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ABSTRACTS

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INVITED SPEAKER ABSTRACTS:

IP1

LESSONS FROM FRACTURE PREVENTION TRIALS

Steven R. Cummings, MD, FACP
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The 1990s generated evidence that a variety of antiresorptive treatments reduce fracture risk more effectively and rapidly than expected from the modest improvements in bone mass. These observations have drawn attention to non-BMD ('bone quality') effects of treatments, but it is not clear how to assess quality in research studies or future clinical practice. While demonstrating the rapid onset of reduction in vertebral fractures, trials have also suggested that potent antiresorptives reach a limit and might have a declining effectiveness in preventing vertebral fractures after the first year or two of therapy. Because trials have been short-term, we may never be sure whether it is helpful or harmful to continue potent anti-resorptive therapy longer than five years.

Trials have shown that antiresorptive therapies generally cannot restore normal bone and fracture risk. The recent trial of PTH has demonstrated that it is possible to improve bone mass, restores architecture and reduces fracture risk raises the possibility that we may be able to treat patients until we reach a goal of normal bone mass and structure and acceptably low risk of fracture. We need a clinical trial comparing this new clinical strategy to the current practice of long term 'maintenance' with antiresorptives.

Fracture trials have, so far, found that four to five years of drug treatment of the millions of women with "osteopenia" is either ineffective or not worthwhile. They have not, and may never, answer whether preservation of bone mass and structure early after menopause produces substantially greater benefit than treating when osteoporosis develops. In aggregate, trials have shown that prevention of fracture in otherwise healthy women with osteoporosis decreases disability somewhat, but does not reduce mortality. At the same time, trials of raloxifene and estrogen have shown that these hormonal treatments for osteoporosis have other effects that are more important than prevention of fractures.

Despite the success of fracture trials, we have realized that there is more to reducing fracture rates in populations than proving that drugs work. Effective treatments are underused, often stopped and too expensive to substantially reduce fracture rates especially in patients and countries with limited funds for health care. Under use and nonadherence will be more easily solved than affordability.

IP2

PARATHYROID HORMONE: A NEW HORIZON IN THE THERAPY OF OSTEOPOROSIS.

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In concept, an anabolic agent is very different from an antiresorptive drug. Anabolics stimulate bone formation whereas antiresorptives reduce bone remodelling. The time during which bone formation is stimulated preferentially over bone resorption can be viewed as the "anabolic window". Parathyroid hormone (PTH), an anabolic agent currently under intensive investigation, is used at low dose and once daily, an approach that minimizes its catabolic potential and maximizes its anabolic potential. With PTH therapy in women and men with osteoporosis, increases in lumbar spine and in hip density are seen. Forearm bone density does not change or falls slightly. Bone formation markers rise quickly followed thereafter by increases in bone resorption markers, a kinetic pattern consistent with the concept of an anabolic window. Vertebral and non-vertebral fractures are reduced significantly by over 60% and 50% respectively. Analyses of bone biopsies before and after therapy with PTH show enhanced connectivity among trabecular plates. Cancellous bone volume is increased. Surprisingly, cortical wall thickness is increased. This latter observation would seem to be at variance with the fact that cortical bone density does not change or is slightly reduced. However, geometrical properties of bone at cortical sites are also changed by virtue of the fact that periosteal bone apposition is greatly stimulated. The result is greater wall thickness and larger cross-sectional area. These findings predict greater bone strength that has now actually been confirmed in primate models. PTH marks the first agent in a new paradigm of therapy for osteoporosis. Questions such as who are the ideal candidates for therapy, whether antiresorptive agents should be used before, during, or after PTH therapy, and whether retreatment protocols are likely to be indicated after an initial treatment period with PTH, are all being addressed at this time in ongoing studies.

IP3

UNRAVELLING THE COMPLEXITIES OF HUMAN OSTEOCLAST DIFFERENTIATION.

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Osteoclasts normally form in the bone marrow in the presence of multiple cell types including those of the osteoblast/stromal, haematopoietic, immunological and angiogenic lineages. These cells can directly effect osteoclast generation and function and can also mediate the effects of local and systemic humeral factors. In this complex environment it is difficult to determine the mechanism of effects produced by treatments and other manipulations.

The availability of recombinant soluble RANKL and human M-CSF has allowed the in vitro generation of human osteoclasts from readily available haematopoietic sources such as peripheral and cord blood mononuclear cells (PBMC and CBMC) as well as $CD14^+$ cells isolated from PBMC and CFU-GM derived from CBMC. Importantly, the osteoclasts can be produced and studied in cultures free of modulating cells such as osteoblasts and lymphocytes. However, co-cultures with other cells can be used when investigating the modulating effects of these cells.

Unstimulated total, $CD3^+$ and $CD4^+$, but not $CD8^+$, $CD19^+$ nor $CD16^+$ lymphocytes potently inhibit osteoclastogenesis when co-cultured with osteoclast precursors treated with RANKL and M-CSF. This inhibition is prevented by co-treatment with dexamethasone, which paradoxically, is itself a direct inhibitor in the absence of added lymphocytes. A similar paradox exists with 1,25 dihydroxyvitamin D which promotes osteoclastogenesis in the presence of osteoblasts (by up-regulation of RANKL expression by osteoblasts) yet is a potent direct inhibitor in the absence of osteoblasts. Parathyroid hormone also has opposite direct and indirect (osteoblast-mediated) effects and it is likely that many other examples of this phenomenon exist.

Significant differences exist in the regulation of murine and human osteoclastogenesis. In murine models, TNFα promotes osteoclast generation in the presence of M-CSF, independent of RANKL, and has a synergist effect in the presence of low concentrations of RANKL. However, in our human models we find that TNFα does not promote significant osteoclastogenesis in the absence of RANKL (<2% of that induced by RANKL) and is not synergistic. TGFβ has been reported to enhance RANKL/M-CSF-induced osteoclastogenesis in murine systems but inhibits in our human models.

The cell biology and molecular tools now exist to define the complex cellular interactions and molecular mechanisms involved in osteoclast differentiation. While murine models provide valuable information and have certain advantages (eg the availability of transgenic animals), because of species differences, it will be important to also investigate using human models.

IP4

MOLECULAR INTERACTIONS IN PATHOLOGICAL BONE LOSS

David R Haynes, Department of Pathology, University of Adelaide, Australia.

Over the past decade there have been major advances in our understanding of the factors that regulate osteoclast formation. It is now apparent that receptor activator for NFκβ (RANK), its ligand, RANKL (also known as TRANCE, osteoclast differentiation factor and osteoprotegerin (OPG) ligand) and the RANKL inhibitor, OPG, are the major factors influencing osteoclast formation. While much is known about the way these molecules influence normal bone physiology, comparatively little is known about the activity of these molecules in human disease. However, there is now growing evidence that RANK-RANKL interactions stimulate osteoclast formation in major bone loss pathologies. This presentation reviews our recent work using a variety of techniques, including immunohistological and *in situ* staining techniques to compare, for the first time, expression of the above factors in several major human bone loss pathologies. These pathologies are, aseptic peri-prosthetic bone lysis, rheumatoid arthritis and alveolar bone loss in periodontal disease. Immunohistological analysis and *in situ* hybridisation was carried out on frozen human tissue biopsy specimens. Sections were evaluated by computer-assisted image analysis and semi-quantitative analysis. Overall, our results showed that there was a both an elevation in the expression of RANKL and a reduction in OPG expression in the tissues adjacent to bone loss in three different diseases. *In situ* hybridisation indicated that the cells expressing RANKL or OPG mRNA were likely to be producing the same protein. These studies show that OPG and RANKL are likely to be key molecules regulating bone loss in a variety of diseases and that therapeutic intervention that targets these molecules may be helpful in treating pathological bone loss.

IP5

CELLULAR AND MOLECULAR MECHANISMS OF BONE LOSS IN RHEUMATOID ARTHRITIS: RELATIONSHIP TO PHYSIOLOGIC BONE RESORPTION.

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Focal marginal joint erosions represent the radiographic hallmark of rheumatoid arthritis (RA). These bone changes are localized to the joint margins, but also are present in the subchondral bone adjacent to the bone marrow space into which the inflamed synovial tissues have extended. Because progressive destruction of the articular bone contributes significantly to joint dysfunction and disability in patients with RA there is considerable interest in developing a better understanding of the cellular and molecular mechanisms involved in this process and in designing therapies that can arrest these events. Previous analysis of joint tissues from patients with RA indicate that osteoclasts appear to be the major cell type responsible for the focal bone erosions. Recent data from animal models of inflammatory arthritis, including adjuvant arthritis (Kong et al. Nature 1999; 402:304), serum transfer arthritis (Pettit et al. Am J Pathol 2001;159:1689) and TNF-transgenic mice with spontaneous arthritis (Redlich et al. Arthritis Rheum 2002; 46:785), provide further evidence implicating osteoclasts in the pathogenesis of focal bone erosions. These studies and other in vivo and in vitro models have helped to identify the cytokines and inflammatory mediators that are involved in the recruitment and activation of bone resorbing cells associated with inflammatory arthritis. Among these factors, receptor activator of NF-kβ ligand (RANKL), appears to play a critical role. These findings provide a rational framework for developing targeted therapies that can specifically inhibit or slow the progressive focal bone destruction associated with the rheumatoid synovial lesion and serve as a paradigm for dissecting the mechanisms responsible for pathologic bone loss associated with other inflammatory disorders that affect skeletal tissues.

IP6

THE ROLE OF FGF AND FGFR IN THE CONTROL OF OSTEOBLAST PHENOTYPE.

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The effects of growth factors, particularly fibroblast growth factors (FGFs), on osteogenesis are contributing to the design of novel therapeutic techniques for bone repair. It has been well demonstrated that FGFs once bound to their specific receptors (FGFRs), are capable of stimulating the proliferation and differentiation of cells of the osteoblast lineage. The importance of the FGF/FGFR complex has been further verified by FGFR-linked skeletal disorders. Insight into the expression patterns of specific FGFRs and FGFs during osteoblast development will significantly improve the therapies available for the treatment of osteogenic disorders.

Cultured MC3T3-E1 cells (murine preosteoblasts) were grown for up to 30 days. Every 5^{th} day, cultures were assayed for proliferation, differentiation and maturation status using a combination of bromodeoxyuridine (BrdU) incorporation, protein and gene expression for alkaline phosphatase (AP), osteopontin (OP), and osteocalcin (OC). At each time point, changes in the gene expression of FGF and their receptors (FGFR1-4) were also examined and their expression compared to the developmental status.

Our results show that MC3T3-E1 cultures have an extensive proliferative capacity for up to 10 days, followed by a drop in proliferation and a subsequent rise in alkaline phosphatase, osteopontin and osteocalcin expression (markers for osteoblast differentiation and maturation). In phase with this switch from a proliferative to a differentiative phenotype, was a change in the expression profiles of the FGFRs. These findings are of use in facilitating the application of osteoblasts to bone reconstruction. We are uncovering novel ways of using fibroblast growth factor signalling and biomaterials to regulate osteoblast development that aims to develop ways for improving bone repair.

IP7

BONE HISTOMORPHOMETRY: UNFOLDING TISSUE LEVEL SECRETS ESSENTIAL IN SKELETAL PHYSIOLOGY AND DISORDERS

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By 1950 intensive studies of the skeleton's biochemistry, molecular and cellular biology had begun but the hidden assumption, neither realised nor verbalised, was that skeletons lacked tissue-level structures. At this time Harold Frost devised a method to prepare undecalcified bone sections that enabled the study of bone by bright field and ultra violet light microscopy, to study the skeleton's tissue-level organisation. After almost 30 years, in 1980 it was accepted that skeletal histomorphometry would enable the study of bone dynamics in all bones and diseases to reveal how drugs and skeletal loading affected these dynamics. Until histomorphometry was widely accepted as a valuable investigative tool, skeletal physiologists had overlooked the tissue-level organisation, so they had assumed bone diseases stemmed directly from disturbances in osteoblasts and/or osteoclasts, or in their regulation by humoral and genetic factors, with no "middle man". Histomorphometry is a powerful tool for mining hidden physiology, why things exist and/or work in the way they do, in the intact skeleton's tissue-level.

It took years to realise that BMU-based remodelling exists to: 1) to replace primary spongiosa with secondary spongiosa during endochondral ossification, 2) to replace fracture callus with lamellar bone, 3) to remove mechanically unneeded bone and more recently, 4) to repair fatigue/microdamage. Bone histomorphometry is the only tool available to study the tissue-level organisation of bone and is an essential tool in mapping gene expression to bone morphology and understanding the skeletal consequences of anatomical structure-function relationships.

IP8

A HISTOPATHOLOGIST'S EXPERIENCE AND PRACTICE IN THE USE OF BONE HISTOMORPHOMETRY

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A twenty-two year practice in metabolic bone biopsy diagnosis using histomorphometry is described with particular reference to the most recent 10 years.

Bone biopsies have usually been transiliac, double tetracycline labelled where possible and embedded in methylmethacrylate. Bone sections have been cut on a Jung K, heavy duty microtome and sections stained using Goldners' modified trichrome , Von Kossa and toluidine blue and where applicable a stain for aluminium has been used. For the first 10 years of the metabolic bone biopsy practice "manual" histomorphometry was employed involving the use of a Zeiss integrating microscope eyepiece graticule and a haematology bone cell counter to register "hits" on desired bone features. Since 1990 "Osteomeasure" software in a personal computer linked to a digitizing tablet has been used.

In the decade 1990-1999, 144 bone biopsies were submitted for analysis. Approximately 30% of biopsies were studied for renal osteodystrophy, 24% of biopsies were for osteoporosis and 22% for osteomalacia or mineralization defect. Remaining biopsies were taken for a variety of purposes, including drug effect monitoring.

Bone histomorphometry in my experience has proved to be a useful enhancement to qualitative histological examination of bone biopsies submitted for assessment of metabolic bone disease, refining diagnoses that influence treatment decisions and monitoring treatment trials.

IP9

PHASE-CONTRAST X-RAY IMAGING OF BONE

DE Myers[1], M Waltham[2] and AW Stevenson[3].
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Various imaging techniques are used for assessment of bone quality to aid clinical investigations and the study of metabolic bone diseases. A novel technique, termed phase-contrast X-ray (PCX) imaging, has been developed. Phase shift variations in the X-rays emerging from the sample manifest as intensity variations that are recorded digitally using imaging plates (1).

PCX imaging enables acquisition of high-resolution images and, in bone samples, the digital images can be used for quantitative assessment and for presentation as photographic images. Fine microstructures in bone can be resolved, particularly features of woven bone in the trabecular region, a reactive region in metabolic bone diseases. This technology enables assessment of various biological samples and represents a novel methodology for the assessment of diseases of bone. In animal models, tumour metastases to bone and fine features of lesions can be discerned due to the X-ray phase shifts that occur at object density boundaries at bone interfaces.

PCX imaging shows potential for new applications in diverse biomedical fields including imaging of excised and processed pathology samples (e.g. tumours/organs), tumour detection and definition of tumour-induced bone lysis. In the field of metabolic bone research, techniques under development include qualitative analysis of tumour metastases to bone in animal models (2) and quantitative analysis of long-bone microstructures in animal models of osteoporosis. While PCX imaging using synchrotron-radiation techniques has been applied in other orthopaedic studies (3), we have achieved PCX imaging without using a synchrotron. In conclusion, we believe that PCX imaging has the potential to become a routine procedure in metabolic bone research, diagnostics and monitoring of therapeutic intervention in disease.

1. Wilkins SW, Gureyev TE, Gao D, Pogany A, Stevenson AW Nature (1996) 384 335-8
2. Waltham M, Stevenson AW, Javni J, Gao D, Williams ED *et al.* Proc. Am. Assoc. Cancer Res. 91st Ann meeting, New Orleans, 2001
3. Mori K, Sekine N, Sato H, *et al.*, Synchrotron Rad (2002) 9 143-7

IP10

LINKING THE CELLULAR AND TISSUE RESPONSES OF MECHANICAL LOADING TO PRACTICAL OUTCOMES.

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Loading determines the length and shape of bone during growth. The section of bone (diaphysis, metaphysis or epiphysis), the surface of bone (periosteal or endocotical) and the age and maturity of bone will influence how the skeletal responds to loading. In addition bones within the skeleton will respond differently to loading depending on the MES and the background loading history (i.e. weight bearing versus non-weight bearing bones).

Constant load is required for the template for longitudinal growth to be fulfilled. The bone lengthens in unloading and shortens when overloaded. The osteocyte has been implicated as the key messenger for the bones adaptive responses to loading (1). The osteocyte signalling sets the threshold for maintaining BMD in bones being exposed to different loading strains. The osteocyte participates in the development of bone shape in response to loading by coordinating site-specific recruitment of osteoblasts and/or osteoclasts.

At the tissue level the results of animal studies inform about the combinations of rate, frequency, magnitude and duration and the response to loading. Such prescription detail about the human skeleton is limited; the field has been driven by the response to generic loading regimes expressed as BMC or BMD, with little indication of the associated change in bone structure or strength. Generic assessments of BMD also provide little information about where the loaded bone has responded. Recent work has shown that there is heterogeneity in the surface-specific response to exercise at the mid and distal humerus (2).

The response to loading is also influenced by the hormonal and nutrient status of the individual. The skeletal response to loading is enhanced in post-menopasual women when combined with estrogen. The level of calcium intake also appears to influence the loading response at the femur.

In summary investigation of the skeletal response to loading at the cellular, macroscopic and tissue level is essential for informing the practical application of exercise as a tool for osteoporosis prevention.

1. Burr et al., J Musculoskel Neuron Interaction 2002; 2(3): 264-7
2. Bass et al., JBMR (In press)

IP11

IMMOBILISATION, EFFECTS UPON THE SKELETON - FROM EMPIRICAL APPROACHES TO BEST PRACTICE.

Jillian Clark, Hampstead Rehabilitation Centre, Adelaide University.

A body of experimental and clinical evidence supports the proposition that periods of immobilisation and in particular, skeletal unloading lead to bone loss. However, gaps in understanding of the determinants of immobilisation bone loss have led to a lack of consensus about the skeletal management of clinical populations[1]. In a climate of longevity medicine, where rehabilitation and falls prevention programmes are mainstays of management, it is important to define the efficacy of accepted interventions and acknowledge the limitations of resource-intensive regimes. The present health position- a reflection of the repertoire of prescriptive knowledge- led to our research interests in the natural history of immobilisation bone loss (IBL), following catastrophic trauma and illness.

Observations of this population indicate that bone loss may be predictable during a period of immobilisation from peak bone mass to skeletal fragility leading to notions of early predictors and pre-clinical pathways. The profiles of metabolic activity are consistent with earlier histological reports of an increase of marrow adipogenesis for both immobilised and aging populations[2,3], the hormonal profiles of immobilised populations[4] and more recent evidence from experimental systems, of central hormonal actions,[5] upon bone mass.

Additionally, population surveys have identified the potential socio-economic impact of declining functional mobility, for this and other countries. Clearly the management of these issues is complex and will not be addressed by a single approach or therapy.

1. Consensus Statement, MJA, 167, S4-15, 1997
2. Mineure et al Calcif. Tiss. Res,, 17,57-73, 1974.
3. Rozman et al Exp Haematol., 17, 34-37, 1989.
4. Baumann et al Hormone and Metabolic Research, 28, 723-6, 1996
5. Hertzog et al Proc. of 22nd Annual Meeting, ANS, 2002

IP12

ESTROGEN RECEPTORS AND THEIR SPECIFIC CONTRIBUTIONS TO BONE STRUCTURE; INSIGHTS FROM KNOCKOUT MICE

Natalie A Sims

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The role of estradiol in maintaining trabecular bone volume is well established and while two mammalian estradiol receptors (ERα and ERβ) have been described in bone, their relative roles in maintaining bone mass have not been fully described. Additionally, non-ER-mediated mechanisms of estrogen action in bone have been proposed. To determine contributions of each ER and the role of non-ER-mediated mechanisms, the bone phenotype in single and double ER knockout (KO) mice and the effects of gonadectomy and estradiol treatment have been studied in a number of laboratories using different KO mice. In females, full knockouts of both estrogen receptors results in low bone mass, which is not reduced further by ovariectomy, indicating that osteoprotective effects of estradiol are ablated by the deletion of both ERs. Furthermore, estradiol treatment did not alter either bone mass indicating that both effects require the presence of one or both ERs. In female ERβKOs, as in wild type males and females, estradiol prevents gonadectomy-induced bone loss, indicating that ERα is the major receptor mediating estradiol effects in bone. No response to estradiol is detected in male ERαKOs, indicating that ERα is the sole regulator of bone response to estradiol in males. In contrast, female ERαKOs respond mildly to estradiol with incomplete protection against ovariectomy-induced bone loss and a dose-dependent reduction in bone turnover, indicating that ERβ also mediates some estradiol effects in bone in females. These data confirm that ERα is the major mediator of estrogen osteoprotection *in vivo*, but that ERβ also plays a role in the maintenance of trabecular bone in female mice.

IP13

THE SECONDARY PREVENTION OF FRAGILITY FRACTURES: RESPONSIBILITIES AND LOGISTICS.

L.G. Cleland, Rheumatology Unit, Royal Adelaide Hospital, Adelaide. SA.

Fragility fractures have reached epidemic proportions in the frail elderly with adverse trends likely to continue as more people survive into advanced age. The management of an epidemic involves a multifaceted approach, which encompasses interventions that alter unfavourable physiology, as well as cultural, environmental and, technological change. There are a number of relatively simple public health approaches that could reduce risk of fractures if implemented systematically. However, lack of commitment by professionals and failure to communicate consensus on core issues and to put in place as default procedures, effective logistic arrangements, continue to militate against containment of the epidemic. Matters that need to be addressed include sentinel events (eg. first fractures), avoidable financial barriers, unhealthy nutritional and lifestyle practices and use of inexpensive external hip protectors in those at special risk.

IP15

WORKSHOP: HORMONE REPLACEMENT THERAPY

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Estrogen is essential to development and maintenance of bone mass in women and men. Loss of estrogen production causes a transient increase in the rate of bone loss according to most studies and postmenopausal women who have no detectable estradiol have somewhat lower bone density and an increased risk of hip and spine fractures compared with women who have low but detectable levels.

Randomized trials have consistently shown that estrogen therapy improves bone mass. The HERS trial, the largest trial of hormone therapy (HRT) before July, 2002 found that HRT did not reduce the risk of fractures. However, the larger Women's Health Initiative (WHI) found that a mean 5 years estrogen plus medroxyprogesterone reduced the risk of all fractures combined by 24% (95% confidence interval 0.69 to 0.85) and reduced hip and vertebral fracture risk by 34%. These benefits were somewhat greater for this women.

However, HERS and WHI showed that HRT causes more harm than benefit in WHI. In women without a previous diagnosis of heart disease, E+P increases the risk of CHD by 29%, stroke by 40% and venous thromboembolic disease (VTE) by 2.1-fold. The risks were even greater when the analysis was limited to women who took at least 80% of their study medication: 51% for CHD, 67% for stroke and 3.3-fold for VTE. HRT increased invasive breast cancer 26%, an effect that began in the 4th year of treatment but decreased colon cancer risk 37%. Although HRT did not increase overall mortality there were about 2 more harmful than beneficial events per year for every 1,000 women who were assigned to HRT.

The workshop will use cases to address these and other questions: Should HRT (E+P) ever be started for fracture prevention? If so, under what circumstances? Would lower doses and different preparations be safer and more effective? What advice should be given to women who have been taking HRT for many years? What alternatives can be suggested for managment of hot flashes and other conditions for which HRT has been used?

IP17

OPPORTUNITIES FOR THE FUTURE: REBUILDING BONE, NOT JUST STOPPING THE ROT.

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Although antiresorptive agents are the mainstay of therapy for osteoporosis, they do not directly address a major challenge, namely to improve bone quality as well as bone quantity. This challenge is being met with anabolic agents, in which therapeutic principles differ fundamentally from those that govern antiresorptive agents. Rather than slowing bone turnover, anabolic agents stimulate the processes associated with bone formation. If bone formation is stimulated preferentially and to a greater extent than bone resorption, at least over a reasonable period of time, bone will be formed. If the bone that is formed is normal, anabolic agents have the potential to restore microarchitectural abnormalities that are, in large measure, responsible for skeletal fragility. There is an additional key conceptual feature of anabolics best illustrated by parathyroid hormone, a new and promising anabolic agent. Bone strength is a function not only of bone mineral and its microarchitectural integrity, but it is also dictated, to a certain extent, by bone geometry. If a therapeutic agent, for example, improves the cross sectional diameter of bone, the strength of that bone, for the same amount of bone mineral density, will be greater. Additionally, if an anabolic agent changes the ratio of the outer to the inner diameter of bone, that change too will improve bone strength. For parathyroid hormone, there is evidence in clinical trials and in animal studies that these geometrical properties of bone are influenced favourably. Thus, bone strength is improved not only by greater skeletal density, but also by improved inter-plate connectivity and favourable geometric parameters. With the advent of anabolic agents to treat osteoporosis, the potential for restoration of the skeleton, not merely for maintenance of it, is clear. Treatment of osteoporosis is thus moving quickly to a new paradigm based upon improving bone quality, not just bone quantity.

IP19

OSTEOPOROSIS: EXERCISE AND BONE FRAGILITY.

MR Forwood, School of Biomedical Sciences, Department of Anatomy and Developmental Biology, The University of Queensland, Brisbane Qld 4072.

Individuals diagnosed with osteoporosis have a high risk of skeletal injury. Regular physical activity may contribute to prevention of osteoporosis, but the efficacy of exercise intervention once the disease is established has not been rigorously investigated. The primary benefit of exercise on the bones of adults is conservation, not acquisition. In elderly individuals, physical activity can reduce the rate of bone loss, and improved fitness and muscle strength contributes to prevention of falls, and a lower risk of fracture. Exercise goals for osteoporosis should include pain reduction, increased mobility and improvements in muscle endurance, balance and stability. These are worthwhile end-points because they prevent falls and improve quality of life. In conjunction with advice to increase dietary calcium, exercise plays a significant part of a lifestyle prescription for reducing fractures in later life. In postmenopausal women, it is less effective than oestrogen for maintaining BMD, and should be regarded as part of an overall treatment strategy.

IP20

ASSESSING FALLS RISK

A/Professor Stephen Lord, Prince of Wales Medical Research Institute, Sydney, NSW.

Until recently, most studies of osteoporotic fractures have focused on bone mineral density as the major risk factor. However, in addition to the numerous published reports on the strong relationship between bone mineral density and fracture risk, there are other complementary and independent fracture risk factors. These factors include falls risk factors such as impairments of vision, sensation, strength and balance, the type of fall and patient thinness and frailty. As over 90% of hip fractures result from a fall, and because many falls risk factors are also fracture risk factors, it is important to accurately identify older people at increased risk of falling.

Many falls risk screens have now been devised. These range from simple questionnaires to comprehensive medical, physical, psychosocial and environmental evaluations. Short screens have advantages in that they are low cost, take only a short time to administer and are technically simple. However such screens have only moderate sensitivity and specificity and go little way in identifying the underlying causes of falls in each patient. More comprehensive screens give more information about the causes of falls, and identify factors amenable to intervention. In this presentation, a range of screening tools will be described including the FallScreen program developed at the Prince of Wales Medical Research Institute. FallScreen makes use of normative data derived from large population studies for assessing vision, peripheral sensation, lower limb strength, reaction time and balance. This information is then used to derive a falls risk index score and a physiological profile for identifying specific physiological deficits that require targeted interventions for reducing falls risk.

IP21

DIET, VITAMIN D AND BONE STRENGTH

G Jones

Menzies Centre for Population Health Research, Hobart.

The aim of this presentation is to review evidence relating vitamin D and fruit and vegetable intake to bone metabolism, density and fractures.

Vitamin D is a seco-steroid which is essential for bone and probably joint homeostasis. It is primarily sourced from ultraviolet light exposure and, to a lesser extent, diet. Severe deficiency results in osteomalacia in adults and rickets in children. These clinical syndromes are rare in Australia. However, subclinical vitamin D deficiency appears to be much more common. The definition is controversial but is best defined as the level of serum 25OHD below which bone homeostasis is impaired. Estimates in the literature range from 40 to 110 nmol/l. Tasmanian data support a conservative definition of 50 nmol/l. If this level is considered the cutpoint for deficiency, then virtually all institutionalised subjects are deficient. In addition, a high proportion of medical inpatients are deficient in Perth. In the more Southern latitudes, studies have revealed high rates of deficiency in Tasmania (10% of 8 year olds, 68% of 16 year olds and 85% of community living elderly) and Geelong (45% of premenopausal women). However, there have been few studies aimed at identifying a level above which fracture risk is minimised. Such studies are required before policies on sun exposure or food supplementation can be developed in Australia.

Recently studies have suggested that fruit and vegetable intake may be important for both osteoporosis prevention and bone development. There are many components that may have biologically relevant actions. These include potassium intake, vegetable protein, vitamin C, vitamin K, phytoestrogens and as yet unrecognised compounds. Further longitudinal studies and trials are required at this stage but these are exciting developments.

IP22

A COMMUNITY-BASED APPROACH TO FRACTURE PREVENTION

Douglas S.C.Brown Box 2386, Mt. Gambier SA 5290, Limestone Coast Division of General Practice, Millicent, South Australia

Fractures in the elderly have a dominant place in the cost of injury in Australia. In June 1999, the Limestone Coast Division of General Practice and the Regional Community Health Service began a 3-year collaborative project to lower the incidence of falls and to lessen the extent of falls injury in the some 7550 elderly (65 years and over) in the Lower South East of South Australia. The project attempted to cover both primary and secondary prevention within the three settings of community, residential care accommodation, and hospitals, using a multi-faceted intervention program. Regional reporting of falls and falls injury and the Action Plan began on April 1, 2000 (April Falls Day). The two-year total was some 2100 reported falls and 109 reported fractures. Over the two years, there was no overall reduction in falls; but a marked reduction in hospital admissions and in all fractures, and a significant reduction in hip fractures compared to the previous years' average. This program has saved the State Government a conservative estimate of $400,000 in two years. The Action Plan, what went well and what didn't, and the database analysis will be presented with particular reference to fracture and fracture prevention.

IP23

NEW OPPORTUNITES IN THE ORTHOPAEDIC MANAGEMENT OF FRAGILITY FRACTURES

LR Ferris, Wakefield Orthopaedic Clinic and Modbury Hospital, Adelaide, South Australia

The role of Orthopaedic surgeons in secondary prevention and some of the newer treatment options which are or will be available for treatment of common fragility fractures will be presented. These options include the establishment of clinical teams and protocols with Orthopaedic departments, the concepts of stabilizing vertebral fractures, the role of the new bisphosphonates in peri-operative management and the risk/reward of different implants to stabilize fractures.

ORAL ABSTRACTS:

O1

FEMORAL NECK BONE LOSS PREDICTS RISK OF HIP FRACTURE IN ELDERLY WOMEN

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In postmenopausal women, measurement of femoral neck bone mineral density (FNBMD) predicts hip fracture risk, and improvement in BMD with antiresorptive therapy are associated with decreased fracture rate. However, it is not clear whether short-term bone loss is an independent risk factor for hip fracture. To address this question, 724 elderly women of the Dubbo Osteoporosis Epidemiology Study were studied.

The women aged 60+ years (as at June 1989), had been followed for a median of 8.6 years, and had had at least two FNBMD measurements prior to the fracture event. During the follow-up period, 43 women suffered a hip fracture, who had multiple measurements of BMD prior to the event. Apart from lower baseline BMD and higher age, the [mean ± SD] annual rate of bone loss for fracture women was –2.1 ± 4.2 %, significantly greater than in non-fracture women (–0.76 ± 2.8%; p = 0.005). Lumbar spine (LS) BMD among hip fracture women also decreased at a significantly higher rate than among those without a fracture (–1.1 ± 3.4% vs. +0.1 ± 2.02%; p <0.01). In Cox's proportional hazards model (univariate analysis), each 5% decrease in FNBMD and LSBMD was associated with a 1.8-fold (1.2 – 2.8) and 2.8-fold (1.5 – 5.4) increase in hip fracture risk, respectively. In multivariate analysis, after adjusting for age in a proportional hazards model, each 0.1 g/cm^2 loss of FNBMD was associated with a relative risk (RR) of 3.1 (95% CI: 1.6 – 6.1) for fractures at the hip, comparable to a 0.1 g/cm^2 difference in baseline hip fractures (RR 3.6, 95% CI: 2.7 – 4.8).

These results suggest that bone loss at the femoral neck is an independent predictor of hip fracture risk in elderly women, and help explain why a modest improvement in BMD induced by antiresorptive therapy is associated with greater reduction in fracture risk that is predicted from cross-sectional BMD studies.

