the structural bone changes associated with AED treatment.

Methods: Five strains (n=60 per strain; 30 per diet) of adult mice were placed on a 0 or 4 g/kg valproate (VPA) diet for 16 weeks. Following treatment *in vivo* BMC measures were acquired using dual-energy x-ray absorptiometry; *ex vivo* volumetric bone measures and structural changes were assessed using peripheral quantitative computed tomography (measurement taken 5%, 15% and 50% distal to the tibial growth plate) and histomorphometry (proximal tibia).

Results: Compared with controls, C3H/HeJ animals treated with VPA had reductions of 9.1% ($p<0.01$) in BMC; 10.7% ($p<0.01$) in trabecular density; 19.6% ($p<0.05$) in trabecular volume; 14.3% ($p<0.04$) in trabecular number; and a 19.9% increase in trabecular separation ($p<0.05$). No differences were found in the A/J strain.

Conclusion: We successfully identified and validated the first animal model of AED-induced bone loss, identifying both sensitive and resistant mouse strains. This model provides a powerful tool to investigate the pathophysiology of AED-associated bone disease and will facilitate the design of therapeutic strategies for its treatment and prevention.

OR25

RUNX2 trinucleotide repeat mutations are associated with decreased bone density and altered protein function

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The RUNX2 transcription factor is essential for osteoblast differentiation and chondrocyte maturation. A unique feature of RUNX2 is the polyglutamine and polyalanine (poly Q/A) domain encoded by a trinucleotide repeat sequence. Such repeat sequences are prone to strand slippage resulting in high mutation rates. We hypothesized that mutations within the RUNX2 poly Q/A domain would exist and these variants would be associated with altered bone density and protein function. 4361 DNA samples were obtained from multiple epidemiological studies of bone density. A total of 21 subjects were identified as being heterozygous for a wild type 23Q/17A allele and a poly Q/A repeat variant allele. Deletions (15Q and 16Q) and insertions (30Q and 23A) were identified. Collectively Q/A repeat variants presented with significantly lower Femoral Neck BMD (DEXA) displaying a 0.65 SD decrease ($n = 21$, $p = 0.0004$) and lower bone density as measured by quantitative ultrasound of similar magnitude (-0.79 SD, $n = 8$, $p = 0.031$). Functional analyses revealed the RUNX2 mutants did not have any obvious changes in DNA binding capacities. However, reporter gene analysis using the mouse osteocalcin promoter revealed significant decreases in the transactivation function of 16Q and 30Q. In addition the 16Q and 30Q RUNX2 proteins displayed defective nuclear localisation compared to WT. We have identified a new class of functionally relevant RUNX2 variants that occur at collective frequency of ~0.5%. These mutations are associated with significantly lower bone density and altered protein function.

OR26

TWEAK inhibits human osteoblast differentiation and modulates the activity of TNF-α on osteoblast behaviour, in part through mitogen activated kinase (MAPK) induction of sclerostin expression

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The TNF superfamily member TWEAK is pro-angiogenic, pro-inflammatory and proliferative for endothelial and other cell types. We recently showed that TWEAK is a novel mediator of mouse collagen-induced arthritis (CIA)(1). Our current studies have demonstrated that human primary osteoblasts (NHBC) express both high levels of the TWEAK receptor, as well as TWEAK mRNA and protein. In this study, we investigated the functional effects on TWEAK *in vitro*, as well as the pathways involved in TWEAK signaling and compared these to the classic inflammatory mediator, TNFα.

Exposure to TWEAK resulted in increased NHBC proliferation, an effect also seen with TNFα, prolonged suppression of osteogenesis-related genes and inhibition of *in vitro* mineralisation. TNFα displayed only transient suppression of these genes, with an eventual osteogenic effect. Interestingly, TWEAK and TWEAK/TNFα induced the expression of the Wnt signalling inhibitor, sclerostin, a possible mechanism for the observed inhibitory effect of TWEAK on NHBC differentiation and *in vitro* mineralisation.

Signalling experiments on serum-starved cells revealed that TWEAK elicited phosphorylation of c-terminal Jun kinase (JNK), while TNFα predominantly induced ERK1/2 phosphorylation. MAPK inhibition studies showed that TWEAK-induced sclerostin transcription was strongly dependent on JNK phosphorylation and to a lesser extent on phosphorylation of ERK1/2. The mitogenic effect of TNFα was strongly phospho-ERK1/2 dependent, however the TWEAK response was only partially dependent. Our results suggest that TWEAK is a physiologic regulator of human osteoblast activity and it may alone, or in combination with TNFα, confer a defective osteoblast phenotype in pathologic conditions, such as rheumatoid arthritis.

1. Perper SJ, Browning B, Burkly LC, Weng S, Gao C, Giza K, Su L, Tarilonte L, Crowell T, Rajman L, Runkel L, Scott M, Atkins GJ, Findlay DM, Zheng TS, Hess H 2006 TWEAK is a novel arthritogenic mediator. J Immunol **177**(4)**:**2610-20.

OR27

Potential role of Rab3D-calmodulin interaction in osteoclastic bone resorption

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Osteoclastic bone resorption is a highly dynamic process that requires the tight ordering of intracellular trafficking events in order to maintain the structural and functional polarization of the ruffled border and basolateral domains. Rab3 proteins are a subfamily of GTPases, known to mediate membrane transport in eukaryotic cells and play a role in exocytosis. Our recent data indicates that Rab3D modulates a post-TGN trafficking step that is required for osteoclastic bone resorption (1). Here, to identify down-stream regulatory molecules of Rab3D, we have performed a yeast two-hybrid screen. Amongst several candidate Rab3D-interacting proteins identified, Rab3D was found to associate with calmodulin, an established regulator of osteoclastic bone resorption. As an

initial effort to better define the interaction between Rab3D and calmodulin, we generated several mutants of Rab3D which interfere with the GDP/GTP nucleotide exchange (Rab3DQ81L, Rab3DN135I) and/or membrane attachment of Rab3D (Rab3D-CXC). By *in vivo* bioluminescence resonance energy transfer (BRET) assay, Calmodulin was found to associate equivalently with wild-type Rab3D as well as Rab3DN135I and Rab3DCXC variants. Overexpression of constitutively-active Rab3D (Rab3DQ81L) enhanced this interaction suggesting that the active form of Rab3D (i.e. GTP-bound) might recruit additional effector molecules which further potentiate its binding to calmodulin. In an attempt to address the impact of calmodulin activity on Rab3D-calmodulin interaction and osteoclastic bone resorption, we performed complementary BRET and *in vitro* bone resorption assays in the presence of the calmodulin inhibitor, calmidazolium chloride. Interestingly, we show that suppression of calmodulin activity via calmidazolium chloride impairs the association of Rab3D with calmodulin, an affect that correlates with a disruption in osteoclastic bone resorption. We propose that the recruitment of calmodulin by Rab3D might be an important requirement for osteoclast-mediated bone resorption.

1. Pavlos, N.J., Xu, J, Riedel, D., Yeoh, J.S.G., Teitelbaum, S.L., Papadimitriou, J.M., Jahn, R., Ross, F.P., Zheng, M.H. (2005) *Mol. Cell. Biol* **25**, 5253-5269

MOST OUTSTANDING CLINICAL ABSTRACT

OR28

Prediction of vertebral body bone strength: the contribution of individual trabecular elements

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Micro-CT imaging enables measurement of bone microarchitecture and subsequently mechanical strength of the same sample. It is possible using micro-CT data to perform morphometric analysis on individual rod and plate bone trabeculae using a volumetric spatial decomposition algorithm and hence determine their contribution to bone strength.

Forty eight pairs of vertebral bodies were harvested from 12 human cadavers and bone cubes (10mmx10mmx10mm) were obtained. After micro-CT imaging, the volumetric spatial decomposition algorithm was applied. Mean rod and plate thickness (<Ro.Th>, <Pl.Th>) mean rod and plate length (<Ro.L>, <Pl.L>), mean rod and plate volume (<Ro.V>, <Pl.V>), BV/TV, total rod volume (Ro.BV/TV) and total plate volume (Pl.BV/TV) were calculated for each sample. Bone strength was measured in compression, where one sample from each pair was tested supero-inferiorly (on-axis) and the paired sample was tested antero-posteriorly (off-axis).

BV/TV was the strongest predictor of on-axis ($r^2=0.77$, $p<0.0001$) and off-axis strength ($r^2=0.54$, $p<0.0001$), respectively. Prediction of on-axis strength was improved to $r^2=0.90$ with the addition of <Ro.L>, <Pl.Th> and Pl.BV/TV. Prediction of off-axis strength was improved to $r^2=0.92$ with the addition of <Ro.V>, <Ro.L> and the ratio of <Ro.V> to <Pl.V>.

Microarchitectural measures of individual trabeculae that contribute to bone strength have been identified. In addition to the contribution of BV/TV, trabecular rod morphology contributes 38% to prediction of off-axis strength, whereas measures of trabecular plate and rod morphology contribute 13% to prediction of on-axis strength. Decomposing vertebral body bone architecture into its constituent elements enables identification of the critical components that determine bone strength.

MOST OUTSTANDING BASIC ABSTRACT

OR29

Osteoblast-targeted disruption of glucocorticoid signalling delays intramembranous bone development *in vivo*

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Transgenic expression of 11beta-hydroxysteroid dehydrogenase type 2, driven by a 2.3kb collagen type I promoter (Col2.3-HSD2), abrogates intracellular glucocorticoid signalling in mature osteoblasts. To investigate the effects of osteoblast-targeted disruption of glucocorticoid signalling on early intramembranous and endochondral skeletal development, we analysed calvaria and long bones of wild-type (WT) and Col2.3-HSD2 transgenic (tg) mice aged 0, 1, 7, 10 and 14 days.

HSD2 mRNA and protein expression was present in all tested bones of transgenic mice but absent in those of WT mice. In marked contrast to WT littermates, tg mice had poorly-formed parietal bones with substantial cranial cartilage present at day 0. The cranial cartilage plate was present in all tg mice but absent in WT mice at day 1. By day 7, the cranial cartilage plate was reduced in size but still present in tg animals. Transgenic calvaria appeared normal by day 14. TUNEL staining indicated reduced chondrocyte apoptosis and immunohistochemical analyses showed reduced protein expression of MT1-MMP, an enzyme essential for calvarial cartilage removal, in the cranial cartilage of tg mice. In contrast, no phenotype was observed in the long bones of tg mice.

Our results indicate that osteoblast-targeted disruption of glucocorticoid signalling results in delayed intramembranous bone development without affecting endochondral bone. These findings further suggest that osteoblasts regulate cranial cartilage removal in neonatal mice, a function that appears to be glucocorticoid-dependent and mediated through activation of chondrocytic MT1-MMP expression. Our studies point to a novel role for both glucocorticoid and osteoblasts in intramembranous bone development.

OR30

A RCT of the effects of vitamin D and calcium on bone structure and muscle strength in older women with vitamin D insufficiency

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Aim: To evaluate the relative importance of vitamin D and calcium treatment on bone and muscle in patients with vitamin D insufficiency.
Methods: 302 elderly women (age 77.2±4.6 years) with a serum 25OHD concentration less than 60 nmol/L participated in a 1 year randomised, double-blind, placebo controlled trial. Subjects received 1000 mg calcium citrate per day and 1000 IU ergocalciferol (Vitamin D) or identical placebo (Calcium).
Results: At baseline 25OHD was 44.7±12.6 nmol/L, this increased in the Vitamin D group but not the Calcium group (59.8±13.8 vs 45.0±13.3 nmol/L, P<0.001). At 1 year the Calcium group significantly improved hip and total body BMD (+0.18±0.22% and +0.42±0.21% P<0.05 cf baseline) respectively, as did the Vitamin D group (hip +0.52±0.26%; whole body BMD +0.44±0.22% P<0.05 cf baseline) with no inter group differences. P1NP, a measure of bone turnover, decreased in both groups (Vitamin D -3.71±4.40%; Calcium -5.52±3.79% P<0.05 cf baseline) with no inter group differences. However in those with baseline hip muscle strength values in the lowest tertile Vitamin D improved muscle strength compared to Calcium (hip extensors 22.6±9.5%; hip adductors 13.5±6.75% P<0.05 cf Calcium).
Conclusion: Elderly women with low vitamin D status have no extra benefit from vitamin D on bone structure or turnover compared to calcium alone. However vitamin D treatment but not calcium improved hip muscle strength in those with low baseline values. Calcium citrate supplementation should be recommended for all elderly women for bone benefit, vitamin D should be added to those with insufficiency to assist muscle function.

OR31

Osteal macrophages: novel regulators of bone formation

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Resident tissue macrophages dwell in many tissues and are important in immunity, homeostasis and repair. We have observed mature macrophages (referred to as OsteoMacs) on the surface of human trabecular bone. Detailed analysis of F4/80$^+$ (resident tissue macrophage marker) OsteoMac distribution in mouse bones demonstrated that these cells are present in all bone lining tissues. Strikingly, 77±1.6% of the endosteal cortical bone forming osteoblast (OB, collagen type I$^+$ and osteocalcin$^+$) surface was covered by a F4/80$^+$ cell 'canopy', suggesting these cells regulate OB function. We examined the effect of OsteoMacs on OB functional responses to elevated extracellular calcium (eCa^{2+}), as eCa^{2+} is unique to the bone microenvironment and induces macrophage expression of the osteo-inductive molecule BMP-2. Using a co-culture system of macrophages with OBs, in either direct contact or separated using transwells, we demonstrated that OB mineralization occurred in response to eCa^{2+} only in the presence of macrophages. Conditioned medium from eCa^{2+} stimulated macrophages also induced mineralization of OB cultures, indicating that macrophages produce a soluble factor that enhances OB mineralization. However addition of recombinant noggin or soluble BMPR1 to co-cultures did not inhibit macrophage-induced OB mineralization, indicating that BMP-2 is not the osteo-inductive molecule. These observations suggest macrophages detect changes in eCa^{2+} and consequently produce soluble factor(s) that drive OB mineralization. We propose that OsteoMacs are an integral component of osteal tissues and that they play a novel role in bone homeostasis through regulating OB function and orchestrating bone formation.

P – POSTER PRESENTATIONS

P01

OPG and a variant of OPG, EGX-010, prevent bone loss in the ovariectomised rat model

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Although osteoprotogerin (OPG) inhibits bone resorption, administration of OPG also interferes with the TRAIL cancer cell surveillance system. An OPG variant, EGX-010, differing by one amino acid from OPG, has been shown not to interfere with TRAIL signaling. The aim of this project was to compare the potency *in vivo* of EGX-010 with the OPG in inhibiting bone loss due to ovariectomy. Ten week old female mice (N=60) were assigned to ovariectomy (OVX) or Sham-OVX groups. Prior to operation, tibias from each animal were scanned by micro-CT followed by daily injections of either EGX-010 (1mg or 5mg/kgBW/day), OPG (1mg or 5mg/kgBW/day) or vehicle for 3 days. Injections continued for 14 days post-operations before tibias were repeat scanned by micro-CT and killed. Serum crosslaps and alkaline phosphatase were markedly reduced by both OPG and EGX-010 treatments at 1mg and 5mg/kgBW/day, suggesting that resorption and formation processes were both reduced. At the concentration of 1mg/kgBW/day, both compounds significantly increased bone volume when compared to untreated OVX and Sham-OVX animals. In the EGX-010-treated animals, 50% increase in BV/TV was due to increased Tb.Th and Tb.N. Only 20% increase in BV/TV with 5mg/kgBW/day EGX-010 was due to greater inhibition of bone formation due to the higher dose. Osteoclast surface measurements, however, were not reduced by either OPG or EGX-010 treatment, despite inhibited resorptive activity, suggesting that existing osteoclasts are inhibited from resorbing bone. In conclusion, EGX-010 has equal potency to OPG in preventing bone loss and may prove to be an important treatment of metabolic bone diseases.

P02

14-3-3 protein isoforms interact differentially with the calcium-sensing receptor intracellular tail

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The main role of the calcium-sensing receptor (CaR) is to maintain calcium homeostasis but the receptor also participates in other functions such as cell proliferation and apoptosis mediated by various intracellular signalling pathways. To provide insight into mechanisms that control CaR signalling, yeast two-hybrid (Y2H) studies in our laboratory, using the CaR tail as bait, identified a number of interacting proteins including the 14-3-3 isoforms zeta and theta. 14-3-3 proteins are ubiquitously expressed and highly conserved, and are emerging as a group of multifunctional adapter proteins with a recognised role as chaperones. 14-3-3 proteins bind to numerous partner proteins having a preference for phosphorylated targets. Y2H deletion mapping studies have delineated the interaction region for 14-3-3 zeta to residues 923-1078 in the CaR tail. By contrast, the 14-3-3 theta interaction site is confined to residues 865-923 in the CaR tail. This region of 14-3-3 theta interaction contains a consensus 14-3-3 binding motif that includes a phosphorylated serine, residue 895. *In vivo* interaction between the CaR and 14-3-3 theta has been demonstrated by co-immunoprecipitation in mammalian cells. In addition, a direct *in vitro* interaction has been confirmed between 14-3-3 theta and the CaR tail using pulldown assays. Site-directed mutagenesis and subsequent co-immunoprecipitation experiments have shown that the phosphoserine residue (S895) in the identified consensus motif in the CaR tail is not primarily responsible for mediating CaR and 14-3-3 theta interaction. Differential binding of the 14-3-3 isoforms to the CaR tail points to differences in the way these isoforms might influence CaR-mediated signalling.

P03

Serum testosterone in elderly men measured by liquid chromatography-tandem mass spectrometry and radioimmunoassay: concordance and effect on epidemiologic association

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Serum testosterone is used as a measure to define androgenic status and its association with health outcomes. Radioimmunoassay (RIA) and liquid chromatography-tandem mass spectrometry (MS) are common methods for measuring testosterone. This study examined the concordance between testosterone measured by RIA and MS, and the effect of this concordance on the study of association between testosterone and fracture risk.
Testosterone was measured by RIA (RIA-T) and MS (MS-T) in 593 men aged 60+ years, who had participated in the Dubbo Osteoporosis Epidemiology Study between 1989 and 2005 during which fracture incidence was recorded. The coefficient of concordance between RIA-T and MS-T was 0.67 ($p<0.001$). The Deming's regression equation (assuming equal variance) was log(RIA-TT)=-0.52+1.19*log(MS-TT), with the coefficient of correlation being 0.75. On average, RIA-T were significantly lower than MS-T by 5% (0.36 pmol/L), with 95% confidence limit being 68% lower to 178% higher (12.2 pmol/L lower to 11.5 pmol/L higher) than MS-T. Among men with low levels of MS-T, RIA-T tended to over-estimate MS-T by around 5%, while among those with high

levels of MS-T, RIA-T tended to under-estimate MS-T by around 25%. Each SD of log MS-T was significantly associated with fracture risk with hazard ratio [HR] being 1.37 (95% CI: 1.15, 1.60), which was slightly higher than the association observed between log RIA-T and fracture risk (HR: 1.19; 95% CI: 1.02, 1.40).
These data suggest that there was a modest concordance between MS-T and RIA-T, and this had minor effect on the assessment of associations between testosterone and fracture in epidemiologic studies.

P04

The vitamin K homologue menatetrenone (K2) provides the optimal conditions for the differentiation of primary human osteoblasts into osteocyte-like cells

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Vitamin K homologues are currently under study for their roles in bone metabolism and as potential therapeutic agents for skeletal diseases. Current data suggest that vitamin K2 has a protective role in post-menopausal bone loss, alone and in combination with anti-resorptives. Vitamin K is a known cofactor in the conversion of peptide-bound glutamate residues to γ-carboxyglutamate (Gla) residues in target proteins, such as osteocalcin (OCN) whose Gla residues confer high affinity binding to hydroxyapatite. We have investigated the effects of phytonadione (K1) and menatetrenone (K2) on primary human osteoblasts (NHBC) derived from adult trabecular bone. Both homologues promoted *in vitro* mineralisation, with K2 reproducibly increasing apposition of calcium into cell monolayers to approximately two-fold that of controls. Increased apposition of hydroxyapatite was demonstrated by EDS analysis. Vitamin K also time-dependently increased the transcription of mature osteoblast/osteocyte markers, including sclerostin and dentin matrix protein (DMP)-1. Incubation with K1 or K2 resulted in an increased number of viable cells after long-term culture, suggesting that both K2 and K1 promote the survival of mature osteoblastic cells. K2 at 5 μM promoted cell associated mineral apposition in a 3D collagen culture. Furthermore, these cells had the morphological appearance of osteocytes. Taken together, the results are consistent with vitamin K, in particular K2, inducing a maturational effect on human osteoblasts, thereby increasing the prevalence and survival of an osteocyte-like phenotype.

P05

Evidence that human cartilage and chondrocyte do not express calcitonin receptor

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Calcitonin has been recently shown to have a direct protective effect on articular cartilage against joint degenerative disease. It has been proposed that calcitonin might act through the calcitonin receptor (CTR) to activate the cyclic AMP pathway and protect type II collagen degradation. In this study, we examined the presence of the calcitonin receptor in human articular cartilage and chondrocytes and investigated the potential pharmacological effects and transduction pathway of salmon calcitonin in human chondrocytes. Five human articular cartilage samples were examined for the expression of the CTR by polymerase chain reaction (PCR), immunostaining and Western blotting. Cyclic AMP levels in human chondrocyte stimulated with salmon calcitonin were measured by ELISA. The effect of salmon calcitonin on the gene expression profiles, including aggrecan, type II collagen, matrix metalloproteinase (MMP)-1, MMP-3 and MMP-13, of human chondrocytes was also examined by Real-time PCR. It was shown that CTR was not detectable in human cartilage and chondrocytes. The cAMP level in human chondrocytes *in vitro* was significantly increased by forskolin (100μM) by >10 folds ($P<0.001$), but was not induced by salmon calcitonin (10^-7M, 10^-8M, 10^-9M). Real-time PCR demonstrated that salmon calcitonin tended to reduce the gene expression of MMPs, yet without statistical significance. In contrast to previous reports, our data showed that human cartilage and chondrocytes do not express calcitonin receptors. There was no direct effect of salmon calcitonin on human chondrocytes. It suggests that the chondroprotective effect of calcitonin observed *in vivo* may be indirect via its effect on subchondral bone resorptive activity.

P06

Characterisation of RANKL gene regulation in human T cells

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Activated T cells are a pathologic source of RANKL (receptor activator of NF-κB ligand) and stimulate osteoclastogenesis in vitro and in vivo. Given the critical role of RANKL in osteoclastogenesis it is essential we understand its gene regulation.
AIM- Investigate RANKL gene regulation in human T cells to identify strategies to prevent pathologic RANKL expression.
Methods/results: *In silico* and *in vitro* analysis revealed 3 major RANKL mRNA transcripts, including the original membrane RANKL (NM_00369.1) and 2 novel transcripts encoding a secreted RANKL protein. Expression of these transcripts was examined in activated primary human T cells and secreted RANKL mRNA variants were the predominantly expressed transcripts. Using specific inhibitors, signal transduction pathways important for RANKL mRNA expression were investigated. When primary human T cells were activated in the presence of cyclosporin A (calcineurin inhibitor), RANKL expression was ablated, suggesting the calcineurin/NFAT signalling pathway is critical for RANKL mRNA expression. Similarly, SB2390963 (p38MAPK inhibitor)

attenuated RANKL expression by 50%, suggesting the p38MAPK/AP-1 pathway, is also important for RANKL mRNA expression. Promoter analysis revealed NFAT/AP-1 sites in secreted and membrane RANKL putative promoters and standard promoter reporter assays confirmed sensitivity to cyclosporin A.
Conclusions: Activated T cells predominantly produce secreted RANKL, which negates the requirement for direct cell contact in the regulation of osteoclastogenesis, and may be a distinguishing mechanism between physiologic and pathologic bone resorption. We also provide evidence suggesting RANKL expression in T cells is regulated by NFAT/AP-1 transcription factors, indicating yet another role for these transcription factors in osteoclast biology.

P07

Progressive recruitment of amino acids with successively larger side-chains contribute to the calcium-sensing receptor's extreme sensitivity to Ca^{2+}

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The calcium-sensing receptor (CaR) is activated not only by elevated extracellular Ca^{2+} concentration (Ca^{2+}_o), but also allosterically by L-amino acids. Previous studies have shown that L-amino acids require a threshold Ca^{2+}_o concentration for their activating effects; however, Ca^{2+}_o threshold concentrations for different amino acids have not been clearly defined. In the current study, we have compared the Ca^{2+}_o requirements CaR activation by amino acids with small and large side-chains. We used two cell model systems: CaR-expressing HEK293 cells and normal human parathyroid cells and find that the Ca^{2+}_o threshold for activation is dependent on side chain size. Fura 2 loaded HEK-CaR cells were exposed to L-Ala or L-Phe over the Ca^{2+}_o concentration range, 0.5 – 3.0 mM. In the absence of amino acids, the Ca^{2+}_o threshold for intracellular Ca^{2+} oscillations was 2.0 mM. In the presence of L-Ala, the Ca^{2+}_o threshold fell to 0.8 mM; however, in the presence of L-Phe it was clearly higher at around 1.2 mM. Data for the smallest amino acid Gly resembled that for L-Ala and data for the bulky hydrophobic amino acid L-Trp resembled that of L-Phe, indicating a positive relationship between side-chain size and the Ca^{2+}_o threshold. Interestingly, although L-Ala and Gly had lower Ca^{2+}_o thresholds for activation, L-Phe and L-Trp were significantly more potent, suggesting that once bound, the activators with the largest side-chains are the slowest to dissociate from the VFT binding site. Similar results were obtained in fura 2 loaded parathyroid cells. The results indicate that as the Ca^{2+}_o rises from the sub-physiological level of 1.0 mM to the supra-physiological level of 1.5 mM additional amino acids are recruited to the CaR's amino acid binding site to markedly enhance the receptor's sensitivity to Ca^{2+}_o.

