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ABSTRACTS

Oral abstracts are listed in order of their presentation.

IP	Invited Speaker
O	Oral
P	Poster

Invited Speaker Abstracts

IP1 18.8.04: 0830 – 0915 hours

SEX HORMONES AND THE SKELETON

S Khosla
Division of Endocrinology, Metabolism & Nutrition, Mayo Clinic College of Medicine, USA

Estrogen has long been recognized as playing a key role in regulating bone metabolism in women. While conventional wisdom long held that testosterone played the analogous role in men, the finding of impaired bone mass acquisition in the estrogen receptor and aromatase negative males has triggered a major paradigm shift in our thinking about sex steroid regulation of the male skeleton. Moreover, since adult men have serum estrogen levels intermediate between those found either in pre- or postmenopausal women, studies in men have provided novel information on the *in vivo* dose response relationships between estrogen and bone metabolism that may also be applicable to women. Using direct interventional studies, we have demonstrated that estrogen plays a significant, and perhaps dominant, role in regulating bone resorption in men, while both estrogen and testosterone are important for the maintenance of bone formation. In addition, a number of cross-sectional and longitudinal studies have demonstrated that serum estrogen levels correlate better than serum testosterone levels with bone mass and with rates of bone loss in men. Findings from two studies are consistent with a "threshold" estradiol level below which the male skeleton appears to become estrogen deficient, and variations in the estrogen receptor alpha genotype may modulate the relationship between circulating estrogen levels and bone mass/bone loss in men. Testosterone, however, is clearly also important for bone: it serves as the precursor for estrogen, has some anti-resorptive activity, is important for the maintenance of bone formation, and likely enhances periosteal apposition, leading to increased bone size and the resulting reduced fracture risk in men.

IP2 18.8.04: 0915 – 0940 hours

INSIGHTS FROM ERKO MODELS: WHAT DO THEY TELL US ABOUT ESTROGEN RECEPTOR FUNCTION IN BONE?

NA Sims
Department of Medicine, St. Vincent's Hospital, Melbourne, VIC

Maintenance of bone mass by estradiol is well established, but the roles of the two mammalian estradiol receptors (ERα and ERβ), and non-ER-mediated mechanisms remain poorly understood. A number of groups have studied bone phenotypes of ER knockout mice, but the phenotypes observed have not been identical. To determine contributions of each ER and non-ER-mediated mechanisms, gonadectomy and estradiol treatment have also been studied in these knockouts, yet again, the results have been complicated by differences in the knockouts used. In all female double ER KOs studied, bone mass was low, and was not reduced further by ovariectomy, indicating that any protective effect of estrogen is ilost by deletion of the two receptors. In full double ER KOs, estradiol treatment did not alter either bone mass or ovarian size, indicating that both effects require the presence of one or both ERs. In contrast, in double ER KOs that express a truncated form of the ERα, mild responses to estradiol were observed in bone and ovary, suggesting a role for this truncated receptor in vivo. The use of gonadectomy and estradiol treatment in single ER KOs revealed that, in female mice, while ERα is the major receptor mediating osteoprotective effects of estradiol, ERβ is also able to mediate a bone-protective effect of estradiol. In males, however, it appears that only ERα regulates of bone response to estradiol. Testosterone treatment is unaffected by the absence of either ER, confirming that aromatisation is not required for an anabolic effect of testosterone in bone. More recently, selective ligands for the ERs have confirmed a role for both ERα and ERβ in bone.

IP3 18.8.04: 1430 – 1500 hours

OSTEOBLAST DIFFERENTIATION

G Karsenty
Baylor College of Medicine, Houston, Texas, USA

Coffin-Lowry Syndrome (CLS) is an X-linked mental retardation condition associated with skeletal abnormalities. The gene mutated in CLS, *RSK2*, encodes a growth factor-regulated kinase. However, the cellular and molecular bases of the skeletal abnormalities associated with CLS remain unknown. Here, we show that RSK2 is required for osteoblast differentiation and function. We identify the transcription factor ATF4 as a critical substrate of RSK2 that is required for the timely onset of osteoblast differentiation, for terminal differentiation of osteoblasts, and for osteoblast-specific gene expression. Additionally, RSK2 and ATF4 posttranscriptionally regulated the synthesis of Type I collagen, the main constituent of the bone matrix.

Accordingly, *Atf4*-deficiency results in delayed bone formation during embryonic development and low bone mass throughout postnatal life. These findings identify ATF4 as a critical regulator of osteoblast differentiation and function, and indicate that lack of ATF4 phosphorylation by RSK2 may contribute to the skeletal phenotype of CLS.

IP4 18.8.04: 1500 – 1530 hours

REGULATION OF BONE DEVELOPMENT – BLIND GENES VERSUS FUNCTIONAL ADAPTATION

F Rauch
Shriners Hospital and Department of Pediatrics, McGill University, Montreal, Canada

A large number of molecular, cellular and epidemiological factors have been implicated in the regulation of bone development. A major unsolved problem is how to integrate these disparate findings into a concept that explains the development of bone as an organ. Events on the organ level are often simply presented as the cumulative effect of all factors that individually are known to influence bone development. In such a cumulative model it must be assumed that each bone cell carries the construction plan of the entire skeletal anatomy in its genes. One problem with this scenario is that it would require an astronomical amount of positional information to be stored in the genome. An alternative model views the development of bone shape and strength as an adaptive process. The genome only provides positional information for the basic outline of the skeleton. Thereafter, bone cell action is coordinated by the mechanical requirements of the bone. When mechanical challenges exceed an acceptable level (the 'mechanostat setpoint'), bone tissue is added at the location where it is mechanically necessary. The main mechanical challenges during growth result from increases in bone length and muscle force. Hormones, nutrition and environmental factors exert an effect on bone either directly by modifying the mechanostat system or indirectly by influencing longitudinal bone growth or muscle force. Predictions based on this model are in accordance with observations on prenatal, early postnatal and pubertal bone development.

IP5 18.8.04: 1530 – 1600 hours

PTH AND ARRESTINS

SL Ferrari
Division of Bone Diseases, WHO Collaborating Center for Osteoporosis Prevention, Geneva University Hospital, Switzerland

Parathyroid hormone (PTH) stimulates bone resorption to maintain serum calcium levels. Yet, intermittent PTH administration is an anabolic therapy for osteoporosis. By promoting G protein coupled receptors uncoupling from G proteins and internalization of agonist-receptor complexes, cytoplasmic arrestins inhibit Gs- and Gq-mediated signaling. Accordingly, activation of PTH/PTHrP receptor without concomitant recruitment of ß-arrestins nor receptor endocytosis, as caused by PTHrP-derived agonists modified on position 1, results in sustained cAMP signaling, and furthermore in complete desensitization upon re-challenge with the agonist in vitro.

To examine the role of ß-arrestins in the regulation of bone turnover and modeling/remodeling of skeletal architecture, we investigated ß-arrestin2 KO mice and their wildtype (WT) littermates. ß-arrestin2 KO mice are viable, fertile, and show no gross phenotypic abnormalities, including normal body size, skeletal morphology, and growth plate organization. In contrast, ß-arrestin2 KO mice post-natally develop significantly lower bone mass, altered cortical and trabecular bone architecture, increased bone resorption relative to formation, and increased serum calcium response to PTH. Moreover, intermittent PTH administration results in lesser net bone mass gain in ß-arrestin2 KO mice, particularly in the trabecular compartment, as explained by increased indices of bone resorption compared to WT. To understand the molecular mechanisms implicated in this phenomenon, we investigated primary osteoblastic cells, and found that PTH-stimulated cAMP signaling is increased and sustained, whereas osteoprotegerin (OPG) mRNA expression is inhibited in ß-arrestin2 KO mice.

Altogether these results demonstrate that the effects of PTH on bone turnover and architecture depend on signaling regulation by ß-arrestin2.

IP6 18.8.04: 1600 – 1630 hours

THE BONE REMODELING COMPARTMENT: A POSSIBLE COUPLING STRUCTURE

EF Eriksen
Medical Director, Eli Lilly & Co, Indianapolis, USA

Bone Remodeling takes place at discrete sites on the trabecular surface and in cortical Haversian systems. Until recently, remodeling sites, be it resorptive or formative, were supposed to be in direct contact with the marrow space. Moreover, several hypotheses pertaining to osteoclastic and osteoblastic differentiation assumed free exposure to the marrow microenvironment. Due to improvements in tissue preparation techniques we were able to demonstrate that most resorptive and formative sites are actually covered by a dome-shaped layer of flattened cells. This structure, the Bone Remodeling Compartment (BRC), comprises a specialized vascular structure, lined on one side by a layer of flattened cells, and on the other side by the bone surface and the cells covering it. Immuno-cytochemical analyses have shown that the dome shaped cell layer exhibits histochemical and immuno-cytochemical reactions similar to lining cells of adjacent quiescent surfaces. The cells are positive for alkaline phosphatase, osteocalcin, OPG, BMP, TGFß etc). During normal bone remodeling, osteoblasts generally appear after osteoclasts and osteoclast precursors have disappeared. However, the BRC comprises a structure, where osteoclast differentiation, which demands direct cell to cell contact between osteoblast and osteoclast precursors, may take place. The osteoblast in this case would be the lining cell layer. The BRC may also be involved in mechanotransduction as a structure intimately connected to osteocyte network.

IP7 19.8.04: 0830 – 0900 hours

BODY WEIGHT AND BONE DENSITY

IR Reid
University of Auckland, New Zealand

Body weight impacts on both bone turnover and bone density, and is therefore an important risk factor for vertebral and hip fractures, ranking in importance alongside that of age. The effect of body weight is probably contributed to by both fat mass and lean mass, though in postmenopausal women, fat mass has been more consistently demonstrated to be important. A number of mechanisms for the fat-bone relationship exist and include the effect of soft tissue mass on skeletal loading, the association of fat mass with the secretion of bone active hormones from the pancreatic beta cell (including insulin, amylin, and preptin), and the secretion of bone active hormones (eg, estrogens and leptin) from the adipocyte. These factors alone probably do not fully explain the observed clinical associations, and further study of the actions on bone of novel hormones related to nutrition is an important area of further research. An understanding of this aspect of bone biology may open the way for new treatments of osteoporosis. More immediately, the role of weight maintenance in the prevention of osteoporosis is an important public health message that needs to be more widely appreciated.

IP8 19.8.04: 0900 – 0925 hours

GROWTH HORMONE, IGFS AND BONE: A PROMISE YET UNFULFILLED

S Khosla
Division of Endocrinology, Metabolism & Nutrition, Mayo Clinic College of Medicine, USA

Puberty is associated with a marked increase in bone and muscle mass, driven in large part by activation of the growth hormone (GH)-insulin-like growth factor (IGF) axis. By contrast, senescence is characterized by significant loss of bone and muscle mass (osteopenia and sarcopenia, respectively) associated with declining GH and IGF-I production. This has led to the longstanding, plausible, and yet unproven hypothesis that treatment of aging individuals with GH or IGFs could reverse osteopenia and sarcopenia without significant adverse side-effects. A number of small, randomized trials of GH therapy of aging individuals have been conducted over the past decade and the results have been equivocal, at best. However, a recent randomized, placebo controlled trial of 80 postmenopausal women on estrogen therapy found remarkable increases in bone mineral content (BMC) and bone mineral density (BMD) at several skeletal sites after 48 months, with GH having been administered for 36 months, consistent with a delayed and extended effect of GH on bone. Lean mass also increased significantly. There is even more limited data on the use of IGF-I as an anabolic agent, in large part due to significant, dose-dependent side effects. Since IGF binding proteins (IGFBPs) can both modulate IGF action as well as serve to potentially transport/target IGFs to particular tissues, combinations of IGF-1 and IGFBP-3 have been used in animal and in a small human study. Finally, based on findings in the rare syndrome of hepatitis C-associated osteosclerosis, we have suggested that a combination of IGF-II (or its precursor, IGF-IIE) and IGFBP-2 may be effective in targeting IGFs to bone, with subsequent anabolic effects. In summary, the GH-IGF axis remains a promising, but as yet unproven, target for novel anabolic approaches to treat age-related osteopenia and sarcopenia.

IP9 19.8.04: 0925 – 0950 hours

Y RECEPTORS IN THE CENTRAL CONTROL OF BONE

PA Baldock

Bone and Mineral Program, Garvan Institute, Sydney, NSW

Recent studies have highlighted the interactions between the central nervous system and bone with the hypothalamus emerging as a regulator of osteoblastic function. Along with studies of mice lacking the adipocytic hormone leptin, Y receptor deletion models have provided evidence for potent control of osteoblast activity via this region of the brain.

Y receptors (Y1, Y2, Y4, Y5, y6) mediate the actions of several neuropeptides through diverse distributions in both central and peripheral nervous tissue, with roles in many organ systems. Initial studies in Y2 deleted mice revealed a large and rapidly inducible increase in cancellous bone volume and osteoblast activity in response to specific deletion of hypothalamic Y2 receptors of adult mice. Specificity of individual Y receptor pathways was evident in Y4 deleted mice, which did not display a bone phenotype. In contrast, Y2Y4 double deleted mice displayed a synergistic elevation in bone volume. This change however, was only evident in male mice, suggesting the possibility of hormonal interactions with these neuronal pathways. Interaction of the Y2 receptor-mediated and leptin pathways was investigated in models in which hypothalamic neuropeptide levels were elevated but peripheral leptin ranged from absent to obese levels. These studies suggest that the Y2 and leptin pathways can act distinctly from one another in the control of osteoblast activity.

Y receptor function represents a novel avenue for investigation of potent bone anabolic pathways.

IP10 19.8.04: 1130 – 1200 hours

GENETIC MANIPULATION OF HUMAN BONE MARROW STROMAL STEM CELLS FOR ENHANCED REGENERATION OF SKELETAL TISSUES

S Gronthos
Mesenchymal Stem Cell Group, Division of Haematology, Institute of Medical and Veterinary Science, Adelaide, SA

Postnatal bone marrow stromal stem cells (BMSSC) have the capacity to develop into a variety of different cell types including bone, fat, cartilage, myelosupportive stroma, skeletal/cardiac muscle and neural-like cells. However, studies have shown that *ex vivo* expanded BMSSCs display a gradual decrease in their capacity to proliferate and differentiate into various tissues following successive subculture, and has greatly limited their use in tissue engineering applications. Work in our laboratory has developed an immunoselection protocol to purify human BMSSCs populations directly from bone marrow aspirates, allowing for the first time the ability to properly characterise BMSSC as they exist *in vivo*. From these studies, BMSSC displayed a marked altered gene expression pattern *in vitro* for various markers such as telomerase. The loss of telomerase activity by cultured BMSSCs was found to have a significant impact on the life-span of BMSSC following ex vivo expansion. Recent studies have shown that enforced expression of telomerase activity in cultured BMSSCs significantly increased their life span *in vitro* and greatly enhanced the potential of these cells to form bone *in vivo*. Telomerase expressing BMSSCs were also found to express higher levels of the STRO-1 antigen, indicating a maintenance of stem cell populations following *ex vivo* expansion. Collectively, these studies define the properties of human BMSSC *in vivo* and help elucidate the fundamental conditions necessary to maintain and expand primitive stem cell populations *ex vivo,* in order to develop novel tissue engineering and gene therapy strategies.

IP11 19.8.04: 1130 – 1200 hours

THE MATERIAL AND STRUCTURAL BASIS OF BONE STRENGTH: THE ANATOMY OF FRACTURE PREVENTION BY REMODELLING SUPPRESSANTS

E Seeman
Austin Hospital, The University of Melbourne, Melbourne, VIC

Bone must be stiff for movement against gravity yet flexible for energy absorption in impact loading, and light for speed yet strong for loading. These contradictory properties are met by variable impregnation of type 1 collagen with mineral with fashioning of this mineralised tissue into hollow tubes for lever function and cancellous vertebral bodies for spring-like function. The material and structural properties decay because of age-related abnormalities in remodelling rate and balance. High remodelling reduces tissue mineral content and stiffness, low remodelling rates makes bone too stiff increasing micro-damage burden. Remodelling imbalance produced by reduced bone formation and increased bone resorption in each BMU produces bone loss, architectural disruption while limited periosteal apposition compensates incompletely. The anti-resorptive drugs reduce the rate of remodelling which slows bone loss; more time is available to restore tissue mineral content. They reduce the volume of bone resorbed in each remodelling cycle slowing trabecular thinning, loss of connectivity and progression of cortical porosity. High dose bisphosphonates in animals is associated with micro-damage, reduced bone tissue toughness but not whole bone strength. A better understanding of purpose of remodelling, the extent to which remodelling should be suppressed and individuals may increase anti-fracture efficacy.

IP12 19.8.04: 1330 – 1415 hours

GENETIC DETERMINANTS OF THE SPATIAL RESTRICTION OF EXTRACELLULAR MATRIX MINERALISATION

G Karsenty
Baylor College of Medicine, Houston, Texas, USA

Extracellular matrix (ECM) mineralization is physiological in bone and pathological in soft tissues. The mechanisms determining the spatial restriction of ECM mineralization are poorly understood yet their elucidation could help preventing pathological mineralizations. Here we show that a normal extracellular phosphate concentration is required for bone mineralization, while lowering this concentration prevents it. However, raising extracellular phosphate concentration is not sufficient to induce pathological mineralization because of the presence in all ECMs of pyrophosphate, a physiological inhibitor of mineralization. ECM mineralization occurs only in bone because of the exclusive coordinate expression in osteoblasts of *Type I collagen* and of *Tnap* that encodes an enzyme cleaving pyrophosphate. This dual requirement explains why *Tnap* ectopic expression in another *Type I collagen*-expressing cell leads to pathological mineralization. This study reveals that co-expression in osteoblasts of otherwise broadly expressed genes is necessary and sufficient for bone mineralization and suggest means to prevent pathological mineralization.

IP13 19.8.04: 1710 – 1740 hours

THE IMMUNE SYSTEM AND BONE REMODELLING

M Gillespie
St. Vincent's Institute of Medical Research, NSW

In determining the fundamental mechanisms of bone formation and resorption, most attention has been paid to the role of the either the osteoblast or osteoclast. It is well accepted that communication networks exist between these cells that are pivotal to the formation and activation of the osteoclast, and that the osteoclast also modulates osteoblast behaviour. However, studies into the action of cells of the immune system, particularly T, B and NK cells, to modulate the activity of either bone formation or resorption are in their infancy. These cells powerfully influence the growth and development of bone cells through the action of lymphocyte-derived cytokines. Several T cell-derived cytokines including IFN-γ, IL-4, IL-10, IL-13, OCIL, sFRPs and GM-CSF act upon the osteoblast or directly upon osteoclast precursors to inhibit osteoclast formation, whilst RANKL, IL-6, IL-7 and IL-17 act to stimulate this process.

Both activated and naïve T lymphocytes participate in bone remodeling in normal physiology and in inflammatory diseases such as arthritis, where T cell production of TNFα and soluble RANKL are fundamental drivers of this process.

The capacity of naïve T cells to influence bone remodeling is now emerging. Using genetically altered mice, both histomorphometric analyses and the use of targeted cellular populations *ex vivo*, have demonstrated how several cytokines that elicit their actions through T cells have differing affects upon normal bone structure or cellular function. Studies with IL-12, IL-18, and IL-12+IL-18 null mice and the defects in bone architecture will be discussed.

IP14 19.8.04: 1710 – 1740 hours

STRUCTURAL AND MATERIAL BASIS OF BONE STRENGTH AND ITS DECAY

NL Fazzalari
Bone and Joint Research Laboratory, Institute of Medical and Veterinary Science and Hanson Institute, Adelaide, SA

The mechanical function of bone, in the skeleton, is to sustain the loads applied by physiological motion. This optimisation relates to both the material properties of bones and their cross-sectional and structural geometry. If the bone is too small or its structure degraded, if the bony material properties have been degraded, the bone is prone to fail under physiological load. This mechanical perspective of osteoporotic fracture has resulted in a strong interest in the concept of bone quality. This term includes characteristics of bone, such as: trabecular architecture, the rate and extent of bone turnover, the organic and inorganic composition of bone matrix, the type and amount of collagen cross links, the degree of matrix mineralization, microdamage accumulation, and cell viability. These characteristics of bone quality can influence the mechanical effect of complex physiological loads on the skeleton.

For mechanical analysis, it is necessary to consider separately axial load, bending and torque, and superimpose these to reconstruct the physiological load. Axial load produces compression or tension. However, when the compression load is eccentric bending is produced. The effects of bending are more important than the effects of axial loading. In addition, long bones are loaded in torsion and often when overloaded result in spiral fractures.

Material properties are generally described by the relationship between both normalised load and deformation. The *Elastic modulus* is the first and most important mechanical characteristic of a material. The relationship between the axial deformation and deformation perpendicular to the loading axis is given by *Poisson's ratio*. Shear load is due to off axis loading. The relationship between the shear stress and shear deformation is a third mechanical characteristic of material, the *modulus of rigidity*. Elastic modulus, Poisson's ratio and modulus of rigidity are three related material characteristics.

There is an urgent need to evaluate the theoretical bases of how structural and material characteristics of bone relate to bone strength and fracture risk.

IP15 20.8.04: 0900 – 0930 hours

TREATMENT OF ADVANCED PROSTATE CANCER

F Saad
Universite de Montreal, CHUM/Hopital Notre-Dame, Montreal, Quebec, Canada

Prostate cancer often metastasizes to bone during disease progression. Patients who develop bone metastases have a high risk of developing skeletal complications, including pathologic fractures, spinal cord compression, and severe bone pain. Bisphosphonate therapy is the standard of care for the prevention of skeletal complications in patients with bone lesions from other forms of primary cancers, such as multiple myeloma and breast cancer. Until recently, however, no bisphosphonate had ever demonstrated objective clinical benefit in patients with prostate cancer and osteoblastic bone lesions. A recent multicenter, randomized, placebo-controlled trial found zoledronic acid (4 mg) to be a safe and effective therapy in patients with bone metastases from hormone-refractory prostate cancer. Zoledronic acid significantly reduced the proportion of patients who experienced skeletal complications and extended the time to first skeletal complication. Further, zoledronic acid significantly reduced the risk of skeletal complications over the 24-month study and provided consistent reductions in bone pain compared with placebo. Another area of research is in bone loss due to androgen deprivation therapy, often necessary in the treatment of prostate cancer. The role of IV bisphosphonates in this area has shown to be effective. Recently published studies demonstrated that pamidronate IV was able to prevent bone loss and that zoledronic acid was able to not only prevent bone loss but also to increase bone density in men undergoing androgen deprivation therapy. Zoledronic acid is an important advancement in the care of patients with prostate cancer metastatic to bone. The role of bisphosphonates in the treatment of patients with prostate cancer continues to evolve.

IP16 20.8.04: 0930 – 1000 hours

TNF FAMILY MEMBERS AND MALIGNANCY

CR Dunstan
Bone Biology Laboratory, ANZAC Research Institute, Concord, NSW

Osteoblast lineage cells are known to mediate the effects on osteoclasts of many pro-resorptive hormones and cytokines including cancer related cytokines such as PTHrP and MIP1alpha. TNF receptor and TNF family members, Receptor Activator of NF kappa B (RANK), and osteoprotegerin (OPG), and their common ligand, RANK Ligand (RANKL) have a central role in this action. RANKL binds to RANK on osteoclasts and their precursors to promote differentiation, activation and survival. OPG acts as a decoy receptor which when bound to RANKL prevents its interactions with RANK.

The development of the mammary gland for lactation is also dependent on RANKL/RANK signalling. RANKL acts as a proliferative factor mediating the actions of prolactin and progesterone on the developing lobular alveolar structures. Other osteotropic factors such as PTHrP, IGF1 and mCSF are also important for normal lactation. Presence of these common signalling molecules in bone may support breast cancer metastasis and explain the propensity of breast cancer to metastasise to bone. Multiple myeloma cells appear to increase bone resorption by up-regulating RANKL expression and by increasing OPG clearance through binding between syndecan-1 and the OPG heparin binding domain.

Blocking RANKL/RANK interactions has been shown to reduce bone resorption in patients with metastatic breast cancer and with multiple myeloma and is a potential therapeutic approach to limit tumour growth and induction of bone destruction in these diseases

IP17 20.8.04: 1000 – 1030 hours

BISPHOSPHONATE USE IN ORTHOPAEDIC THERAPIES

DG Little
Orthopaedic Research and Biotechnology, The Children's Hospital at Westmead, Sydney, NSW

Bisphosphonates have potent effects on bone, but only recently have bisphosphonates been seen as potential therapeutic agents in orthopaedic surgery. Preclinical work has focussed on the potential of bisphosphonates as adjunctive therapy in joint arthroplasty, distraction osteogenesis, fracture repair and in osteonecrosis.

Animal studies have shown that nitrogen-containing bisphosphonates (N-BP) can improve initial fixation of materials used in joint arthroplasty, as well as prevent particle-induced osteolysis and reverse it once it is present. Translation of these effects clinically is unproven, but the potential benefit is clear.

There is much interest in adjunctive therapy for fracture healing, and recently anabolic agents have become available for clinical use. However, when an endogenous anabolic response is already present, this can be optimised by bisphosphonate mediated control of catabolism. We have performed multiple pre-clinical studies using zoledronic acid (ZA) in distraction osteogenesis and fracture repair. Combination anabolic / anti -catabolic therapy has also proven to be synergistic in a non-union model.

Osteonecrosis is a complex disorder affecting children and adults. When a critical structure like the femoral head is affected, pain and disability result, usually leading to the need for surgery or arthroplasty. Our preclinical work shows that ZA can improve outcomes in osteonecrosis. Other preclinical work with N-BPs is also supportive, as are initial clinical experiences.

Multiple opportunities exist for the development of bisphosphonate use in orthopaedics, however none of the indications have yet passed through the rigorous clinical trials required to translate pre-clinical experiments to the bedside.

IP18 20.8.04: 1100 – 1130 hours

MOLECULAR MECHANISMS IN ANABOLIC THERAPY

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

Bone formation results from a complex cascade of events that involves proliferation of primitive mesenchymal cells, differentiation into osteoblast precursor cells (osteoprogenitor, preosteoblast), maturation of osteoblasts, formation of matrix, and finally mineralization. It is highly likely that in some forms of osteoporosis, deficient bone formation results from impaired osteoblast replenishment from precursors, and even from a deficiency of progenitors. Recent studies of control of osteoblast differentiation have provided valuable new insights, including identification of the roles of several key transcription factors in osteoblast differentiation.

The anabolic effect of PTH is dependent upon intermittent administration, but when an elevated PTH level is maintained even for a few hours it initiates processes leading to new osteoclast formation, and the consequent resorption over-rides the effects of activating genes that direct bone formation. The observation that concurrent treatment with bisphosphonates impairs the anabolic response to PTH, adds to other clues that osteoclast activity is necessary to complement the direct effect that PTH has in promoting differentiation of committed osteoblast precursors. This might involve the generation of a coupling factor from osteoclasts that are transiently activated by RANKL in response to PTH.

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

IP19 20.8.04: 1130 – 1200 hours

WNT/LRP5 MUTATIONS AND OSTEOPOROSIS

SL Ferrari
Division of Bone Diseases, WHO Collaborating Center for Osteoporosis Prevention, Geneva University Hospital, Switzerland

LRP5 is a member of the low-density lipoprotein (LDL) receptor-related family that mediates Wnt signaling. Transgenic mouse models have demonstrated the importance of LRP5 in bone formation and bone mass acquisition. Several gain-of-function mutations in LRP5 have been identified, that cause dominantly inherited high bone mass (HBM) and sclerosing bone dysplasias. Among them, the G171V mutation confers resistance to Dkk1, an endogenous inhibitor of Wnt/LRP5 signaling. Loss-of-function mutations in LRP5 are responsible for osteoporosis pseudoglioma (OPPG), an autosomal recessive disorder characterized by low bone mass, spontaneous fractures and blindness.