O2

HORMONE REPLACEMENT THERAPY AND NON-VERTEBRAL FRACTURE RISK: GEELONG OSTEOPOROSIS STUDY.

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Most data supporting the anti-fracture efficacy of HRT are provided by observational studies. In this case-control study, we aimed to determine whether HRT decreases the risk of fracture and to evaluate the association between HRT and BMD. Cases (n=262) were women with non-pathological, non-vertebral incident fracture (MVA excluded, 1994-6); controls (n=365) were randomly-selected women without incident fracture during the same period (all aged 60-80 yr). Fracture sites (n) included: hip (40), Colles' (63), humerus (34), forearm (32), ankle (24), tibia/fibula (17), foot (17), others (42). The exposure of interest was current use of HRT for at least 6 months and median duration of use was 5 yr (IQR 3-10).

There were 20 HRT-users among the cases and 49 among controls (age 68.4±5.8 vs 67.3±5.6 yr, P=0.5). Age-adjusted OR for fracture associated with HRT was 0.55 (95% CI 0.32-0.96). BMD-adjusted OR were 0.57 (0.33-0.99) for the spine, 0.59 (0.34-1.02) for the femoral neck, 0.55 (0.31-1.00) for the whole body, 0.63 (0.35-1.11) for the ultradistal-forearm, and 0.63 (0.36-1.11) for the mid-forearm. HRT was associated with 3-8% greater age- and weight-adjusted BMD (P<0.05 at all sites, except the femoral neck, P=0.2). Dietary calcium, activity, alcohol intake, smoking, use of glucocorticoids and calcium/vitamin D supplements were not significant confounders. The effects were more pronounced among women who started HRT within 10 years of menopause (9 cases, 26 controls): adjusted OR for fracture 0.47 (0.21-1.04), with greater BMD ranging from 6% at the whole body to 19% at the ultradistal-forearm ($P \leq 0.005$, all sites).

Our results indicate that HRT confers a 45% reduction in fracture risk. This effect operates only partially through increased BMD and may be greater if therapy is started within 10 years of menopause.

O3

A DELETION IN THE GENE ENCODING OPG CAUSES AN IDIOPATHIC HYPERPHOSPHATASIA PHENOTYPE

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We have investigated the molecular basis of a bone disease in a family with Idiopathic Hyperphosphatasia (MIM 239000). Three of the 9 children in this family were affected, with long bone deformities and sensorineural deafness. A genome-wide search suggested linkage to chromosome 8q24. The gene *TNFRSF11B* encoding osteoprotegerin (OPG) which lies in this region was an obvious candidate. Sequencing of this gene in DNA from members of the family identified an in-frame 3 base pair deletion causing the loss of an aspartate residue at position 182, within a highly conserved sequence of TNF-receptor like region. All 3 affected siblings were homozygous for the mutation.

To determine whether this mutation causes loss of function of OPG, wild-type and mutant OPG cDNA were cloned and expressed in HEK293 cells. Western blot analysis demonstrated that the mutant protein had a lower electrophoretic mobility than the wild type. Following deglycosylation both proteins appeared to be of similar size, suggesting that the mutant protein is hyper-glycosylated. The importance of glycosylation for OPG function is currently under investigation. The biological activity of the mutant OPG was compared to that of the wild type in murine bone marrow cultures and neonatal calvarial organ cultures. We demonstrated that the deletion of aspartate 182 results in loss of function as unlike the wild type, the mutant OPG protein could not inhibit osteoclastogenesis and bone resorption in these assays.

These data indicate that the absence of functional OPG protein in the patients results in the severe phenotype, and is the first description of a disease-causing mutation in OPG.

O4

SINGLE BASE-PAIR DELETION OF GENE ENCODING *SEQUESTOSOME 1* (*SQSTM/P62*) IN PAGET'S DISEASE OF BONE

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Paget's disease of bone (PDB) is a common disorder of unknown cause. This disease is characterised by focal and disorganised increase of bone turnover, associated with pain, deformity, fracture, neurological compression and osteosarcoma. There is evidence that PDB has a strong genetic component demonstrating linkage to a number of different chromosomes. In this study we wish to characterise the genetic basis of PDB. We conducted a genomewide scan with 61 individuals from a large family with multiple family members affected with PDB. The family was typed for 474 markers at an average distance of 10 cM. Initial linkage analysis suggested evidence of linkage to chromosomes 7, 19, 18 and 5. Fine mapping, linkage analysis and haplotyping of chromosome 7 and 19 failed to support linkage in these regions. Further analysis on chromosome 18q23 revealed linkage in only a branch of the family with a lower age of onset.

In this study we report on the fine mapping and linkage analyses of the entire family to the region on chromosome 5q35-qter (*PDB3*) with a peak multipoint LOD score of 6.77. Sequence analysis of the gene encoding the ubiquitin-binding protein *sequestosome 1* (*SQSTM/p62*) revealed a single base pair deletion (1215delC) segregating with the affected members of the pedigree. This deletion resulted in an early termination of the protein at position 394, removing the ubiquitin-associated domain (394-440).

O5

1,25 DIHYDROXYVITAMIN D CONTRIBUTES TO PHOTOPROTECTION IN SKIN CELLS.

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1,25Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) is best known for its ability to increase gut calcium and phosphate absorption. The vitamin D hormone is produced in the skin but its functions there have not been fully evaluated. We have previously reported that $1,25(OH)_2D_3$ protects keratinocytes and melanocytes from UV-induced cell death. The hypotheses that $1,25(OH)_2D_3$ also protects skin fibroblasts from UV-induced cell death and, for all skin cells, does so without increased DNA damage in surviving cells, were tested. Skin fibroblasts, melanocytes and keratinocytes were cultured and irradiated with a UVA and UVB source filtered through cellulose acetate, which removes all wavelengths below 290nm. DNA damage was measured by image analysis after staining with a specific antibody for cyclobutane pyrimidine dimers (CPD).

Pre-treatment for 24h with 10^{-10} to 10^{-8}M $1,25(OH)_2D_3$, dose-dependently decreased the loss of fibroblasts after UV exposure compared with vehicle control. In cells from 3 donors, cell loss was halved. DNA damage, assessed by CPD, was reduced at all time points up to 48h following UV irradiation in the presence of $1,25(OH)_2D_3$, compared with vehicle control. In keratinocytes, melanocytes and fibroblasts, treatment with $1,25(OH)_2D_3$ 10^{-12} to 10^{-8} M resulted in a dose-dependent decrease in detection of CPD in surviving cells. These results indicate that $1,25(OH)_2D_3$, which can be formed from vitamin D locally in skin(1) probably acts as an intrinsic part of the normal skin defence system to protect epidermal cells from UV-induced cell death.

1. Lehmann B et al 2001 J Invest Dermatol 117:1179-85.

O6

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

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Receptor activator of NF-kB ligand (RANKL) is a key factor necessary for osteoclast differentiation and activation. This study investigates the inhibitory effects of RANKL mutants on RANKL-induced osteoclast differentiation and activation. A series of truncated regions of the TNF-like core domain of RANKL were expressed and affinity-purified, and their biological activities examined using $RAW_{264.7}$ cells and isolated rat osteoclasts. GST-rRANKL (aa160-318) containing the full TNF-like core region had the highest capability to induce the formation of osteoclast-like cells from $RAW_{264.7}$ cells whereas GST-rRANKL (aa239-318), (aa160-268), (aa160-291), (aa246-318) had a reduction of osteoclast inductivity. Competition studies showed that GST-rRANKL (aa239-318), (aa160-268), (aa160-291), (aa246-318) inhibit RANKL (aa160-318) induced osteoclast formation in various degrees. Among them, RANKL mutant (aa246-318) was the most potent one. Furthermore, RANKL mutant (aa246-318) significantly blocked RANKL (aa160-318) induced osteoclastic bone resorption in isolated rat osteoclast cultures. An *in vitro* protein-protein interaction showed that RANKL mutants had reduced binding to Flag tagged RANK in a GST pull down assay. In addition, RANKL mutants showed reduced effect on the activation of NF-kB, ERK and JNKs signalling pathways. In short, our results suggest that RANKL mutants can be used to inhibit RANKL-induced osteoclast differentiation and activation and may have therapeutic effects on osteolysis.

O7

EFFECTS OF MILK SUPPLEMENTATION ON BONE MINERAL ACCRETION AND BIO-MARKERS OF BONE TURNOVER IN CHINESE ADOLESCENT GIRLS

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A 24 months double-blind, randomised, controlled milk supplementation trial was carried out in 757 Beijing girls aged 10 years to study the effects of milk supplementation on bone mineral accretion and bone biomarkers. Subjects were randomised to three groups. Group 1 subjects received 330 ml Ca fortified UHT milk (containing 560 mg Ca), and Group 2 subjects received 330 ml of the same milk fortified additionally with 8 μg vitamin D on every school day. Group 3 subjects were controls. The supplement was given on a total of 319 days over 24 months. Measurements made at baseline, mid- and end-trial were: anthropometry, pubertal stage, dietary intakes, BMC and BMD of distal forearm (DF) and proximal forearm (PF) in all subjects; total body (TB) BMC and BMD in half of the subjects. Levels of bone ALP, OC, DPD, PTH, and IGF-I were measured in 50 subjects from each group at baseline and 80 subjects from each group at mid- and end-trial. At end-trial, the two supplemented groups in comparison with controls, had 3.2-5.0% extra gains in TBBMD ($P \leq 0.001$), 1.6-2.8% in size-adjusted TBBMC ($P<0.01$), 10.1-10.2% in PFBMD ($P<0.001$), and 1.5-2.4% in size-adjusted PFBMC ($P<0.01$). Decreased bone turnover rates were observed in the supplemented groups at mid-trial but not end-trial. Group 2 had lower PTH concentrations than controls at both mid- ($P<0.001$) and end-trial ($P=0.001$). Supplemented groups had significantly higher IGF-I levels than controls at end-trial ($P<0.05$). In conclusion, the present study showed that milk supplementation for 24 months had positive effects on bone mineral accretion in Chinese adolescent girls, possibly mediated by reduced rates of bone turnover and increased IGF-I levels in supplemented subjects.

O8

REGULATION OF *FGF23* EXPRESSION IN BONE CELLS

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The inherited phosphate wasting condition autosomal dominant hypophosphataemic rickets (ADHR) is associated with missense mutations in *Fibroblast Growth Factor 23* (*FGF23*) (1). Tumours associated with oncogenic osteomalacia (OOM), a tumour-induced phosphate wasting condition, express FGF23 (2). FGF23 may also be involved in X-linked hypophosphataemic rickets (HYP), an inherited phosphate wasting disorder. The involvement of FGF23 in these phosphate wasting disorders indicates that it may have a role in normal phosphate homeostasis.

Low levels of *FGF23* mRNA expression were detected in human bone and in a primary human osteoblast-like bone cell strain. Changes in *FGF23* expression levels were observed in the bone cells after treatment with phosphate. Expression of *FGF23* mRNA was detectable after treatment with high phosphate concentrations, while expression was undetectable following incubation in low and normal phosphate concentrations. The bone cells also showed detectable *FGF23* mRNA expression with mineralisation induced by multilayering and 5mM β-glycerophosphate, with no detectable expression in nonmineralised cultures.

This study is the first reported example of regulation of *FGF23* expression. Expression of *FGF23* mRNA in bone and renal tissues, as previously reported, is consistent with a role for FGF23 in regulation of mineral metabolism. This is supported by the increased expression of *FGF23* mRNA in bone cells in response to high phosphate, and in mineralised bone cells. These results raise the possibility that *FGF23* may be a local regulator of bone cell function.

(1) ADHR Consortium (2000) Nat Gen 26:345-348
(2) White, KE et al. (2001) J Clin Endocrinol & Metab 86:497-500

O9

ENDOGENOUS ESTROGEN PREDICTS INCIDENT FRACTURE IN ELDERLY WOMEN.

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A decline in endogenous estrogen concentration occurs after menopause that accelerates bone loss. We examined the relationship between endogenous estrogen production, BMD and incident fracture in elderly women. A population-based sample of 1462 women was followed for 3 years with mean age 75±3years and weight 69±12kg. One-year hip BMD was measured using DEXA (Hologic 4500A) and Z scores were calculated. Incident clinical fractures were determined from x-ray reports after the event. At 3 years 10% had sustained ≥1 incident fractures. The fracture group had significantly lower concentrations of serum estradiol (25±12 vs 27±14 pmol/L), free estradiol index (FEI) (502±379 vs 651±551 pmol/nmol) and total hip BMD (775±130 vs 813±123 mg/cm^2) than the non-fracture group. After adjustment for age, weight and treatment, incident fracture was predicted by FEI (RR per SD below the mean, 1.478: 95%CI 1.131-1.933) (P=0.004). After further adjustment for hip BMD the effect of FEI was not significant (P=0.135). Of these women, 34 sustained a vertebral fracture (RR per SD decrease in FEI, 2.222: 95%CI 1.147-4.306) (P=0.018) and 99 sustained a peripheral fracture (RR per SD decrease in FEI, 1.437: 95%CI 1.062-1.944) (P=0.019) after adjustment for age, weight and treatment. After adjustment of hip BMD the effect of FEI was no longer significant for vertebral (P=0.183) or peripheral fracture (P=0.256). Hip BMD predicted peripheral fracture (RR per SD below mean, 1.369: 95%CI 1.103-1.850) (P=0.041) but not vertebral fracture (P=0.162). Low FEI increases the probability of having an incident fracture as a result of decreasing BMD. These data confirm the importance of postmenopausal estrogen concentration in the pathogenesis of osteoporosis in elderly women.

O10

SERUM VITAMIN D AND FALLS IN OLDER WOMEN IN RESIDENTIAL CARE IN AUSTRALIA.

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There is uncertainty about the prevalence of vitamin D deficiency in older people in residential care, and the influence that vitamin D status may have on their falls incidence. From a previous investigation we found an association between vitamin D deficiency and retrospective falls in older people in residential care. The aim of this investigation was to examine prospectively the possible association between Vitamin D levels and the prevalence of falls in a population of older people in residential care.

Serum 25-hydroxyvitamin D (25D) levels and recognised risk factors for falls were measured in Australian women living in residential care (667 in low level and 953 in high level care; mean age 83.7 years) and followed for an average 145 and 168 days, respectively. Falls were recorded prospectively in diaries completed monthly by residential care staff. Vitamin D deficiency (25D level below 25nmol/l) was present in 144 (22%) women in low level care and 428 (45%) in high level care. After excluding 358 bed bound residents, and adjusting for weight, cognitive status, psychotropic drug use, previous Colles fracture and the presence of wandering behaviour, serum 25D level remained inversely associated with risk to first fall. The adjusted odds ratio was 0.74 (0.59-0.94) P = 0.01.

Vitamin D deficiency is common in residential care in Australia. A low level of serum Vitamin D is an independent predictor of incident falls. Correction of this nutritional deficiency may have more benefits than prevention of fractures alone.

O11

THE NOVEL HORMONE, PREPTIN, IS ANOTHER BONE-ACTIVE PRODUCT OF THE PANCREATIC β-CELL

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Several hormones that regulate nutritional status also impact on bone metabolism. Preptin is a recently isolated 34-amino acid peptide hormone that is co-secreted with insulin and amylin from the pancreatic â-cells. Preptin corresponds to Asp^{69}- Leu^{102} of proIGF-II.

We have assessed preptin's activities on bone. Preptin, like insulin and amylin, dose-dependently stimulates the proliferation (cell number and DNA synthesis), of primary fetal rat osteoblasts and osteoblast-like cell lines at periphysiological concentrations ($>10^{-11}$M). In addition, thymidine incorporation is stimulated in murine neonatal calvarial organ culture, likely reflecting the proliferation of cells from the osteoblast lineage. Preptin does not affect bone resorption in this model. The mitogenic effect of preptin on osteoblasts appears to be dependent upon signaling via p42/44 MAP kinases. Preptin induces phosphorylation of p42/p44 MAP kinases in osteoblastic cells in a dose-dependent manner (10^{-8}-10^{-10}M), as assessed by immunoblotting. The proliferative effects of preptin on primary osteoblasts were blocked when the cells were pre-treated with either of the MAP kinase kinase inhibitors PD-98059 or U-0126. We also assessed preptin's effects on primary osteoblast apoptosis induced by serum deprivation. Apoptotic cells were detected by light microscopy using a modified TUNEL assay. Preptin had anti-apoptotic effects, at 10^{-8}M with treatment/control ratio of 0.78 ± 0.08.

In conclusion, preptin is a novel bone-active factor secreted from the pancreatic β-cell that may act in concert with the other β-cell hormones, insulin and amylin, to stimulate bone formation in hyperinsulinemic states, such as obesity.

O12

MANAGEMENT OF ACUTE OSTEOPOROTIC VERTEBRAL FRACTURES: A NON RANDOMISED TRIAL COMPARING PERCUTANEOUS VERTEBROPLASTY TO CONSERVATIVE THERAPY.

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There are no published data comparing percutaneous vertebroplasty (PV) to conservative therapy (CT) for acute osteoporotic vertebral compression fractures.

In this nonrandomised study, we enrolled 79 consecutive osteoporotic patients over a 12 month period, presenting with severe back pain due to acute vertebral fractures. There were 55 patients treated by PV and 24 by CT alone. Their osteoporotic risk factors and bone densitometry was measured at baseline, while the patients' pain score and level of function was recorded on presentation, at 24 hours, 6 weeks and 6-12 months post therapy.

There were 24 men and 55 women, aged 51-93 years, followed for 215 days (range 57-399). Patients treated by PV did not differ from those treated by CT, with respect to their biochemistry, bone densitometry and radiological evidence of multiple pre-existing vertebral fractures. A significant decrease in the patients' pain score from 19 to 9 (-53%, P=0.0001) and an improvement in their physical functioning from 14 to 18 (+29%, P=0.0001) was noted at 24 hours after PV. Thirteen patients (24%) were able to cease their analgesia after the procedure. By contrast symptomatic improvement in the patients treated by CT was delayed. A decrease in their pain score from 20 to 7 (-65%, P=0.0001) and an improvement in their physical functioning from 13 to 17 (+31%, P=0.01) was only evident at 6 weeks post therapy.

In experienced hands, PV is safe and effective for treating acute osteoporotic vertebral compression fractures. It allows for the prompt relief of pain and a rapid rehabilitation.

O13

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

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Currently, there is no effective medical treatment for Perthes disease (avascular necrosis) in childhood. We hypothesised that zoledronic acid (ZA), a potent bisphosphonate, could have a positive effect by maintaining the sphericity of the femoral head in a previously established model of Perthes disease - the spontaneously hypertensive rat (SHR). One hundred and twenty 4-week old rats were divided into three groups: saline monthly (40); 3 doses of 0.05 mg/kg ZA monthly (40); or 10 doses of 0.015 mg/kg ZA weekly (40). Rats were euthanased at 15 weeks.

53% of control femoral heads showed radiographic signs of osteonecrosis. Radiographs revealed less osteopenia and improved sphericity of the femoral heads in treated groups. The sphericity analysis documented a 50%-100% increase in number of spherical heads in the ZA treated animals compared to saline treated animals ($p<0.05$). Epiphyseal Quotient (EQ) was significantly improved in both treatment groups ($p<0.01$). The proportion of "flat" heads (EQ < 0.40) was also significantly reduced from 45% to 16% ($p<0.01$). DXA showed treated femoral head BMD was increased 18% in the Weekly group and 21% in the Monthly group over controls ($p<0.01$).

48% of saline femoral heads showed significant lucent areas within the epiphysis, while the rate of lucencies was reduced to 20% in the treated groups ($p<0.01$). Observational histology indicated retention of the calcified cartilage core in treated animals such that a framework remained for new bone formation.

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

O14

CARBOHYDRATE BINDING SPECIFICITY OF OSTEOCLAST INHIBITORY LECTIN

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Osteoclast Inhibitory Lectin (OCIL) is a type II membrane-bound of 207aa containing a C-lectin domain in its extracellular domain, that inhibits osteoclast formation in vitro. In order to determine the carbohydrate binding specificity of OCIL, we established an ELISA based assay where sugars were covalently linked to free amine groups on the surface of wells. Binding of recombinant GST-OCIL was carried out and determined using an anti-GST antibody. OCIL bound to high molecular weight sulfated glycosaminoglycans (GAGs) carrageenan, fucoidan and dextran sulfate in dose dependent manner. No significant binding was seen for unsulfated dextran, suggesting that the presence of anionic sulfate groups was important for interaction. Independence of anionic charge alone was observed, since OCIL did not bind to hyaluronic acid. The low molecular weight sulfated GAGs, chondroitin sulfate A, chondroitin sulfate C, dermatan sulfate, heparan sulfate, heparin, and keratan sulfate showed no specific affinity for OCIL, while only weak interactions were observed for some monosaccharides.

OCIL inhibited osteoclast formation in murine bone marrow macrophage precursor (BMMP) cells cultured with M-CSF and RANKL. In BMMPs treated with fucosidase (to remove terminal fucose residues) OCIL inhibition of osteoclast formation was reduced to approximately 50%. In BMMPs treated with neuraminidase as a control (to remove sialic acid residues), the inhibitory action of OCIL was not ablated and complete inhibition was noted. This suggests that OCIL's inhibitory action may be mediated by specific sugar binding.

In summary, OCIL exhibited binding to high molecular weight GAGs that contain anionic sulfate groups. Fucosidase treatment of BMMP cells partially ablated subsequent OCIL inhibitory action on osteoclast formation, suggesting a role for specific sugar binding in the function of OCIL inhibition of osteoclastogenesis.

O15

MANUFACTURER DISCORDANCE IN LUMBAR SPINE T SCORES

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Differences in the DXA reference ranges used in the proximal femur have been previously reported by ourselves and by other researchers. This issue has been addressed by the recommendation of adoption of the NHANES III reference range. In the lumbar spine however there is no recommended reference range for interpretation of BMD and the use of different reference populations by different manufacturers could potentially result in inconsistencies in the diagnosis of lumbar spine osteopenia or osteoporosis. To address this question we studied 59 women who had lumbar spine BMD measured on the same day using Lunar DPXL and Norland Excell scanners. Absolute BMD values measured by the two scanners were highly correlated (r = 0 98, p<0.0001). However when lumbar spine BMD was expressed as T scores (number of standard deviations from the young reference population), the instruments assigned significantly different values (p<0.0001, Table). Significant differences were also seen (p<0.005) when BMD was expressed with respect to age matched values (Z scores).

	BMD-g/cm^2 mean (SD)	SBMD mean	T Score mean (SD)	Absolute T mean (SD)	Z Score mean (SD)
Lunar	1.013 (0.17)	9646	-1.56 (1.42)	1.79 (1.11)	-0.43 (1.20)
Norl	0.890 (0.15)	9577	-1.19 (0.99)	1.35 (0.76)	-0.68 (0.93)

and partly to different young normal standard deviations employed in calculating the T scores.

In summary, this study has shown that in the lumbar spine two commonly used DXA scanners provide comparable absolute BMD values, when converted to sBMD, but there are significant differences in derived T scores due primarily to differences in the young reference populations. There is an urgent need for standardization of the reference ranges used in the lumbar spine.

O16

RACIAL, BUT NOT SEX, DIFFERENCES IN VERTEBRAL BODY VOLUMETRIC BONE MINERAL DENSITY IN ASIAN AND CAUCASIAN WOMEN AND MEN.

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Asians have lower vertebral body (VB) aBMD than Caucasians. We hypothesized that the vertebral fracture rate in Asians and Caucasians is similar because of scaling; a reduced muscular loading on a commensurately smaller VB in Asians will produce a similar ratio in load/strength–ie a similar fracture risk index (FRI). We studied 94 healthy Asians (Chinese ancestry, 58 females, 36 males) and 396 healthy Caucasians (304 females, 92 males) aged 18 to 43 yrs. VB cross-sectional area (CSA), aBMD and volumetric BMD (vBMD) were measured by using DXA by PA and lateral scanning. We calculated vertebral body compressive stress (load/CSA) and FRI during bending forward. Results (mean ± sem). VB aBMD was 20% higher in Asian men than Asian women because CSA was 26% greater in men than women (10.45 ± 0.25 vs 8.29 ± 0.12 cm^2); there was no sex difference in vBMD (0.289 ± 0.008 vs 0.274 ± 0.007 g/cm^3, NS), stress (329.5 ± 11.0 vs 316.1 ± 6.2 N/cm^2, NS), and FRI (0.32 ± 0.01 vs 0.37 ± 0.02, NS). Similar figures were presented in Caucasian men and women. Compared to Caucasians, VB CSA was 12% lower in Asian than Caucasian men and 13% lower in Asian than Caucasian women. By contrast, VB vBMD was 14% higher in Asian than Caucasian men, and 10% higher in Asian than Caucasian women. There was no different in load per unit CSA between Asian and Caucasians in either gender. However, both Caucasian men and women had higher FRI than Asian men and women, respectively (men 0.41 ± 0.02 vs 0.32 ± 0.01; women 0.42 ± 0.01 vs 0.37 ± 0.02, both p < 0.01). In summary and conclusion, (i) The higher aBMD in Asian men than women is due to the larger bone size, vBMD was no different between gender. (ii) The load per unit CSA is no different between genders and races, suggesting that the smaller bone is loaded by a proportionally smaller load. (iii) At peak, Asians achieved a smaller bone size but higher vBMD than Caucasians, conferring a lower risk of vertebral fractures.

O17

MORTALITY ASSOCIATED WITH PRIMARY HYPERPARATHYROIDISM

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The aim of the study was to evaluate survival in all patients with primary hyperparathyroidism seen at the Royal North Shore Hospital and Sydney Hospital between 1961 and 1994. 561 patients were retrospectively studied: 448 had surgery (SX) and 113 did not have surgery and remained hypercalcaemic (NSX). 124 patients died during the 33-year period of observation. The death rate of the hyperparathyroid (HPT) group was compared with that of the state population (CTRL) of the appropriate time period. The HPT and CTRL populations were matched for age, sex, year observation was begun, and duration of observation. The death rate of the HPT population was significantly greater than CTRL ($p<0.00001$). The relative risk of death for the SX group, adjusted for age, sex and period of diagnosis, was 0.67 (95% C.I. 0.38-1.18), not significantly different from the NSX group. In the total HPT group, there was no significant difference in the death rate between those with Calcium (Ca) >3.00mmol/L compared with Ca <3.00mmol/L. In the SX group, risk factors significantly associated with death were diabetes ($p=0.001$), kidney stones ($p=0.001$), CCF ($p=0.02$), CAD ($p=0.03$), and hypertension ($p=0.044$). In the NSX group, death was significantly associated with high PTH ($p=0.001$), CAD ($p=0.004$) and kidney stones ($p=0.035$). This study does not support the concept that surgery for primary hyperparathyroidism confers a survival benefit even though the severity of the disease, as judged by the Ca and PTH, was greater in the SX group.

O18

SUBJECTS WITH TYPE II DIABETES HAVE HIGHER BONE MASS BUT NORMAL FRACTURE RISK COMPARED WITH NON DIABETIC CONTROLS.

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It is not clear whether people with Type II Diabetes Mellitus (DM) have an increased fracture risk. These subjects have associated obesity and higher bone mass would be expected. Nevertheless it is not clear whether fracture risk is influenced by diabetes. We examined the relationship between DM, BMD and incident fracture. A population-based sample of 1462 women was followed for 3 years. A diagnosis of DM (n=91) was ascertained from the medical practitioner history at baseline. We measured weight, DEXA bone mineral density (BMD) and quantitative ultrasound (BUA, SOS and stiffness). Incident fracture was ascertained from radiology reports. At 3 years 10% had sustained ≥1 incident fracture. Results were analysed using Student's *t*-test, multiple regression and $\div^2$. The diabetic and non-diabetic groups had the same mean age 75±3years. Weight was greater in DM (74.7±16.4 vs 68.3±12.2 kg $p<0.0001$). Both BMD (858±16 vs 808±12mg/cm^2 $p<0.01$) and QUS (BUA 103±9 vs 100±8 dB/MHz $p<0.001$; SOS 1520±28 vs 1512±25 m/sec $p<0.01$; stiffness 75±13 vs 70±11 $p=0.001$) were greater in DM. After adjustment for age and weight, those with diabetes had a significantly higher BUA ($P=0.026$), SOS ($P=0.014$) and Stiffness ($P=0.01$) but not hip BMD. There was no difference in incident fracture rate between the diabetic (8.8%) and non-diabetic group (10.5%) after adjustment for age, weight and BMD using logistic regression analysis. We conclude that Type II diabetes is associated with a higher bone mass. Greater bone mass in diabetic subjects does not confer a lower fracture risk than people without diabetes suggesting impaired bone quality.

O19

GENETIC AND ENVIRONMENTAL VARIATION IN BALANCE AND GAIT PERFORMANCE: A TWIN STUDY.

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A combination of low bone mineral density (BMD), increase in postural sway, physical inactivity, muscle weakness, and falls contributes to an increased risk of osteoporotic hip fractures. Epidemiological studies have reported a familial aggregation of hip fracture risk, where a maternal history of hip fracture doubles a woman's hip fracture risk.

A classical twin study was performed to determine the extent of genetic and environmental sources of variation in falls risk in 56 monozygotic (MZ) and 65 dizygotic (DZ) female twin pairs aged 46-82 years, by performing validated clinical and laboratory tests of gait and balance. Within-pair MZ correlations were generally higher compared with DZ within-pair correlations, in particular with the Human Activity Profile (HAP) [rMZ=0.50 (95%CI=0.27-0.67); rDZ=0.16 (95%CI=-0.09-0.39); p=0.04], Stride Analyser [rMZ=0.58 (95%CI=0.38-0.73); rDZ=0.09 (95%CI=-0.17-0.33); p=0.002] and on the Chattecx Balance System [rMZ=0.42 (95%CI=0.18-0.61); rDZ=0.03 (95%CI=-0.22-0.28); p=0.03]. This suggests the presence of moderate to strong genetic effect. It was estimated that the within-pair correlations were explained 67.6-99.8% by additive genetic factors.

These findings suggest that a clinically significant proportion of the familial aggregation of hip fractures in older women may be explained by the heritability of gait and balance function.

O20

DIFFERING RISK FACTORS FOR FALLS IN OLDER PEOPLE LIVING IN HOSTEL AND NURSING HOME CARE WHO CAN AND CANNOT STAND.

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As over 90% of hip fractures in the elderly involve a fall, it is important to identify falls risk factors in this group. We conducted a prospective cohort study involving 1000 people aged 65 to 103 years (mean age 85.0, SD=7.4) living in hostels and nursing homes in northern Sydney to determine risk factors for falls and injurious falls in this at-risk population.

There was a strong non-linear association between standing balance and falls, with falling rates lowest in those with the best and worst balance. Many health and physical function-related factors were significantly associated with falls in those who could stand. Significant and independent risk factors determined from multivariate negative binomial analyses included increased age, male gender, high resident care classification levels, daytime incontinence, Parkinson's disease, psychoactive medication use, and poor ability to rise from a chair. In contrast, quite different risk factors were evident in those who could not stand, with a number of risk factors identified in community-dwelling older people (previous stroke, reduced ability to rise from a chair, slow reaction times) associated with a decreased risk of falls. In this group, significant and independent risk factors for falls were hostel (rather than nursing home) care, poor health status, psychoactive medication use and not being chair fast. Similar patterns were evident for injurious falls.

The findings indicate that there are differing risk factors for falls in those who can and cannot stand in hostel and nursing home residents, and provide important information for developing fall risk assessments and falls prevention strategies in these populations.

O21

PATHOGENESIS OF FOCAL BONE EROSION IN A SERUM TRANSFER MODEL OF ARTHRITIS.

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There is considerable evidence that osteoclasts (OC) contribute significantly to focal bone erosion in inflammatory arthritis. RANKL is an essential factor for OC differentiation. Additionally, TNF-α has been demonstrated to facilitate OC differentiation and both TNF-α and IL-1 increase OC activity. To determine the requirement of RANKL, IL-1 or TNF-α for bone erosion in inflammatory arthritis, we generated serum transfer arthritis in RANKL-/-, IL-1 receptor 1(r1)-/- and TNF-α-/- mice and assessed the degree of inflammation and bone erosion clinically and histologically. Kinetics of TNF-α and IL-1 mRNA expression in serum transfer arthritis in C57Bl/6 mice was assessed by real time-PCR. In arthritic ankle tissue, TNF-α and IL-1 mRNA were induced by >600 fold and >13000 fold respectively compared to control tissues. No inflammation was observed in serum injected IL-1r1-/- mice. Inflammation was comparable in arthritic RANKL-/- and control mice[1]. A proportion of TNF-α-/- mice developed inflammation. Bone erosion in arthritic RANKL-/- mice was dramatically reduced compared to arthritic control mice[1] and was absent in IL-1r1-/- mice. Bone erosions were present and proportionate to the degree of inflammation in arthritic TNF-α-/-. This study demonstrates that IL-1 is necessary for the development of serum transfer arthritis, whereas TNF-α is important but not required. TNF-α was not required for bone erosion. In contrast, RANKL was necessary for OC formation and bone erosion[1], even though TNF-α and IL-1 expression is induced in this model of arthritis.