P08

Bone turnover and loss is significantly associated with inflammatory biomarkers in older adults: a longitudinal study

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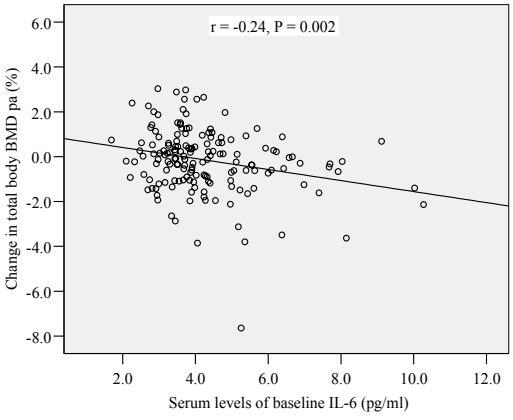
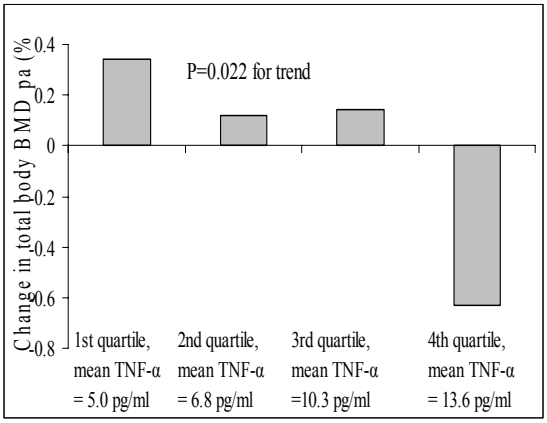
Objective: To determine the associations between serum inflammatory markers and changes in bone mineral density (BMD) and urinary pyridinoline/creatinine ratio (PYR/Cr) over 2.9 years in older subjects.
Methods: A total of 168 randomly selected subjects (mean 63 years, range 52-78, and 48% female) were studied. Total body, lumber spine and total hip bone mineral density (BMD) was measured by DXA at baseline (mean T-score: -0.18 to -0.61) and 2.9 years later. Serum high-sensitivity C-reactive protein (hs-CRP), interleukin (IL)-6, and tumor necrosis factor- α (TNF-α) and urinary PYR and Cr were also measured on both occasions.
Results: The mean annual change in BMD was -0.15% to -0.34%. In multivariable analysis, change in total body and spine BMD was negatively associated with baseline hs-CRP, IL-6 and TNF-α, as well as change in hs-CRP and IL-6 (all P<0.05). If these markers were put in the same predictive model, only associations of IL-6 remained largely unchanged. Change in hip BMD was significantly associated with baseline IL-6 in men and change in IL-6 in women. Lastly, baseline PYR/Cr ratio was positively associated with baseline TNF-α (P<0.01) but not the other markers; however, change in PYR/Cr ratio was positively associated with baseline IL-6, hs-CRP and change in IL-6 and hs-CRP (all P<0.05) in women, not in men.





Conclusions: Variation within the generally low levels of inflammatory markers especially IL-6 observed in this study predicts bone

loss and bone turnover suggesting targeted anti-inflammatory therapy may be an important strategy for the prevention of osteoporosis.

P09

Saturated fatty acids inhibit osteoclastogenesis

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There is a growing literature exploring the links between lipids and bone. Total body fat mass is positively related to bone density and fracture risk. There is also evidence that fat ingestion influences bone turnover. Although some effects are mediated by endocrine mechanisms, it is possible that lipids might act directly on bone. To address this question, we have used primary cultures of osteoblasts, bone marrow and neonatal mouse calvariae.

Saturated fatty acids, assessed at 0.1, 1.0 and 10 μg/mL, inhibit osteoclastogenesis in bone marrow cultures. This effect is related to fatty acid chain length, and is maximal for palmitic acid (C16) (160 ± 15 vs 324 ± 22 osteoclasts/well; $P<0.01$). The introduction of two or more double bonds abrogates the effect, and may even lead to its reversal. ω3 and ω6 fatty acids have comparable low activity. Primary cultures of fetal rat osteoblasts display modest stimulation of thymidine incorporation, indicating that this is not a non-specific toxic effect. There is also no effect of these fatty acids on bone resorption in neonatal mouse calvariae, indicating that the effect is on osteoclastogenesis rather than on the activity of mature osteoclasts.

Our findings demonstrate that fatty acids can suppress osteoclastogenesis, as well as stimulating osteoblast proliferation. This provides a novel link between lipid and bone metabolism, which might contribute to the positive relationship between adiposity and bone density, as well as providing novel targets for pharmaceutical and nutriceutical development.

P10

Cxcl1 is a target of PTH/PTHrP in committed osteoblasts

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The parathyroid hormone receptor (PTHR1) functions in the regulation of bone mass in an autocrine /paracrine (PTHrP) or endocrine (PTH) manner. In this study, a mouse gene micro-array was performed to determine novel PTH-induced signaling molecules involved in bone biology. A mouse bone marrow stromal cell line committed to the osteoblast lineage (4b10 cells) was treated with PTH(1-34) or PTHrP(1-141) for 1, 6 and 24 hours, RNA was extracted and used to probe the Affymetrix whole mouse genome micro-array. The resulting 15272 probes were screened for intracellular signalling molecules expressed >2.5 fold in response to PTH(1-34). A total of 101 probes (targeting 43 genes) were determined. Of interest is a novel PTH target gene that has emerged from this study, the CXC chemokine ligand 1 (Cxcl1) also commonly known as keratinocyte cytokine and suggested to be the mouse homolog of interleukin-8. Cxcl1 was induced 13.2 fold in response to 1 hour PTH(1-34) treatment and this induction has been validated by real-time RT-PCR using independent RNA from 4b10 cells (15- to 40-fold) and mouse calvarial osteoblast cells (up-to 300-fold). The effects of transcriptional and translational blockers suggest that Cxcl1 is an immediate primary response gene of PTH. The mechanism for Cxcl1 induction by PTH and its potential role in bone biology is being investigated.

P11

Glucocorticoids stimulate Wnt expression in mature osteoblasts

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Glucocorticoids (GC) and Wnt/beta-catenin signalling have both been shown to promote osteoblastic differentiation. However, a direct relationship between these signalling pathways has not been fully established.

Transgenic (tg) overexpression of 11beta-hydroxysteroid dehydrogenase type 2 (HSD2), a GC inactivating enzyme, under the control of a 2.3kb collagen type I promoter (Col2.3-HSD2) abrogates intracellular GC signalling in mature osteoblasts. Previously, we have demonstrated that calvarial cell cultures derived from Col2.3-HSD2-tg mice exhibit reduced osteoblastogenesis and retarded Wnt/beta-catenin signalling. Here, we used calvarial cultures of Col2.3-GFP and Col2.3-GFP-HSD2 tg mice to investigate the GC-dependent regulation of Wnts in mature osteoblasts by isolating GFP positive cells using FACS to yield mature osteoblasts. These were then grown in serum free media and treated with 10^{-8}M corticosterone. In Col2.3-GFP cultures, mRNA expression (by real time PCR) of Wnt7b was upregulated by 4 and 3.5-fold, and of Wnt10b by 2.5 and 3-fold, compared to controls, following 2 and 4 hrs of treatment, respectively. Up-regulation of Wnt7b and Wnt10b expression was blocked by the addition of cycloheximide, suggesting the requirement of de novo protein synthesis. Col2.3-GFP-HSD2 tg cultures have low expression of either Wnt and the addition of 10^{-8}M corticosterone had no effect on Wnt expressions. In contrast to mature osteoblasts, enriched precursor cells derived from either WT or tg calvaria have only a low expression of Wnt10b and Wnt7b was not detected.

Overall, intact GC signalling in mature osteoblasts, in association with transcriptional regulatory complexes, is essential to stimulate Wnt expression and hence osteoblastic differentiation.

P12

Transgenic disruption of glucocorticoid signalling in mature osteoblasts attenuates KRN serum-induced arthritis *in vivo*

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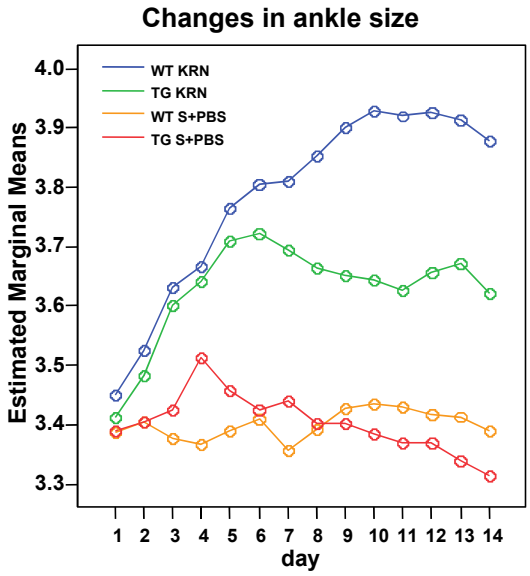
Transgenic over-expression of 11beta-hydroxysteroid dehydrogenase type 2, controlled by a 2.3Kb collagen type I promoter (Col2.3-HSD2-tg), abrogates intracellular glucocorticoid signalling exclusively in mature osteoblasts. Since glucocorticoids are important immune modulators, we investigated the impact of osteoblast-targeted disruption of glucocorticoid signalling on joint inflammation and bone catabolism using the KRN serum transfer model of autoimmune arthritis.

KRN arthritis was induced in 5-week-old male Col2.3-HSD2-tg mice (tg-KRN, n=28) and wild-type (WT) littermates (WT-KRN, n=27). Controls (12 tg and 13 WT) received normal serum or PBS. Body weight and ankle size (clinical arthritis, scored 0-12) were assessed daily from induction (day 0) to day 14. IL-1beta, IL-2, IL-4, IL-6, IL-10, IL-12, TNF-alpha, IFN-gamma, G-CSF, M-CSF and corticosterone serum levels were determined on days 7 and 14. End-point bone volume of the tibia was assessed using micro-computerised tomography.

Both tg-KRN and WT-KRN developed acute arthritis; however, the inflammatory response was significantly blunted in tg-KRN from day 7 (Fig. 1). On day 14, tibia bone volume was significantly reduced in WT-KRN but not in tg-KRN versus controls. On day 14, serum TNF-alpha, IL-6 and IL-12 levels were significantly lower in tg-KRN versus tg. Mean serum M-CSF levels were significantly lower on day 14 versus day 7 in tg-KRN but not in WT-KRN. However, cytokine, corticosterone or ACTH levels in tg-KRN and WT-KRN did not differ at any time-point, suggesting that the inflammatory process was attenuated through local mediators in Col2.3-HSD2-tg mice.

We conclude that osteoblasts can modulate local inflammatory responses significantly via a glucocorticoid-dependent pathway.



P13

Potential involvement of ephrinb2 in the anabolic action of PTH in osteoblasts

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The aim of the study was to identify paracrine factors under the control of PTH and PTHrP using the mouse marrow stromal cell line, Kusa4b10, that acquires osteoblast features under differentiating conditions. After the appearance of functional PTHR1 cells were treated with either PTH(1-34) or PTHrP(1-141) for 1, 6 and 24 hours and RNA subjected to Affymetrix whole genome mouse arrays. The data was analysed statistically by SAM and genes that were differentially expressed greater than 1.5 fold investigated. Among genes belonging to the family of ephrins and their receptors, ephrinB2 and EphB2 were up-regulated. These responses were validated using independently prepared RNA from differentiated Kusa4b10 cells, UMR106-01 rat osteogenic sarcoma cells, and primary mouse calvarial osteoblasts, as well as *in vivo* using RNA from metaphyseal bone of 3 week-old rats following a single PTH injection. Stimulation of ephrinB2 mRNA was maximal by 6 hours with a 3-4 fold response while EphB2 was increased 2 fold *in vitro* after 6 hours. This effect was dose-dependent and sensitivity increased with inhibition of cAMP phosphodiesterase. Constitutive expression remained constant throughout differentiation. Western blotting showed a sustained increase in ephrinB2 protein following PTH treatment. Recent evidence implicates osteoclast-derived ephrinB2 acting through its receptor, EphB4, on osteoblasts to promote osteoblast differentiation, and suppression of osteoclastogenesis through reverse signaling. The present data raise the possibility that ephrinB2 might act in a paracrine or autocrine manner in osteoblasts under the influence of PTH, and provide a local event related to the anabolic action of PTH.

P14

Strontium ranelate promotes osteoblast differentiation resulting in an enhanced osteocyte-like phenotype in mineralised cultures of human primary osteoblasts

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Strontium ranelate (SR) is a new treatment for osteoporosis that reduces the risk of hip and vertebral fractures in post-menopausal women. Clinical studies, and studies in animal models, have demonstrated that SR increases bone formation, while decreasing bone resorption. While there is increasing evidence of an important role of osteocytes in bone metabolism, the ability of SR to modulate osteocyte differentiation and activity has not been studied. The aim of this study was therefore to investigate the effect of SR in adult primary human osteoblasts (NHBC) in culture, and their differentiation into osteocyte-like cells.
NHBC were cultured under conditions permissive for *in vitro* mineralisation in the presence of SR (1-10 mM Sr^{2+}). Cultures were assayed for *in vitro* mineralization, expression of human osteoblast differentiation markers, alkaline phosphatase (AP) and STRO-1, and the expression of osteoblast differentiation genes, including the osteocyte genes dentin matrix protein (DMP)-1 and sclerostin.
SR time- and dose-dependently increased the percentage of cells that were negative for AP and STRO-1 expression, a phenotype consistent with that of osteocyte-like cells. Concomitant with SR-induced mineralisation, DMP-1 transcription increased (up to 150-fold) in a time- and dose-dependent manner. Similarly, mineralizing cultures also expressed sclerostin mRNA, which together with DMP-1 expression, is strongly suggestive of the presence of increased numbers of osteocyte-like cells. SR also elicited a marked OPG secretory response. This study provides new insight into the mechanism of action of SR on cells of the osteoblast lineage, and suggests the possibility that SR promotes the differentiation and/or survival of osteocytes.

P15

Effects of a phosphodiesterase 4 (PDE4) inhibitor on bone mass: mechanism of action in human osteoblasts

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Glucocorticoids (GCs) are used for the treatment COPD, however, GCs may be associated with decreased bone density. Alternative anti-inflammatory agents such as PDE4 inhibitors are under investigation for COPD. In a mouse model, the specific PDE4 inhibitor rolipram enhanced bone mass, though the cellular mechanism of action is unclear.
This study investigated the targeted PDE4 inhibitor roflumilast for its potential to modulate human bone cell formation.
Human osteoblastic MG63 cells were cultured in DMEM supplemented with 10% FBS and adapted to serum-free medium for 24 h before adding experimental agents. Roflumilast was tested at 10^{-10} to 10^{-6}M. Replicative responses of cells to roflumilast were measured by tritiated thymidine incorporation. Differential expression of receptor activator of NFκB-ligand (RANKL) and osteoprotegerin (OPG), which regulate osteoclast generation, was observed using real-time PCR. Human peripheral blood mononuclear cells were cultured on glass coverslips. Differentiation to osteoclasts was induced by RANKL and human macrophage-colony stimulating factor.
Cell replication significantly increased with 10^{-7}M and 10^{-6}M roflumilast ($p<0.001$) comparable to control. Alkaline phosphatase, measured by enzyme activity, increased 2 to 3-fold at 10^{-7}M and 10^{-6}M ($p<0.001$), with some increase at 10^{-10}M. RANKL/OPG mRNA expression ratio was significantly decreased at 10^{-7} and 10^{-6}M roflumilast ($p<0.001$). Up to 10^{-6}M roflumilast osteoblast survival after stress (doxorubicin) was not affected. Over 21 day-cultures, osteoclast generation was blunted with lower doses of roflumilast while at 10^{-7}M and 10^{-6}M bone-resorbing cells increased.
In conclusion, the PDE4 inhibitor roflumilast promoted proliferation and differentiation of osteoblasts and decreased the resorption signals produced by these cells at therapeutic concentrations. Higher roflumilast concentrations facilitated osteoclast production in the presence of fixed, high osteoclast-generating signals. Thus, PDE4 inhibitors may be advantageous to GC with regard to effects on bone mass.

P16

Macrophages persist within primary osteoblast cultures and enhance osteoblast mineralisation

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Primary calvarial osteoblasts (OBs) have been extensively used to characterise osteoblast differentiation *in vitro.* However, the relative heterogeneity of these osteoblast cultures has been largely overlooked when ascribing responsibility for functional outcomes based on experimental findings. The aim of this study was to investigate whether macrophages are retained in osteoblast cultures and if they influence osteoblast function. Microarray analysis of differentiating calvarial OB cultures (day 5, 14 and 21) demonstrated large numbers of macrophage-associated genes at all time points and that OB cultures clustered with macrophages using hierarchical clustering analysis (symatlas.gnf.org). Immunocytochemistry and flow cytometry confirmed that calvarial OB preparations co-isolated F4/80$^+$ mature macrophages that persist and expand during OB differentiation *in vitro*. Multiple passaging didn't eliminate macrophages from these cultures. Bone explant cultures, an alternative approach to generating primary OBs, were similarly shown to contain F4/80$^+$ macrophages. It was the macrophages within bone explant cultures that selectively respond to bacterial stimulus LPS with induction and secretion of TNF. To delineate the cooperative and distinct functional roles of macrophages and OBs, we used magnetic-assisted cell sorting to generate highly enriched calvarial OBs that no longer contained macrophages. Strikingly, macrophage removal resulted in both significantly decreased osteocalcin mRNA expression and *in vitro* mineralisation in enriched OB cultures. In conclusion, we show that macrophages contribute to osteoblast full functional differentiation and promote osteoblast mineralisation *in vitro*. These data provide compelling support for a new role for mature tissue macrophages in the regulation of bone homeostasis.

P17

G-CSF enhances osteoclast differentiation and function

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Osteoclasts (OCs) are critical protagonists for mobilizing the endosteal niche of hematopoietic stem-cells. Granulocyte Colony Stimulating Factor (G-CSF), a widely applied stem-cell mobilizer, possesses a clinically documented risk of bone-loss. If OC activity is required for stem-cell mobilization, G-CSF and potentially other stem-cell mobilizers should enhance OC differentiation and bone-resorption.

In *ex vivo* human OCs, G-CSF stimulated mature bone-resorption activity, and increased early OC size and number. Treatment with RANKL and G-CSF resulted in larger OCs by 7 days when compared to RANKL treated cells ($p=0.0004$). Once RANKL treated OCs had matured (14d) they did not significantly differ in size from OCs treated with G-CSF, suggesting exogenous G-CSF accelerates early OC formation. G-CSF treatment increased TRAP+ multi-nuclear cell counts (7d $p=0.00005$ and 14d $p=0.013$). Using real time PCR analysis, TRAP mRNA content was greater in G-CSF treated OCs, consistent with the increased number of TRAP+ cells ($p=0.007$). Continual RANKL and G-CSF treatment (21 days) increased bone-resorption activity measured by pit area on dentine slices ($p=0.012$). Similarly, addition of G-CSF stimulated bone-resorption in mature purified OCs (G-CSF-naïve), suggesting presence of G-CSF receptor on mature OCs. Gene array showed RANKL induced G-CSF receptor in OCs (12 fold), consistent with the hypothesis that OCs respond to G-CSF as a result of RANKL mediated induction of G-CSF receptor.

In summary, G-CSF increases osteoclast bone-resorption, TRAP expression, cell-size and multinucleation. The osteopenic effect of G-CSF therapy and endosteal hematopoietic stem-cell mobilization may be regulated by the same phenomenon; G-CSF stimulation of osteoclast differentiation and bone-resorption.

P18

The osteogenic sensitivity of skeletal myoblasts corresponds to an increased expression of bone morphogenetic protein receptors

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Numerous studies have demonstrated that muscle cells are capable of adopting an osteogenic profile upon stimulation with bone morphogenetic proteins (BMPs). However, the relative sensitivity of muscle cells versus other cell types to BMP treatment and the mechanism underlying the differing cell sensitivities requires further clarification. To address these issues, we have undertaken a comprehensive *in vitro* study where myoblasts, fibroblasts, and osteoprogenitors were treated in parallel with BMP-2. This study utilized both established cell lines as well as primary cells cultured from C57BL6/J mice.

Osteoprogenitors could be differentiated to express bone markers in the absence of BMP-2 treatment, although markers were greatly enhanced by BMP-2. Myoblasts treated with BMP-2 adopted an osteogenic phenotype, expressing ALP and producing a mineralized matrix. In contrast, fibroblasts responded poorly to BMP-2. We hypothesised that this varying sensitivity may result from differences in the level of BMP receptors (BMPRs). Quantitative real-time PCR revealed that BMPRs were robustly expressed in myoblasts and osteoprogenitors, but not in fibroblasts. BMP-2 treatment caused a further upregulation of BMPRs in myoblasts, indicative of a positive feedback loop, but not in osteoprogenitors.

These findings confirm that muscle cells have a strong and specific capacity to respond to BMP-2 and suggest that this is directly related to their expression of BMP receptors. Our data also indicates that BMP-2 regulation of BMP receptors may be contextually dependent on cell type. These findings have important implications in adding to our current understanding between BMP receptors and the actions of the BMP signalling cascade.

P19

Abnormal chondrocyte and osteoclast morphology in mice with collagenase-resistant type II collagen

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'Knockin mice' with type II collagen resistant to cleavage by collagenases have shorter long bones than wildtype mice (Gauci et al, 2007, Proc. ORS, USA). Between birth and 6 weeks, the growth plate is elongated, and unusually for mice, partial growth plate closure is observed by 6 weeks. The aim of this study was to investigate the morphology of chondrocytes in growth cartilage from collagen II knockin mice. Tibiae from knockin and wildtype mice aged 3 days, 3 weeks and 6 weeks were examined by electron microscopy. At all ages, chondrocytes of knockin mice appeared to undergo normal hypertrophy, with a dramatic increase in volume and apparent dissolution of cytoplasmic contents. As they approached the ossification front, however, rather than dying the chondrocytes appeared to undergo dedifferentiation; their cytoplasm showed a density typical of non-hypertrophic chondrocytes and contained abnormally high numbers of mitochondria. At 6 weeks, dedifferentiated chondrocytes in the lacunae adjacent to the ossification front showed a dramatic increase in the abundance of lysosomes, as did the invading osteoclasts. From 3 weeks, a small number of erythrocytes and haemopoietic cells were visible in the middle of the growth plate, surrounded by dying chondrocytes; these cells appeared to be the precursors of the partial growth plate closure observed at 6 weeks. These observations indicate that physiological death of hypertrophic chondrocytes is dependent on collagenolytic cleavage of the cartilage matrix surrounding them. Chondrocytes and osteoclasts appear to adapt to the failure of normal collagenolysis by increasing their formation of lysosomes.

P20

CRELD2: a potentially novel thrombin-interacting protein and its expression by osteoblasts

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Thrombin participates in the regulation of many physiological and pathological processes via the thrombin-responsive members of the protease-activated receptor (PAR) family of seven transmembrane domain G-protein-coupled receptors. Previously we have demonstrated that thrombin inhibits osteoblast apoptosis by a mechanism independent of the thrombin-responsive PARs but which is dependent upon thrombin's proteolytic activity. In this study we have used yeast-2-hybrid screening to attempt to identify novel thrombin-interacting proteins expressed by osteoblasts. Yeast-2-hybrid screening of an E14.5 day embryonic mouse cDNA library identified 12 clones that interacted with a bait protein consisting of thrombin, containing an active site mutation, fused to the DNA binding domain of the GAL4 transcription factor. Of these clones only one, cysteine-rich with EGF-like domains 2 (CRELD2), gene encoded a protein that has been described as being located extracellularly. PCR analysis of the expression confirmed that CRELD2, along with a second closely related gene CRELD1, was expressed by primary calvarial osteoblasts. Further analysis of the protein sequence of CRELD1 and CRELD2 using the Predictor of Protease Specificity tool, suggests that both these proteins contain potential thrombin cleavage sites. These results suggest that CRELD2 may act to mediate thrombin's PAR-independent effects on osteoblasts.

P21

Rab3D recruits the dynein complex to secretory vesicles in osteoclasts through direct interaction with Tctex-1

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Targeted intracellular trafficking of osteolytic enzymes and degraded bone matrix substrates to/from the ruffled border of bone-resorbing osteoclasts requires coordinated interplay between carrier vesicles, motor proteins and the cytoskeletal network. We have previously shown that Rab3D, an exocytic-related small GTPase, regulates a vesicle transport step that is required for the maintenance of the ruffled border membrane and bone resorption. Here, to investigate the complement of effectors through which Rab3D elicits its biological function we have employed a yeast two-hybrid system to screen for candidate Rab3D-interacting proteins. Using Rab3D as "bait" we identified Tctex-1, a 14 kDa light chain of the dynein motor complex. Specific interaction between Rab3D and Tctex-1 was confirmed by GST-pull-down, immunoprecipitation and colocalization studies. In addition, two-hybrid analyses revealed that Tctex-1 specifically associates with Rab3 family members via an interaction with the Switch II/GTP-binding motif. Consistently, bioluminescence resonance energy transfer (BRET) analyses demonstrated that Tctex-1 preferentially binds to the active (GTP-bound) forms of Rab3s *in vivo*. Overexpression of wild-type and constitutively active Rab3D led to the recruitment of Tctex-1 and other subunits of the dynein/dynactin complex to secretory vesicle membranes. Furthermore, depolymerisation of the microtubule network disrupted the spatial distribution of Rab3D-bearing vesicles and impaired Rab3D-Tctex-1 interaction *in vivo.* These data demonstrate that Tctex-1 is a *bona fide* Rab3 effector and suggest that Tctex-1 may cooperate with Rab3D to recruit the dynein motor complex to membrane micro-domains, thereby regulating the sorting and microtubule-dependent targeting/recycling of post-Golgi secretory vesicles to/from the ruffled border during osteoclastic bone resorption.