A QTL for bone mineral density (BMD) in the general population was mapped at 11q12-13, the LRP5 locus. A population-based study of five LRP5 polymorphisms with allele frequencies >2% found that a missense substitution in exon 9 (c.2047G>A, p.V667M) and haplotypes based on exon 9 and exon 18 (c.4037C>T, p.A1330V) alleles were significantly associated with bone mass and projected area of vertebrae at the lumbar spine in adult males, but not females, accounting for up to 15% of the population variance for these traits in men. Moreover, 1-year changes in lumbar spine bone mass and size in pre-pubertal boys were also significantly associated with these LRP5 variants, suggesting that LRP5 polymorphisms could contribute to the risk of spine osteoporosis in men by influencing vertebral bone growth during childhood. In a case-control study, exon 9A and exon 18T alleles were over-represented among men with idiopathic osteoporosis, and the odds for fractures were greater than 2 among carriers of the 9A-18T haplotype. Further analyses in two large populations from Framingham and the Netherlands confirm association of BMD with LRP5 polymorphisms, particularly exon 18 SNP in men.

In summary, LRP5 genetic variation influences bone mass in the general population, underscoring the potential of pharmacologically manipulating the Wnt/LRP5 pathway for osteoporosis treatment.

IP20 20.8.04: 1200 – 1230 hours

TREATMENT OF OSTEOPOROSIS IN WOMEN – WHY, WHOM, WHEN AND WHAT DRUG?

E Seeman
Austin Hospital, University of Melbourne, Melbourne, VIC

Why? (i) Spine and hip fractures increase morbidity, mortality and cost. (ii) the burden of fractures is increasing, (iii) bone loss accelerates with age (iv) effective treatments are available. *Whom and when?* The most important factor is an individual's absolute risk for fracture – this increases with age, lower BMD, prior or incident fracture. Treating fewer older persons (> 60 years) at high risk than many younger persons at low risk ensures those likely to benefit receive treatment and those unlikely to benefit, don't. *What drug? Spine fractures* The most rigorously studied drugs are alendronate, risedronate, raloxifene, PTH and strontium ranelate (SR). These drugs reduce the risk of symptomatic and asymptomatic single fractures by about 40-50% and multiple fractures by about 80-90%. The benefits are reported in 6-18 months of treatment. Evidence is available for anti-fracture efficacy with raloxifene and SR in women with osteopenia. *Non-spine fractures* Alendronate, risedronate, SR and hormone replacement therapy (HRT) have been reported to reduce hip fractures in community dwelling women. PTH has been reported to reduce the risk of non-vertebral, not hip, fractures. Raloxifene has been reported to reduce the risk of non-vertebral in a post-hoc sub-analysis. Calcium plus vitamin D and hip protectors have been reported to reduce hip fractures in nursing home and institutionalised women. PTH use is likely to be limited to severe osteoporosis. HRT is not recommended for fracture risk reduction in women without postmenopausal symptoms. Credible evidence for anti-fracture efficacy of calcitonin, fluoride, anabolic steroids, or active vitamin D metabolites is lacking. There is no evidence that combining bisphosphonates with raloxifene or HRT reduces fractures more than either drug alone. *How long?* It remains unclear as to whether anti-fracture efficacy is sustained beyond 5 years. High bisphosphonate doses in animals resulted in micro-damage, a decline in bone toughness but no decrease in overall bone strength. The relevance of these studies to humans is uncertain. Recurrence of bone loss is likely to occur sooner with cessation of HRT or raloxifene than bisphosphonates. Women and men with fragility fractures should be treated with rigorously investigated agents.

IP21 20.8.04: 1230 – 1300 hours

MONITORING OSTEOPOROSIS TREATMENT

MJ Seibel
ANZAC Research Institute, Bone Research Program, concord, NSW

A number of agents have been demonstrated to reduce the risk of osteoporotic fracture. The question in this presentation is whether it is possible, and if yes, how to best monitor the effect of these agents in the individual patient.

Aside from not monitoring at all, the following approaches are currently used to assess therapeutic efficacy: clinical presentation (e.g. incident fractures, pain, height loss); measurement of changes in bone mineral density (BMD); measurement of changes in bone turnover (BTO).

There is considerable controversy over which outcome measurements, response thresholds and schedules are most appropriate to gauge the effects of anti-resorptive or anabolic treatments on bone health. For example, it is unclear how much increase in BMD is necessary, or how much change in BTO is sufficient to preserve or restore bone strength and to reduce fracture risk. Is bigger always better, or can too much of a good thing be bad, even in bone? Are there thresholds above or beyond the skeletal response to treatment differs? How does baseline BMD or BTO affect therapeutic outcomes?

While there is general agreement on the necessity of monitoring treatment, its "how" and "when" still needs careful consideration. Outcome measures such as BMD or BTO may be used as tools for dialogue with the patient. Also, a lack of

response may alert to the assessment of compliance or to a re-evaluation for secondary causes of osteoporosis. Newer imaging techniques, such as MRI or micro-CT may, in the future, offer additional options for monitoring treatment effects on bone structure and architecture.

Oral Abstracts

O1 18.8.04: 0940 – 0950 hours

OSTEOCLAST INHIBITORY LECTIN (OCIL) IS REQUIRED FOR NORMAL BONE REMODELLING *IN VIVO* AND INHIBITS OSTEOBLAST FUNCTION *IN VITRO*

A Nakamura[1], JMW Quinn[1], NA Sims[2], CT Gange[1], H Zhou[1], V Kartsogiannis[1], EH Allan[1], TJ Martin[1], KW Ng[3] & MT Gillespie[1]

[1] St. Vincent's Institute, Fitzroy, VIC and Departments of [2] Medicine and [3] Diabetes and Endocrinology, The University of Melbourne, St. Vincent's Hospital, Fitzroy, VIC

OCIL, a C-type lectin expressed by osteoblasts which binds sulphated glycosaminoglycans and NK cell receptors, inhibits osteoclast differentiation *in vitro*. To investigate the roles of OCIL in bone metabolism we generated OCIL null mice (*ocil*$^{-/-}$) and examined their bones by histomorphometry. We also investigated recombinant OCIL effects on mineralisation and osteoblast differentiation marker expression by primary osteoblasts and pre-osteoblastic KUSA O cells.

10 week old *Ocil*$^{-/-}$ tibial trabecular bone volume and cortical thickness were significantly lower (22% and 15% respectively) relative to age matched wild type mice. This osteopenia was associated with high bone turnover, with 153% higher osteoclast surface (OcS/BS) and approximately treble the osteoblast surface (ObS/BS) and osteoid volume (OV/BV) of controls. *In vitro*, OCIL profoundly inhibited mineralisation by osteoblasts and KUSA O cells treated with ascorbate and β-glycerophosphate for 21 days. KUSA O osteopontin, alkaline phosphatase, BSP and osterix mRNA levels were unaffected by OCIL, but osteocalcin expression was strongly and dose-dependently inhibited within 3 days, even in cells treated with ascorbate prior to OCIL addition. OCIL also reduced adipocyte differentiation of KUSA O cells. OCIL expression was elevated in KUSA O clonal sub-lines that were unable to mineralise, differentiate into adipocytes or express osteocalcin, and BMP-2 treatment of KUSA O cells decreased OCIL mRNA expression.

The action of OCIL to reduce osteoblast function (possibly related to reduced osteocalcin production), the previously described OCIL inhibition of osteoclastogenesis and the high turnover osteopenia in *ocil*$^{-/-}$ mice confirm that OCIL plays an important role in normal bone metabolism.

O2 18.8.04: 0950 – 1000 hours

THROMBIN STIMULATES OSTEOCLAST DIFFERENTIATION BY ACTIVATION OF PROTEASE-ACTIVATED RECEPTOR-1

S Sivagurunathan[1], PD Campbell[2], R Smith[2], CN Pagel[1], RN Pike[2] & EJ Mackie[1]

[1] School of Veterinary Science, University of Melbourne, Parkville, VIC

[2] Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC

Thrombin is a serine proteinase that has a critical role in blood coagulation but also has hormone-like effects on cells. Many responses to thrombin are mediated by the protease-activated receptor (PAR) family of G-protein coupled receptors. Thrombin stimulates osteoclastic bone resorption in organ culture, though little is known about the mechanism of thrombin's effect, and whether it is exerted on osteoclast differentiation or activity of mature osteoclasts. Two known thrombin receptors are expressed by mouse osteoblasts, PAR-1 and PAR-4. The aim of this study was to identify whether thrombin can regulate osteoclastic differentiation, and if so, whether it is mediated by PAR-1. Mouse bone marrow cultures were used to examine thrombin's effect on osteoclast differentiation. Thrombin markedly stimulated the formation of multinucleated tartrate-resistant acid phosphatase-positive cells (TRAP+ MNC). A specific PAR-1 activating peptide (AP) was also shown to enhance the formation of TRAP+ MNC. A concentration of 100 nM thrombin stimulated a maximal increase in the number of TRAP+ MNC, and the number of TRAP+ MNC formed in response to thrombin was at least 70% of the number formed in response to 10 nM parathyroid hormone (PTH). The numbers of nuclei per osteoclast in thrombin-treated cultures (7 ± 0.60) and PAR-1 AP-treated cultures (5 ± 0.24) were lower than in PTH-treated cultures (18 ± 0.37). In cultures prepared from PAR-1-null mice, neither thrombin nor the PAR-1AP was able to stimulate osteoclast differentiation. These results suggest that thrombin stimulates osteoclastic differentiation and that the effect is mediated by PAR-1.

O3 18.8.04: 1000 – 1010 hours

INHIBITION OF BREAST CANCER GROWTH IN BONE USING TRAIL THERAPY

LM Thai, A Labrinidis, S Hay, V Liapis, S Bouralexis, K Weldon, BJ Coventry, DM Findlay & A Evdokiou

Department of Orthopaedics & Trauma, University of Adelaide, Hanson Institute, Adelaide, SA

TRAIL is a promising molecule that induces cell death through apoptosis in transformed but not normal cells in numerous *in vitro* and *in vivo* studies. TRAIL signals through two 'agonistic' (TRAIL-R1 and -R2) and has three 'antagonistic' (TRAIL-R3, -R4, and soluble OPG) receptors. Using the estrogen-independent breast cancer cell line MDA-MB-231 and a TRAIL-resistant sub-line of the same cell line in an *in vitro* 'cytotoxic' assay system, our laboratory has demonstrated that clinically relevant chemotherapeutic drug including, doxorubicin, cisplatin, and etoposide, in combination with TRAIL, has a synergistic or additive effect in killing these cells. More importantly, the synergistic effect was also observed in the TRAIL-resistant sub-line. Our initial receptor profiling revealed no differences in TRAIL surface receptor expression. We believe resistance to TRAIL in these cells is attributed to differences in the level of expression of intracellular anti-apoptotic signaling proteins and work is in progress to identify these proteins. Using an animal model of direct transplantation of breast cancer cells into the tibiae of athymic nude mice, we report here for the first time, the efficacy of TRAIL in inhibiting breast cancer growth and associated bone destruction. Animals inoculated with MDA-MB231 cells and left untreated reproducibly developed large lesions that invaded the marrow cavity and began to erode the cortical bone as assessed by radiography, micro computed tomography and histology. In contrast, animals treated with TRAIL for four weeks showed significant conservation of the tibiae with reduced areas of osteolysis. Further *in vivo* studies using combinations of TRAIL and chemotherapy are planned. Our studies will provide invaluable insights into TRAIL signaling biology and the mechanism by which some cancer cells may evade TRAIL-induced death.

O4 18.8.04: 1010 – 1020 hours

TARGETING PRIMARY PREVENTION OF FRACTURE IN OSTEOPOROTIC WOMEN: GEELONG OSTEOPOROSIS STUDY

MJ Henry, MA Kotowicz, JA Pasco, KM Sanders, E Seeman & GC Nicholson
The University of Melbourne, Department of Clinical and Biomedical Sciences, Barwon Health, Geelong, VIC

In Australia, subsidised bisphosphonate or SERM anti-fracture therapy is only available for secondary prevention. However, primary prevention in those with markedly reduced bone density may be appropriate. The aim of this case-control study was to determine T-Score thresholds among women with no previous fracture (NoFrac) that are associated with fracture risk equivalent to those with a previous fracture and osteoporosis at the lumbar spine or femoral neck (FracOP). Cases were 291 women (mean age 72yr, range 50-93) from a low-trauma fracture (hip, spine, humerus and wrist) cohort (n=668) who had BMD measurements and completed study questionnaires. Controls were 823 women (median age 70yr, range 50-94) drawn from a random population-based sample recruited during the same time period. BMD was measured at the femoral neck and spine (Lunar DPX-L), and self-reported adult low-trauma fractures recorded. Optimal cut-points for fracture within the NoFrac group, stratified by age and BMD, were determined by discriminant analysis and fracture risk scores were calculated. Logistic regression determined the fracture risk score threshold that produced odds for fracture equivalent to that of the FracOP group.

Table 1: T-Scores of equivalent fracture risk (NoFrac *vs* FracOP)

	50-59yr	60-69yr	70-79yr	≥80yr
Spine	-4.2	-3.5	-2.8	-2.0
Femoral Neck	-4.7	-4.0	-3.2	-2.5

These thresholds identify an additional 8.8% of the Australian female population for whom treatment may be cost-effective. Primary prevention of fracture with bisphosphonate or SERM therapy should be considered in women meeting these age and T-Score criteria.

O5 18.8.04: 1020 – 1030 hours

BONE RESORPTION AND OSTEOPOROTIC FRACTURES IN ELDERLY MEN: THE DUBBO OSTEOPOROSIS EPIDEMIOLOGY STUDY

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

Approximately one third of osteoporotic fractures occur in men. Among the potential risk factors for fragility fractures, bone turnover is considered an important determinant. The association between fracture risk and rates of bone turnover has not been well established in men. We examined this relationship in elderly community-dwelling men.

This case-cohort control study included 50 men with incident low-trauma fractures (cases) and 101 men without fracture (controls), aged 71±5.2 yrs (mean±SD) who have been prospectively followed in the Dubbo Osteoporosis Epidemiology Study for a median of 6.3 yrs (range, 2-13 yrs). Bone mineral density at the lumbar spine (LSBMD) and at the femoral neck (FNBMD), and markers of bone turnover were measured at baseline. Bone resorption was assessed by serum carboxyterminal cross-linked telopeptides of type I collagen (S-ICTP, S-CTX). Bone formation was assessed by serum aminoterminal propeptide of type I procollagen (S-PINP).

At baseline and compared to controls, cases had lower BMD, both at the femoral neck and the spine, lower dietary calcium intake, and higher S-ICTP levels. Age, BMI, FNBMD/yr, smoking habits, S-CTX and S-PINP did not differ between groups. Based upon univariate regression analysis, S-ICTP (RR 2.2, 95% CI, 1.5-3.2), FNBMD (RR 1.5, 95% CI, 1.0-2.1), LSBMD (RR 1.5, 95% CI, 1.1-2.2), and age (RR 1.4, 95% CI, 1.0-1.9) were all associated with increased risk of fracture. In multivariate logistic regression analyses, only S-ICTP (RR 2.3, 95% CI, 1.4-3.5) and FNBMD (RR 1.8, 95% CI, 1.2-2.9) remained independent predictors of fracture risk in men. Men within the highest quartile of S-ICTP had a 2.8-fold (95%CI 1.4-5.4) increased risk of fracture compared with men with levels in the lowest quartile. The incidence of osteoporotic fractures was 10 times higher in men with high S-ICTP and low FNBMD as compared to men with low S-ICTP and high FNBMD. Of the fracture risk in the population, 31% was attributable to high S-ICTP and/or low FNBMD, and S-ICTP contributed 24% to the estimated risk.

In conclusion, high bone resorption is associated with an increased risk of osteoporotic fracture in elderly men, independent of BMD. Combining measurements of BMD and bone turnover improved fracture prediction in this cohort. In populations and studies were biological variability (i.e. fasting state, diurnal variation) can not be controlled, S-ICTP appears to be a more robust marker of future fracture risk than S-CTX.

O6 18.8.04: 1100 – 1115 hours

IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF AN OSTEOCLAST-DERIVED OSTEOBLASTIC FACTOR (ODOF): NOVEL GROWTH FACTOR IN BONE

T Phan, R Han, V Smuts, MH Zheng & J Xu
Molecular Orthopaedic Laboratory, School of Surgery and Pathology, The University of Western Australia, Nedlands, WA

Bone is a living tissue and is maintained by the coordinate action of osteoblasts and osteoclasts. Intercellular communication between osteoblasts and osteoclasts is the quintessential mechanism in bone remodelling. Here we report the identification and functional characterization of an Osteoclast-Derived Osteoblastic Factor (ODOF), which is expressed by osteoclasts, binds specifically to osteoblasts and elevates cytosolic calcium ($[Ca^{2+}]_i$) resulting in the proliferation of osteoblastic cells. The ODOF gene was identified in $RAW_{264.7}$ cell-derived osteoclasts utilising a PCR-selected subtractive hybridisation screening process. Further investigations using reverse transcriptase PCR, revealed that ODOF mRNA was up-regulated during RANKL-induced osteoclastogenesis but was not expressed in osteoblasts or osteoblast-like cells. Recombinant His-tagged ODOF was subsequently produced and labelled with ^{125}I to ascertain its binding profile. The protein exhibits highly specific binding to primary calvarial osteoblasts with a binding affinity of 1.7±0.4nM and $2.7x10^4$±306 receptors per cell but not with osteoclasts and their precursor cells. Functional studies demonstrated that ODOF stimulates an increase in the proliferation of osteoblastic cells. Additionally, ODOF induces the formation of the bone matrix *in vitro*. In contrast, the protein did not promote osteoclastogenesis, osteoclast survival or bone resorption. Mechanistic analysis revealed that ODOF, alone, elevates intracellular $[Ca^{2+}]_i$ through the PLC-IP3 induced depletion of calcium stores. Moreover, western blot analysis reveals that ODOF induces the activation of the PI3K-Akt and ERK pathways in osteoblasts. Taken together, our results provide evidence for a novel cross-talk mechanism between the osteoclast and osteoblast and indicate that osteoclasts play a role in regulating osteoblastic growth and proliferation.

O7 18.8.04: 1115 – 1130 hours

Y-RECEPTOR AND LEPTIN INTERACTIONS IN THE CENTRAL REGULATION OF BONE FORMATION AND MARROW FAT

SJ Allison[1], PA Baldock[1], A Sainsbury[2], RF Enriquez[1], M Couzens[2], H Herzog[2] & EM Gardiner[1]
[1] *Bone and Mineral Research Program, Garvan Institute of Medical Research, Sydney NSW*
[2] *Neurobiology Program, Garvan Institute of Medical Research, Sydney NSW*

The neuropeptide Y (NPY) Y2 receptor and leptin regulate cancellous bone volume via central mechanisms, with increased bone formation in Y2KO and leptin-deficient obese (*ob/ob*) mice. Leptin and NPY interact to regulate peripheral fat deposition; however, whether they interact to regulate marrow fat and bone formation is unknown. Marrow adipocyte and bone formation were determined in distal femora of wildtype, Y1, Y2, *ob/ob*, Y1ob, and Y2ob deleted mice. Values are: mean ± SEM.

Cancellous bone volume, (BV/TV) was increased in Y1KO (13.4%±1.8), Y2KO (12.4%±1.7) and *ob/ob* (10.3%±1.0) compared to wildtype (5.3%±0.5). BV/TV was reduced in Y1ob (8.5%±1.4) and in Y2ob (9.9%±1.0), compared to Y1KO and Y2KO respectively. These changes were consistent with reduced mineral apposition rate in Y1ob (1.46±0.1μm/d vs 1.8±0.1μm/d in Y1KO), but greater osteoclast surface in Y2ob (12.6%±1.8 vs 6.0%±1.5 in Y2KO).

Marrow adipocyte number was increased 80-fold in *ob/ob* mice (268±63 vs wildtype 3.3±1.2). This increase was significantly reduced in Y1ob (130±32) and Y2ob (76±23), with Y2ob not different from wild type.

Thus, deletion of leptin signalling attenuated the bone phenotype of Y1 and Y2KO, although by differing cellular pathways, suggesting distinct functional interactions in their antiosteogenic responses. Y receptors also mediated the effects of leptin deficiency on marrow fat albeit to differing extents. These data therefore demonstrate that leptin and Y-receptor pathways interact in the regulation of both bone formation and marrow adipogenesis, with specific actions of specific Y receptors.

O8 18.8.04: 1130 – 1145 hours

GENE EXPRESSION IN THE PAGETIC OSTEOBLAST

U Bava[1], D Naot[1], KE Callon[1], IR Reid[1], R Pitto[2], S McCowan[3], T Cundy[1] & J Cornish[1]
[1] *Department of Medicine, University of Auckland, Auckland, New Zealand*
[2] *Department of Surgery, University of Auckland, Auckland, New Zealand*
[3] *Department of Orthopaedics, Auckland Hospital, Auckland, New Zealand*

Paget's disease is characterised by focal areas of increased bone resorption coupled to increased and disorganised bone formation. Pagetic osteoclasts have been studied extensively however, due to the integral cross-talk between osteoclasts and osteoblasts, we propose that pagetic osteoblasts also play a role in the pathogenesis of Paget's disease. To identify any differences between pagetic and non-pagetic osteoblasts, the expression levels of 13 genes were investigated in primary cultures of human osteoblasts and bone marrow stromal cells. Gene expression levels were determined using quantitative Taqman® Real-Time PCR technology with 18S ribosomal RNA as an internal control.

Twenty-two non-pagetic osteoblast samples and 21 non-pagetic stromal samples were compared against nine pagetic osteoblast samples and 10 pagetic stromal samples, respectively. MIP-1alpha, RANK and TNF-alpha were only detected in stromal cells. BCL-2, IL-6, SHIP, M-CSF, IL-11, OPG, VEGF, RANKL, COX-2 and IL-beta were detected in both osteoblasts and stromal cells. In pagetic osteoblasts, IL-6 expression was increased (P=0.0148). OPG expression increased in pagetic stromal cells (P=0.031) and there was a tendency for RANKL to decrease in both pagetic osteoblasts and pagetic stromal cells. This resulted in a significant decrease of RANKL/OPG in both pagetic stromal cells (P=0.0037) and pagetic osteoblasts (P=0.013). No significant changes were seen in the other genes investigated.

Increased levels of IL-6 possibly reflect the over-active pagetic bone microenvironment. The decreased RANKL/OPG ratio displays conditions that suppress osteoclastogenesis, suggesting that the RANKL/OPG system is compensatory to another mechanism that increases osteoclast number and activity within the active pagetic lesion.

O9 18.8.04: 1145 – 1200 hours

CONTROVERSIES IN CALCIUM-SENSING IN BONE

S Atwa[1],TC Brennan[1], AD Conigrave[2] & RS Mason[1]
[1] *School Of Medical Sciences and Institute for Biomedical Research,* [2] *School of Molecular and Microbial Biosciences, University of Sydney, NSW*

Both the calcium–sensing receptor (CaSR) and the metabotropic glutamate receptor (mGluR) belong to sub-group C of the G-protein-coupled receptor family. The CaSR senses extracellular calcium and is involved in calcium ion homeostasis. Whether calcium sensing by bone cells is via the CaSR remains controversial. The aims of this study were to determine whether human osteoblasts express detectable CaSR; whether the specific CaSR-activating type-II calcimimetic NPS R-467 influences bone cell proliferation or function and whether the calcium response might be affected by agents which modulate one or other mGluR sub-types. Human osteoblast-like cells from trabecular ends of long bones and osteosarcoma MG-63 cells were used. For experiments, cells were changed to serum-free mixture of DMEM without calcium, and Hams F12 medium. This medium was then supplemented with $CaCl_2$ to achieve various concentrations in the range 0 – 3 mM. Cell proliferation was measured by [3H] thymidine incorporation. Receptor activator of NF-κB-ligand (RANK-L) and osteoprotegerin (OPG) expression were determined by real-time RT-PCR. The CaSR was detected in osteoblasts by immunohistochemistry and RT-PCR. Ca^{2+} (0.5–3 mM) and another CaR activator, Gd^{3+} (5 and 50 μM) stimulated proliferation 4 to 6 fold. The specific CaSR calcimimetic R-467, not previously shown to affect bone cells, enhanced Ca^{2+}-dependent suppression of RANK-L: OPG ratios, but unexpectedly stereoselectively suppressed Ca^{2+}-dependent increases in cell proliferation. DCGIV, an agonist of mGluR group II receptors, suppressed Ca^{2+}-induced osteoblast proliferation.

These data provide evidence supporting the presence and functional activity of the CaSR in bone cells, albeit with atypical properties, and also point to potential involvement of glutamate receptors in modulating the calcium response.

O10 18.8.04: 1200 – 1215 hours

ZOLEDRONIC ACID TREATMENT DOES NOT DELAY THE PROCESS OF ENDOCHONDRAL OSSIFICATION IN A RAT FRACTURE MODEL

MM McDonald, SK Dulai & DG Little
Orthopaedic Research Department, The Children's Hospital Westmead, Sydney, NSW

While osteoclasts are required for bone remodelling, we speculated that their role in the removal of cartilaginous matrix may be redundant, making them unnecessary for soft callus removal during fracture repair. We tested this in a closed rat fracture model treated with zoledronic acid (ZA).

Three treatment groups were analysed (n=90): weekly saline (Saline), Weekly ZA 0.005mg/kg, and Bolus ZA 0.025mg/kg. Doses commenced at 1 week. 10 rats per group were culled at 2, 4 and 6 weeks for X-ray, QCT, histology and histomorphometry.

X-rays at 2, 4 and 6 weeks revealed no difference in fracture union between groups. QCT at 2 weeks showed BMC was increased 16% in Weekly ZA and 19% in Bolus ZA over Saline ($p<0.01$). By 4 weeks, BMC was increased 57% and 33%, and at 6 weeks 80% and 64% respectively ($p<0.01$). Callus volume did not differ between treatment groups at 2 or 4 weeks but was 35% higher for Weekly ZA and 28% higher for Bolus ZA at 6 weeks ($p<0.05$).

At 2 weeks, callus histomorphometry showed 9-10% cartilage content for all treatment groups. At 4 weeks this was reduced to 1-2% for all groups and by 6 weeks no cartilaginous callus remained.

In summary, ZA blocked bone resorption leading to increased bony callus volume, but without delaying the removal of soft callus cartilage. These data support our hypothesis that ZA does not delay endochondral ossification prior to bone remodelling and support the clinical safety of employing ZA during fracture repair.

O11 18.8.04: 1215 – 1230 hours

SESQUITERPENE LACTONE PARTHENOLIDE BLOCKS LIPOPOLYSACCHARIDE INDUCED OSTEOLYSIS VIA THE SUPPRESSION OF NF-κB ACTIVITY

KHY Man[1], MH Zheng[1], HT Feng[1], JH Steer[2], DA Joyce[2] & J Xu[1]
[1] Molecular Orthopaedic Laboratory, School of Surgery and Pathology, and Western Australia Medical Research Institute, [2] Pharmacology Unit, School of Medicine and Pharmacology, University of Western Australia, Nedlands, WA

Aim and Introduction:
Osteolysis induced by bacterial infection underlies many bone diseases. Drugs that inhibit LPS-induced osteolysis are critical in the prevention of bone destruction in infective bone diseases. Here we investigated the potential effect of an herbal extract, parthenolide (PAR) on LPS-induced osteolysis.

Materials and Methods:
The LPS-induced osteolysis in the mouse calvarian model was used to examine the effect of PAR in vivo. RANKL-induced osteoclast differentiation from RAW $_{264.7}$ cells and bone resorption were used to assess the effect of PAR in vitro. Assays for NF-κB activation, p65 translocation and IκB-α degradation were employed to determine the mechanism of action of PAR in osteoclasts and their precursors. Flow cytometry and confocal microscopic analysis were used to examine cell apoptosis. Semiquantitative RT-PCR were employed to determine PAR effect on RANK and TRAF6 gene expression.