1. A. R. PETTIT, H. JI ET AL. 2001. AM. J. PATHOL. 159: 1689-99.

O22

A DIRECT ROLE FOR RANKL EXPRESSION BY HUMAN MYELOMA PLASMA CELLS IN THE OSTEOLYSIS ASSOCIATED WITH MULTIPLE MYELOMA

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Multiple myeloma (MM) is an incurable B cell malignancy, characterised by the presence of a monoclonal population of myeloma plasma cells (MPC), often associated with focal osteolytic lesions throughout the axial skeleton. The precise mechanisms responsible for this bone pathology remain unclear, however, it is generally accepted that it is due to MPC-mediated disruption of the normal equilibrium between bone formation by osteoblasts and bone resorption by osteoclasts (OC). The aim of this study was to examine the involvement of RANKL and its antagonist, osteoprotegerin (OPG) in MM biology. Most human MM cell lines tested expressed RANKL at both the mRNA and protein level. RANKL expression was also detected by flow cytometry in MM patient-derived BM cells, including purified $CD38^{++}CD45^{+}$ and $CD38^{++}CD45^{-}$ MPC subpopulations, using two independent anti-RANKL antibodies. Co-culture of FACS-sorted patient-derived $CD38^{++}CD45^{+}$ and $CD38^{++}CD45^{-}$ MPC with adherent human peripheral blood mononuclear cells, resulted in the formation of multinucleate, TRAP-positive OC-like cells capable of forming resorption lacunae on slices of dentine. Our data suggest that RANKL expression by MPC confers on them the ability to participate directly in the formation of OC. Moreover, high expression of membrane-associated RANKL by $CD38^{++}$ cells is associated with the presence of radiological lesions in multiple bones in individuals with MM ($p<0.025$), suggesting that MPC-expressed RANKL has a direct role in the formation of focal osteolytic lesions characteristic of this disease.

O23

GM-CSF INHIBITS OSTEOCLASTOGENESIS AND PROMOTES DENDRITIC CELL DIFFERENTIATION FROM HUMAN CFU-GM.

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Osteoclasts (OC) and dendritic cells (DC) differentiate from haematopoietic cells of the myelomonocytic lineage. However, how and when their precursor cells diverge from this lineage is unclear. GM-CSF is essential in the early differentiation of precursors and for DC terminal differentiation, although its precise role in osteoclastogenesis remains controversial. To investigate this role, we have used a human osteoclastogenesis assay employing CFU-GM colonies derived from umbilical cord blood mononuclear cells (CBMC). CBMC were cultured for 10d in semi-solid media containing GM-CSF, IL-3 and SCF. The resultant colonies were then pooled and the cells were cultured for a further 7-14d with dentine slices and human M-CSF, with and without soluble RANKL and/or GM-CSF.

Co-treatment with GM-CSF and RANKL dose-dependently inhibited OC number and dentine resorption (IC_{50} 0.1ng/mL) with 96% inhibition of both at 10ng/mL. Under these conditions, DC cluster formation was abundant. Pre-treatment with GM-CSF for 7d prior to RANKL exposure for a further 7d inhibited OC formation by 55%. However, in these cultures, ongoing treatment with GM-CSF after the addition of RANKL completely inhibited OC generation. GM-CSF had no effect on OC number or resorption when added to mature OC (d10), but did result in the formation of multiple DC clusters.

These results demonstrate that GM-CSF inhibits human osteoclastogenesis by diverting differentiation of early precursors towards DC. However, the potent switching effect of GM-CSF is dependent on the presence of RANKL. GM-CSF does not inhibit mature OC function.

O24

AN INVERSE RELATIONSHIP BETWEEN THE OSTEOGENIC AND OSTEOCLASTOGENIC PHENOTYPE IN HUMAN OSTEOBLASTS

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Normal human bone-derived cells (NHBC) are a mixed population of osteoblast-like cells, defined in terms of their osteogenic phenotype using the cell surface markers STRO-1 and alkaline phosphatase (AP). We compared the cell surface expression of STRO-1 and AP with that of molecules central to osteoclast (OC) formation, namely RANKL and OPG, to examine the dual functionality of the human osteoblastic stromal cell in both forming bone and supporting osteoclastogenesis.

NHBC were sorted by FACS into STRO-1^{bright} and STRO-1^{dull} populations. The expression of RANKL and OPG mRNA was examined in each population in response to $1,25(OH)_2$vitaminD_3 (vitD_3) and dexamethasone (DEX). STRO-1 positivity did not correlate with the basal level of RANKL or OPG mRNA, however, vitD_3 stimulated an increase in the RANKL:OPG mRNA ratio to a greater extent in the STRO-1^{bright} cells than the STRO-1^{dull} cells. This suggests a greater sensitivity of immature OB to pro-osteoclastogenic factors. VitD_3 treatment of mixed NHBC also increased the percentage of immature (STRO-1^+/AP^-, STRO-1^+/AP^+) populations. DEX treatment alone had little effect on the expression of RANKL, downregulated OPG mRNA, and had a profound effect on the maturation of mixed NHBC, as indicated by an increase in the %AP-expressing cells. The RANKL:OPG mRNA ratio decreased as a function of increased maturation of the cultures. Our findings suggest that immature OB are more responsive to pro-osteoclastic stimuli than mature OB, and suggest that the dual functionality of OB in supporting OC formation or forming bone is a function of the maturation state of the same lineage of cells.

O25

MODULATION OF OSTEOCLASTOGENESIS BY CD4+ CELLS

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Peripheral blood mononuclear cells (PBMC) are used routinely for osteoclast (OC) formation assays. We have observed that there is considerable variability in OC yield from different PBMC donors, possibly related to lymphocyte status. To determine whether CD4+ and CD8+ lymphocytes are involved, a series of OC formation experiments were performed using lymphocyte-depleted PBMC or addition of purified lymphocytes in assays utilising either CD14+ PBMC or CFU-GM cells as OC precursors.

PBMC (10^6 cells) or CD14+ PBMC (10^5 cells) were cultured for 3 wks on bone slices in α-MEM (10%FBS) with M-CSF (25 ng/ml) and sRANKL (125 ng/ml). In CFU-GM experiments $4x10^4$ cells per well were cultured on dentine slices under the same conditions for 2 wks. CD4+ depletion of PBMC caused a significant increase in OC number (56±9 OC per dentine slice to 312±17, $p<0.001$). However, removal of CD8+ or CD19+ lymphocytes did not promote OC formation. Further, addition of purified CD4+ cells in either the CD14+ PBMC or CFU-GM precursor models, caused significant inhibition of OC formation and, in some assays, total abrogation of OC formation. Supernatants from CD4+ cultures caused a concentration-responsive inhibition of M-CSF/sRANKL-induced OC formation from CD14+ PBMC ($1.4x10^6$ cells required for 50% inhibition). Various lymphocyte cytokine products including OPG, GM-CSF, IFNγ, interleukins and others, are potential candidates of these responses. In these experiments IL-3, IL-4, OPG and GM-CSF caused concentration-responsive inhibition of osteoclastogenesis.

In conclusion, we have shown that removal of CD4+ lymphocytes from PBMC increases OC yield in this model and that soluble products derived from lymphocytes may be responsible for the variability noted in PBMC OC formation assays.

O26

A NOVEL INTERACTION OF DYNEIN LIGHT CHAIN TCTEX-1 WITH RAB3 AND ITS IMPLICATION IN SMALL G PROTEIN-MEDIATED VESICLE TRANSPORT IN OSTEOCLASTS

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The small G proteins have long been speculated to regulate the association of microtubule-based organelle transport. Rab3D proteins, small GTP-binding proteins of the Ras superfamily play important roles in vesicle transport in non-neuron cells including osteoclasts. Yeast two hybrid screening resulted in the identification of a novel interaction between Rab3D and Tctex-1, a cytoplamic dynein light chain protein. In-vitro protein-protein interaction analyses confirmed a specific binding of Rab3D to Tctex-1. The interacting domain of Rab3D with Tctex-1 was mapped to amino acids 74 to 95. In addition, Tctex-1 did not interact with Rab5 and Rab6, indicating that the interaction of Tctex-1 with Rab3 is subfamily-specific. Furthermore, bioluminescence resonance energy transfer (BRET) assays showed that Tctex-1 interacts with Rab3D, Rab3D/Q81L and Rab3A but its interaction with Rab3D/N135I was largely reduced in vivo, indicating that the interaction of Tctex-1 with Rab3 is GTP dependent. Confocol microscropy analysis reveals that Rab3D is partially colocalized with Tctex-1 and β-microtubulin as well as with TGN network, but not with lysotracker and dextran. Moreover, Nocodazole treatment that disrupted microbtubulin resulted in a dispersion of Rab3D-EYFP bearing vesicles. These results suggest that Tctex-1 direct the microtublues-based movement of Rab3-bearing vesicles through its interaction with Rab3.

O27

VITAMIN D METABOLISM IN THE BONE: DISTINCT REGULATION BY SERUM CALCITONIN AND CALCIUM

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Although, the regulation of 1,25D (1,25-dihydroxyvitamin D) synthesis in the kidney by 1α-hydroxylase (CYP27b1) and the catabolism of 1,25D by 24-hydroxylase (CYP24) is established, the regulation of these genes in bone is relatively unknown. The absolute quantities of CYP27b1 and CYP24 mRNA were examined by real-time RT-PCR in the kidneys and bones of female Sprague-Dawley rats aged 3 weeks to 2 years and in 9-month-old vitamin D-replete (D(+)) and vitamin D-deplete (D(-)) rats fed either a 0.1% Ca (LC) or 1% Ca (HC) diet for 3 months. Under normocalcemic conditions, circulating 1,25D levels decreased with age, which was associated with both a reduction in kidney CYP27b1 mRNA (R^2=0.72) and an increase in CYP24 mRNA expression (R^2=0.71). Serum calcitonin was positively correlated with kidney CYP24 mRNA levels (R^2=0.81). Only under hypocalcemic condition did the high levels of serum PTH induce kidney CYP27b1 mRNA expression by 80x fold ($p<0.001$). In contrast, bone CYP27b1 mRNA expression together with CYP24 mRNA, was elevated only during rapid growth and development and in animals fed HC diet. Bone CYP27b1 mRNA was not correlated with serum PTH but was positively correlated with serum calcium levels during ageing (R^2=0.34) and in the D(-) animals (R^2=0.69). During normocalcemia, bone CYP27b1 mRNA levels were positively correlated with serum calcitonin levels (R^2=0.65). Furthermore, bone CYP27b1 mRNA expression was strongly coupled with bone CYP24 mRNA expression in all animals (R^2=0.85) suggesting that 1,25D production in the bone is a major determinant of bone CYP24 activity. These findings suggest that the regulation of CYP27b1 mRNA expression in the bone, possibly by calcitonin, calcium and 1,25D itself, is independent from the regulation of CYP27b1 mRNA expression in the kidney.

O28

THE SYNERGISTIC ACTION OF NEUROPEPTIDE Y Y4 AND Y2 RECEPTORS IN NEUROREGULATION OF BONE MASS.

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Neuropeptide Y (NPY) Y2 receptor has previously been shown to regulate cancellous bone formation in the mouse. Pancreatic polypeptide (PP) the major ligand for NPY Y4 receptor was elevated in these mice, implicating this Y4 receptor in the Y2 KO bone phenotype. This study compared NPY Y2 KO, Y4 KO, Y2/Y4 double KO and PP overexpression to examine the possible roles of these pathways in regulation of bone mass. Bone structure was examined in the distal femoral metaphysis and mid-femoral diaphysis of 4 month old male knockout, transgenic and wildtype mice.

PP overexpression and Y4KO did not affect bone mass. In contrast, deletion of both Y2 and Y4 receptors in Y2/Y4 KO mice had a synergistic effect. BV/TV was elevated compared to Y4 KO (17.4 ±2.1 vs 8.2 ±1.2%) and Y2 KO (12.4 ±1.7%), although the latter 40% difference was not significant. Trabecular number was also elevated (4.9 ±0.4 vs 3.2 ±0.3 /mm) and (4.0 ±0.3 /mm). Trabecular thickness was elevated compared to Y4 KO (34.4 ±2.6 vs 24.7 ±1.9 um) but not Y2 KO (29.9 ±2.0 um). Cortical area was reduced in Y2/Y4 KO compared to Y4 KO (0.91 ±0.03 vs 1.12 ±0.04 mm^2) and Y2 KO (1.09 ±0.05 mm^2), associated with reduced cortical thickness (203 ±4 vs 239 ±5 and 237 ±6 um).

These data suggest that circulating PP levels do not mediated these NPY effects. Furthermore, although the Y4 receptor pathway does not independently regulate bone mass, there is a synergistic interaction between the Y2 and Y4 pathways to reduce cancellous bone volume and increase cortical bone mass.

O29

FACTORS ASSOCIATED WITH BONE TURNOVER AND SHORT TERM GROWTH IN ADOLESCENT BOYS

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The primary aim of this six-week randomised trial was to assess the effect of isoflavone supplementation to the level of the usual Japanese diet on bone turnover markers and short-term growth in adolescent boys. A secondary aim was to assess the effect of sunlight exposure, vitamin D stores, urinary electrolytes, physical activity and winter on bone turnover markers and short-term growth.

There were 136 subjects from a single school (mean age 16 years). All subjects received isoflavone or identical placebo. Sun exposure and physical activity were assessed by questionnaire. Vitamin D stores at baseline were assessed by serum 25OHD (Incstar). Bone turnover was assessed by bone specific alkaline phosphatase (ALKPHASE, BSAP) and urinary pyridinoline cross-links (Pyrilinks-D, PYR) at baseline and follow-up. Height and weight change were measured as were urinary daidzein and genistein.

Despite large increases in urinary daidzein and genistein with supplementation (both $p<0.001$), there was no effect on bone turnover or growth (all $p>0.35$). The mean 25OHD level was low (44nmol/l, 68% <50nmol/l) and significantly associated with BSAP (cutpoint 55nmol/l, $p=0.03$) and PYR ($r=-0.23$, $p<0.01$) but not growth. Both BSAP and PYR increased during the winter and predicted height but not weight change. Urinary sodium was positively associated with PYR after taking short-term growth into account while urinary calcium was negatively associated. Of the physical activity measures, only hours of television watching was significantly negatively associated with short-term height but not weight change ($r=-0.26$, $p=0.003$).

In conclusion, phytoestrogen supplementation has little effect on bone turnover and short-term growth in male children while vitamin D stores, sodium and calcium excretion are associated with bone turnover markers which, in turn, are related to growth. This association is made more complex by the separate impact of growth and homeostasis on bone turnover in children. Television watching in winter has a dose response relationship with short-term growth.

O30

VITAMIN D INSUFFICIENCY AND CALCIUM ABSORPTION IN POSTMENOPAUSAL WOMEN.

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Low levels of serum 25 hydroxyvitamin D (25(OH)D), the major circulating metabolite of vitamin D, are often found in the elderly and are associated with low bone density and hip fractures. Because low 25(OH)D may cause excessive bone loss by impairing intestinal calcium absorption we studied radiocalcium absorption and serum 25(OH)D, 1,25 dihydroxyvitamin D ($1,25(OH)_2D$) and parathyroid hormone (PTH) in 863 postmenopausal women aged 35-94 (mean 64,SD 9) attending our osteoporosis clinics. 25(OH)D was measured by radio-immunoassay, $1,25(OH)_2D$ by radioimmunassay after HPLC, and intact PTH by immunoradiometry. Hourly fractional Ca absorption (α) was measured using 5μCi of ^{45}Ca and 20 mg of calcium carrier, with a blood sample taken one hour after the dose.

In the whole set α was related positively to 25(OH)D ($P<0.01$), $1,25(OH)_2D$ and body weight (both $P<0.001$) and inversely to age ($P<0.001$). By multiple linear regression α was related positively to $1,25(OH)_2D$ and weight (both $P<0.001$) and inversely to age ($P<0.001$), but not to 25(OH)D ($P=0.91$). However, α was lower in patients with 25(OH)D $\leq$ 40 nmol/L than in those with 25(OH)D > 40 nmol/L (0.61(0.30) vs 0.66(0.30) fx/h; $P=0.046$). In the 195 patients with 25(OH)D $\leq$ 40 nmol/L multiple linear regression showed that α was related positively to weight ($P<0.001$), $1,25(OH)_2D$ ($P<0.001$) and 25(OH)D ($P<0.05$) and inversely to age ($P<0.001$).

We conclude that calcium absorption may be influenced by 25(OH)D independently of $1,25(OH)_2D$ when 25(OH)D is low. Whether the low calcium absorption found in women with 25(OH)D $\leq$ 40 nmol/L contributes to their increased bone resorption remains unclear.

O31

OESTRADIOL SUPPRESSES 1,25 DIHYDROXYVITAMIN D-INDUCTION OF THE CYP24 PROMOTER IN AN OSTEOBLAST CELL LINE.

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1,25-dihyroxyvitamin D (1,25D) induces its catabolising enzyme, cytochrome P450-24 (CYP24) in its target tissues, presumably limiting the intracellular actions of the hormone. We have previously shown that oestrogen suppresses the 1,25D-induction of a CYP24 promoter/luciferase reporter gene construct in an intestinal cell line (CaCo-2). The present study used the UMR-106 cell line to investigate the combined effect of 1,25D and 17β-oestradiol (E_2) on the induction of the CYP24/reporter construct in osteoblasts. Cells were treated with E_2 (10^{-9}M) and 1,25D (10^{-9}M). UMR-106 cells were responsive to E_2 as demonstrated by a 2.4(±0.5) fold induction of an ERE promoter/luciferase reporter construct compared to ethanol vehicle ($p<0.05$). 1,25D treatment alone resulted in a 26.2(±8.4)-fold induction of luciferase activity compared to ethanol vehicle ($p<0.001$), while E_2 (10^{-9}M) alone caused no induction above vehicle. E_2 (10^{-9}M) in combination with 1,25D (10^{-9}M) resulted in suppression of the levels achieved with 1,25D alone to 4.3(±0.8) fold above ethanol vehicle ($p<0.05$ Vs 1,25D). These findings are consistent with our previous findings in CaCo-2 cells and support the hypothesis that E_2 modulates vitamin D's induction of CYP24 in osteoblasts, possibly via MAP kinase-dependent co-activation factors. It is likely that suppression of the 1,25D-inducible CYP24 by oestradiol would result in reduced catabolism of 1,25D, protecting its intracellular concentration. Thus a general role for oestradiol in calcium homeostatic tissues may be the maintenance of higher intracellular 1,25D enhancing its action.

O32

REGULATION OF GENE EXPRESSION BY THE CYP27B1 PROMOTER; A TRANSGENIC MOUSE STUDY

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The enzyme 25-hydroxyvitamin D-1α- hydroxylase (CYP27b1), is required for the activation of vitamin D to its biologically active form, 1α,25-dihydroxyvitamin D (1,25D), which plays an important role in the maintenance of plasma calcium levels, normal bone structure and other cellular functions. Although the kidney is considered to be the only significant producer of 1,25D, a number of non-renal tissues and cells have been shown to express the CYP27b1 gene. Little is, however, known about the physiology of CYP27b1 in these cells. To study the regulation of the expression of the CYP27b1 gene in a whole organism, we established a transgenic mouse model that expresses the luciferase reporter gene under the control of the 1.5 kb CYP27b1 promoter. Luciferase activity was detected in the brain, testis, bone marrow and bone tissues in 2 separate transgenic lines. It was undetectable in a number of tissues including liver, ovaries, spleen and gut. Importantly, no luciferase activity was detected in the kidney. Transgenic mice were fed diets containing varying levels of dietary calcium (0.05%, 0.2%, 0.4% and 1%) and phosphate (0.08%, 0.17% and 0.34%) for 4 weeks. No significant effect of these diets was detected on the promoter-directed luciferase activity in the kidneys of these animals. In vitamin D-deficient mice fed a low, 0.1 %, calcium diet for 4 weeks, 2 mice with the lowest serum 25-hydroxyvitamin D levels from a total of 7, demonstrated a slight but detectable up-regulation of the luciferase activity. These findings suggest that the 1.5 kb CYP27b1 promoter is incapable of up-regulating gene expression in the kidney, except under conditions of severe vitamin D-depletion.

O33

AMYLIN IS REQUIRED FOR THE MAINTENANCE OF BONE IN ADULT MICE.

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Amylin or islet amyloid polypeptide stimulates the proliferation of osteoblasts and inhibits osteoclastic bone resorption *in vitro* and *in vivo*. However, its importance in the maintenance of bone volume *in vivo* has not been determined. The aim of this study was to investigate the role of amylin on bone by utilising an amylin deficient mouse model (1). Male and Female control and knockout (KO) mice were sacrificed at 2, 4, 6 and 26 weeks of age. We aim to collect a total of 6 femora from each group and time point. Distal femora were prepared for quantitative histomorphometry using established resin embedding techniques. Trabecular bone volume (BV/TV), trabecular thickness (Tb.Th) and trabecular number (Tb.N) were calculated in the metaphysis using a Quantimet 500 image analysis system. The genotype of each animal was confirmed by PCR on tail DNA. BV/TV was decreased in female and male amylin KO mice compared to controls at 26 weeks of age (Mean ± SD; Female Control: 30.1±3.3, n=2, Female KO: 23.5±2.4, n=5, Male Control: 27.8±3.7, n=3, Male KO: 23.8±2.4 n=6). This decrease in BV/TV in adult amylin KO animals is associated with a reduction in Tb.N with no change in Tb.Th. However, preliminary data suggest that as the amylin KO mice accrue BV/TV from 2 to 6 weeks of age, the Tb.Th in these animals is reduced compared with controls, suggesting an impairment in bone formation. In summary, these preliminary results suggest that amylin is required for the maintenance of trabecular bone volume in adult male and female mice.

(1) Gebre-Medhin S *et al.* 1998 Biochem Biophys Res Comm, 250:271-277.

O34

CYCLOOXYGENASE-2 (COX-2) GENE EXPRESSION INCREASES IN RAT TIBIA 2 HOURS AFTER MECHANICAL LOADING IN VIVO.

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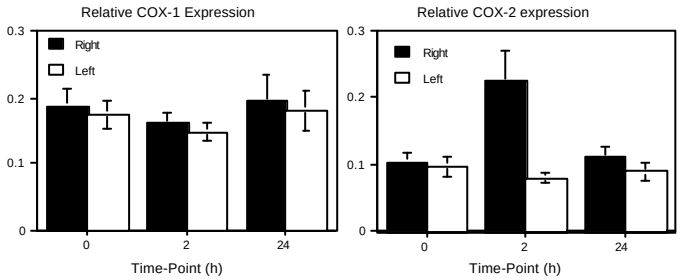
Using pharmacological inhibitors of COX-2, we showed previously that COX-2 is necessary for mechanically-induced bone formation. The aim of the present study was to determine if gene expression for COX-2 was increased in rat tibiae following a single period of 4-point bending in vivo. Right tibiae of 18 rats were subjected to 300 cycles of bending at 2.0 Hz with an applied load of 65 N. At 0, 2 and 24 hr post-loading, tibiae were dissected, frozen and total RNA isolated from a 1 cm region of left and right bones for semi-quantitative RT-PCR analysis for COX-1 and COX-2 mRNA expression relative to GAPDH.



Two hours after mechanical loading, COX-2 mRNA expression was significantly increased in right (loaded) tibiae. By 24 hr, COX-2 expression had returned to levels similar to that in left (control) tibiae. Expression of COX-1 mRNA was not significantly changed by mechanical loading. These data support our hypothesis that COX-2 is a key regulator of mechanically-induced bone formation.

O35

OSTEOPROTEGERIN (OPG) IS LOCALISED TO THE WEIBLE-PALADE BODIES OF HUMAN VASCULAR ENDOTHELIAL CELLS

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OPG has been shown to prevent osteoclast formation and activity, and may have a role in endothelial cell survival and the prevention of arterial calcification. In this study, we show that vascular endothelial cells in situ, and human umbilical vein endothelial cells (HUVEC), express abundant OPG. In HUVEC, OPG is co-localised with P-selectin, within the Weibel-Palade bodies, where it is detectable using an antibody that recognises the dimeric form of human OPG. Treatment of HUVEC with the inflammatory cytokines, TNFα and IL βresulted in a rapid mobilization of OPG protein within 30 minutes of exposure followed by a sustained increase in OPG mRNA and protein expression over 48 hours. Using confocal microscopy we found that OPG secretion coincided with mobilisation of OPG from the Weibel-Palade bodies. Furthermore, frozen sections of active rheumatoid arthritis (RA) pannus tissue revealed a dramatic decrease in endothelial cell OPG protein content compared with similar tissue sampled from patients with inactive RA, consistent with the notion that increased levels of circulating pro-inflammatory mediators such as TNFα and IL-1β results in the egress of OPG from the endothelial cells within the rheumatoid tissue. The intracellular localisation of OPG in HUVEC, together with its rapid and sustained secretory response to inflammatory stimuli, strongly support a modulatory role in inflammation, and are consistent with the notion that the loss of endothelial cell OPG in active RA is due to chronic exposure to inflammatory stimuli.

O36

ALLELES OF RUNX2/CBFA1 GENE ARE ASSOCIATED WITH DIFFERENCES IN BONE MINERAL DENSITY AND RISK OF FRACTURE.

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The aim of this study was to determine if DNA polymorphism within runt-related gene 2 (*RUNX2*)/core binding factor A1 (*CBFA1*) is related to bone mineral density (BMD). *RUNX2* contains a glutamine-alanine repeat where mutations causing cleidocranial dysplasia have been observed. Two common variants were detected within the alanine repeat: an 18 base pair deletion and a synonymous alanine codon polymorphism with alleles, GCA and GCG (noted as A and G alleles, respectively). In addition, rare mutations that may be related to low BMD were observed within the glutamine repeat. In 495 randomly selected women of the Geelong Osteoporosis Study (GOS), the A allele was associated with higher BMD at all sites tested. The effect was maximal at the ultra-distal radius (p=0.001). In a separate fracture study, the A allele was significantly protective against Colles' fracture in elderly women but not spine and hip fracture. The A allele was associated with increased BMD and was protective against a common form of osteoporotic fracture, suggesting that *RUNX2* variants may be related to genetic effects on BMD and osteoporosis.

O37

IDENTIFYING SURVIVAL FACTORS PRODUCED BY OSTEOBLASTS IN RESPONSE TO THROMBIN

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Thrombin has been shown to significantly inhibit apoptosis in serum-starved cultures of the osteoblast-like Saos-2 cell line and primary mouse calvarial osteoblasts. This inhibition has been shown to require thrombin's specific proteolytic activity and is independent of the known thrombin receptors. The current study was undertaken to identify the mechanism by which this inhibition occurs. Soas-2 cells and primary mouse calvarial osteoblasts were serum-starved for 24 hours and treated for a further 36 hours with either fresh serum-free medium or fresh serum-free medium containing 100nM thrombin. After treatment, conditioned medium was collected and RNA extracted from the cultures. Conditioned medium, in which the thrombin had been inactivated, was used to treat fresh serum-starved cultures. RNA was used as template for either RT-PCR or ^{32}P-labelled cDNA probes. Apoptosis assays demonstrated that conditioned medium from thrombin-treated cells inhibited apoptosis in serum-starved osteoblasts almost as well as thrombin. Furthermore, this effect could be abolished by blocking protein synthesis in the conditioning cells, suggesting that a survival factor is produced by osteoblasts in response to thrombin. RT-PCR analysis of the expression of a number of known osteoblast survival factors including FGF-2, PDGFa, TGF- , IGF-1, IGF-2 showed no evidence of regulation of expression in response to thrombin. Microarrays probed with cDNA from control and thrombin-treated cells showed up-regulation of MCSF and down-regulation of IGF binding protein 3 (IGFBP3) in response to thrombin. We consider both MCSF and IGF/IGFBP3 as good candidates for effectors of thrombin's anti-apoptotic activity, and are currently studying the direct effects of expression of these genes on osteoblast cultures in vitro.

O38

ELEVATED OSTEOBLASTIC VDR EXPRESSION INCREASES WNT-FRIZZLED GENE EXPRESSION AND CORTICAL BONE MASS IN TRANSGENIC MICE.

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Wnt glycoproteins signal through frizzled (Fzd) receptors to regulate cell growth and polarity and act in chondrocyte differentiation and bone formation. OSVDR mice, over-expressing vitamin D receptor in mature osteoblastic cells, have enhanced cortical bone formation. Wnt-Fzd gene expression changes in OSVDR were evaluated.

Long bones were collected from OSVDR and wildtype mice 6 hours after 1,25-dihydroxyvitamin D3 (1,25-D) or control treatment. Wnt-Fzd expression changes in tibial midshafts were detected using cDNA arrays, and in marrow-flushed femoral diaphyses by real time RT-PCR. Wnt-Fzd and osteoblast marker distribution was examined by *in situ* hybridisation.

In control OSVDR mice, Wnt5b, Fzd7 and Fzd8 genes were increased (9-, 2- and 3-fold) but not Wnt4. 1,25-D treatment reduced expression of Wnt5b, Fzd7 and Fzd8 genes by 90%, 47% and 60% in OSVDR but not wildtype mice. By contrast, Wnt4 expression was reduced by 1,25-D treatment in both OSVDR and FVB (68% and 56%). Wnt4 expression was observed in chondrocytes, mature osteoblasts of secondary spongiosa, scattered mature periosteal osteoblasts, and a population of alkaline phosphatase positive cells in perichondrium on the posterior aspect of distal metaphysis. Fzd8 expression was also observed in the perichondral cell population, and abundantly in mature periosteal osteoblasts.

Thus Wnt5b, Fzd7 and Fzd8 expression is increased in association with elevated osteoblastic VDR. The acute negative 1,25-D response of these genes may relate to direct transcriptional effects, whereas the differences in control mice may relate to OSVDR effects on osteoblastic differentiation. These data support a role for the Wnt-Fzd pathway in vitamin D regulation of cortical bone formation.

O39

EXTRACELLULAR L-AMINO ACIDS SENSITIZE HUMAN PARATHYROID CELLS TO CALCIUM IONS.

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Dietary protein intake has a positive impact on bone mineral density and the risk of osteoporosis (1). The significance of dietary protein for calcium metabolism is underscored by human studies that demonstrate a negative effect of dietary protein intake on PTH levels and a positive effect of dietary protein intake on gastro-intestinal calcium absorption. The finding that L-amino acids are allosteric activators of the cloned human calcium-sensing receptor (CaR; Genbank: U20759) (2) has the potential to explain several of the physiological phenomena described above. In the current work, we tested and confirmed the prediction that plasma amino acids sensitize human parathyroid cell CaRs to their physiological agonist, Ca^{2+} *in vitro*. Normal (> 20 samples) and adenomatous human parathyroid tissue (> 50 samples) was obtained from male and female patients undergoing thyroid or parathyroid surgery. Parathyroid cells were prepared by collagenase digestion in modified culture media containing plasma-like amino acid mixtures. As predicted, aromatic and aliphatic but not basic or branched chain L-amino acids, stereoselectively enhanced the Ca^{2+}-sensitivity of normal and adenomatous parathyroid cell CaRs as determined by fura2-dependent measurement of cytoplasmic ionized Ca^{2+} concentration and PTH secretion. In the presence of maximally effective concentrations of amino acids (e.g., L-Phe 3 mM, L-Ala 3 mM or post-prandial levels of plasma-like amino acid mixtures), the EC_{50} for extracellular Ca^{2+} shifted from about 3 mM to about 1.5 mM i.e., close to the physiological level of extracellular ionized Ca^{2+} concentration. Comparison of normal and adenomatous human parathyroid cells provides evidence that L-amino acid sensitivity may be reduced in primary hyperparathyroidism.