P22

IL-33 inhibits osteoclast formation indirectly through osteoblastic cells

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IL-33 is a proinflammatory factor that induces Th2 cytokine production. Its receptor is ST2, and a natural soluble form of ST2 has anti-inflammatory actions. Since IL-33 is related to IL-18 we investigated whether IL-33, like IL-18, inhibits osteoclastogenesis in vitro.
We studied IL-33 effects on murine OC formation from the following cell populations containing haemopoietic progenitors: spleen cells, M-CSF dependent bone marrow macrophages (BMM), and RAW264.7 cells. OC formation was stimulated by treatment with recombinant RANKL (100ng/ml) plus M-CSF (30ng/ml), or by co-culture with 1,25 dihydroxyvitamin D3-stimulated calvarial osteoblasts (OBs) or Kusa O stromal cells.
In RANKL/M-CSF stimulated cultures, IL-33 dose dependently inhibited OC formation from spleen cells. However, OC formation from BMM and RAW264.7 cell populations (which contain no lymphocyte or stromal cell components) was not inhibited by IL-33. This suggests IL-33 does not act directly on haemopoietic progenitors to inhibit OC. In co-cultures of BMM with OBs or Kusa O cells, IL-33 dose dependently inhibited OC formation. This suggests an indirect IL-33 action on BMM differentiation through the osteoblastic cells. IL-33 also inhibited OC formation in cultures of BMM and Kusa O cells that were separated by a porous membrane in the presence of 1,25 dihydroxyvitamin D3 and exogenous RANKL and M-CSF.
Our results indicate IL-33 is a novel OC inhibitor that acts on osteoblastic stromal cells to induce a soluble inhibitor of OC formation. This points to a significant role for IL-33 and its receptor ST2 in bone metabolism.

P23

The calcium-sensing receptor is involved in human osteoblast proliferation and function

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We have previously shown that the calcium-sensing receptor (CaR) is present in human osteoblastic MG63 cells and that changes in extracellular Ca^{2+} concentrations are responsible for a variety of physiological effects in these cells. In particular, elevations in extracellular Ca^{2+} result in increases in both proliferation rate and alkaline phosphatase activity, in addition to decreases of the RANKL:OPG mRNA ratio and susceptibility to cell-death. It has recently been shown that the Ca^{2+}-induced increases in proliferation, and the expression of the immediate early genes c-fos and egr-1 in primary rat osteoblasts are mediated via the CaR [1]. Whether these processes are mediated via the CaR in human osteoblasts has remained unclear.
In order to further investigate the role of the CaR in human osteoblasts we have utilized microRNA technology to develop a stable MG63 cell line that expresses lower levels of CaR. A whole-cell ELISA technique was employed to confirm that the stable MG63 cell line that was developed (MG63/CaR-) expressed significantly less CaR protein when compared to the transfected control (MG63/GW, $p<0.05$). Both of these transfected cell lines were cultured in DMEM with 10% FBS for 24 h and then adapted to serum-free medium for 24 h before experimental treatments were added. When cultured in serum free medium in the presence of 1 mM Ca^{2+} over a 5-day period, MG63/CaR- showed a 5-fold decrease in their proliferation rate when compared to MG63/GW ($p<0.001$). Also, the secretion of OPG from MG63/CaR- was shown to be decreased 1.5-fold when compared to MG63/GW after incubation for 24 h in the presence of 1 mM Ca^{2+} ($p<0.01$). These initial findings suggest that signalling from the CaR is responsible, in part, for the proliferation and rate of secretion of important regulatory factors from human osteoblasts.

1. Chattopadhyay N, Quinn SJ, Kifor O, Ye C, Brown EM. (2007) Biochem Pharmacol.

P24

Osteoclast precursor numbers from peripheral blood are increased in patients with the arthritis mutilans form of psoriatic arthritis

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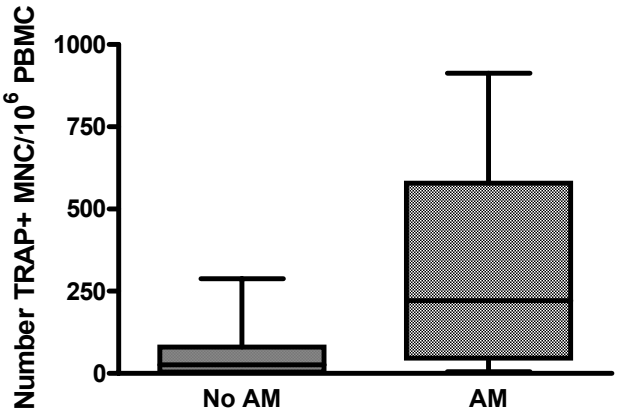
PURPOSE: Increased numbers of osteoclast precursors (OCPs) have been reported in psoriatic arthritis (PsA), possibly associated with abnormalities of bone turnover. Patients with arthritis mutilans (AM) constitute a subset of PsA where bone erosion is most severe. We investigated whether OCP numbers were elevated in radiographically defined AM.
METHODS: Patients with erosive PsA were enrolled (n = 25). Hand and feet radiographs were scored by two observers as AM or not AM, separately and where opinions differed, by consensus. AM was defined as complete erosion of bone on both sides of the joint(s). Peripheral blood mononuclear cells (PBMC) were analyzed for the number of OCPs by flow cytometry (CD14+/CD11b+ cells), and the number of osteoclast-like cells (TRAP positive multinucleated cells) following culture in RANKL and M-CSF. Data were analyzed using Mann Whitney U tests.

RESULTS: There was no difference in the number of CD14+/CD11b+ cells in PBMC from patients with AM and those without AM. Patients with radiographic evidence of AM had higher numbers of TRAP positive multinucleated cells following culture (median 319, range 5-913) than non-AM patients (median 26, range 0-288) (p= 0.058) but the range of values in both groups was wide (Figure).
CONCLUSIONS: Patients with the AM form of erosive PsA appear to have abnormal osteoclast development.



P25
Osteoclast formation is inhibited at therapeutic ranges of NFAT inhibitors FK506 and Cyclosporin A
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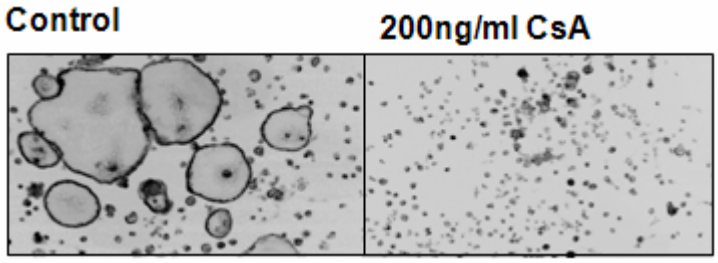
FK506 and Cyclosporin A (CsA) are widely used immunosuppressants that target the activation of NFAT family member proteins. Due to severe side effects including nephrotoxicity and hepatotoxicity, human serum levels for CsA are kept below 700ng (ranging from 100-600ng/ml) and FK506 are kept below 25ng/ml (normal range 5-15ng/ml). These compounds inhibit osteoclast formation and *in vitro* tissue culture doses can be as high as 10000ng/ml for both FK506 and CsA. These values exceed normal serum values by 20-2000 fold. We wished to test if osteoclast differentiation and NFAT-dependent genes were still inhibited by aforementioned therapeutic doses of CsA and FK506.

At doses of between 50 and 500ng/ml of CsA, 50-100% reduction in osteoclast formation and size (number of nuclei per cell) was observed in both human PBMCs and RAW264.7 cells. Gene expression of calcitonin receptor, an NFAT-dependent gene, was reduced by 90% at doses of 200ng or greater of CsA. Moreover, dose-dependant cytotoxic effects were observed at doses of FK506 exceeding 25ng/ml and 1000ng/ml of CsA. At 10000ng/ml of both compounds, almost 100% of cells died.

We show that high levels of CsA/FK506 used are cytotoxic and not necessary to study NFAT in the osteoclast because low levels of CsA/FK506 are sufficient to inhibit both osteoclast formation and NFAT-mediated genes. When using CsA/FK506 for investigating osteoclastogenesis and function, only therapeutic levels of these compounds are recommended.



P26
DMSO potently enhances RANKL-dependant osteoclast formation
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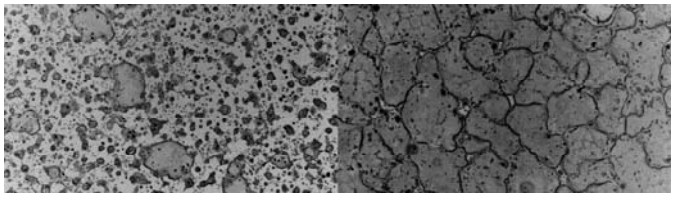
DMSO, a polar oxidant, can induce differentiation of osteoblasts and haematopoietic-based erythroleukemia cells. Since osteoclasts are also of a haematopoietic origin and are responsive to many conditions that osteoblasts respond to, we investigated the effects of DMSO on RANKL-induced osteoclast differentiation. Using RAW264.7 cells treated with RANKL, DMSO generated 5.5 times more osteoclasts than control cells ($p < 0.006$) with maximum formation beginning at 0.35% DMSO. Moreover, DMSO used at concentrations for maximum effect produced osteoclasts that were almost 100% confluent. A dose-response analysis indicated an EC50 of 0.19% DMSO while anything less than or equal to 0.05% DMSO was not significantly different to control cells.

Numerous studies reveal that DMSO-induced differentiation of erythroleukemia cells occurs through phosphorylation and therefore activation of PI3K. Using specific inhibitors for PI3K (LY294002), p38 (SB203580) and MEK1/2 (U0126), we demonstrate that DMSO-enhancement of osteoclastogenesis can be blocked with both LY294002 and SB203580 (both $p < 0.001$) but not U0126 indicating that DMSO targets directly PI3K and p38 or pathways below these signalling proteins but does not function through MEK1/2.

We show that through p38 and PI3K, DMSO can significantly promote osteoclast differentiation. Care must be taken when working with drugs/inhibitors dissolved in DMSO when studying osteoclastogenesis because its potent properties could mask weaker, but still significant effects.



P27

Adenosine receptors can modulate osteoclastogenesis

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Adenosine receptors (ARs) make up a family of four subtypes AR_1, AR_{2A}, AR_{2B} and AR_3. ARs have been reported in monocyte/macrophage cell types and since osteoclasts are derived from the monocyte/macrophage lineage, we investigated whether ARs could play a role in the osteoclast.

In murine bone marrow cells treated with MCSF only (control), only AR2A, AR2B and A3 were found. However on addition of RANKL, AR_1 became expressed, AR_{2A} was upregulated 4 fold while AR2B and A3 did not change significantly. To establish whether ARs could affect osteoclast differentiation, agonists for A_1 (CCPA), A_{2A} (CGS21860), AR_{2B} (NECA) and AR_3 (Cl-IB MECA) were used. At low concentrations, (1-10nM), CGS21680 was able to almost double osteoclast numbers (108 vs 195 respectively for control vs CGS21680, $p = 0.01$) suggesting that the $A2_A$ receptor stimulates this effect. However at higher concentrations (100nM-10uM), NECA increased osteoclast counts to 525 and compared to control (108 osteoclast counts), represented an increase of more than 500% ($p = 0.01$). These findings suggest A_{2A} and A_{2B} are high and low affinity receptors respectively that can enhance osteoclastogenesis.

Next, by eliminating basal adenosine in culture with adenosine deaminase, an increase in the formation of osteoclasts occurred in RANKL-treated cells with CGS21680, NECA or no agonists. It is known that actions of A1/A3 and A2A/A2B oppose one-another therefore it is possible that basal adenosine levels could inhibit osteoclastogenesis via A1/A3 receptors and thus when eliminated, potentiate osteoclastogenesis.

We show that the four known adenosine receptors are expressed in the osteoclast and results attained suggest adenosine receptors can modulate osteoclast differentiation.

P28

The role of the calcitonin receptor in osteoclast formation and function

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Whilst calcitonin is known to inhibit osteoclast (OCL) activity, its effect on OCL formation is less well understood. Our overall aim was to investigate the role of the calcitonin receptor (CTR) in osteoclastogenesis. To address this aim global CTR knock-down and osteoclast specific CTR knock-out mice were generated using the Cre-loxP system. We then compared OCL formation and function in cultures derived *in vitro* from the bone marrows of global CTR knock-down and control animals. For OCL formation assays, cells were stained for tartrate resistant acid phosphatase (TRAP) activity and the number of TRAP+ cells containing 3 or more nuclei were counted. Secondly, immunohistochemistry was used to investigate the effect of calcitonin on actin ring assembly, an essential component of osteoclastic bone resorption. Preliminary results indicate no difference in OCL number between marrow cultures derived from global CTR knock-down mice versus controls (n=2/group, 4 replicates). Treatment of OCL cultures with 0.01uM salmon calcitonin abolished actin ring structures in OCLs derived from control mice whilst global CTR knock-down derived OCLs maintained actin rings. Real time RT-PCR studies are currently being performed to investigate the relative *in vivo* expression of genes associated with osteoclastogenesis. Preliminary data indicate no difference in the ratio of receptor activator of nuclear factor-kB (RANK):receptor activator of nuclear factor-kB (RANKL), RANK:osteoprotegerin (OPG) or RANKL:OPG mRNA in the tibias of osteoclast specific CTR knock-out animals (n=2) and controls (n=8). Taken together, these data are consistent with a view that CTR signalling inhibits OCL activity, but not their formation.

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Wnt signaling antagonists may suppress osteoblast differentiation and function in rheumatoid arthritis

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Osteoclast (OC)-mediated focal bone erosion at the pannus bone-interface is a defining feature of rheumatoid arthritis (RA). The functional capacity of bone-forming osteoblasts (OB) at erosion sites is not known. We hypothesize that (OB)-mediated bone formation is impaired at these sites by factors produced by inflammatory cells in synovium. We utilised the K/BxN serum transfer arthritis model in male C57Bl/6J mice. In situ hybridisation and immunohistochemistry (IHC) for differentiation markers of OBs demonstrated a paucity of mature OBs at the pannus-bone interface. Bone histomorphometric analyses indicated a reduction in mineralized surface/total bone surface (MS/BS, %) at the pannus-bone interface compared to bone surfaces remote from pannus, and in non-arthritic bone. No difference was observed in bone formation parameters in the distal femur (a site remote from inflammation) between arthritic and non-arthritic mice. Wnt signaling is requisite for OB differentiation and antagonists of this pathway are implicated in suppressing OB differentiation in multiple myeloma. qRT-PCR and IHC demonstrated that Wnt signaling antagonists DKK1 and sFRP1 are expressed by cells at sites of focal bone erosion throughout the timecourse of arthritis. Together these results indicate that OB differentiation and function is suppressed at sites of erosion in inflammatory arthritis, contributing to net loss of bone at these sites. DKK1 and sFRP1 are candidate factors for mediating this effect. As proof of principle, a recent study published by Diarra et al., showed that DKK1 blockade in inflammatory arthritis models resulted in protection from focal bone erosion.

Diarra, D., et al (2007) Nature Med. 13(2):156-63

P30

Chondrocyte apoptosis in osteoarthritic knee joint of rabbits

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The presence of chondrocyte apoptosis in the osteoarthritic cartilage has been reported. However, the relative contribution of apoptotic cell death to the pathogenesis of osteoarthritis (OA) is not clearly understood because of the chronic nature of the disease. The aim of this study is to explore the presence and its role of chondrocyte apoptosis in the early stage of OA. The Hulth instability model was performed on left knee joints of 12 rabbits. The right knee joint was set as control. Six rabbits (group 1) were killed 2 weeks after and another 6 rabbits (group 2) 4 weeks after the operation and knee cartilage was sampled from the same location of the knee joint surface of all rabbits. Light microscopy and electromicroscopy were used to examine the morphologic changes in cartilage and chondrocyte. TdT-mediated-dUTP nick end labeling (TUNEL) was used to examine the presence of apoptotic chondrocyte. Cartilage underwent degeneration after operation and more typical in group 2 rabbits. The number of apoptotic chondrocytes was in agreement with the severity of cartilagous morphologic change: significantly more in the group 2 rabbits than in control and in group 1 rabbits ($p<0.05$). In conclusion, chondrocyte apoptosis, not only necrosis, contributes to the decreased chondrocyte number, and consequently, to the pathogenesis of OA.

P31

Ectoderm Neural Cortex 1, a Wnt target gene, is expressed in chondrocytic and osteoblastic cells

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The Wnt pathway is involved in bone mass regulation; however, few Wnt target genes affecting this process have been elucidated. Ectoderm Neural Cortex 1 (ENC1) is a reported Wnt target gene found to be involved in adipocyte differentiation. This study was undertaken to establish a role for ENC1 in bone biology. Human ResGen cDNA arrays were used to profile transcripts of fracture callus tissues in a rabbit model of tibial distraction osteogenesis. Expression of ENC1 was up-regulated 40-fold between 2 and 4 or 6 weeks post surgery, correlating with a change of callus composition from fibroblasts and cartilaginous cells at 2-weeks, to mature osteoblasts and osteocytes in 4- and 6-week callus. ENC1 transcripts were localised to osteoblastic cells and remnant chondrocytes by *in situ* hybridisation of rabbit 4-week callus. In normal mouse bone, ENC1 was expressed in growth plate and articular chondrocytes and in mature periosteal osteoblasts. Quantitative RT-PCR analysis confirmed the expression of ENC1 during the differentiation of primary osteoblastic cultures, and of cultured MC3T3-E1 and Kusa-O 4b10 osteoblastic cells. Expression of ENC1 was also confirmed in osteosarcoma and chondrocytic cell lines. Transient expression of epitope tagged ENC1 in MG63 and SaOS2 osteosarcoma cells and stable over-expression in HEK293 and CHO cell lines showed ENC1 to have a cytoplasmic distribution with staining of a subset of nuclei in MG63 cells. This nuclear distribution of ENC1 was also seen by subcellular fractionation in CHO stable lines. These results indicate that ENC1 is expressed in chondrocytic and osteoblastic cells and may play a role in Wnt regulated osteoblastic differentiation.

P32

Novel biomaterials for skeletal tissue regeneration

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Pseudowollostonite ($CaSiO_3$) ceramics are regarded as a potential bioactive material for bone tissue regeneration due to their osseointegration properties. Major drawbacks of $CaSiO_3$ ceramics are their high degradation and dissolution rates. We hypothesize that chemical modification of $CaSiO_3$ ceramics will improve their physical and biological properties. Bone development and remodeling involves the activities of osteoblasts, osteoclasts and endothelial cells.
The present study aims at: 1) chemically modifying $CaSiO_3$ by incorporating titanium (Ti) 2) and determining it's effect on the proliferation and differentiation of primary human bone cells (HBDC) & OC and human dermal microvascular endothelial cells.
Our data showed that the incorporation of Ti in $CaSiO_3$ resulted in the formation of a new novel material, "Sphene", with improved chemical and biological properties. Sphene resulted in decreasing the ions (Ca & Si) released into the culture medium as well as maintaining its physiological pH values. There was a significant increase in HBDC proliferation and differentiation when cultured on Sphene, compared with $CaSiO_3$ ceramics. Phalloidin staining of HBDC cultured on Sphene showed a distinct and well defined stress fibres and actin containing microfilaments as compared to $CaSiO_3$ where the HBDC showed faint and poorly organized stress fibres. In addition, Ti incorporation into $CaSiO_3$ supported the formation of mature and functional OC, and was found to be conducive to human dermal microvascular endothelial growth. Our results indicate that Sphene ceramics had a significantly improved chemical stability and biological properties compared to that of $CaSiO_3$, suggesting their potential use in skeletal tissue regeneration and as coating onto currently available orthopedic/dental implants.

P33

Osteoclast-associated receptor (OSCAR) is expressed at sites of focal bone resorption adjacent to orthopaedic implants

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Osteoclast Associated Receptor (OSCAR) is a novel member of leucocyte receptor complex (LCR)-encoded family expressed by pre-osteoclasts and mature osteoclasts (OC). Blocking of OSCAR binding to its putative ligand has been shown to inhibit osteoclast formation. To date there are no data available regarding the expression of OSCAR in tissues associated with osteolysis and the objective of this study was to determine if OSCAR is expressed adjacent to focal bone osteolysis near orthopaedic implants. A total of 22 samples (10 Peri-implant tissue and 12 OA synovial tissue) were studied. OSCAR antibodies were a gift from R&D Systems Inc. (Minneapolis, MN, USA). The tissues were analysed using semi-quantitative scoring (SQA), independently by two observers. Non-parametric Mann Whitney-U test was used to test statistical significance. Dual labelling for OSCAR and CD68 expression was also carried out. Strong expression of OSCAR was seen in the majority of multinucleated cells in peri-implant tissues while OA tissues showed very low levels of OSCAR expression. Dual labelling studies revealed that the cells expressing OSCAR also expressed CD68. There was a significant difference in the expression of OSCAR between peri-implant tissue and OA synovial tissue ($p<0.003$). This study shows that OSCAR is expressed at high levels by the numerous CD68 multinucleated cells present in peri-implant tissues. These findings, and recent reports on the role OSCAR may play in osteoclast formation, indicate that OSCAR could be an important mediator of peri-implant osteolysis.

P34

Gene expression of stress fracture healing

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The aim of this study was to undertake temporal gene expression studies of the healing and remodelling process initiated by stress fracture formation following fatigue-loading of the rat ulnar.
Real-time PCR was performed on the left and right ulnae from 48 rats in which a stress fracture was created in the right ulna. Rats were euthanized following 4 hours, 24 hours, 4 days, 7 days and 14 days (n=8/group). A control group did not undergo loading. At 4 hours post fracture there was peak increased mRNA expression ($p < 0.05$), compared to non-loaded controls, for IL-6 (400 fold increase), OPG (11 fold), COX-2 (6 fold) and VEGF (2 fold). At 24 hours there was peak mRNA expression of IL-11 (48 fold). At 4 days there was a significant increase in mRNA expression of SDF-1 (4 fold), SOST (3 fold), and BMP-2 (6 fold). At 7 days there was peak mRNA expression of RANK-L (12 fold). Other genes that showed a marginal increase in mRNA expression were BAX, Bcl-2, Collagen 10, COX-1, IGF-1, Runx-2, TNFα and IL-1, all with 2-3 fold increases, peaking at 4 days post fracture.
These gene expression results demonstrate a clear temporal cascade of important signalling events that occur during healing and remodelling. Dramatic, early up-regulation of IL-6 and IL-11 suggests their central role in signalling events that direct formation of new basic multicellular units to specific sites. Prominent, early, increases in COX-2, VEGF, OPG, SDF-1, BMP-2 and SOST prior to peak expression of RANK-L demonstrates the importance of these factors in mediating and co-ordinating directed remodelling.

P35

Early, soft fracture callus exhibits the smooth muscle-associated traits of stress-relaxation and reverse stress-relaxation: implications for the fracture repair process

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Early, soft bone fracture callus contracts and relaxes *ex vivo* and displays some properties that are typical of smooth muscle[1]. Osteoprogenitor cells of this callus contain much α smooth muscle actin and thus are the likely source of this contractility. Two common traits of smooth muscle are stress-relaxation (SR) and reverse-stress relaxation (RSR). Therefore the aims of the present study were to i) establish whether early callus displayed both SR and RSR and if present ii) explain the possible significance of these phenomena on fracture healing *in vivo.*

The 6th rib was fractured in 15 male rats and calluses removed 7 days later. Force production by calluses was measured under calcium-free Krebs-Henseleit solution (0-Ca^{2+}KH; pH 7.4, 22°C). To study SR, callus tension was rapidly decreased to zero tension (0 μN), released and the force was recorded over the ensuing 30 min. To study RSR in the same preparation, tension was increased to ~100 μN and then immediately released. Responses after release were again analyzed for 30 min.

When immersed in 0-Ca^{2+}KH, all stretched calluses relaxed immediately to values approaching pre-stretch forces i.e. exhibited SR. Each callus responded to a decrease in tension with an immediate contraction followed by a prolonged maintenance period at a force approaching initial callus tension i.e. displayed RSR. The addition of Ca^{2+} to bathing solution (i.e. normal K-H) did not affect force production following brief stretch or brief release.

This report shows that early, soft fracture callus has similar responses to sudden stretch and sudden release from stretch to some smooth muscles. Thus, SR and RSR may re-establish callus static tension after bone fracture which may facilitate fracture repair. This mechanism may be achieved by promoting the differentiation of osteoprogenitor cells to osteoblast-like cells; an outcome previously described where smooth muscle cells are placed under prolonged static tension.

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

P36

PTH treatment increases fracture callus size and strength more effectively in closed than open fractures

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Manipulation of fracture healing with anabolic agents has been explored to improve union rate. Open fractures more often result in delayed or non-unions than closed fractures due to reduced anabolism. PTH has been demonstrated to enhance bone repair in closed fracture models. In a previous study in an open fracture model we have shown that PTH did not improve the union rate, whereas BMP-7 did.

To study the effects of PTH in both these biological situations, rats were dosed with daily PTH at 50ug/kg after surgery for either an open or closed fracture.

All closed fractures healed by 6 weeks. PTH did not improve union rate in open fractures. Saline produced a 30% non-union rate and PTH a 25% non-union rate, as determined radiologically.

Callus BMC was increased 67% and callus volume 52% in closed fractures compared to Saline ($p<0.01$). In open fractures callus BMC was increased 47% and volume 37% with PTH compared to Saline ($p<0.01$).

Fracture callus strength was increased by 60% with PTH treatment in closed fractures ($p<0.05$). Although the union rate was not different, in open fractures PTH produced a 49% increase in fracture strength compared to Saline ($p<0.05$). PTH treated closed fractures were however 114% stronger than PTH treated open fractures ($p<0.05$).

PTH treatment increased callus size and strength in closed fractures. While PTH increased callus strength in open fractures, it did not improve the union rate, confirming our previous results that in the absence of a robust endogenous anabolic response, PTH is less effective.

P37

Mesenchymal precursor cells in ovine lumbar spinal fusion

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Introduction: Autologous bone is a finite resource and harvesting is associated with significant morbidity. This study investigated the safety and efficacy of mesenchymal precursor cells (MPCs) as an alternative to promote solid bone growth in spinal fusion in sheep.

Methods: Fifty mature sheep underwent instrumented posterolateral lumbar spinal fusion using 25M, 75M or 225M commercially prepared allogeneic MPCs and carrier (either MasterGraft Matrix or MasterGraft Granules, Medtronic). Controls received carrier or iliac crest autograft only. Clinical pathology was assessed at monthly intervals and fusion was assessed by ex vivo mechanical testing and CT scan after 3 and 9 months.