Results:
We found PAR (0.5mg/kg and 1mg/kg), injected simultaneously with LPS (25mg/kg) or 3 days later, blocked LPS-induced osteolysis in the mouse calvarian. In vitro studies showed that low concentration PAR (less than 1μM) inhibited in-vitro osteoclastogenesis and bone resorption, whilst higher concentrations (greater than 5μM) dose-dependently triggered apoptosis. Furthermore, PAR inhibited LPS-induced NF-κB activation, p65 translocation and IκB-α degradation in both mature osteoclasts and their precursors. In addition, PAR inhibited NF-κB activation induced by osteoclastogenic factors RANKL, IL-1β or TNF-α, and reduced RANK and TRAF6 expression.

Conclusion:

The NF-κB pathway is known to mediate both osteoclast differentiation and survival. These findings indicate that PAR blocks LPS-induced osteolysis via the suppression of NF-κB activity; suggests a possible therapeutic value in bacteria-induced bone destruction.

O12 18.8.04: 1230 – 1245 hours

FUNCTIONAL RESPONSES OF BONE CELLS TO STIMULATION BY STRONTIUM

TC Brennan[1], MM Muir[1], AD Conigrave[2] & RS Mason[1]
[1] *School of Medical Sciences and Institute of Biomedical Research, University of Sydney, NSW*
[2] *School of Molecular and Microbial Sciences, University of Sydney, NSW.*

The divalent cation, strontium (Sr^{2+}) has been shown to increase bone formation, decrease bone resorption and reduce the risk of experiencing a fracture in postmenopausal women. The mechanisms underlying these actions of Sr^{2+} have not yet been elucidated. This study was aimed at evaluating some of the functional responses of the human osteoblast-like cell line, MG63, to stimulation by Sr^{2+}. MG63 cells were cultured in DMEM supplemented with 10% FBS then adapted to serum-free medium for 24 hours before experimental treatments were added. The tested concentrations of Sr^{2+} were 0.01 to 1 mM in the presence of 1 mM Ca^{2+}. Tritiated thymidine [^{3}H] incorporation was used to measure the proliferative responses of the cells to Sr^{2+}. Cell proliferation increased 2- to 3- fold as a function of increasing concentrations of Sr^{2+} ($P<0.001$). The EC_{50} was $\leq$ 0.1 mM. The differential expression of Receptor Activator of NFκB-ligand (RANKL) and osteoprotegerin (OPG), which regulate osteoclast generation, were also observed using real-time PCR (RT-PCR). The RANKL:OPG mRNA expression ratio was more than halved by 0.1 and 1 mM Sr^{2+} ($P<0.01$). The EC_{50} was $\leq$ 0.1 mM. OPG protein concentrations, determined using ELISA, were significantly increased in the presence of Sr^{2+} ($P<0.05$). Soluble RANKL protein (sRANKL) in culture supernatants was not detected. The data indicate that Sr^{2+} is a potent regulator of bone cell function in vitro as well as in vivo. The molecular target for Sr^{2+} is not yet determined.

O13 18.8.04: 1245 – 1300 hours

SECRETORY PHOSPHOLIPASE-A_2 ($sPLA_2$) INHIBITION PREVENTS OVARIECTOMY-INDUCED BONE LOSS IN ADULT RATS

LS Gregory, WL Kelly & MR Forwood
Department of Anatomy and Developmental Biology, School of Biomedical Sciences, University of Queensland, Brisbane, QLD

Introduction:
Secretory phospholipase A_2 ($sPLA_2$) mediates transcellular prostaglandin (PG) biosynthesis leading to an amplification of the PG signal. Whilst cytosolic PLA_2- ($cPLA_2$) inhibitors and COX-2 inhibitors abolish PGE_2 production induced by IL-1β in osteoblasts *in vitro*, $sPLA_2$ inhibitors affect PGE_2 synthesis only partially. $cPLA_2$ knockout mice have demonstrated an inhibition of osteoclast formation following IL-1 stimulation, however studies investigating $sPLA_2$ inhibition in the bone microenvironment are limited. The objective of this study was to investigate the effect of inhibiting $sPLA_2$ in a model of bone loss in the rat.

Materials & Methods:
Ninety-seven adult female Wistar rats, aged 9.5 $\pm$ 1 month, were ovariectomised (OVX) or sham-operated (sham). Rats commenced treatment 14 days after surgery with vehicle or a $sPLA_2$ inhibitor (KH064) at two doses: 0.4mg/kg/d and 4.0mg/kg/d. Treatment continued daily until rats were sacrificed at 70 or 98 days post-OVX. The right tibiae were harvested, fixed and embedded for structural histomorphometric bone analysis at the proximal tibial metaphysis.

Rsults & Discussion:
Ovariectomy induced a significant increase in bone formation rate (BFR/B.Ar), a significant loss of trabecular bone area and interconnectivity, and a marked increase in percentage resorption surface. KH064 significantly decreased BFR/B.Ar, marginally increased percentage trabecular area, and significantly decreased resorption surface, reversing many of the OVX-induced bone changes.

Conclusion:
Treatment with a $sPLA_2$ inhibitor significantly increased trabecular bone mass and restored trabecular connectivity post-OVX, preventing OVX-induced bone loss. This suggests that cytosolic- and secretory-PLA_2s are both important in the generation of prostaglandins required for catabolic bone metabolism.

O14 19.8.04: 0950 – 1000 hours

AN ASSOCIATION STUDY OF BMP4 POLYMORPHISMS IN POSTMENOPAUSAL WOMEN SHOWS EFFECTS ON BONE MASS

L Ramesh Babu[1,2], SG Wilson[2,3], IM Dick[1,2,3], FMA Islam[1,3], A Devine[1,2,3,4] & RL Prince[1,2,3]
[1] School of Medicine and Pharmacology, University of Western Australia, [2] Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, [3] Western Australian Institute of Medical Research, [4] School of Biomedical and Sports Sciences, Edith Cowan University, Perth, WA

The pathogenesis of osteoporosis is multifactorial with a strong genetic component. Twin and family studies have shown that heritability accounts for 50-80% of the variance in bone mass which is the single most important determinant of osteoporotic fractures.

Although linkage analysis and association studies have identified several candidate genes regulating BMD, they account for only a small percent of this variance. BMPs are multifunctional growth factors belonging to TGF β super family, which promote osteoblast differentiation and bone matrix formation.

We studied the association between polymorphisms of the BMP 4 gene, bone mass (DXA of hip and QUS heel) and prevalent and incident fracture rates over 5 years in a cohort of 1232 postmenopausal women mean age 75 years. Three SNPs (rs 1957860,rs 2855532 and rs17563) in the BMP 4 gene were genotyped by Pyrosequencing™.

Due to a non-synonymous SNP rs 17563 which codes for alanine(C allele) or valine (T allele) patients homozygous for the CC genotype (prevalence 32%) had lower BMD at the total hip site (3.1%(vs TT) and 2.3%(vs CT) p = 0.023), and intertrochanter site (3.7%(vs TT) and 2.8 % (vs CT) p=0.012). These differences persisted after adjustment for body mass and age. No polymorphisms showed association with the prevalence or incidence of clinical osteoporotic fractures.

A polymorphism found in the BMP4 gene, which alters the amino acid sequence, determines low bone mass presumably via an effect on matrix production.

O15 19.8.04: 1030 – 1040 hours

PTHRP AS A MEDIATOR OF DNA REPAIR IN CANCER

N Ilievska, S Bouralexis, RJ Thomas, TJ Martin & MT Gillespie
St. Vincent's Institute of Medical Research, Fitzroy, VIC

Parathyroid hormone-related protein (PTHrP) is widely expressed and its production by cancer metastases in bone promotes osteolysis. PTHrP also affects cellular functions including differentiation, proliferation and apoptosis. We have examined the potential roles of PTHrP upon breast, prostate and osteosarcoma cell lines. PTHrP was found to regulate several DNA repair (XPG, BRCA1, BRCA2 and Rad51), cell cycle (p21 and p53), and apoptosis-related genes. PTHrP peptides 1-34, 1-108, 106-139 or 122-139 did not affect mRNA or protein levels for these target genes, whilst PTHrP peptides (107-139 or 107-111 at 100 nM) encompassing osteostatin (TRSAW: 107-111) regulated DNA repair genes within 4 to 24 hrs: changes were also noted in protein levels. Mutant peptides of osteostatin (TRSPW, TRGAW, PRSAW, YRSAW, TKSAW and TASAW) identified a requirement for Thr107 and Ser109 for activity on DNA repair genes in MCF-10A cells.
Signal transduction pathway blockade revealed that PKA and PKC pathways were required for TRSAW effects on DNA repair genes.

Finally, TRSAW was determined to protect against etoposide induced DNA damage using COMET assays. TRSAW and peptides that elevated DNA repair gene expression protected against etoposide-induced DNA damage, whilst peptides that had no effect upon DNA repair gene expression did not induce DNA repair.

Combined, these data indicate a new function for PTHrP to protect against as well as mediate DNA damage repair, and this activity may well account for the widespread distribution of PTHrP, and its association as a prognostic indicator of survival in patients with breast cancer.

O16 19.8.04: 1040 – 1050 hours

ESSENTIAL ROLE FOR SIAH1A, BUT NOT SIAH2, UBIQUITIN LIGASE IN BONE GROWTH AND REMODELLING

NA Sims[1,2], IJ Frew[3], JMW Quinn[2], CR Walkley[3], RA Dickins[3], LE Purton[3], DDL Bowtell[3] & MT Gillespie[2]
[1] Department of Medicine at St. Vincent's Hospital, Fitzroy, VIC
[2] St. Vincent's Institute, Fitzroy,VIC
[3] Trescowthick Research Laboratories, Peter MacCallum Cancer Institute, East Melbourne, VIC

The seven in absentia homolog (Siah) proteins are E3 ubiquitin ligases that target degradation of protein substrates. Mice have three homologous Siah proteins: Siah1a, 1b and Siah2. Siah1a and b differ by only 6 of 282 amino acids, while Siah2 contains an extended N-terminal region. To characterise the roles of these proteins in bone metabolism in vivo, we analysed *Siah1a* and *Siah2* null mice.

Siah2KO mice are largely phenotypically normal, and while trabecular and cortical structure and bone turnover are normal, bone marrow from *Siah2KO* mice produced more osteoclasts in vitro than wild-type, indicating that *Siah2* regulates osteoclastogenesis, but this is compensated for in its absence.

Siah1aKO mice are growth-retarded and exhibit early lethality. They are also osteopenic; trabecular bone volume was half that of wild type mice. This was associated with low bone formation, including low osteoblast numbers and osteoid volume, while osteoclast numbers were more than doubled. However, haematopoietic osteoclast progenitor numbers and *ex vivo* osteoclast formation from *Siah1aKO* marrow were normal. Furthermore, adoptive transfer of *Siah1aKO* bone marrow into wild type mice failed to induce osteopenia or increase osteoclast numbers. While *ex vivo* osteoblast colony formation was normal in *Siah1aKO* mice, mineralization from these cells was elevated. These findings suggest that, unlike *Siah2, Siah1a* is essential for normal bone metabolism, yet the bone defect in *Siah1a* mutant mice is not due to cell-autonomous requirements for Siah1a in osteoblast or osteoclast formation. Bone metabolism defects in *Siah1aKO* mice appear to be secondary to alterations in an unidentified systemic or paracrine factor.

O17 19.8.04: 1050 – 1100 hours

DIETARY OMEGA-3 OILS STIMULATE BONE FORMATION IN VIVO

HA Morris[1], A Moore[1], A Mulaibrahimovic[1], LG Cleland[1,2] & MJ James[1,2]
[1] Hanson Institute, Adelaide, SA and [2] Rheumatology Unit, Royal Adelaide Hospital, Adelaide, SA

Modern Western diets are rich in omega-6 polyunsaturated fatty acids due to the extensive use of soybean and sunflower oils, whereas fish oils can provide dietary omega-3 polyunsaturated fats. Omega-6 and omega-3 fatty acids are substrates for cyclooxygeanse and lipoxygenase enzymes, each of which can synthesize both omega-6 and omega-3 eicosanoids. Animal models and human studies provide evidence that eicosanoids are involved in modulating bone metabolism. Sprague-Dawley female rats were fed AIN-76 semisynthetic diets containing 1% calcium and either 5% (w/w) sunflower oil (SO) (omega-6 fatty acids) or 5% fish oil (FO) (omega-3 fatty acids) for 4 months. Groups were also ovariectomised and/or fed diets containing 0.1% calcium with the respective oils. At 6 months of age rats were killed following administration of fluorescent labels. Distal femora were prepared for quantitative histomorphometry using established resin embedding techniques. In ovary-intact rats fed 1% calcium+FO, epiphysial BV/TV was increased by 34% over ovary-intact rats fed 1% calcium+SO (Mean; FO 43.2%, SO 32.3%; P= 0.008) as a result of a 29% increase of TbTh (Mean; FO 120 um, SO 93 um, P=0.001). Ovary-intact 1% calcium+FO rats had increased % double-labelled surface compared with ovary-intact 1% calcium SO rats (P<0.03) and increased bone formation rate (P<0.002). No effect of FO on trabecular bone or bone formation variables was detected in animals fed 0.1% calcium or ovariectomised rats. These data suggest that dietary fish oils can stimulate bone formation to increase bone volume in animals with low bone turnover and fed high dietary calcium.

O18 19.8.04: 1110 – 1110 hours

M-CSF PROMOTES PROLIFERATION OF HUMAN OSTEOCLAST PRECURSORS BUT INHIBITS RESORPTION BY MATURE OSTEOCLASTS AND IS NOT NECESSARY FOR RESORPTION ACTIVITY

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

Although M-CSF has an important role in osteoclast (OC) differentiation, conflicting data in animal and human models suggest that timing of exposure and concentration of M-CSF govern its effects. To clarify the role of M-CSF in human osteoclastogenesis, we used a model employing CFU-GM cultured for 14d on dentine slices treated with sRANKL, with and without added human M-CSF.

Time-course experiments with and without nocodazole demonstrated three phases: (1) d0-4, proliferation; (2) d5-7, differentiation and fusion; (3) d8-14, resorption. Endogenous M-CSF production allowed osteoclastogenesis with sRANKL alone but this was completely inhibited with M-CSF neutralising antibody. Continuous treatment with M-CSF for 14d had a biphasic effect on osteoclastogenesis, stimulating formation and resorption in a concentration-dependent manner up to 10-25 ng/mL, but potently inhibiting both at 100 ng/mL. In contrast, treatment with M-CSF after d5 produced no additional increase in OC number or resorption although OC size continued to increase. When treatment with M-CSF (up to 100 ng/mL) was restricted to d0-4, proliferation was stimulated in a concentration-dependent manner. Co-treatment from d5-14 with M-CSF antibody did not effect resorption, indicating that M-CSF is not necessary for resorption. When added to the resorption phase of the culture (d8-14), 50-100 ng/mL M-CSF inhibited resorption by 33%.

These data demonstrate that the timing of exposure and concentration of M-CSF are important factors governing human osteoclastogenesis. M-CSF is essential only in the early phase were it proliferates precursors, but it is not required for resorption to occur and, at higher concentrations, inhibits resorption.

O19 19.8.04: 1110 – 1120 hours

THREE-DIMENSIONAL MORPHOLOGY OF CRACK GROWTH IN CORTICAL BONE: IMPLICATIONS FOR BONE QUALITY

JS Kuliwaba[1,2], KH Zarrinkalam[1], MAB Wallwork[3], B Martin[4] & NL Fazzalari[1,2]
[1] Bone & Joint Research Lab, Div of Tissue Pathology, IMVS & Hanson Institute, [2] Dept of Pathology & [3] Adelaide Microscopy, Uni of Adelaide, Adelaide, SA, [4] Orthopedic Research Lab, UC Davis Medical Center, Sacramento, CA, USA

The material and structural components that determine a bone's resistance to fracture (i.e., bone quality) include: mass, geometry, micro-architecture, composition, mineralisation, and fatigue damage/microcrack accumulation. The process by which microcracks initiate, propagate, and ultimately coalesce leading to failure remains poorly understood. The aim of this study was to examine changes in microcrack morphology during crack propagation in cortical bone. Cracks were mechanically initiated and extended longitudinally in bovine tibial compact tension specimens (25x25x5mm). The sequential application of chelating fluorochromes, xylenol orange(XO) followed by calcein(CA), allowed the 3D nature of microcrack damage at different stages of propagation to be monitored by confocal microscopy. High-resolution confocal images provided a detailed visual description of the crack's '*process zone*', characterised by surface-associated diffuse microdamage surrounding the tip of the crack. This morphological feature represents a mechanism of energy dissipation. A clear distinction existed between the end of the first crack and the commencement of its extension. Confocal images of this '*interface region*' demonstrated that the extended CA-stained crack forms a continuum with the pre-existing crack and propagates through the former XO-stained process zone. 3D-reconstructed confocal images provided evidence for a submicroscopic tissue involvement in fatigue damage, in addition to the potential influence of vascular canals and osteocyte lacunae on its propagation through the bone matrix. This novel technique for monitoring crack growth in bone has provided new insight into crack shape characteristics which will enhance our understanding of the material properties of bone and thus the role of bone quality in predicting fracture risk.

O20 19.8.04: 1120 – 1130 hours

TARTRATE-RESISTANT ACID PHOSPHATASE AND CATHEPSIN K PROMOTERS USED TO DRIVE CRE RECOMBINASE EXPRESSION IN OSTEOCLASTS IN TRANSGENIC MICE

WSM Chiu[1], JF McManus[1], AJ Notini[1], AI Cassady[2], JD Zajac[1] & RA Davey[1]
[1] Department of Medicine, University of Melbourne, Austin Health, Heidelberg, VIC
[2] The Institute for Molecular Bioscience and Department of Biochemistry and Molecular Biology, The University of Queensland, QLD

Two transgenic mouse models have been generated with the aim of providing an *in vivo* tool to help study osteoclast biology. These two transgenic mouse models, namely TRAP (tartrate-resistant acid phosphatase)-Cre and Ctsk (cathepsin K)-Cre, express Cre recombinase under the control of either the TRAP or Ctsk promoter. The functionality of the Cre recombinase in these mice was examined by breeding them with GT-ROSA (R26R) reporter mice. The Cre-mediated recombination was assessed by the presence of β-galactosidase activity, indicated by blue colouration following the staining with X-gal. β-galactosidase activity was detected in all the transgenic mouse lines indicating that the Cre recombinase was functional. Both TRAP-Cre and Ctsk-Cre transgenic lines showed Cre-mediated recombination predominantly in long bones, calvaria, ribs and vertebrae, however, the Ctsk-Cre lines had a more restricted tissue distribution of Cre-mediated recombination compared to the TRAP-Cre lines. For example, recombination was seen in kidney and spleen in the TRAP-Cre lines while in contrast, it was absent in these tissues in the Ctsk-Cre lines. Furthermore, Cre-mediated recombination in Ctsk-Cre lines was only found in osteoclasts in bone but in the TRAP-Cre lines, the recombination was also found in proliferating and/or hypertrophic chondrocytes in addition to osteoclasts. These observations were further supported by expression of Cre recombinase protein, identified by immunohistochemistry.

In conclusion, TRAP-Cre and Ctsk-Cre transgenic mouse models have been generated and validated. They will be a valuable tool for studying the role of target genes in osteoclasts by using the Cre/*loxP* system.

O21 19.8.04: 1030 – 1040 hours

THE EFFECT ON BEHAVIOUR AND BONE MINERAL DENSITY OF INDIVIDUALISED BONE MINERAL DENSITY FEEDBACK AND EDUCATIONAL INTERVENTIONS IN PREMENOPAUSAL WOMEN: A RANDOMISED CONTROLLED TRIAL

T Winzenberg[1], B Oldenburg[3], M Riley[4], S Frendin[2], L De Wit[1] & G Jones[1]
[1] *Menzies Research Institute, Hobart, TAS*
[2] *Department of Health and Human Services, Hobart, TAS*
[3] *Queensland University of Technology, Brisbane, QLD*

This study aimed to determine the effects of individualised bone density (BMD) feedback and different educational interventions on osteoporosis preventive behaviour and BMD in pre-menopausal women over a 2-year period. A population-based sample of 470 healthy women aged 25-44 years (response rate 64%) received BMD feedback according to T-score and were randomly allocated to receive either an osteoporosis information leaflet or an osteoporosis self-management course (OPSMC). We measured changes in dietary calcium intake, calcium supplement use, smoking behaviour, physical activity, endurance fitness, lower limb strength and BMD. Women with low BMD had a greater increase in femoral neck BMD than those with normal BMD (+1.6% p.a. vs. +0.7% p.a., p=0.0001), but there was no difference in lumbar spine BMD change (+0.1% p.a. vs.+0.08% p.a., p=0.9). Both educational interventions had similar increases in BMD (Leaflet = +1.0% p.a., OPSMC = + 1.3% p.a., p=0.4). Femoral neck BMD change was only significantly associated with starting calcium supplements and persistent self-reported change in physical activity levels, with the BMD increase being +1.3 % p.a. (95%CI +0.49, +2.17) and +0.7% p.a. (95%CI +0.22, +1.22) with calcium supplement and physical activity change respectively. Individualised BMD feedback combined with a minimal educational intervention is effective at increasing hip but not spine bone density in premenopausal women. The changes in behaviour through which this was mediated are potentially important in the prevention of other diseases, thus measuring BMD at a young age may have substantial public health benefits, particularly if these changes are sustained.

O22 19.8.04: 1040 – 1050 hours

PLAYING GOLF IS ASSOCIATED WITH INCREASED VERTEBRAL BONE SIZE AND MASS IN POST MENOPAUSAL WOMEN

J Cook[1], K Singleton[2], J Black[2], RM Daly[2] & SL Bass[2]
[1] *School of Physiotherapy, La Trobe University,* [2] *Centre for Physical Activity and Nutrition Research, School of Exercise and Nutrition Sciences, Deakin University, Burwood, VIC*

A golf swing results in lateral bending that imparts shear, compressive and torsional strains on the spine. These forces, together with the rapid changes in the direction of their application and the intermittent nature of the game, create an ideal loading environment for an optimal osteogenic response. Thus we hypothesized that women who play regular golf would have greater lumbar spine bone traits than active controls.

Forty-eight postmenopausal women aged 62.2 (95% CI: 60.2, 64.1) years who had been playing golf for 16.9 (12.9, 21.0) years were compared to 35 active controls 60.7 (58.3, 63.1) years matched for age, height, weight, age of menopause, years post menopause and prior use of HRT. No subjects were currently using HRT. BMD, BMC and bone area were assessed by DXA.

Lumbar spine BMD, BMC and bone area were 11%, 16% and 5% greater in the golfers (table). The greater bone area was due to a 4% wider vertebral body (not height). No differences in BMD were detected at total body or hip.

Lumbar Spine (L1-L4)	Golfers (n=49)	Controls (n=35)
BMD (g/cm^2)	1.138 (1.099, 1.177)**	1.029 (0.971, 1.087)
BMC (g)	63.1 (60.0, 66.2)**	54.6 (50.5, 58.7)
Area (cm)	55.52 (53.98, 57.06) *	53.07 (51.39, 54.74)
Width (cm)	4.10 (4.02, 4.19) *	3.96 (3.87, 4.05)

[mean (95% CI)] **p<0.01, *p<0.05

In summary, playing golf was associated with increased lumbar spine BMC, BMD and bone width. In conclusion, regular golf may be an ideal exercise for reducing the risk of low vertebral bone strength in postmenopausal women.

O23 19.8.04: 1050 – 1100 hours

THE HEAVIER THEY ARE, THE HARDER THEY FALL?

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

The rising prevalence of obesity in Australia causes concern because morbidity risk rises with increasing body mass index (BMI). One advantage of increased body fatness, however, is the positive association with BMD and the potential protection against fracture. We determined the BMI (kg/m^2) and total hip BMD for an age-stratified, random sample of 628 women aged 60-94yr, enrolled in the Geelong Osteoporosis Study, 1994-7. 38.5% were overweight (BMI 25.0-29.9) and 24.6% obese (BMI≥30.0). Subjects were followed until 2002, or until sustaining a fracture (ascertained radiologically), death, or migration from the region.

BMD and BMI were positively correlated (r=0.53) and this remained significant after adjusting for age (both P<0.001). Using Cox proportional hazards models, the relative risk for fracture (RR) increased 1.2-fold for each SD decrease in BMI (RR=1.2, 95%CI 1.0-1.5). Similarly, the RR increased 1.9-fold for each SD decrease in BMD (RR=1.9, 1.6-2.4). Comparing overweight and obese women with women of the same age but normal BMI, overweight and obese women were increasingly protected against fracture, but differences were not significant (overweight RR=0.9, 0.6-1.4; obese RR=0.8, 0.5-1.3). Obesity protects against fracture through increased BMD. When the RR was further adjusted for BMD, a different pattern occurred. For women with the same age and BMD, the obese were at significantly higher risk of fracture than women with normal BMI (overweight RR=1.2, 0.8-1.8; obese RR=1.7, 1.0-3.0).

The cushioning effect of adipose tissue could absorb forces acting on bones. It is also likely that heavier body weights produce greater forces during a fall.

O24 19.8.04: 1100 – 1110 hours

ETHNIC DIFFERENCES IN EFFECT OF CALCIUM SUPPLEMENTATION ON BONE DENSITY IN PERIPUBERTAL GIRLS IN A DOUBLE-BLIND, PLACEBO-CONTROLLED, RANDOMISED TRIAL

C Rodda[1], M Urdampilletta[2], J Hu[2], B Strauss[3], E Briganti[4] & C Gilfillan[3]
Departments of [1]Biochemistry & Molecular Biology, [2]Paediatrics, [3]Medicine, [4]Epidemiology and Preventative Medicine, Monash University and Monash Medical Centre, Clayton, VIC

We proposed that ethnicity may influence the efficacy of calcium supplementation during the peripubertal years to significantly increase rate of bone mass accrual at this critical time. We recruited 93 healthy girls (40 Chinese; 53 Anglocelt) aged between 10 – 12 years, from local schools. Girls were matched for pubertal status and with total BMD within 2% and were randomised to receive either calcium carbonate 1.2 mg (n=45) (Caltrate, Whitehall) or placebo (n=48), using a randomized block design, for 1 - 4 years. Baseline and 6 monthly follow-up data included height, weight, sitting height, pubertal staging, date of menarche. Bone age, DXA total body and lumbar spine BMD and BMC were performed annually (Lunar DPX). Overall calcium supplementation did not increase lumbar spine or total body bone density. Those who participated in the study for more than two years showed significant increase in lumbar spine BMD (0.026g/cm^2, 3.2%, p=0.033). AC girls showed no significant benefit of supplementation, but supplemented Chinese girls showed significant benefit of 0.045 g/cm2 (5.6%, p=0.028) per year advantage over unsupplemented girls at the lumbar spine. Results suggest benefit of calcium supplementation for more than 2 years in Chinese peripubertal girls. These ethnic differences may due to genetic or dietary factors.

O25 19.8.04: 1110 – 1120 hours

HORMONE REPLACEMENT THERAPY AND MEASURES OF HIP STRENGTH: A CO-TWIN ANALYSIS

L Paton[1], T Beck[2], C Nowson[3], C Margerison[3], L Semanick[2], S Kantor[1] & JD Wark[1]
[1] *Department of Medicine, Royal Melbourne Hospital, University of Melbourne, VIC*
[2] *Department of Radiology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA*
[3] *School of Health Sciences, Deakin University, ACT*

Hormone replacement therapy (HRT) has been shown to improve bone density. Our objective was to estimate the difference in measures of bone strength of the proximal femur [hip structural analysis (HSA) in female twin pairs discordant for HRT use.

Forty-two twin pairs (17 monozygotic, 25 dizygotic) were identified, where one twin had more than 6 months of continuous HRT, mean 53 months (range 6 – 276 months), use and the other had no exposure to HRT. HSA strength parameters were evaluated from proximal femur densitometry scans at the narrowest segment of the femoral neck (NN), intertrochanteric (IT) and upper femoral shaft (FS) sites. All data were adjusted for age, height and weight. There were no significant within-pair differences in age, weight, height, dietary calcium intake, BMI, fat mass or lean mass with HRT use.