1. Kerstetter, J *et al.*, Calcif. Tissue Int. 66:313 (2001).
2. Conigrave *et al.*, PNAS, 97:4814-4819 (2000).

O40

CHARACTERIZATION OF COLLAGENASE-3 BINDING AND INTERNALIZATION BY RABBIT CHONDROCYTES

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Collagenase-3 (MMP-13) is an extracellular matrix metalloproteinase that cleaves type II collagen, the major protein component of cartilage, with high specificity. Several studies have identified increased levels of MMP-13 in arthritic synovial fluid. Our laboratory has previously documented a process where by osteoblastic cells remove MMP-13 from the surrounding milieu by binding the enzyme to a specific receptor. The enzyme is then internalized and degraded through the actions of the endocytotic receptor, the low-density lipoprotein receptor-related protein (LRP). Such a mechanism eliminates a destructive enzyme from the extracellular environment. This process of MMP-13 internalization also occurs in chondrocytes and is significantly reduced in osteoarthritic (OA) chondrocytes. We are currently characterizing the internalization of MMP-13 in normal rabbit chondrocytes. Primary rabbit chondrocytes were harvested and RT-PCR was used to assess the cell phenotype during the culture period. ^{125}I-MMP-13 was used to assess the ability of the rabbit chondrocytes to bind MMP-13. Appreciable specific cell-association of MMP-13 was detected after 10 min of exposure to the ligand and equilibrium was obtained after 2 h. After identifying the time to equilibrium we determined whether binding was saturable by incubating the chondrocytes with increasing concentrations of ^{125}I-MMP-13 ranging from 0 to 150 nM at 4°C for 2h. The amount of specifically associated MMP-13 approached saturation at 75 nM, allowing assessment of the receptor kinetics. Finally, we have assessed the ability of rabbit chondrocytes to internalize ^{125}I-MMP-13 over time at 36°C. We are currently exploring the receptors involved in the internalization of MMP-13 by chondrocytes as a first step in identifying how this system is impaired in OA cartilage.

O41

INTERLEUKIN-11 MESSENGER RNA EXPRESSION AND REGIONAL DISTRIBUTION OF CANCELLOUS BONE MICRODAMAGE IN THE HUMAN PROXIMAL FEMUR

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For a given bone mass, fracture risk increases with age. Increased bone fragility may be associated with an accumulation of microdamage (Mdx), due to impaired damage repair mechanisms. Animal studies have demonstrated that Mdx is targeted for repair by bone remodelling.[1] This study describes the *in vivo* distribution of Mdx in different regions of the human proximal femur and presents a preliminary analysis of the relationship between *in vivo* Mdx and mRNA gene expression of the osteotropic cytokine interleukin(IL)-11. Cancellous bone samples, from the subchondral and medial principal compressive (SPC, MPC), and medial to the greater trochanter (MGT) regions of the proximal femur, were obtained from 12 postmortem cases (7 females, 5 males; median age 68 years). *In vivo* Mdx was identified in 70μm sections using the basic fuchsin *en bloc* staining technique. The following were measured: crack length (Cr.Le), crack density (Cr.Dn), crack surface density (Cr.S.Dn), damaged bone fraction (DxV/BV). In addition, total RNA was isolated from each MGT bone sample for semi-quantitative RT-PCR analysis of mRNA expression of IL-11. There was no difference between the regions for Cr.Dn, Cr.S.Dn or DxV/BV, except in the SPC region where Cr.Le was significantly reduced ($p<0.001$). This relatively uniform distribution of Mdx in the proximal femur suggests that the principal components of the femoral cancellous bone network are equally exposed to cyclic deformations resulting in Mdx. A positive association was observed between IL-11 mRNA expression and Cr.Le in the MGT region ($p<0.05$), which is consistent with Mdx providing a stimulus for bone resorption. Future research on elucidating the cellular repair mechanisms of bone microdamage is important, as there is some concern about the long-term pharmacological inhibition of bone remodelling, such as the use of bisphosphonates, leading to accumulation of Mdx,[2] resulting in increased bone fragility.

1. Mori & Burr 1993 *Bone* 14:103-9;
2. Mashiba et al. 2001

O42

ZOLEDRONIC ACID MODULATES BONE REPAIR THROUGH TRANSIENTLY DELAYED REMODELLING

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Bisphosphonates have been shown to increase mineral content, size & strength of fracture callus but the mode by which this occurs is unclear. Inhibition of osteoclastic resorption may lead to callus retention, but bisphosphonates also stimulate *in vitro* bone formation. To investigate the mode, 8wk-old maleNZW rabbits underwent right tibial osteotomy, distraction & consolidation until sacrifice at 2,4,6,18&44wks post surgery. Animals received saline, single dose zoledronic acid (0.1mg/kg at surgery) or double dose zoledronic acid (at surgery&2wks). X-rays were taken at 6,18,44wks. In some animals, BMD&BMC were measured by DXA at 2,4,6,18,44wks. In others histomorphometry was carried out after sacrifice. BMD&BMC increased in all animals between 2&4wks, more so treated animals (33%&53% $p<0.01$). Saline animals lost callus mineral density between 4&6wks (34% $p<0.01$) but treated animals did not. Between 6&44wks BMD&BMC gradually decreased in treated animals & increased in saline group, so that at 18&44wks there was no difference between groups. Trabeculae & cortical bone ends were still apparent in x-rays of treated groups at 18wks. BV/TV & % cartilage was greater in treated groups at 4,6&18wks; $p<0.05$. Tb.N increased by 26% & 53% in treated groups at 4wks & by 28% & 60% at 6wks $p<0.01$.There was no difference in Tb.Th at 2,4,6wks & no change in MAR at later times. Ob.S.% was less in treated animals $p<0.05$. There was a decrease in Oc.S.% (in 2&4wks treated groups $p<0.05$). These data do not support a bisphosphonate-induced increase in bone formation. Rather, the increase in new bone in the callus appears due to retention from decreased resorption. In this study, the clinically valuable increase in callus volume & strength can be explained by bisphosphonate suppression of osteoclastic resorption of bone produced in the exuberant osteoblastic response to fracture.

O43

DETERMINANTS OF TRANSMENOPAUSAL BONE LOSS DIFFER ACCORDING TO SKELETAL SITE

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The menopause transition is characterised by variable rates of bone loss. Identification of women at risk of rapid bone loss could target preventative therapy.

We prospectively assessed biochemical, hormonal and genetic determinants of transmenopausal bone loss at the spine and proximal femur in 409 pre-, peri- and post-menopausal women (post) aged (mean±SD) 50±2.3 yrs. 76 women on HRT were excluded. Spinal (LS) and femoral neck (FN) BMD were measured at baseline and after 25 and 48 months, respectively, in 274 (82%) and 216 (65%) women. Women were grouped according to whether they had remained pre- or post, or changed from pre- to post. Estrogen (ER) and androgen receptor (AR); and aromatase (AROM) genotypes were related to changes in LS and FN BMD.

Changes in LS and FN BMD were highest in the transmenopausal group. ER genotype was associated with bone loss at the spine and FN, while aromatase genotype was associated with increases in spinal and femoral neck BMD. General linear modelling indicated that changes in LS BMD were related to baseline serum estradiol, and urine CTx and Pyr; age; BMI; menopause transition; and AR and aromatase genotypes. Changes in FN BMD were related to baseline serum FSH; age; menopause transition; and ER genotype.

Bone loss from the spine and proximal femur occurs early during the menopause transition. ER and aromatase genotypes are associated with bone loss in a site-specific manner and also have interactions with serum oestradiol and FSH, respectively. Interactions between ER and aromatase genotypes and sex hormone levels may influence the rate of transmenopausal bone loss.

O44

THE ROLE OF BONE MINERAL DENSITY AND METACARPAL MORPHOMETRY IN CHILDREN WITH UPPER LIMB FRACTURES: A POPULATION-BASED CASE CONTROL STUDY

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Upper limb fractures in children are very common but the causes are incompletely understood. The aim of this population-based case-control study was to investigate the role of bone mass, risk taking, co-ordination, growth and other factors in the aetiology of upper limb fractures in children aged 9-16 years. The current study refers to bone mass and size variables only. Bone mass (BMD and BMAD) was measured by DXA in total body, lumbar spine and femur neck. Metacarpal index (MI) was assessed using standard techniques. .

A total of 642 subjects were recruited. Of these, there were 321 incident fracture cases identified from the Tasmanian fracture registry and 321 individually matched randomly selected controls. Fracture sites were as follows (hand: n= 91, wrist and forearm: n= 190, upper arm: n= 40)

Table : Odds Ratios for bone mass and MI by subgroup

	Odds Ratio (95% CI): per SD decrease	
	LS BMAD	**MI**
All	1.40(1.18~1.66)	1.31(1.11~1.53)
Males	1.39(1.13~1.71)	1.33(1.09~1.62)
Females	1.43(1.07~1.90)	1.27(0.96~1.67)
Hand	1.15(0.87~1.52)	1.19(0.88~1.61)
Wrist and forearm	1.62(1.26~2.07)	1.49(1.19~1.87)
Upper arm	1.37(0.91~2.06)	1.03(0.66~1.43)

A child with osteopaenia (Z<-1.0) had a much higher risk of fracture (OR 1.74 [1.15~2.64] for upper limb fracture and 3.25 [1.72~5.75] for wrist and forearm fracture). No particular BMD site was statistically better than any other. Both BMAD and MI remained significant predictors of wrist and forearm fracture in multivariate modelling, most likely reflecting a contribution of both cortical and trabecular bone to fracture risk in adolescence. In conclusion, bone mass is a risk factor for wrist and forearm fracture in both boys and girls but not other upper limb fractures. The magnitude of this relationship is somewhat weaker than in adults but suggests that increasing peak bone mass will prevent fractures in children.

O45

DO DIFFERENCES IN NUTRIENT INTAKE PREDICT DIFFERENCES IN BONE MASS AND DIMENSIONS IN BOYS: A CO-TWIN CONTROL STUDY

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The proportion of variance attributable to genetic factors can be determined using the classic twin model, which assumes similarities in lifestyle between twin pairs independent of zygosity. To evaluate the veracity of this assumption we studied protein and calcium intakes in 40 MZ and 42 DZ male twin pairs aged 11.3 ± 2.9 years (range 7-20 years). Bone mass and dimensions, and body composition were measured using dual energy x-ray absorptiometry (DXA). Dietary intake was assessed using 3-day weighed food diaries, and analysed using Food Works Nutrition Program (Version 2.10). Anthropometry was measured using standard methods. Similarities within pairs were assessed using Pearson's correlation. The extent to which within pair differences in bone mass could be accounted for by within pair differences in nutrient intake was determined using multiple linear regression through the origin. Data was analysed using StatView (version 4.51).

MZ and DZ twins did not differ in mean age, bone mass, anthropometry or calcium, protein and energy intakes. Age-adjusted correlations for anthropometry and bone mass ranged from r = 0.88-0.96 for MZ pairs and r = 0.64-0.70 for DZ twins (all $P < 0.01$). Age-adjusted correlations for calcium, protein and energy intakes ranged from r = 0.78 - 0.87, for MZ pairs and r = 0.31 – 0.41 for DZ pairs (all $P < 0.01$). Within pair differences in protein intake was a significant predictor of within pair differences in total body ($P < 0.01$) and appendicular BMC ($p < 0.01$), but not axial BMC. Within pair differences in calcium predicted within pair differences in TB BMC ($P < 0.06$). Within pair differences in calcium intake predicted within pair differences in endosteal, but not periosteal widths even after adjusting for within pair differences in size ($P < 0.05$).

MZ pairs differ less in their dietary intake than do DZ pairs, which can be partly explained by their greater similarity in skeletal traits. Protein intake may be a more important factor in bone mass accrual at the appendicular than axial skeleton.

O46

RACIAL DIFFERENCES IN HIP FRACTURE RISK ARE ESTABLISHED DURING GROWTH: STRUCTURAL AND BIOMECHANICAL DETERMINANTS OF STRENGTH AT THE PROXIMAL FEMUR IN ASIANS AND CAUCASIANS

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The lower areal BMD (aBMD) in Asians than Caucasians is largely due to their smaller bone size. To determine the structural and biomechanical basis for the lower incidence of hip fractures in Asians than Caucasians, we used DXA derived bone mass and dimensional data (width) to estimate endocortical diameter, mean cortical thickness, and section modulus at the femoral neck (FN) and calculated the buckling ratio, an estimate of the relative cortical thickness (subperiosteal radius/cortical thickness) in 42 healthy Asian men and 63 Asian women (Chinese ancestry), 101 healthy Caucasian men and 264 Caucasian premenopausal women aged 18 to 43 years. We hypothesized that Asians have a narrow bone with a thicker cortex at peak conferring a lower risk of hip fracture than Caucasians. FN width and cortical thickness were 11% and 10% less in Asian than Caucasian men respectively (corresponding figures were 16% and 7% less in Asian than Caucasian women). Section modulus (a measure of bending strength) was 29% lower in Asian than Caucasian men, and 36% lower in Asian than Caucasian women (all $p < 0.01$). These differences remained significant after adjusting for height and weight. Buckling ratio was lower in Asian than Caucasian women (7.1 ± 0.2 vs. 7.8 ± 0.1, $p < 0.01$), not in men, suggesting that at peak, Asian women achieved a relatively thicker cortex in a narrow femoral neck than Caucasian women. Sex differences in FN width were about 20% whereas sex differences in cortical thickness were only 6% in Asian men versus Asian women, and the corresponding figures were 13% vs 9% in Caucasians. We infer that the relatively thicker cortices within the narrower FN in Asian women at peak may produce a more structurally stable femoral neck in old age protect against the increased risk of hip fracture.

O47

OBESE CHILDREN AND ADOLESCENTS HAVE LOW SPINAL BONE MINERAL CONTENT FOR THEIR WEIGHT: A DXA STUDY OF 362 FRACTURE-FREE SUBJECTS.

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Heavy children require stronger bones than leaner children. The present cross-sectional observational study was undertaken to examine the magnitude of compensatory increases in the bone mineral content (bmc) and area of the lumbar spine shown by overweight and obese children and adolescents. Vertebral area (cm2) and BMC (g) of vertebrae l2-4 were measured by dual energy x-ray absorptiometry in 202 boys and 160 girls aged 3-19 years using a lunar dpx-l scanner. Subjects were categorized as of normal weight, overweight or obese using recent international cutoffs for body mass index. Compared to children of healthy weight our overweight and obese children had lower vertebral BMC for their bone area, body height, body weight and tanner stage of pubertal development: ratios and 95 % CI for overweight and obese groups were; girls 0.92 (0.87-0.97) and 0.88 (95% ci 0.80-0.96); boys 0.96 (95% ci 0.91-1.02, ns) and 0.87 (95% ci 0.78-0.96), respectively. Spinal area was low in overweight and obese girls compared with girls of healthy weight but overweight and obese boys had enlarged their vertebral area appropriately for their increased body size. We conclude that during growth overweight and obese children do not increase their spinal BMC to fully compensate for their excessive weight. Limiting excessive adiposity in childhood and adolescence may help to avoid excessive loading and stresses on the lumbar spine during growth.(Grant Support: Health Research Council of New Zealand).

O48

NO EVIDENCE OF AN EXERCISE-CALCIUM INTERACTION AT LOADED SITES: A RANDOMISED CONTROLLED STUDY IN BOYS.

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It is unknown if the skeletal effect of exercise may be enhanced when combined with increased dietary calcium. We conducted a single blind, prospective, randomised controlled study to test the following hypothesis: at the loaded sites, exercise and calcium will produce greater benefits than exercise or calcium alone. Eighty-nine boys aged 9.0 (1.1) years (mean (SD)) of Tanner stages 1 or 2 were randomly assigned to one of four intervention groups: 1) moderate impact exercise+calcium (n=20), 2) moderate impact exercise+placebo (n=21), 3) low impact exercise+calcium (n=22) or 4) low impact exercise+placebo (n=26). The 9-mth school-based program consisted of 20 min. of moderate or low impact exercise 3 times/wk. Participants also received ~800 mg/day calcium (milk based calcium-fortified foods) or an equivalent food without additional calcium. Total and regional BMC, anthropometry, sexual maturity, dietary calcium intake using a 3-day food record and physical activity levels (questionnaire) were assessed at baseline and post-intervention. Interaction between exercise and calcium was determined by ANOVA (4-group analysis). The main effects were determined by ANOVA (2 group analysis): exercise (groups 1 & 2 vs 3 & 4) and calcium (groups 1 & 3 vs 2 & 4). ANCOVA was used to control for baseline confounding factors - BMI, sexual maturity, regional BMC, dietary calcium intake and physical activity level. At baseline, no differences were reported between the intervention groups. There was no significant calcium-exercise interaction on BMC gain at any site. For the unadjusted analysis a main effect of calcium was detected at the femur: additional calcium resulted in a 2.6% greater increase in BMC (mean±SE, 3.9±1.2 g, $p<0.01$). A main effect of exercise was detected at the loaded sites: moderate impact exercise resulted in a 1.9% and 3.2% greater increase in BMC at the femur (2.5±1.2 g, $p<0.05$) and tibia (2.9±1.4 g, $p<0.05$). There was no effect of additional calcium or moderate impact exercise at lumbar spine, humerus or radius. Similar results were found after adjusting for baseline confounding variables. In conclusion, in pre- and early pubertal boys greater gains in bone mass at the femur may be achieved with either short bouts of moderate impact exercise and increased dietary calcium; there was no evidence of an exercise-calcium interaction.

POSTER ABSTRACTS:

P1

EXPRESSION OF DOMINANT-NEGATIVE RAB3D IMPAIRS OSTEOCLAST-MEDIATED BONE RESORPTION

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Osteoclasts are terminally differentiated polykaryons responsible for bone matrix degradation, a processes mediated by a highly specialised and convoluted membrane domain. This ruffled domain is formed by the rapid fusion of acidified intracellular vesicles to the bone-apposed plasmalemma, an event analogous to regulated secretion in other mammalian systems. While it is clearly evident that intense vesicular trafficking is required to sustain the constant flux of bone resorbing enzymes and matrix components between the ruffled border and its respective basolateral domains, the molecular machinery that govern these dynamic processes remain to be defined. In search of novel molecules related to the control of exocytosis in osteoclasts we have recently identified the small Rab GTP-binding protein Rab3D as a putative regulator of secretory vesicle transport in osteoclasts. Here, using a combination confocal and video time-lapse microscopy Rab3D was found to localise and direct post-trans-Golgi (TGN) vesicular transport along the secretory pathway. In addition, in an attempt to delineate the biological function of Rab3D we have generated a number of RAW.C4/osteoclast precursor stable cell lines over-expressing EYFP-tagged wild-type Rab3D and two Rab3D mutants deficient in GTPase activity. Expression dominant-negative Rab3DN135I restricted Rab3D trafficking at the TGN in osteoclasts and their mononuclear precursors. Moreover, analysis of the bone resorption phenotype by SEM revealed that osteoclasts over-expressing dominant-negative N135I displayed impaired resorption capacity. In all, our results implicate a functional role for Rab3D in exocytotic vesicle transport in osteoclasts during bone resorption.

P2

ANALYSIS OF THE L-AMINOACID BINDING SITE ON THE CALCIUM-SENSING RECEPTOR BY CHIMERIC RECEPTORS

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The human calcium-sensing receptor (CaR) responds to Ca^{2+} ions acting as its physiological agonist. In addition, L-amino acids act as allosteric activators of the cloned CaR (1) although the location of the amino acid binding site is uncertain. We have now used chimeric receptors constructed by domain shuffling between the human CaR and rat metabotropic glutamate receptor type-1 (mGlu) to investigate the location of the amino acid binding site. In particular, we examined whether the amino acid binding site is located in the N-terminal bilobed Venus fly-trap (VFT) domain or the cys-rich region (CRR) of the extracellular head, or in the transmembrane region (TMR). The following chimeric receptors (VFT: CRR:TMR-tail) were either constructed *de novo* or kindly provided by Dr Edward Nemeth, NPS Pharmaceuticals (Salt Lake City, UT, USA): CaR/ CaR/ mGlu-tail; CaR/ mGlu/ mGlu-tail; mGlu/ mGlu/ CaR-tail; mGlu/ CaR/ CaR-tail. The chimeric receptors were expressed in HEK-293 cells and receptor activity was assessed by Ca^{2+} mobilization as detected by aequorin luminescence or fura2 fluorescence. Receptors that exhibited an mGlu VFT domain were inactive when expressed in HEK-293 cells i.e., they failed to respond to glutamate or quisqualate. On the other hand, receptors that exhibited a CaR VFT domain responded robustly to Ca^{2+} ions. Replacement of the CaR transmembrane domain, for example in CaR/CaR/mGlu-tail or CaR/mGlu/mGlu-tail eliminated the allosteric response to R-467 but allosteric activation by L-amino acids was retained. The data indicate that the CaR's amino acid binding site is distinct from that for type-II calcimimetics and lies in the extracellular domain, most probably in the VFT domain.

1. Conigrave AD, Quinn SJ, Brown EM (2000) L-amino acid sensing by the extracellular Ca^{2+}-sensing receptor. PNAS 97:4814-4819.

P3

EXPRESSION AND LOCALIZATION OF EXTRACELLULAR MATRIX METALLOPROTEINASE INDUCER IN GIANT CELL TUMOUR OF BONE

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Matrix metallproteinases (MMPs), a regulator in tumour invasion and metastasis are induced by extracellular matrix metalloproteinase inducer (EMMPRIN). Giant cell tumour of bone (GCT) is a benign neoplasm but locally aggressive. In this study, we determined the protein and mRNA expression of EMMPRIN in the tumours and proposed that EMMPRIN is associated with the biological aggressiveness of GCT. To examine the expression and localization of EMMPRIN in GCT, we performed in-situ hybridization (ISH) using antisense EMMPRIN riboprobe, immunohistorchemistry (IH) with the mAb 8G6 against human EMMPRIN and semiqautitative RT-PCR. Our results indicated that EMMPRIN mRNA was expressed in all GCT. The level of EMMPRIN mRNA expression is associated with the clinical stage of GCT. High level of EMMPRIN mRNA expression was observed in GCT with advanced stage III. In all of 17 cases tested, there is a great significant ($p \ll 0.01$) difference of EMMPRIN expression between GCT stage I & II and GCT stage III. Both ISH and IH demonstrated that EMMPRIN expression is present at the multinuclear osteoclast-like giant cells of GCT and immunostaining showed strong positive signals on the cell membrane of giant cells. Interestingly, the stromal tumour cells were also positive but the level of expression is weaker. Our studies suggest that EMMPRIN mRNA and protein are synthesized in GCT. Moreover, its expression levels are significantly correlated with aggressive potential of the tumour progression. In short, EMMPRIN may be a regulatory factor involved in processing MMP-dependent tumour behaviors of GCT.

P4

PROTEASE-ACTIVATED RECEPTOR-2 AND MEDIATORS OF OSTEOCLAST DIFFERENTIATION

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Protease-activated receptor-2 (PAR-2), which is expressed by osteoblasts, is activated specifically by a small number of tissue proteases. PAR-2 is also activated by a peptide (RAP) which corresponds to the 'tethered ligand' created by cleavage of the extracellular N-terminal domain. In mouse bone marrow cultures, RAP inhibits osteoclast differentiation induced by parathyroid hormone (PTH) or 1,25 dihydroxyvitamin D_3 ($1,25D_3$). Semi-quantitative RT-PCR was used to investigate expression of mediators of osteoclast differentiation in mouse bone marrow cultures and primary calvarial osteoblast cultures treated with RAP. In bone marrow and osteoblast cultures treated with PTH or $1,25D_3$, RAP inhibited expression of RANKL; in osteoblast cultures, RAP also stimulated osteoprotegerin expression. In bone marrow cultures treated with PTH or $1,25D_3$, activation of PAR-2 led to reduced expression of both the constitutive and inducible prostaglandin H synthases (PGHS-1 and –2). In osteoblasts treated with PTH, activation of PAR-2 caused a suppression of PGHS-2 expression. RAP inhibited PTH- or $1,25D_3$-induced expression of interleukin-6 in bone marrow cultures but not in osteoblast cultures. RAP also inhibited expression of RANK in PTH- or $1,25D_3$-treated bone marrow cells, which may be mediated by a direct effect on osteoclast precursors, since the RAW264.7 osteoclastogenic macrophage cell line was shown to express PAR-2. These observations indicate that PAR-2 activation inhibits osteoclast differentiation by affecting multiple mediators of the effects of PTH and $1,25D_3$.

P5

INVESTIGATION INTO THE PRESENCE OF ANDROGEN RECEPTOR IN OSTEOCLAST-LIKE CELLS AND RAT BONE

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Evidence exists to suggest that androgens may inhibit the formation and/or function of osteoclasts, however this is controversial and the underlying mechanism for this action is not well understood. The aim of this study is to identify androgen receptor (AR) mRNA and protein in mouse osteoclast-like cells generated *in vitro* and rat bone. RAW264.7 cells were cultured for up to 7 days in the presence of 50ng/ml RANKL and 10^{-8}M dihydrotestosterone (DHT), testosterone (T) or vehicle (V). At day 0, 3 and 7, cells were harvested and RNA extracted. RT-PCR was performed for the mouse AR, tartrate resistant acid phosphatase (TRAP) and calcitonin receptor (CTR) genes. Results from AR RT-PCR were confirmed by Southern blot analysis. Nine female Sprague-Dawley rats were sacrificed at 4.5 months of age. Femurs were collected, decalcified and paraffin embedded for immunohistochemistry and *in situ* hybridisation. Large multinucleated cells, generated following RANKL treatment of RAW264.7 cells, expressed TRAP and CTR mRNA. AR mRNA was not detected at any stage during osteoclastogenesis and this was not affected by treatment with DHT or T. AR protein was localised in the cytoplasm and nucleus of large multinucleated cells found within resorption pits by immunohistochemistry. These cells were confirmed to be mature osteoclasts following staining for TRAP enzyme activity and the identification of CTR using a specific rabbit polyclonal antibody. The results from these studies indicate that AR protein is expressed in mature osteoclast cells *in vivo*. Furthermore the use of osteoclast-like cells derived from RAW264.7 cells *in vitro* is not an appropriate model for the study of androgen action on osteoclasts.

P6

STUDIES ON THE RECEPTORS MEDIATING RESPONSES OF OSTEOBLASTS TO THROMBIN

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Thrombin is a multifunctional serine protease, which plays a critical role in thrombosis and haemostasis, as well as having hormone-like effects on a diverse range of cells. In osteoblasts, thrombin stimulates proliferation, but decreases alkaline phosphatase (AP) activity and apoptosis. Three thrombin receptors have so far been identified, protease activated receptor (PAR)-1, PAR-3 and PAR-4, and we have previously demonstrated that osteoblasts express PAR-1.

We have studied the effect of thrombin on osteoblast proliferation, differentiation and apoptosis to determine which of the thrombin receptors are responsible for these actions. RT-PCR was used to show that the osteoblasts isolated from mouse calvariae express PAR-1 and PAR-4, but not PAR-3. Synthetic peptides which specifically activate PAR-1 (TFFLR-NH_2) and PAR-4 (AYPGKF-NH_2) were used to identify which receptor is responsible for thrombin's effects on primary mouse calvarial osteoblasts. Both TFFLR-NH_2 and AYPGKF- NH_2 stimulated substantial calcium responses. TFFLR-NH_2 was as effective as thrombin at reducing AP activity and stimulating proliferation (as measured by 5-bromo-2'-deoxyuridine incorporation). AYPGKF-NH_2, however, had no effect on either AP activity or proliferation. Neither peptide was able to mimic thrombin's effect on apoptosis, and thrombin was as effective at inhibiting apoptosis in cells isolated from PAR-1-null mice as in wild type cells. The results demonstrate that thrombin stimulates proliferation and inhibits differentiation of osteoblasts through activation of PAR-1, while thrombin inhibition of osteoblast apoptosis is independent of known thrombin receptors. It is likely that thrombin through PAR-1-dependent and -independent effects on osteoblast behaviour plays a critical role in bone healing.

P7

FACTORS ASSOCIATED WITH TOTAL CALCIUM INTAKE IN PREMENOPAUSAL WOMEN: A CROSS-SECTIONAL STUDY.

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It is well recognized that most women in Australia do not achieve the recommended daily intake of calcium. However, the reasons behind this are unclear. The aim of this study was to describe sociodemographic factors associated with calcium intake. We studied 467 healthy women aged 25-44 years who were randomly selected from the electoral role. Subjects were excluded if they were pregnant or lactating or had a previous bone density. Data collected included: anthropometrics (age, height , weight, body mass index, body composition); smoking history; breastfeeding history; physical activity (by questionnaire, muscle strength and endurance fitness); family history of osteoporosis and or fracture; history of fracture in the subjects; socioeconomic factors; osteoporosis knowledge (by questionnaire); osteoporosis self efficacy (by questionnaire) and bone density. Calcium intake was assessed by the Angus food frequency questionnaire.

Average daily total calcium intake was 448 mg (range 84 to 3285). In univariate analysis there were significant correlations between calcium intake and education level, number of children, osteoporosis knowledge, osteoporosis self efficacy and fat mass (all $p<0.05$). In multivariate analysis, these remained significant with the exception of osteoporosis efficacy. There were no significant correlations between calcium intake and age, family history of fracture, family history of osteoporosis, smoking history, current smoking or employment status. In conclusion, women with lower levels of education and osteoporosis knowledge and higher fat mass and greater numbers of children have poorer calcium intakes. These results provide information that will help in targeting programs aimed at increasing calcium intake in women.

P8

ALLOSTERIC ACTIVATION OF CALCIUM-SENSING RECEPTOR-STIMULATED MAP-KINASE ACTIVITY BY L-AMINO ACIDS.

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We have recently shown that L-amino acids are allosteric activators of the cloned human calcium-sensing receptor (CaR; ref. 1) as detected by intracellular Ca^{2+} mobilization in fura2-loaded cells. In the present study we have examined whether L-amino acids may also activate other intracellular signaling pathways by testing the effects of CaR-active amino acids on the activation of p42 and p44 MAP-kinase. HEK-293 cells that stably expressed the human CaR were exposed to various concentrations of Ca^{2+} (0.5 mM to 5 mM) for 0 -10 min in the presence or absence of CaR active amino acids (e.g., L-Phe 10 mM) and then lysed in the presence of inhibitors of protease and protein phosphatase activity. The cell lysate was then separated by SDS-PAGE and electro-transferred to nitrocellulose or PVDF membranes. The membranes were then developed by exposure to an antibody that is selective for the phosphorylated/active form of MAP kinase followed by an alkaline phosphatase-conjugated secondary antibody and enzyme substrate. Equivalence of loading was controlled by an antibody that recognises total MAP kinase. In the presence of active amino acids, the Ca^{2+} sensitivity of CaR was left-shifted from an EC_{50} of about 4 mM to about 2 mM. The results indicate that amino acid-dependent modulation of the CaR can influence intracellular signaling pathways that are coupled to the regulation of growth and differentiation.

1. Conigrave AD, Quinn SJ, Brown EM (2000) L-amino acid sensing by the extracellular Ca^{2+}-sensing receptor. PNAS 97:4814-4819.

P9

THE NITROGEN-CONTAINING BISPHOSPHONATE, ZOLEDRONIC ACID SENSITISES OSTEOGENIC SARCOMA CELLS TO APO2L/TRAIL-INDUCED CELL DEATH.

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We have previously shown that the third generation nitrogen-containing bisphosphonate, zoledronic acid (ZOL) induces cell death of human osteogenic sarcoma (OS) cells by a caspase independent mechanism whereas, induction of apoptosis by the newly recognised TNF family member, Apo2L/TRAIL, is caspase dependent.

The aim of this study was to investigate the effect of ZOL in combination with Apo2L/TRAIL in a panel of human OS cell lines (BTK-143, MG-63, SJSA-1, G-292, SAOS-2, HOS). We found that ZOL when used alone, reduced cell number in a dose and time dependent manner, due either to a cytostatic response as a result of cell cycle arrest in S-phase or the induction of apoptosis. ZOL in combination with Apo2L/TRAIL resulted in a significantly greater induction of cell death than for either agent alone. More importantly we found that OS cells that are normally resistant to Apo2L/TRAIL, are sensitised by ZOL to the apoptotic effects of Apo2L/TRAIL. The addition of broad-spectrum caspase inhibitor, z-VAD-fmk, prevented the Apo2L/TRAIL-mediated apoptosis but not that induced by ZOL. Addition of geranylgeraniol, an intermediate of the mevalonate pathway, suppressed the ZOL-induced apoptosis and blocked the ability of ZOL to sensitise OS cells to Apo2L/TRAIL-induced cell death.

This suggests that geranylgeranylated proteins may be involved in regulating Apo2L/TRAIL receptor-mediated cell death in the combined treatment. The intracellular mechanisms by which ZOL sensitises OS cells to Apo2L/TRAIL-induced apoptosis are currently not known. However by understanding the pathways activated by these two non-toxic agents and exploiting their use, there is exciting potential for their use to manage osteosarcomas clinically.

P10

AUTOSOMAL DOMINANT HYPOCALCEMIA - A NOVEL ACTIVATING MUTATION (E604K) OF THE CALCIUM-SENSING RECEPTOR.