Results: There were no adverse clinical findings. CT scans showed moderate to good fusion in all subjects after 3 months with more solid fusion after 9 months. Supplementation of the carriers with MPCs promoted fusion to the same extent as autologous

bone graft although there was no clear dose response, consistent with the mechanical testing data. Fusion was more complete with MasterGraft Matrix than with MasterGraft granules.
Discussion: MPCs promote early development of spinal fusion equivalent to autologous bone graft in this ovine model. Given the potential for significant clinical problems with autologous grafting consideration must be given to using MPCs in this setting.

P38
Examination of pagetic osteoblasts by transmission electron microscopy
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Paget's disease of bone is a focal disorder characterised by increased bone resorption coupled to increased and disorganised bone formation. The pagetic osteoclast is considered to be the cause of this disease and this cell has been studied extensively. However, due to the critical role that osteoblasts play during osteoclast development and activity in normal bone biology, we have studied the pagetic osteoblast and its potential role in the development of Paget's disease. Transmission electron microscopy (TEM) was used to examine possible morphological changes between pagetic and non-pagetic osteoblasts.
Trabecular bone explants were used to culture both pagetic and non-pagetic osteoblasts. Nine trabecular bone samples were acquired from six pagetic patients and three non-pagetic patients. Two pagetic patients also provided bone samples from unaffected skeletal sites. All pagetic bone samples were confirmed to be from pagetic sites.
For ultrastructure analysis, osteoblasts originating from primary cultures were grown to confluency on plastic coverslips, fixed, embedded in resin, and processed for examination by TEM. Several individual osteoblasts were examined from each sample.
Non-pagetic osteoblasts displayed typical and expected ultrastructural features. In comparison, pagetic osteoblasts contained extensive rough endoplasmic reticulum (RER) in long multiple and parallel arrays, and vesicles were frequently larger and more abundant.
Our results suggest that pagetic osteoblasts are morphologically different to non-pagetic osteoblasts. The features observed may be an indication of over-activity, likely contributing to the pathogenesis of Paget's disease.

P39
Species variation in bone mineral investigated using pyrolysis and X-ray diffraction analysis
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Bone is a complex material and despite many years of research into its composition and structure, its exact nature remains poorly defined. In particular, the understanding of bone mineral is limited by uncertainties and is surrounded by much debate. An enhanced knowledge of the nature of bone is of considerable importance to a wide range of research disciplines and practical applications. However, an obstacle in the analysis of bone mineral is the poorly crystalline and composite nature of this material. The study presented here has developed and used a combined method of pyrolysis and X-ray diffraction (XRD) analysis to overcome such analytical difficulties. The work has investigated variation in bone mineral chemistry for a range of animal species. The results demonstrate that consistent, quantifiable interspecies variation exists. Furthermore, human bone has been shown to be distinct from all other species studied and species can be reliably discriminated using the proposed method. These insights are of particular relevance to; research into the optimisation of biomaterials, the understanding of bone disease and aging, forensic and archaeological identification of skeletal remains and, numerous quality assurance agencies concerning food production, waster disposal and land remediation.

P40
V-ATPase subunit d2 promoter is regulated by NFAT in osteoclasts
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Vacuolar adenosine triphosphatase (V-ATPase) proton pumps play an essential role in the acidification of the bone matrix during osteoclast-mediated bone resorption. Recently, mice lacking the V-ATPase d2 subunit have been shown to be osteopetrotic due to defective osteoclasts (Lee et al., Nature Med, 2006). Here, to investigate the role of RANKL in the transcriptional regulation of the d2 gene we have cloned and characterized its putative promoter region. Bioinformatic analysis of the cloned 3 kb d2 promoter region revealed several candidate transcription factor binding sites including NFATc1, a key transcription factor for osteoclastogenesis. To explore the influence of RANKL on d2 transcription, we generated a series of d2 promoter constructs using the pGL-3 reporter plasmid. Using luciferase assays, the d2 promoter was found to be induced by RANKL stimulation and/or NFATc1 overexpression. Furthermore, targeted mutagenesis of the putative NFAT transcription binding sites was found to significantly reduce the luciferase activity as induced by NFATc1 overexpression. By semi-quantitative RT-PCR, expression of d2 and NFATc1 was found to be strongly up-regulated by RANKL but not by other pro-osteoclastic factors including TNF, LPS and M-CSF. Interestingly, the RANKL-induced expression pattern of NFATc1 appeared to precede that of d2 during osteoclastogenesis. Consistently, addition of the NFATc1 inhibitor cyclosporin A was found to blunt the mRNA expression of d2 induced by RANKL in

RAW264.7 cells. Finally, chromatin immunoprecipitation (ChIP) assays demonstrate that NFATc1 forms a complex with the d2 promoter. We propose that NFATc1 is an important regulator of d2 transcription during RANKL-induced osteoclastogenesis.

P41

Effects of dietary antioxidants on human osteoclast formation and function

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Epidemiological studies have demonstrated a link between dietary consumption of antioxidants, plasma antioxidant levels and bone density. Antioxidants have been shown to inhibit osteoclast (OC) formation and resorptive function in animal models, however, there is a lack of data in human *in vitro* systems. The aim of this study was to assess the effect of several common dietary antioxidants on human OC formation and function.
CFU-GM-derived OC precursors were cultured on dentine with sRANKL (125ng/mL) and hM-CSF (25ng/mL) for 14d and co-treated with the dietary anti-oxidants ascorbic acid, resveratrol, quercetin, catechin and vitamin E.
Catechin had no effect on OC formation and function. The remaining 4 antioxidants all inhibited these parameters but displayed differering potencies. Resveratrol and quercetin were the most potent, inhibiting both OC formation and resorption at 30μM. Ascorbic acid inhibited OC formation and resorption with maximal effects observed at 2.5mM. Vitamin E had a partial inhibitory effect on formation at 50μM and resorption at 100μM.
We have demonstrated that dietary antioxidants are capable of *in vitro* regulation of OC formation and resorption. The variability in the potency of the different antioxidants is likely to be due to differences in their mechanisms of action These findings support a potential role for dietary antioxidants in the maintenance of bone density.

P42

Human parathyroid hormone (1-34) restores ovariectomy-Induced cortical and cancellous bone loss within axial and appendicular skeletal sites of C57BL/6J mice

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Parathyroid hormone (PTH) is an anabolic agent now used to treat osteoporosis and when administered intermittently, stimulates bone formation and reverses the bone loss associated with oestrogen deficiency. However, the tissue and cellular responsivity to PTH in the skeleton is still not well understood. In this study we have used high resolution μCT to assess the efficacy of intermittent PTH treatment on several skeletal sites in C57BL/6J mice. Mice were ovariectomized (OVX) or sham operated and after 7 weeks treated intermittently with human PTH(1-34) or vehicle for 4 weeks. μCT analysis was performed on sections of the proximal tibia, distal femur and lumbar vertebral body. Eleven weeks of oestrogen deficiency induced a 33% loss of cancellous bone at the tibia, 24% loss in the femur and 26% loss in the vertebrae and was accompanied by substantial disruption to the trabecular network. Four weeks of hPTH (1-34) treatment was able to compensate significantly for OVX induced bone loss in the tibia and vertebrae but not in the femur, with a greater increase in the proximal tibia (33%) than in the vertebral body (15%). In all bones hPTH (1-34) improved trabecular architecture by significantly increasing trabecular thickness. In addition, hPTH (1-34) markedly enhanced cortical bone volume in the femoral and tibial diaphysis to that above sham and OVX vehicle treated mice. In conclusion, we found that OVX-induced bone loss is significantly restored after intermittent PTH treatment in C57B/l6J mice although PTH's anabolic response appears to vary between axial and appendicular skeletal sites.

P43

Is persistance with alendronate better in the Waikato region and New Zealand wide than international published rates ?

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Continuing long term treatment for an asymptomatic condition is always a challenge. Studies suggest only about a third of patients persist with alendronate to 2 years even if given ongoing encouragement. [CANDOO data]
Using encrypted national health index data supplied by the New Zealand Health Dept I have tracked prescriptions for alendronate issued to individual patients over the years 2002 to 2005. Compliance for a particular year has been assumed if a prescription was filled in the year both proceeding and following the index year.
Compliance in the Waikato was 71% over 2003 and 64% over 2004. New Zealand wide compliance was 72% over 2003 and 64% over 2004.
Compliance rates in New Zealand are higher than international reported rates. This may reflect local enthusiasm for monitoring response to therapy by DEXA.

P44

XXY mice have an osteoporotic phenotype

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Klinefelter's syndrome is the most common chromosomal aneuploidy in men (XXY karyotype, 1:600 live births) and is characterised by infertility, androgen deficiency, cognitive impairment and osteoporosis. Whether skeletal changes are due to sex hormone deficiency or, at least in part, to gene over-dosage, cannot be easily determined in humans.
To address this issue, we generated XXY mice through a complex 4-generation breeding scheme. Eight intact XXY mice, 9 XY littermate controls, 8 castrated XXY mice and 8 castrated XY littermate controls were sacrificed at one year of age. Castration occurred 6 months prior to sacrifice. Tibiae were examined by μCT and histomorphometry. Blood testosterone was assayed by radioimmunoassay.
Compared to intact XY controls, XXY mice had a lower bone volume (6.8±1.2 vs 8.8±1.7%, mean±SD, P=0.01) and trabeculae were thinner (50±4 vs. 57±5μm, P=0.007). Trabecular separation (270±20 vs. 270±20μm), osteoclast number relative to bone surface (2.4±1.0 vs 2.7±1.5/ mm^2) and blood testosterone concentrations (5.3±4.7 vs 2.5±3.9 ng/mL) were similar. Castration drastically decreased bone volume, trabecular thickness, trabecular separation and blood testosterone concentrations, however effects were similar in XY and XXY mice.
In conclusion, XXY mice replicate many features of human Klinefelter's syndrome making them a useful model for studying bone. Testosterone deficiency may not fully explain the bone phenotype in the non-castrate state as XXY mice show both reduced bone volume and similar blood testosterone levels. These data suggest that novel genes, that escape X inactivation, contribute to bone loss and may provide unique molecular targets for the management of osteoporosis.

P45

Generation of bone forming osteoblasts derived from Wharton's Jelly mesenchymal stem cells – effect of oxygen tension

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Wharton's Jelly-derived Mesenchymal Stem Cells (WJ-MSC) have been shown to differentiate into bone forming osteoblasts (OB) in conventional tissue culture under atmospheric oxygen (O_2). O_2 tensions experienced by OB in bone, however, are much lower. The purpose of this study was to examine the effect of varying O_2 tensions on WJ-MSC proliferation and OB formation.
WJ-MSC were isolated from the Wharton's Jelly of human umbilical cord and cultured in varying O_2 levels. Proliferation was assessed as days taken to reach confluence and OB formation was assessed in WJ-MSC cultured in the presence or absence of osteogenic factors for 30d. OB phenotype was assessed by determining alkaline phosphatase activity and extent of bone mineralisation using Alizarin Red or von Kossa staining.
Decreasing O_2 tension from 20% to 5% increased proliferation of WJ-MSC by 50% - 200%.
Culturing WJ-MSC in osteogenic media resulted in significant increases in alkaline phosphatase activity and bone mineralisation at 5%, 10% and 20% O_2. However, basal levels of these parameters also increased in the absence of osteogenic factors at 10% and 20% O_2, but not at 5%.
In conclusion we have shown that functional OB can be generated from WJ-MSC. O_2 tension affects the process of WJ-MSC proliferation and maturation into OB *in vitro,* and OB can be generated in low O_2 reflective of the bone micro-environment.

P46

The novel c-fms inhibitor Cyc11645 potently inhibits human osteoclast formation and function

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The proliferation, survival and differentiation of cells of the myelomonocyte lineage is dependent on M-CSF signaling via the receptor tyrosine kinase, c-fms. Cyc11645 is a novel heteroaromatic compound with anti-tyrosine kinase activity. This study investigated the kinase specificity and *in vitro* effects on target cells of Cyc11645, and compared it to existing tyrosine kinase inhibitors GW2580 and Imatinib.
Protein kinase assays were performed using the Amplified Luminescence Proximity Homogenous Assay screening platform. Cell survival was determined in mouse bone marrow macrophages (BMM) over 96h and proliferation in the hM-CSF-dependent mouse myelogenous cell line M-NFS-60. Human osteoclast (OC) generation and function was assessed in CFU-GM-derived cells treated with sRANKL (125ng/mL) and hM-CSF (25ng/mL) for 14d. Resorbing activity of OC was assessed on dentine.
CYC11645 potently inhibited c-fms, c-kit and PDGFR® kinases (IC_{50} 0.15nM, 0.63nM and 24 nM respectively) and was inactive against 34 other kinases. Cell survival was inhibited in BMM in the presence of 20 ng/ml of hM-CSF (0.14μM) and proliferation inhibited in M-NFS-60 cells (0.15μM). Cytotoxic effects were absent in HepG2 and HEK293 cells (> 20μM). Cyc11645 potently inhibited OC formation (0.29μM) and resorption (0.29μM) compared to GW2580 (0.80μM and 0.36μM) and Imatinib, which had no effect on either parameter up to 1.5μM.
We have demonstrated that Cyc11645 is a highly potent inhibitor of c-fms kinase that has potent inhibitory effects on myelomonocytic cells *in vitro*. Cyc11645 is also a highly effective inhibitor of human OC generation and resorbing activity, supporting its role as a potential anti-resorptive therapy.

P47

No evidence of measles virus RNA or somatic SQSTM1 mutations in bone cells from patients with Paget's disease

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Paget's disease is a bone disease with uncertain etiology. SQSTM1 mutations have been identified in familial and sporadic Paget's disease, and long-term paramyxoviral infection may be associated with the disease. However, the focal nature of Paget's disease is not understood.

We have collected RNA from cultured osteoblasts and bone marrow from 22 patients with Paget's disease, and from control patients. Differential gene expression identified several changes in these samples, including increased Dkk1 and IL-6 in pagetic cells compared to controls.

Somatic mutations within the pagetic lesion could be responsible for the focal nature of the disease. To investigate this possibility, SQSTM1 cDNA was sequenced in all patients with Paget's disease. More sensitive detection of the common P392L mutation was also performed using allelic discrimination. The wild-type sequence was found using both techniques in all but one patient, who was heterozygous for the P392L mutation. Sequencing DNA from peripheral blood in this subject indicated a germ-line mutation. We conclude that somatic mutations for SQSTM1 are not commonly present in Paget's disease.

The RNA was also used to identify evidence of measles virus involvement in Paget's disease. Blinded analysis failed to detect measles virus nucleocapsid or matrix genes in any of the samples using nested RT-PCR. This suggests that long term measles virus infection is not commonly present in New Zealand patients.

The results indicate that pagetic lesions are different from normal bone in terms of gene expression, but do not commonly contain somatic SQSTM1 mutations or measles virus infection.

P48

Adenomatous human parathyroid cells exhibit decreased sensitivity to L-amino acids

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The Ca^{2+}-sensing receptor (CaR) is activated by extracellular Ca^{2+} (Ca^{2+}_o) and L-amino acids. L-amino acids stimulate intracellular Ca^{2+} mobilization and suppress PTH secretion from normal human parathyroid cells and these effects arise from enhanced Ca^{2+}_o sensitivity. Parathyroid adenomatous cells exhibit reduced sensitivity to Ca^{2+}_o, raising the possibility that impaired L-amino acid activation of the CaR contributes to impaired control of PTH secretion. In the current study, we compared the amino acid sensitivities of samples of normal and adenomatous human parathyroid tissue under guidelines established by the local hospital committees. Human parathyroid cells were prepared by collagenase digestion and loaded with Fura-2 AM for analysis of intracellular Ca^{2+} mobilization or perifused for analysis of PTH secretion. L-Phe markedly left-shifted Ca^{2+}_o-dependent mobilization of intracellular Ca^{2+} in normal parathyroid cells as described previously. In the absence of amino acids, the EC_{50} for Ca^{2+}_o was 3.9 ± 0.5 mM (n = 4); in the presence of 3 mM L-Phe, it was 1.6 ± 0.1 mM (n = 4). Adenomatous cells, however, exhibited impaired amino acid sensitivity: the EC_{50} values for Ca^{2+}_o in the absence and presence of 3 mM L-Phe were 3.6 ± 0.1 mM (n = 4) and 2.3 ± 0.1 mM (n = 4) respectively. In studies of PTH secretion, normal and adenomatous cells were exposed to Ca^{2+}_o in the range 0.8 – 1.8 mM. Normal cells exhibited suppression of PTH secretion in the Ca^{2+}_o range 0.8 – 1.2 mM. Adenomatous cells, however, were unresponsive to 5 mM L-Phe in this Ca^{2+}_o range but sensitive to L-Phe at substantially higher Ca^{2+}_o concentrations (1.4 – 1.8 mM). The data support the hypothesis that impaired amino acid sensitivity in human adenomatous parathyroid cells contributes to the loss of Ca^{2+}_o-dependent feedback control of PTH secretion in primary hyperparathyroidism.

P49

Basic milk proteins in the lactoferrin fraction augment anabolic bone activity

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The basic protein fraction of bovine milk contains not only growth factors such as IGFs and TGFs, which are important to the growth and development of bone, but also proteins such as cystatin, kininogen fragment and lactoferrin. These have all been implicated in the maintenance of bone health. Lactoferrin, in particular, has been shown to have potent effects on bone growth, acting on bone formation through proliferation and survival of osteoblasts, and on bone resorption through attenuation of osteoclast development. Lactoferrin is isolated from milk as a high ionic strength salt eluate from cation exchange processes. Such eluates, although generally of high purity, are likely to contain other high isoelectric point proteins such as the angiogenins (angiogenin-1 and ribonuclease-4) and fibroblast growth factor binding protein (FGFBP).

In the current study, we investigated the contribution these proteins make to the overall bone cell activity of bovine lactoferrin preparations. The proteins were separately purified from the lactoferrin fraction of bovine milk and their activities assessed in primary fetal rat osteoblast and mouse bone marrow cultures.

The basic proteins, angiogenin-1, ribonuclease-4 and FGFBP, significantly stimulated osteoblast proliferation ($p<0.001$). The lactoferrin fraction, which includes the above basic proteins, had a more potent mitogenic effect on osteoblasts than pure lactoferrin. Furthermore, angiogenin-1 and ribonuclease-4 potently inhibited osteoclastogenesis. These mechanisms appear, in part,

to act through the low density lipoprotein receptor 1, since RAP, a specific inhibitor of this receptor, significantly abrogated the inhibitory effect of these peptides.

P50

Reduced bone mineral content, size, and strength caused by intrauterine growth restriction are corrected by an improved postnatal lactational environment in female offspring

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Aims: Intrauterine growth restriction and accelerated postnatal growth program adult diseases. Uteroplacental restriction impairs prenatal and postnatal nutrition and growth in the rat. We have used cross-fostering to assess the influences of prenatal and postnatal nutrition on adult bone.
Study Design: Bilateral uterine vessel ligation (Restricted, R) or sham surgery (Control, C) was performed on gestational day 18 in WKY rats. Control, Reduced (RED; reducing litter size of C to match R) and Restricted pups were cross-fostered onto C or R mothers 1 day after birth. Femur length, dimensions, mineral content and density were measured using pQCT (femur midshaft) at 6 months. Biochemical markers of bone turnover (BMBT) and sex steroid concentrations were measured.
Results: Restricted (R-on-R, R-on-C) pups were born lighter than Controls with males, but not females, remaining smaller than C-on-C pups. Pups born of normal weight grew slowly during lactation then accelerated after weaning when suckled on an R mother (RED-on-R). Cortical bone mineral content, dimensions and strength were reduced in R-on-R and Red-on-R males and females, with lower density in Red-on-R females. Cross-fostering a Restricted female (but not male) onto a mother with normal lactation (R-on-C) restored bone parameters. Estrogen and testosterone were reduced in R-on-R, with no changes in BMBT.
Conclusions: Being born small, or accelerated growth after weaning, programs bone deficits which were corrected by improving postnatal nutrition (R-on-C) in females, highlighting sex specific programming and the importance of postnatal nutrition. This may have implications for reducing fracture risk for individuals born small.

P51

Effect of oxygen tension on human osteoclast formation and function

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Oxygen (O_2) tension has been shown to play an important role in osteoclast (OC) formation and function. O_2 tensions in bone are much lower than those used in conventional tissue culture. The aim of this study was to investigate the effect of varying O_2 tension on human OC formation and activity.
CFU-GM-derived OC precursors were generated in either 5% or 20% O_2. OC formation was assessed in CFU-GM cultured on dentine with sRANKL (125ng/mL) and hM-CSF (25ng/mL) for 14d. To quantify mature OC function, OC generated on plastic were detached, re-settled onto dentine and cultured for 72h.
Reducing O_2 tension from 20% to 5% increased precursor proliferation. Precursors generated at 20% O_2 and then incubated at 20% O_2, efficiently formed resorbing OC, whereas formation was poor at 2-10% O_2. In contrast, precursors generated at 5% O_2 efficiently formed OC when incubated at 5% O_2, but not at 20% O_2. When mature OC generated in 20% O_2 were re-settled onto dentine and cultured in decreasing O_2 levels, OC number and size progressively decreased but resorption was biphasic, peaking at 10% O_2. Alternatively, mature OC generated at 5% O_2 survived and resorbed efficiently between 2-10% O_2, but not at 20% O_2.
We have shown that: 1. O_2 tension affects OC precursor proliferation, formation and resorptive function *in vitro*; 2. OC precursors and OC can be efficiently generated in low O_2 reflective of the bone micro-environment; 3. Changes in O_2 are poorly tolerated, possibly related to effects on endogenous redox/anti-oxidant systems.

P52

State-of-the-art mathematical modeling approaches applied to bone biology

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Mathematical modeling provides a powerful tool for description of complex biological systems such as bone tissue and allows one to test various experimental and theoretical hypotheses "in-silico". This paper will present a variety of distinct mathematical modeling approaches which can be applied to advance our current knowledge on bone biology at various scales of observation. Examples have been chosen based on our current research projects.
The first example deals with modeling the dynamic response of bone cells within the framework of ordinary differential equations. It is now well established that cells of osteoblastic and osteoclastic origin "cross-talk" via the RANK-RANKL-OPG pathway. Many factors have been identified increasing the RANKL/OPG ratio and, hence, bone resorption including parathyroid hormone, prostaglandins, interleukins, and vitamin D_3. Recently we and others proposed integrated mathematical models describing interactions of various cell lines of the osteoblastic and osteoclastic lineage utilizing the RANK-RANKL-OPG pathway together with growth factors [1]. Many characteristics of bone diseases can be qualitatively reproduced by these models. Furthermore, application of these models to the design of drug therapies allows identification of optimal strategies to restore bone mass.

The second example deals with modeling of tumor morphology and phenotypic evolution driven by selective pressure from the microenvironment [2]. For this problem the chosen mathematical model is a coupled continuum-discrete model where the continuum part is employed to model the evolution of variables of the microenvironment such as oxygen concentration and extracellular matrix macromolecule concentration. The discrete part accounts for cell motility, cells proliferation, differentiation and apoptosis.

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P53

Fatty acids act directly on osteoclast-like cells

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There is a growing amount of evidence to suggest that free fatty acids (FFA) have profound influences in bone cell biology. Fat mass is related to bone density and to fracture risk. In feeding experiments, fat ingestion influences bone turnover and while these effects could be the result of other endocrine mechanisms, e.g., insulin, leptin, etc., a direct connection between FFA and bone cells is also possible. The purposes of this study were to evaluate effects of FFA on osteoclast-like cells.
We have recently demonstrated that some saturated FFA significantly inhibit osteoclastogenesis in bone marrow cultures. Recently, a family of G protein-coupled receptors (GPR) have been identified through which FFA act. In this study we screened bone cells for these GPRs and identified, using real time RT-PCR, that GPR120 was present in osteoblast-like cells as well as osteoclast-like cells. In addition, GPR40, 41 and 43 were also present in osteoclast-like cells. To test the possibility of a direct effect of FFA to inhibit osteoclastogenesis, we chose the most reactive saturated FFA in the bone marrow culture, palmitic acid (C16:0) and stearic acid (C18:0) and investigated their effect on RAW264 cells at 3 concentrations (0.1, 1 and 10 μg/ml). Analysis of the number of TRAP-positive multinucleated cells indicated that both palmitic and stearic acids significantly inhibited osteoclastogenesis at 10 μg/ml ($P < 0.01$, by Dunnett's test).
These results demonstrate that some saturated fatty acids can directly inhibit osteoclastogenesis and could potentially lead to the development of novel anti-resorptive treatment for osteoporosis.

P54

Micro-CT analysis of tibiae in leptin receptor-deficient mice

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Leptin is a major hormonal product of the adipocyte which regulates appetite and reproductive function through its hypothalamic receptors. It has now become clear that leptin receptors are much more widely distributed than just the hypothalamus, and the skeleton has emerged as an important site of action of leptin.
The signalling form of the leptin receptor has been found in several cell types including human osteoblasts, rat osteoblasts and human chondrocytes. *In vitro* we have shown leptin to an anabolic factor, stimulating osteoblast proliferation and inhibiting osteoclastogenesis. Leptin increases bone mass and reduces bone fragility when administered peripherally but has an indirect inhibitory effect on bone mass via the hypothalamus when administered directly into the central nervous system.
Data from animal models where there is an absence of either leptin production (ob/ob) or its receptor (db/db) have been contradictory. In this study we compared the bone phenotype of leptin receptor-deficient (db/db) and wild-type (WT) mice. Micro-CT analysis was done on proximal tibiae using a Skyscan 1172 scanner. Db/db mice had significantly reduced trabecular bone volume, trabecular thickness and trabecular number and a higher degree of trabecular separation. Cortical bone was also significantly lower in db/db animals in volume, cross-sectional thickness and perimeter.
These results demonstrate that in the absence of leptin signalling there is reduced bone mass indicating that leptin indeed acts *in vivo* as a bone anabolic factor, mimicking the *in vitro* results.

P55

Histomorphological investigation of the mechanisms of the bone loss in an osteoporotic sheep model

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Introduction: Certain critical limitations of the rodent models suggest that a large animal is needed to investigate the pathophysiology of osteoporosis. Sheep could be suitable but there is scant information about their bone physiology. The aim of this study was to investigate the mechanisms of bone loss in a sheep osteoporosis model.
Methods: Osteoporosis was induced in five mature ewes using a combination of bilateral ovariectomy, injections of dexamethasone and low calcium diet for six months. Three normal sheep were used as a control. After sacrifice trabecular bone from the iliac crest (IC), the proximal femur (PF) and the lumbar spine (LS) were taken for histomorphometry studies. Data were tested using ANOVA and Tukey-Kramer.