At the NN region there were significant within-pair differences in HSA derived parameters as follows comparing HRT users vs HRT non-users, respectively: cross-sectional moment of inertia (1.61 vs 1.46 cm^4, p = 0.003), subperiosteal width (2.90

cm vs 2.84 cm, p = 0.018), section modulus (1.02 cm^3 vs 0.96 cm^3, p = 0.016) and estimated endocortical diameter (2.62 cm vs 2.56 cm, p = 0.031). There were no significant differences in NN aBMD or HSA derived parameters including aBMD at the IT or FS regions.

These results suggest that HRT in routine clinical use may increase the bending and torsional strength of the bone in the NN region independently of bone mineral density.

O26 19.8.04: 1120 – 1130 hours

EARLY EXPERIENCE OF BISPHOSPHONATE USE IN ADOLESCENTS WITH OSTEONECROSIS

DG Little, RR Brown, E Goergens & CT Cowell
Children's Hospital at Westmead, Sydney, NSW

Traumatic osteonecrosis of the femoral head in adolescents has a poor prognosis due to collapse and degenerative change. We hypothesised that early bisphosphonate treatment could allow revascularisation and repair with maintenance of joint congruity.

Nine patients with osteonecrosis were treated with intravenous pamidronate (Aredia, Novartis) commencing within a mean 1 month of diagnosis (5 to 91days). There were 7 boys and 2 girls with mean age 13 years. The dosing protocol has evolved to 1.5 mg/kg second monthly for 18 months. Mean follow up is 25.3 months (18 to 38 months) with all patients followed for more 18 months. Six patients who presented after unstable slipped epiphysis, 2 with femoral neck fracture and one traumatic hip dislocation.

Eight patients are pain free. The mean Harris Hip score is 96.8 (91.7-100). Seven of 9 patients show no significant resorption of the femoral head at follow up. Of the two patients with significant resorption, one patient began to resorb on a lower dose of bisphosphonate, early in the series. The other patient had resorption of a section of the femoral head, which had not re-vascularised by 18 months. These two hips are pain free and functional in the short term, but their deformity is expected to bring about early osteoarthritis in adult life.

This early experience lays the foundation for prospective clinical trials of bisphosphonate therapy in adolescents with osteonecrosis. It appears that bisphosphonate treatment protocols for adolescents will need to be prolonged to around 18 months.

O27 19.8.04

THE BONE PROTECTION PROJECT: AN AUDIT OF IN HOSPITAL INVESTIGATION AND MANAGEMENT OF OSTEOPOROSIS FOLLOWING MINIMAL TRAUMA FRACTURE (MTF)

CP Gilfillan
Endocrine and Diabetes Research Centre, Frankston Hospital, VIC

The Bone Protection Project was a project undertaken in 6 sites across Australia with the aim of establishing rates at which secondary preventative measures were implemented following MTF. The study has the additional aim of developing protocols to improve the initiation of proven anti-fracture treatment is such patients. 595 patients with MTF were subject to file audit. 76% were women and 24% men. Fracture incidence increased with age peaking in the 80's in both men and women. Hip fracture was the most common fracture (38%) followed by wrist in women (27%). Only a small number of clinical vertebral fractures were seen in this inpatient population. 36% of patient had a previous fracture, but despite this only 7% were taking calcium and only 1% taking vitamin D supplement. Only 8.6% were on any bisphosphonate. Whist in hospital 19% had a serum calcium measured but only 4% had their vitamin D status assessed. Only 3% had any osteoporosis therapy commenced in hospital. No patients were referred to endocrine, rheumatology or metabolic bone disease clinics for follow-up. At Frankston Hospital we have followed up 130 patients by telephone 12 months after their fracture. Of the 130 contacted, 9 (6.7%) were deceased, 26 (19.8% of total) patients had undergone a DEXA scan, 19 (14.4%) had been commenced on an osteoporosis drug. 5 patients (3.7%) were on calcium supplementation, only 2 (1.5%) were on vitamin D. We intend to randomly assign patients to a protocol of in hospital assessment, DEXA scanning, treatment initiation and telephone follow-up to see whether this poor rate of secondary prevention of MTF can be improved.

O28 19.8.04

RPAH FIRST FRACTURE CLINIC

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

Previous work at many centres has shown that patients who suffer their first osteoporotic fracture are inadequately managed in terms of osteoporosis care. Currently available therapies have been shown to greatly reduce the risk of subsequent fracture in patients who have already fractured. We have set up a clinic to assess patients presenting to the Orthopaedic Fracture Clinic, to assess BMD, detect underlying contributors to osteoporosis, and to instigate therapy where appropriate. A dedicated Osteoporosis Nurse (ON) attends Orthopaedic Fracture Clinics and identifies patients with low trauma fractures. A standardised history is taken and BMD and appropriate blood testing is performed, with the results entered into a custom-built computer database. Patients are then assessed by a medical officer and treatment commenced if appropriate. The ON then telephones the patient after one month to check compliance and assess problems, and arrangements are made for BMD and review in one year. Of the first 110 patients completing assessment, 40% (29%) were osteoporotic at the lumbar spine (femur) with the majority of the remainder osteopenic. Inadequate calcium intake and suboptimal vitamin D were present in most patients. Therapy was commenced in most patients (calcium 56%, vitamin D 61% and oral or intravenous bisphosphonate therapy in 72%). We expect that this intervention will reduce incidence of future fracture and thereby minimise associated morbidity, mortality and economic cost.

Start-up funding for our ON has been provided by MSD.

O29 19.8.04: 1415 – 1425 hours

OPG MUTATIONS THAT CAUSE IDIOPATHIC HYPERPHOSPHATASIA IMPAIR OPG PROTEIN SECRETION

D Naot, CA Middleton-Hardie, H Cundy, IR Reid, T Cundy & J Cornish
Department of Medicine, University of Auckland, Auckland, New Zealand

Genetic studies have recently established a link between mutations in OPG and idiopathic hyperphosphatasia (IH). IH is a rare bone disease characterised by increased bone turnover, with considerable phenotypic variation among affected families. The aim of our study was to investigate genotype-phenotype correlation between specific mutations, the function of the mutant proteins and the clinical presentation of the patients.

The patients were grouped into mild, intermediate and severe phenotype according to clinical, biochemical and radiographic data. We have produced constructs corresponding to four mutations: two from patients with intermediate disease (OPGΔD182 and OPGF117L) and two with severe disease, where a cysteine residue is replaced with arginine (OPGC65R) or tyrosine (OPGC87Y). When expressed in HEK293 cells, none of the constructs had altered rates of cell proliferation, and measurement of OPG mRNA levels by real-time PCR demonstrated that all constructs were transcribed with comparable efficiency. Similar levels of OPGF117L protein, compared to wild-type OPG, were secreted into the medium. OPGΔD182 had lower secretion levels, while only very low levels of OPGC65R and OPGC87Y could be detected in the medium. OPGΔD182 is hyperglycosylated and is detected on gels as a much larger glycoprotein, while all the other mutants are similar in size to the wild-type OPG.

The various OPG mutations identified in IH families and the phenotypic variation of the disease offer a unique opportunity for a structure-function study of OPG. Our investigations suggest an impaired intracellular processing of some of the OPG mutations, particularly the ones associated with the severe IH phenotype.

O30 19.8.04: 1425 – 1435 hours

L-AMINO ACID REGULATION OF PARATHYROID HORMONE SECRETION

AD Conigrave[1], H-C Mun[1], L Delbridge[2], M Wilkinson[2], SJ Quinn[3] & EM Brown[3]
[1] School of Molecular and Microbial Sciences, University of Sydney, NSW, Departments of Surgery and Endocrinology, [2] Royal North Shore Hospital, St Leonards, NSW, [3] Brigham and Womens Hospital and Harvard Medical School, Boston, MA, USA

We previously showed that L-amino acids activate the extracellular Ca^{2+}-sensing receptor (CaR) in HEK-293 cells (Conigrave et al.., 2000). We have now completed an analysis of amino acid effects on CaR activation and PTH secretion from normal human parathyroid cells and conclude that L-amino acids are physiological regulators of PTH secretion. Samples of normal human parathyroid transplants were obtained at neck surgery according to institutional ethics guidelines. Viable parathyroid cells were prepared by collagenase digestion. For studies of receptor dependent mobilization of cytoplasmic free Ca^{2+} concentration, cells were loaded with fura-2 and mounted in a microfluorimetry apparatus. For studies of PTH secretion, around 50,000 cells were loaded into a column of Sephadex G-25 (nominal cut-off 5 kDa) and perifused with physiological saline solutions containing a plasma-like amino acid mixture at 1.5 mL/min at 37 C. For each solution, three 2 min samples were collected and stored at –80 ˚C prior to analysis for intact PTH using an Immulite 2000 Autoanalyser. L-amino acids including the aromatics, L-Phe, L-Trp and L-His and the aliphatic, L-Ala stereoselectively activated intracellular Ca^{2+} mobilization, enhanced receptor sensitvity to extracellular Ca^{2+} concentration and suppressed PTH secretion. CaR-inactive amino acids including L-Arg and L-Leu were without effect. In addition, a physiologically

relevant increase in the fold concentration of a plasma-like amino acid mixture (from 1x to 2x) activated Ca^{2+} mobilization and suppressed PTH secretion. The data support the conclusion that amino acids acutely and reversibly regulate PTH secretion and thus whole body calcium metabolism.

Conigrave, A.D., Quinn, S.J., Brown E.M. (2000) PNAS 97, 4814-9.

O31 19.8.04: 1435 – 1445 hours

IL-13 INHIBITS OSTEOCLAST DIFFERENTIATION AND ENHANCES DENDRITIC CELL FORMATION IN HUMAN CFU-GM CULTURES

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

Interleukin-13 (IL-13) is a pleiotropic cytokine expressed by T-helper and CD8+ cells that down-modulates macrophages and promotes dendritic cell (DC) differentiation of monocytes. It has been reported to inhibit murine osteoclast differentiation. The aim of this study was to characterise the effects of IL-13 on osteoclastogenesis in a human model.

Human osteoclasts were generated by culturing CFU-GM with dentine slices in the presence of human M-CSF (25 ng/ml) and soluble RANKL (125 ng/ml) for 14 days. Co-treatment with IL-13 caused a dose-dependant inhibition of osteoclast formation and resorption with maximal inhibition of 98% at 10 ng/ml, EC_{50} 3 ng/ml. Time-course experiments showed that the potent inhibitory effect of IL-13 was only seen if treatment was commenced in the first 2 days and continued for at least 7 days. However, a disproportionate inhibition of resorption was seen even when IL-13 treatment was not begun until day 5, or begun at day 0 and removed before day 7. Cultures treated with IL-13 developed clusters of CD1a-positive cells, consistent with DC formation and showed a 10-fold down-regulation of cFos at 3 days.

Thus IL-13, in the presence of M-CSF and RANKL, is a potent direct inhibitor of osteoclastogenesis from human CFU-GM, instead promoting DC differentiation. These results provide another mechanism by which lymphocytes can modulate osteoclast differentiation. The effect of IL-13 is similar to that of GM-CSF but not IL-4, which inhibits osteoclastogenesis without DC formation, despite the sharing of a predominant signaling chain (IL-4Rα) by IL-4 and IL-13 receptors.

O32 19.8.04: 1445 – 1455 hours

LACTOFERRIN RECEPTORS AND SIGNALLING PATHWAYS IN OSTEOBLASTS

Q Zhu[1], A Grey[1], T Banovic[1], K Callon[1], M Watson[1], J Bracegirdle[1], K Palmano[2], IR Reid[1] & J Cornish[1]
[1] *Department of Medicine, University of Auckland, Auckland, New Zealand*
[2] *Fonterra Research Centre, Palmerston North, New Zealand*

Lactoferrin (Lf) is an 80 kDa glycoprotein that stimulates bone formation *in vivo*. *In vitro*, Lf activates p42/44 MAP kinase and PI3K-dependent Akt signalling, actions which may underpin its ability to promote the proliferation and survival of osteoblastic cells.

Lf binds to LRP1 and LRP2/megalin, two endocytic members of the low density lipoprotein receptor family that includes LRP5 and LRP6, which are known to regulate osteoblast function. LRP1 and LRP2 are expressed in several osteoblastic cell types. In primary rat osteoblastic cells, the LRP1/2 inhibitor receptor associated protein (RAP) blocks endocytosis of lactoferrin, and abrogates lactoferrin-induced p42/44 MAP kinase signalling and mitogenesis. Lactoferrin-induced mitogenesis is also inhibited by an antibody to LRP1. In SaOS-2 cells, which express LRP1 but not LRP2, lactoferrin-induced proliferation and p42/44 MAP kinase signalling are sensitive to RAP, and Lf-induced activation of p42/44 MAP kinase signalling is inhibited by expression of dominant negative Ras. The mitogenic response of LRP1-null fibroblastic cells to lactoferrin is substantially reduced compared to that of cells expressing wild-type LRP1. Thus, mitogenic signalling through LRP1 via Ras to p42/44 MAP kinases contributes to the anabolic effects of lactoferrin on osteoblastic cells. LRP1 also mediates the internalization of Lf by osteoblastic cells. However, the endocytic and signalling functions of LRP1 are independent, since lactoferrin can activate mitogenic signalling in conditions in which endocytosis is inhibited. These data identify a mechanism by which lactoferrin exerts its anabolic effects in bone, and suggest that a third LRP receptor (LRP1) may signal osteoblast anabolism.

O33 19.8.04: 1455 – 1505 hours

BONE DENSITY RELEVANCE AND INTERPRETATION IN CAUCASIAN CHILDREN AGED 9-16 YEARS OF AGE: INSIGHTS FROM A POPULATION BASED FRACTURE STUDY

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

The interpretation of bone density measurement in children is more difficult than in adults due to a number of factors. This study asked two questions: Is there a preferred bone density measurement site or type using DXA for fracture risk in children; and what is the best way to interpret bone density in children? This population based case control study included 321 upper limb fracture cases and 321 class and sex matched randomly selected controls. Bone density at the hip, spine, total body and arm was measured by a Hologic QDR2000 densitometer (DXA) and examined as bone area (BA), bone mineral content (BMC), bone mineral density (BMD), bone mineral apparent density (BMAD) and BMD/lean mass (BMDLM). The only DXA variables that were consistently significantly associated with total fracture risk in both boys and girls were spine BMD (AUC 0.56-0.58) and spine BMAD (AUC all 0.59) for total upper limb fractures and BMAD at the spine (AUC 0.60-0.63) and hip (AUC 0.58-0.60) for wrist and forearm fractures. No significant associations were observed for BA and BMDLM and inconsistent associations for BMC and other BMD sites. In controls only, all DXA variables were associated with age, height and weight but the weakest association was with BMAD. In conclusion, spine BMAD has the strongest and most consistent association with upper limb fracture risk in this sample of children. The associations with age imply that age and sex specific Z scores will be most appropriate for interpretation of DXA measures in children.

O34 19.8.04: 1505 – 1515 hours

ASYMPTOMATIC VERTEBRAL DEFORMITY AS MAJOR RISK FACTOR FOR SUBSEQUENT FRACTURES AND MORTALITY: A 14-YEAR PROSPECTIVE STUDY

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

The aim of the study was to examine the inter-association between asymptomatic vertebral deformity, osteoporotic fractures and risk of mortality in elderly men and women.

In 1992, radiographs (X-ray) at the lumbar spine were performed on 300 individuals (113 men and 187 women) aged 60 or above at baseline, who were randomly selected from the Dubbo Osteoporosis Epidemiology Study. The presence of vertebral deformity was defined as a reduction of at least 3 standard deviations (SD) from same-sex normals for each vertebrae. Baseline bone mineral density (BMD) was measured by DXA. Incidence of mortality and atraumatic fractures was ascertained during the study period of 1989 and 2003.

At baseline, the prevalence of asymptomatic vertebral deformity was 31% in men and 17% in women. During the follow-up period, subjects with vertebral deformity had a significantly higher risk of all types of osteoporotic fractures than those without vertebral deformity (44% vs. 29%; relative risk (*RR*), 2.1 [95%CI, 1.2-3.7]), particularly symptomatic vertebral fracture (*RR,* 7.4 [3.0-17.7]). Mortality rate was highest after a symptomatic fracture among those with vertebral deformity (*RR*, 9.6 [3.0-30.6]). These associations remained virtually unchanged after adjusting for age, BMD and gender.

Therefore, vertebral deformity was a strong predictor of subsequent risk of fractures, particularly symptomatic vertebral fracture, and a risk factor for fracture-associated mortality in both elderly men and women.

O35 19.8.04: 1515 – 1525 hours

SHOULD ALL OLDER PEOPLE IN RESIDENTIAL CARE RECEIVE VITAMIN D TO PREVENT FALLS? RESULTS OF A RANDOMISED TRIAL

L Flicker[1], R MacInnis[2], M Stein[3], S Scherer[4], K Mead[5], C Nowson[6], J Thomas[1], C Lowndes[5], JL Hopper[5] & JD Wark[5]
[1] University of WA, Perth, [2] The Cancer Council Victoria, Melbourne, [3] Royal Melbourne Hospital, Melbourne, [4] Royal Freemasons' Homes, Melbourne, [5] University of Melbourne, Melbourne, [6] Deakin University, Melbourne, VIC

The aim of this study was to test whether vitamin D supplementation could reduce the risk of falls and fractures in a residential care setting.

2454 nursing home and 1797 hostel residents were approached. Subjects with 25-hydroxyvitamin D (25D) levels less than 25 nmol/l or greater than 90 nmol/l were excluded. Subjects were randomised to ergocalciferol (initially 10,000 IU per week, then 1,000 IU per day) or matching placebo. All subjects received 600 mg of elemental calcium as calcium carbonate. Residents, institutional and study staff were blinded to treatment allocation. Logistic regression and negative binomial models were used to estimate the effect of vitamin D supplementation.

601 subjects entered the intervention phase. The two randomised groups had similar baseline characteristics. The Odds Ratio (OR) [95% CI] for the effect of vitamin D supplementation on the risk of ever falling was 0.85 [0.61-1.17] and was 0.72 [0.42-1.25] on the risk of ever fracturing. The incident rate ratio between groups for falls was 0.78 [0.60-1.00]. Restricting the analyses to the 534 subjects whose compliance with D therapy was ≥ 50% produced an OR of 0.71 [0.50-1.00] for ever falling and of 0.68 [0.42-1.25] for ever fracturing. The incident rate ratio between groups for falls was 0.67 [0.52-0.88].

We conclude that vitamin D supplementation reduces the risk of falls and possibly of fractures in older people in residential care, whose 25D level is greater than 25 nmol/l. This effect was apparently additional to any effect of calcium supplementation but may depend on calcium.

O36 19.8.04: 1525 – 1535 hours

THE FEMORAL NECK IS ELLIPSOID. ASSUMPTION OF CIRCULARITY INTRODUCES ERRORS IN FN VOLUME, VOLUMETRIC BONE MINERAL DENSITY, AND BONE STRENGTH

R Zebaze[1], F Welsh[2], S Iuliano-Burns, A Evans, J Konstantynowicz & E Seeman[1]
[1] *Austin Hospital, University of Melbourne, Melbourne, VIC*
[2] *University of Aberdeen, Aberdeen, UK*

Comparisons of femoral neck dimensions, volumetric BMD and bone strength between sexes, races, patients with and without fractures use estimates derived from bone densitometry. However, the assumptions under which these parameters are calculated remain untested. Volumetric BMD calculation assumes that the FN is a parallelepipedal ($vBMD_p$) or a cylinder structure ($vBMD_c$). We used DXA in 26 Caucasian female cadavers mean age of 69.2 (range 29 - 85 years) and directly measured FN dimensions using a caliper and Archimedes principle. FN diameter measured by DXA as an average FN 3.37 ± 0.4 or mid-FN value 3.37 ± 0.1 were no different from FN width measured ex-vivo 3.51 ± 0.13 cm. Femoral axis length and neck shaft angle measured by DXA and directly did not differ (9.61 ± 0.2 vs 9.65 ± 0.22 cm and 123.5 ± 0.21° vs 123.6 ± 0.19°respectively). However, FN depth was always lower than the width by 25% (range 14 - 36%). Thus, DXA underestimated FN vBMD by 41% for $BMAD_p$ (0.214 ± 0.02 vs 0.364 ± 0.03 g/cm^3) to 20% for $BMAD_c$ (0.272 ±0.01 vs 0.364 ± 0.03 g/cm^3). Smaller bones were more circular, bigger bones were more ellipsoid so the bigger the bone, the worse the FN vBMD under-estimation. Adjustments of the DXA estimates assuming that the FN was ellipsoidal ($BMAD_e$) with the bigger axis being the FN width and the smaller axis being the depth (0.75 depth/width) resulted in no difference between $BMAD_e$ and FN vBMD (0.359 ± 0.02 vs 0.364 ± 0.03 g/cm^3). We infer that DXA overestimates volume, and underestimates FN vBMD and this may have an impact on fracture prediction. (ii) Estimates assuming a cuboidal structure should be abandoned in favour of an ellipsoidal structure.

O37 19.8.04: 1600 – 1610 hours

VITAMIN D METABOLISM IN HUMAN OSTEOBLASTS

GJ Atkins[1], PH Anderson[2], K Welldon[1], ACW Zannettino[3], PD O'Loughlin[2], HA Morris[4] & DM Findlay[1]
[1] *Department of Orthopaedics and Trauma, University of Adelaide, Adelaide, SA*
[2] *Division of Endocrinology, Institute of Medical and Veterinary Science, Adelaide, SA*
[3] *Division of Haematology, Institute of Medical and Veterinary Science, Adelaide, SA*
[1-4] *Hanson Institute, SA*

Circulating 1α,25-dihydroxyvitamin D_3 (1,25D) derives from conversion of 25-hydroxyvitamin D_3 (25D) by the kidney 1α-hydroxylase (CYP27B1). 1,25D exerts a number of effects on human osteoblasts, including inhibition of proliferation and differentiation, and effects on gene expression, including stimulation of RANKL,[(1)] osteocalcin (OCN) and bone sialoprotein (BSP). We have examined vitamin D_3 metabolism in human osteoblastic cells, and found that primary normal human osteoblasts (NHBC) up-regulate the negative regulator of 1,25D, the 24-hydroxylase (CYP24), in response to 1,25D exposure. Additionally, NHBC expressed CYP27B1 mRNA encoding the 1α-hydroxylase, implying that human osteoblasts are capable of metabolising 25D into 1,25D. We have investigated this possibility and found that NHBC exposed to physiological concentrations of 25D (10 – 100 nM) in the absence of serum, exhibit up-regulated transcription of the downstream genes RANKL and OCN. Unlike 1,25D, 25D did not elicit a vigorous CYP24 response except at high concentrations (10^{-7} – 10^{-6} M). Consistent with this, NHBC treated with high concentrations of 25D secreted detectable 1,25D into the culture supernatant. We also found that NHBC express the 25-hydroxylase, and treatment with 1-hydroxyvitamin D_3 (1D), resulted in a gene expression response qualitatively similar to 25D. Inhibition of CYP activity using ketoconazole (10 μM) resulted in an elevated response to 1,25D, probably due to inhibition of the catabolic activity of CYP24. Results to date indicate that the activity of 1D is CYP-dependent, since ketoconazole abolished its effects. However, 25D effects at low concentrations were unaffected by ketoconazole, indicating that 25D may have direct effects in osteoblasts independent of its conversion to 1,25D. Our results suggest that vitamin D_3 metabolism represents an intrinsic autocrine/paracrine pathway in these cells. Thus, vitamin D metabolites may regulate key functions in human osteoblasts independently of circulating levels of 1,25D.

1.Atkins GJ, et al. 2003 RANKL expression is related to the differentiation state of human osteoblasts. J Bone Miner Res **18:**1088.

O38 19.8.04: 1610 – 1620 hours

PROSTAGLANDIN E_2 MEDIATES IL-6 PRODUCTION BY THROMBIN-STIMULATED OSTEOBLASTS

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

We have previously shown that treatment of serum-deprived or dexamethasone-treated primary mouse calvarial osteoblasts with 100 nM thrombin significantly reduces apoptosis in these cultures and that this effect is not mediated by any of the known thrombin receptors (PAR-1, -3 and –4) but is mediated by a secreted inhibitor of apoptosis.

In this current study we have used quantitative PCR and ELISA/EIA to study the expression of IL-6, cyclooxygenase-2 (COX-2) and prostaglandin E_2 (PGE_2) following thrombin treatment of PAR-1 null and wild-type primary osteoblasts. Six hours after thrombin treatment of PAR-1 null osteoblasts, expression of COX-2 mRNA had increased approximately 5 fold over levels in untreated cultures. Correspondingly, PGE_2 levels as well as IL-6 mRNA and protein were also significantly elevated in comparison to untreated wild-type and PAR-1 null cultures at this time point. Interestingly, pre-treatment of cultures with COX inhibitors such as indomethacin not only prevented the increase in PGE_2 in medium conditioned by thrombin-treated osteoblasts but also the increase in IL-6. Furthermore treatment of serum-deprived wild-type primary osteoblast cultures with1 μM PGE_2 caused a significant increase in the levels of IL-6 in medium conditioned by serum-deprived osteoblasts.

These results suggest that thrombin stimulates COX-2-mediated PGE_2 production by serum-deprived osteoblasts by a mechanism at least partially independent of PAR-1, and that the rise in PGE_2 causes the synthesis and secretion of IL-6 by serum-deprived osteoblasts. We are currently investigating what role these events may play in the inhibition of osteoblast apoptosis by thrombin.

O39 19.8.04: 1620 – 1630 hours

HYDROGEN PEROXIDE ACCELERATES HUMAN OSTEOCLAST DIFFERENTIATION AND UP-REGULATES THIOREDOXIN

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

Signaling pathways important for osteoclast (OC) differentiation, including NF-κB and AP-1, are enhanced by the endogenous redox-sensitive thiol, thioredoxin. We have investigated the role of redox in OC differentiation using the ROS-donor H_2O_2.

Human osteoclasts were generated by culture of CFU-GM with dentine slices for 14 days with sRANKL and hM-CSF (25 ng/mL). In the presence of 125 ng/mL sRANKL, co-treatment with 1 nM H_2O_2 increased OC number and size by 70-80% at 7 days. Resorption was maximally increased by 140% by 10 nM H_2O_2. In the presence of 31 ng/mL sRANKL, H_2O_2 dose-dependently increased OC number and resorption with maximum effects at 100 nM (+170% and +200%, respectively). Time-course studies showed that H_2O_2 was only necessary for the first 24 hours of culture to achieve a maximum stimulatory effect on osteoclastogenesis. The resorption rate of mature OC was not increased by H_2O_2 treatment. Treatment of precursors with 100 nM H_2O_2 produced a 4-fold increase in thioredoxin mRNA at 12 hours as quantified by real-time PCR.

These results show that oxidative stress accelerates early differentiation events in human OC precursors in synergy with sRANKL, and up-regulates thioredoxin expression. The latter is likely to facilitate RANKL/RANK signaling and mediate the effect of H_2O_2. In this *in vitro* model, OC generation is finite because a finite number of precursors are present. However, *in vivo*, oxidative stress is likely to produce a steady-state of increased OC numbers.

O40 19.8.04: 1630 – 1640 hours

EXPRESSION OF TENASCIN-W AS A POTENTIAL TENASCIN-C REPLACEMENT IN THE SKELETON OF TENASCIN-C KNOCKOUT MICE

BI Morrison[1], A Scherberich[2], R Chiquet-Ehrismann[2], CN Pagel[1] & EJ Mackie[1]
[1] *School of Veterinary Science, University of Melbourne, Parkville, VIC*
[2] *Friedrich Miescher-Institut, Basel, Switzerland*

The tenascin (TN) family of extracellular matrix proteins, consisting of five identified members (C, R, X, W, and Y), has been linked to a variety of developmental and regulatory roles in various species. TN-C and TN-W are the only tenascins that have been identified in skeletal tissue. Contrary to initial predictions, TN-C knockout (TN-C -/-) mice appear to have normal skeletal development. It is hypothesized that TN-W may act in a compensatory role in the TN-C -/- mouse. Immunohistochemical staining for TN-C and TN-W has been performed on embryos at embryonic day (E) 12, 14, 16, and 18, and on hindlimbs from 4-, 8-, and 12-week-old TN-C +/+ and TN-C -/- mice. Adjacent sections were stained for alkaline phosphatase activity, a marker of osteoblast differentiation. TN-W expression was detected from E12 in developing membrane bones of the skull and from E14 in the perichondrium of developing endochondral bones. From E16, TN-W expression was also observed in tendinous insertions on developing bones. At all postnatal ages examined, TN-W was only detected at periosteal surfaces, and appeared to be enriched at sites of insertion of muscle on bone. The intensity and distribution of staining for TN-W and TN-C did not appear to differ between the TN-C +/+ and TN-C -/- mice at any stage of development. The results so far point to a role for TN-W in bone development and maintenance but no evidence has pointed to a compensatory effect of TN-W in the TN-C -/- mouse.