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The control of parathyroid hormone (PTH) secretion by extracellular calcium is mediated by the calcium sensing receptor (CaR). Autosomal dominant hypocalcemia (ADH), characterised by hypocalcemia in the context of inappropriately low levels of PTH as well as calcitriol-induced hypercalciuria has been linked to activating mutations of the CaR. Here, we report a novel activating mutation in the Cys-rich region of the CaR in a family with ADH. The missense mutation E604K co-segregated with hypocalcemia in all seven individuals for whom DNA was available. Two unaffected, normocalcemic members of the family did not exhibit the mutation. The molecular impact of the mutation on two key components of the signalling response (Ca^{2+} mobilization and MAP-kinase activity) was assessed in HEK-293 cells transfected with cDNA corresponding to either the wild-type CaR or the E604K mutation. There was a significant left-wards shift in the concentration response curves for extracellular Ca^{2+} in both assays. We conclude that E604K is a novel natural activating mutation of the CaR that has an impact on diverse intracellular signalling pathways. Furthermore, these findings suggest that the C-terminal end of the Cys-rich region normally acts to suppress receptor activity in the context of low extracellular Ca^{2+} concentrations.

P11

REGULATION OF TBP-2 GENE EXPRESSION MODULATES OSTEOCLAST DIFFERENTIATION.

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Using gene array analysis, we found that treatment of human PBMC with RANKL down regulates thioredoxin (TRX) binding protein-2 (TBP-2) expression. TBP-2 is a negative regulator of TRX, a small protein with a redox-active dithiol active site. TRX is involved in a variety of cellular processes and enhances DNA binding of transcription factors such as NFκB and AP-1. Our hypothesis was that regulation of TBP-2 gene expression using adenoviral gene transfer would, in a model employing human CFU-GM precursors treated with sRANKL and M-CSF, modulate osteoclastogenesis.

The adenoviral lacZ expression vector (AdenoX, Clontech) achieved infection efficiencies of >80% at days 4 to 12. Over-expression of TBP-2 using a sense construct resulted in a 75% reduction in $TRAP^+$ cells after 14d compared to lacZ control. There was a 50% reduction in the number of $TRAP^+$ multinuclear cells and they were 66% smaller and contained 50% fewer nuclei. Resorption was inhibited by 75%. In contrast, inhibition of TBP-2 expression using an anti-sense construct resulted in an increase in the size, number of nuclei and survival of $TRAP^+$ multinuclear cells and stimulation of resorption by 150%.

We have shown that adenoviral vectors can be used to stably infect human osteoclast precursor cells at high efficiency. We have also shown that TBP-2 over-expression inhibits, and under-expression stimulates, osteoclast differentiation. This is consistent with the known importance of the redox sensitive transcription factors NFκB and AP-1 in osteoclast differentiation.

P12

DUAL HIP BMD DISCORDANCE DUE TO OSTEOARTHRITIS., McGrath F, Freund J, Pocock N, St Vincent's Hospital, Sydney

There is generally a high correlation between Bone Mineral Density (BMD) in the proximal femora. Some patients however demonstrate significant BMD discordance between the hips. The aetiology of this discordance is not always evident. Previous studies (1) have suggested that hip osteoarthritis (OA) is associated with an increase in proximal femur BMD. This however conflicts with our own observations in patients with clinical OA. We set out to test the hypothesis that unilateral painful OA of the hip or knee, could result in a reduction in ipsi-lateral proximal femur BMD.

Patients were entered into the study if they had significant painful OA of one hip or knee, based on the patient's history, and as well had a significant discordance in total proximal femur (TPF) BMD based on a difference between the hips exceeding the precision error ($2\sqrt{2}xCV$). In total 30 patients (27 females, 3 males) were studied with an age range of 47 - 84 years; (mean 67, SD 12.6). The difference in TPF BMD in these subjects ranged from 4.8 to 9.6% (mean 6.5%). To correct for gender, TPF BMD T-scores in the affected leg were compared to the T-scores in the unaffected leg using a paired t-test. The mean TPF T score of the affected legs was –1.86 (SD 0.87), while the mean TPF T score of the unaffected legs was –1.32 (SD 0.93) ($p< 0.0001$). In all but one patient the BMD in the affected leg was lower than in the unaffected leg

Conclusion. Unilateral lower limb painful OA may be one cause of the observed discordance in TPF BMD in some patients. In these patients the affected side is significantly lower than the unaffected side and hence may be at increased risk of fracture. Altered weight bearing is considered the most likely mechanism. If these data are confirmed, DXA operators may need to alter their scanning protocols appropriately.

1. Nevitt MC et al. 1995. Radiographic osteoarthritis of the hip and bone mineral density. Arthritis & Rheumatism; 38(7):907-16.

P13

MECHANICAL STRESS REDUCES THE LEVEL OF VITAMIN D-24 HYDROXYLASE INDUCTION IN UMR-106 CELLS.

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The 1,25-dihydroxyvitamin D-24-hydroxylase (P450c24) plays an important role in vitamin D metabolism and function in kidney and bone, which are target tissues for 1,25-dihydroxyvitamin D (1,25D). P450c24 expression and activity is stimulated by 1,25 D in all tissues examined. We have studied the effects of mechanical stress on P450c24 expression in UMR-106 osteoblastic cells which were cultured in D-MEM with 0,1,10 nM 1,25D. Half the cells were mechanically stressed using the Flexer plate system using a 1 second cycle exerting 0 to 15 % elongation over a 6 hour period. Following culture and stressing, mRNA was extracted from cells for RT-PCR and Northern blot analyses for P450c24 and alkaline phosphatase mRNA. RT-PCR results demonstrated a 1,25D dose dependent increase for P450c24 mRNA. However 1,25D induction of P450c24 was suppressed in cells subjected to mechanical stress. In contrast, expression of alkaline phosphatase mRNA, a marker of osteoblast maturation, was increased by mechanical stress. Northern blot analysis confirmed the data obtained by RT-PCR. These results suggest that mechanical stress effects UMR 106 cells by reducing 1,25D catabolism and stimulating alkaline phosphatase expression consistent with induction of osteoblast maturation.

P14

ELEVATED OSTEOBLASTIC VITAMIN D RECEPTOR REDUCES OSTEOCLASTIC RESPONSE TO PROLONGED DIETARY CALCIUM RESTRICTION IN TRANSGENIC MOUSE MODEL.

PA Baldock, GP Thomas, K Henderson, JA Eisman, EM Gardiner. Bone and Mineral Research Program, Garvan Institute of Medical Research, Darlinghurst Sydney, NSW 2010.

To amplify the osteoblastic actions of 1,25-dihydroxyvitamin D3, vitamin D receptor (VDR) levels were elevated exclusively in mature cells of the osteoblastic lineage in a transgenic mouse model (OSVDR). As previously reported, bone loss after 1 month of calcium (Ca) restriction was reduced in OSVDR mice. These observations have now been extended to examine the long-term kinetics of this protective effect.

Three month old OSVDR and wildtype (FVB/N) mice were divided into High (1%) and Low (0.1%) Ca diets. Groups were collected at baseline, then 4 and 6 months of age. The 4th caudal vertebrae were analysed for trabecular architecture and turnover.

Cancellous bone volume (%) was greater in OSVDR than FVB/N across the study (23.1 ±0.9 vs 18.9 ±0.8, $P<0.0001$). This elevation was evident in all groups except the 6 month Low Ca group, in which OSVDR bone volume was reduced to FVB/N levels. This decline was not the result of bone resorption, as osteoclast surface (%) was significantly reduced in OSVDR across the study (14.8 ±1.0 vs 11.4 ±0.7, $P<0.005$), most evident in the Low Ca groups. The decline in OSVDR bone volume was consistent with altered bone formation rate ($um^2/um/d$), which was reduced in the 6 month Low Ca group in OSVDR (0.18 ±0.02 vs 0.06 ±0.03, $P<0.005$) but not in FVB/N (0.14 ±0.02 vs 0.11 ±0.05).

These data suggest that elevated VDR in mature osteoblastic cells results in persistently reduced bone resorption despite extended calcium restriction. Furthermore, reduced bone formation can contribute significantly to net bone loss during calcium restriction.

P15

GENDER DIFFERENCES IN VOLUMETRIC BONE DENSITY: A STUDY ON OPPOSITE – SEX TWINS

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The aims of the study were to compare gender differences in bone mass (BMC), areal BMD (aBMD), volumetric BMD (vBMD).

DEXA derived BMC, aBMD, vBMD at the third lumbar vertebra (L3), femoral neck (FN) and forearm were compared between 82 opposite sex pairs aged 18 – 80. Volumetric BMD was calculated by dividing BMC by the volume of the skeletal sites of interest. FN volume and volume (V) at 4 regions of the forearm were estimated by the formula: $V = \eth (d/2)^2 h$. The region of interest is assumed to be a cylinder and d = the estimated diameter and h is the height of the region of interest). Vertebral volume of the L3 vertebra was estimated using $V=A^{3/2}$, where A is the projected area of the third lumbar vertebra obtained by PA DEXA scanning.

BMC was significantly higher in males, particularly at the forearm sites (20.1% - 45.3%). For aBMD the gender differences remained significant at all sites except the spine. The average differences in aBMD were not as great as the differences in BMC (2.2% - 23.7%). The differences in vBMD however followed a different pattern. FN and L3 vBMD were significantly higher in females (4.8% and 0.6% respectively), while forearm BMD was higher in males (1.3% to 5.4%). The differences at the forearm sites however reached statistical significance at only one of the 4 regions.

Comparing aBMD values between males and females, when females in general have smaller skeleton than males can result in misleading conclusions. A comparison of vBMD between men and women may be a more accurate reflection of gender differences in bone density.

P16

EFFECT OF FIBROBLAST GROWTH FACTOR-8a (FGF-8a) ON OSTEOBLAST AND OSTEOCLAST PROLIFERATION, DIFFERENTIATION AND FUNCTION.

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The fibroblast growth factors (FGFs) are a group of structurally related peptides with at least 23 members identified so far. They are involved in many biological processes and some of them are active in bone. However, the effects of FGF-8 on bone tissue are unknown. Recent studies have shown that FGF-8 stimulated chondrocyte proliferation and differentiation, and enhanced the expression of core binding factor A1 in a fibroblast cell line, suggesting a potential role in bone metabolism.

Cell proliferation (^{3}H-thymidine incorporation) and bone resorption (^{45}Ca release) were measured in osteoblast cultures and mouse calvarial organ cultures. To assess the effect of FGF-8a on osteoclast formation, mouse bone marrow cells were cultured for 8 days followed by staining for tartrate resistant acid phosphatase (TRAP)-positive cells. TRAP-positive cells with three or more nuclei were counted. In rat isolated osteoclast cultures, osteoclast activity was scored as a ratio of the number of pits to the number of osteoclasts per bone slice.

Murine recombinant FGF-8a at 25 ng/ml or greater stimulated the proliferation of rat primary osteoblasts and UMR-106 cells. It significantly inhibited osteoclast formation at 0.05 to 25 ng/ml in mouse bone marrow cultures, but this effect was lost at 100 ng/ml. FGF-8a had no effect on bone resorption in calvarial organ cultures and isolated osteoclast cultures, suggesting that FGF-8a does not directly effect the mature osteoclast. These results indicate for the first time that FGF-8a might have a role in bone cell proliferation and differentiation. Its function in regulating osteoblast differentiation and bone formation is still under investigation.

P17

CLINICALLY DETECTED KYPHOSIS AS A PREDICTOR OF VERTEBRAL FRACTURE IN ELDERLY WOMEN

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Vertebral fractures impair physical function and quality of life in older women and are associated with increased mortality. The aim of this study was to determine the relationship between a clinical measure of kyphosis and underlying fracture and to determine the clinical utility of kyphosis as a predictor of fracture.

435 women aged over 70 years were recruited from the population using the electoral role. The kyphosis index (KI) was calculated from flexicurve ruler measurements using previously described methods. The presence of fractures were determined from morphometric x-ray absorptiometry (MXA) using a Hologic 4500A bone densitometer.

The mean age of the patients was 75±3 years, one or more anterior wedge fractures, crush fractures and central fractures were present in 14% 13% and 5% of the subjects respectively. The mean KI was 14.4±3.4. When the group was split into tertiles of KI the odds ratio (OR) of having an anterior wedge fracture was 3 and 9 times greater in the second and third tertiles compared to those in the lowest tertile. The OR's for crush and central fractures were not significant. Utilising ROC analysis, at a sensitivity of 80% for the detection of an anterior wedge fracture (equivalent to a KI of 14.2 or greater), the predictive power of a positive test was 25% and the predictive power of a negative test was 95%.

The results from this study indicate that kyphosis is related to the presence of anterior wedge vertebral fractures, but not central collapse or crush fractures. Furthermore, kyphosis is a clinically useful indicator of the presence of anterior wedge vertebral fracture, which is an independent risk factor for future fracturing.

P18

BONE STRUCTURE IN DAUGHTERS OF WOMEN WITH HIP FRACTURE

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Women with hip fracture and their daughters have increased femoral neck (FN) diameter and reduced volumetric bone mineral density (vBMD). To determine whether the reduced vBMD is the result of a thinner cortex, bone mass and bone width were measured at the FN using DXA and to estimate endosteal diameter, mean cortical thickness and section modulus in 35 postmenopausal women with hip fracture and in their 48 premenopausal daughters. We calculated the buckling ratio (index of structural instability). The data were expressed as the Z-score (mean±SE) adjusted for age and weight, using a normal population of 262 premenopausal and 370 postmenopausal women. FN width and endosteal diameter were greater in women with hip fracture (Z=0.84±0.27 and 0.98±0.26, respectively) and in their daughters (0.46±0.13 and 0.38±0.13, respectively). FN cortical thickness was reduced in women with hip fracture (–0.75±0.11,) and in their daughters (–0.29±0.13, $p < 0.05$). FN section modulus was normal in fracture cases but was increased in their daughters (0.54±0.15 SD). Buckling ratio was increased in fracture cases(1.54±0.18 SD), and not in their daughters. We infer that the larger FN diameter in the daughters of women with hip fractures is accompanied by a thinner cortical thickness conferring a structural disadvantage as endocortical resorption erodes the thin cortex in which increasing the risk of buckling in old age.

P19

MELATONIN PROMOTES BONE FORMATION IN THE CULTURE OF OSTEOBLASTS ISOLATED FROM AGED RATS

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Melatonin released from the pineal gland regulates a variety of physiological processes including circadian rhythms. It is known that the secretion of melatonin is decreased during an aging process, and when administrated to aged animals, it is capable of increasing their life span. Although little is known about the biological consequences of diminished melatonin production during an aging process, it is likely to have a profound effect on a variety of systems *in vivo*. The purpose of this study is to investigate the effects of melatonin on bone formation by osteoblasts and to assess whether melatonin acts as an anti-aging agent in regard to bone metabolism.

Osteoblasts were enzymatically isolated from calvariae of 90-week-old female Wistar rats. Melatonin increased bone formation markers, alkaline phosphatase activity, collagen synthesis, bone nodule formation, calcium content, and osteocalcin expression. To determine whether melatonin-induced bone formation is mediated through the trasmembrane receptor, the cells were treated with luzindole (a competitive inhibitor of binding of melatonin to the receptor) and pertussis toxin (an uncoupler of G_i protein from adenylate cyclase and phosphatidylinositol-specific phospholipase C). Both luzindole and pertussis toxin were shown to reduce melatonin-induced bone formation. Melatonin decreased cAMP production and increased inositol triphosphate production and both luzindole and pertussis toxin decreased these effects. In conclusion, melatonin acts as a promoter for bone formation due to the inhibition of adenylate cyclase and the activation of phosphatidylinositol-specific phospholipase C in the cells isolated from aged rats, in addition, this study shows a possibility that melatonin may act as an anti-aging agent in regard to bone metabolism.

P20

MODULATORY ROLE OF NITRIC OXIDE ON FACTORS INVOLVED IN FRACTURE HEALING

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Fracture healing is a complex process involving numerous factors. Nitric oxide (NO), generated by nitric oxide synthases (iNOS, eNOS, bNOS), was reported by us to modulate rat femoral fracture healing. The purpose of this study was to investigate the interaction between NO and other factors involved in bone repair and mechanisms by which NO may effect fracture healing, utilizing a callus explant gene therapy model.

Tissues from a day-10 rat femoral fracture callus explant culture were transfected with human iNOS or eNOS gene carried by an adenovirus vector (Adv-HNOS), NOS inhibitor NMMA (negative control), NO donor SNAP (positive control), or were incubated in unconditioned medium as a baseline control. RT-PCR and western blot were used to detect the human NOS expression in rat tissue and competitive PCR was used to quantitate the changes in gene expression of transforming growth factor beta (TGF-beta), basic fibroblast growth factor (bFGF), alkaline phosphatase (ALP) and osteocalcin (OCN) under endogenous and exogenous NO regulation.

We found both human iNOS and eNOS mRNA and protein expression in rat callus after 3 and 24 hours adenovirus transfection. NOS inhibition resulted in a 50% reduction in ALP and 25% reduction in bFGF expression, while SNAP treatment and Adv-HNOS transfection enhanced ALP and bFGF expression. In contrast, OCN and TGF-beta gene expression were enhanced by NOS inhibition and were decreased by 60% and 45% when treated by SNAP, and also decreased by Adv-HNOS transfection.

These findings suggest that NO may effect fracture repair by up-regulating ALP and bFGF gene expression and down-regulating TGF-beta and OCN gene expression in fracture callus.

P21

FUNCTIONAL ANALYSIS OF CALCIUM-SENSING RECEPTOR MUTATIONS BY AEQUORIN LUMINESCENCE AND MAP KINASE-LUCIFERASE.

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The human calcium-sensing receptor (CaR) is a G-protein coupled receptor that plays an essential role in regulating serum calcium by inhibiting parathyroid hormone secretion from the parathyroids and decreasing renal Ca^{2+} reabsorption. Mutations in the CaR gene are associated with familial hypocalciuric hypercalcemia (FHH) or autosomal dominant hypocalcemia (ADH) depending upon whether they are inactivating or activating, respectively. To investigate whether high-throughput assays could be applied to the functional analysis of CaR mutations, we developed two robust and technically simple assays for assessing CaR activity. The first assay uses the photoprotein aequorin as a sensitive detector of intracellular Ca^{2+} while the second makes use of a MAP-kinase-luciferase reporter system to evaluate CaR-stimulated MAP-kinase activity. Initially, we tested known inactivating (eg: E297K, S170A) and activating (eg: L125P) mutations to confirm the utility of the assays. Recently, we have employed the assays to probe the effect of new mutations isolated from families with FHH (D216G) and ADH (E604K), and to assess the molecular impact of changes to ligand binding sites. The results obtained indicate that these new assays will be useful for the rapid screening of mutant CaR activity on two different intracellular signalling pathways.

P22

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

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Bone mineral density (BMD) is largely determined by genetic factors. However, the nature of the genes regulating BMD variation in different types of bone has not been well documented. This study was designed to fill that gap in knowledge by specifically testing the hypothesis of a major gene (MG) in the determination of BMD variation.

BMD at the lumbar spine and femoral neck was measured by DXA (LUNAR Corp, Madison, WI) in 330 men and 413 women, aged between 18 and 90 years, who were participants of the Dubbo Osteoporosis Genetic Study. The participants were from 250 nuclear families, including 5 large pedigrees with 325 individuals, who were identified through an index case with moderately high femoral neck BMD (Z score $\geq$ 1.28). Complex segregation analysis, based on tests of hypotheses regarding the fit of Mendelian segregation ratios for BMD in families, were performed to test the major gene hypothesis.

After adjusting for age and body weight, genetic factors accounted for up to 72% of the total variation in BMD. The multifactorial (polygenic) model did not fit the data adequately (χ^2=26; df=8; p=0.003). However, the best fit model suggested the Mendelian transmission of a major gene locus with significant residual correlations among sibs (χ^2=4.9; df=7; p=0.67). This most parsimonious genetic model suggested that up to 42% of the genetic variance was attributable to the MG effect. Further analyses suggest the existence of residual polygenic effects when adjusting for sex, body weight and age.

These findings support the hypothesis that a large component of the variance of BMD is under genetic control, and that there exists a major gene locus influencing BMD. These results also provide evidence that study of large pedigrees identified via moderately high BMD probands may increase the probability of finding a major gene for BMD variation.

P23

DXA AFTER WRIST FRACTURE - IS IT COST EFFECTIVE?

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Wrist and forearm fracture is common (15%) in females over 50. Those with wrist fractures may have low bone density at the vertebrae and hip. To examine the cost of efficacy of DEXA scans in people over the age of 65 years with a wrist fracture the model assumed the cost of DXA was borne by the ACC and subsequent reduction in wrist, vertebral and hip fracture in 1 and 3 years resulted in savings to the ACC. The model includes 9 inputs to estimate the number of fractures that may be prevented by screening and the associated financial costs and benefits of the programme. The inputs include the number in this age group who could be screened and the associated annual fracture risk for this group. Additionally, the percentage screened who are identified as in a high-risk group (and therefore appropriate for a treatment intervention) and the associated risk ratio for this group relative to the remaining screened patients are used. 3 inputs relate to potential treatment. These include the percentage who are eligible for treatment; the percentage who are likely to be compliant with treatment and the associated annual proportional risk-reduction for those compliant with treatment. The two financial variables included in the model are the cost of screening an individual and the annual cost of treating a single fracture. ACC receives claims from over 21,000 people with wrist and forearm fractures. Only 20% of these occur over the age of 65. The number of upper/lower arm (F/D) and hand/wrist (H/W) fractures was 26% and 15% over 65 years of age respectively. No net benefit was observed at one year. Data adjusted for ongoing costs to the ACC of wrist, vertebral and hip fracture showed ACC funding for DEXA scans in this group started to achieve cost benefits at 4 years. After 10 years considerable cost benefits appear to be achieved.

P24

CALCITONIN DECREASES THE ADHERENCE AND SURVIVAL OF HEK-293 CELLS BY A CASPASE-INDEPENDENT MECHANISM

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We recently reported that CT can profoundly inhibit the growth of HEK-293 cells transfected with the hCTR. We also obtained preliminary evidence that suggested a role for CT in cell survival, which appeared to be dependent on the cell growth conditions. In the present study we have investigated the pro-apoptotic action of CT in conditions of low serum concentration.

We have found that CT treatment of HEK-293 cells stably transfected with the insert-negative form of the human CTR (HR12 cells) caused a time-dependent decrease in cell number associated with loss of cellular attachment. Loss of cellular adherence in CT-treated cultures caused programmed cell death (PCD) in serum deplete, but not serum replete, conditions. PCD was shown by condensation and cleavage of nuclear DNA, failure of cells to exclude trypan blue dye, annexin V staining, and appearance of hypodiploid cells and cell fragments in FACS analysis. The accumulation of non-adherent cells and cell death was concomitant with increased intracellular activity of caspase-3. However, inhibition of caspase activation in HR12 cells did not prevent CT-mediated loss of attachment and did not maintain the viability of non-adherent cells, indicating that caspase activation accompanied, but was probably not causal, of the loss of cell viability. The Erk1/2 inhibitor, PD 98059, inhibited the loss of cellular adherence and the consequent reduction in cell numbers.

These results demonstrate that CT can negatively affect cell survival and identify roles for cell adherence and MAPK activation in this process.

P25

REGIONAL DIFFERENCES IN VERTEBRAL BODY CORTICAL BONE.

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A study of the regional differences in vertebral body cortical bone was proposed. It is unclear whether the cortical bone of vertebral bodies changes in relation to the level of the vertebral column. Understanding any changes in the cortical bone may assist the prevention or treatment of vertebral crush fracture.

Histoquantitation of para-sagittal vertebral body slices of each vertebral body was used to assess the extent of cortical bone presence. Autopsy specimens totalling 27 lumbar spine T12-L5 (19 male, 8 female) were used. Specimens were analysed using a Quantimet Image Analyser with which measurements were taken of the ratio of bone volume (BV) to total volume (TV) of cortical bone along each margin of each vertebral body. Statistical analyses then allowed the effective cord length (mean chord length x BV/TV) and porosity (1-BV/TV) to be calculated. These data were then compared to ascertain regional differences in cortical bone.

The amount of cortical bone present in the vertebral bodies of weight-bearing functional units differs throughout the vertebral column. Superior vertebral bodies have greater effective chord lengths (T12 = 520μm±235) than inferior vertebral bodies (L5 = 342μm±190). Cortical bone also has increased porosity in the inferior vertebral bodies (T12 = 0.170±0.091 vs. L5 0.263±0.145). Regional differences within the vertebral bodies were also observed and will be discussed.

Analyses of cortical bone thickness in various regions of the weight-bearing vertebral column revealed distinct differences. These data may be of great importance for the assessment and treatment of vertebral crush fractures, as they suggest that certain regions of the vertebral column may be more susceptible to fracture.

P26

EXPRESSION OF FGF23 BY A TUMOUR AND BY CULTURED TUMOUR CELLS FROM AN ONCOGENIC OSTEOMALACIA PATIENT.

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Fibroblast Growth Factor 23 (FGF23), originally identified as the gene mutated in autosomal dominant hypophosphataemic rickets (1), is a candidate for the phosphate-wasting factor secreted by oncogenic osteomalacia (OOM) tumours. In this study, FGF23 was examined in a patient who presented with symptoms and signs typical of OOM.

FGF23, measured by commercial assay, was elevated in pre-operative serum from the patient at 209 RU/ml (normal range 68+/-SE8). Significant phosphate uptake inhibitory bioactivity was detected in the serum, as previously described in other OOM patients. (2). Following surgical excision of a giant cell tumour from the pelvis, the patient's symptoms resolved and serum phosphate and $1,25(OH)_2D$ (which peaked at 410 mmol/L post-operatively) normalised. *FGF23* mRNA expression was detected by RT-PCR in tumour tissue and in cells cultured from the tumour. Inhibition of renal phosphate uptake was detected on several occasions by bioassay using conditioned media from the tumour cells and was also demonstrated with media from cells transfected with FGF23.

These results are consistent with the proposal that FGF23 is involved in OOM and with previous reports of overexpression of FGF23 in other OOM tumours (3). The measurement of FGF23 in serum may be useful in screening patients for OOM and other phosphate wasting conditions.

1. ADHR Consortium 2000. Nat Genet 26:345-348.
2. Nelson et al. 2001. Europ J Endo 145:469-476.
3. White et al. 2001. J Clin Endo Metab. 86:497-500.

P27

EFFECTS OF ANTIRESORPTIVE TREATMENT THERAPIES ON BONE STRUCTURE IN THE RAT

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Antiresorptive treatment therapies exhibit marked efficacy for reducing risk of fracture, although a significant corresponding increase in BMD has not been demonstrated. The mechanisms by which this effect is achieved have not been clearly established. We have studied the effects of antiresorptive treatment therapies on bone remodelling in the rat. All rats were fed a 0.4% calcium diet and either Sham operated (SHAM) or ovariectomised (OVX) at 3 months of age. At 6 months, 42 female Sprague Dawley rats were either killed (SHAM and OVX) or assigned to one of 5 treatment groups including SHAM vehicle, OVX vehicle, oestradiol (E_2) (2.5 µg/kg BW/day), or calcium supplements (0.8% and 1.6%). Femora were processed and embedded in resin for measurement of static histomorphometric variables [epiphyseal trabecular bone volume (BV/TV), trabecular number (Tb.N), and trabecular thickness (Tb.Th)], bone formation rate (BFR) and osteoclast surface (Oc.S). Treatment of OVX with E_2 restored BV/TV to SHAM levels while calcium supplements demonstrated a positive dose effect with 1.6% achieving SHAM levels. Tb.N continued to decrease in vehicle-treated OVX, which was reversed by E_2 treatment, while only a marginal effect was obtained with calcium supplements. In contrast, 1.6% calcium markedly increased Tb.Th to levels above SHAM vehicle values but the effect of E_2 did not achieve statistical significance. Both treatments reduced Oc.S and BFR to equivalent levels. Both E2 and dietary calcium supplements reduce bone metabolism and improve trabecular bone volume. However, they appear to affect microarchitecture by different mechanisms.

P28

LACTOFERRIN: A NOVEL ANABOLIC FACTOR IN BONE- AN IN VITRO AND IN VIVO STUDY

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Lactoferrin is an iron-binding glycoprotein found in milk and other exocrine secretions in mammals and released from the granules of neutrophils during inflammation. Physiological effects of lactoferrin involve more than simple chelation of iron and include: antimicrobial activity, stimulatory and inhibitory effects on cell growth and differentiation, regulation of myelopoiesis, cytokine production and embryonic development. We focused our research on this multipotent molecule and demonstrated lactoferrin to be an anabolic factor in bone.

In vitro, lactoferrin stimulates the proliferation of primary fetal rat osteoblasts, osteoblast-like cell lines and primary ovine chondrocytes at concentrations above 0.1 µg/ml. DNA synthesis is also stimulated in murine neonatal calvarial organ culture, likely reflecting the proliferation of cells of the osteoblast lineage. Lactoferrin is also a potent osteoblast survival factor. In TUNEL and DNA fragmentation assays, lactoferrin decreased apoptosis induced by serum withdrawal by up to 70%. Lactoferrin activates MAP kinase signalling in osteoblasts in a time and dose-dependent manner. It also has powerful effects on osteoclastogenesis, decreasing osteoclast development at concentrations >1 µg/ml in a murine bone marrow culture system, but did not alter bone resorption in calvarial organ culture, suggesting that it does not influence mature osteoclast function. *In vivo,* local injection of lactoferrin above the calvariae in adult mice resulted in significant increase in bone area and dynamic histomorphometric indices of bone formation after dosing for 5 days.

These data demonstrate, for the first time, that the milk-derived lactoferrin is anabolic to bone *in vivo*, an effect that is consequent upon its potent proliferative and anti-apoptotic actions in osteoblasts, and its ability to inhibit osteoclastogenesis. We could therefore speculate that lactoferrin may have a physiological role in bone growth and healing, and a potential therapeutic role in osteoporosis.

P29

'WHO' GUIDELINES ARE INSENSITIVE: GEELONG OSTEOPOROSIS STUDY. KM Sanders[1], E Seeman[2], MJ Henry[1], MA Kotowicz[1], JA Pasco[1], GC Nicholson[1]. The University of Melbourne, [1]Dept Clinical and Biomedical Sciences, Barwon Health; [2]Dept Medicine, Austin and Repatriation Medical Centre; Victoria

This analysis aimed to identify women aged 50 years and over at high risk of fracture. We compared women with recent fracture resulting from a fall (n=432) with women randomly selected who had not fractured in the postmenopausal period (n=604).

Age-standardised prevalence of risk factors

Risk factor[1]	**% fracture (cases)**	**% non-fracture (controls)**
Osteoporosis (o/p: T score ≤ -2.5)	40	26
Osteopenia (T score < -1.0)	43	50
Z score: ≤ -0.5	67	44
Z score: ≤ -0.8	56	31
Z score: ≤ -1.0	38	20
Fallen in past year [2] 'faller'	33	18
'Faller' + o/p	13	6

[1] BMD at the spine and femoral neck. [2] Fallen in the past year apart from the fall that resulted in fracture for the cases.

Only 40% of women with fragility fractures have o/p suggesting that a diagnosis of o/p is an insensitive method of identifying women with fracture. A screening program based on this criterion would fail to identify 60% of cases having a fracture. While the combination of osteoporosis and 'faller' does identify a small high-risk group, most women who fractured did not have osteoporosis and had not recalled falling in the past year. Further analysis of the BMD with other information is needed to separate women with and without fracture. Such information could improve clinicians' ability to also target intervention to the greater proportion of women at higher risk of fracture but with a T score above the WHO cut-off for osteoporosis of –2.5.

P30

OSTEOPOROSIS AWARENESS IN GENERAL PRACTISE: ARE PATIENTS ADEQUATELY MANAGED?

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One in 2 woman and 1 in 3 men over 60 years of age living in Australia will sustain an osteoporotic fracture. Many of these fractures are often treated without the patient being investigated for osteoporosis. We performed a 12 month survey (Nov 2000 to Oct 2001) to determine the awareness of osteoporosis in our community.

Patients were recruited from 18 private radiology practices in the Southern Sydney area. Patients 45 years and older diagnosed with radiological evidence of a minimal trauma fracture were invited to participate. They were provided with (i) a questionairre, (ii) a stamped addressed return-envelope and (iii) an osteoporosis information card. Compliance by the busy practices was maintained by monthly contact.