Result: There was significant bone loss after the treatment ($p<0.05$). OS/BS and BFR reduced over 6 and 2.5 fold at IC, over 7 and 1.4 fold at LS and over 3 and 2.2 fold at PF. ES/BS and the number of osteoclasts/mm^2 were increased over 61% and 26% at IC, and over 67% and 14% at PF ($p<0.05$). In LS, ES/BS increased over 590% but the number of osteoclasts/mm^2 decreased by 5%.
Conclusion: The extent of histomorphological changes varied at different anatomical sites. Reduction of the BFR relative to OS/BS suggested that maturation of osteoblasts may be affected by the treatment more than osteoid formation. The greater increase of ES/BS relative to the number of osteoclasts/mm^2 suggests delayed coupling of bone resorption and bone formation, rather than a real increase in osteoclast activity.

P56

Do mutations in *SH3BP2* cause giant cell tumours?

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Background: Cherubism, a condition in which giant cell tumours develop in the maxilla and mandible at the time of tooth eruption, is due to mutations in a 6AA area of exon 9 of *SH3BP2* (c-Abl SH3 domain-binding protein 2), a member of the scaffold, anchoring and adaptor molecule family connecting receptor-activating signals to downstream effectors by assembling, targeting and regulating signal molecules. Whether this pathway is involved in development of other multinucleated giant cell lesions (including aneurysmal bone cysts, central giant cell granulomata and other giant cell tumours) is not known.
Methods: Tumour samples from giant cell tumours of the bone were identified from the Princess Alexandra Tissue Tumour bank, University of Queensland (12 samples) and from the bone histomorphology department of the Nuffield Department of Surgery, Oxford (5 samples). Ethical approval was available for both tissue resources.
DNA was extracted and the exons for *SH3BP2* amplified. The entire gene was sequenced (all exons and intron/exon boundaries) using conventional sequencing methods using an ABI 3130 Genetic Analyser, BigDye 3.1 technology and Seqscape software.
Results: No mutations were identified in exon 9 of SH3BP2, corresponding to the mutated area in cherubism. Sequencing variants were identified in the remaining exons and intron/exon regions, the significance of which is yet to be determined.
Conclusion: In this cohort of tumour samples, mutations were not found in exon 9 of *SH3BP2*.

P57

Tartrate-resistant acid phosphatase 5 as a marker for pulmonary metastasis in osteosarcoma

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Osteosarcoma (OS) is the most common primary bone tumour in children and young adults, and the second highest cause of cancer-related death in this age group, with a relative 5-year survival rate of only 24%. In spite of aggressive chemotherapy, disease-free survival has not improved significantly in the past 20 years, and 50% of patients will subsequently develop fatal pulmonary metastasis. We undertook a pilot microarray study aimed at determining whether gene expression profiling could be used to predict patient outcome in order to (i) modify the management of "poor responders" to a more aggressive or experimental stream, and (ii) to identify potential therapeutic targets. We examined the expression of 41,000 genes in 25 OS patient biopsies in duplicate and correlated this to the development of metastasis. This analysis identified tartrate-resistant acid phosphatase 5 (ACP5, TRAP), a classic marker of active osteoclasts, which predicts, with 90% accuracy, those patients that will progress to lung metastasis. These results have been confirmed by immunohistochemistry in the original patient biopsies. Furthermore, within the metastatic patient group, the time to detectable metastasis is directly correlated to the expression level of ACP5. Interestingly, we found no evidence that lack of ACP5 expression was due to an abundance of OPG or a lack of RANKL expression in the metastatic tumours, compared with non-metastatic tumours or non-malignant bone. These preliminary findings suggest that ACP5 regulation may contribute to OS metastasis. If so, modulators of ACP5 expression/activity may have important therapeutic benefits in patients at risk of metastasis.

P58

Multimodal imaging analysis of tumour progression and bone destruction in a murine model of multiple myeloma: efficacy of Apo2L/TRAIL

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This study evaluates the use of multimodal imaging to measure the efficacy of recombinant soluble Apo2/TRAIL on tumour progression and bone destruction in a xenotransplanted tumour model of human multiple myeloma. The majority of multiple myeloma cell lines are resistant to the cytotoxic effects of Apo2L/TRAIL in-vitro whereas, RPMI-8226 myeloma cells are highly sensitive. *In-vitro*, Apo2L/TRAIL caused a dose-dependent increase in RPMI-8226 cell apoptosis after treatment for 24 hr concomitant with processing and activation of caspase 8/10, caspase 9, caspase 3 and the cleavage of Bid and PARP. To examine the

anti-tumour activity of Apo2L/TRAIL, *in-vivo* we established a mouse model in which RPMI-8226 cells tagged with a triple reporter gene construct (NES-HSV-tk/GFP/Luc) were transplanted directly into the tibial cavity of nude mice. These cancer cells reproducibly establish growth in the marrow cavity and produce osteolytic lesions in the area of injection. Tumour progression and bone destruction with and without Apo2L/TRAIL treatment were monitored using D-luciferin-induced bioluminescence, micro-computer tomography (μCT) and histology. In animals transplanted with RPMI-8226 multiple myeloma cells and were left untreated, we observed a gradual increase in D-luciferin-based bioluminescence concomitant with detectable osteolytic lesions that invaded the marrow cavity and eroded the cortical bone. Progressive bioluminescence imaging in live animals demonstrated that tumour growth was inhibited with Apo2L/treatment and μCT analysis in vivo provided a method to quantify bone loss and its prevention by Apo2L/TRAIL treatment.

P59

Zoledronic acid modulates the expression of ADAMTS-1 and -5 in chondrocytes

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Introduction: Bisphosphonates (BP) are widely used for the treatment of osteoporosis, osteoarthritis and Rheumatoid arthritis and have been shown to be effective as anti-inflammatory agents. However, little is known about the effect BP has on articular cartilage damage in osteoarthritis. This study aims to determine the role of Zoledronic Acid (ZA) in cartilage remodeling. We examined the in vitro responses of various does of ZA on cartilage anabolic and catabolic gene expression profile.
Materials & Methods: Articular cartilage chondrocytes from 6 months old ovine knee cartilage were treated for 24 hrs with ZA at concentrations of 0, 5, 10 & 20 μM. *In vitro* mRNA expression of aggrecan, MMP-13, collagens I & II, ADAMTS-1, -4 & -5, were evaluated using quantitative reverse transcriptase real time polymerase chain reaction (qRT-PCR) with ovine specific primers and were expressed relative to total RNA.
Results: qRT-PCR revealed no change in the expression of aggrecan, MMP-13, collagens I & II. In contrast, significant downregulation of ADAMTS -1 and -5 in chondrocytes stimulated with 5, 10 and 20 μM ZA was found.
Conclusion: Increased ADAMTS-1 and -5 expressions are associated with progressive cartilage degeneration in osteoarthritis. The downregulation of ADAMTS -1 and -5 in this study, suggest a potential role for ZA in modulating in vitro cartilage degradation.

P60

Are the skeletal benefits of calcium-vitamin D_3 fortified milk sustained following withdrawal of supplementation in older men? An 18 month follow-up study

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In our previous 2 year RCT, we reported that calcium-vitamin D_3 fortified milk stopped or slowed bone loss at a number of clinically relevant skeletal sites in older men (Daly et al. JBMR 21:397-405, 2006). The aim of this study was to determine whether the skeletal benefits were sustained after supplementation withdrawal. Of the 149 men aged >50 years who completed the intervention, 109 were followed for an additional 18 months during which no fortified milk was provided. The two groups remained matched for age, height, weight, and physical activity at the beginning of the follow-up, and there was no difference in dietary calcium at the end of the study (mean±SD: 923±332 mg/day). Comparison of the mean changes from baseline between the groups after adjusting for baseline age, BMD and change in weight revealed that the net benefits of the fortified milk on FN and UD-radius BMD at the end of the intervention (between group differences: 1.8% $p<0.001$ and 1.5%, $p<0.01$, respectively) were sustained at 18 months follow-up (1.4% and 1.1%, both $p<0.05$). Similar results were observed at the total hip, but the between group differences were not significant in this subgroup of 109 men (intervention 0.8%, $p=0.16$; follow-up 0.7%, $p=0.18$). At the lumbar spine, the trend for a beneficial effect of the fortified milk on BMD (1.0%, $p=0.1$) was lost at follow-up (-0.1%). In conclusion, this study indicates that there are some residual benefits of calcium-vitamin D_3 fortified milk on BMD in older men following withdrawal of supplementation.

P61

Hip radiographic osteoarthritis predicts total hip bone density loss over 2 years: the Tasmania Older Adult Cohort (TASOAC) Study

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Objective. To determine if older subjects with hip radiographic OA have greater loss of total hip bone mineral density (BMD) over 2 years.
Methods. A total of 620 subjects (mean 63 years, range 51-80, 50% female) were included in this study. Right and left hip superior and axial joint space narrowing (JSN) (0-3), acetabular and femoral osteophytes (0-3), subchondral sclerosis (0-3), and subchondral lucencies (0-3) were assessed at baseline using Altman's atlas. Total hip BMD was measured at baseline and follow-up using a Hologic Delphi densitometer.
Results. Hip radiographic OA (score of any above feature > 0) was common (40%) in this population. After adjustment for age, sex, body mass index, hip pain and physical function, baseline total hip BMD was negatively associated with right and left hip superior

JSN (both P=0.02) and left axial JSN (P=0.007). Percentage change in total hip BMD was predicted by both right (β = - 0.5% per grade, P=0.003) and left hip axial JSN (β = - 0.5% per grade, P=0.01) and right hip superior JSN (β = - 0.3% per grade, P=0.06). This change was also associated with right hip femoral subchondral sclerosis (β = -1.4% per grade, P=0.02) and lucencies (β = -4.3% per grade, P=0.035). No associations were determined between hip osteophytes and change in total hip BMD.
Conclusions. Older subjects with radiographic hip OA particularly JSN have higher total hip bone loss over 2 years, suggesting prevention of bone loss should be considered in hip OA at risk subjects.

P62
Effects of isoflavone on surgically menopaused women
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Objective: To evaluate the effects of isoflavone intake on estrogen deprivated symptoms in surgically menopaused women.
Methods: Premenopausal women who took total hysterectomy with both adnexectomy were randomized into three groups. One group took conjugated equine estrogen 0.625 mg daily for 12 weeks after surgery, the second group took isoflavone 100 mg #3 daily for 12 weeks after surgery, and the third group took no medication for controlled group. Questionnaires about the acute menopausal symptoms, incontinence score, blood lipid profile, bone turnover marker were checked before and 12 weeks after surgery.
Results: Kupperman's index and insentience score were showed less increased rate than control group. Total cholesterol and triglyceride were increased in all groups and HDL cholesterol was increased in estrogen and isoflavone groups. Osteocalcin was decreased in estrogen and isoflavone groups, ICTP was decreased in estrogen group, and increased in the other groups.
Conclusion: Isoflavone was effective to acute postmenopusal symptoms, urogenital atrophy and bone turnover.

P63
The effect of vitamin D3 addition to bone mineral density in postmenopausal women
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Objective: To determine the effect of the addition of Vit. D3 (1,25-Dihydroxychole calciferol D3) to the conventional postmenopausal hormone replacement therapy on bone mineral density(BMD)
Design: A 2-year retrospective, randomized study
Setting: Department of Obstetrics and Gynecology of Catholic university hospital
Patients: 388 postmenopausal women were recruited and divided into 5 groups according to treatment regimen; A: conjugated estrogens only treated group(n=146), B: conjugated estrogens and progesterone treated group(n=103), C: conjugated estrogens and Vit.D3 treated group (n=36), D: conjugated estrogens, progesterone and Vit.D3 treated group (n=41), E: control group (n=60).
Methods: The bone mineral density of the lumbar spines and femoral neck were determined by dual-energy X-ray absorptiometry(DEXA) every 2 years.
Statistics: The difference between before and after treatment was determined by paired t-test. The comparison among the groups were determined by one way ANOVA test and student's t-test
Results: The addition of progesterone to estrogen showed insignificant increase in the lumbar and femoral neck BMD. The addition of Vit. D3 compared with conventional hormone replacement therapy insignificantly influenced bone density in women with initially normal BMD, but definitely increase in women with initially osteopenic and osteoporotic BMD of femoral neck rather than lumar spine($p<0.05$).
Conclusion: The use of Vit. D3 combined with postmenopausal estrogen replacement effects the increase of BMD in low bone density than normal bone density, especially femoral neck.

P64
Is regular table tennis activity associated with increased bone and muscle strength and improved balance in elderly Asian men and women?
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Background: Physical activity programs benefit bone health and lower falls risk in the elderly. However, there are no published studies of the effects of table tennis(TT) on fracture risk.
Aims: To investigate the association of regular TT(>1 hour/week for over 1 year) in older Asian Australians with measures of fracture risk, by comparing areal bone mineral density(aBMD), bone geometry and strength, lower limb strength and balance function, comparing TT players with non- players.
Methods: 77 healthy ambulatory men and women of Chinese descent, living in Melbourne, age 50-80y, were recruited. ABMD was measured at the femoral neck(FN), total hip(TH), lumbar spine(LS) and distal 1/3 radius(FA) [cross-sectional independent t-test was used, and results adjusted for age, height and weight]; and peripheral quantitative computed tomography(pQCT) of both forearms(4%,30% sites) and tibia(4%,33% sites). Validated tests of balance and strength were performed: Kinetic Communicator dynamometer (KIN-COM), Nicholas Manual Muscle Tester(NMMT). Chattecx Balance System(CBS), Neurocom and Lord's balance test(LBT)(for postural stability).
Results:S ignificant mean differences in adjusted aBMD were seen:for TT-player(n=45) vs non-TT-players(n=32), FN:+0.052g/cm^2,p=0.003; TH:+0.057g/cm^2,p=0.006 and LS:+0.084g/cm^2,p=0.007.There was no significant FA difference between

players and non-players. The mean cortical area of distal 33% radius in players' dominant arm was 3.2±3.5mm2 (p=0.007)greater than their non-dominant arm. Isometric knee extensor and hip abductor strength was 44±44 Nm(p=0.03) and 1.42±1.58 kg(p=0.048) greater among TT players in 11 matched player/non-player pairs.
Conclusion: TT players had significantly greater adjusted aBMD than non-players at clinically-relevant sites, which may reduce fracture risk in this population. Prospective studies of the skeletal benefits of table tennis are warranted.

P65

Complementary and alternative medicine use by patients with osteoporosis: the CAMEO study

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Complementary and alternative medicine (CAM) therapies have become increasingly popular, but their use by patients with osteoporosis is unknown.
We performed a prospective, questionnaire-based study to determine the prevalence and patterns of use of CAM therapies in 100 outpatients with osteoporosis. The use of CAM was reported by 68 patients (68%) with osteoporosis. The most frequent were acupuncture (33%), megavitamins (26%), yoga (19%) and Tai chi (19%). 21% tried more than one form of CAM therapy. 59% thought their osteoporosis improved with these approaches.
Those who used CAM were slightly older (mean age 69 vs. 64 years, p = 0.036) compared to CAM non-users. There was no significant difference between CAM users and non-users in terms of their gender, income level, education level and duration of osteoporosis.
The most common reasons for using CAM for patients were having a holistic approach to their healthcare (41%) and inadequate pain control (32%).
Most CAM users (88%) paid for their therapies out of pocket. In an average month, the majority (84%) spent up to 100 Australian dollars, with 61% spending less than 50 and almost a quarter spending between 50 and 100 Australian dollars.
Whilst 88% of patients who used CAM reported that their treating doctors were aware of their CAM usage, more than quarter (27%) did not consult their GP or specialist prior to starting CAM.
We conclude that there is a high prevalence of CAM use in our patients with osteoporosis, and over half of them reported that their symptoms improved.

P66

A cohort study of the effects of protein intake on body composition

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[4]School of Exercise, Biomedical and Health Sciences, Edith Cowan University

Background: The long term effects of protein intake on muscle mass and bone density in the elderly are not clear. Studies show that low protein intake is associated with loss of muscle mass, and protein supplementation can increase bone density in hip fracture patients.
Objective: The aim was to study the relationship between protein intake at baseline and lean mass and bone density 60 months later in elderly women.
Method: This population based longitudinal study of 863 women aged 75 ± 3 y assessed protein intake at baseline by a food frequency questionnaire developed by the Victorian Cancer Council. Body composition was assessed by whole body DXA (Hologic 4500A) at 60 months.
Results: The baseline protein intake was 81 ± 28 g (1.22 ± 0.45 g/kg body weight) and it contributed 19±2% of total energy intake. The baseline BMI was 26.8 ± 4.4 kg/m^2. At 60 months the bone-free lean body mass was 36.39 ± 4.67 kg, and whole body BMD (minus head) was 0.84 ± 0.09 g/cm^2. The baseline protein intake was positively correlated with bone-free lean body mass (R = 0.182, P < 0.001) and BMD (R = 0.135, P < 0.001) before and after adjustment for age and body size. Similar correlations were found for limb bone-free lean body mass, which is largely muscle and for limb BMD.
Conclusion: High protein intake has a small long term beneficial effect on muscle and bone structure in elderly women.

P67

Bone loss prior to fracture: Geelong Osteoporosis Study

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We have previously reported that a fracture does not affect the long-term pattern of bone loss. Using a case-crossover study design, we aimed to determine whether accelerated bone loss precedes fracture in postmenopausal women. In this study, women with incident fracture who had 3 serial biennial BMD measurements were identified. From the 3 measurements, 2 rates of change in BMD (ΔBMD) were determined: the ΔBMD during the period immediately prior to fracture (ΔBMD2) and the previous ΔBMD that did not result in fracture (ΔBMD1).
Incident fracture cases included prospective low trauma, radiologically confirmed fractures (n=34, aged 61-87 yr): hip (7), forearm (10), rib (4), pelvis (1), humerus (5), tibia/fibula/femur (7). BMD was measured (Lunar DPX-L) at spine (SP), femoral neck (FN),

whole body (WB) and ultradistal forearm (UD). A non-fracture group was also randomly selected from the community (n=249, aged 61-87).
Using matched-analysis, differences between ΔBMD2 and ΔBMD1 were compared.

Rates of change in BMD (g/cm²/2 year) (mean ± SE)			
	Cases		
	ΔBMD1	ΔBMD2	P
SP	0.019 ± 0.008	0.018 ± 0.01	0.88
FN	-0.008 ± 0.01	-0.027 ± 0.01	0.27
WB	-0.003 ± 0.005	-0.008 ± 0.004	0.42
UD	0.001 ± 0.003	-0.011 ± 0.003	0.03

Significantly accelerated bone loss immediately prior to fracture was observed at the UD site only, although it may occur at all sites except SP. The difference in ΔBMD at the UD for fracture cases was compared with that in women with no fracture. The difference among fracture cases was greater than the difference observed in women with no fracture (ΔBMD2-ΔBMD1, fracture vs no fracture -0.012±0.01 vs 0.003±0.001 (g/cm²/2year), p=0.01). This pattern persisted after adjusting for baseline BMD.
Although we have not investigated risk factors for fracture other than BMD, these data suggest accelerated bone loss occurs prior to fracture.

P68

Risk of fracture in elderly men and women with cancer

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Although it has been suggested that survivors of breast cancer have increased risk of fracture, it is not clear whether cancer is a risk factor of fracture. The present study sought to examine the association between cancer and fracture risk in elderly women and men.
A sample of 1362 aged 60+ years as at 1989 of Caucasian background from the Dubbo Osteoporosis Epidemiology Study had been followed for 17 years. The incidence of cancer and fracture were recorded during the follow-up period. Baseline BMD (GE Lunar Corp, MI) was obtained from all participants. Survival analysis was conducted in individuals whose diagnosis of cancer occurred before a fracture. During the follow-up period, there were 152 women and 120 men who were diagnosed with cancer before sustaining a fracture. Among whom 59 (38%) of women had breast or ovarian cancer (BROVC), and 42 (35%) of men had prostate cancer (PC).
After adjusting for age at diagnosis and baseline BMD, women with BROVC had lower risk of hip fracture (hazard ratio: 0.35, 95% CI: 0.05-2.54) but increased risk of clinical vertebral fracture (HR 1.88; 1.05-3.39). Men with PC had significantly higher risk of hip fracture compared to those without cancer (HR 3.83, 1.34-10.98). There was no significant association between non-BROVC or non-PC and fracture risk.
These results suggest that the relationship between cancer and fracture is sex- and site-dependent, such that women with breast cancer had lower risk of hip fracture but increased risk of vertebral fracture whereas men with prostate cancer was associated with increased hip fracture.

Source of financial support: National Health and Medical Research Council, Australia

P69

Bone mineral density and hip fracture: a revisit with time-varying effects

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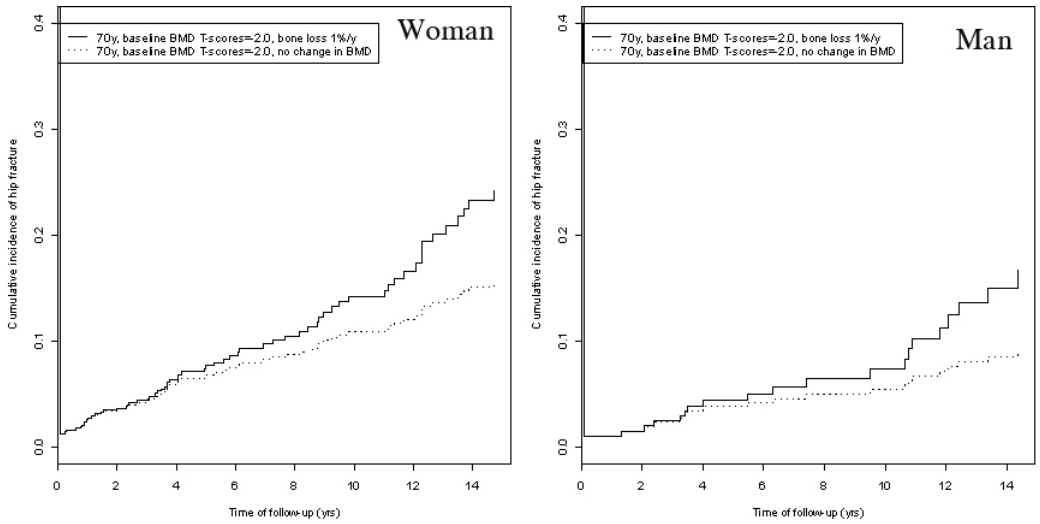
All previous studies of association between BMD and fracture were based on a single measurement, and assumed that BMD do not change, which is clearly untenable since BMD is known to decrease with advancing age. The present study sought to estimate the magnitude of association between BMD and hip fracture risk taking into account the time-dependent bone loss.
A cohort of 782 women and 566 men aged 60+ years who have had at least two BMD measurements preceding a hip fracture was studied. The individuals have been followed-up for a median period of 15 years with an average of 5 BMD measurements (GE Lunar DPX) per subject. During the follow-up period, 77 women and 23 men had sustained at least one hip fracture. The Cox's proportional hazards model with time-varying covariates was used to evaluate the absolute risk of fracture for an individual conditioned on the individual's age, history of prior fracture and fall, and BMD measurements.
For a given risk profile, in addition to baseline BMD, bone loss further increased the risk of hip fracture in an individual. For example, a 70-year old woman with baseline BMD T-score of -2.0, and with a prior fracture and a fall is estimated to have the 5-year and 10-year risk of hip fracture of 6.8% and 10.6%, respectively. However, conditional on an annual bone loss of 1%, her 5-year and 10-year risks of hip fracture increase to 7.7% and 14.3%, respectively. Using this time-varying approach, it is possible to construct a full profile of BMD and fracture risk for an individual.
Thus, the magnitude of association between BMD and fracture risk was underestimated in previous studies. These data suggest that to accurately assess the risk of hip fracture in an individual, the dynamic nature of BMD should be considered.
Source of financial support: National Health and Medical Research Council, Australia



P70

Development of a nomogram for individualizing the absolute risk and time to subsequent fracture

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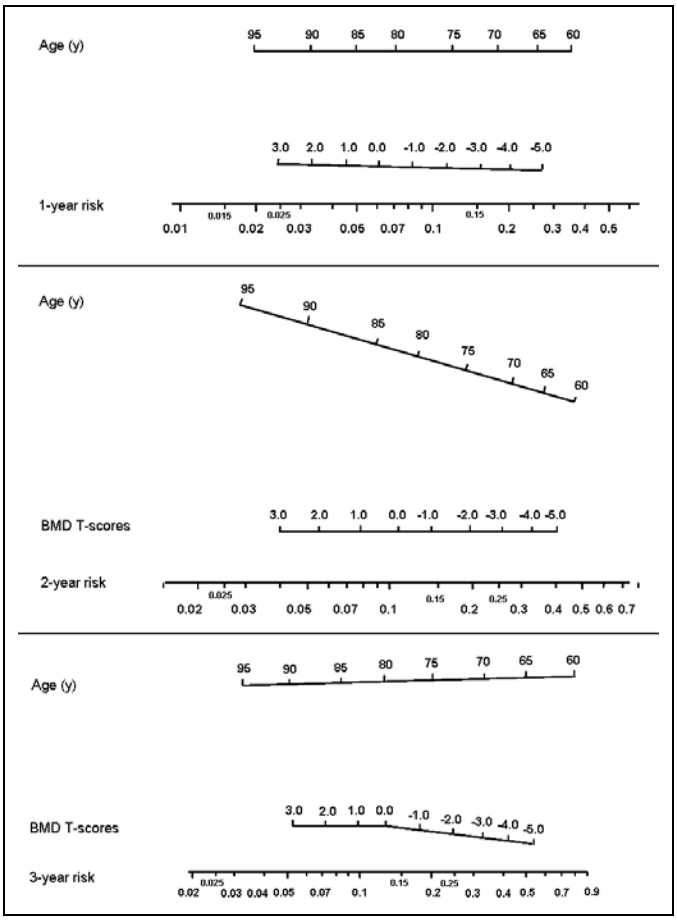
Although it is well known that individuals with a prior fracture have increased risk of subsequent fracture, it is not known which factors are associated with the time to and the risk of subsequent fractures. This study sought to develop a nomogram for individualizing the risk of and time to the subsequent fractures for individuals with an existing incident fracture.