O41 19.8.04: 1640 – 1650 hours

N-ACETYLCYSTEINE INHIBITS HUMAN OSTEOCLAST DIFFERENTIATION VIA MODULATION OF THE THIOREDOXIN SYSTEM

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

Redox-sensitive cell signaling regulates many cellular processes. Signaling pathways required for Osteoclast (OC) differentiation, including AP-1 and NF-κB, are under redox regulation and their activity is enhanced by the ubiquitous and multifunctional thiol protein, thioredoxin (Trx).

We have used a human osteoclastogenesis model to examine the effect of the ROS-scavenger N-acetylcysteine (NAC) on osteoclast differentiation and resorption activity. Human CFU-GM precursors cultured in the presence of human M-CSF and soluble RANKL differentiate into resorbing osteoclasts in 10-14 days while in the presence of M-CSF alone, macrophages develop. The resorptive activity of mature OC was measured on dentine substrate. Western Blots were used to measure OC expression of Trx and its negative regulator, Trx binding protein-2 (TBP-2).

At 7 days, expression of Trx protein was higher, and expression of TBP-2 lower, in cultures treated with RANKL and M-CSF compared to those treated with M-CSF alone. In cultures treated with RANKL and M-CSF, co-treatment with 10 mM NAC, reduced Trx and increased TBP-2 expression. Additionally, in 14d osteoclastogenesis cultures, NAC dose-dependently reduced OC number and resorption (IC_{50} ~1mM) with 10mM producing a maximum effect on both formation (-99.3%) and resorption (-99.94%). Osteoclast size was also dose-dependently reduced.

Our results show that Trx and TBP-2 are differentially regulated in osteoclast compared to macrophage differentiation and that NAC treatment is associated with down-regulation of Trx and up-regulation of TBP-2. Furthermore, NAC potently inhibits osteoclast differentiation. This inhibitory effect is probably mediated by inhibition of RANKL/RANK signaling secondary to modulation of Trx and its inhibitor TBP-2.

O42 19.8.04: 1650 – 1700 hours

GENERATION OF A CALCITONIN RECEPTOR (CTR) LOXP MOUSE LINE FOR TARGETED DELETION IN OSTEOCLASTS

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

Our aim is to investigate the physiological control of bone resorption and formation by calcitonin (CT), by generating mouse models in which the calcitonin receptor (CTR) is either deleted specifically in osteoclasts or in all tissues.

Exons 13 and 14 of the CTR, encoding the seventh transmembrane domain and C-terminus, were cloned from a SV129/J mouse genomic library. A CTR-*lox*P targeting construct was generated. The targeting construct was electroporated into

SV129/J ES cells and 500 neomycin resistant colonies screened by PCR for homologous recombination events. Six targeted ES cell clones were identified. One was injected into SV129/J blastocysts, resulting in 3 chimeric offspring. Chimeras were backcrossed to C57Bl/6 mice to confirm germline transmission.

Homozygous CTR-*lox*P mice were bred with CMV-Cre mice, which express Cre almost ubiquitously, to generate global CTR knockouts (KO). We have demonstrated Cre-mediated deletion of exons 13 and 14 of the CTR in the bones of CTR KO mice. We are currently assessing the effect of deleting exons 13 and 14 of the CTR on receptor function by measuring the serum calcium response of these CTR KO mice to CT. The bone phenotype of CTR KO mice is being determined by bone histomorphometry. Mouse models in which the CTR is deleted specifically in osteoclasts will be generated by breeding the CTR-*lox*P mice with TRAP-Cre and Ctsk-Cre mice (1). In conclusion, these mouse models will provide valuable insight into the regulation of bone formation and resorption by CT.

(1) WSM Chiu, JF McManus, AJ Notini, AI Cassady, JD Zajac, RA Davey. 2004 Transgenic mice that express Cre recombinase in osteoclasts. Genesis (in press).

O43 19.8.04: 1700 – 1710 hours

THE EFFECT OF LACTOFERRIN ON RANKL AND OPG mRNA LEVELS IN BONE CELLS

PC Tong, D Naot, U Bava, JM Lin, M Watson, KE Callon, IR Reid & J Cornish
Department of Medicine, The University of Auckland, Auckland, New Zealand

Lactoferrin is an 80 kDa iron-binding glycoprotein that belongs to the transferrin family. High levels of lactoferrin are found in breast milk, epithelial secretions, and in the secondary granules of neutrophils. In the serum, lactoferrin circulates at concentrations of 2-7 μg/ml. Lactoferrin is a multifunctional protein with antimicrobial activity, it affects embryonic development, myelopoiesis, cell adhesion, cytokine production and it also has anti-inflammatory and immunoregulatory activities.

We have recently established that lactoferrin is anabolic to bone at physiological concentrations. Lactoferrin stimulates the proliferation, survival and differentiation of primary osteoblasts, and also acts as a potent inhibitor of osteoclastogenesis. One possible mechanism for the inhibition of osteoclast formation is by affecting the levels of gene expression in the osteoblast to decrease the RANKL/OPG ratio. We used real-time PCR to study the effects of lactoferrin on RANKL and OPG gene expression in two in vitro models; a primary human osteoblast cell culture and a mixed cell population derived from murine bone marrow. In the human osteoblast cultures lactoferrin induced an increase in the levels of RANKL mRNA. In contrast, in the bone marrow cultures where RANKL expression is induced by vitamin D, lactoferrin had an inhibitory effect on RANKL induction. In both experimental systems lactoferrin treatment produced a small decrease in the levels of OPG.

Our results suggest that in the bone marrow environment, where the pre-osteoblasts are in close contact with other cell populations, inhibition of RANKL expression might be one pathway by which lactoferrin inhibits osteoclastogenesis.

O44 19.8.04: 1600 – 1610 hours

PREDICTION OF HIP FRACTURE IN THE ELDERLY BY FALL-RELATED FACTORS

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

The aim of this study was to examine the contribution of fall-related factors to the prediction of hip fracture in the elderly.

Serial femoral neck BMD, postural stability, and quadriceps strength measurements were obtained in 1649 (960 women) participants in the Dubbo Osteoporosis Epidemiology Study. Incidence of hip fractures was ascertained during the study period of 1989 and 2003. During the 14-year follow-up period, 115 participants have sustained a hip fracture. Each SD reduction in FNBMD was associated with a 3.8-fold (95%CI, 3.5-4.7) increased risk of hip fracture in women and a 3.3-fold (2.5-4.1) in men, independent of age. Individuals with the highest tertile of postural sway and lowest tertile of quadriceps strength had a relative risk of hip fracture of 2.7 (1.6-4.5) and 3.0 (1.3-6.8), respectively, after adjustment for FNBMD and sex. In addition, a fall during the preceding 12 months and a prior low trauma fracture were independent predictors of hip fracture. For each level of BMD, the risk of hip fracture increased exponentially with the number of these risk factors. Individuals with at least 3 risk factors had the highest risk of hip fracture [RR= 4.0 (2.5-6.0)] compared to those with <3 risk factors, independent of BMD.

Postural instability, quadriceps weakness and a prior fall and a prior fracture are BMD-independent predictors of hip fracture in both men and women. These risk factors could be incorporated into an assessment model for prediction of hip fracture in the elderly population.

O45 19.8.04: 1610 – 1620 hours

GENETIC AND ENVIRONMENTAL CONTRIBUTIONS TO HIP STRENGTH: IN FEMALE TWINS AGED 18 AND OVER

L Paton[1], TJ Beck[2], L Semanick[2], S Kantor[1], R Teh[1], C Nowson[3] & JD Wark[1]
Department of Medicine[1], Neuroscience[2], University of Melbourne, Melbourne, VIC

We postulated that, like BMD, the variation in HSA-derived biomechanical parameters may have a significant genetic component which may help explain the familial aggregation of fractures.

Proximal femur scans from 238 monozygotic and 270 dizygotic twin pairs with a mean (SD) age of 42.2 (14.7), range 18 – 89 years, were analyzed. HSA provided measures of BMD (g/cm^2), cross-sectional area (CSA, cm^2), subperiosteal width (cm), section modulus (cm^3), average cortical thickness (cm) and buckling ratio (BR) at the narrowest region of the femoral neck (NN), intertrochanteric (IT), and femoral shaft (FS) regions. [Mechanosensitivity index (MI) was calculated for the NN [((section modulus/moment arm of the NN)/lean body mass)), where the moment arm is the NN length*sin(180-neck shaft angle)]]. The intraclass correlations for rMZ and rDZ twin pairs were used to estimate the proportion of variance due to genetic (G = 2*(rMZ – rDZ).

There was a moderate to strong genetic influence on almost all HSA-derived parameters which persisted after height and height-weight adjustment. Particularly at the NN, the strength of the genetic influence appeared to be less in the 45+ age group(section modulus G = 0.27) compared with 18 –45 years (section modulus G = 0.91) while the environmental component of variation appeared to be greater in the older group.

The finding suggest that environmental exposure may be more important than genes in determining hip fracture risk.

Table. The range in genetic variance for HSA parameters for the NN, IT and SF regions adjusted for age and age-height-weight.

	Age		Age-height-weight	
	18 – 45 years	45+ years	18 – 45 years	45+ years
Section modulus	0.75 – 0.94	0.22 – 0.58	0.52 – 0.91	0.27 – 0.71
Cross-sectional area	0.79 – 0.90	0.48 – 0.73	0.72 – 0.88	0.45 – 0.74
Subperiosteal width	0.48 – 0.77	0.28 – 0.76	0.27 – 0.738	0.35 – 0.81
Endocortical diameter	0.33 – 0.63	0.22 – 0.90	0.30 – 0.60	0.37 – 0.84
Average cortical thickness	0.57 – 0.70	0.28 – 0.554	0.51 – 0.61	0.16 – 0.77

O46 19.8.04: 1620 – 1630 hours

THE CHARACTERISTICS OF BONE FRACTURES IN POLISH ADOLESCENTS AGED 16 – 20 YEARS. ANTHROPOMETRIC, CONSTITUTIONAL AND ENVIRONMENTAL CONDITIONS

J Konstantynowicz, I Bialokoz-Kalinowska, R Motkowski, P Abramowicz, J Piotrowska-Jastrzebska, J Sienkiewicz & E Seeman
Department of Endocrinology, University of Melbourne, Austin Hospital, Heidelberg, VIC
Department of Pediatrics and Auxology, Department of Public Health, Medical University of Bialystok, Children's University Hospital 'Dr. L.Zamenhof', Bialystok, Poland

The aim of the study was to identify the relationship between family and anthropometric factors, lifestyle patterns and fracture occurrence in adolescents. The total of 1246 persons aged 16.3 - 20.6 years (539 boys, 707 girls) completed a parental assisted lifestyle and medical questionnaire. 869 reported to be fracture-free (FF) while 377 (30.26%) had had fractures, of which 145 adolescents reported multiple fracture events (MF). More boys had fractures than girls (35.6% vs 24.9%, p=0.00003). The fractures were: wrist and distal forearm - 82.4%, forearm - 11.3%, humerus - 3.1%, tibia - 2.3% and femoral shaft and neck - 0.88%. The MF-group had greater weight, height and BMI than the FF-group. The greater weight of

fathers and lower BMI of mothers were also found in the MF-group. The fractures in the MF subjects were determined in 46% by the fractures in mothers (but not fathers) and fractures in siblings. Teenagers with MF reported the access to a home computer for a longer time than those without fractures and more hours spent daily at the computer. Additionally, MF-subjects reported spending more time in physical education and participating in team sports. 18.6% of MF admitted to cow's milk – avoidance for years, whereas only12.4% of FF declared milk-free diets. Conclusions: Adolescents presenting with multiple bone fractures were heavier and taller than the age-matched fracture-free subjects. The strongest predictors of fractures in teenagers were fractures in their mothers and siblings. A high level of physical activity and computer use predisposed the subjects to fractures.

O47 19.8.04: 1630 – 1640 hours

BALANCE PERFORMANCE, MUSCLE STRENGTH AND GAIT FUNCTION IN WOMEN: EFFECTS OF GENES AND ENVIRONMENT

N El Haber[1,2], K Hill[2], A-M Cassano[1,2], JS Cui[3,4], R MacInnis[1], JL Hopper[3] & JD Wark[1]
[1] *Department of Medicine, Royal Melbourne Hospital, University of Melbourne, Parkville, VIC*
[2] *National Ageing Research Institute, Parkville, VIC*
[3] *Centre for Genetic Epidemiology, University of Melbourne, Parkville, VIC*
[4] *Mathematical and Information Sciences Division, CSIRO, Clayton, VIC*

A twin study was performed to determine the heritability of balance, gait and strength in 93 monozygotic (MZ) and 83 dizygotic (DZ) female twin pairs aged 21.0-82.5 years. Validated clinical and laboratory measures of balance including Lord's Balance Test (LBT) and Chattecx Balance System (CBS) with static and dynamic conditions, muscle strength and gait analysis were performed. Variance component analyses were conducted to estimate the genetic, shared environment and individual specific components of the total variance for each trait using the software FISHER.

MZ within pair correlations (rMZ) were consistently greater than DZ within pair correlations (rDZ) on measures of balance and gait indicating the presence of additive genetic effects. For muscle strength measures rMZ and rDZ were strong to moderate, strongly suggestive of common environmental effects. Proportions of variance explained by additive genetic effects on the CBS in the anterior posterior perturbations with the distractor task accounted for 35.5%, on the LBT with eyes closed on the foam accounted for 30.4%. Genetic modelling explained 88% of variance in walking speed and common environmental effects explained 30-78% of variance in muscle strength measures, depending on muscle type.

This study provides important information bearing on the genetic epidemiology of falls, fractures and other falls-related outcomes. The findings indicate a likely genetic influence on the performance of validated tests of balance and gait, and environmental influences on muscle strength function.

O48 19.8.04: 1640 – 1650 hours

MECHANOSENSITIVITY OF THE FEMORAL NECK IN FEMALES IS NOT INCREASED DURING THE ADOLESCENT GROWTH SPURT

MR Forwood, DA Bailey, TJ Beck, RL Mirwald, ADG Baxter-Jones & K Uusi-Rasi
School of Biomedical Sciences, The University of Queensland, Brisbane, QLD
College of Kinesiology, University of Saskatchewan, Saskatoon, Canada
Department of Radiology, Johns Hopkins University School of Medicine, Baltimore, USA
School of Human Movement Studies, The University of Queensland, Brisbane, QLD

During adolescence, it remains equivocal whether sex differences in bone strength can be attributed to factors other than size; and if skeletal mechanosensitivity in females is altered by oestrogen. To examine this, we used DXA to apply hip structural analysis to femoral necks of 70 boys and 68 girls from a 6-year longitudinal study in Canadian children. Cross sectional area (NkCSA, an index of axial strength), subperiosteal width (NkSPW) and section modulus (NkZ, an index of bending strength) were analysed using hierarchical (random effects) modelling. Biological age (BA) was defined as years from peak height velocity (PHV). Controlling BA, stature and total-body lean mass, boys had significantly higher NkZ at all maturity levels. Controlling height and lean for NkCSA demonstrated a significant independent sex by BA interaction effect (NkCSA was greater in boys prior to PHV, but higher in girls after PHV). The significant sex difference in Z was relatively small and close to the error of measurement, suggesting it may have relatively little biological significance. The sex difference in bending strength was therefore explained by anthropometric differences. In contrast to recent hypotheses, we concluded that the NkCSA:lean ratio did not imply altered mechanosensitivity in girls because bending dominates loading at the neck, and the NkZ:lean ratio remained similar between the sexes throughout adolescence. That is, despite the greater NkCSA in girls, the bone is strategically placed to resist bending; hence, the bones of girls and boys adapt to mechanical challenges in a similar way.

O49 19.8.04: 1650 – 1700 hours

QUANTITATIVE ULTRASOUND OF THE CALCANEUS BUT NOT BIOCHEMICAL MARKERS OF BONE TURNOVER ARE ASSOCIATED WITH FRACTURES IN FRAIL OLDER PEOPLE WITH VITAMIN D DEFICIENCY: THE FREE STUDY

JS Chen, MJ Seibel, LM March, ID Cameron, RG Cumming & PN Sambrook
Institute of Bone & Joint Research, Department of Public Health, ANZAC Research Institute, University of Sydney, Sydney, NSW

Background:
Biochemical markers of bone turnover have been reported to predict fracture risk independent of bone mass in postmenopausal women. We investigated their use in predicting fractures in frail elderly living in residential care.

Methods:
Cases were the first 159 respondents who had sustained a fracture where serum samples were available. For each case, a control was selected based on gender, age, institution type and follow-up period. We measured one bone resorption marker (ICTP) and two bone formation markers (BSAP and PINP) in serum. BUA was measured in the calcaneus using a McCue Cuba II machine.

Results:
Mean age of subjects was 86.8 (86% female). 35% were living in nursing homes and 62% of subjects had hypovitaminosis D (25OH D < 31 nmol/L). There was no difference between cases and controls in mean serum 25OH D or PTH. There were significant differences between cases and controls for BUA ($p < 0.01$). However, no significant differences were detected for either the resorption marker or the two formation markers between fracture cases and controls. This was also the case after stratifying for age, gender and renal function. However 75% of ICTP values were above the postmenopausal range. Significant correlates with increased ICTP, PINP and BSAP included higher PTH, lower BUA and older age.

Conclusion:
In the frail elderly with vitamin D deficiency, BUA but not bone turnover markers are associated with fractures. The lack of a biochemical response may be because in this vitamin deficient population, bone resorption is already disturbed, possibly confounding the relationship between bone turnover and fractures seen in younger subjects.

O50 19.8.04: 1700 – 1710 hours

LOSS OF FEMORAL NECK BONE MASS WITH RAPID WEIGHT LOSS IS MORE STRONGLY RELATED TO BASELINE FAT MASS THAN CHANGE IN BODY COMPOSITION

KM Sanders[1], GK Kiroff[2] & GC Nicholson[1]
[1] *The University of Melbourne, Department of Clinical and Biomedical Sciences: Barwon Health, VIC*
[2] *St John of God Hospital; Geelong, VIC*

This study aimed to identify predictors of femoral neck (FN) bone loss accompanying large decreases in body weight. Subjects (20 women, 4 men) undergoing lap-band gastric surgery for morbid obesity had baseline and serial six-monthly DEXA scans for two years. Biochemistry was measured at baseline. The group characteristics were (median, range): age 44, 25-60 years; initial weight 117, 90-174 kg; BMI 41, 34-56; weight loss 23, 7-51 kg; BMI reduction 8, 2-20. The FN bone loss was BMC -3.0% (median -0.17g, range -0.89 to +0.55g) and BMD -2.7%, equivalent to 0.25 SD change and was best predicted by baseline truncal fat (R^2 adj 26%, p=0.008). A 10kg difference in baseline truncal fat equated to a 0.31g decrease in FN BMC. Baseline total body fat, and serum SHBG in females, were less strong predictors (R^2 adj 8%, p=0.10, 10.4%, p=0.12 respectively). Age, gender and serum estradiol, PTH, 25-OH vitamin D and C-peptide levels were not significant. The bone loss was not predicted by changes in weight, total body or truncal lean mass.

Bone losses associated with lap-band surgery are sufficient to increase hip fracture risk, but in this study appear to be related more to baseline measures than change in body composition. Although methodological problems in the serial DEXA analysis may influence results, initial BMI was a better predictor FN bone loss than change in BMI (p=0.18 and p=0.6, respectively). Common genetic determinants of fat and bone mass and/or dietary changes may partially determine FN bone loss accompanying significant weight loss.

Poster Abstracts

P1

ANALYSIS OF HYPERTROPHY AND PHYSIOLOGICAL CELL DEATH IN THREE-DIMENSIONAL CULTURES OF EQUINE FOETAL CHONDROCYTES

YA Ahmed, L Tatarczuch, K Alkhodair, CN Pagel & EJ Mackie
School of Veterinary Science, University of Melbourne, Parkville, VIC

Terminal differentiation of chondrocytes into hypertrophic cells is an obligatory step in the pathway of endochondral ossification. It has recently been demonstrated that hypertrophic chondrocytes die by a process of physiological cell death (PCD) distinct from apoptosis (Roach and Clarke, 2000; J. Bone Joint Surg. 82: 601-13). Cells with two different types of morphology have been described: 'paralysed' and 'dark' chondrocytes. The current study was undertaken to investigate whether equine chondrocytes grown in three dimensional culture could be induced to undergo the same modes of PCD as seen in growth cartilage *in vivo*. Chondrocytes from cartilaginous extremities of long bones of equine foetuses were isolated using collagenase, and cultured as pellets in centrifuge tubes. After 0, 7, 14, 21 and 28 days the pellets were collected for histological examination by light and electron microscopy. By day 7, cells in the pellets were organized into a cartilage-like tissue surrounded by a perichondrium-like layer of flattened cells and contained hypertrophic chondrocytes resembling those seen *in vivo.* At days 7 and 14, many paralysed chondrocytes (about 246 and 132 cells/ section, respectively) were present but at days 21 and 28, dark chondrocytes were the predominant form of dying cells (about 54 and 150 cells/ section, respectively). Apoptotic cells were present at all stages of culture. In this culture system, the patterns of chondrocytes death reflect those previously described in growth cartilage *in vivo*.

P2

COMPARISON OF OSTEOARTHRITIC AND NORMAL TRABECULAR BONE FROM THE INTERTROCHANTERIC REGION OF THE PROXIMAL FEMUR BY cDNA MICROARRAY ANALYSIS

B Hopwood[1,5], S Gronthos[2,5], JS Kuliwaba[1,3,5], DM Findlay[4,5] & NL Fazzalari[1,5]
[1] Bone & Joint Research Group, Division of Tissue Pathology and [2] Mesenchymal Stem Cell Group, Division of Haematology, Institute of Medical and Veterinary Science, Departments of [3] Pathology and [4] Orthopaedics & Trauma, University of Adelaide, [5] Hanson Institute, Adelaide, SA

Osteoarthritis (OA) is a common age-related joint disease resulting in progressive degenerative damage to articular cartilage. The aetiology of primary OA has not yet been determined. However, there is evidence supporting the hypothesis that primary OA might actually be a disease affecting bone remodelling in addition to a disease directly affecting cartilage. We set out to investigate whether differences in gene expression between OA and normal individuals could be identified in trabecular bone distal to the hip joint, specifically the intertrochanteric region of the proximal femur. Total RNA from 3 pairs of age- and sex-matched OA and normal control (CTL) bone samples was reverse-transcribed and radioactively labeled to generate cDNA probes, and then hybridized with the Research Genetics human gene microarray filter GF211. The microarrays were analyzed using the PATHWAYS software package. The CTL and OA samples were found to have similar levels of gene expression for more than 4000 known human genes. However, forty-one genes were identified that were two fold or more differentially expressed between all three CTL - OA sample pairs. Three genes; Vascular endothelial growth factor (VEGF) receptor 1 (FLT1), Plexin B1 (PLXNB1) and Small inducible cytokine A2 (SCYA2) were confirmed to be consistently expressed at lower levels in OA, using semi quantitative RT-PCR analysis of twenty CTL - OA bone sample pairs. These three genes have potentially interesting roles in bone remodelling, including angiogenesis, osteoblast recruitment and differentiation, and osteoclastogenesis. Down-regulation of these genes is consistent with the involvement of bone in the pathogenesis of OA.

P3

CYCLOOXYGENASE-2 (COX-2) INHIBITION PREVENTS OVARIECTOMY-INDUCED BONE LOSS IN ADULT RATS

LS Gregory, WL Kelly & MR Forwood
Department of Anatomy and Developmental Biology, School of Biomedical Sciences, University of Queensland, Brisbane, QLD

Introduction:
The inhibition of COX-2 can retard bone adaptation, preventing loading-induced bone formation and delaying healing in bone fractures. Bone resorption induced by IL-1 and IL-6 occurs via stimulation of COX-2 dependent PGE_2 production in

osteoblasts. The effect of COX-2 inhibition on post-menopausal bone loss is suggested by epidemiological studies, but has not been tested experimentally. This project aimed to investigate the role of COX-2 in catabolic bone events.

Materials & Methods:
One hundred and two adult female Wistar rats, aged 9.5 $\pm$ 1 month, were ovariectomised (OVX) or sham-operated (sham). Rats commenced treatment 14 days after surgery with vehicle or a COX-2 inhibitor (DFU @ 0.02mg/kg/d and 2.0mg/kg/d). Treatment continued daily until rats were sacrificed at 70 or 98 days post-OVX. The right tibiae were harvested, fixed and embedded for structural histomorphometric bone analysis at the proximal tibial metaphysis.

Results & Discussion:
Ovariectomy resulted in a significant increase in bone formation rate (BFR/B.Ar), a significant loss of trabecular bone area and interconnectivity, and a marked increase in percentage resorption surface. Both doses of DFU markedly prevented the loss of bone structural parameters such that many returned to sham control levels. Compared to vehicle-treated controls, DFU-treated rats had significantly greater trabecular area, improved matrix architecture, significantly reduced BFR/B.Ar and marginally decreased resorption surface.

Conclusion:
Treatment with a COX-2 inhibitor maintained trabecular integrity and slowed bone turnover to prevent the deterioration in bone architecture associated with ovariectomy. This suggests a role of COX-2 in the control of catabolic bone adaptive responses.

P4

SYNTHESIS OF 1,25(OH)D_3 IN OSTEOBLASTS: AN AUTOCRINE OR PARACRINE ROLE

PH Anderson[1], K Welldon[2], DM Findlay[2], HA Morris[1] & GJ Atkins[2]
[1] *Endocrine Bone Research, Institute of Medical and Veterinary Science, Adelaide, SA*
[2] *Division of Orthopaedics and Trauma, University of Adelaide, Adelaide, SA*
[1,2] *Hanson Institute, Adelaide, SA*

Although the vitamin D metabolising enzymes, 25D-1α-hydroxylase (CYP27B1) and 25D-24-hydroxylase (CYP24) are present in bone cells, including osteoblasts, little is known of their regulation and function. The effect of aging on the mRNA levels for CYP27B1 and CYP24 in femoral bone was studied in S-D rats aged between 3 weeks and 2 years. CYP27B1 mRNA levels were 4 to 5 fold higher in animals aged between 3 and 15 week when compared to adult animals aged 26 weeks and older. Bone CYP24 mRNA levels were positively correlated with bone CYP27B1 mRNA levels (R^2=0.74), and not with circulating 1,25D levels, suggesting that 1,25D produced in the bone has autocrine or paracrine activity. CYP27B1 mRNA levels were correlated positively with trabecula number (R^2=0.49) and negatively with trabeculae thickness (R^2=0.46) in the femoral epiphysis, suggesting that locally synthesised 1,25D may mediate bone formation. To investigate this, primary normal human osteoblasts (NHBC) were cultured for 30 days in a osteogenic medium. CYP27B1 mRNA levels were 2-fold higher in late-stage cultures containing mineralised NHBC when compared to early, non-mineralised cultures. Conversely, CYP24 mRNA levels in early stage, 1,25D-treated cultures were 10-fold higher when compared to the level of induction in late-stage cultures. In addition, when NHBC were cultured in medium lacking 1,25D, the level of induction of CYP24 mRNA expression by acute 1,25D (20nM) administration decreased dramatically as NHBC increased in maturity. This suggests that CYP27B1 and CYP24 mRNA expression are regulated to increase the production of 1,25D in osteoblasts with maturation, possibly to facilitate the mineralisation process.