Data was available on 175 women and 75 men, aged 45 to 86 yrs. Outcome assessment was possible in 161 (64%) subjects. In this group, there were 32 spinal, 116 upper limb, 55 lower limb and 24 rib fractures. BMD was requested in 73 (56%) women and 9 (30%) men. Osteoporosis of the lumbar spine and/or a femoral neck (defined as BMD t-score value < –2.5) was noted in 46 (63%) women and 5 (56%) men. All 46 (100%) women, but none of the 5 men with osteoporosis were receiving anti-osteoporotic treatments.

Our data suggests that osteoporosis awareness and fracture risk assessment remains a problem in general practice. Despite encouragement, active participation by the private radiology practices was limited. Furthermore, 49% of patients who sustained a fracture did not undergo BMD, despite being informed of their possible osteoporosis risk (via an information card). A public awareness campaign, highlighting the need for bone densitometry in men and women 45 years and older who sustain minimal trauma fractures in order to diagnose osteoporosis, is required.

P31

SECONDARY PREVENTION IN THE MANAGEMENT OF OSTEOPOROTIC FRACTURE

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Risk factors for osteoporotic fracture include low bone density, frequent falls and a previous fracture from minimal trauma. This study investigated whether patients with Colles fracture from minimal trauma were subsequently identified, assessed and treated for their elevated risk of subsequent fracture.

Medical records at Sir Charles Gairdner Hospital from August 1999 to July 2000 were audited and 111 patients who had sustained a Colles fracture from minimal trauma were identified. Questionnaires were posted to participants to determine whether any assessment or treatment was undertaken outside the hospital system.

From documentation in the records, 9% (10/111) had their bone mineral density (BMD) assessed, 15% (17/111) were receiving medical therapy for osteoporosis, 7% (8/111) had their falls risk assessed and 51% (58/111) were seen by a physiotherapist. Of the 58 who received physiotherapy, 76% (44/58) received upper limb exercises but only 19% (11/58) received lower limb or balance exercises.

Results from the questionnaire, sent 1-2 years after the fracture, showed 37%(18/48) had BMD assessed and 27% (13/48) were receiving medical therapy for osteoporosis. Thirty-five per cent (17/48) of patients recalled being advised to increase their calcium intake. Twenty-seven per cent (13/48) reported more than one fall in the past 12 months and of these 62% (8/13) had been seen by a physiotherapist. Forty-six per cent (6/13) reported having their balance assessed and 54% (7/13) reported having a home visit for assessment.

Our data support anecdotal impressions that many patients with Colles fracture are not receiving secondary prevention for osteoporotic fracture. Consequently they may remain at unnecessarily elevated risk for subsequent fracture.

P32

LOW CALCIUM ABSORPTION IN POSTMENOPAUSAL WOMEN IS ASSOCIATED WITH VERTEBRAL RATHER THAN PERIPHERAL FRACTURES

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Low calcium absorption in postmenopausal women with vertebral compression was first reported in 1973 [1] and has since been shown to respond to treatment with calcitriol [2]. There have been no studies of calcium absorption in patients with peripheral fractures.

We report a series of 549 untreated postmenopausal women whose investigations included the measurement of radiocalcium absorption (α) [3], visual assessment of radiographs of the lateral lumbar and thoracic breathing spine and a record of all peripheral fractures from minor trauma since the menopause. The principal data are shown in the Table. (The mean normal α is 0.75/hr (0.50-1.00)).

	No fracture	**Peripheral only**	**Spine only**	**Both**
Number of cases	157	86	141	165
Age (yrs) (SD)	62.5 (7.22)	63.9 (8.10)	66.4 (9.10)	68.7 (7.42)
Alpha (fx/hr) (SD) (age-corrected)	0.72 (0.31)	0.65 (0.31)	0.58 (0.31)[a]	0.59 (0.32)[a]

[a]P<0.001 *vs* no fracture

After adjustment for age, alpha was not significantly lower in peripheral fracture cases than no-fracture cases nor significantly lower in vertebral than in peripheral fracture cases but it was significantly lower in both spinal fracture groups than in the no-fracture group (P<0.001). There was no difference in alpha between the vertebral fracture groups with and without peripheral fractures.

1. Gallagher JC, et al. Clinical Endocrinology & Metabolism 1973;2:293-315.
2. Need AG, et al. Calcified Tissue International 1997;61:6-9.
3. Nordin BEC, et al. Journal of Nuclear Medicine 1998;39:108-113.

P33

AGE-DEPENDENT VARIATION IN GENETIC-ENVIRONMENTAL DETERMINANTS OF THE ASSOCIATION BETWEEN LEAN MASS AND BONE DENSITY.

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Cross-sectional associations between lean mass (LM) and bone mineral density (BMD) have been reported. The genetic and environmental basis of these associations remains controversial. We studied the extent to which genetic and environmental factors explained LM-BMD associations in female twins (760 monozygotic pairs, MZ; 770 dizygotic pairs, DZ) aged 8 to 89 years, divided into young (8-16), mid-range (17-44) and older (45-89 years) groups. Age- and age-height-adjusted lumbar spine, total hip and total body bone mineral measures were moderately/highly heritable in adult twins (Falconer estimate (h) = 0.50 – 0.83). Age-height-adjusted LM had an h = 0.60-0.61 in adult twins. Analysis of age- and age-height-adjusted cross-trait correlations (LM-BMD) within-individuals (WI) and within MZ and DZ pairs revealed that: In young twins, within-pair shared environment plus additive genetic(AG) factors (mostly related to height) explained a moderate/high LM-BMD correlation (WI r = 0.60-0.87). In the mid-range twins, AG factors (partly related to height) largely explained a moderate LM-BMD correlation (WI r = 0.43 – 0.76). In older twins, AG factors (not related to height) plus an effect of the twins' environment for the hip explained a moderate LM-BMD correlation (WI r = 0.42-0.76).

By studying female twins it was found that LM and BMD were moderately/highly correlated at all ages, AG factors were important determinants of the LM-BMD association, with strong evidence for a role of height-related genes in young twins. Environmental factors contributed to the LM-BMD association, in keeping with evidence that experimental interventions can increase both LM and BMD.

P34

DETERMINANTS OF FEAR OF FALLING AT 3 YEARS IN ELDERLY WOMEN: EFFECTS OF INCIDENT FRACTURE.

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We examined the relationship between baseline fear of falling (FoF) and incident fracture on future FoF, falls and restriction of activity in an elderly population. 1290 women were recruited from a population based sample of females ≥70 yrs who were followed prospectively for 3 years. Baseline mean age and weight were 75±3 years and 68±12kg respectively. Incident clinical fractures were determined from x-ray reports after the event. At 3 years 10% had sustained ≥1 incident fractures. Questionnaires relating to i) falling episodes ii) FoF, iii) household and outdoor activity restriction due to FoF, iv) walking aid use and vi) lack of physical activity were administered at baseline and 36 months.

Logistic regression analysis demonstrated that compared to the non-fracture group, those subjects who sustained an incident fracture were more likely at 36 months to report FoF (R.R 1.8: 95%CI 1.2-2.7) (P=0.004) and restrict outdoor activity (R.R 1.6: 95%CI 1.0-2.5) (P=0.042) after adjustment for age, weight, socioeconomic status (SES) and baseline FoF and outdoor activity respectively. Subjects with baseline FoF, were more likely at 36 months, to report a fall (R.R 2.3: 95%CI 1.6-3.1) (P=0.001), have FoF (R.R 9.4: 95%CI 7.0-12.7) (P=0.001), restrict household (R.R 2.2: 95%CI 1.6-3.1) (P=0.001), outdoor (R.R 3.6: 95%CI 2.5-5.0) (P=0.001) and physical activity (R.R 1.6: 95%CI 1.1-2.1) (P=0.006) and use a walking aid (R.R 2.5: 95%CI 1.6-3.9) (P=0.001) after adjustment for age, weight, SES and respective baseline co-variates.

These data suggest that fracture predicts future FoF and restriction of outdoor activity. However, FoF has a greater effect on functioning than fracture. Identification of FoF post fracture is important in the rehabilitation process.

P35

REFERENCE RANGE DATA FOR ADOLESCENT GIRLS.

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Bone density studies in paediatric populations are being recognised to be of clinical value particularly given the importance of bone mass accrual during childhood and adolescence to future fracture propensity. In order to undertake a study of bone density in adolescent females with chronic illness a local reference population (WA) was recruited and compared to both the available manufacturer's data and a large database developed in the USA (Bachrach et al, 1999). The local BMD data was evaluated using Hologic and USA reference data to calculate site specific Z-scores.

Normal females (n=111) aged 12 to 18 years were recruited from local Western Australian schools. BMD of the spine and hip was measured by pencil beam DXA (Hologic QDR 2000). Tanner stage was also determined. The mean BMD at each site was modelled using linear, quadratic or exponential regressions. The requirement for age-specific standard deviation (SD) was tested by regressing the absolute residuals from the fit against age (Altman, 1993). The best regression fit for the spine BMD was linear and for the hip sites was exponential. Comparison with other data sets is shown in the table.

Total spine: mean (SD) g/cm^2

Age (yrs)	***12***	***14***	***16***	***18***
WA	0.841 (0.120)	0.911 (0.120)	0.981 (0.120)	1.051 (0.120)
USA	0.816 (0.122)	0.973 (0.127)	1.023 (0.116)	1.041 (0.105)
Hologic	0.784 (0.094)	0.895 (0.103)	1.010 (0.110)	1.015 (0.110)

Total Hip: mean (SD) g/cm^2

WA	0.694 (0.090)	0.921 (0.090)	0.943 (0.090)	0.945 (0.090)
USA	0.803 (0.119)	0.924 (0.124)	0.953 (0.121)	0.958 (0.110)

Unlike the Hologic & USA fitted models, the SD for spine and hip BMD local reference range (RR) did not vary significantly with age. The difference between z-scores (WA vs Hologic) for subjects with low spine BMD differed between ± 0.4 when plotted against the average. Local spine BMD RR produced z-values that were significantly higher ($p<0.001$) than USA. In the development of paediatric RR for spine and hip BMD, it is crucial to use large datasets and sophisticated statistical modelling techniques.

P36

VALIDATING THE OSTEOPOROSIS SELF-ASSESSMENT TOOL (OST) IN NEW ZEALAND.

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Osteoporosis is an important public health concern in New Zealand. Currently no osteoporosis risk assessment tool is available for identifying osteoporotic women in this coountry. The Osteoporosis Self-Assessment Tool (OST), an index based on age and weight to identify osteoporotic women, was developed in Asia and validated in North American and European cohorts.

The present study assesses performance of the OST in identifying postmenopausal women at risk of osteoporosis (femoral neck BMD T ≤ -2.5) in New Zealand.

Performance of the OST was assessed using retrospective data on a sample of 1,351 postmenopausal women ages 50 and over in New Zealand. The index was calculated using the OST algorithm by subtracting patient's age from patient's weight and multiplying that by 0.2. Sensitivity and specificity were evaluated to assess performance of the index and determine an appropriate cut-off point for identifying osteoporotic women. A receiver operating characteristic (ROC) curve was generated, and the area under the curve (AUC) was inspected.

The mean (SD) T-score was –1.23 (1.12) while 11.7% of the sample were osteoporotic (femoral neck T-score ≤-2.5). The mean age and weight were 73.5 years (SD=4.3 years) and 66.6 kg (SD=11.4 kg) respectively. Using a cut-off point of –1 yielded a sensitivity of 84.9% and specificity of 43.4%. The ROC curve showed an AUC 0.74 for the OST index. Based on the index, three risk categories were identified: high risk patients (index <= -5, 47.8% were osteoporotic), moderate risk patients (-5<index<=-1, 12.7% were osteoporotic) and low risk patients (index>-1, 4.4% were osteoporotic).

The OST performed adequately in identifying the vast majority of osteoporotic patients in New Zealand.

P37

MEASUREMENT OF PHALANGEAL BONE DENSITY USING DIGITAL RADIOGRAPHIC ABSORPTIOMETRY.

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The recent National Osteoporosis Risk Assessment study [1], has further defined the important role of peripheral BMD measurements in fracture risk prediction. One of the most facile and rapid assessments of peripheral BMD is at the fingers using digital radiographic absorptiometry (RA), as no gel or preparation is necessary, and scan time is «1s. Moreover phalangeal bone density has been shown to be a significant predictor of future hip fracture risk [2]. In this study we evaluated the 'Alexa' instrument (Alara Inc), which measures BMD in the middle phalanges of the 2^{nd}, 3^{rd} and 4^{th} digits of the non-dominant hand, and reports the result as a mean BMD with the respective T- and Z-scores. Precision was assessed by twice daily measurements (n=20) for a 55 yr woman, over a 30 day span; at a mean BMD of 51.27 mg/cm^2, total variability was 0.576^2, with $S^2_{within\text{-}day}$ » $S^2_{between\text{-}day}$. The 3^{rd} digit was associated with the lowest variance (0.362^2) and the 2^{nd} with the highest (1.158^2). In 5.6% of all women (n=195) studied a single digit was excluded from the final result due to failure to identify the middle phalange. The prevalence of phalangeal osteoporosis was 21%, compared to 24.1 and 16.8% at the spine (L_2-L_4) and femur neck respectively (determined by DXA). The gamma index was used to measure the pair-wise association between finger, spine and hip BMDs, after converting T-scores into four categories – high, normal, low and very-low BMD. The G values ranged from 0.63 – 0.68, and there was no statistically significant difference between any of the sites. Digital RA therefore has a potential role, particularly in the primary care setting, for identifying women at high fracture risk.

Siris ES et al. JAMA 2001;286:2815-22
Mussolino ME et al. Arch Intern Med 1997;157:433-8

P38

HOW MANY CHILDREN REPORT NO BONE FRACTURES THROUGHOUT GROWTH? A STUDY OF 600 SUBJECTS STUDIED AT REGULAR INTERVALS BETWEEN BIRTH AND 18 YEARS OF AGE.

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Broken bones are common adverse events in childhood and adolescence. However, because comprehensive longitudinal information is hard to obtain it has not to our knowledge previously been established how many children experience single fractures, multiple fractures or no fractures throughout the growth period. Between l972 and 1973 a birth cohort of children was enrolled in the Dunedin Multidisciplinary Health and Development Study. We report the lifelong fracture history of 601 participating children (289 girls, 312 boys) who were seen at every phase of the study from age 5 to the usual age of attaining peak bone mass. Fracture histories were taken at 5, 7, 9, 11, 13, l5 and l8 years of age. In total 291 subjects reported 498 bone fractures, the peak periods for fracture occurring between 11-14 years of age. Although 76 (26.2%) of the girls and 96 (30.9%) of the boys sustained only a single fracture, 46 (15.9%) of the girls and 73 (23.4%) of the boys reported they had broken a bone on more than one occasion. Fractures of the wrist were the most common skeletal site affected (n=120 fractures) and a high proportion of children (22 (47.8%) girls and 39 (53.4%) boys) with multiple fractures had suffered one or more wrist fractures. However, in our study sample life-table analysis showed that 57.9% (95% CI 51.9-63.2 of the girls and 45.7% (95% CI 40.2-51.3) of the boys had reported no fractures throughout growth. (We thank Dr R Poulton for permission to use the Dunedin Multidisciplinary Health and Development Study data and the New Zealand Health Research Council for support)

P39

PARITY, LACTATION, HIP BONE MINERAL DENSITY AND GEOMETRY IN SRI LANKAN WOMEN

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Hip geometry, independent of bone mineral density (BMD) is a risk factor for hip fracture. Despite low BMD, Asian women have favorable hip geometry when compared with women from USA. This study examines the effect of age, parity and period of breast feeding on hip BMD and geometry in a cohort of Sri Lankan women.

235 women with mean age of 54.6 (10.8) years, referred for densitometry for clinical indications were analyzed. The number of pregnancies (mean 3.7± 2.1) including miscarriages of > 20 weeks of gestation and total period of breast feeding (period during which child was breast fed more than four times a day) were recorded (mean 5.5 ± 4.2 years). Non-dominant hip was scanned with Norland Eclipse XR machine. Femoral neck width (FNW) was measured at the narrowest neck. The neck shaft angle (NSA) was measured by software automatically. All analyses were corrected for age and weight and height.

FNBMD showed significant partial correlation with FNW ($r=-0.30$, $p<0.01$) and NSA ($r=-0.20$, $P<0.05$). Trochanteric BMD also correlated with FNW ($r=-0.27$, $p<0.01$) and NSA ($r=-0.16$, $p<0.05$). Parity and breast-feeding period showed no significant correlation with hip BMD, FNW or NSA. Age increase by 10 years was associated with expansion of FNW by 2mm ($p=0.012$), narrowing of NSA by 0.50 (0.063) and reduction of FN BMD and trocanteric BMD by 0.05gm/cm2 ($p<0.001$) and 0.03gm/cm2 ($p<0.001$). No significant difference in mean BMD at FN or trochanter and FNW was seen between parous ($n=200$) and nulliparous ($n=30$) women. Mean NSA of parous women was 2.38 degrees broader ($p=0.05$) than nulliparous women. No significant difference in BMD or indices of hip geometry was seen in four quartiles of period of breast-feeding.

Changes in hip geometry may compensate the age related bone loss seen at hip. Pregnancy and lactation had no clear effect on BMD or hip geometry. However study had only a limited power (16%) to determine this association.

P40

DETERMINANTS OF LONGITUDINAL CHANGE IN BONE MASS N ELDERLY WOMEN – A TWIN STUDY.

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Age-dependent changes in bone mineral may be influenced by body composition. In this follow-up study of older female twins we assessed the change in bone mass with increasing age and effects of changes in body composition on this loss.

Initially, 79 pairs of female twins over the age of 60 years were recruited during 1991-93; 103 subjects returned for at least 1 follow-up visit. Analysis was performed on the interval, between the first and last visit. The mean (3.9 to 6.9) interval was 5.75 years. The mean (SD) age at baseline and final were 67.3 (5.8) and 73.05 (5.0) years, respectively. The annualized change in: bone mass was expressed as the percentage change from baseline, annual change in body composition was expressed as the unit change/year (Hologic QDR 1000W). There was no significant change in lumbar spine (LS) BMD. There was a significant decrease in BMD of 0.5% per year at both the proximal femur (PF) and femoral neck. Total forearm decreased by 0.8% per year. There was a modest increase in fat of 0.15 kg per year. Mean total body bone mineral content (TB BMC) and lean mass were stable over the study period. Change in lean mass (kg/year) was associated with annual % change in BMD (Beta ± SE) of the LS (0.61 ± 0.30), PF (0.66 ± 0.28) and TB BMC (0.68 ± 0.27) all $p<0.05$. The annual change in fat mass was associated with the % change in TB BMC (0.87 ± 0.15).

This longitudinal study demonstrated a decrease in hip BMD which was associated with a change in lean mass. There was no evidence for a change in LS BMD over time. The change in fat was associated with the change in TB BMC.

P41

MONITORING THE RESPONSE TO ALENDRONATE IN THE PROXIMAL FEMUR.

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Osteoarthritis (OA) is common in the lumbar spine and often leads to spurious evaluation of Bone Mineral Density (BMD). Hence in patients with osteoporosis and lumbar spine (LS) OA, difficulty may occur in using the LS for monitoring response to therapy. We set out to determine which Proximal Femur (PF) site is optimal for monitoring change in LS BMD following therapy. We performed a retrospective study of patients who had undergone longitudinal DXA studies for monitoring the response to Alendronate therapy.

A total of 53 patients (4 males, 49 females) were included. All patients reported taking Alendronate (Fosamax) for a minimum of 2 years. BMD was measured by DXA (Lunar Expert) in the LS, total proximal femur (TPF), femoral neck (FN) and trochanter (Troc), both before commencement of Alendronate, and again after at least 2 years of treatment. Correlations between LS BMD % change and BMD % change in each of the PF sites were calculated in all 53 patients, and separately in 'responders', defined as patients who had an increase in LS BMD of at least 5% (n=32).

	All pts (n=53)	Responders (n=32)
LS change vs TPF change	r=0.52 (p<.0001)	r=0.45 (p=.0097)
LS change vs FN change	r=0.35 (p=.0092)	r=0.27 (p=.1329)
LS change vs Troc change	r=0.39 (p=.0035)	r=0.32 (p=.0747)

Conclusion: In the PF, change in BMD calculated from TPF measurements, demonstrate the best correlation to the change in LS BMD, following Alendronate therapy. Hence in patients with LS OA, the TPF may be the region of interest of preference for monitoring response to therapy.

P42

THE RELIABILITY OF DXA, AT THE INDIVIDUAL LEVEL, IN ASSESSING SIGNIFICANT BONE DENSITY CHANGE

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Cummings et al [1], after evaluating individual results for two large placebo-controlled clinical trials (alendronate and raloxifene), concluded that DXA BMD results, inconsistent with treatment expectations, should be discounted. In response, Bonnick [2] suggested that the latter conclusion misinterpreted the concept of "regression to the mean", and neglected to address requirements for a statistically significant change. However both these viewpoints fail to understand the basic tenets of analytical performance, and in the DXA case, the frequent incidence of "outliers" [3]. To further expand these claims we have reviewed findings for 80 subjects, who had 6 – 8 serial DXA BMD measurements, performed at the spine and hip, during a 4.2 – 7.7 year period. A mean decrease of approximately 50% in the standard error of the estimate (S_{yx}) about the trendline (BMD vs time) could be achieved by removing a single select data point. Moreover, S_{yx} associated with spine BMD determinations, was greater than that for the hip, highlighting that the variability is not simply an operator effect. Within the individual time series, there were clearly defined examples of "turning points" (peaks and troughs) where successive BMD changes were statistically significant but biologically implausible. Accordingly, relying on differences between follow-up BMD measurements to assess bone status has major limitations. Detection of a systematic trend within a patient's BMD series was also quite dependent on the indice used. The issues raised by this study, and previously by other authors, have to be addressed if DXA measurements are to be practically implemented at the individual level.

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P43

LIFESTYLE DETERMINANTS OF BONE MASS, SIZE, AND BENDING STRENGTH IN OLDER MEN. M Brown[1], R Daly[1], S Bass[1], C Nowson[1], E Briganti[2]. [1]School of Health Sciences, Deakin University, Melbourne; [2]Dept of Epidemiology and Preventive Medicine, Monash University, Melbourne, AUSTRALIA.

Osteoporosis is a major public health problem in men. However, little is known about the effects of lifestyle factors on bone fragility in older men. Bone fragility is determined by bone mass, size and the structural distribution of bone. We asked, what are the predictors of FN bone mass, size and distribution in older men? We measured FN BMC, BMD, CSA and CSMI (DXA, hip strength analysis) in middle aged and older men (n=144) aged 49-87 yrs (mean ± SD, 61.8 ± 7.6 yrs). Explanatory variables included age; height, weight, FM and LM; exercise, smoking, calcium, tea, coffee and alcohol intake; history of fx (45 yrs), hypertension, diabetes and asthma; use of medication and family history of osteoporosis. The initial regression model included explanatory variables with a p-value <0.20 on univariate analysis. The final model was determined by purposeful backward stepwise regression using the log likelihood ratio test, with a p-value <0.10 considered significant (Table). The total variance for FN bone traits explained by these models ranged from 17-34%.

β-coefficients	BMC g	BMD g/cm^2	CSA mm^2	CSMI mm^4
Weight, per kg	+0.050	+0.005	+1.5	-
Height, per cm	-	-	+0.6	+183.4
Fat mass, per kg	-0.040	-0.004	-1.3	-
Lean mass, per kg	-	-	-	+276.2
Hypertension	-0.409	-0.061	-16.5	-
Fx history	-	-0.056	-	-
Adjusted R^2	31.3%	16.7%	31.5%	34.3%

In summary, lean mass and height were significant predictors of bending strength, but not bone mass. In contrast, hypertension, fat mass and weight were significant predictors of BMC, BMD and CSA, but not bending strength. In conclusion, lean mass and height appear to be the most important determinants of bone fragility in older men.

P44

HIP FRACTURE PATIENTS: DO OSTEOPOROSIS MANAGEMENT PROTOCOLS AND AVAILABILITY OF BONE DENSITY MEASUREMENT CHANGE PRESCRIBING PRACTICE?

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Hip fracture patients are at high risk of subsequent fracture. Previous studies[(1)] showed poor investigations and treatment of these osteoporotic subjects. An investigation and treatment protocol was introduced into the orthogeriatric ward together with ultrasound and DEXA measurements available on site (also as an outpatient). Compliance with the protocol was measured in 193 patients retrospectively. Treatment advice to the primary care physician was assessed.

Blood tests were measured in 63% of patients and bone mineral density in 78% (136 ultrasound, 13 DEXA). Vitamin D levels were low in over 95% of patients and the T-score was less than -2.5 in 84% of patients and osteopenic range in 12%. Treatment advice was not adhered to in over 50% of patients. Treatment with Calcium supplements occurred in (7%), Calcium supplements and Vitamin D (23%) and both (69%). Fewer patients were on Bisphosphonates and/or Hormone Replacement Therapy. Although significant improvement with investigations and advice for osteoporosis treatment were achieved in the hospital environment, greater than 50% of patients received either no or inappropriate treatment from their primary care physicians. A cost benefit analysis needs to be undertaken to address the efficacy of such treatment and advice protocols.

(1) Wilkinson et al, NZMed J;114;329-31

P45

A CORE TEST BATTERY FOR THE PREDICTION OF FUTURE HIP FRACTURES IN WOMEN: REFINING THE ASSESSMENT OF BALANCE AND OTHER RELATED MEASURES.

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The maintenance of balance is essential for daily activities. Adequate assessment can be difficult as there is no single balance test that represents the complex nature of the balance system. Reduced balance performance in women over 65 years old is correlated with increased falls. Ninety-percent of hip fractures result from falls. Fracture risk, however, is determined by the interaction of several factors, including low bone mineral density, poor postural stability, physical inactivity, muscle weakness and falls. Assessment of balance, muscle strength and physical activity may therefore be important in the prevention of hip fractures.

A range of validated clinical and laboratory tests assessing gait, body sway, activity levels and muscle strength were performed on 236 subjects aged 46-82 years. A factor analysis of 24 measures from the Chattecx Balance System, stride analyser, Lord's balance test, step test, one-legged stance test and the Human Activity Profile (HAP) questionnaire, resulted in the extraction of 7 factors. This accounted for 71.6% of the variance in global balance. In reducing order of importance these factors were: 1) Muscle strength (ankle, knee, hip), 2) perturbation measures (stable and anterio-posterior),
3) Lord' balance test, 4) HAP, 5) one-legged stance test, 6) step test, 7) perturbation (medio-lateral). Session duration can be shortened from 90 to 45 minutes by data reduction methods involving a factor analysis. This could have significant clinical application, providing a core test battery for refining balance assessment for prediction of hip fracture risk and targeting of preventive interventions.

P46

BONE DENSITY AS A PREDICTOR OF OSTEOPOROTIC FRACTURE

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An important clinical role for DXA is in the detection of patients at risk of fracture. Using a case control design 515 healthy control women without fracture were matched for age to two fracture population 128 hip fracture patients and 178 patients with clinical spine fracture. The sensitivity and specificity for DXA at various hip and spine sites for the detection of fracture was calculated using ROC analysis and the theoretical proportions of the Australian female population between ages 50 and 89 that would be classified as osteoporotic on these criteria were calculated.

Overall there was no significant advantage at any bone density site in the sensitivity or specificity for hip or spine fracture detection. The bone density values corresponding to the T scores from various reference ranges including NHANESIII were evaluated for their sensitivity and specificity for the detection of hip and spine fracture. These analyses indicated that the method of calculation of the T scores and the nature of the reference populations employed in determining the reference range had large effects on sensitivity and specificity for detection of hip and spine fracture, and on the proportion of women in the population classified as osteoporotic. The use of particular T score values without consideration of the effects on the proportion of the fracture population detected and the proportion of the whole population classified as osteoporotic is unwise. In general depending on the reference population used to calculate the T score the value corresponding to T –2.0 appeared to produce increase detection of fracture subjects at the expense of classifying a larger proportion of the population as osteoporotic.

P47

ARE POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS BEING TREATED? S Korn, JA Pasco, MJ Henry, GC Nicholson, MA Kotowicz. The University of Melbourne, Department of Clinical and Biomedical Sciences, Barwon Health, Australia.

Postmenopausal women with low bone mineral density (BMD) and a history of fracture are at a high risk of subsequent fracture and should be treated. We aimed to assess whether women with high fracture risk are receiving appropriate management. The Geelong Osteoporosis Study recruited a random sample of women from the Barwon Statistical Division, 1994-7. All subjects received their baseline BMD report for the PA-spine. Follow-up data was documented for 592 postmenopausal women (median age=70yr, range 55 to 94, median time to follow up 2.05yr). Self-reported low-trauma adult fracture (20+yr), discussion of BMD results with a doctor and interventions were documented by questionnaire.

Among women who returned for follow-up, 24% (141/592) had osteoporosis on BMD criteria ie. T-score ≤-2.5, 68% (402/592) discussed results with their doctor and 30% (119/402) received treatment. Of those with osteoporosis, 93% (131/141) were previously unaware of the condition, with 24% (34/141) not seeking medical advice. Among women who sought medical advice, treatment was initiated for 69% (73/106) of women with osteoporosis, 23% (40/174) with osteopenia (T-score ≤-1 to <2.5), and 17% (10/59) with osteopenia and fracture. Women who were prescribed HRT/bisphosphonates ± calcium were younger than those prescribed calcium/calcitriol/vitamin D (median age 65, range 55 to 85 vs 71, range 56-94, p=0.008), and had similar T-scores (median -2.78, range –4.31 to 0.07 vs –2.62, range –4.87 to –0.09, p=0.6). Compared with osteoporotic women, osteopenic women with fracture were less likely to receive HRT/bisphosphonates ± Ca (OR 0.27 CI 0.05-1.37, p=0.1). OR (95% CI) for intervention are tabulated.

Group of interest	**Comparison**	**Intervention**	**P**
Osteopenia	Normal	4.90 (2.11-11.36)	<0.001
Osteopenia	Osteoporosis	0.15 (0.09-0.25)	<0.001
Osteopenia + fracture	Normal	2.93 (1.03-8.31)	0.043
Osteopenia + fracture	Osteoporosis	0.09 (0.04-0.21)	<0.001

Women with severe bone deficits are likely to receive medical intervention. Older women with low bone mass, or women with low trauma fracture and low bone mass, are at increased risk of fracture, but their treatment is, at best, conservative.

P48

PREVALENCE OF COELIAC DISEASE IN OSTEOPOROSIS

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Coeliac Disease (CD) is a relatively common disorder. A recent epidemiologic study in New Zealand has shown the prevalence of CD in the adult population to be 1.2%(1). CD is associated with low Bone Mineral Density (BMD) (2) and institution of a gluten free diet can improve BMD(3). In a Swedish study of 92 patients with idiopathic osteoporosis, CD was confirmed by intestinal biopsy in 3 patients, and routine screening for CD in the osteoporotic population was proposed (4). This notion was not supported by a Canadian study in which there was no evidence of increased prevalence of CD among asymptomatic patients with low BMD (5). We set out to establish the prevalence of undiagnosed CD in patients with low BMD attending an Osteoporosis Clinic in Sydney.

138 consecutive patients with low BMD, defined as a Lumbar Spine or Femoral neck T score <-1.0 were screened with Antigliadin (AGA) and Anti-Endomysial (EMA) Antibodies. 27 patients had positive IgA and/or IgG AGA but negative EMA. 8 have had small bowel biopsy to date, and all have been negative. 1 patient with positive EMA has biopsy proven CD. This suggests a prevalence of undiagnosed CD of at least 0.7% in adults with low BMD. EMA is more specific than AGA and is probably the screening test of choice in the osteoporotic population.

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P49

LIMITED UTILITY OF CLINICAL RISK INDICES IN THE IDENTIFICATION OF POSTMENOPAUSAL WOMEN WITH FRACTURE

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Bone mineral density (BMD) is the primary predictor of fracture, and is utilised in the definition of osteoporosis. Mass screening for osteoporosis is however currently not recommended. The primary objective of this study was to develop, validate and assess a simple, non-invasive scoring system to identify women at high risk of fracture.

Using the baseline data of the Dubbo Osteoporosis Epidemiology Study, a sample of 1256 women aged 60 or above was randomly divided into a development cohort (n = 846) and a validation cohort (n = 410). Low BMD (measured by DXA (LUNAR Corp, Madison, WI) was defined as a T-score of 2.0 or 2.5 standard deviations below the mean for young normal women) at either the femoral neck or lumbar spine. Incident fractures were identified by X-ray records. Logistic regression model was used to derive the DOEScore in the development cohort, and the performance of this score was then assessed in the validation cohort.