Data were analyzed from 2252 (1377 women) participants aged 60+ at baseline. Between 1989-2006, 520 women and 200 men had sustained an incident fracture, among whom 193 women and 74 men sustained a subsequent fracture; 41 women and 13 men had 3 or more re-fractures. Baseline BMD and clinical data were recorded in all subjects.

Results of a "counting process" analysis model suggested that the time from the first to a subsequent fracture was significantly shorter than the time from no-fracture to the first fracture. Consequently, the 3-year and 5-year risks of subsequent fracture were comparable to the 5-year and 10-year risks of first incident fracture, respectively.

A multivariable nomogram was developed for individualizing the absolute risk of subsequent fracture based on an individual's age and BMD. For example, the 5-year risk of first fracture for a 60-year old woman with T-score of -2.5 was ~17%, which was equivalent to the 5-year risk of subsequent fracture the same age woman with T-scores of -1.5.

The data suggest that the time from the first incident fracture to a second fracture was shorter than the time to the first fracture. By providing individual risk profiles, the model is potentially beneficial for identifying the high-risk individuals for intervention.



P71

Hip radiographic image-derived morphometric & trabecular structural phenotypes in a large group of dizygotic twin pairs: methodology

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Genetic linkage analysis of twins phenotyped for multiple traits is a proven approach for gene discovery (Wilson *et al.* Eur J Hum Genet. 2006 14(3):340-8). Studies of osteoporotic fracture in families show it is under genetic control. However BMD is an incomplete descriptor of bone fragility, not accounting for shape or trabecular structure, which are aspects of "bone quality"; a problematic contributor to bone strength. Structural analysis of plain radiographs favourably discriminates hip-fracture cases. This study of female twins (n= 650) examines new methods for determination of gross morphometric and trabecular-structural phenotypes from pelvic radiographs.

Proximal femoral (PF) images were digitised (600 dpi) and subjected to an algorithm that established a femoral neck (FN) axis by determination of femoral-head centre and midpoint of FN. Femoral shaft (FS) axis was determined analogously. Derived variables described: gross shape of PF, its cortical structure plus several 12x12mm regions-of-interest (ROI) for image "texture" analyses, positioned relative to PF. Fast Fourier transform (FFT) power spatial-frequency spectra were determined for each ROI using a novel thresholding algorithm based on maximisation of the power-spectral variance coefficient between all angles; comparing favourably with traditional noise-reduction methods.

Intra- and inter-operator reproducibilities (CV%; n=100 in duplicate) were similar, but strongly dependent on the phenotypic variable: FH diameter (0.8%), bone widths (1-1.5%), ROI position (<1%), inferior and superior FN cortical thicknesses (8-10%).

These improved semi-automated methods are suitable for application to large groups of proximal femoral images and potentially provide new and important phenotypes for the study of bone structure and fracture.

P72

Association between LRP5 gene and bone mineral density: a meta-analysis

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The present study was aimed at examining the association between LRP5 gene polymorphism and BMD by using meta-analysis.

All published studies in English on the association between LRP5 gene and osteoporosis-related phenotypes, including BMD and fracture were identified by a systematic electronic search. Phenotype data were summarized for individual studies by LRP5

genotype, and a synthesis of data was performed with both fixed-effects and random-effects meta-analyses. Among the selected studies, 6 studies used the A1330V polymorphism and have reported actual BMD which were available for analysis.
The 6 eligible studies consisted of 8935 (6889 women) individuals, aged between 20-70 years. The overall distribution of genotype frequencies was: AA, 68%, AV and VV, 32%. However, the genotype frequency varied significantly within as well as between ethnicities. Lumbar spine BMD among individuals with the AA genotype was on average 0.02g/cm^2 (95% CI: 0.01-0.03) higher than those with either AV or VV genotype. Similarly, femoral neck BMD among AA genotype carriers was 0.01g/cm^2 (0.001-0.02) higher than those without the genotype. While there was no significant heterogeneity in the association between the A1330V polymorphism and lumbar spine BMD ($p = 0.59$), the association was heterogeneous for femoral neck BMD ($p = 0.04$).
These results suggest that although the LRP5 is in linkage with BMD in high bone mass pedigrees, its effect on BMD in the general population was modest. The clinical value of this gene in the assessment of osteoporosis and fracture risk is uncertain.

Source of financial support: National Health and Medical Research Council, Australia

P73

Incremental prognosis of osteoporotic fractures by genetic marker: contribution of the collagen I alpha 1 gene

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The present study was aimed at developing a nomogram-based prognostic model for predicting 5-y and 10-y of fracture risks in elderly women by incorporating genetic marker information.
COLIA1 genotypes (GG, GT and TT) were determined in 915 women of Caucasian background aged 60+ years as of 1989, who have been followed for up to 17 years. During the follow-up period, osteoporotic fractures were recorded. Femoral neck bone mineral density (FNBMD) was measured by DXA (GE-LUNAR) at baseline and expressed as T-scores. A series of nomograms for predicting 5-year and 10-year risk of fractures including any fracture, hip and vertebral fractures for an individual woman were developed by using the Cox's proportional hazards model.
The proportion of individuals with GG, GT and TT genotypes in the population of 63%, 32% and 5%, respectively, which was consistent with Hardy-Weinberg equilibrium law. After adjusting for age and FNBMD, the risk ratio for COLIA1 TT (vs. G allele) and any, hip and vertebral fractures were 3.07 (1.47-6.43), 7.07 (2.37-21.14) and 4.45 (1.89-10.47), respectively. The area under the curves for prediction models for any fracture, hip and vertebral fractures with COLIA1 genotype were significantly increased by 0.03 to 0.07 compared to the model without the genotype. A 60-year old woman with the TT genotype had an absolute fracture risk equivalent to an 80-year old woman without the TT genotype.
These results indicate that the incorporation of COLIA1 genotype could increase the prognostic value of fracture risk over and above model with BMD and age.

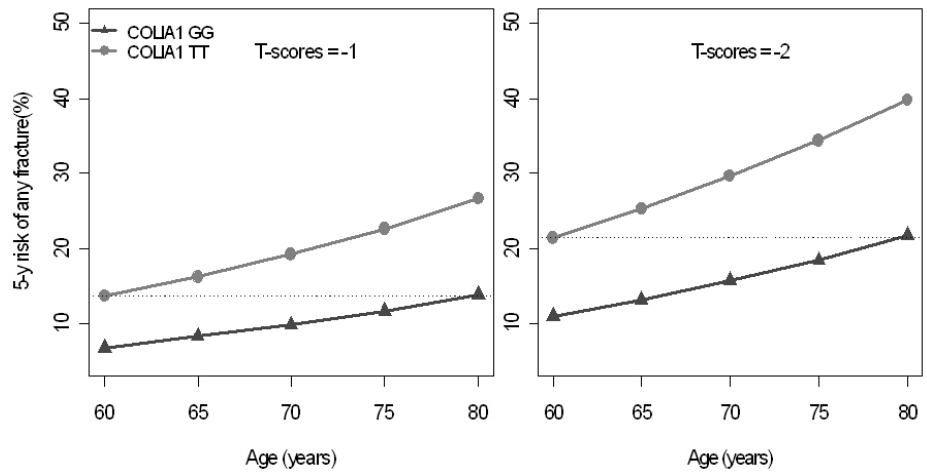

Figure: 5-y risk of any fracture in women, stratified by age, BMD and COLIA1 genotype.

Source of financial support: National Health and Medical Research Council, Australia

P73A

Bone mineral homeostasis and bone mineral density In Vitamin D sufficient and deficient patients with hyperthyroidism

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Aims and Objectives: To assess parameters related to bone mineral homeostasis such as calcium, phosphorous, alkaline phosphatase, 25-hydroxy vitamin D [25 (OH) D], parathyroid hormone (PTH) and bone mineral density in patients with thyrotoxicosis and varying degrees of vitamin D deficiency.
Materials and Methods: The study subjects included 30 consecutive patients with hyperthyroidism attending endocrine clinic of All India Institute of Medical Sciences, (AIIMS), New Delhi. Twenty-seven of 30 study subjects had Graves' disease, two had toxic

multinodular goitre (MNG) and one had solitary toxic nodule (STN). All these patients were tested for parameters for bone homeostasis i.e. serum calcium, phosphorous, alkaline phosphatase, vitamin D and PTH levels. Serum T4, serum 25(OH)D estimation was done by radioimmuno assay. Serum intact PTH and TSH concentration were measured by immunoradiometric assay using commercial kits.

Bone mineral density was measured using Hologic DR 4500A densitometer. Bone mineral density was measured at both hips, both forearms and lumbar spine (L_2-L_4) using anteroposterior view. Based upon serum 25(OH) D levels observed in healthy soldiers in an earlier study from our center, hyperthyroid subjects were stratified into vitamin D sufficient and deficient group. A cut off level of 10 ng/ml was used to designate vitamin D deficient group.

Results: Based upon the cut off value of vitamin D deficiency at 10 ug/dl eight patients had vitamin D deficiency and 22 were vitamin D sufficient. In vitamin D deficient group PTH levels were significantly higher as compared to vitamin D sufficient group. The mean BMD scores were in the osteoporotic range at left hip and left forearm and in the osteopenic range at lumber spine. However, in vitamin D sufficient mean BMD scores were in the oseteopenic range at all sites (Table).

Bone mineral homeostasis and related indices in vitamin D deficient and vitamin D sufficient patients with hyperthyroidism.

Parameters	Vit-D deficient	Vit-D sufficient	P value
N	**8**	**22**	-
Age (yrs)	36.5±8.9	33.5±8.7	0.43
BMI (kg/m^2)	18.9±3.8	19.0±3.5	0.96
Serum Calcium (mmol/l)	2.17±0.18	2.26±0.15	0.39
Serum PO_4 (mmol/l)	1.27±0.18	1.27±0.18	0.97
Serum alkaline phosphatase (KA units)	13.9±5.20	12.23±4.46	0.37
Serum i-PTH (pg/ml)	**31.2±16.3**	**18.9±13.1**	**0.041**
Serum 25 (OH) D (ng/ml)	**6.6±1.3**	**18.4±5.4**	**<0.001**
Left Hip-T score	**-2.65±1.13(8)**	**-1.64±1.04(22)**	**0.0289**
Left Hip – Z score	-2.13±1.21(8)	-1.17±1.07(22)	0.0514
Left forearm – T score	**-3.04±1.3(6)**	**-1.27±1.66(19)**	**0.026**
Left forearm – Z score	**-2.85±1.24(6)**	**-0.91±1.6(19)**	**0.0157**
Lumbar spine-T score	-1.83±1.71(8)	-1.60±0.79(22)	0.72
Lumbar spine-Z score	-1.6±1.69(8)	1.38±0.88(22)	0.64

Number of patients studied are given in parenthesis after mean ± SD data.

Conclusion: Patients with hyperthyroidism have significant bone loss as compared to Caucasians. Concomitant vitamin D deficiency exacerbates bone loss in these patient with hyperthyroidism. Further studies are required to assess reversibility of bone loss after treatment with antithyroid drugs and effects of calcium and vitamin D supplementation on recovery of bone loss in patients with hyperthyroidism from vitamin D deficient population.

P74

Children experiencing fractures during growth have higher adiposity than those remaining fracture-free: an 8-year study of 142 New Zealand girls

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This study compares the anthropometry, bone and body composition at maturity of girls experiencing incident fractures (Group 2, n = 39 girls having 59 new fractures), and girls who had fractures at baseline but no further fractures (Group 3, n = 44) with values in girls remaining fracture-free throughout growth (Group 1, n = 59). Fractures were confirmed radiologically. Anthropometry (height, weight, body mass index) and DXA measurements (Lunar DPXL) at the forearm, lumbar spine, hip and total body were taken at baseline, 4 years later and 8 years later. The mean (SD) ages of the 142 participants at baseline and study end were 9.61(2.88) and 17.62 (2.91) years. At study end girls with any history of fracture (groups 2 and 3) had 11.1% higher fat mass ($P < 0.035$) than the fracture-free group, though the groups did not differ in height, lean mass or bone area. Group 3 girls achieved higher final total body bone mineral content (BMC) for age (+5.5%, $P < 0.001$) than fracture-free girls but Group 2 girls did not. Consequently girls experiencing incident fractures (Group 2) had lower final BMC for their height and weight than fracture-free participants (-3.0 %, $P < 0.039$) whereas Group 3 girls had higher values (+2%). We conclude that during growth girls who remain fracture-free have lower adiposity than those who sustain fractures. Moreover, girls experiencing incident fractures had lower BMC for their height and weight than fracture-free participants, suggesting their bone mineral accrual has not increased adequately for their anthropometry.

P75

A cross-sectional study of factors associated with skeletal age deviation

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Objective: Skeletal age deviation (SAD) is associated with bone mass and fracture risk in children but factors determining this are unknown. The aim of this population based cross-sectional study was to describe the factors associated with SAD.
Methods: A convenience sample of 640 male and female 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: Subjects with a SAD >0 had significantly higher height, weight and Tanner stage compared to subjects with a SAD <0. Bone free lean mass, fat mass and grip strength were positively associated with SAD. In multivariate analysis, ever smoking and use of inhaled corticosteroids were negatively associated with SAD while milk drinking was positively associated with SAD. There was no significant association between sunlight exposure, television watching, light or strenuous exercise and SAD.
Conclusions: This study suggests body composition, strength, diet, ever smoking and inhaled corticosteroid use may modify bone maturity relative to age and thus affect 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.

P76

Relationship of total body fat mass to bone area in New Zealand five-year-olds: a DXA study of the FLAME birth cohort

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Clark et al [1] found that fat mass was a positive determinant of bone mass and size independently of lean mass, in a birth cohort population of prepubertal British children aged 9.9 yr. Our study was undertaken to ascertain whether this relationship is also present in younger children. Height, weight and body mass index were measured and a total body DXA scan (Lunar DPX-L) was performed close to their fifth birthday on 194 children (81 girls,113 boys) participating in the Dunedin birth cohort FLAME study. Relationships of total body fat mass and lean mass to total body less head (TBLH) area and TBLH BMC were evaluated using linear regression as in the British Study. Girls had greater mean fat mass (3.9 vs 3.2 kg) and lower lean mass (14.5 vs 15.2 kg) than boys ($P<0.001$), but heights, weights and TBLH bone area (603 vs 626 cm2) were similar. Fat mass was an independent predictor of TBLH bone area ($R^2=0.79$, $P<0.001$) and TBLH BMC ($R^2=0.74$,$P<0.001$) in data adjusted for SES, ethnic group, lean mass and height in New Zealand five-year-olds. Associations between fat mass and BMC diminished after adjusting for bone area. We conclude that at age 5 years children have 9.4cm^2 (girls) and 12.8cm^2 (boys) higher TBLH bone area (wider bones) per kg fat mass than children of the same sex, height and lean mass, suggesting some association between fat mass and periosteal bone expansion.

[1] Clark EM et al. J Clin Endocrinol Metab 91: 2534-2541, 2006.

P77

Risk of subsequent fracture depends on bone mineral density and fracture type: a 17-year prospective study

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Half of all fractures occur in people with non-osteoporotic BMD. Although osteoporotic fracture increases re-fracture risk, there is no information on re-fracture risk at different bone density levels. This study examined re-fracture risk according to BMD for all types of osteoporotic fractures in 1390 women and 877 men aged 60+ from the Dubbo Osteoporosis Epidemiology Study (April 1989 - May 2007).
Fractures were classified into hip, vertebral, major (proximal humerus, distal femur, proximal tibia, pelvis, multiple rib) and minor (all other peripheral fractures excluding digits). Subsequent fracture rates were compared with initial fracture rate of a BMD-matched group.
There were 369 incident fractures in women and 133 in men, followed by 228 subsequent fractures in women and 52 in men (3124 and 983 person-years, respectively).
Absolute re-fracture risks were highest for women and men with osteoporosis [86 (74-100) and 161 (108-240); RR: 2.1 (1.7-2.6) and 6.9 (4.0-12.0), respectively] and still increased for osteopenia [45 (34-60) and 47 (31-73); RR: 2.1 (1.5-3.0) and 3.4 (2.1-5.6) respectively], and normal BMD [RR: 2.1 (1.7-2.6) and 2.1 (1.0-4.8) respectively].
Re-fracture risk increased with decreasing T-scores for all fracture types. Hip and vertebral fractures were associated with the highest re-fracture risk, but this risk was also increased for osteopenic and osteoporotic major fractures and osteoporotic minor fractures.
Thus, there was an overall increased BMD-standardised re-fracture risk following initial fractures in women and men for all levels of BMD. Randomised clinical trials should be conducted to examine treatment outcomes for those with osteopenia or normal BMD.

P78

Bone mineral density in quitters compared with continuing smokers at one year: the Quitline cohort study

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Rationale: Smoking is a key lifestyle risk factor for osteoporosis. There is only indirect evidence supporting reversibility of smoking-associated bone disease after smoking cessation. Therefore, we are investigating whether smoking cessation over 1 year is associated with increases in bone mineral measures compared with continuing smokers.
Methods: 103 smokers (42 males, 61 females) who contacted Quit Victoria to quit smoking, smoking >15 cigarettes a day, with mean (SD) lifetime smoking 21.2 (13) pack years, age 52.7 (9) years, height 168.1 (8.9) cm and weight 73.8 (13.6) kg have been studied to 1 year. Baseline questionnaires assessed health and lifestyle. Areal BMD of hip (TH) and lumbar spine (LS) was measured with dual energy x-ray absorptiometry (Hologic QDR 4500A) at baseline and 12 months. Data were adjusted for age, height and weight. Results (g/cm2) were expressed as mean ± SD change (continuing smokers vs. abstainers). Independent t tests were used to assess difference between groups.
Results: No significant difference ($p>0.05$) was seen in continuing smokers (n=69) compared to abstainers (n=34) in mean BMD change from baseline to 12 months for: TH (-0.021 ± 0.131 vs. -0.027 ± 0.064), LS (-0.024 ± 0.025 vs. -0.029 ± 0.029).
Conclusion: There was no early recovery in bone mineral measures demonstrated in abstainers compared to current smokers, at 12 months. Ongoing observations will be made in our cohort of 420 Quitline callers. These studies have major implications for policy and practice in fracture prevention in relation to smoking.

P79

The -1260C/A polymorphism in the 1-alpha hydroxylase gene (*CYP27B1*) promoter is associated with fracture risk in the frail elderly (the FREE Study)

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The *CYP27B1* gene encodes the mitochondrial enzyme 1-alpha-hydroxylase that is central to mineral ion homeostasis through its conversion of 25-hydroxyvitamin D to its active 1,25-dihydroxylated metabolite. The present study sought to assess the contribution of common variants at the *CYP27B1* gene to fracture risk in an elderly frail population.
Genotypes of -1260C/A and +2838T/C *CYP27B1* polymorphisms and the vitamin D receptor (VDR) BsmI polymorphism were determined in 615 individuals aged between 65 and 101 years (average: 86). The incidence of any fracture (n=135) was ascertained between 1999 and 2006 with average length of follow-up being 2 years. Quantitative ultrasound measurements (QUS) and PTH were measured in all individuals. The association between the polymorphisms and fracture risk was analyzed by the Cox's proportional hazards model.
In each polymorphism, the genotypic distribution was consistent with the Hardy-Weinberg's law. Individuals homozygous for the C allele of the *CYP27B1* -1260 polymorphism had a 2.04-fold (95%CI: 1.04-4.00) increased risk of fracture as compared to those homozygous for the A allele, either before of after adjusting for age, PTH and QUS measurements. The number of fracture cases that can be attributed to the variation at this polymorphism was 11.4%. Fracture risk was not significantly associated with the *CYP27B1* +2838 (p=0.15) or *VDR* BsmI genotypes (p=0.29 respectively). In transient transfection studies, a reporter gene downstream of the –1260(A)-containing promoter was more highly expressed that that containing the C allele.
These data suggest that a common variation within the *CYP27B1* promoter is functionally associated with fracture risk in the elderly.

P80

Osteoporosis: use of bisphosphonates in the Australian population

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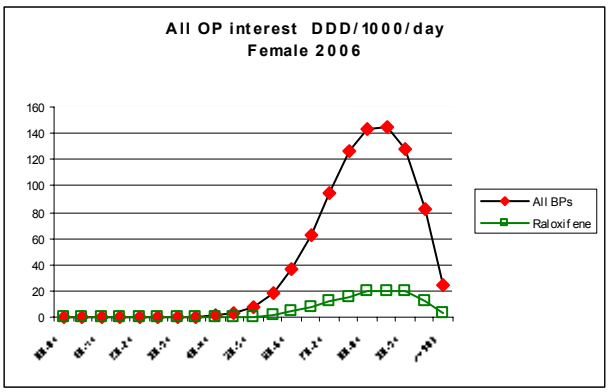
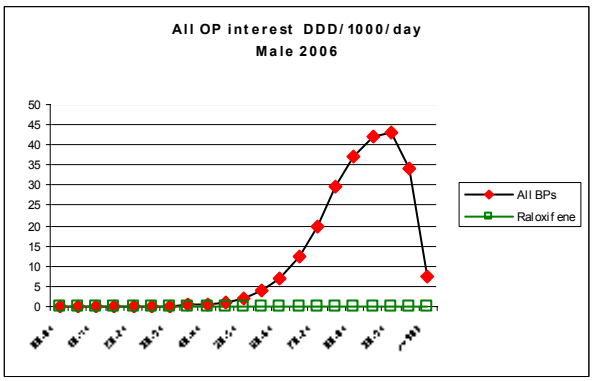
Background: Fracture rates in the elderly are high. The use of antiresorptive medications in patients with fracture is low (18% for women with fracture, and even lower in men with fracture (Eisman 2004)).
Objectives: To examine trends in prescribing of bisphosphonates and raloxifene in the Australian population.
Methods: Government (Medicare Australia) databases were analysed for script and cost data for bisphosphonates and raloxifene from introduction in 1996 (age and gender from 2002). Prescription counts were converted to DDD/1000 population/day with Australian Bureau of Statistics population data.
Results: Total subsidised bisphosphonate use increased from their introduction in 1997 (raloxifene in 1996) from 2.2 DDD/1000/day in June 2000 to 12.3 DDD/1000/day in June 2006. 75.1% of prescriptions were for alendronate, 24.6% for risedronate, and remainder etidronate, at June 2006, with similar proportion of costs. Raloxifene was 1.32 DDD/1000/day in June 2006. Weekly forms of alendronate and risedronate (introduced May 2001 and February 2003 respectively) resulted in substantial increase in prescribing. Peak age of use of BPs and raloxifene is 80-90 years in women and 85-95 years in men. 14% of women and 4% of men aged 80-90 are on an antiresorptive medication. Average $ per DDD/1000/day was AUD$991,366 in 2006.
Private usage of bisphosphonates was 0.48 DDD/1000/day in June 2006.

Conclusions: There has been substantial increases in prescriptions for bisphosphonates, with rapid adoption of the weekly forms. However, only a small proportion of the elderly population is prescribed antiresorptive medication, despite reported high prevalence of fracture.





P81

Body composition and bone mass: childhood to adolescence

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Aim: To describe the association between prepubertal body composition, change in body composition and change in bone mass during adolescence.
Methods: 183 subjects had DXA derived measures of lean mass (LM), fat mass (FM) and bone mass at age 8 and age 16 years. Bone mineral content (BMC), areal bone mineral density (aBMD) and bone mineral apparent density (BMAD) were assessed at the spine, hip, and total body by a Hologic QDR2000 densitometer.
Results: LM at age 8 predicted all adolescent DXA measures in males, but only total body BMC in females, independent of LM at follow-up. FM at age 8 predicted adolescent body BMC and aBMD in both sexes, independent of FM at follow-up.
Change in LM was positively associated with change in DXA measures at all sites, except spine and body BMAD in females (R^2 = 10-41%), independent of baseline LM. In contrast, change in FM was inversely associated with change in spine and hip BMC, aBMD at all sites, and body BMAD in males (R^2 = 5-22%), but only with change in spine BMC in females (R^2 = 18%), independent of baseline FM.
Conclusion: In both sexes, LM at age 8 was predictive of adolescent bone mass. Increase in LM from childhood to adolescence was strongly associated with increases in bone mass in both sexes, independent of change in height. The inverse association with change in FM in males most likely reflects the fact that those who gain excess weight during puberty are less active.

P82

Post-fracture excess of mortality in elderly women and men after adjusting for background mortality risk

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Although elderly individuals with a fracture are known to be at increased risk of mortality, the excess of mortality in these individuals relative to the expected survival in the general population has never been systematically examined. The present study was aimed at estimating the post-fracture excess of mortality by taking into account the longitudinal changes in survival in the general population.
The incidence of mortality after a fracture among 1119 men and 1842 women in the Dubbo Osteoporosis Epidemiology Study was ascertained between 1989 and 2006. The mortality data were then analysed in relation to the age and sex-specific lifetables of the Australian population from 1901 to 2006 by the relative survival model.
During the follow-up period 539 women and 210 men had sustained a fracture. During the same period, after adjustment for background mortality, the observed/expected deaths (risk ratio) after a fracture increased in both sexes. One-year mortality after a hip fracture increased by 3.4-fold in men, which was higher than in women (1.3-fold). For clinical vertebral fracture, the one-year post-fracture mortality in men and women increased by 2.2-fold and 1.4-fold, respectively. Given the current incidence of fractures (142,420) in Australia, it was estimated that the annual number of excess deaths associated with fracture was ~2400 and 450 in men and women, respectively.
Thus, the excess of deaths after a fracture in men was higher than in women after adjusting for background mortality risk. However, these data also underscore the public health significance of fracture at the national level.