P5

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

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

Transforming growth factor-beta (TGF-β) and 1,25 dihydroxyvitamin D (1,25D) can each regulate osteoblast proliferation and differentiation. 1,25D inhibits osteoblast proliferation and can stimulate mineralization while TGF-β stimulates proliferation and inhibits development of the mature osteoblast phenotype. Production of 1,25D is catalysed by the enzyme 25-hydroxyvitamin D_3-1α-hydroxylase (CYP27B1). The aim of this study was to investigate the regulation of CYP27B1 gene expression in the osteoblast-like cell line ROS 17/2.8. Control of basal promoter activity was analysed by transiently transfecting CYP27B1 promoter-luciferase constructs into ROS 17/2.8 and measuring luciferase activity. Constructs included truncations of the CYP27B1 5' flanking region (1.5kb). Control of endogenous gene expression was analysed by

treating cultures with TGF-β followed by real time RT-PCR on cell extracts. Maximal CYP27B1 promoter activity was detected in the -997 base pair (bp) construct and decreased by 35% and 65% in the -305 and -1501 bp constructs respectively, suggesting that an enhancer region lies beyond the basal promoter between -531 and -997 bp with a repressive region between -997 and -1501 bp. The enhancer region contains two putative Ets-1 protein-binding sites, mutations of which decreased luciferase activity by 60% and 40%. Treatment of cultures with TGF-β (1ng/ml) reduced both endogenous CYP27B1 gene expression and luciferase activity by approximately 50%. The TGF-β responsive element was localised to between -305 and -531 bp. These results demonstrate that regulation of the CYP27B1 gene in ROS 17/2.8 involves a complex interaction of enhancer and repressor elements, which can be down regulated by TGF-β.

P6

ENDOCYTOSIS OF LACTOFERRIN BY OSTEOBLASTS

M Watson[1], KE Callon[1], A Zhu[1], JM Ross[2], IR Reid[1], D Naot[1], AB Grey[1] & J Cornish[1]
[1]Dept. of Medicine, [2]Dept. of Anatomy with Radiology, University of Auckland, Auckland, New Zealand

Lactoferrin (LF) is an 80kDa glycoprotein that belongs to the transferrin family and is present in milk and other exocrine secretions in mammals. We have previously reported that LF, at physiological concentrations, stimulates the mitogenesis, differentiation and survival of osteoblasts *in vitro*. In addition, the local injection of LF in mice calvaria increases new bone formation *in vivo*.

We have demonstrated that the activation of p42/44 MAPK signalling and the proliferative effects of LF in osteoblast-like cells are mediated through LRP1, a member of the LDL receptor-related family of proteins.

In this study we have confirmed that LF is endocytosed by primary rat osteoblasts using confocal laser scanning microscopy. The addition of fluorescently labelled LF resulted in an intracellular vesicular pattern of staining, characteristic of endocytosis. Co-staining with a membrane dye (DiI) revealed co-localisation of LF with intracellular vesicles. Inhibition of endocytosis (using a hypertonic medium or placing the cells at 4°C) resulted in the absence of intracellular LF. The specific LRP inhibitor, RAP, also blocked LF endocytosis. Studies using ^{125}I-bLF demonstrate that LF binds specifically to osteoblastic cells and is internalised by a RAP-sensitive mechanism.

Further work in our group has shown that endocytosis and MAPK signalling in osteoblasts operate independently. Binding, but not endocytosis, appears to be necessary for the mitogenic effect of LF.

The role of LF internalisation in bone cell function is yet to be determined.

P7

OSTEOCLAST RESORPTION OF HYDROXYAPATITE BONE SUBSTITUTE MATERIALS

H Lucas[1], H Zreiqat[2], KA Gross[3], C Holding[1], DM Findlay[1], M Wallwork[1] & DR Haynes[1]
[1] University of Adelaide, Adelaide, SA
[2] University of New South Wales, Sydney, NSW
[3] Monash University, Melbourne, VIC

Hydroxyapatite (HA) is used extensively in orthopaedics for bone augmentation, fracture repair, and to improve bone bonding to metal implants. HA materials are resorbed over time and replaced by new bone. While there are many studies investigating how osteoblasts interact with HA there are very few studies of osteoclast interaction with HA.

10mm discs of whale tooth dentine, sintered HA and HA coated titanium alloy were seeded with human peripheral blood mononuclear cells. Cells were cultured in the presence of macrophage colony stimulating factor, Dexamethasone, and Vitamin D_3 for 14 days. On Day 7 recombinant RANKL at 100ng/ml was added. Scanning electron microscopy and confocal microscopy were used to evaluate osteoclast formation and resorption lacunae after cells were removed.

Precursors of osteoclasts in the peripheral blood attached to all the substrates and osteoclasts formed. Imprints left by osteoclasts on the different materials were markedly different. The lacunae on the HA were much shallower than on dentine. On sintered HA imprints were poorly defined with a granular structure. On coated HA the imprints were slightly raised cracked surface with deep crevices. The imprints appeared to expose the granular or crystalline structure of the sintered and coated substrates respectively.

Our results show while osteoclasts may form, they reveal the microstructure of the HA materials. Osteoclast action on these materials can modify the surface for osteoblast deposition. These changes influenced by the different HA substrates is likely to modulate bone metabolism, and hence osseointegration of the bone substitute material.

P8

PARATHYROID HORMONE PEPTIDE (1-34) DERIVED FROM THE JAPANESE PUFFER FISH STIMULATES BONE FORMATION IN YOUNG MALE RATS

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

Parathyroid hormone (PTH), when administered intermittently, is a proven anabolic agent in the promotion of bone growth in animals and humans. This has been shown for both the full-length PTH hormone and PTH peptide sourced from either humans or rats. Here we demonstrate that PTH peptide derived from the Japanese puffer fish *Fugu rubripes* (Fugu) is also capable of increasing bone formation. We compared the effect of Fugu PTH peptide with human PTH peptide in the proximal tibiae of 3-4 week old male Sprague Dawley rats. Twelve rats were injected subcutaneously with either 3 or 10 ug per 100g of body weight of Fugu PTH peptide (1-34), human PTH peptide (1-34) or vehicle, daily for 30 days. At the end of this period, rat tibiae were harvested and assessed by bone histomorphometry. The high dose of Fugu PTH peptide resulted in significant increases in metaphyseal trabecular bone volume ($p<0.05$), number ($p<0.05$) and thickness ($p<0.05$), and osteoblast number ($p<0.05$) and surface ($p<0.05$) compared with controls. There was also a trend towards a decrease in osteoclast number. These results reflect an increase in bone formation. This increase however did not reach that obtained with either of the doses of human PTH peptide. The effects of Fugu PTH on bone appear to be the result of an increase in osteoblast number and possibly a decrease in osteoclastogenesis. In conclusion PTH peptide (1-34) derived from *Fugu rubripes* is effective in increasing bone mass in young male rats.

P9

MODIFIED AND UN-MODIFIED GELATINE SPONGES AS A SCAFFOLD FOR OSTEOBLASTS CELLS

R Rohanizadeh[1], MV Swain[2] & RS Mason[1]
[1] *Department of Physiology, Institute of Biomedical Research, University of Sydney, NSW*
[2] *Biomaterials Unit, Faculty of Dentistry, University of Sydney, NSW*

Because of its flexibility, biocompatibility, and biodegradability, gelatine sponge could be used as a scaffold for cell growth in tissue regeneration or as a drug carrier in different orthopaedic or dental applications.

Aim:
To determine osteoblast proliferation and integration in modified and un-modified gelatine sponges.

Design:
Three scaffolds were studied: gelatine sponge (Gelfoam®), sponge/mineral (apatite) composite, and sponge/polymer (poly-lactide-*co*-glycolide) composite. Plastic coverslip was used as control. Sponges were characterized using Scanning Electron Microscopy and X-ray diffraction. Cell number (DNA content), and cell-replication rate (thymidine assay) were measured after 24h, 3 days, 1, and 2 weeks of osteoblasts (MG-63) culture. Cell penetration into the sponges was determined using H&E staining.

Results:
Cell number was higher in the control group (2D plastic) than in the different sponges (3D scaffold) after 24h. With increasing the culture time, the cells grew faster in the sponges and at 2 weeks, the cell number was higher in different sponges than that of the control (20% higher). The cells number was higher in un-modified sponge and sponge/mineral than that of sponge/polymer composite. Cell proliferation correlated with cell number and was higher in sponges than control after 1 week of culture. Staining with H&E demonstrated the ability of cells to penetrate into the porosities of the 3mm sponges, while for 120mm sponges 80% of the cells were present on top area of the sponges.

Conclusion:
Gelatine sponges supported the cell growth. The presence of calcium phosphate crystals in the sponge favoured cell proliferation.

P10

TRANSGENIC MICE OVER-EXPRESSING 11ß-HYDROXYSTEROID DEHYDROGENASE TYPE 2 (11ßHSD2) UNDER THE COL1a(I) PROMOTER PROVIDE A MODEL TO TEST DIRECT EFFECTS OF EXOGENOUS CORTICOSTEROIDS ON OSTEOBLASTS

KJ Brennan, H Zhou, J de Winter, CR Dunstan & MJ Seibel
Bone Biology Unit, ANZAC Research Institute, Sydney, NSW

Endogenous Glucocorticoid (GC) levels are systemically regulated via the hypothalamic-pituitary-adrenal axis. Two isoforms of 11ßHSD modulate cytoplasmic GC levels. Type 1 (11ßHSD1) predominantly converts inactive cortisone to active cortisol while 11ßHSD2 unidirectionally catalyses conversion of active GCs to inactive metabolites. The relative activities of 11ßHSD1 and 11ßHSD2 determine the availability of active ligand. Over-expression of 11ßHSD2 in osteoblasts should provide bone protection from GC effects.

Four week old transgenic male mice or wild type littermates over-expressing 11ßHSD2 under the Col1a(I) 2.3 kb promoter were treated with vehicle or corticosterone (0,5, 2, or 10mg/kg/d, n = 4 - 5) for 14 days. Bones were removed for assessment of 11ßHSD 1 and 2 expression and for histomorphometry.

Endogenous 11ßHSD2 expression was not detectable in the bones of wild type mice. The 11ßHSD2 transgene was abundantly and stably expressed in tibia of transgenic mice and was unchanged by corticosterone treatment. 11ßHSD1 was constitutively expressed in the bones of mice and was not altered by transgene expression or corticosterone dosing.

11ßHSD2 transgenic mice tended to have a lower bone volume compared to wild type in vertebral bodies and proximal tibiae. Wild type mice lost proportionally more bone in the proximal tibia than transgenic mice at the 2mg/kg dose, though this effect was lost at the highest dose. Osteoclast and osteoblast surfaces were similar at all doses as were bone formation and bone resorption markers.

11ßHSD2 transgenic mice provide a potential model for evaluating the osteoblast specific actions of both endogenous and exogenous glucocorticoids.

P11

MODEL-INDEPENDENT 3D DESCRIPTORS OF CANCELLOUS BONE QUALITY: AN IMPROVED EXPLANATORY MODEL FOR A SURROGATE OF TRABECULAR STRENGTH

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

The advent of high-resolution micro computed tomography (Micro-CT) enables measures of architectural bone quality to be obtained from voxel-based 3D representations of cancellous bone.

Thoracolumbar vertebral bodies (T6, T8, T10, L1, L3 and L5) were obtained from 4 cadavers. Three-dimensional anaglyphs were obtained by scanning electron microscope (SEM) imaging at a spatial resolution of 7.83 μm. Micro-CT imaging was subsequently performed on the specimens at a spatial resolution of 15.63 μm with a Skyscan 1072.

The load to buckling index (Buckling index) of trabecular rods was calculated from trabecular rod thickness and trabecular rod length obtained from anaglyphs. From the 3D voxel-based datasets, BV/TV, Tb.Th, Tb.Sp, Tb.N, Trabecular bone pattern factor (TBPf), Structural model index (SMI), Euler-Poincare number (Euler number) and Degree of anisotropy (DA) were obtained. Multiple regression analysis was performed to determine what combination of parameters best explains the variability in trabecular rod strength.

BV/TV is the best individual explanatory parameter for trabecular strength (r^2=0.51, $p<0.0001$). Multiple regression analysis shows an improvement in explanatory power for trabecular rod strength from 51% for BV/TV alone to 75% when Euler number, TBPf and SMI are added to BV/TV in the statistical model.

In this study, a composite explanatory model of vertebral body strength is proposed. Multiple regression analysis shows that the amount of variability in the Buckling index attributable to BV/TV can be improved from 51% to 75% with the addition of morphological parameters to the statistical model. Further work, where mechanical testing of cancellous bone to measure strength prior to conducting a similar morphological analysis on micro-CT images will enable validation of the results presented in this study.

P12

STUDIES ON TRANSCRIPTION FACTORS EXPRESSED DURING ENDOCHONDRAL OSSIFICATION OF EQUINE ARTICULAR-EPIPHYSEAL GROWTH CARTILAGE

K Alkhodair, Y Ahmed, CN Pagel & EJ Mackie
School of Veterinary Science, University of Melbourne, Parkville,VIC

Osteochondrosis is one of many diseases affecting cartilage in growing animals and humans, and results from failure of chondrocyte hypertrophy, and thus failure of the endochondral ossification process in articular-epiphyseal growth cartilage (AEGC). Our aim is to further investigate expression of various factors during the normal process of endochondral ossification, which will help in understanding how this disease occurs. Sox9 and Runx2 are transcription factors that are thought to play important roles in chondrocyte differentiation and hypertrophy. Samples were collected from AEGC of growing horses. Chondrocytes were isolated in monolayer culture and the RNA was extracted from cultured cells as well as from cartilage tissue. RT-PCR was carried out on RNA from chondrocyte cultures and cartilage tissue to detect expression of Sox9, Runx2 and Collagen types I, II and X. Expression of Collagen type II, a chondrocyte marker, was detected in both cell culture and cartilage tissue. Expression of Sox9, Runx2 and Collagen types I and X were not detected in monolayer cell culture, but were detected in cartilage tissue. All these factors have been cloned and sequenced, and the sequence results confirmed against equine gene sequences. Previously unpublished 435bp sequence of equine Runx2 has been generated. Riboprobes will be synthesised from these clones to be used in *in situ* hybridisation studies to investigate expression of Sox9 and Runx2 in normal AEGC and in osteochondrotic lesions.

P13

IS INCREASED BONE TURNOVER CONTRIBUTING TO HUMAN FEMORAL FRACTURES?

H Tsangari[1], JS Kuliwaba[1,3] , DM Findlay[2] & NL Fazzalari[1,3]
[1] Bone & Joint Research Laboratory, Division of Tissue Path, IMVS and Hanson Institute, Adelaide, SA
[2] Department of Orthopaedics & Trauma & [3] Department of Pathology, University of Adelaide, Adelaide, SA

Fragility fractures result from reductions in the amount, quality and architecture of bone, yet morphometric investigations have been limited. The aim of this study was to assess whether there are differences in the morphology and remodelling of femoral cancellous bone and gene expression of osteoclastogenic factors from fractured neck of femur (#NOF) and control individuals. Femoral cancellous bone cores were obtained from 13 autopsy cases (median age 77yrs) with no evidence of skeletal pathology, and from 18 patients (median age 80yrs) undergoing surgery for a #NOF. Bone samples were processed undecalcified into resin and a subset of cases was used for mRNA expression analysis. Histomorphometry revealed no significant difference in bone volume or trabecular architecture between the #NOF and control groups (BV/TV[%]: 7.8 [5.7-9.8] vs. 6.3 [4.4-7.1]; p=NS). Static indices of bone turnover, eroded and osteoid surface, were not significantly different between the #NOF and control groups. However, we have recently reported an increase in the osteoclastogenic influence at this skeletal site in hip fracture patients (Tsangari *et al*., 2004, Bone, in press). Intriguingly, molecular histmorphometry revealed a positive association between trabecular number and mRNA expression of the osteoclastic influences IL-6 and RANK, in the fracture group (r=0.65, p<0.05 and r=0.62, p<0.05). These data suggest that increases in the osteoclastic stimuli may be associated with a change in the trabecular connectivity, contributing to an increase in fracture risk. Ongoing investigations into the gene expression patterns will further elucidate the molecular mechanisms of fragility fracture.

P14

TRANSACTIVATION RESPONSE OF VDRB1 IS PROMOTER- CELL- AND LIGAND-SPECIFIC

C Fong, LM Esteban, SJ Allison, JL Flanagan, D Amr*, C Liddle*, JA Eisman & EM Gardiner
Bone and Mineral Research Program, Garvan Institute of Medical Research, Sydney, NSW
** Department of Clinical Pharmacology and Toxicology, Westmead Hospital, Westmead, NSW*

The vitamin D receptor (VDR) mediates the effects of $1,25(OH)_2D_3$, the active form of vitamin D. The human VDRB1 isoform differs from the originally described VDRA by an N-terminal extension of 50 amino acids. We investigated cell-, promoter- and ligand-specific transactivation by VDRB1.

Relative activities of the VDR isoforms on two cytochrome P450 gene promoters, CYP24 and CYP3A4, were studied in COS1 and HEK293 cell lines. Transactivation was induced by $1,25(OH)_2D_3$ or the secondary bile acid, lithocholic acid (LCA), a recently reported VDR activator.

On CYP24, the VDRB1 response to 1,25$(OH)_2D_3$ was greater (130%) than that of VDRA but similar in response to LCA in COS1 cells. In HEK293 cells, the isoforms had similar activity in response to 1,25$(OH)_2D_3$, but with LCA VDRB1 was less active (68%) than VDRA. On CYP3A4 the activity of VDRB1 was lower (60-75%) than that of VDRA in response to either ligand in either cell line. In gel shift assays VDR:DNA complex formation was stronger in the presence of 1,25$(OH)_2D_3$ than with LCA. Mutant analysis indicated that both the 1,25$(OH)_2D_3$- and LCA-regulated activities were dependent on a functional ligand-dependent activation function (AF-2) domain.

These studies support the concept that the differential VDRB1 transactivation activity is dependent on promoter- and cellular- context and the nature of the ligand.

P15

MUTATIONAL ANALYSIS CLARIFIES MECHANISM FOR DISTINCT TRANSACTIVATION PROPERTIES OF THE VITAMIN D RECEPTOR, VDRB1

LM Esteban, SJ Allison, C Fong, JL Flanagan, JA Eisman & EM Gardiner
Bone and Mineral Research Program. Garvan Institute, Sydney, NSW

The biological actions of 1,25 $(OH)_2D_3$, the active form of vitamin D, are mediated by the vitamin D receptor (VDR). There are two protein isoforms of the human VDR which differ in the length of their N-terminus or A/B region (VDRA and VDRB1 with 23 and 73 amino acids respectively). The N-terminal extension of VDRB1 is responsible for differences in transactivation capacity and subcellular localisation. VDRB1 has stronger AF-1 (ligand-independent activation function) activity and its A/B region interacts with coregulators as well as its own C-terminus more efficiently than VDRA.

We undertook mutational analysis of this region to study the contribution of individual amino acids to the differential activity of VDRB1 and to functions of its A/B region. Specific point mutations targeted charged residues, aromatic amino acids, and two potential N-myristoylation sites (Gly). Transactivation of the rat CYP24 promoter and AF-1 activity of the isolated A/B domain of these mutants were compared to wildtype VDRB1 activity.

Amino acids D10 and G20, and several basic residues appeared to be critical for the differences between VDRA and VDRB1 in transactivation and AF-1 functions. In contrast, mutations in aromatic residues affected transactivation and AF-1 functions in opposite directions, suggesting that charged and aromatic residues may be differentially involved in interactions with coregulators and the VDR C-terminal AF-2 domain. These findings are consistent with observations that relative transactivation by VDRA and VDRB1 vary according to cellular context.

P16

PATTERNS OF PHYSIOLOGICAL CELL DEATH IN EQUINE HYPERTROPHIC CHONDROCYTES

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

Chondrocytes in growth cartilage undergo a series of progressive changes, which involve proliferation, hypertrophic differentiation and cell death, leading to replacement of cartilage by bone. It is generally accepted that hypertrophic chondrocytes die by apoptosis, however some recent studies have indicated that physiological cell death of chondrocytes is not confined to apoptosis. An ultrastructural study to characterise the morphology of physiological cell death of chondrocytes in articular–epiphyseal growth cartilage (AEGC) from horses was undertaken. Specimens of AEGC were collected from the lateral trochlear ridge of the femur and from distal tibia during foetal and postnatal growth. Based on the ultrastructural appearance, two types of dying chondrocytes have been identified, ‘dark’ chondrocytes and ’light’ chondrocytes. The dark chondrocytes were characterised by a dark nucleus with small, irregular patches of condensed chromatin and their electron dense cytoplasm gradually extruded into extracellular space. The light chondrocytes also contained a condensed nucleus but their cytoplasm and organelles appeared to be undergoing gradual disintegration within a preserved cellular membrane. Comparison of samples from different ages showed a distinct change in the types of chondrocyte present. Morphological changes in both types of dying chondrocyte were different from classical apoptosis, indicating that death of chondrocytes in growth cartilage involves another mechanism of physiological cell death. In the tibial samples, in which growth had almost ceased, chondrocytes with distinct morphological changes of the nucleus suggestive of apoptosis were observed. It is possible that apoptosis is responsible for elimination of chondrocytes in adult cartilage.

P17

DEVELOPMENT AND USE OF AN ANTIBODY TO FGF23

M Mirams[1,2], BG Robinson[2], R Clifton-Bligh[2], RS Mason[1] & AE Nelson[1,3]
[1] *Dept of Physiology, Institute for Biomedical Research, University of Sydney, NSW*
[2] *Cancer Genetics Dept, Kolling Institute of Medical Research, Royal North Shore Hospital, Sydney, NSW*
[3] *Garvan Institute of Medical Research, Sydney, NSW*

Fibroblast Growth Factor 23 (FGF23) is a possible novel phosphate regulating factor. Mutations in FGF23 have been associated with autosomal dominant hypophosphataemic rickets and elevated serum FGF23 has been detected in patients with oncogenic osteomalacia (OOM), an acquired phosphate wasting disorder. The aim was to raise polyclonal antibodies against a peptide epitope of FGF23.

An 18 residue peptide was designed at the C-terminal end of FGF23, a region with no sequence homology to other known FGFs. Antiserum was raised in a rabbit, and the antibody titre was tested by dot-blot immunoassay to detect the peptide. Using antiserum from the terminal bleed, 0.1 ng of the FGF23 peptide was detected. The antiserum was successfully used in Western blotting to detect full-length FGF23. Full length FGF23, 35kD, and processed, 20kD, FGF23 was detected in conditioned media from HEK293 cells transfected with FGF23 expression plasmid, but not in conditioned medium from HEK293 transfected with vector control. The antiserum was successfully used in immunohistochemical staining of tissue samples.

Sections from a confirmed OOM tumour, a benign giant cell tumour of bone, stained positively for FGF23 using the antiserum, while a control mesenchymal tumour, a Schwannoma, was negative. Other negative tissues tested included normal human bone marrow. In the positively stained tumour, staining was granular and cytoplasmic. Not all cells in the tumour were stained. The antiserum appeared at least as sensitive in Western blotting and immunohistochemistry as an FGF23 peptide antibody (Jonsson et al., NEJM 2003 348:1656-1663) used in a commercial FGF23 ELISA.

P18

LIMITED EXPOSURE OF OSTEOBLASTS TO HIGH DOSES OF ZOLEDRONIC ACID DOES NOT ADVERSELY AFFECT CELL SURVIVAL OR OSTEOGENIC DIFFERENTIATION

A Schindeler, C Godfrey, M McDonald & DG Little
Orthopaedic Research and Biotechnology, The Children's Hospital at Westmead, Sydney, NSW

Zoledronic acid (ZA) is a potent nitrogen-containing bisphosphonate (N-BP) used in the treatment of osteoporosis and diseases of high bone turnover. Once administered, ZA binds rapidly and strongly to bone where it suppresses osteoclast-mediated bone resorption. Coxon *et al* recently showed that osteoclasts grown on N-BP-treated dentine could release bound N-BP and internalise it within intracellular vesicles. In contrast, non-resorbing cells (J774 macrophages) grown on N-BP-treated dentine were incapable of releasing and internalising bound N-BP [1]. Numerous studies have described the effects of ZA and other N-BPs on osteoblasts *in vitro,* generally indicating that ZA augments osteogenic differentiation at low concentrations while reducing proliferation and survival at higher doses. However, the work of Coxon *et al* raises the possibility that osteoblasts may not internalise ZA trapped at the bone surface, thus questioning the validity of the methodology of such studies.

To better model the effects of systemic N-BP treatment (as seen by osteoblasts during a clinical dosing), we exposed differentiating MC3T3-E1 osteoblasts briefly to ZA and then cultured them normally for 1-3 weeks. Osteoblasts proved highly resistant to all short ZA treatment regimes, even when utilising doses of ZA that prevented mineralisation and/or induced cell death when administered for prolonged periods (i.e. 10-50μM). Moreover, osteoblast survival was unaffected when cells were grown on ZA-treated hydroxyapatite, suggesting that these osteoblasts could not release bound ZA. Together with the results of Coxon *et al*, these data may lead to more physiologically relevant models of N-BP treatment being adopted in osteoblast culture systems.

[1] Coxon FP, Langton J and Rogers MJ. "Visualisation of the uptake of a novel fluorescent bisphosphonate by resorbing osteoclasts and J774 cells" Bone 34 Supp. 1, S51 (2004).

P20

THE EFFECTS OF EXOGENOUS GALANIN APPLICATION ON BONE FORMATION IN MOUSE CALVARIA

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

Concentrations of the neuropeptide, galanin (GAL) increase in callus after bone fracture[1]. One role for this increase in GAL concentration may be to suppress inflammation (a known action of GAL) and thus facilitate normal bone formation. Therefore, our aim was to determine whether application of GAL to mouse calvaria influenced both inflammation and bone formation.

5 groups of 4 week-old mice were used: non-injected controls, vehicle-injected controls and 3 groups injected with GAL (0.2, 2 and 20 ng). Solutions were injected subcutaneously in 10 µl volumes every day onto calvaria for 2 weeks.

Real time polymerase chain reaction analysis of calvaria demonstrated that expression of the inflammatory cytokine, interleukin-1 β was increased in vehicle-injected controls ($p = 0.07$) and decreased in 20 ng GAL injected mice ($p = 0.0002$) compared to non-injected controls. Thus, this implicates a possible anti-inflammatory role of GAL.

Vehicle-injected controls exhibited a higher expression of the following genes compared with non-injected controls: collagen type I ($p < 0.05$), GAL ($p = 0.07$) and GAL receptors 1 and 2 ($p < 0.01$). The 20 ng GAL injection regimen showed similar expression levels of these genes to non-injected controls.

Dynamic histomorphometric analysis of calvarial parietal bone showed that vehicle-injected controls had a lower mineral apposition rate (MAR) compared to non-injected controls ($p < 0.001$), suggesting that inflammation caused by repeated daily injections inhibited MAR. In mice receiving 20 ng GAL, however, MARs were similar to non-injected controls, indicating that this dose of GAL offset the reduced MAR exhibited by vehicle-injected controls.

These results suggest that exogenous application of GAL may counteract the adverse effects of inflammation on normal bone formation.

1. McDonald *et al.,* 2003. Bone 33: 788-797.

P21

GALANIN AND GALANIN RECEPTOR EXPRESSION IN UMR106.01 CELLS

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

The neuropeptide galanin (GAL) acts on a number of tissues including the immune, nervous and gastrointestinal systems. GAL possesses three receptors, GALR1, GALR2 and GALR3, all of which belong to the G protein-coupled receptor superfamily. The activation of these receptors by GAL initiates intracellular secondary messengers that influence a number of physiological processes including mitogenesis, apoptosis and nerve regeneration.