Approximately 57% and 40% of women (in both cohorts) had T-scores <-2.0 and T scores <-2.5, respectively. Age, body weight, and a history of fracture were significantly related to BMD at both the femoral neck and lumbar spine; and the three variables were used in the development of the DOEScore. When applied to the validation cohort, the sensitivity and specificity of DOEScore were 0.82 and 0.52, respectively for selecting women with T scores < -2.5; the area under the receiver operating characteristic curve (ROC) was 0.75. These goodness-of-fit indices were comparable to, or better than, those obtained by the FOSTA, SOFSURF and ORAI score systems. However, when the DOEScore was applied to identify women with incident fractures, the sensitivity and specificity were 0.52 and 0.49, respectively, with an area under the ROC curve of 0.48. Clinical risk scores are sensitive in the identification of women likely to have low BMD albeit with low specificity, but they can not identify women who will have a fracture.

P50

EFFECTS OF LIFESTYLE ON CHANGES IN BONE MINERAL DENSITY IN YOUNG WOMEN.

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The purpose of this study was to examine relationships between changes in BMD (Hologic QDR 1000w) during the young adult years and childbearing, habitual physical activity (PA) and dietary calcium intake (Ca), ascertained by questionnaire. Sixty-two women, of mean (SD) age 27.8 (1.0) years, were reassessed 9.4 (0.9) years after participating in a 2-year calcium supplementation trial.

Calcium supplementation for 2 years during adolescence had no effect on BMD of these young adult women (p>0.35). Mean (SD) annualised percent changes in BMD were +0.36 (0.60, p<0.001), -0.40 (0.62, p<0.001), -0.34 (0.60, p<0.001), +0.122 (0.58, p=0.1) and –0.06 (0.54, p=0.3) for the L1-4, neck of femur (neck), trochanter (troch), intertrochanter (inter) and total hip (hip) sites respectively. Lifestyle predictors (childbearing (yes=8), Ca and PA) of change in BMD at each site were determined using stepwise regression analysis after correction for the covariates baseline BMD and original intervention group entered first as a block. See table for standardised beta coefficients for the resultant equations for each site.

	Block entered		Entered stepwise		
	Baseline BMD	**Group**	***Childbearing***	***Ca***	***PA***
L1-4	-0.120	0.034	-0.400 *	-0.268	**0.156*
Neck	***-0.294 ****	***0.131***	***-0.262 ****	**-0.181**	**0.209**
Troch	-0.308 *	0.085	-0.308 *	*-0.208*	*0.196*
Inter	***-0.297 ****	***0.076***	***-0.263 ****	**-0.184**	***0.265 ****
Hip	**-0.335 ***	**0.080**	**-0.269 ***	**-0.200**	**0.256 ***

** p 0.05 and Italics indicated value not accepted in model.*

Physical activity had a small but significant favourable effect at two hip sites, whereas, dietary calcium had a negative effect on the increase in BMD at the spine. Childbearing had unfavourable effects on changes in BMD at all sites.

P51

HORMONE REPLACEMENT THERAPY AND SOFT TISSUE COMPOSITION INTERACTIVELY INFLUENCE BONE MINERAL DENSITY IN FEMALE TWINS.

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Hormone replacement therapy (HRT) significantly, though variably increases bone mineral density (BMD) in postmenopausal women. In studies of female twins, within-pair differences in BMD were associated with fat mass (FM) and lean mass (LM) with the possibility of soft tissue composition influence on the HRT response. Therefore, we sought potential interactive effects of HRT use and soft tissue composition as determinants of BMD.

Lumbar spine (LS) BMD, total and regional FM and LM were measured by dual-energy Xray absorptiometry in 166 pairs of healthy female twins aged 30-65 years. 39 pairs (Group 1) were discordant for HRT use and 127 pairs (Group 2) were concordant for use or non-use of HRT. Multivariate linear regression with the within-pair differences in that abdominal FM, total FM, total LM and height as predictors, indicated the within-pair difference in abdominal FM was the strongest predictor of within-pair difference in LS BMD (independently of within-pair difference in FM in Group 1), where it explained 28% of the BMD difference. Within-pair difference (SE) in LS BMD increased 16.4(7.9)% ($P = 0.046$) per kg abdominal fat in Group 1, in contrast to 0.45(3.1)% ($P = 0.886$) in Group 2. There was no within-pair difference in FM or LM in either group.

These findings point to an interactive effect of soft tissue composition on BMD with HRT use, and need to be further analysed using the Maximum Likelihood Random Effect regression model, which takes into account the possible random effects that may influence the within-pair difference in twins.

P52

FOREARM BONE SIZE AND WRIST FRACTURES: GEELONG OSTEOPOROSIS STUDY.

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Smaller vertebral volume has been associated with spine fracture[1] whereas wider femoral neck dimensions has been associated with fracture at the hip[2]. We have investigated the relationship between bone size and fracture at the wrist (ICD-9 code 813.4).

Women sustaining low trauma wrist fractures of the dominant arm were recruited from an area surrounding Geelong (n=56, aged 37-87yr). A non-fracture group (n=842, aged 37-91yr) was established from the same region. BMD was measured at the ultradistal forearm on the non-dominant side using a Lunar DPX-L densitometer with median time of measurement 68 days post fracture. Width of wrist was the average measurement from the scan of both the radius and ulna. Non-fallers reported never or rarely falling over the past 12 months whereas fallers had few, several or regular falls.

After adjusting for age, women with wrist fractures were taller (161.4 ± 0.8cm vs 159.8 ± 0.2cm, $p = 0.06$) and had wider wrists ($3.71\text{cm} \pm 0.04$ vs $3.65\text{cm} \pm 0.01$, $p=0.20$) but there was no significant difference in weight ($p = 0.63$). Height and wrist size were weakly correlated ($r=0.3$) and the relationship between wrist size and fracture was weaker after adjusting for height ($p = 0.63$). In those with wrist fractures BMC was 14% lower (1.414 ± 0.050g vs 1.648 ± 0.013g, $p=0.00$) after adjusting for width of wrist. The OR for fracture associated with falls was 1.7 (95%CI 0.9-3.2, $p=0.14$); and after adjusting for BMC and wrist width 1.5 (0.8-2.8, $p=0.27$).

These data suggest that a larger skeleton, lower bone mineral density and a tendency to fall, all predispose to wrist fracture.

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P53

SELF-REPORTED TOOTH LOSS AND BONE MINERAL DENSITY AMONG ASIAN WOMEN

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Previous studies have shown an association between low bone mineral density (BMD) and tooth loss and these studies have been done in European countries. We examined for the same association in a group of postmenopausal women in Sri Lanka, where social habits, malnutrition and chronic infections contribute to poor oral hygiene.

52 postmenopausal women with mean age of 59 (±7.7) years, referred for densitometry for the suspicion of osteoporosis were included in the analysis. They were free of other bone active diseases and medications. The number of teeth lost or removed after menopause was recorded. BMD of the lumbar spine (L2-L4) and non-dominant hip was determined using the Norland Eclipse XR machine. Partial correlations between tooth loss and BMD (controlling age), and comparison of mean BMD of four quartiles of tooth loss were done.

Tooth lost had a median of 3 with IQR of 0 to 8. No correlation was found between tooth loss and BMD of spine ($r=0.005$, $p=0.97$), femoral neck ($r=0.20$, $p=0.21$) or trochanteric area($r=0.05$, $p=0.74$). In quartile analysis, mean BMD at spine, femoral neck and trochanter showed a gradual decline with highest mean BMD among women with less tooth loss. In the highest quartile, mean BMD at spine, femoral neck and trochanter were 8.9%, 1.9% and 4.5% lower respectively, than those of the lowest quartile and these differences were not statistically significant.

Although lower BMD was found among women who lost more teeth in comparison to women who lost fewer teeth, no significant association was found between tooth loss and BMD in this cohort of women. Poor oral hygiene due to social habits, chronic periodontal diseases and nutritional deficiencies, which are more common in developing countries, may have disturbed the association seen in other studies.

P54

COST-EFFECTIVENESS OF ALENDRONATE FOR THE TREATMENT OF PATIENTS WITH LOW BMD AND NO PRIOR FRACTURE IN AUSTRALIA.

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Bisphosphonate use, including alendronate, on the Australian Pharmaceutical Benefits Scheme is currently restricted and not available for patients with low BMD without prior fracture.

This study undertakes an economic evaluation of the use of alendronate as add-on therapy to standard management (vitamin D and/or calcium) for patients aged 70 years of age with low BMD (< -2.5 SD from mean) with no prior fracture in Australia.

A Markov model was used to depict patients progression from having low BMD and no prior fracture to a series of health states based on incidence of hip and vertebral fractures to death over a 30-year period. The model conservatively assumed a cumulative incidence of 2 hip and/or vertebral fractures per patient. Direct medical costs were based on Australian survey data. Outcomes (fractures avoided, life years gained, QALYs gained) were based on the Fracture Intervention Trial and Australian and international estimates of population fracture incidence, fracture-related and all-cause mortality and utility scores. Costs and outcomes were discounted at 5% annually.

Average cost per patient for alendronate plus standard management and the standard management alone were AUD$8,500 and AUD$6,484 respectively. The average gain in survival was 0.146 QALYs with alendroante. Incremental cost per QALY gained with alendronate was AUD$13,808. Results were sensitive to estimates of alendronate effectiveness, assumptions on the impact of an initial fracture on subsequent fractures and fracture-related mortality.

The use of alendronate as add-on therapy to standard management for patients with low BMD without prior fracture is cost-effective.

P55

COMPARISON OF LUNAR DPX-L AND PRODIGY DUAL X-RAY ABSORPTIOMETRY MACHINES.

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Repeat BMD measurements are currently being performed on patients attending osteoporosis clinics. The impact of a change in machine, to the ability to interpret the change in BMD results requires clarification. We studied the ability of two different LUNAR densitometers (DPX-L and Prodigy) to reproduce BMD estimates. A Hologic spine phantom was scanned 20 times on each machine. The average BMD for the DPX-L was 1.1904gm/cm^2 and the Prodigy was 1.1702gm/cm^2. The Co-efficient of variance for each machine was 0.31% and 0.26%, respectively. Fifty patients lumber spine (L2-4) and femoral neck BMD was assessed on both machines on the same day. The mean difference between the two machines for L2-4 was 2.19% with a range of -8.16% to 11.57%. The mean difference for the femoral neck was 4.85% with a range of –6.01% to 34.94%. This shows the BMD differences between the two machines varied considerably in both the spine and hip measurements for each patient, with BMD measurements on the Prodigy lower than the DPX-L. We found that the reproducibility of lumbar spine and femoral neck bone density data between the two machines was quite variable and could not be reliably used for assessing treatment response. It is suggested that at the time of transfer to a new densitometer, patients will need to be evaluated on both machines simultaneously to re-establish their baseline BMD for any future comparison.

P56

THE RELATIONSHIP BETWEEN BODY COMPOSITION AND BONE MASS.IN FEMALES ADOLESCENTS WITH ANOREXIC NERVOSA

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Anorexia nervosa is a complex psychological disorder characterised by a relentless pursuit of thinness and frequently severe medical complications. The aim was to determine body composition and bone mass in 66 young female adolescents with anorexia nervosa (AN) + eating disorder not otherwise specified (EDNOS) with restricting subtype). Subjects underwent whole body DEXA scan and anthropometric assessment. Body mass index (wt/ht^2) (AN 14.9±1.4; EDNOS 17.0±2.1; $p< 0.001$), lean (AN 33.3±4.8 kg; EDNOS 36.0±3.3 kg; $p<0.05$) and fat mass (AN 6.6±2.5 kg; EDNOS 8.7±3.7 kg; $p<0.01$) were lower for AN compared with EDNOS ($p<0.05$). There was no difference in the age, height or duration of dieting but there was, a difference in duration of amenorrhoea. (AN 11.3±11.6 m; EDNOS 5.4±6.1; $p<0.05$). Regional or whole body BMD did not differ between the AN and EDNOS group, except at the pelvic region which was lower for the AN group AN 1.04±0.10, EDNOS 1.07±0.07; $p<0.05$), suggesting a site specific effect of bone loss. The effect of BMI, total fat, bone-free lean, age, Tanner Stage, duration of dieting and duration of amenorrhoea on body composition was determined using stepwise multiple regression. Bone-free lean mass and duration of amenorrhoea were the only significant predictors of whole body BMD (r^2=0.44 SEE 0.07) and pelvic BMD (r^2=0.41 SEE 0.11). As lean tissue mass decreased and duration of amenorrheoa increased, there was a reduction in bone mass. This suggests that other variables not determined, such as hormonal levels, physical activity and dietary intake may further explain the variability of BMD in young adolescents with anorexia nervosa.

P57

FEMORAL NECK GEOMETRY AND HIP FRACTURE RISK: GEELONG OSTEOPOROSIS STUDY.

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To determine the relationship between femoral neck geometry and risk of hip fracture, we compared the femoral neck dimensions of 62 women with a hip fracture to those of 608 controls.

Measurements were made from DEXA scans and included: femoral neck width (FNW), femoral neck axis length (FNAL) and femoral shaft width (FSW). Baseline characteristics were: median age 79 y (64-92) and 73 y (60-94), median weight 59.9 kg (41.3-90.0) and 64.5 kg (35.2-117.2), mean height 157.4 ±0.8 cm and 156.8±0.3 cm respectively for the fracture and non-fracture groups. The results are in mm as mean±SE:

	AGE ADJUSTED		BMC ADJUSTED	
	Fracture	Non-fracture	Fracture	Non-fracture
FNW	31.8±0.3	30.3±0.1*	32.3±0.3	30.3±0.1*
FNAL	97.3±0.8	96.0±0.3^	97.9±0.8	95.9±0.3**
FSW	31.8±0.3	30.4±0.1*	32.2±0.3	30.3±0.1*

*p<0.001;*p<0.05;^p≤0.10;BMC-bone mineral content

After adjusting for age or BMC, the FNW (+4.7-6%), FNAL (+1.3-2%) and FSW (+4.4-5.9%) were higher in the fracture group. The pattern for bone widths (but not FNAL) persisted after adjusting for height. We conclude that women with hip fractures have wider femoral necks and shafts than controls. There is also a trend towards an increase in their femoral neck length. Alteration in hip geometry is associated with risk of hip fracture.

P58

THE EFFECT OF VARYING TISSUE COMPOSITION ON BMD MEASUREMENTS

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DEXA systems rely on soft tissue adjacent to the bone for soft tissue calculation and asymmetry of fat or air may lead to errors. This study examined the effect of varying the amount and position of fat and air on BMD measurements in a phantom.

A LUNAR aluminium spine phantom was scanned, using 1cm perspex plates, fat slabs or air at varying thicknesses to change the composition of the phantom. The total tissue depth was maintained at 15cm. Five different configurations of tissue (uniform, superior, inferior, lateral & medio-lateral) were used, with varying amounts of air and fat in each. Three scans in each configuration were performed on a LUNAR DPX-L (pencil beam) and a LUNAR Expert XL (fan beam) in standard lumbar spine modes.

The mean BMD, SD and CV for L2-L4 were calculated for each scan set. Mean CV for all configurations was better than 0.5% for both scanners. With uniform tissue distribution, the Expert variation from expected BMD demonstrated a significant, but irregular change ($p<0.05$) in BMD as fat and air increased. The superior and medio-lateral air/fat configuration demonstrated the smallest change from reference BMD ($p<0.05$). The DPX-L showed a steady increase in BMD with increase fat and air in uniform distribution ($p<0.01$). The greatest effect was seen with the lateral configuration with large amounts of fat and air.

The distribution of fat and air in the tissue anterior to the site of measurement may contribute to an erroneous BMD.

P59

EFFECT OF VARYING TISSUE COMPOSITION IN A PHANTOM ON LUMBAR SPINE BMD MEASUREMENTS: A COMPARISON BETWEEN TWO PENCIL BEAM DENSITOMETERS.

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In the estimation of BMD, DEXA systems extrapolate soft tissue measurements adjacent to the bone for correction values. The presence and asymmetry of fat or air in this tissue may lead to errors. The aim was to examine the effect on measured BMD caused by varying the amount and position of fat and air in a phantom model, using two pencil beam densitometers.

The phantom model uses an aluminium spine phantom, perspex plates (tissue equivalent), fat plates and air, with constant total tissue depth of 15cm. The fat and air were positioned: uniformly, superiorly, inferiorly, laterally and medio-laterally in varying depths. Three scans of each configuration were performed with a Lunar DPX-L and a Hologic QDR-1000 densitometer, in standard lumbar spine modes.

The mean BMD, SD and CV were calculated for each set. Mean CV was better than 0.4% for both scanners. The DPX-L demonstrated an increase in BMD proportional to the increase in fat and air in the uniform distribution ($p<0.01$). The lateral configuration caused the greatest changes, whilst the superior configuration caused the least changes ($p>0.05$). The QDR-1000 also showed changes caused by changes in the uniform configuration ($p<0.05$), however the lateral configuration produced the greatest changes ($p<0.011$).

The distribution of fat and air in a phantom model has been shown to change measured BMD. These are most significant with asymmetric distribution of fat and air.

P60

COMBINED USE OF HORMONE REPLACEMENT THERAPY AND CALCITONIN IN POSTMENOPAUSAL WOMEN WITH BONE MASS LOSS.

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Statement of purpose: determining if the combined use of hormone replacement therapy and calcitonin influences on bone mass loss in postmenopausal women.

Statement of method: we studied for 18 months 23 women who were 47 to 62 years old at base line, were within 3 and 10 years of menopause, and had a bone mineral density at the lumbar spine between 150 mg/cc and 50 mg/cc measured by the QBMAP system with a spiral CT Picker PQ-S densitometer at L2, L3, L4 and L5. Of all the women, 10 were assigned to transdermal therapy with 50 μg/day of 17β-oestradiol on a intermitent cyclic regimen (28 out of 35 days) combined with 100 mg/day of micronized progesterone, and 13 were treated with the same hormone replacement therapy plus 200 UI of intranasal calcitonin. The SPSS programme was used for statiscal analysis.

Summary of results: The characteristics of the women recruited for both groups were similar. Mean mineral bone density at the lumbar spine was between 1 and 3 DS below the mean value for 30 years old normal premenopausal women. After a treatment of one and a half years statistically significant difference was found among the groups with and without calcitonin as for the bone mineral density at the lumbar spine.

Conclusions: it is necessary to carry out a wider study but it seems that the 200 UI calcitonin contribute advantages when it is combined with hormone replacement therapy to decrease the bone mass loss in postmenopausal women at least at lumbar spine.

P61

THE OSTEODYSTROPHY OF MUCOLIPIDOSIS TYPE 3

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Mucolipidosis type 3 (ML3, MIM 252600) is a lysosomal storage disorder arising from deficiency of the enzyme N-acetylglycosaminyl-1-phosphotransferase. Its function is to add the mannose-6-phosphate (M6P) recognition marker to other enzymes, permitting their intracellular trafficking to lysosomes. In the absence of the marker enzymes 'leak' out of the cell from the cytoplasm, whilst intracellular lysosomal enzyme activity is reduced. The accumulation of unprocessed macromolecules results in tissue damage. Skeletal complications are a prominent feature of ML3. We have investigated two siblings with ML3 (age 19 and 15) who had chronic pain and loss of mobility from severe destructive lesions of the hips and spine. Radiographs demonstrated marked erosive destruction of the upper femora and vertebral bodies. Biochemical markers suggested that bone turnover was accelerated. Bone biopsies showed intense subperiosteal osteoclastic bone resorption (affecting 100% of this surface) whereas trabecular resorption was not increased. The increase in subperiosteal osteoclastic activity is paradoxical, given that many osteoclastic enzymes require the M6P marker. In view of these findings, and the efficacy of bisphosphonate treatment in ameliorating skeletal complications in another lysosomal storage disorder, Gaucher disease, treatment with intravenous pamidronate (1mg/kg/month) was tried. Clinically, the treatment produced dramatic effects in reducing bone pain and improved mobility, but biochemical, radiographic and histological data suggested that the pathological bone resorption was not completely suppressed after one year's treatment. Intravenous bisphosphonates offer useful clinical benefit to patients with ML3, but higher than conventional doses might be needed to arrest the destruction of bone.

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BONE MINERAL DENSITY AND FRACTURE EFFECTS OF THE TGFß T869 AND CYP19 TTTA VNTR POLYMORPHISMS PREDICT BONE DENSITY AND FRACTURE. Dick, IM Devine, A Dhaliwal,SS, Li, S, Prince, RL Departments of Medicine, University of WA, Endocrinology and Diabetes, Sir Charles Gairdner Hospital and the Western Australian Institute for Medical Research

Osteoporosis is a disease that is strongly genetically determined and polymorphisms present in a range of candidate genes may be involved. In a population based study of 1337 caucasian women over age 70 we examined the genetic polymorphisms TGFß T869 in codon 10 and CYP19 TTTA VNTR in intron 4. The TGF β C allele was observed in 50% of the subjects and was associated with reduced hip BMD at all sites (2.8% total hip, 2.4% femoral neck, 2.6% intertrochanter, 3.4% trochanter) compared to the TGF β TT genotype. The TGF β C allele was also associated with a reduction in the calcaneal quantitative ultrasound (QUS) parameters BUA, SOS and stiffness of 0.87%, 0.26% and 2.4% compared to the TGF β TT genotype. A Cyp19 (TTTA)n > 12 was observed in 63.1% of the subjects and was associated with reduced hip BMD (1.9% total hip, 1.7% femoral neck, 2.0% intertrochanter, 2.3% trochanter) but was not associated with a reduction in calcaneal quantitative ultrasound . After adjustment for weight in an ANOVA model, the effect of the The TGF β C allele was no longer significant except at the trochanter but the QUS parameters remained unchanged. The Cyp19 (TTTA)n > 12 effect on BMD remained significant after adjustment for weight.

A TGF β C allele was associated with an increase in osteoporosis (T score < 2.5 S.D. OR 2.18 95% CI 1.21-3.91) and prevalent fracture (1.37 95% CI 1.02-1.85). Despite its effect on hip BMD a Cyp19 (TTTA)n > 12 was not associated with osteoporosis or fracture. After adjustment for BMD and QUS stiffness, the association of the TGF β C allele with prevalent fracture was no longer statistically significant (O.R. 1.42, 95% C.I. 0.99-2.0). This indicates that the effect of the C allele on fracture was through the association between a reduction in BMD and QUS stiffness with fracture.

P63

ROLE OF THE AROMATASE GENE (CYP19) IN MALE BONE MINERAL DENSITY DETERMINATION.

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The importance of the aromatase enzyme in bone physiology has been revealed in recent years by the study of men with aromatase deficiency who have osteoporosis. The individuals grow taller due to failure of epiphyseal closure, and have reduced bone mineral density (BMD). We have recently shown that polymorphism in the aromatase gene *(CYP19*) is associated with adult male height[1], resulting in a 2-3 cm difference in height.

In this study, we have performed a preliminary analysis of the role of *CYP19* in BMD to determine whether the *CYP19* polymorphism previously associated with adult male height is also associated with BMD. We grouped 61 healthy adult males with known bone mineral densities by genotype of a single nucleotide polymorphism (SNP) in exon 3 of *CYP19.* Two alleles were detected, termed 'A' and 'B' . Differences in BMD between genotype groups were tested for using ANOVA.

Under a model of a dominant effect of allele 'A', the *CYP19* SNP was associated with Ward's BMD [Genotype 'AA': 0.669 g/cm^2, SD: 0.148 (n = 21) vs genotypes 'AB' and 'BB': 0.741 g/cm^2, SD:0.127 (n = 40), P = 0.05]. A similar (but not yet statistically significant) pattern was also observed for femoral neck BMD [Genotype 'AA': 0.873 g/cm^2, SD:0.092 (n = 21) vs genotypes 'AB' and 'BB': 0.913 g/cm^2, SD:0.082 (n = 40), P = 0.09].

Variation in the gene encoding aromatase may be involved in determining BMD in normal adult males. Studies in larger populations are now required to confirm these results.

[1] JA Ellis, M Stebbing, SB Harrap. 2001. J Clin Endocrinol Metab 86:4147-4150.

P64

ANDROGEN RECEPTOR CAG REPEAT POLYMORPHISM AND BONE MASS IN FEMALES AGED 8-17 YEARS.

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Studies suggest that androgens may have a regulatory role in bone metabolism, potentially via the androgen receptor on the osteoblast. Exon A of the androgen receptor contains a polymorphic CAG triplet repeat, with a normal range of 11 – 32 repeats. CAG triplet number may influence androgen action. The aim of this investigation was to determine whether there was a difference in bone mass in healthy young females related to average number of CAG repeats.

Cross-sectional data were obtained from 190 females with a mean (SD) age of 12.7 (2.2) years. The CAG repeat number was determined by PCR amplification of genomic DNA; the product bands were sized on a 6% polyacrylamide gel. Data were stratified into four groups by percentiles of an individual's average number of allelic CAG repeats. The average (SEM) number of CAG repeats were; group 1: 19.2 (0.014), group 2: 20.9 (0.04), group 3: 22.2 (0.04) and group 4: 24.8 (0.22) ($P < 0.01$ compared to group 1). There was no difference in weight, height, total lean and fat mass between the groups. There was a significant age difference in the groups 3 and 4 compared to group 1. Hip BMD was lower in groups 3 and 4 (both 0.83 (0.02) compared to group 1 (0.90 (0.02), $P < 0.05$. There was no difference in lumbar spine BMD or total body BMC between group with increasing number of CAG repeats.

After adjustments for age in relation to menarche, for height and for body composition (total fat or lean mass), there was no difference in BMD or BMC between the groups. From this cross-sectional analysis of healthy young females there was no difference in BMD with increasing number of CAG repeats after adjustment was made for age and soft tissue composition.

P65

XBAI POLYMORPHISMS OF THE ESTROGEN RECEPTOR GENE AND BONE MINERAL DENSITY AND BMI IN BEIJING HAN WOMEN .

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Objective: To study the relationship between XbaI polymorphisms of the estrogen receptor (ER) gene and bone mineral density in Beijing Han Women.

Methods: Bone mineral density (BMD) was measured at lumbar spine, proximal femur and arm by dual energy X-ray absorptiometry (DEXA) and ER gene was determined by polymerase chain reaction - restriction fragment length polymorphism (PCR - RFLP) in 179 Beijing Han Women. Results: The frequency distribution of the ER genotype was 0.302, 0.464 and 0.234 in Beijing Han women. There was a significant difference between premenopausal and postmenopausal women in frequency of the XbaI genotype. The BMD of lumbar spine in postmenopausal women (0.836 +/- 0.18g/cm2) was significant lower than that in premenopausal women (1.038 +/- 0.14g/cm2) ($P<0.01$) and the incidence of osteoporosis in postmenopausal women was 54.3 % .

Conclusion: Frequency of XbaI genotype of ER gene in Han women was apparently different from that in other races and menopause effected the frequency of XbaI genotype. The XbaI genotype of ER gene was not associated with BMD. However the weight and BMI of xx genotype was significantly higher than that of XX and Xx genotypes in Beijing Han women.

P66

MARKERS OF BONE TURNOVER ARE NOT PREDICTIVE OF BONE METASTASES IN BREAST CANCER PATIENTS

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Markers of bone turnover are often elevated in breast cancer patients with bone metastases (BM). To test whether bone markers could be used as early indicators of developing BM, we prospectively followed 112 postmenopausal women operated for primary breast cancer. At the time of diagnosis/ study inclusion, none of the women had BM, other skeletal disease or bone active drugs. During follow-up (range 0.6 – 4 yrs., median 30 mo.), patients were seen every 3 months and timed blood/ urine specimens were obtained. Eleven patients developed BM (BM+) and each of these were matched to 4 women free of BM (BM-). The markers measured were serum (s) calcium, sTAP, sBAP, sOC, sPICP, urinary (u) PYD, uDPD, uNTX, sNTX, uCTX, sCTX. All analyses were done in single batches after study end.

Results: At any given point in time (including baseline), marker levels in the BM+ group did not differ significantly from those in the BM- group. Marker levels at baseline did not predict the later development of BM (OR 0.14 -1.01, all NS). 93% of all changes in bone markers were below the least significant change, as defined in an independent group of similar patients. The remaining 7% of values could not be associated in a consistent pattern with the occurrence of BM.

Conclusion: In patients with breast cancer, biochemical markers of bone turnover can not be used to predict or diagnose incident BM. This lack in diagnostic validity is mainly attributable to the high overall and long-term variability of the currently used bone markers.

P67

VITAMIN D DEFICIENCY AMONG HIP FRACTURE PATIENTS ADMITTED FOR REHABILITATION.

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We measured serum 25OHD, PTH, albumin and corrected calcium in 15 male (median age 81 yr, IQR 74-87) and 49 female (87 yr, 81-90) hip fracture cases, admitted to a rehabilitation centre in Geelong (38°S) during 2001. 53 were living independently and 11 came from hostels. Data were compared with randomly-selected women (n=510) enrolled in the Geelong Osteoporosis Study, spanning similar ages. Median time between fracture event and biochemistry was 13 days.

Among hip fracture cases, median 25OHD levels were similar in men and women (25 [IQR 16-54] vs 24 [IQR 17-33] nmol/L, p=0.5). Corresponding values for log-transformed PTH (lnPTH) were equivalent to PTH values of 5.8 [4.1-7.7] vs 6.1 [3.8-8.7] pmol/L, p=0.7. Correlations between age and 25OHD (r= -0.1, p=0.4), age and lnPTH (r=0.2, p=0.1), and 25OHD and lnPTH (r=0.02, p=0.9) were weak. Prevalence of low 25OHD (<28 nmol/L) was 61%, and raised PTH (>6.5 pmol/L) was 40%. Age-adjusted body weight was lower among hip fracture women compared with the random sample (mean [95%CI] 62.9 [58.9-66.9] vs 67.3 [66.1-68.4] kg, p=0.04). A similar pattern was observed for albumin (30.3 [29.2-31.5] vs 39.8 [39.5-40.1] g/L, p<0.001), 25OHD (35 [26-43] vs 63 [61-66] nmol/L, p<0.001) and PTH (4.0 [3.4-4.7] vs 5.0 [4.8-5.2] pmol/L, p=0.02). No difference was observed for calcium (2.29 [2.26-2.32] vs 2.29 [2.28-2.30] mmol/L, p=0.9). Age-adjusted OR for hip fracture associated with low 25OHD was 10.3 [95%CI 4.9-21.9].

These post-fracture data are consistent with hip fracture patients being frail (lower body weight and serum albumin) and having a high prevalence of low 25OHD, similar to nursing home residents[1] (61% vs 52%, p=0.2). Their consistently poor 25OHD status was not always associated with elevated PTH. The possibility these changes are a consequence of the fracture event cannot be excluded.

[1]Stein MS, et al. Clin Endocrinol 1996;44:375

P68

25-HYDROXYVITAMIN D AND PARATHYROID HORMONE LEVELS IN YOUNG FEMALE TWINS AGED 10 TO 16 YEARS OF AGE.

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There are few reference data available for 25-hydroxyvitamin D (25-OH-D) and parathyroid hormone (PTH) serum levels in adolescent healthy females in Australia. The aim of this investigation was to determine levels of 25-OH-D and PTH in healthy young females at different times of the year in South-Eastern Australia.

Serum was collected from 43 individual female twins (20 non-identical pair (DZ) and 3 individuals), with a mean (SD) age of 12.8 (1.8). Sample collection took place over a 12 month period. Stored serum samples were used to determine levels of 25-OH-D (ng/ml) by 125I RIA method (DiaSorin) and PTH (pmol/L) by an intact PTH assay (Biomediq). The mean (SD), median and range for 25-OH-D and PTH levels were 69.9 (16.62), 70, 32 - 100 and 1.8 (1.8), 1.10, 0.1 – 7.0, respectively. The 25-OH-D and PTH measurements were grouped by month of sample collection. Group 1 represented November to April while group 2 represented May to September. From the 43 25-OH-D measures, 4 were <50 ng/ml; none were < 25 ng/ml. The corresponding PTH were not significantly elevated. 25-OH-D levels were significantly different (P=0.002) compared by period of collection (group 1: 76.40(14.23) *vs.* group 2: 60.89 (15.77)). 25-OH-D and PTH were not significantly correlated. Age was not correlated with PTH and was moderately correlated with 25-OH-D (r = -0.3, P = 0.04). The within-pair correlations for 25-0H-D and PTH were r = 0.74 and r = 0.88, both p<0.001, respectively.

There was significant seasonal variation in 25-OH-D but there was no evidence of an increase in PTH levels with lower 25-OH-D levels. If it is assumed that a 25-OH-D of 50 nmol/l represents vitamin D “adequacy”, vitamin D insufficiency is uncommon in healthy females during their major growth phase.

P69

IS THERE A GENDER DIFFERENCE IN FOOD INDUCED CHANGES IN SERUM C-TELOPEPTIDES?