P83

Development of a nomogram for making decision concerning measurement of bone mineral density for an individual

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There is at present no data for guiding the decision to measure bone mineral density (BMD) for an individual. The present study sought to examine the natural progression to osteoporosis and/or fracture in non-osteoporotic individuals, and thus provide data for making decision of BMD measurement.
This study is part of the ongoing longitudinal Dubbo Osteoporosis Epidemiological study, in which women and men aged 60+ as at 1989 were followed for 17 years. During the follow-up period, BMD measurement was measured bi-annully, and the incidence of fracture was recorded and confirmed by X-ray. The study was limited to 838 women and 594 men whose baseline BMD T-scores were greater than -2.5 (ie, non-osteoporosis).
During the follow-up period, 273 (33%) women and 116 (20%) men became osteoporotic at the femoral neck or sustained a low trauma fracture. Using Cox's survival model, a nomogram was developed to estimate the 5-year and 10-year risks of osteoporosis or fracture for an individual conditioned on the individual's current BMD and age. The 5-year risk of osteoporosis or fracture for a 60 years old woman with T-score=-2.4 was 13%, which is equivalent to the risk for an 80 years old woman with T-score=-1. The nomogram can individualise the risk of osteoporosis or fracture by using actual, not categorized, BMD value.
These results suggest that the decision to measure BMD in an individual should be based on the individual's risk of osteoporosis or fracture, and the nomogram presented here can help identify those high-risk individuals.

P84

Pacific Island adolescents have greater bone mineral content and bone area than age-and size-matched New Zealand Europeans

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Pacific Island adults have larger, denser skeletons than Europeans, though this is not evident in prepubertal children[1]. The present study set out to determine whether NZ Pacific Island teenagers (40 females, 40 males, aged 15-18 years living in Dunedin) differed in bone size, bone mineral content (BMC) or bone mineral density (BMD) from a) NZ European teenagers matched for age and height, or b) NZ European adults matched for height and weight, and to examine the associations of bone measures to body composition. Total body composition, bone size (cm^2), BMC (g) and BMD (g/cm^2) were measured for all participants (DXA, Lunar DPX-L).
Pacific Island males and females had greater lean mass and weighed 15kg more than the European teenagers ($P<0.001$). Only Pacific Island males had greater lean mass than weight-matched adult males ($P<0.05$). Pacific Island teenagers had greater BMC and bone area ($P<0.002$) compared with both age- and size-matched Europeans. Pacific Island BMD was greater than in age-matched Europeans but was similar to the size-matched, adult Europeans. In weight-adjusted data Pacific Island teenagers had 241g more BMC, and $58cm^2$ greater bone area than European teenagers ($P<0.01$).
We conclude that Pacific Island teenagers develop large skeletons early since they have higher bone mass and area than NZ Europeans even than adults of similar height and weight.

1. Grant AM et al. Calcif Tiss Int 2005; 76: 397-403.

P85

Bone mineral density in a population-based sample of Australian men: Geelong Osteoporosis Study (GOS)

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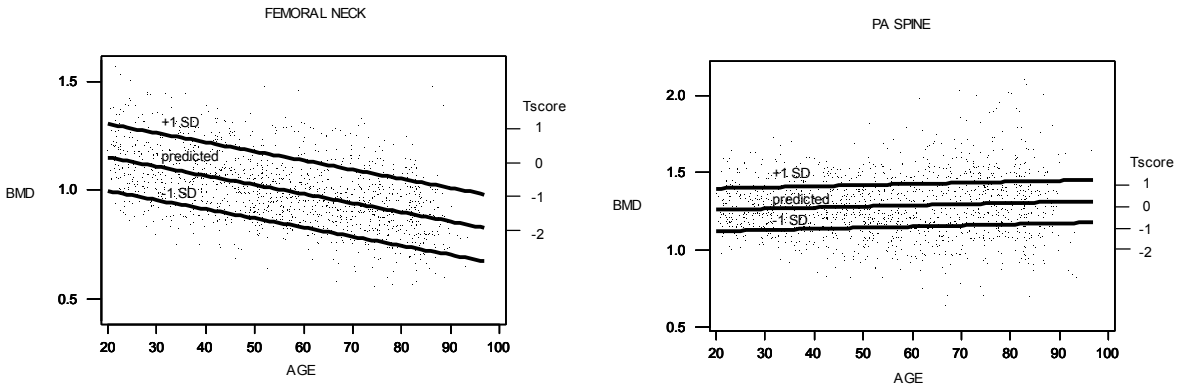
There are few published studies reporting normative BMD in men and very few derived from random population-based studies. The most widely used BMD reference range has been supplied by NHANES (Looker et al, 1998), which provided data for the proximal femur only. This study documents BMD across the full adult age range in men randomly-selected from the general community. This Australian data is compared to that of NHANES.

An age-stratified random population-based sample of men (n=1,467 20-93yr participation rate 67%) was recruited from Commonwealth electoral rolls. BMD was measured at the PA spine, proximal femur, whole body and forearm using a Lunar densitometer and investigated for patterns of bone loss across the adult age range. Femoral neck BMD was converted to Hologic units (Lu et al, 2001) and compared to NHANES (Looker et al, 1998).
Age-related changes in BMD were best predicted by a linear relationship at the femoral neck and PA spine and by quadratic functions at the whole body and forearm. The steady decrease at the femoral neck equated to $0.004g/cm^2$ decrease in BMD per year while on average BMD increased at the PA spine by $0.0007g/cm^2$ per year. Comparison of the GOS and NHANES femoral neck BMD showed no difference for those aged 20-79yr. Differences in BMD (3.8%) observed for men aged 80yr and older may be attributed to the utilization of conversion equations developed for women aged 20-79yr.



P86

Breastfeeding predicts both bone mass and fractures in adolescents: a 16 year longitudinal study

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Aim: We have previously reported that breastfeeding was associated with bone mass and fractures in 8 year old children. The aim of this study was to determine whether breastfeeding was independently associated with bone mineral density (BMD) and fracture in 16-year-old children.

Methods: 415 subjects (150 girls and 265 boys) participated in the study in 2004-05.

Breastfeeding intention at birth and actual breastfeeding at 1 and 3 months were assessed by questionnaire in 1988/9.

Dual X-ray Absorptiometry [DXA] measures were performed at age 16 at spine, hip, radius and total body using a Hologic Delphi densitometer. Fractures were assessed by self-report with X-ray confirmation.

Results: Adolescents who were breastfed (for 25 days or more) had higher bone mineral density at the spine (2.34% higher, 95%CI 0.09, 4.58), hip (2.57% higher, 95%CI 0.04, 5.10) and total body (3.00% higher, 95%CI 1.24, 4.75), compared with those who were bottle fed. This changed little after adjustment for body size and other confounders. No effect of duration of gestation was observed. They also had a lower risk of having any fracture (RR 0.69, 95%CI 0.49, 0.99). Breastfeeding was not associated with number of fractures.

Conclusion: Breastfeeding in early life may program bone development and thus increase peak bone mass and protect against fracture suggesting that osteoporosis prevention programs need to start very early in the life cycle.

P87

The burden of fracture imposed by men in a major regional hospital

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This study aimed to analyse the fracture burden imposed by men at the Geelong Hospital, the only large public teaching hospital in the study region. Men (20yr+) who were identified by X-rays as having a fracture during the years 2005-6 were included. Patterns of hospital admission, surgery and trauma were investigated by age (young <60yr, older >60yr).

We identified 2,103 men, aged 20-97yr, with at least one incident fracture, with 70% aged <60yr. A smaller number but greater proportion of older men were hospitalised compared to young men (44%, (282/641) vs 29% (419/1462), p<0.001). In-hospital mortality was 6% (16/282) for older men and 0.2% (1/419) for young men. Over the two years, older men accumulated 6,925 bed days in hospital (median 13 days/person, IQR 4-35), compared to 2,199 bed days for young men (median 3 days/person, IQR 1-5). Data on surgical intervention was available for 1104 men. Older men had lower numbers and prevalence of surgery compared to young men (34% (104/309) vs 45% (355/795) p<0.001).

Trauma level was determined for 1,726 cases: 656 low and 1080 high. Low trauma fracture included spontaneous fracture and those resulting from falls <standing height, overexertion or strenuous movement. Fracture was attributable to high trauma in 78% (911/1,173) of young men and 30% (169/563) of older men (p<0.001).

Although older men accounted for a third of the fracture numbers, they accumulated more inpatient bed days. Young men accounted for the greater number of surgical procedures due to the large number of incident fracture cases.

P88

Polymorphisms within *ARHGEF3* are associated with bone mineral density in women

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Introduction: The 3p21 region of the genome has been identified as containing a QTL for BMD in multiple studies. The *ARHGEF3* gene is situated within this region and is a strong functional candidate for regulation of BMD via effects on the cytoskeleton of osteoblasts and osteoclasts. The aims of this study were to evaluate the role of variations in the *ARHGEF3* gene sequence on DXA evaluated spine and hip bone mass in women.

Materials and Methods: 24 single nucleotide polymorphisms (SNPs) in *ARHGEF3* were genotyped (Illumina Golden Gate assay) in a discovery cohort of 768 women from the GENOS sib study. A cohort of 811 postmenopausal women was used in a replication study using MALDI-ToF mass spectrometry. Analysis of the BMD DXA spine and hip data (Hologic) from the discovery cohort was performed using the software FBAT and UNPHASED. Analysis of the data from the replication cohort was performed using one-way ANOVA and ANCOVA.

Results: Significant associations with BMD were observed for 5 SNPs in the discovery cohort (BMD Z score $P = 0.0007–0.042$) and confirmed in a 3-SNP haplotype analysis ($P = 0.003–0.04$). The most significantly predictive SNP, rs7646054, was genotyped in the replication cohort confirming an association with BMD (*A* allele: BMD spine Z score +0.22 $P = 0.007$; hip Z score +0.26 $P = 0.014$).

Conclusions: Genetic variation in *ARHGEF3* plays an important role in the determination of bone structure in Caucasian women.

P89

Prediction of fracture risk by LRP5 gene polymorphisms: a haplotype analysis in a population-based study

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Polymorphisms of the low density lipoprotein receptor related protein 5 (LRP5) gene have been shown to be linked to or associated with bone mineral density (BMD), but their association with fracture risk is not known. The present study sought to assess the association between LRP5 gene polymorphisms and fracture risk in women by using haplotype analysis.

Haplotype tagging single nucleotide polymorphims (in order rs314776, rs3736228, rs4988320, rs4988321, rs556442, rs587808 and rs660925) of LRP5 gene was determined in a sample of 1286 (821 women) participants from a population-based cohort. Bone mineral density (BMD) was measured at baseline. During the follow-up period of 1989-2006, 125 men and 338 women sustained at least one fracture.

There were no statistically significant association between any single SNP and BMD or fracture risk. However, haplotype analysis showed that three haplotypes [CCAGAAGC (3% in the population), CCGGAGA (3%) and TCGGGAG (2%)] were associated with variation in BMD. Furthermore, carriers of haplotype CTGGGGA (6% in the population) had an increased risk of fracture (RR: 1.4, 95% CI: 1.1-3.0), hip fracture (2.2, 1.0-4.9) and wrist fracture (2.4, 1.1-4.9), after adjusting for age and BMD. There was no significant association between any haplotype and BMD or fracture risk in men. The proportion of fracture cases that is attributable to the haplotype was 2.3%.

These results suggest that common genetic variation at the LRP5 gene was modestly associated with fracture risk in women independent of its association with BMD. The present study also suggests that a haplotype-based analysis is more powerful than a SNP-based analysis in the detection of association.

Source of financial support: National Heath and Medical Research Council, Australia

P90

Knowledge of osteoporosis among tertiary students in Vietnam

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Knowledge is considered as important component in any preventative program; however, assessment of osteoporosis knowledge has not been well studied. The aim of this study was to assess the knowledge of osteoporosis among tertiary students in Vietnam.

A sample of 305 students aged 23 years were randomly invited from 5 universities/colleges (Medicine, 40%; Pharmacy, 12%; Poly-techniques, 17%; Journalism, 17%; and Pedagogy, 14%). Each student was administered a Vietnamese version of the OPQ questionnaire, which consists of 20 items with four components: general knowledge (GK), risk factors (RF), nutrition and exercise (N&E) and treatment (T). Each question was scored as 1 for correct answer and 0 for incorrect answer. Analysis was based on a multivariable log-binomial regression.

The median proportion of correct answers (for 20 items) was 65% (inter-quartile range, IQR: 45-75). The median of proportion of correct answers for GK was 80% (IQR: 60-100), RF 63% (IQR: 50-75), N&E 75% (IQR: 50-100) and T 33% (IQR: 0-67%). Medical and pharmacy students were more knowledgeable than other students, with the proportion of >75% correct answers being 55% vs. 7%. The degree of osteoporosis knowledge among medical and pharmacy students was 7.3 times (95% CI: 3.7-14.6) higher than non-medical and pharmacy students.

University/college students in Vietnam had good knowledge of osteoporosis. However, they had poor knowledge of treatment. These findings imply that there is a need for educational program via newspapers and radio to impart the osteoporosis knowledge in the general public and university students.

P91

Characteristics of men with morphometric vertebral fracture: Geelong Osteoporosis Study

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In this cross-sectional study we compared characteristics of men with and without morphometric vertebral fracture (MVF). Using vertebral morphometry from lateral scans (Lunar Prodigy, T10-12, L1-4) moderate and severe wedge, biconcave or compression deformities (>25% height reduction) were identified as MVFs in an age-stratified, random sample of 556 men (60-93yr).
Of the 55 men with MVFs (26L, 35T), 93% were unaware of the condition. Prevalence: 60-69yr 4.7%, 70-79yr 10.0%, 80+yr 14.6% and 8.1% overall. Men with MVF were older (mean 78.3 (95%CI 76.2-80.3) vs 74.5 (73.8-75.2) yr, p=0.001), had lower weight and BMI. No difference was detected in armspan and, although half the men with MVF had self-reported height loss, there was only weak evidence that height differences persisted after adjusting for age. No differences in age- and weight-adjusted BMD were observed for PA-spine or femoral neck, but adjusted BMD was lower at the whole body, UD-forearm and mid-forearm.
Among 55 men with MVF: 7(13%) reported paternal and 11(20%) maternal history of osteoporosis [age-adjusted OR=1.92 (0.80-4.61; p=0.1) paternal and 1.37 (0.67-2.78; p=0.4) maternal]; 8(14%) reported falling more than once during the previous 12-months [OR=1.18 (0.52-2.66; p=0.7); 11(20%) had a history of low trauma, nonvertebral fracture since age 45yr [OR=1.64 (0.80-3.38, p=0.2]; osteoporosis was identified in 5(9%) at the spine and 10(18%) at the mid-forearm; two men with MVF had bilateral hip replacements but among the remaining 53, 9(17%) had osteoporosis at the femoral neck.
Although most MVFs appear asymptomatic, several characteristics exist that distinguish men with, from men without, MVF.

Adjusted mean (95%CI)	MVF	No MVF	p
Weight (kg)*	77.0 (73.5-80.5)	81.3 (80.2-82.5)	0.02
Height (cm)*	170.7 (168.9-172.4)	172.1 (171.5-172.7)	0.1
BMI (kg/m^2)*	26.3 (25.2-27.4)	27.4 (27.1-27.8)	0.06
Armspan (cm)*	176.0 (173.9-178.0)	176.7 (176.0-177.4)	0.5
BMD (g/cm^2)**			
PA-spine	1.284 (1.223-1.344)	1.317 (1.297-1.337)	0.3
femoral neck	0.915 (0.879-0.952)	0.927 (0.915-0.939)	0.5
whole body	1.200 (1.173-1.227)	1.225 (1.216-1.234)	0.08
UD-forearm	0.369 (0.351-0.387)	0.390 (0.384-0.396)	0.03
mid forearm	0.765 (0.743-0.788)	0.793 (0.786-0.861)	0.02

P92

Vitamin D status and effects on postural control between fallers and non-fallers

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Aim: Vitamin D insufficiency has been linked to falls. This case control study examined differences in measures of neuromuscular control in fallers and non-fallers to identify functional components associated with falling and to determine those associated with 25OHD status.
Methods: Independent community living women aged over 70 years were selected,100 had fallen in the last year (fallers) and 100 had not (controls). Subjects underwent a neuromuscular test battery to assess performance in areas of the sensory system (tactile sensitivity, joint position sense), motor system (ankle, knee and hip strength), and integrated neuromuscular function (reaction time, sway, timed up and go (TUAG)). Plasma 25OHD concentrations were assessed by RIA (Diasorin).
Results: Fallers had significantly lower 25OHD values than controls (46.5±15.8 and 69.5±20.0 nmol/L respectively $P < 0.001$). Fallers as a group had worse ankle, knee and hip strength, hand reaction time, sway and TUAG than controls. Fallers but not controls had low 25OHD values associated with reduced hip flexor, extensor and abductor muscle strength ($R = 0.28$, 0.20 and 0.22 $P < 0.05$) respectively but no other functional tests.

Conclusions: These data show that fallers are characterised by a variety of neuromuscular deficits. However vitamin D insufficiency was only associated with hip muscle strength in the falling population presumably because the higher levels of 25OHD found in the control population were sufficient to remove 25OHD status as a determinant of muscle strength. Clearly there are many neuromuscular deficits in the falling population that are not vitamin D related.

P93

Calcium and vitamin D supplementation prevents hip bone loss in elderly ambulant Australian women

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Background: There are few long-term studies comparing the effects of calcium alone and calcium plus vitamin D versus placebo on bone loss prevention and bone turnover in elderly people.
Aim: In a 5-year randomised controlled double-blind trial, we evaluated the benefits of calcium supplementation with or without vitamin D on hip BMD and bone turnover markers in 120 elderly postmenopausal women aged 74.8 ± 2.6 years at baseline.
Methods: Participants were randomised to receive either 1000 IU vitamin D and 1200 mg calcium (CalD), 1200 mg calcium and placebo vitamin D (Ca) or both placebos (placebo) per day over the 5 years. Primary endpoints were effects on total hip BMD and bone turnover makers.
Results: Both Ca and CalD groups had significantly less loss in total hip BMD than the placebo group at 1 year (-0.04% and -0.17% vs -1.27%, P<0.05) and the effect was maintained in the CalD group at 3 and 5 years. Compared to the placebo group, both Ca and CalD groups had significantly lower plasma total alkaline phosphatase concentrations (6.8-11.3%, P≤0.02) at 1 year, and significantly lower urinary DPD/Cr ratios at 1 and 3 years (15.6-34.5%, P≤0.05). These effects were only maintained in the CalD group at 5 years.
Conclusion: Addition of vitamin D to calcium may have long term beneficial effects on bone structure in older postmenopausal women living in a sunny climate, which were probably mediated by the long term effects of calcium plus vitamin D on reducing the rate of bone turnover.

P94

Fracture risk index after falling: a selection strategy for fracture reduction through falls prevention

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Aim: This study aims to develop and evaluate a fracture risk index after falling for use in the frail elderly.
Methods: We assessed risk factors at baseline and prospectively recoded falls and falls-related fractures in 2005 institutionalized elderly. Each fall was treated as a study unit and a fracture as an outcome. Fracture score was calculated by multiplying the RRs of all independent risk factors in a resident.
Results: During the follow-up, 6646 falls were reported by 1342 residents and 308 fractures by 270 residents, giving an incidence rate of 214 and 9.9 per 100 person years for falls and fractures respectively. The independent fracture risk factors after falling were lower broadband ultrasound attenuation, lower weight, longer lower leg length, better balance, hostel residence, a history of fracture and lack of reporting a fall in the past year. Fracture rate was 1.5 and 10.4 per 100 falls for falls with the lowest and highest quintile of the score respectively, a multiplicative increase of 7.1. The risk score multiplied by a falls risk score (calculated using the falls risk index proposed by us[1]) could identify 31% of all residents (fallers and non-fallers) with falls and fracture incidence rates of 247 and 16.6 per 100 person years respectively. These rates were 27% and 126% higher in falls and fractures respectively compared to the rest of the sample. They accounted for 52% of the total fractures.
Conclusions: The index could help to rationalize the provision of falls prevention programs in the frail elderly.

1. Chen JS, March LM, Schwarz J, et al: A multivariate regression model predicted falls in residents living in intermediate hostel care. J Clin.Epidemiol. 58:503-508, 2005

P95

Feedback on calcium intake is an effective tool to increase intake in older women

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Ensuring adequate calcium intake in older women is a challenge. Many women find the recommended 3 to 4 daily serves of dairy foods difficult and long-term adherence of supplements is often poor. Using a short validated food frequency questionnaire[1] (FFQ) we assessed calcium intake of 70+ year-old (70-97 years) women annually for three years.
Participants completed the FFQ yearly as part of a RCT investigating the effect of annual high dose vitamin D_3 on falls and fractures (Vital D Study). Participants and their doctor then received a letter advising if the calcium intake was adequate (diet plus supplements >800mg/day[2]) or inadequate. Participants also receive a dairy brochure.
Baseline calcium assessment occurred at recruitment (1035±10mg/day; Mean±SEM). Of the 2,238 participants, 79% (n=1756) were notified of an adequate calcium intake. When retested, this proportion increased significantly to 84% (1718/2038) at 12 months and 88% (802/916) at 24 months (p<0.000, 12 and 24 months). At 12 months, 72% of the group reporting that they had increased their calcium intake over the past year (n=309) had been successful.

A significant proportion of participants notified of inadequate calcium intake at baseline (n=481) increased to an adequate intake by 12 months (39%; 166/429) and 24 months (52%; 99/192) (p=0.003, 12 and 24 months). Annual feedback of calcium intake, plus information on increasing calcium through diet and/or supplementation, has a cumulative positive effect in this large group of older women.

[1]Angus R et al J Am Diet Assoc 1989; [2]Devine A et al JBMR 2004.

P96

Is BMD a surrogate measure of longterm exposure of risk factors for breast and bowel cancer?

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The risk of breast cancer has been associated with a longer lifetime estrogen exposure and prospective studies have reported higher bone density in postmenopausal women with breast cancer. Recently calcium supplementation has been associated with reduced recurrence of adenomatous polyps, the common precursor of bowel cancer. We hypothesised that BMD would be lower in those with bowel cancer or adenomatous polyps while women with breast cancer would have higher BMD compared with controls.

We measured aBMD (Lunar) in adults recently diagnosed with breast, bowel cancer or adenomatous polyps (time between diagnosis and BMD: cases-breast: 4.8±3.5 months and bowel cancer/polyps: 5.1±3.6 months). BMD (spine; hip sites; TBBMC and forearm sites) was compared with a random sample of the population (no cancer) from the Geelong Osteoporosis Study. The analyses used ANCOVA with adjustment for age and weight.

In premenopausal women there was no association between BMD and breast cancer at any site (cases=48; controls=242: all $p>0.5$). Postmenopausal breast cancer cases had higher BMD at the spine and forearm ($p<0.05$) and a trend for higher TB BMC ($p=0.10$) (cases=230; controls=372). In contrast females with bowel cancer/polyps had lower BMD (spine; TB BMC and trochanter, $p<0.05$). BMD did not differ in males with and without cancer/polyps although male cases>80kg tended to have lower TB BMC (cases=90; controls=435).

Our BMD findings are consistent with estrogenic risk factors for breast cancer and low calcium status for bowel cancer in females although further analysis in males may suggest a different risk profile for obese and non-obese men.

P97

A genome-wide ENU mutagenesis screen for skeletal abnormalities – a new resource for Australian bone researchers

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To fully characterise skeletal regulation and the underlying genetic basis for skeletal disease a combination of both genotype- and phenotype-based approaches is required.

One phenotypic based approach is the generation of systemic mutations throughout the genome of mice using ethyl-nitrosourea (ENU). ENU mutagenesis generates point mutations approximately 1 in every 1000 gametes for any given gene. Mutations induced include loss-of and gain-of function, viable hypomorphs of lethal complementation groups, antimorphs thereby complementing and extending the information provided by targeted gene disruptions in genotype driven approaches.

Funding has been obtained to undertake a genome wide ENU mutagenesis screen at the MRC Mammalian Genetics Unit in the UK. This screen will specifically target diseases of the skeleton and calcium and phosphate handling such as osteoporosis, osteoarthritis (OA), gout, chondrocalcinosis, Paget's disease, parathyroid disorders and kidney stones. Screening will be undertaken by X-ray, DEXA and serum biochemistry to identify skeletal malformaties and ectopic mineral deposits, extremes of BMD/BMC variation and serum irregularities. A dominant screen of 8,000 F1 mutants and a recessive screen of 3000 F3 mutants will be completed over three years. Mutagenesis and initial screening has commenced with interesting mutants already identified for further investigation with an expected screening rate of approximately 10 mutants per week.

Taking the very conservative assumption that approximately 0.5-1% of the screened F1 mutants will carry a mutation of interest, then we can expect to generate around 55-110 new mouse models of skeletal disease which will be available to Australian researchers collaboratively.

P98

The combined influence of bone, muscle and fat on gait, balance and falls in older adults

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Aim: Bone, muscle and fat mass may play a role in gait, balance and falls. To investigate relationships between these variables, healthy older adults were recruited from an ongoing randomized controlled trial of Tai Chi as a falls prevention intervention.

Methods: 183 subjects (49 males, 134 females age 72.7 ± 6yr) were recruited from the main study. Timed up and go (TUG), 30s chair stand (CS) and number of falls (NF) in the previous year were collected at baseline. Total and appendicular skeletal muscle mass (ASM), fat mass, total, neck of femur and lumbar spine bone mineralization were assessed using DXA.

Results: ASM was positively correlated to total body bone mineral density (r=0.61) and lumbar bone mineral content (r=0.56). The neck of the femur t-score in males with high fat/low ASM was significantly poorer compared to females (-2.2 ± 0.6 vs -1.4 ± 1.0

p=0.0009). Osteoporosis in the neck of the femur and low ASM were independently associated with lower CS score (p=0.005, p<0.0001, respectively) with a trend towards an interaction with fat (p=0.061).
Conclusion: Low ASM and osteoporosis appear related to lower gait and balance scores and the combination of low ASM and high fat may be a risk factor for femoral osteoporosis in males. Larger trials that are balanced for sex are needed to adequately assess these complex relationships.

P99

Is quantitative ultrasound a useful monitoring test for antiepileptic drug - associated bone disease?