We hypothesize that GAL is involved in skeletal metabolism. GAL and GALR1 have been located in bone and cartilage, and concentrations of GAL increase in fracture callus compared to intact bone[1]. To this stage, however, no reports have documented whether GAL or its receptors are expressed in osteoblastic cell lines. Therefore, the aims of these experiments were to determine whether GAL and its receptors were expressed in UMR106.01 cells, and if so, whether GAL treatment to these cells induced changes in the expression of these genes.

Real time polymerase chain reaction analysis identified the presence and levels of all four genes with GALR2 expression being significantly more abundant than GAL, GALR3 and GALR1 ($p < 0.001$). Immunohistochemistry confirmed the presence of GAL- and GALR-like immunoreactivity in the cells, thus substantiating that mRNA expression translated into protein.

Although GAL treatment (10 nM, 100 nM and 1000 nM) for either 2h or 24 h, produced no change in the levels of expression of these genes, previous studies in neuroblastoma cells with similarly high GAL2 expression have reported that GAL stimulates apoptosis and inhibits cell proliferation. Therefore, future studies will investigate analogous actions of GAL on various osteoblastic cell lines.

In conclusion, the expression of GAL and all its receptors in osteoblast-like cells has functional implications for osseous metabolism.

1. McDonald *et al.,* 2003. Bone 33: 788-797.

P22

NOVEL ACTIVATORS OF THE CALCIUM-SENSING RECEPTOR ACTIVATE RENAL CALCIUM AND WATER EXCRETION

HC Lok & AD Conigrave
School of Molecular and Microbial Biosciences, University of Sydney, NSW

Recently, two new classes of calcium-sensing receptor (CaR) activators have been identified. Type-II calcimimetics sensitize the CaR to calcium ions. More recently, several sub-classes of L-amino acids have been shown to act as allosteric activators of the CaR. We have now examined the impact of intravenously administered L-amino acids or the type-II calcimimetic, NPS R-467 on renal calcium and water excretion. Female Wistar rats (200-300 g) were anaesthetized with halothane then catherized. Both jugular veins were cannulated and the animals were infused (4 mL per hour) with an isotonic physiological saline solution. After a 60 min equilibration period, a continuous infusion of amino acids was commenced. Blood samples (0.3 mL) were collected at regular intervals for analysis of creatinine, osmolality, total calcium and various amino acids. Urine samples were collected at 15 min intervals to assess flow rate, osmolality and the concentrations of creatinine, calcium, phosphate and amino acids. The type-II calcimimetic R-467 enhanced urinary calcium excretion (by approximately 3 fold) and urinary flow rate. In addition, R-467 suppressed urinary osmolality consistent with an inhibitory action of the CaR on vasopressin-induced water reabsorption in the collecting ducts. R-467 also lowered serum total calcium levels. Infusions of the CaR-active L-amino acid, L-Phe sufficient to raise the serum level from 0.05 mM to about 2 mM, also elevated calcium excretion (by about 2-fold) and urinary flow rate, and suppressed urinary osmolality. Taken together the data are consistent with the idea that novel activators of the CaR mimic the effects of elevated Ca^{2+} on urinary calcium excretion, flow rate and osmolality.

P23

RECEPTOR ACTIVATOR OF NUCLEAR FACTOR KAPPA B (RANK) AND TUMOUR NECROSIS FACTOR ALPHA (TNFα) EXPRESSION IN TISSUES OBTAINED FROM SITES OF PERI-IMPLANT OSTEOLYSIS CHARACTERISED BY COMPUTED TOMOGRAPHY (CT)

CA Holding[1], KR Shrestha[2], K Dharmapatni[1], R Stamenkov[3], DW Howie[3], DM Findlay[3], MA McGee[3], GJ Atkins[3] & DR Haynes[1]
[1] *Department of Pathology, Adelaide University, SA*
[2] *Department of Orthopaedics and Trauma, Queen Elizabeth Hospital, SA*
[3] *Department of Orthopaedics and Trauma, Adelaide University, and Hanson Institute, SA*

Introduction:
RANKL and TNFα are key factors regulating bone resorption in several bone loss pathologies. RANKL is a crucial factor in osteoclastogenesis and TNFα can stimulate both inflammatory responses and synergise with RANKL. The aim of this study was to investigate the expression of the RANKL receptor, RANK, and TNFα in tissues surrounding peri-prosthetic osteolysis, in patients undergoing total hip replacement.

Methods/Results:
Immunohistochemical analysis of formalin fixed tissue sections from 8 patients undergoing revision of total hip prosthesis was performed using monoclonal antibodies directed against RANK and TNFα (R&D Systems). Tissue sampled from patients could be related to bone lesions imaged using high-resolution spiral multislice CT, with a metal artefact suppression protocol. CT also enabled measurement of the volume of osteolytic lesions around titanium cementless acetabular and femoral components of total hip arthroplasties. Control tissue consisted of synovial tissue samples taken from patients at primary joint replacement surgery for osteoarthritis. Sections were evaluated by light microscopy, and polarised light was used to detect polyethylene particles, which were abundant in these tissues. Both RANK and TNFα were strongly expressed in revision tissues adjacent to osteolytic lesions and were associated with large multinucleated osteoclast-like cells. Control tissue stained very weakly for both RANK and TNFα.

Conclusion:
This study demonstrated that the expression of both RANK and TNFα is strongly elevated in tissues from sites of peri-implant osteolysis. These molecules were associated with large multinucleated cells and the presence of abundant small polyethylene particles, implicating wear of this material as a potential cause of peri-prosthetic osteolysis.

P24

INTERACTION OF GTP-BINDING RAB3D WITH TCTEX-1, A LIGHT CHAIN OF THE CYTOPLASMIC DYEIN MICROTUBULE MOTOR COMPLEX

NJ Pavlos[1], J Xu[1], A Carrello[1], K Kroeger[2], K Ednet[2] & MH Zheng[1]
[1] *Unit of Orthopaedics, School of Surgery and Pathology, University of Western Australia, Nedlands, WA*
[2] *Western Australia Institute for Medical Research, QEII Medical Centre, Nedlands, WA*

Rab3D belongs to the family of small GTPases involved in the regulation of exocytosis. Previously we have shown that Rab3D is required for the maintenance of the osteoclastic resorptive organelle, namely the ruffled border membrane. Here, to further delineate the mechanism(s) underlying this phenomenon, we have employed a yeast two-hybrid system to identify potential Rab3D interacting proteins. Screening a mouse embryonic cDNA library, 5 clones (M-18, -30, -37, -39 and -40) were positively identified which specifically interacted with the N-terminus of Rab3D. Database searches identified these clones as mouse Tctex-1, a 14 kDa light chain of the multimeric cytoplasmic dynein motor complex. A specific interaction between Rab3D and Tctex-1 was confirmed by GST-pull down and co-localisation studies. Truncation analyses mapped the Tctex-1 binding site to the switch II/GTP-binding motif of Rab3D (amino acids 74-95). Consistently, bioluminescence resonance energy transfer (BRET) analysis demonstrated that Tctex-1 preferentially associated with the GTP-bound conformation form of Rab3D in live cells. When overexpressed, Flag-Tctex-1, GFP-dynamitin, or antisense-Tctex-1 disrupted the spatial distribution of Rab3D *in vivo*. Additionally, Rab3D-secretory granules localise to microtubules and are redistributed by nocodazole treatment into β-COPI-positive Golgi mini-stacks in transfected COS-1 cells. These data lend support to the notion that Rab GTPases and molecular motor proteins act in concert to regulate directional membrane transport and furthermore suggest that Rab3D may functions to recruit and regulate the activity of cytoplasmic dynein Tctex-1, controlling the sorting and microtubule-dependent targeting of post-Golgi secretory granules to the ruffled border membrane during osteoclastic bone resorption.

P25

OSTEOSCLEROSIS IN MICE LACKING RAB3D IS RELATED TO DISRUPTIONS IN POST-TGN VESICLE TRAFFICKING IN OSTEOCLASTS

NJ Pavlos[1], J Xu[1], D Riedel[3], SL Teitelbaum[2], R Jahn[3], FP Ross[2] & MH Zheng[1]
[1] *Unit of Orthopaedics, School of Surgery and Pathology, University of Western Australia, Perth, WA*
[2] *Department of Pathology, Washington University School of Medicine, St. Louis, USA*
[3] *Department of Neurobiology, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany*

Intracellular membrane trafficking is essential to osteoclast function however little is known about the nature and regulation of the transport pathways that govern its structural and functional polarisation. We have recently reported the existence of the small exocytic-related Rab3D GTPase in osteoclasts. Here, to shed light on the possible involvement of Rab3D in osteoclast physiology we examined Rab3D-deficient mice for skeletal anomalies. Strikingly, we identify an osteosclerosis phenotype in these mice; with bones from these animals exhibiting increased total volume, increased trabecular number and reduced trabecular separation. Basal osteoclast numbers were normal, however, the total eroded surface was significantly reduced, suggesting that the resorptive defect was due to attenuated osteoclast activity rather than a disruption in osteoclast formation. Consistently, ultrastructural analyses revealed that Rab3D$^{-/-}$ osteoclasts exhibit irregular regulated borders. To further delineate the molecular mechanism(s) underlying this resorption deficiency, we expressed a series of enhanced yellow fluorescent (EYFP)-tagged wild-type and mutant Rab3D fusion chimeras in osteoclasts and examined their subcellular localisations and affects on bone resorption. Rab3D was found to associate with the *trans*-Golgi network (TGN) and an as yet undefined subset of post-TGN vesicles of non-endosomal/lysosomal origin. Moreover, while overexpression of Rab3D wild-type and its mutants had no affect osteoclastogenesis or cellular attachment, expression of the GTP-binding deficient Rab3DN135I mutant, which was largely restricted to the TGN, profoundly impaired osteoclastic bone resorption. These data document the existence of a novel Rab3D-mediated post-TGN trafficking pathway that is required for the maintenance of the ruffled border membrane during physiological bone metabolism.

P26

A HUMAN OSTEOCLAST FORMING CO-CULTURE

ACW Zannettino[2], C Vincent[1], P Kostakis[1,2], K Welldon[1], DM Findlay[1] & GJ Atkins[1]
[1] *Department of Orthopaedics and Trauma, University of Adelaide, Adelaide, SA*
[2] *Division of Haematology, Institute of Medical and Veterinary Science, Adelaide, SA*
[1-2] *Hanson Institute, SA*

Normal human trabecular bone-derived osteoblasts (NHBC) have been well characterised in terms of their ability to form a mineralised matrix, but not with respect to support of osteoclastogenesis. NHBC were tested for their ability to support osteoclast formation under continuous treatment with either 1α,25vitamin-D_3 or parathyroid hormone (PTH), in the presence of dexamethasone, from normal human osteoclast precursors using a medium previously used to support human osteoclast formation on a stroma of murine ST-2 cells. We also tested osteoclast formation in serum-free media. NHBC phenotypes were assessed by flow cytometry and expression of RANKL and OPG mRNA were examined by real-time PCR. Osteoclast formation was assessed by both histochemical staining for TRAP and by scanning electron microscopy for resorption pit formation. NHBC supported human osteoclast formation in a defined serum-free medium (SDM). Osteoclast formation in co-cultures occurred from $CD34^+$ or $CD14^+$ bone marrow mononuclear cells (BMMC), or $CD14^+$ peripheral-blood mononuclear cell (PBMC) precursors, in the absence of exogenous recombinant RANKL/M-CSF. Compared with osteoclastogenesis stimulated by recombinant RANKL/M-CSF or co-culture with murine cell lines as stromal support, the human-human co-culture model is likely to be more analogous to physiological osteoclast differentiation. This novel model of adult human primary osteoblasts driving human osteoclast differentiation will enable the detailed study of the role of both cell types in this process.

P27

INTERACTION BETWEEN WNT SIGNALLING PATHWAY AND $1,25(OH)_2D_3$ IN OSTEOBLAST-LIKE MC3T3-E1 CELLS

Y Shi, JA Eisman & EM Gardiner
Bone and Mineral Research Program, Garvan Institute of Medical Research, Sydney, NSW

Wnt/ ß-catenin signalling pathway regulates cell proliferation, differentiation and bone formation by stabilization of ß-catenin and increased LEF/Tcf-dependent gene expression. Lithium Chloride(LiCl) mimics this effect. Also, active hormone vitamin D($1,25(OH)_2D_3$), regulates gene expression through vitamin D receptor (VDR), which can interact with ß-catenin and alter Tcf-mediated transcription in some colon cancer cell lines. The present study examined the interaction between the Wnt/ ß –catenin pathway and $1,25(OH)_2D_3$ in the MC3T3-E1 osteoblastic cell line.

In MC3T3-E1 cells transiently transfected with a VDR expression vector and ß-catenin/Tcf responsive (TOPFLASH) reporter construct, treatment with $1,25(OH)_2D_3$ caused a dose-dependent repression of reporter activity, whereas LiCl(10mM) increased Tcf activity, but, combined treatment with both $1,25(OH)_2D_3$ and LiCl reduced activity lower than $1,25(OH)_2D_3$ alone. Prior to onset of mineralization(16day culture with ascorbic acid and ß-glycerophosphate), $1,25(OH)_2D_3$ inhibited cell proliferation(BrdU incorporation) and viable cell number dose-responsively. LiCl had no effect either parameter, but combined treatment reduced these values below $1,25(OH)_2D_3$ alone. In long term (28 days) mineralising culture, continuous treatment with $1,25(OH)_2D_3$ (10nM) reduced cell density and slowed cell differentiation, as evidenced by reduced alkaline phosphatase, Von Kossa and Alizarin red staining patterns. Continuous exposure to LiCl also reduced differentiation but, unlike $1,25(OH)_2D_3$ treatment, was associated with increased cell density. Combined treatment reduced cell density below the LiCl level, and reduced differentiation compared to either treatment alone. Our results suggest an interaction between $1,25(OH)_2D_3$ and Wnt response pathways in regulation of both osteoblast proliferation and differentiation.

P28

EVIDENCE OF RECIPROCAL REGULATION BETWEEN THE HIGH EXTRACELLULAR CALCIUM AND RANKL SIGNAL TRANSDUCTION PATHWAYS IN RAW CELL DERIVED OSTEOCLASTS

J Xu[1], C Wang[1], R Han[1], N Pavlos[1], T Phan[1], JH Steer[2], AJ Bakker[3], DA Joyce[2] & MH Zheng[1]
[1] Molecular Orthopaedic Laboratory, [2] Pharmacology Unit, [3] Physiology Unit, The University of Western Australia, WA

Aims:
In this study, we aim to investigate the role of high extracellular Ca^{2+} in receptor activator of NF-kB ligand (RANKL)-mediated osteoclast survival and their functional interrelationship.

Methods:
RAW cells derived osteoclast survival assay, NF-kB and AP-1 reporter gene assays, Western blot analysis and Ca^{2+} measurement.

Results:
We show that RANKL enhances osteoclast tolerance to high extracellular Ca^{2+} by protecting the cell from high extracellular Ca^{2+}-induced cell death in a dose dependent manner. We have provided evidence that RANKL does this by attenuating high extracellular Ca^{2+}-induced Ca^{2+} elevations. Moreover, we have found that high extracellular Ca^{2+}-induced cell death was partially inhibited by a caspase-3 inhibitor, suggesting that a caspase-3-mediated apoptosis is involved. Conversely, we have demonstrated that high extracellular Ca^{2+} desensitizes the RANKL-induced activation of NF-kB and c-Jun N-terminal

kinase (JNK), and inhibits constitutive and RANKL-stimulated ERK phosphorylation, indicating a negatively feed-back mechanism via specific RANKL signaling pathways.

Conclusion:
This study provides evidence for a reciprocal regulation between high extracellular Ca^{2+} and RANKL signaling in RAW cell derived osteoclasts. Our data imply a cross talk mechanism of extracellular Ca^{2+} on osteoclast survival through the regulation of RANKL.

P29

THE CALCINEURIN-INHIBITOR *TACROLIMUS* (FK506) IS A POTENT SUPPRESSOR OF HUMAN OSTEOCLAST DIFFERENTIATION

CM Lopez, JM Hodge, MJ Constable, CJ Aitken & GC Nicholson.
The University of Melbourne, Dept of Clinical and Biomedical Sciences: Barwon Health, VIC

The transcription factor Nuclear Factor of Activated T cells cytoplasmic-1 (NFATc1) is activated in response to RANK signaling during osteoclast (OC) differentiation [1,2]. To establish the effects of NFATc1 inhibition on OC differentiation, the immunosuppressant *tacrolimus* (FK506) was used. FK506 and its binding protein (FKBP) bind to calcineurin, inhibiting its ability to dephosphorylate and activate NFATc1.

In a human osteoclastogenesis model employing CFU-GM as precursors, cells were treated with M-CSF and sRANKL, or M-CSF alone. Real time PCR showed that sRANKL induced NFATc1 expression at 7 and 14 days. Western blot demonstrated that proteins of 93 kDa and 112 kDa, corresponding to the NFATc1/A isoforms, were increased in response to sRANKL. Co-treatment with FK506 for 14 days dose-dependently inhibited OC generation by 70% and dentine resorption by 90% at 100 ng/mL. Time-course studies showed that exposure to FK506 for the first 24h of the culture was sufficient to produce this inhibitory effect. Late treatment from 10 days had no significant effect, whereas treatment from 7 days had an intermediate effect of formation and resorption. In mature OC treated with FK506 100 ng/mL for 48 hours, RANKL-induced NFATc1 activation was reduced by approximately 70%.

FK506 is a potent irreversible inhibitor of early OC differentiation and inhibits activation of NFATc1 by sRANKL. These results demonstrate the crucial role of calcineurin-NFAT interactions in osteoclastogenesis.

[1] Ishida N *et al* 2002 J Biol Chem 277:41147
[2] Takayanagi H *et al* 2002 Dev Cell 3:889

P30

GENE EXPRESSION PROFILING IN CALLUS TISSUE FROM PATIENTS WITH NEUROFIBROMOTOSIS 1-ASSOCIATED PSEUDOARTHROSIS OF THE TIBIA

EJ Smith[1,2], EM Gardiner[2], N Elmonoufy[1], CE Johnston II[1] & R Welch[1]
[1] *Seay Centre for Musculoskeletal Research, Texas Scottish Rite Hospital for Children, Dallas, Texas, USA*
[2] *Garvan Institute of Medical Research, Sydney, NSW*

Congenital pseudoarthrosis of the tibia (CPT), involving non-union after fracture, continues to be one of the most difficult orthopaedic conditions to treat. At least fifty percent of CPT patients carry an inactivating mutation in the Neurofibromin (NF-1) tumor suppressor gene which normally suppresses p21RAS mediated cell proliferation. While the molecular mechanisms of tumor initiation in neurofibromatosis-1 are beginning to be understood, the role of NF-1 and underlying molecular mechanisms of CPT are unknown. To gain insight into potential mechanisms in CPT non-union we conducted expression profiling in CPT callus tissue.

Expression profiling was performed on biotin-labeled cRNA from 4 CPT callus and 4 control bone samples, using Affymetrix HU133A oligonucleotide arrays. Genespring 6.0 analysis was used to compare absolute expression values of CPT callus to control bone, followed by cross-referencing to expression profiles from models of bone repair.

Parametric statistical group comparisons revealed 116 (1.2%) transcripts differentially expressed between CPT callus and control bone ($p<0.05$). Of these, 100 transcripts were up-regulated and 16 down-regulated ($p<0.05$). Among these were 2 up-regulated fibroblast-related, 6 chondrogenic/cartilaginous matrix-related of which 2 were up-regulated >50-fold, 4 adipocyte-related, a neuronal cell transcription factor induced by BMP signaling and 4 down-regulated bone matrix genes. This expression profile was not seen in other bone repair models.

The transcript profile seen in this study suggests a CPT callus mixed cell population with fibroblastic, chondrogenic and/or adipogenic, rather than bone forming phenotypes. This is consistent with CPT callus histology in which there is an immature matrix and fibrous dysplasia.

P31

FOUR NOVEL MUTATIONS IN THE CALCIUM-SENSING RECEPTOR ASSOCIATED WITH FAMILIAL HYPOCALCIURIC HYPERCALCAEMIA

I Stewart[1], N Lewis[2], A McElduff[3], D Chipps[4], A Poynten[5], P Clifton-Bligh[3], A Conigrave[2], B Robinson[1,3] & R Clifton-Bligh[1,3]
[1] Department of Cancer Genetics, Kolling Institute, Royal North Shore Hospital; [2] Department of Biochemistry, University of Sydney; [3] Department of Endocrinology, Royal North Shore Hospital; [4] Department of Endocrinology, Westmead Hospital; [5] Liverpool, NSW

Familial hypocalciuric hypercalcaemia (FHH, OMIM 145980) has been associated with heterozygous inactivating mutations in the calcium-sensing receptor gene (*CaSR*) that impair calcium feedback in parathyroid cells (causing hypercalcaemia with normal or elevated serum PTH concentrations) and in the renal tubule (leading to hypocalciuria). We have identified four novel *CaSR* mutations in FHH kindreds: an asparagine to serine mutation at codon 89 (N189S), aspartate to glycine at codon 216 (D216G), cysteine to tyrosine at codon 546 (C546Y), and cysteine to arginine at codon 765 (C765R). The function of these mutant receptors was assessed by calcium-dependent MAP kinase activation. Some mutations either abolished (C765R) or markedly reduced (D216G) response to extracellular calcium, whereas the function of N189S was not detectably different from the wild-type receptor in this assay. In vitro receptor function paralleled clinical behaviour in particular with respect to the degree of hypocalciuria, and indeed one individual with N189S mutation demonstrated normal urinary calcium excretion (1.4%). Our study emphasises the need to consider FHH in the differential diagnosis of hypercalcaemia. Although a fractional urinary calcium excretion of less than 1% usually distinguishes FHH from primary hyperparathyroidism, normal urinary calcium excretion may occur if the FHH mutation is functionally mild.

P32

CALCIUM ABSORPTION AND VITAMIN D METABOLITES IN HIP FRACTURE SUBJECTS

AG Need[1,2,3], M Metz[1], PD O'Loughlin[1,3], M Horowitz[2,3] & BEC Nordin[1,2,3]
Division of Clinical Biochemistry[1], Institute of Medical and Veterinary Science, Department of Medicine[2], Royal Adelaide Hospital and Hanson Institute, Adelaide, SA

Impaired intestinal calcium absorption has been reported in hip fracture cases [1] and claimed to increase the risk of hip fractures prospectively [2] but the relation between this impairment and circulating vitamin D metabolites has not been examined. We have measured fractional radiocalcium absorption (α)[3] and serum 25 hydroxyvitamin D (25D) and 1,25 dihydroxyvitamin D (1,25D) in 56 ambulant outpatients (48 female, 8 male) of mean age 67(SD11) yr with prevalent hip fracture, and in the same number of age and sex matched controls.

Mean α was significantly lower in the fracture subjects (0.46(0.18) vs 0.63(0.22):$P<0.001$) as was serum 1,25D (89(46) vs 106(36) pmol/L:$P=0.036$), but 25D did not differ significantly between the groups (54(27) vs 61(23) nmol/L:$P=0.17$). α was significantly related to 1,25D in both groups but not related to 25D in either group. Using the relation between α and 1,25D in the controls ($\alpha=0.36+0.0026$x1,25D: $r=0.41$,$P=0.002$) as the reference line we calculated that the lower 1,25D in the fracture cases (17 pmol/L) could only account for 20% of their calcium absorption deficit.

The results suggest that the calcium absorption deficit in cases of hip fracture is largely due to a diminished intestinal response to circulating 1,25D.

1. Nordin et al, Osteoporos Int 2004;15:27-31
2. Ensrud KE et al, Ann Intern Med 2000;132:345-353
3. Nordin BEC et al, J Nucl Med 1998;39:108-113

P33

BONE MINERAL AND BONE SIZE: COMPARISONS OF PREPUBERTAL PACIFIC ISLAND AND EUROPEAN CHILDREN LIVING IN NEW ZEALAND

AM Grant[1], FK Gordon[2], EL Ferguson[2], SM Williams[3], T-E Henry[2], VM Toafa[2], BE Guthrie[2] & A Goulding[1]
Departments of Medical & Surgical Sciences[1], Human Nutrition[2] and Preventive and Social Medicine[3], University of Otago, Dunedin, New Zealand

Polynesian adults have larger bones and greater bone mineral density than Caucasians. However, no previous studies have been undertaken to determine whether differences are present in childhood. This study aimed to determine whether prepubertal NZ Pacific Island and NZ European children differ in bone size, bone mineral content (BMC) or bone mineral density (BMD), and to examine the associations of bone measures to lean mass, fat mass and chronological age.

Forty-one Pacific children (both parents of Pacific Island descent) and 38 European children aged 3 to 7 years living in New Zealand were recruited. Heights and weights were determined by simple anthropometry. Body composition, bone size and BMC (g) were measured by dual energy x-ray absorptiometry (DXA) of the total body and the non-dominant forearm (Lunar DPX-L).

In data adjusted for age and gender, Pacific children had significantly ($P<0.05$) greater height (3%) and weight (16%), greater BMC in the total body (12%), ultradistal radius (16%) and 33% radius (8%), and greater total body bone area (10%) than European children. However adjustments for body weight, in particular lean mass, eliminated differences between Pacific and European children in every bone measure.

We attribute the larger bone area and BMC of young Pacific children to their greater body size. This study shows that unlike adults, prepubertal Pacific children do not have greater bone size or BMC for their weight. (Study support: Pacific Island Advisory Council & The Otago Medical Research Foundation)

P34

SITE-SPECIFIC BONE CHANGES OF YOUNG MILK-AVOIDERS OVER TWO YEARS

JEP Rockell, SM Williams, RW Taylor, AM Grant, IE Jones & A Goulding
Department of Medical and Surgical Sciences, University of Otago Medical School, Dunedin, New Zealand

No previous longitudinal studies of anthropometry, body composition or bone have been reported in children with a history of chronic milk avoidance although they are known to be short and fracture-prone with low calcium intakes and small, poorly mineralised skeletons. The present study was undertaken to determine whether improvements occurred in z scores for height, body mass index (BMI), and total and regional bone measurements over 2 years in 46 such children aged 6.1 $\pm$ 2.0 years (mean $\pm$ SD) at baseline. Body composition, bone mineral content (BMC) and areal bone mineral density (BMD) were measured by dual energy x-ray absorptiometry (Lunar DPX-L). Volumetric bone mineral apparent density (BMAD) values were calculated. Although calcium intakes had improved and some catch-up in height had taken place the group remained significantly shorter than the reference population (z scores -0.39 $\pm$1.14) with elevated BMI (z scores 0.46 $\pm$ 1.0) due to high adiposity. The z scores for BMD had improved to lie within the normal range at predominantly cortical sites (33% radius, neck of femur and hip trochanter) but had worsened at predominantly trabecular sites (ultradistal radius and lumbar spine) where values were below the reference group ($P< 0.05$). Similarly although volumetric BMAD z scores had normalized at the 33% radius, spinal BMAD values remained below the reference population at follow-up (-0.67 $\pm$ 1.12, $P <0.001$). Our results demonstrate persisting height reduction, overweight and osteopenia at the ultradistal radius and lumbar spine in young milk-avoiders over 2 years. (Grant support: Fonterra, HRC).

P35

SKELETAL AGE DEVIATION: ASSOCIATION WITH BONE MASS AND FRACTURE RISK IN CHILDREN

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

There is no data describing the association between skeletal age deviation (SAD, defined as the difference between bone age and chronological age) and either bone mass or fracture. The aim of this study was to describe the association between SAD, bone density and upper limb fracture risk in male and female children aged 9-16 years. We studied a total of 321 fracture cases and 321 randomly selected individually matched controls. Skeletal age was assessed by standard atlas using the Tanner Whitehouse 2 method. Bone mineral density (BMD) was measured by DXA and metacarpal index (MI). There were no significant differences in mean skeletal age or chronological age between fracture cases and controls. However, SAD was associated with total, hand and female fracture risk (all $p<0.05$). The fracture associations became non-significant after adjustment for BMD and MI in all subgroups with the exception of hand fractures (OR 0.67/year, 95% CI 0.47-0.96). SAD was also positively associated with BMD at all sites ($r = 0.33$-0.35, all $p<0.05$) and MI ($r = 0.20$, $p<0.05$). The strength of association reduced but remained significant after adjustment for body size, sexual maturity, age and sex. SAD was associated with milk intake, birth weight, grip strength, ever smoking and inhaled corticosteroid usage. In conclusion, SAD is positively associated with measures of bone strength and negatively associated with upper limb fracture risk (especially the

hand) in children. SAD is simple to measure and gives additional information to DXA and MI regarding bone health and fracture risk in children.