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Calcium salts given in oral doses of more than 400 mg suppress both PTH and biochemical markers of bone resorption. It is now well established that feeding suppresses serum C telopeptides (sCTX)[1], but gender differences have not been reported. We compared male and female sCTX responses to milk and to mixed meal ingestion.

Forty five people, 16 men and 29 women, over 55 years, consumed 400 ml of high calcium skim milk powder, containing 1200mg of calcium, after a 12 hour fast. Blood samples were taken at baseline and hourly for 8 hours after the milk. Two plain biscuits were eaten after 1.25 and 6.25 hours and a light lunch (energy content 1510kJ, 68% CHO, 21% fat, 9% protein, and calcium 35mg) was consumed 3.25 hours after the milk. Serum parathyroid hormone (PTH), sCTX and serum calcium (sCa), adjusted for albumin, were measured and results are expressed as LS Mean ± SEM. Comparisons between time and sex were evaluated using repeated measures analysis of variance.

There was no significant difference between men and women for either sPTH or sCa any time point. The sCTX values decreased after the milk drink, and this decrease was not different between men and women. The women also had an abrupt dip in sCTX 1.25 hours after lunch, but this was not seen in the men, (change from baseline
-0.22 ± 0.016 ng/ml vs -0.17 ± 0.021 ng/ml, $P<0.05$).

The abrupt dip in the sCTX in women is consistent with previous reports that sCTX is strongly influenced by feeding[1]. However, our data suggest that sCTX in men is not influenced by feeding to the same extent. It would be interesting to do further studies in men, and to explore the reasons for these gender differences.

1.Bjarnason NH et al, 2002. Bone 30(1): 307-13.

P70

THE EFFECTS OF PROPENTOFYLLINE ON BONE IN OVARIECTOMIZED RATS: A PILOT STUDY.

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Bone remodelling is under the influence of nerves and the neuropeptides that they produce. Nerve growth factor (NGF) is involved in maintenance of nerves and has local, positive effects on bone formation. Propentofylline (PPF) is a drug that can induce the synthesis of NGF. PPF may therefore have beneficial effects in the treatment of diseases such as osteoporosis. This pilot project looked at the effects of PPF on bone in an ovariectomized (OVX) rat model.

Adult female Sprague-Dawley rats were used. 7 rats were sham-operated and subcutaneously injected with saline (sham), 8 animals were OVX and given saline, and 8 rats were OVX and administered PPF (10 mg/kg body of weight/day). Rats were killed after 6 weeks. NGF concentrations were measured in bone using ELISA. Histomorphometric analyses of proximal tibial metaphyseal trabecular bone were undertaken.

Femoral NGF concentrations were 21% lower in OVX rats compared to shams ($p = 0.07$). Treatment with PPF caused an 18% increase in femoral NGF concentrations compared to OVX rats but this difference was also not significant. OVX+PPF resulted in a 62% increase in trabecular volume compared to OVX rats ($p < 0.01$), however, this was still a 40% reduction in bone volume when compared to shams ($p < 0.01$). Mineral apposition rate (MAR) was higher in the OVX group compared to both sham ($p < 0.001$) and OVX+PPF groups ($p < 0.001$). MARs were comparable between sham and OVX+PPF groups.

In summary, PPF treatment to OVX rats lead to a significant reduction in trabecular bone loss, however, the mechanisms are unclear as to how PPF caused this alteration to bone remodelling.

P71

GALANIN CONCENTRATIONS AND LOCALIZATION IN NORMAL AND FRACTURED BONE IN THE RAT SUGGEST A ROLE FOR THE PEPTIDE IN SKELETAL METABOLISM

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Galanin (GAL) is a pleiotropic peptide synthesized by many cell types and participates in a range of physiological functions as diverse as anti-nociception and mitosis. GAL is upregulated in sensory nerves in non-osseous tissues after axotomy and sensory nerves have a role in both bone remodelling and fracture repair. It is not known, however, whether GAL is produced in either normal or fractured bone, or by which cells. Therefore, this study assessed the presence, concentration and localization of GAL in both normal and fractured ribs of rats, using radioimmunoassay and immunohistochemistry.

Adult, male Sprague-Dawley rats were used. The fracture group had their left 6th rib fractured and calluses analysed at days 7 and 14. Matching controls did not undergo fracture. Callus GAL concentrations were ~30-fold ($p < 0.01$) and ~17-fold ($p < 0.01$) higher in callus than in control ribs at days 7 and 14 respectively. In costal cartilage of normal rib, GAL-like immunoreactivity (GAL-LI) was detected in some but not all chondrocytes. Similarly, in fracture calluses, GAL-LI was also found in some but not all chondrocytes as well as in osteoprogenitor cells, osteoblasts and periosteal matrix.

These results suggest GAL may participate in normal skeletal metabolism and that the peptide is upregulated during fracture repair. The data also indicate that osseous GAL may not be solely produced by peripheral nerves but may also be synthesized by skeletal-associated cells.

P72

THE VALUE OF BONE MINERAL DENSITY AND BIOCHEMICAL MARKERS FOR BONE TURNOVER IN THE FEMALE OVARIECTOMISED RAT

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In the evaluation of the effect of ovariectomy (OVX) in the female rat model, bone mineral density (BMD) measurements as well as biochemical markers of bone turnover are used. In this study, both measurements were compared to investigate the validity and agreement between the various parameters.

Female Sprague Dawley rats were ovariectomised or sham operated on, at 6 months of age. They were then fed a semisynthetic casein based diet for 4 months. Blood was sampled from animals at 6, 8 and 10 months and *ex vivo* BMD measurements were done of the lumbar spine and right femur at age 10 months. Plasma samples were assayed for biochemical markers of bone turnover (osteocalcin) and bone resorption (RatLaps; C-telopeptide of type 1 collagen).

At age 10 months, the *ex vivo* BMD measurements of both sites were significantly different between sham and OVX (0.273 vs 0.243 g/cm^2; $p=0.0001$ for right femur and 0.242 vs 0.198 g/cm^2; $p = 0.0001$ for lumbar spine). RatLaps as well as osteocalcin increased significantly over 4 months after OVX indicating increase in bone turnover and resorption. There was a significant positive correlation between RatLaps and osteocalcin at 10 months (0.688; $p<0.001$) and a negative correlation between both markers and the BMD values, indicating that as turnover and resorption increased, bone density decreased.

From this study we have shown that markers and BMD complement each other. We recommend for the OVX rat model, that BMD is used as the primary outcome and a bone marker as the secondary outcome. A four month period after OVX is sufficient to see significant differences in both BMD and bone markers.

P73

CALCIUM BIOAVAILABILITY FROM SKIM MILK FORTIFIED WITH CALCIUM CARBONATE OR MILK CALCIUM.

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Absorbability of calcium from milk and a variety of calcium salts has been a significant focus of studies over recent years. There is general agreement that the best source of calcium is from food, because bone, like other tissues, requires balanced nutrition. Milk, in particular, is an effective source, reflecting its high content of calcium and its supply of vitamin D, necessary for calcium absorption and bone health.

In the present study, the bioavailability of calcium from skim milk powder fortified with calcium carbonate or milk calcium was compared. Four week old male Sprague Dawley rats were randomised into 2 groups of 15 each. The animals were fed a skim milk powder base diet containing 0.5% calcium as calcium carbonate or as milk calcium. Animals were fed the test diets for 8 weeks and then sacrificed. Primary outcomes were bone mineral density, bone calcium content, bone breaking strength and urinary excretion of collagen cross-links, a measure of bone resorption.

Results showed that there was no significant difference in any of the measured parameters. The implication is that the kind of calcium salt used for fortification is not the determining factor for bioavailability. This agrees with human studies that have shown that the various salts are absorbed to similar extents.

P74

MICROARRAY ANALYSIS OF RANKL STIMULATED OSTEOCLASTOGENESIS.

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Macrophage colony stimulating factor (M-CSF) stimulates the differentiation of macrophage-like cells from monocytes. Addition of recombinant receptor activator of NFkB ligand (sRANKL) with M-CSF results in differentiation of osteoclast-like cells (OCLs). Total RNA from monocytes treated with M-CSF alone and monocytes treated with RANKL and M-CSF was isolated at various treatment times. cDNA hybridisation experiments against the arrays revealed regulated genes.

Genes that showed significant regulation in monocytes treated with M-CSF and RANKL compared with monocytes treated with M-CSF alone represent genes that are targets for RANKL-specific regulation. Apart from traditional genes associated with osteoclasts, a number of other regulated genes were identified including transcription factors. The regulation of cysteine protease cathepsin K and the cysteine protease inhibitors cystatin C and cystatin B were confirmed by real-time PCR. Cathepsin K relative expression peaked at one week treatment, while cystatin C expression peaked at two weeks and cystatin B showed a steady increase in expression. Genes that are regulated by the action of RANKL during osteoclastogenesis may provide potential therapeutic targets for bone diseases like osteoporosis.

P75

A NOVEL POTENT DOMINANT NEGATIVE MUTANT OF THE CALCIUM-SENSING RECEPTOR.

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Inactivating mutations of the calcium-sensing receptor (CaR) gene are responsible for the inherited disorders familial hypocalciuric hypercalcemia (FHH) and neonatal severe hyperparathyroidism (NSHPT), leading to a generalised resistance of the receptor to extracellular Ca^{2+} ions. The majority of mutations to date have been identified in the extracellular domain, some of which, for example R185Q and R795W, have been shown *in vitro* to have a dominant negative effect on wild type CaR activity. To investigate the physiological role of the CaR, it would be useful to produce a dominant negative receptor that lacks residual receptor activity. Using site-directed mutagenesis, we have produced such a CaR mutant (R185Q/R795W) by combining two known dominant negative mutations. Dominant-negative effects of this double mutant on CaR-dependent Ca^{2+} mobilization and MAP kinase activity were demonstrated in transfected HEK-293 cells. Furthermore, the double mutant was devoid of a functional CaR response over a wide range of extracellular Ca^{2+} concentrations (0.5 – 20 mM). The data support the conclusion that the R185Q/R795W double mutant may be used to test the physiological significance of the wild-type CaR in cell systems and, possibly, transgenic animals.

P76

NOVEL GROWTH FACTOR COMPLEX STIMULATES OSTEOBLAST PROLIFERATION.

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Accelerating implant osseointegration may reduce the rehabilitation period and inconvenience for patients undergoing uncemented joint replacement or procedures using graft substitute materials. We propose that this may be achieved using a bioactive coating to promote bone formation by stimulating the natural repair process.

A patented protein complex containing insulin-like growth factor II (IGF-II) and vitronectin has been tested for this application. Proliferation of SaOS-2 osteoblast-like cells on variously coated surfaces was measured by ^{3}H-leucine incorporation.

IGF-II–vitronectin complex-treated surfaces exhibit dose-dependent enhanced cell proliferation over untreated controls. This increase is comparable to, or exceeds, the effect of pre-treating with foetal calf serum. Pre-adsorption of vitronectin is required for maximal IGF-II effects.

These results, with vitronectin's ability to adsorb readily to a variety of surfaces [1,2], suggest that vitronectin is an attractive vehicle to deliver a growth factor to cells via a biomaterial surface. Furthermore, the stimulation of osteoblastic cells by this complex suggests application to biomaterial surfaces to accelerate osseointegration.

1. DJ Fabrizius-Homan and SL Cooper (1991) J Biomater Sci Polym Ed 3, 27-47.
2. CR Howlett et al. (1994) Biomaterials 15, 213-222.

P77

THE EXPRESSION AND DISTRIBUTION OF DRM/GREMLIN IN RAT EMBRYO

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Genes up-regulated under the stimulative condition were investigated in calvarial cells from rat embryos or 5-week-old rats. One of the up-regulated genes was gremlin, which is a member of DAN family characterized by a cystein knot motif and antagonistic effect on BMP function. Gremlin was found to be transiently expressed during matrix maturation period of the culture by northern hybridization. The results suggests that it regulates bone formation by suppressing the effect of BMP to avoid excess formation of bone. Tissue distribution of gremlin was also examined in the area of rat embryo undergoing endochondral ossification by *in situ* hybridisation. A 1.9 kb gremlin fragment was cloned into pGEM-T Easy vector, and transcribed using T7 and SP6 RNA polymerase with DIG-11-UTP. Antisense and sense probe was hybridized to 10-micro-meter thick frozen section of 20.5 dpc (days post conception) rat embryos. Hybridized signals were mainly detected in the zone of proliferative and mature chondocytes, but not in the calcifying zone. The expression of gremlin in the endochondral ossification process suggests that it also regulates proliferation and differentiation of rat embryo chondrocytes by affecting BMP function. We are now investigating the distribution of gremlin protein in bone microenvironment using a recombinant gremlin antibody.

P78

AGE-RELATED CHANGES IN THE EFFECT OF ALUMINIUM ON BONE FORMATION

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The accumulation of aluminum (Al) in bone has been associated with the development of osteomalacia due to a defect in the mineralization of bone with an accumulation of osteoid. However, the mechanisms underlying these changes are still not known. The effects of prostaglandin E_2 (PGE_2) are mediated by four characterized receptors, EP_1-EP_4. EP_1 is coupled with intracellular Ca^{2+} transport, whereas EP_2 and EP_4 are coupled with cAMP production through the activation of Gs protein and adenylate cyclase. We have shown previously that an EP_1 agonist stimulates the differentiation of osteoblasts and an EP_2/EP_4 agonist has an opposite effect on osteoblasts. The purpose of this study is to examine the effects of Al on the differentiation of osteoblasts through PGE_2 receptors, and age-related changes in these effects. Osteoblasts were enzymatically isolated from calvariae of 25-week-old (mature) and 90-week-old (aged) female Wistar rats. In both cells, EP_1, EP_2 and EP_4 were expressed. The expression of EP_1 was decreased with aging, whereas EP_2 and EP_4 were constantly expressed. An exposure to Al resulted in a dose-dependent increase in ALP activity, collagen synthesis, and bone nodule formation in the cells isolated from aged rats. By contrast, Al had no significant effect in the cells isolated from mature rats. In both cells, Al decreased EP_2/EP_4 agonist-induced suppression of bone formation due to the decrease in cAMP production but had no effect on folskolin (an adenylate cyclase activator)-induced suppression. Al had no effect on EP_1 agonist-induced stimulation of bone formation in both cells. In conclusion, Al increases bone formation due to modulate the interaction between EP_2/EP_4 and Gs protein in the cells isolated from aged rats.

P79

PQCT DIAPHYSEAL BONE CHANGES ASSOCIATED WITH TRAINING EXERCISE IN THOROUGHBRED HORSES

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Fourteen female thoroughbred horses were bred and raised at pasture until seven of them began conventional training for racing. The other seven remained in small paddock enclosures. The distance and time the trained horses worked each day were recorded, and work was expressed as metres worked at one of three gait velocities. At the end of training, the diaphyses of the left and right third metacarpal and third metatarsal bones were scanned using pQCT (XCT2000, Stratec, Pforzheim), and cortical content, density, area, periosteal circumference, and bone strength index were determined.

The scan sites were 40% of bone length distal to the carpometacarpal or tarsometarsal joints respectively in front and hind limbs, 40% proximal to the distal condylar surface, or at mid-diaphyseal level. Differences in training exercise and bone parameters between control and exercised groups were analysed. Exercise volume was similar in the 7 trained horses until twice weekly galloping began in the ninth week of training, after which the high exercise subgroup (4 horses) galloped 4450 metres at 14.6m/s, and cantered 41600-47600m at 8.4m/s, and the medium exercise subgroup (3 horses) galloped only, 0, 1400, or 1800 metres, and cantered 0, 14000, or 24400 metres.

For all parameters except bone density, there were significant difference between moderate and high exercise, and not between control and medium exercise. Bone density increased stepwise between the groups. The results indicate that data from individuals within treatment groups should be carefully examined. Despite low numbers, highly significant differences were evident. Higher exercise load resulted in the metacarpal and metatarsal diaphyses increasing size, density, and strength.

P80

PHBV AS A BONE SUBSTITUTE IMPLANT

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For over a decade, metals and ceramics have been investigated as potential tools for healing bone defects, whether as replacements, delivery vehicles or as frameworks for the attachment, growth and differentiation of cells. However, a more desirable scaffold is one that is both biodegradable and biocompatible whilst releasing bioactive substances that control osteoblast-matrix interactions and subsequently bone formation. The synthetic polymer poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), a biosynthesized thermoplastic polymer may be the biomimetic solution for this objective. Stem cells, isolated from the bone marrow, have long been recognised for their potential to differentiate along a variety of mesenchymal lineages, including the osteoblast lineage. We hypothesised that PHBV would prove a suitable material capable of supporting stem cell attachment, growth and differentiation along the osteogenic lineage with and without bone-specific extracellular matrix (ECM) proteins.

This project utilised a combination of ELISA assays, immunocytofluorescence, RT-PCR and histochemical staining to examine stem cell attachment, growth and differentiation.

Our results indicate that environmental cues have a positive influence on stem cell attachment, growth and subsequent differentiation. In particular, stem cells preferentially attach to PHBV when compared to common ECM proteins. Furthermore, PHBV favours stem cell commitment, growth and differentitation along the osteoblast lineage.

In addition to its mechanical properties and slow degradation rates, these results support the examination of PHBV as a potential implant for bone tissue engineering.

P81

QUANTITATIVE ULTRASOUND DOES NOT PREDICT MECHANICAL DAMAGE IN BOVINE CANCELLOUS BONE

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Compression induced reduced elastic modulus (E) in human calcaneal cancellous bone is not predicted by QUS variables SOS or BUA (1); implying QUS might not detect onset of bone fragility in absence of changes in microarchitecture. This study imposed similar mechanical conditioning on bovine cancellous bone, to examine whether changes in E were predicted by changes in QUS variables.

Sixty specimens (15 mm diam. & length) were extracted from bovine femoral condyles, cleaned, dried, rehydrated, randomised to 6 groups and mechanically tested; (a) "preconditioning", 14 compression cycles at 0.4% strain, with measurement of initial E; (b) "damage" (strain rate 0.0004 s^{-1}) for each of 6 strain groups (0.4%, 1.0%, 1.2%, 1.5%, 2.0%, 2.2%); (c) "post-damage", one cycle at 0.4% strain, measuring final E. Measurements: (i) vBMD (ii) custom SOS and BUA; before & after mechanical conditioning. SOS poorly correlated (corr.) with "bar wave" velocity, $v = SQRT(E/\rho)$, (R^2=0.054, $p<0.05$). vBMD negatively corr. with BUA (R^2=0.37, $p<0.001$) and positively with E (R^2=0.13, $p<0.005$). This behaviour of BUA, in contrast with the calcaneus (1), is consistent with its inverse parabolic dependence on vBMD (2) and the higher vBMD of bovine bone. SOS was corr. with vBMD (R^2=0.42, $p<0.001$). With increasing strain, E fell from baseline (-33.9+/- 4.2[SEM]% for 1% strain, -59.0+/-5.3% for 1.2%strain) then rose (-19.0+/-8.1%, +6.9+/-14.1%, +4..4+/-13.6% for 1.5, 2.0 & 2.2% strains, respectively). There was no corr. between changes in E and those in SOS or BUA.

We conclude that strain-induced response of elastic modulus in bovine cancellous bone is more complex than in calcaneus (1), but similarly to the latter, changes in SOS or BUA do not predict those changes in E induced by mechanical damage. 1. Nicholson & Bouxsein, 2000 JBMR 15:2467; 2. Han et al. 1996 Osteo Int 64:291.

P82

VERTEBRAL BODY BONE ARCHITECTURE MEASUREMENT USING 3D ANAGLYPHS.

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Macerated sagittal slices of T12 and L1 vertebral bodies from 15 subjects were examined in a scanning electron microscope. These individuals (8 men and 7 women) had no clinical history of crush fractures and were chosen to represent each decade of adult life. 3D anaglyphs were created of nine contiguous fields per slice by recording two digital images (the second tilted 5^o). The anaglyphs were viewed through red/green stereo glasses and the length ($Tb.Le_{rods}$) and thickness ($Tb.Th_{rods}$) of trabecular rods measured using an image analyser.

1884 rod thickness measurements were made with a mean $Tb.Th_{rods}$ of 118 ± 31μm, with male rod thickness (122±32μm) significantly greater than female (112±29μm, $p<0.001$). 1547 rod length measurements were made with a mean $Tb.Le_{rods}$ of 565±168μm, and male rod length (575±164μm) was significantly greater than female (555±170μm, $p<0.02$).

Age related changes showed that $Tb.Th_{rods}$ decreases significantly with age in the males while $Tb.Th_{rods}$ in women showed a trend to increase. $Tb.Le_{rods}$ increased significantly with age in females, but not in men. A load-to-buckling index (L^2/R^4) showed decrease with age for males concomitant with $Tb.Th_{rods}$ decrease. However, in females load-to-buckling was constant through out life despite an increase in rod length. This implies that small (not significant) increases in rod thickness can result in large changes in rod strength.

Direct measurements from 3D anaglyphs provide "real" sizes for trabecular rods. The determination of ranges for rod thickness and length will allow more accurate finite element modelling and better estimates of fracture risk. The current, preliminary data show that there are age-related changes to cancellous bone architecture, which may influence bone quality and risk of crush fracture.

P83

AGE-RELATED DIMORPHISM IN STRUCTURAL COMPLEXITY OF CANCELLOUS BONE. IH Parkinson, NL Fazzalari. Div of Tissue Path, Inst of Medical and Veterinary Science and Dept of Pathology, University of Adelaide. South Australia.

Mass is not the sole determinant of the complex structure of cancellous bone.

Eight skeletal sites were analysed from skeletally normal subjects. Under 50 years of age, 40 subjects with a mean age of 31 years. Fifty years of age and over, 73 subjects with a mean age of 72 years.

Fractal dimensions, BV/TV, BS/BV, Tb.Sp and TBPf were determined. Regression analysis was performed to determine which structural parameter of cancellous bone correlated best with each fractal dimension.

Fractal 1 for the under 50 year old group is significantly correlated with TBPf ($r=-0.25$, $p<0.009$) and for the 50 year old and over group is significantly correlated with BS/BV ($r=0.29$, $p<0.0001$). Fractal 2 is significantly correlated with BV/TV for both groups ($r=0.87$, $p<0.0001$ and $r=0.95$, $p<0.0001$, respectively). Fractal 3 for the under 50 year old group is significantly correlated with BV/TV ($r=0.44$, $p<0.0001$) and for the 50 year old and over group is significantly correlated with Tb.Sp ($r=-0.68$, $p<0.0001$).

The fractal dimensions, as measures of morphological complexity at different scales, are related to specific structural parameters of cancellous bone. In the younger group, BV/TV or TBPf correlate with all fractal dimensions. In the older group, parameters influenced by bone turnover (BS/BV) and spatial arrangement (Tb.Sp) as well as BV/TV correlate with the fractal dimensions. The age-related dimorphism shows that differences in cancellous bone architecture can be identified and 'assigned' to specific structural components. These data show that fractal dimensions describe the structural complexity of specific 'bone compartments' within cancellous bone and older individuals have a greater sensitivity to architectural change as BV/TV decreases.

P84

BILATERAL SYMMETRY OF THE HUMAN METACARPAL:
THE VALIDITY OF MATCHED PAIRING FOR BIOMECHANICAL ASSESSMENT

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The clinical results of metacarpophalangeal (MCP) joint replacement have been unsatisfactory and have higher failure rates when compared with the outcomes of hip and knee replacement. A contributing cause to early loosening may be poor design but there has been limited in vitro testing of MCP prostheses. The number of cadaver metacarpals required for biomechanical testing can be prohibitive. The purpose of this study was to assess the suitability of using bilateral pairing in biomechanical studies of the metacarpal.

Seven pairs of human second metacarpals were extracted at autopsy. Metaphyseal areas, diaphyseal cortical areas, second moments of area, average periosteal and medullary radii and bone densities were measured for each of the metacarpals using computed tomography and bone densitometry. Polar Fourier regression was used to assess the morphometry of sectional periosteal and endosteal boundaries.

Mean differences between left-right parameters were small (<5%) and similar to the degree of experimental precision. There were strong significant left-right correlations for the morphometric, geometric and densitometric parameters considered.

There is a high degree of bilateral symmetry between paired metacarpals and the use of bilateral pairing results in an important reduction in sample size.

P85

ENHANCED RADIOGRAPHY OF THE SKELETONS AND TEETH OF AUSTRALIAN ANIMALS USING PHASE-CONTRAST X-RAY IMAGING (PCI)

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This work investigated the utility of a new X-ray system, phase-contrast X-ray imaging (PCI), for identifying skeletal and dental features in a range of Australian animals. Developed in Australia, PCI uses a micro-focal X-ray tube to generate images. These contain an absorption component (as with conventional radiography) as well as a phase-contrast component that provides superior definition of object boundaries, even in adjacent structures of similar density.

A variety of hard tissue specimens were imaged using PCI, with or without attached soft tissue. These included dense objects varying greatly in size (such as a dog-sized thylacine skull, a battery of horse teeth in mandibular bone, and a battery of mouse teeth), whole small animals (a 30 cm copperhead snake; a 29 cm Johnson's crocodile), and small thin skull specimens (a 6 x 3 cm bandicoot skull; a 1.5 x 0.6 cm bat skull). Appropriate X-ray exposure settings were used, with source-to-object and object-to-image distances adjusted for different levels of magnification. Images were captured at 25 um pixel size on phosphor imaging plates (Fuji, Japan) and scanned with a BAS 5000 scanner (Fuji) to obtain a digital file.

PCI produced X-ray images of superior resolution that clearly differentiated the structures of interest in large hard-tissue specimens. The technique also resulted in spectacular whole and magnified images of small animals, isolated skulls, and other small or tiny (<1 cm) objects. Hence, one immediate research application for PCI is the provision of extremely detailed radiographs showing skeletal changes in rodents used for genetic 'knock-out' or similar studies.

P86

THE EFFECT OF TERG-A-ZYME ON THE BREAKING STRENGTH OF BONE

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After collection of bone specimens for research purposes, extraneous tissue is often cleared prior to experimentation. Removal of soft tissue is laborious and time consuming. It has been suggested that Terg-a-zyme™, a concentrated anionic detergent with a protease enzyme, could assist in this. The aim of this study was to investigate the effects of Terg-a-zyme™ on the mechanical integrity of bone.

Fresh whole lamb femora were randomly allocated to a control group (n=10) (sealed in plastic at 4°C) or to immersion at ambient temperature (n=10) in a 2% Terg-a-zyme™ solution for 48 hours. The soft tissue attachments on the femora were stripped on day 0 in the control group and at the end of day 2 in the study group. A 35mm section was cut from the mid-shaft of the femur. Beams of 2mm thickness were then cut in the axial plane from the central medial and lateral cortices using a Leitz 1600 sawing microtome. The breaking stress of the beams was determined using four-point bending on a custom built jig and Mecmesin Imperial 1000™ materials testing machine. After correction for minor differences in the width and thickness of the beams, the breaking stress values were analysed using the Student's t-test.

Removal of soft tissue attachments from the femora exposed to Terg-a-zyme™ was quicker and less labor intensive than for the controls. There was no statistically significant difference in the breaking stress of the beams in the control and study groups (mean ± stdev: 2.42E+08 ± 3.74E+07, 2.33E+08 ± 2.46E+07 N/m^2). Terg-a-zyme™ has a dramatic impact on the ease of cleaning bone without any apparent negative impact on the breaking stress of bone.

P87

A SYSTEM AND MODEL FOR CELL-FRIENDLY BONE GRAFT TOOLING DEVELOPMENT

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Tissue trauma during the harvesting of bone autograft can kill cells within the graft material. A model and measurement system for the cutting process has been developed that will improve understanding of how tool geometry, cutting speed, and thrust force affect graft morphology and cutting temperatures.

A standard drill press has been instrumented for *in vitro* measurement of cutting forces (Dynamometer Type 5007, Kistler Ag.) and feedrate (LVDT, Schaevitz 500 DC). Several prototype tools, including a commercial cutter (Acumed, Ca., USA) have been compared. All tests were performed using fresh bovine cancellous bone.

When feed rate is plotted against thrust load, three zones can be identified: Zone I) no cutting (sub-critical thrust load), Zone II) increasing feed rate and torque, and Zone III) a feed rate plateau (little or no increase in feed rate as thrust load is increased). At low thrust loads (start of Zone II) we obtain small flake-like chips. As load is increased the chips become thicker and resemble carpet-like tufts. High rotational speeds also favour tuft formation. Cutting the bone into small flake-like chips will subject cells to significant mechanical disruption that could result in shearing damage to cell walls. Our model predicts that at high thrust loads there will be large frictional losses. We propose that cell survival can be improved by avoiding these cutting extremes.

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P88

DIVERSITY AND NOMENCLATURE OF VERTEBRATE EXOSTOSES.

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Hereditary multiple exostosis is a dominantly inherited genetic disorder characterised by benign cartilage-capped bone tumours known as osteochondromas or exostoses. These develop primarily on long bones of effected individuals between early childhood and puberty and lead to varying degrees of orthopaedic deformity that may require surgical intervention.

Mutations in the putative tumour suppressor genes *EXT1* and *EXT2* have been show to be associated with this disorder. These genes encode the proteins exostosin-1 and exostosin-2 respectively, both of which are involved in heparan sulphate biosynthesis. Disturbed heparan sulphate biosynthesis interferes with hedgehog diffusion and thus may interfere with hedgehog/parathyroid hormone-related protein signalling and chondrocyte differentiation.

In contrast with the pathological process in humans is the normal development of exostoses in some amphibians and reptiles. In these animals exostoses arise in early ontogeny as part of normal developmental processes and also possibly in response to mechanical stresses. *EXT* genes are highly conserved with homologues of the human *EXT* genes identified in mice, the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*. Thus it is proposed that spatial and/or temporal regulation of these genes may be involved in the development of these non-pathological exostoses.

We will present examples of exostoses in humans, amphibians and reptiles and propose a nomenclature system to differentiate between pathological and non-pathological exostoses.

P89

THE MODELLING OF BENDING DEFORMATIONS AND STRESSES IN THE HUMAN FEMORAL MID-SHAFT, TAKING ACCOUNT OF POROSITY DISTRIBUTION

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The objective of this work was to develop a model for the bending stress in the cortex of the mid-shaft of the femur. The model was to include the effects of variations in cross-sectional geometry and the distribution of porosity within the cortex. The completed model was intended to help with studies of age-related changes in the bending performance of the shaft of the femur.

Equations for the bending of a beam can only be solved by assuming a uniform cross-section. In the work described here this is achieved by dividing the length of the femoral shaft into around five sections of approximately uniform cross-section. The sections may vary in length. The bending formulas can then be integrated over each length and summed to obtain the total deflection. Within each section the model allows for an arbitrary number of porosity zones.

The model was verified by comparing the observed and calculated deformations in proximal femurs loaded as simple cantilever beams. The test specimens came from the collection of human femora held by the School of Dental Science. Good agreement was found between the predicted and measured deflections. This modelling method shows promise for use in detailed studies of the effects of age changes in cross-sectional geometry and porosity distribution. For the femoral shaft, this model can be implemented using a spreadsheet program running on a modestly powerful PC.

P90

OVER-EXPRESSION OF NATIVE OPG BY BREAST CANCER CELLS ENHANCES TUMOR GROWTH IN BONE.

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Osteoprotegerin (OPG) acts as a decoy receptor for RANKL, preventing it binding to its receptor, RANK, thereby inhibiting osteoclast formation and activation. Since the local production of OPG may also be a means to limit tumor expansion in bone by preventing osteoclastogenesis, we transfected MCF-7 parental cells and MCF-7 cells overexpressing PTHrP with full-length cDNA for OPG in pCEP4. In vitro analysis demonstrated that over-expression of OPG in MCF-7 cells resulted in increased cell growth, in the presence or absence of PTHrP. Parental, vector control and MCF-7 cells over-expressing PTHrP, OPG or PTHrP plus OPG were injected into the proximal tibiae of athymic nude mice. The animals were monitored for 2.5 weeks, after which they were sacrificed and their limbs were assessed by radiology and histology. No osteolysis was observed radiologically following inoculation of the MCF-7 parental cells or the MCF-7 cells over-expressing OPG, although small intramedullary tumors were evident histologically in the latter group. Surprisingly, the MCF-7 cells over-expressing PTHrP and OPG developed larger tibial tumors than the MCF-7 cells over-expressing PTHrP alone (18.6 $\pm$ 3.1 % osteolysis as determined by radiology, compared with 9.0 $\pm$ 2.2 %, $p < 0.05$). The tumors overexpressing OPG also exhibited a change in histology. This increased tumor growth afforded by the over-expression of OPG was abrogated by treatment with OPG-Fc (2.5 mg/kg/day, subcutaneous) resulting in inhibition of tumor growth.

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

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