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We investigated the utility of calcaneal quantitative ultrasound (QUS) in antiepileptic drug (AED)-induced bone disease, by correlating QUS with established methods [dual energy x-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT)].
Nineteen gender-matched twin (n=10) and sibling pairs (n=9) were recruited, where AED-users had received treatment >12months. QUS [broadband ultrasound attenuation (BUA), velocity of sound (VOS)] and areal bone mineral density (aBMD) measurements of the lumbar spine (LS), trochanter (TR) and total hip (TH) were taken. Fifteen pairs had pQCT measurements of the non-dominant tibia at 4% and 38% sites.
BUA and VOS measurements were highly reproducible with kappa scores of 0.936 and 0.965 respectively. No significant within-pair differences were detected for QUS between AED-users and non-users. Correlations were performed in pooled data. Mean BUA correlated strongly with aBMD: LS (r=0.637, p<0.01), TR (r=0.679, p<0.01) and TH (r=0.684, p<0.01). Mean VOS correlated with LS (r=0.518, p<0.01) and TH (r=0.469, p<0.01) and moderately with TR aBMD (r=0.407, p<0.05). Ipsilateral BUA and VOS correlated strongly with pQCT trabecular density with r=0.750 (p<0.01) and r=0.725 (p<0.01) respectively, but not with cortical density or tibial cortical thickness. BUA also correlated with polar and axial stress strain index (measures of bone strength) (r=0.421-0.547). However when above correlations were performed by AED status some differences were reported between AED-users and non-users.
QUS parameters, particularly BUA, correlated strongly with several DXA and pQCT parameters in AED-users and non-users. These results suggest that QUS parameters reflect BMD and measures of bone strength in these subjects.

P100

pQCT measures are associated with fractures in dialysis patients

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Aim: To determine whether fracture is associated with cortical or trabecular measurements by pQCT in dialysis patients.
Methods: We prospectively recruited 47 end-stage renal failure patients (30 men and 17 women), 45 on haemodialysis (HD) and 2 on peritoneal dialysis (PD). We confirmed low-trauma non-vertebral fractures (n=11) since starting dialysis, and prevalent vertebral fractures (n=7) were identified by morphometry of lateral spine x-rays. There were 34 fractures in 21 patients. pQCT measurements (Stratec XCT 3000) of the radius from the non-dialysis forearm and contralateral tibia included trabecular and cortical density, and cortical area. We also measured BMD by DXA (Norland) at the lumbar spine and femoral neck. Other variables included age, gender, weight, cause of renal disease, months on dialysis, smoking status and history of parathyroidectomy. We assessed statistical differences between fracture and non-fracture groups using Mann-Whitney and Chi square tests.
Results: The mean age was 63.98±11.48 years (SD) years with a range of 26-84 years, median weight was 71(89.60-55.50) kg (interquartile range), and median duration on dialysis was 24 (60.50-16) months. The fracture group had lower cortical area at either radius (p=0.03) or tibia (p=0.006), lower radial trabecular density (p=0.03) and tibial trabecular density (p=0.04), lower weight (p=0.03) and proportionally more women (p=0.03). No significant differences were found with any DXA parameter, serum iPTH or age.
Conclusion: pQCT measurements of cortical area and trabecular density at the tibia and radius were associated with prevalent fractures in dialysis patients.

P101

Methodological issues in measuring knee subchondral bone density in the tibia

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Aim: To test the reproducibility and validity of subchondral bone mineral density (BMD) measurements using six different measurement techniques.
Methods: A random sample of 50 participants had subchondral BMD assessed using DXA scans, where anthropometric, pain, MRI and radiographic data were also available. The six methods of measuring techniques were defined as: 1) the midpoint of one intercondylar spine, extending across the tibial surface and descending 10mm; from the midpoint of the two intercondylar spines to 2) the top of the spine descending 20mm, 3) 10 to 20mm beneath the top of the tibial spine; (4-6) from the tibial surface descending, 4) 10mm, 5) 15mm, and 6) 20mm.

Results: All six methods were found to have high Intra-class correlations (ICC), ranging from 0.97 – 1.00. Subchondral BMD was higher in males (methods 2, 3, and 4) and higher in those with medial tibial osteophytes (methods 1, 3 and 4). In addition, medial tibial cartilage defects were correlated with BMD (methods 3 and 4). Method 2, which takes into account the intercondylar spine, was the only method that correlated with medial tibial bone size. Measuring subchondral BMD using method 4 displayed the most significant correlations including medial joint space narrowing and medial femoral osteophytes.
Conclusion: Subchondral BMD can be measured reproducibly with all six methods. Method 4 has the best predictive validity but it's only marginally superior to methods 2, 3 and 5. However, method 2 adds additional information on tibial bone size, suggesting two measures are necessary in clinical studies.

P102

Repeatability of micro-CT machine (Viva-CT 40)

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Objective: The aim of this study was to determine the reproducibility of a micro-CT (Scanco Viva CT-40) for measurement of simple material (pure water) to complex structure (bone).
Material and Methods: We determined the repeatability of measurements of trabecular and cortical parameters at the femur of 12 weeks old mice at the distal metaphysis and midshaft corresponding to15% and 44% of femur length up from its knee joint surface, respectively. In addition, we scanned the water-filled tube, machine-specific phantom consisting of 4 rods with different density of 100, 200, 400, and 800mgHA/ccm^3, respectively. The repeatability was determined after 5 repeated measurements and was expressed as the coefficient of variation (SD/mean*100%).
Results: The repeatability for cortical bone structural indices was 1.3%, 0.35%, 1.01% respectively for total density, cortical density, and cortical thickness. The CV% of trabecular structural indices was 1.73%, 1.17%, 5.01% and 1.83% respectively for, trabecular thickness, number, connectivity density, and separation. The CV% (SD) of water density was 3.45% (0.28). For phantoms with a rod density of 100, 200, 400 and 800mgHA/cm^3, the CVs were 0.48%, 0.50%, 0.09% and 0.11% respectively.
Discussion: This study provides an extensive evaluation of the repeatability errors of viva-CT from pure material to complex structure. We conclude that this μCT machine has good repeatability for the assessment of cortical and trabecular bone density and architectural characterization in mice femurs.

Parameters	Mean	SD	CV%
Water phantom (mgHA/cm^3)	8.33	0.28	3.45
Phantom-100 (mgHA/cm^3)	196	0.9	0.48
Phantom-200 (mgHA/cm^3)	315	1.5	0.50
Phantom-400 (mgHA/cm^3)	540	0.51	0.09
Phantom-800 (mgHA/cm^3)	943	1.0	0.11
Cortical density- midshaft (/mm^3)	1169	4.18	0.35
Cortical thickness- midshaft (mm)	0.204	0.002	1.01
Total density- metaphysic (mgHA/cm^3)	384	5.1	1.3
Trabecular connectivity density (/mm^3)	117	5.88	5.01
Trabecular thickness (mm)	0.125	0.002	1.73
Trabecular number (/mm)	5.88	0.069	1.17
Trabecular separation (mm)	0.139	0.02	1.83

P103

Anti-epileptic drug usage and bone mineral density: a treatment discordant matched twin and sibling pair study

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Aim: Patients taking anti-epileptic drugs (AEDs) have increased risk of fractures and reduced bone mineral density (BMD). Causes and risk factors for these associations are uncertain. We investigated effects of AED-usage on BMD utilizing a treatment-discordant twin/sibling matched pair design (AED-user vs. non-user, where non-user had no history of epilepsy or AED-use). We examined clinically relevant subgroups: (A) current use, (B) polytherapy and (C) epilepsy as AED treatment indication.
Methods: 56 pairs (46 current, 10 past AED-users; 47 female, 9 male; 21 MZ, 21 DZ twins, 14 sib pairs within 3 years of age) with mean (SD) age 45.6 (15.3)y were studied. BMD (Hologic 4500A or 1000W) measured at lumbar spine (LS), total hip (TH), femoral neck (FN), and total forearm (FA). Data adjusted for age, height, weight. Lifetime duration (LDT) and number (LNM) of AEDs calculated. Paired t-tests results expressed as within-pair percentage differences, relative to AED-non-user.
Results: Overall group: No significant within-pair difference in age, height, weight, total fat or lean mass. Significant within-pair differences (AED-user vs. non-user) seen in BMD at TH -4.2%(p=0.02), LS -4.0%(p=0.03) and in subgroups: (A) Current: TH -5.4%(p=0.01), FN -4.3%(p=0.045), LS -4.8%(p=0.03); (B) Polytherapy [(n=14, mean(SD) LMN 3.27(1.94), mean LDT 20.8y(10.3)]: FA -3.4%(p=0.049), TH -8.9%(p=0.04), FN -9.7%(p=0.00); (C) Epilepsy (n=41, LDT(SD) 20.8(13.7)yrs): TH -5.35%(p=0.01), FN -4.29%(p=0.046), LS -4.8%(p=0.03)

Conclusion: Patients taking AEDs for epilepsy and particularly taking polytherapy have reduced BMD when compared to their gender-matched twin/sibling. Whether this reflects epilepsy severity or duration and number of AEDs used requires further investigation.

P104

Monitoring bone growth using quantitative ultrasound in comparison with DXA and pQCT

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Different quantitative ultrasound (QUS) modalities have been developed for bone assessment. The usefulness of two QUS modalities for monitoring bone growth in pubertal girls was examined in comparison to DXA and pQCT. This was a 2yrs longitudinal study involving 258 10-13yrs pubertal girls and 9 37-43yrs healthy adults. Calcaneal broadband ultrasound attenuation (BUA) was assessed using QUS-2 (Quidel, Santa Clara, CA), speed of sound (SOS) of tibial shaft by Omnisense (Sunlight Technologies), apparent vBMD of tibial shaft using pQCT (XCT2000, Stratec), and femur neck (FN) and lumbar spine 2-4 (LS) aBMD by DXA (Prodigy, GE). Over 2-yrs in girls, FN and LS aBMD showed the largest increases (17±8% and 20±8%, respectively), followed by tibial vBMD and calcaneal BUA (10±5% and 9±9%, respectively). There was no apparent change in tibial SOS (2±3%). The larger increase in FN and LS aBMD was accounted for by the change in body size. The change of calcaneal BUA correlated significantly with the change of FN and LS aBMD and tibial vBMD (r=0.24-0.40). At the matched site, SOS correlated only with cortical vBMD, not with cortical thickness or apparent vBMD. The long-term reproducibility, assessed using the concordance correlation of young adults' pre-post measurements (2-yrs interval), was substantially lower in tibial SOS than calcaneal QUS, tibial vBMD, femur neck and lumbar spine aBMD (0.65 vs. 0.97, 0.95, 0.98 and 0.96). In conclusion, the transverse transmission method-derived BUA, not the axial transmission method-derived SOS, is a sensitive and reliable tool comparable to DXA and pQCT for monitoring bone growth.

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The structural strength of a femoral cross section varies according to its position along its length

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It is held that increasing bone length (L) increases strain producing adaptations by greater periosteal apposition, total cross sectional area (TCSA) and section modulus (Z, estimated bending strength). Minimizing strain can occur by modifying cross sectional (CS) shape, cortical and trabecular bone distribution and curvatures of the whole bone axes. We examined the association between femur L and its cross sectional (CS) indices of bending and compressive strength [Z, total bone area (TBA) and TCSA] using HR-pQCT (Scanco) at 30% (subtrochanteric) and 70% (supracondylar) of L in 23 post-mortem specimens from individuals aged 67-94 years. Correlations between L and TCSA were not significant (r = 0.35 NS). Correlations between L and Z and TBA ranged from 0.47 to 0.53 (all $p<0.05$). In a multivariate model (Z, TBA, TCSA), at the subtrochanteric region, correlations were found between TBA and L ($r = 0.47$, $p < 0.05$), Z and TCSA did not correlate with L. At the supracondylar region, Z ($r = 0.47$, $p < 0.05$), but neither TCSA or TBA, correlated with L.
Structural adaptations vary along the femur and are functions of prevailing loads. Longer bones need not be wider and bending strength is not necessarily greater throughout their lengths. Peak strains may be greater distally producing greater adaptations by periosteal apposition. Understanding whole bone strength requires an understanding of regional loading needs. Single estimates of strength may not be an appropriate indicator of whole bone strength.

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Reproducibilty of micro-CT for assessing *in vivo* bone structure

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Background High-resolution peripheral quantitative computed tomography (HR-pQCT, voxel size=82µm) provides images with resolution around 100 micron allowing detailed assessment of the macroarchitecture of bone. To assess the precision of this method we repeated measurements in samples to derive the least significant change (LSC) to determine the utility of the method. We evaluated the precision of HR-pQCT (XtremeCT; Scanco Medical AG, Basserdorf, Switzerland) in determining bone density and bone macro-architecture parameters.
Materials and Methods In 15 volunteers free of disease known to affect bone the non-dominant forearm was scanned 3 times with repositioning at a single visit. Scanning parameters, evaluation and reconstruction of 3D images were performed using the default mode provided by the manufacturer. The reproducibility is expressed as coefficient of variation (%CV = SD/Mean*100) and the LSC as $2\sqrt{2}$*%CV. Data is presented as %CV (LSC) for each interested variable.
Results The reproducibility and LSC of the Xtreme pQCT for 3-D assessment of ultra distal radius bone density and structure, including apparent volumetric BMD (vBMD), trabecular compartment vBMD, cortical vBMD [%CV(LSC)] were 0.86(2.39), 0.90(2.50), and 0.60(1.67) respectively. The reproducibility and LSC of the ratio of trabecular bone volume to tissue volume and cortical thickness were 1.73(4.81) and 1.40(3.88) respectively.
Conclusion The technique is precise for in vivo bone density and macrostructure assessment. Studies are underway assessing the precision and LSC of microarchitecture parameters.

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Back-scattered electron imaging of bone mineralisation in osteoarthritis

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Osteoarthritis affects a large proportion of people in our society. There is controversy over the degree and nature of bone changes in this disease with the presentation of heterogeneous tissue-level morphology. Studies of subchondral bone have shown increased bone volume and decreased bone mineralization, but more distal sites have not been systematically investigated. We have used a quantitative back-scattered electron imaging technique to analyse intertrochanteric bone samples from 22 patients (11 male 70±12 years, 11 female 68±13 years) undergoing total hip replacement for osteoarthritis, compared with 20 normal post mortem controls(12 male 60±14 years, 9 female 63±14 years). Bone samples were fixed, embedded in methylmetacrylate resin, polished, carbon coated and examined in a Philips XL20 scanning electron microscope which was calibrated with carbon/aluminium standards. Data were pooled to create a mineralisation distribution for each of the groups as well as analysing the weight percent calcium and bone volume data for individuals. The mineralisation of the OA group was less than that of the control group (24.2 versus 25.3 wt%Ca). Statistical analysis of individual's values revealed a significant lowering of the weight percent calcium in osteoarthritis (24.4 versus 25.1 wt%Ca, $p<0.001$) concomitant with a significant increase in bone volume (10.4% versus 7.6%, $p<0.02$). These results are similar to those reported for subchondral bone in osteoarthritis and imply an increased rate of bone turnover associated with a net positive gain in bone volume. These findings at a site distal to the primary disease process have implications for systemic changes of bone metabolism in OA.

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Relative contribution of differences in body composition to racial and sex differences in bone mineral density in Chinese and Caucasians

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We studied 258 Chinese (182 females) and 391 Caucasians (282 females) aged 18-45 years to determine whether racial differences in total body bone mineral density (TBBMD) are explained by racial differences in body composition. TBBMD, fat mass (FM), and lean mass (LM) were measured by DXA (Lunar).
The proportion of FM was similar in Chinese and Caucasian women (31.3 vs. 34.8%, $p < 0.01$) and men (20.8 vs. 21.8%, NS). TBBMD was 1.0% lower in Chinese than Caucasian women ($p=0.09$). Adjusted for FM and LM, Chinese women had a 1.6% higher TBBMD than Caucasians ($p < 0.05$). FM and LM accounted for 42.4% and 57.6% the racial difference in total BMD in women respectively. The 3.1% lower TBBMD in Chinese than Caucasians men disappeared after FM and LM adjustment. FM and LM accounted for 14.0% and 86.0% of the racial difference in TBBMD in men.
In Chinese, TBBMD was 3.7% lower in women than men. After FM and LM adjustment, women had a 3.8% higher TBBMD than men. FM and LM accounted for 10.4% and 89.6% of the sex difference in TBBMD in Chinese. In Caucasians, the 5.7% lower TBBMD in women than men disappeared after adjustment. FM and LM accounted for 11.0% and 89.0% of TBBMD variance in Caucasians.
The difference in LM not FM accounts for most racial and sex differences in TBBMD. Whether this is due to shared genetic determinants in bone and muscle mass or a causal relationship produced by loading is not known.

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The femoral neck is no drinking straw: heterogeneity in structure is not captured using the Hip Structural Analysis

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To determine whether single indices derived using hip structural analysis (HSA) adequately reflect FN structure and strength, we measured cortical thickness (C.Th) at every degree around the perimeter of each of the ~ 200 cross sections (CS) along the FN, total cross sectional area (CSA), and section modulus (Z) directly from micro–CT images obtained in specimens from 13 Caucasian females (mean age 69 years). The structure of the narrow neck (NN) and mid-FN were also examined. A single C.Th failed to capture the structural diversity within a CS. The CV (%) of C.Th around the CS averaged 80% (range 54 – 130%; depending on the CS). Along the FN, the structure of one CS only modestly resembled an adjacent one. For example, two CS around the mid-FN separated by less 1mm differed in Z by 12 to 30%. In a region of ± 1 mm to the mid-FN, in ~ 90% of cases, there was a greater than 10% difference in C.Th between two adjacent CS. The NN used as reference varied between specimens, was more distal in individuals with larger NSA ($r = 0.53$, $p<0.05$) and corresponded to the mid-FN in only 2 of 13 specimens; indices at the NN and mid-FN were not comparable. Interpretation of FN structure using HSA protocols inadequately reflects its structural diversity and strength and should be viewed with scepticism. More refined methods to quantify FN structural diversity may help define the structural basis of FN strength, better predict fracture and treatments effects.

P110

The time-course of response to three high oral cholecalciferol doses

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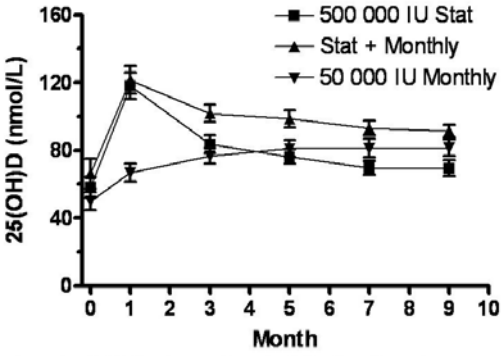
Monthly or loading doses of calciferol are often more convenient than daily doses and are commonly used to maintain vitamin D status in elderly populations. The magnitude and time-course of response to these have not been well characterised since most studies have supplemented with doses of 400-800 IU/day. We compared 25-hydroxyvitamin D [25(OH)D] response to three high-dose vitamin D_3 schedules. Sixty-three people, aged 81.6±6.7 years (mean±sd), were recruited on discharge from hospital. They were randomly assigned to supplementation with either a single dose of 500,000 IU (Stat), 500,000 IU stat plus 50,000 IU monthly for eight months (Stat+Monthly), or 50,000 IU monthly alone (Monthly), in a double-blind trial. Baseline 25(OH)D was 58±32 nmol/L and was not different between groups. Mixed-model ANOVA of 25(OH)D levels throughout the study showed significant Month, Dose, and Month x Dose interaction effects (P<0.001, see figure). The greatest integrated 25(OH)D response was for those in the Stat+Monthly group: 25,800±1100 day.nmol/L compared to 22,400±1300 day.nmol/L and 20,100±1100 day.nmol/L in the Stat and Monthly groups respectively (P=0.004). It took five months for the Monthly group to reach a 25(OH)D plateau of approximately 80±20 nmol/L, the peak being at 27±3 weeks compared to 8±2 weeks in both the Stat and Stat+Monthly groups (P<0.001). After 9 months only the Stat+Monthly and Monthly groups maintained mean levels above 80 nmol/L. If rapid attainment of recommended levels of 25(OH)D is desired in frail elderly patients, a loading dose is recommended. Subsequent regular supplementation is necessary to sustain these levels.



Levels of 25-hydroxyvitamin D by treatment group
Values are mean ± SEM

P111

Increases in adiposity and areal BMD in young women: Geelong Osteoporosis Study

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In this study we investigated the effect of the obesity epidemic on young adult women. Two randomly-selected groups of women of the same age (20-29 yr), measured a decade apart, (baseline 1994-7, n=210; 10 yr follow up 2004-7, n=138) had body composition assessed and compared as part of their involvement in the Geelong Osteoporosis Study (GOS). The two groups were mutually exclusive. Areal BMD was measured at the PA spine (SP) and femoral neck (FN) (Lunar DPX-L). Anthropometric measurements (weight, height, waist and hip circumference) were recorded; lean and fat tissue measurements determined from whole body scans. Compared with the 1994-7 group, all indices of adiposity increased in the 2004-7 group: weight +6.4%, fat tissue +11.7%, lean tissue +3.7%, waist circumference 3.7%, waist/hip ratio +4.0%.

Body composition (median (IQR) or mean±SE)			
	1994-7	2004-7	P
Weight (kg)	62.9(56.9,72.2)	66.9(59.9,79.1)	0.00
Fat (kg)	20.6(15.6,29.5)	23.0(16.9,33.5)	0.05
Lean (kg)	39.7±0.3	41.2±0.4	0.00
Waist (cm)	75.2(69.8,82.8)	78.0(71.0,88.5)	0.01
W/H ratio	0.75(0.72,0.79)	0.78(0.73,0.83)	0.00
BMD (g/cm^2)			
SP	1.23±0.01	1.28±0.01	0.00
FN	1.02±0.01	1.05±0.01	0.06

Increases in BMD were also observed: SP +3.7%, FN +2.7%. Weight confounded the association at the SP and it was also an effect modifier at the FN. Change in weight-adjusted SP BMD was +3.2% (1.236±0.010 vs 1.276±0.012; p=0.01). Weight-adjusted FN BMD increased for women with low weight but decreased for women with high weight; for example at 55 kg, the change in BMD was +5.2% and at 85 kg, –1.6%.

The association between increased weight and increased BMD is likely to be mediated by skeletal loading and/or humoral factors. In this study, both weight-dependent and independent changes in BMD may reflect differences in lifestyle, bone size or structure that have emerged in conjunction with the obesity epidemic.

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Biocompability performance of bone allografts irradiated at standard and lower doses

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Gamma radiation is commonly used to sterilise bone allografts, but the effect of radiation on bone allograft biocompability is not well documented. Therefore, the aim of this study is to investigate what changes occur in the biocompatibility of bone allographs over a range of gamma radiation dosages.

Methods: In this study, cortical bone sections (100 µm thickness) were irradiated at 0, 5, 10, 15, 20, and 25 kGy. These irradiated bone sections were then assayed using the following set of biocompatibility tests. ***Macrophage based assay:*** The pro-inflammatory murine macrophage indicator cells (RAW264.7-ELAM-GFP) were seeded at 3 x 10^5 cells/bone slice and were incubated at 37°C for 24 hours. Cells are then flushed off the bone and analysed for GFP expression with a FACS-Calibur flow cytometer. ***Proliferation assay:*** Murine fibroblast/preosteoblastic MC3T3-E1 cells was seeded at 2 x 10^4 cells/slice and incubated at 37°C for 2 and 4 days and an MTT of cellular proliferation was performed. ***Attachment assay***: An MC3T3 cell suspension was seeded onto bone slices at 2 x 10^4 cells/well, and incubated at 37°C for 2 hours, the slices were washed and an MTT assay was performed on the adherent cells. ***Osteoclast assay:*** bone marrow cells from Balb/c mice were cultured with bone slices at 37°C for 7 days in the presence of CSF-1 and RANKL. Attached cells were fixed and TRAP stained. The number of TRAP+ multinucleated cells was counted.

Results: There was no significant difference by ANOVA in the response of ELAM cells to bone sections sterilised at 0, 5, 10, 15, 20 or 25 kGy. Similarity, neither the attachment nor the proliferation of MC3T3-E1 cells was statistically significantly different between irradiated and control groups.

Conclusions: The use of gamma irradiation for sterilisation at standard or lower doses does not alter biocompability performance of bone allografts as determined by these in vitro assays.

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Chronic anti-epileptic drug treatment is associated with lower balance function test performance - an AED-discordant matched twin and sibling pair study

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Aim: Patients taking AEDs for epilepsy have increased fracture risk, potentially due to bone disease and falls during seizure and at other times. We examined effects of AED-use on muscle strength, gait and balance utilizing a matched twin/sibling AED-discordant pair model.

Methods: 27 same-sex pairs (5 monozygous, 5 dizygous twin, 17 sib pairs; 11 male, 16 female); mean(SD) age 45.5(16.1) assessed. For AED-users, mean(SD) treatment duration was 22.7(16.6) years; AED-indication- epilepsy 26 cases, migraine 1 case. Validated tests predictive of falls risk performed: Chattecx Balance System(CBS) [stable or moving platform, +/-distraction task], Lord's Balance Test(LBT); Kincom, Nicholas Manual Muscle tester (muscle strength); Gaitrite® measures. Human Activity Profile and relevant serum AED-levels measured. Results adjusted: Kincom data for weight; LBT for height; CBS for height: sway index (SI)cm/m; left-right sway (LR)cm/m.

Results: No significant within-pair differences in age, height, weight, strength, or gait measures. AED users had significantly lower activity scores [maximal activity score -3.8 (p=0.05), adjusted activity score -9.0 (p=0.00)] and poorer balance on a number of CBS measures (p≤0.05): Stable platform+distraction: LR +0.38 (p=0.01); anteroposterior moving platform: SI +0.24 (p=0.01), LR +0.37 (p=0.02); mediolateral moving platform(MLMP): LR +0.63 (p=0.02); MLMP+distraction: SI +0.18 (p=0.05), LR +0.82 (p=0.01) and Single leg stance (SLS): stable platform SI +0.16 (p=0.04), LR +0.50 (p=0.04). Valproate level above therapeutic range: 1 patient.

Conclusion: Balance function is inferior in chronic AED users compared to their matched twin/sibling. This impaired balance may increase falls risk and contribute to increased risk of fractures in AED users.

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