P36

AUTOSOMAL DOMINANT PATTERN OF INHERITANCE IN AN AUSTRALIAN FAMILY WITH LOW BONE MINERAL DENSITY

C Meier[1], M Kennerson[2], G Nicholson[2] & MJ Seibel[1]
[1] *Bone Research Program, ANZAC Research Institute, University Sydney, Concord, NSW*
[2] *Neurobiology Laboratory, ANZAC Research Institute, University Sydney, and Molecular Medicine Laboratory, Concord Hospital, Concord, NSW*

Low bone mineral density (BMD) is a major risk factor for osteoporotic fractures. Based on family and twin data it is estimated that 60-80% of the variance in BMD is attributable to genetic factors.

We have identified a family of Anglo-German derivation (21 members, 3 generations, aged 19-86 years) in which 14 members are affected by low BMD at the spine and/or the femoral neck. The 1st generation consists of a female patient aged 86 yrs presenting with multiple low trauma fractures, a lumber spine BMD (LSBMD) T-Score of –4.1 SD (Z-Score –2.2 SD) and a femoral neck BMD (FNBMD) T-Score of –7.4 SD (Z-Score –5.5 SD). In the 2nd generation, all four members (2 females, 2 men, median age 58.2 yrs) are affected by low BMD (LSBMD: median T-score –3.1 SD, median Z-Score –2.8 SD; FNBMD: T-Score –1.8 SD, Z-Score –1.2 SD). Two members presented with prevalent fragility fractures. In the 3rd generation, 9 members (56%, 3 females, 6 males, age 30.7 yrs) had low BMD (LSBMD: T-Score –1.7 SD, Z-Score –1.4 SD; FNBMD: T-Score –1.3 SD, Z-Score –1.0 SD), whereas 7 individuals (3 females, 4 males; age 26.6 yrs) had normal BMD. One member of the 3rd generation was diagnosed with a fragility fracture. Secondary causes of osteoporosis were excluded in all affected individuals. Segregation of low BMD in this family is consistent with an autosomal dominant mode of inheritance.

In simulation studies using the SIMLINK program and four allele markers (n=450) a theoretical mean maximum logarithm of odds (LOD) score of 5.34 was obtained at a recombination fraction (θ) of 0.0. Simulation data also indicated the family had the power to exclude 10.2 cM on either side of an unlinked marker. Two-point linkage analyses excluded mutations in the genes for *COL1A1* (D17S1795, LOD score -2.91, θ of 0.10) and *COL1A2* (D7S3050, LOD score -3.29, θ of 0.10). In addition, the *LRP5* gene has been excluded by linkage analysis (D11S987, LOD score -2.16, θ of 0.5). Based on the autosomal dominant inheritance pattern of low BMD it seems likely that in this family, accrual and maintenance of BMD is under the predominant control of a single gene. A 10 cM genome wide screen is presently under way.

In summary, the study of this family has the statistical power to define a locus on a single chromosome demonstrating that an individual gene produces the observed phenotype.

P37

A COMPARISON OF HEALTHY, YOUNG ADULT MALE VERSUS FEMALE BROADBAND ULTRASOUND ATTENUATION OF THE CALCANEUS VIA QUANTITATIVE ULTRASOUND ANALYSIS

BK Weeks & BR Beck
School of Physiotherapy and Exercise Science, Griffith University, Gold Coast, QLD

Background:
Quantitative ultrasonometry (QUS) is a relatively new method used to determine bone mass. Few existing QUS devices contain male normative databases. Consequently, male measures must be compared with female data to predict fracture risk. As the sex differences in bone mass and size are well known, this approach is inappropriate. Furthermore, while peak bone mass is achieved by young adulthood, normative QUS data is typically available only for older individuals.

Methods:
A total of 147 male and female Caucasians aged 19 to 25 years were recruited. Broadband ultrasound attenuation (BUA) of both calcanei was measured (QUS-2, Quidel Corp, CA). Daily calcium consumption, weight-bearing exercise, smoking habits, alcohol intake, medications and menstrual history were recorded.

Results:
Male BUA was significantly greater than female (100.8 ±2.1 versus 90.1 ±1.5, respectively, p=0.001). No significant effect of age or side-dominance on BUA could be demonstrated for either sex. Weight-bearing exercise (p=0.022), height (p=0.035), weight, (p=0.018), and BMI (p=0.041) contributed to variance between male BUAs. Weight-bearing exercise (p=0.003), and

weight (p=0.005) determined BUA variation between women. Short-term BUA measurement precision was 2.2% for men and 1.2% for women.

Conclusions:
We conclude that young adult men have considerably greater BUA than age-matched women, and that BUA does not increase substantially between 19 and 25 years. Our findings indicate a need for the inclusion of young adult and male databases in QUS devices if the technology is to be used equitably for the prevention, diagnosis and monitoring of osteoporosis in both sexes.

P38

PERSISTENCE WITH RISEDRONATE TREATMENT IN THE ACTNOW™ PATIENT SUPPORT PROGRAMME

J Robertson & S McKechnie
Medical Department, Aventis Pharma, Lane Cove, NSW

The long-term persistence of treatment and daily/weekly compliance with antiresorptive therapy in patients with osteoporosis represents a major hurdle in the prevention of fractures and chronic disability. Published data suggests only approximately 50% of patients remain persistent with osteoporosis therapy after 12 months[1]. The ActNow™ patient support programme was developed by Aventis Pharma in 2001 to address this problem and ensure patients were educated about the importance of remaining on therapy (persistence) and administering the tablet correctly (compliance). The aim of this study is to evaluate the proportion of patients enrolled in ActNow and who are still taking risedronate (Actonel) 18 months after initial enrolment (persistence).

ActNow patients receive regular mailed information and newsletters. They are also contacted by phone by trained nurses at enrolment then at 1, 2, 6, 12, 18 months. Patients are invited to discuss treatment or osteoporosis-specific issues. Patient demographics, treatment history and self reported risedronate persistence data were recorded following patient consent at each phone contact.

3111 patients enrolled into the ActNow programme from 1st February 2001 to 31st July 2002. Average patient age was 71.2 years, 37.4% were taking concomitant calcium supplementation and 3.3% were male. At the time of this analysis, complete data were available for 1973 patients (63.4%). Reasons for dropout included death, lost to follow up, or early change of medication for reasons other than side effects. Of these evaluable patients, 1528 patients (76%)(SD 11%) reported taking Actonel at 18 months after initial enrolment.

Patients who enrol in the ActNow Programme achieve 18-month risedronate persistence rates of approximately 76%. Limitations of this study include the lack of a control group, self-reporting and possible selection bias created by voluntary enrolment.

1. Ettinger et al. American Journal of Managed Care. 1998; 4, 1377-1382

P39

GENDER DIFFERENCES IN RELATIONSHIPS BETWEEN BODY COMPOSITION COMPONENTS, THEIR DISTRIBUTION AND BONE MINERAL DENSITY: AN OPPOSITE SEX TWIN STUDY

J Makovey[1], P Sambrook[1] & V Naganathan[1,2]
[1] *Institute of Bone and Joint Research, Royal North Shore Hospital, University of Sydney, Sydney, NSW*
[2] *Centre for Education and Research on Ageing, Concord Hospital, University of Sydney, Sydney, NSW*

Background:
Numerous studies indicate that bone mineral density (BMD) is closely related to body mass and its components. Most studies have examined these relationships in women with little attention given to how these relationships differ by gender.

Aims:
The aims of the present study were to use the opposite sex twin model to determine if there were gender differences in the relationship between body composition and its relation to BMD and how any such differences were influenced by age.

Methods:
We measured body composition and bone mass by dual energy x-ray absorptiometry in 93 pairs of opposite sex twins. To examine the effect of age, they were divided into two age groups: under 50 years old (45 pairs) and over 50 years old (48 pairs).

Results:
Lean mass (LM) had stronger positive relationships with bone variables in both genders at all ages than fat mass. Fat mass (FM) had positive relationships with total body, lumbar and hip BMD in women under age 50, but not over 50. In general, use of volumetric regional BMD did not improve associations with fat mass or lean mass. There was no significant relationship between FM and total or regional BMD in men under age 50, but men over 50 showed positive relationships between FM measures and total and some regional BMD measures. Central adiposity showed a positive relationship with BMD in men over 50 and women under 50.

Conclusion:
Fat mass (FM) and lean mass (LM) and their distribution in the body have different relations with BMD in men and women with ageing.

Key words: Bone mineral density, body composition, fat mass, lean mass

P40

IMPLEMENTATION OF AN EVIDENCE-BASED PHYSIOTHERAPY SERVICE FOR OSTEOPOROSIS

T Egerton[1], SL Mitchell[2] & G Creed[3]
[1] *University of Queensland, Brisbane, QLD*
[2] *Glasgow Royal Infirmary, Glasgow, Scotland, UK*
[3] *North Glasgow Hospitals NHS Trust, Scotland, UK*

Introduction:
Following the publication of evidence-based guidelines for the management of osteoporosis[1], a management protocol was established in Glasgow. It involved liaison with four DEXA scanning services, 23 hospital and community physiotherapy outpatient departments and the council sports services.

Methods:
One additional part-time physiotherapist position was funded. Patients were referred to physiotherapy following diagnosis, assessed, managed individually for musculoskeletal pain and given either individual supervised exercises, a home programme or referral to a 12-week, physiotherapist-led group exercise programme at one of three hospital sites. Long-term self-management of pain and ongoing exercise was emphasised, along with education on calcium rich diet, back care and community support available.

Results:
Over 6-months, 189 patients were referred to the physiotherapy service. Of those, 16% (30 patients) were treated for pain and 37% (69 patients) were referred to the group exercise programme. The first 41 patients who completed the group programme were reassessed:

Measure:	**Proportion of patients recording improvement:**	**p-value**
Tragus to wall (posture)	76%	p = 0.000
Chest expansion	41%	p = 0.021
One leg stand (balance)	71%	p = 0.001
Six minute walk (aerobic capacity)	84%	p = 0.000

Seventy-four patients who attended at least one physiotherapist led group exercise session were sent a satisfaction questionnaire (response rate 69%):

- 92% enjoyed the exercise
- 69% exercising more now than before
- 49% better balance & less likely to fall
- 49% feel stronger
- 47% feel than can do more now

Conclusions:
This evidence-based service is an example of successful collaboration between diagnostic, primary care and community services. Osteoporosis patients in Glasgow now have access to a much needed and effective physiotherapy service.

Reference:
[1] Chartered Society of Physiotherapy (1999), Physiotherapy Guidelines for the Management of Osteoporosis. Chartered Society of Physiotherapy, London.

P41

DETAILED ANALYSIS OF RISK FACTORS FOR BONE LOSS IN AN AUSTRALIAN CROHN'S DISEASE (CD) POPULATION

N Lee[1], G Radford-Smith[2], J Wong[3], M Forwood[4] & D Taaffe[1]
[1] *School of Human Movement Studies, The University of Queensland, QLD*
[2] *Department of Gastroenterology, Royal Brisbane and Women's Hospital, QLD*
[3] *Department of Medicine, The University of Queensland, QLD*
[4] *Anatomy and Developmental Biology, The University of Queensland, QLD*

Aim:
To assess the importance of anthropometric and disease-related variables for development of osteoporosis in an Australian CD population.

Methods:
62 participants with CD aged 24-72 years underwent BMD of total body, spine (L2-L4), proximal femur and forearm using DEXA. Ultrasound parameters of the heel were assessed using QUS. Risk factors evaluated included lifestyle, disease characteristics, muscle strength, and body composition.

Results:
32 patients (52%) had normal BMD, 20 (32%) were osteopenic and 10 (16%) were osteoporotic. In univariate analysis, greater % lean mass ($p=0.04$) and colonic disease ($p=0.04$) were predictive of lower spine BMD, whilst BMI ($p=0.01$) and greater alcoholic drinks/yr ($p=0.03$) were related to higher spine T-score. At the hip, greater age ($p=0.01$) and BMI ($p<0.01$) were predictive of lower and higher BMD, respectively. Advancing age ($p<0.01$) and lower grip strength ($p=0.04$) were predictive of total body BMD, whilst higher age ($p<0.01$) and lower BMI were predictive of lower forearm BMD. When controlled for age, sex and BMI, colonic disease remained independently predictive of spine BMD ($p<0.1$), whilst higher grip strength remained a moderate predictor of greater total body BMD ($p<0.1$). BMI remained a positive predictor of spine BMD after adjusting for age and weight ($p = 0.01$).

Conclusion:
These results indicate a high prevalence of compromised skeletal status in our Australian CD population. The association between colonic disease and BMD may implicate a number of factors including a low BMI and greater steroid burden in patients with extensive colonic disease. Analysis of other clinical variables is continuing.

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SIMILAR TRUNK LENGTH BUT SHORTER LEG LENGTH IN CHINESE THAN CAUCASIANS: IMPLICATION FOR RACIAL DIFFERENCES IN HIP FRACTURE RATES

XF Wang, Y Duan & E Seeman
Department of Endocrinology and Medicine, Austin Hospital, The University of Melbourne, Melbourne, VIC

The basis for the lower hip fracture rate in Chinese than Caucasians is unknown. We asked: (i) are there racial differences in trunk length or leg length? (ii) Do Chinese have shorter femoral neck axis length (FNAL) after adjustment for femur length? We measured standing and sitting height, leg length, femur length and FNAL in 239 healthy Chinese (162 females) and 542 Caucasians (403 females) aged 18-45 years living in Melbourne, Australia. In both women and men, Chinese had a 5.8-6.3cm (~3.5%) shorter stature than Caucasians due to their shorter leg length (~85%) not sitting height. There was a lower ratio of leg length/standing height in Chinese than Caucasians in both sexes. In a subgroup of Chinese and Caucasians matched by standing height, Chinese had shorter leg length but greater sitting height than Caucasians. FNAL was shorter in Chinese than Caucasians before (~7.4%) and after (~3.8%) adjusted for their shorter femur length in both sexes. In a subgroup of Chinese and Caucasians matched by femur length, Chinese had 3.5% shorter FNAL than Caucasians in both sexes. Racial differences in standing height is predominantly on the leg, not trunk, length. Chinese have a shorter FNAL relative to their femur length. We infer that the lower body segment length and shorter FNAL in Chinese may contribute to the lower hip fracture rates in Chinese than Caucasians.

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THE FRACTURE RISK INDEX AND BMD AS PREDICTORS OF VERTEBRAL STRUCTURAL FAILURE

Y Duan[1], F Duboeuf[2], F Munoz[2], PD Delmas[2] & E Seeman[1]
[1] *Department of Endocrinology, Austin Health, The University of Melbourne, VIC*
[2] *INSERM Research Unit, Lyon, France*

Structural failure becomes increasingly likely as the load on bone exceeds the bone's ability to withstand it. The vertebral fracture risk index (FRI) expresses the risk for structural failure as a ratio of the load per unit area/strength and so should be a more sensitive predictor of fracture than spine aBMD or vBMD, surrogates of bone strength alone. To address this issue, we analysed the results of a case-control study of 89 women with vertebral fractures and 306 controls in Melbourne, and a 10-year prospective study in which 30 women had incident vertebral fractures were compared with 150 controls in Lyon, France. The FRI and vBMD of the L3 vertebral body and spine aBMD were derived using DXA. In the cross-sectional analysis, each SD increase in FRI was associated with 2.1-fold (95% CI, 1.55-2.73) increased fracture risk, while each SD decrease in aBMD or vBMD was associated with 4.0-fold risk (95% CI, 2.69-6.18 & 2.65-6.94, respectively). Using ROC analysis, the FRI was less sensitive and specific than aBMD in discriminating cases (area under ROC, 0.76 vs 0.84, $p < 0.01$). The FRI and vBMD did not differ (0.76 vs 0.79, NS). In the prospective data set, the FRI was not predictive [HR, 1.20 (95% CI: 0.9-1.7)] in contrast to aBMD [HR, 2.4 (95% CI:1.5-3.8)] and vBMD [HR, 2.1 (95% CI, 1.39-3.17)]. We concluded that applying a biomechanical index such as FRI is no better in discriminating fracture cases than conventional aBMD or vBMD. The FRI may not predict incident vertebral fractures.

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INTRA-VERTEBRAL BONE MINERAL DENSITY ASSESSMENT USING DXA: A PILOT STUDY

AM Briggs[1], JD Wark[2], S Kantor[2], N Fazzalari[3], AM Greig[1] & KL Bennell[1]
[1] *Centre for Health, Exercise and Sports Medicine; School of Physiotherapy; and* [2] *Department of Medicine, University of Melbourne, VIC*
[3] *Institute of Medical and Veterinary Science,SA*

Introduction:
Analysis of apparent BMD of the hip and spine has often been used to predict fracture risk and evaluate responses to pharmacotherapy in individuals with osteoporosis. Studies have identified differences in the prevalence rate of vertebral fracture among individuals with comparable BMD. This finding may suggest that current assessments of BMD lack specificity or that factors other than BMD may be involved. Assessment of whole vertebral body BMD using DXA is unable to detect any regional differences in mineral distribution within the vertebral body, since subregional variability is not assessed.

Aim:
The aim of this pilot study was to determine the accuracy of DXA to measure apparent subregional BMD in lumbar vertebral bodies L2-L4.

Methods:
A cadaver spine was scanned in a water bath using AP and laterally orientated projections. Seven subregions were selected within the vertebral body and apparent BMD was calculated for each. The spine was then sectioned for histomorphometric analysis at the same subregions.

Results:
There was a significant difference between regions at all three lumbar vertebrae, confirmed with a one-way repeated measures ANOVA ($p<0.001$). *Post hoc* tests showed significant between-region differences ($p<0.05$). Importantly, the anterior third and central region of the vertebral body consistently showed the lowest apparent BMD. Qualitatively, good agreement between DXA and histomorphometry was established.

Conclusion:
This finding is consistent with previously published *ex vivo* data and may help to explain the aetiology of anterior wedge fracture commonly observed in individuals with spinal osteoporosis. Future work will see DXA findings correlated with subregional histomorphometry and ultimately with clinical outcomes.

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INTRA-RATER AND INTER-RATER PRECISION OF MEASURING APPARENT BONE MINERAL DENSITY IN VERTEBRAL SUBREGIONS USING SUPINE LATERAL DUAL ENERGY X-RAY ABSORPTIOMETRY (DXA) *IN VIVO*

AM Briggs[1], JD Wark[2], S Kantor[2], R Son Teh[2], AM Greig[1] & KL Bennell[1]
[1] *Centre for Health, Exercise and Sports Medicine; School of Physiotherapy and* [2] *Department of Medicine; University of Melbourne, VIC*

Introduction:
DXA is one of the most commonly used clinical tools to measure apparent BMD of the lumbar spine *in vivo*. Measurement of apparent BMD in subregions within the vertebral body from a lateral scan may help to explain the complex aetiology underlying osteoporotic vertebral fracture.

Aim:
The primary aim of this study was to assess the intra and inter-rater precisions of selecting subregions within the vertebral body from a lateral DXA scan. A secondary aim was to determine if significant between-region differences in BMD could be identified.

Methods:
A group of 10 young, healthy volunteers (mean age = 24yrs, sd = 5.7) were scanned on one occasion using a supine-lateral approach. Subregional BMD was analysed six times at L2 by 3 independent raters. Intra-rater precision error in selecting the subregions was expressed as the %CV for each subregion. An ICC statistic was used to reflect intra and inter-rater agreement and correspondence. The difference in apparent BMD between subregions was tested with a one-way repeated measures ANOVA and *post hoc* tests.

Results:
The mean intra-rater error ranged from 0.50-3.68% across the seven regions of interest. Analysis of intra-rater precision yielded a model 1,1 ICC of 0.97-0.99 and inter-rater precision analysis yielded a model 2,1 ICC of 0.80-0.98. A significant between region difference in BMD was found at both L2 and L3 (p<0.0001).

Conclusion:
Excellent intra-rater and good to excellent inter-rater precision exists for selecting vertebral subregions from lateral DXA scans in a young cohort. DXA can identify significant between region differences in vertebral BMD.

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EDUCATING MOTHERS CAN ALTER CHILDREN'S OSTEOPOROSIS PREVENTIVE BEHAVIOURS

T Winzenberg[1], B Oldenburg[3], S Frendin[2], L De Wit[1] & G Jones[1]
[1] *Menzies Research Institute, Hobart, TAS*
[2] *Department of Health and Human Services, Hobart, TAS*
[3] *Queensland University of Technology, Brisbane, QLD*

Childhood represents a unique opportunity to increase bone mass. However, data on methods of changing osteoporosis preventive behaviour in children is sparse. This study aimed to assess whether an educational intervention delivered to mothers might impact on their children. As part of a randomized trial in 470 premenopausal women examining the impact of bone density feedback with either an osteoporosis information leaflet, or a group education intervention, we assessed mothers self-report of calcium intake and physical activity change in their children. Change in children's calcium intake at 2 years was associated with having a child aged less than 18 years (OR 4.3, 95%CI 1.1, 16.7), receiving small group education (OR 2.3, 95%CI 1.4,3.8) and receiving feedback of a low T-score result (OR 2.0, 95%CI 1.2,3.3). Increases in children's calcium intake were more likely in mothers who commenced calcium supplements (OR 2.6, 95%CI 1.0, 6.8) or increased their own self-reported physical activity (OR 2.2, 95%CI 1.3, 3.7). Mothers who reported increases in their own physical activity were also more likely to report increases in that of their children (OR 2.7, 95% CI 1.5, 5.0). In conclusion, both BMD feedback and small group education aimed specifically at women are effective at inducing self-reported osteoporosis preventive behaviour change in their children. While this effect is most obvious if the woman alters her own behaviour, there is also an independent effect on the child regardless of behavioural change in the mother.

P48

EVALUATION OF A PHARMACIST PERFORMING OSTEOPOROSIS SCREENING IN RURAL PHARMACIES USING QUANTITATIVE HEEL ULTRASOUND (QUS)

M Naunton[1], GM Peterson[1] & G Jones[2]
[1] Tasmanian School of Pharmacy, [2] Menzies Research Institute, University of Tasmania, Hobart, TAS

Aim:
To assess the role of a pharmacist, in screening elderly rural women (>65 years) for risk of osteoporosis and to assess whether those found to be at risk seek further help and treatment from their GP following the screening.

Methods:
Women were recruited from 6 rural pharmacies in Tasmania. Subjects had heel bone density measured by QUS (Sahara device) and were educated on risk factors for osteoporosis. Results were forwarded to each subject's GP. Subjects were followed-up at 3-months to assess outcomes.

Results:
345 women (median age = 71) were recruited and underwent screening. The median calcium intake was 812 mg/day. Approximately 20% of women were shown to be at high-risk for osteoporosis (T-score ≤ -1.8). 191 subjects (55%) were referred to their GP for further assessment (T-score ≤ -1 or previous low trauma fractures). At follow-up, 68% had discussed their results with their GP and 11% had undergone further DEXA testing with 4% having further investigations planned. Over one-third (30% calcium, 6% bisphosphonate, 6% vitamin D) of women screened commenced a medication to prevent/treat osteoporosis and two-thirds indicated they had made lifestyle changes.

Conclusion:
Screening for osteoporosis in rural community pharmacies with QUS may be an acceptable first step to identify women at risk of future fracture where DXA scanning is not available. The screening was well received by the subjects, pharmacists and GPs.

P49

ZOLEDRONIC ACID PRODUCES HIGHER AND MORE RAPID THERAPEUTIC RESPONSE RATES VERSUS RISEDRONATE IN PATIENTS WITH PAGET'S DISEASE

I Reid[1], J Brown[2], D Hosking[3], W Fraser[4], K Lyles[5], P Hunt[6], NS Levitt[7], G Tsoukas[8], P Richardson[9], M Luchi[10], Y Saidi[11], G Su[10], K Zelenakas[10] & P Miller[12]
[1]University of Auckland, Auckland, New Zealand, [2]Le Centre Hospitalier Universitaire de Quebec, Sainte-Foy, Quebec, Canada, [3]Nottingham City Hospital, Nottingham, UK, [4]Royal Liverpool University Hospital, Liverpool, UK, [5]Duke University Medical Center, Durham, NC, USA, [6]Christchurch Hospital, Christchurch, New Zealand, [7]University of Cape Town, Cape Town, South Africa, [8]McGill University Health Center, Montreal, Quebec, Canada, [9]Novartis Pharma K.K., Tokyo, Japan, [10]Novartis Pharmaceuticals Corporation, NJ, USA, [11]Novartis Pharma AG, Basel, Switzerland, [12]Colorado Center for Bone Research, Lakewood, CO, USA

Bisphosphonates have revolutionised the treatment of Paget's disease. However, the need remains for faster onset of action, longer duration of effect, and improved dosing regimens. Zoledronic acid (ZA) might potentially fulfill these needs. Our objective was to assess the efficacy and safety of ZA in patients with confirmed Paget's disease over 6 months in a randomised controlled trial comparing one 15-minute infusion of ZA 5 mg (n=88) with oral risedronate 30 mg/day for 60 days (n=90). At entry, patients had serum alkaline phosphatase (SAP) levels ≥ 2X ULN (mean 429 IU/L). Mean age was 70, 33% were male, and 39% were treatment naïve. The primary efficacy end point was therapeutic response at 6 months, defined as a ≥ 75% reduction in SAP excess or its normalisation. Response was achieved in 95% of ZA patients compared to 75% of risedronate patients (*P*<.001). Response rates for ZA were consistent across all demographic and disease severity subgroups, which included age, gender, race, baseline SAP levels, and treatment history. At 6 months, the median percent decreases from baseline in the ZA and risedronate groups, respectively, were: SAP, 80.1% and 68.8%; P1NP, 90.8% and 80.8%; serum βCTX, 77.1% and 45.7%; and urine αCTX, 91.3% and 76.5% (all *P*<.001). Within 3 days of initiating drug, influenza-like symptoms occurred in 14% of ZA and 6% of risedronate patients. Other adverse event rates were similar between groups. We conclude that ZA achieves higher and more rapid rates of therapeutic response than risedronate.

P50

INCREASED BODY WEIGHT IN PRIMARY HYPERPARATHYROIDISM: IMPLICATIONS FOR DISEASE ASSOCIATIONS

MJ Bolland, AB Grey, GD Gamble & IR Reid
Department of Medicine, University of Auckland, Auckland, New Zealand

Although PHPT is frequently asymptomatic, it has been associated with increased prevalence of hypertension, insulin resistance, dyslipidaemia, cardiovascular mortality, and cancer. Previously, we reported that patients with PHPT are heavier than age-matched controls, but further studies addressing this issue have not occurred. Increased body weight potentially could explain these observed associations. We have performed a meta-analysis to determine whether patients with PHPT are heavier than age- and sex-comparable eucalcemic controls.

MEDLINE was searched for English-language studies published between 1975 and 2003. 17 studies met pre-determined eligibility criteria. Data regarding weight, BMI, serum calcium and PTH were extracted from each study, pooled, and meta-analysed using weighted and standard mean difference analyses.

Subjects with PHPT were on average 3.34kg (95%CI 1.97–4.71, P<0.00001) heavier than controls in 13 studies reporting body weight. In 4 studies reporting BMI, subjects with PHPT had an increased BMI of 1.13kg/m^2 (-0.29–2.55, P=0.12) compared to controls. Standard mean difference analysis showed that patients with PHPT have an increased weight or BMI of 0.3 standard deviations (0.19–0.40, P<0.00001).

A weight difference of this magnitude is likely to be of clinical significance. Data from prospective studies predict an increased BMI of 1.1kg/m^2 would increase the risk of diabetes, hypertension, ischaemic heart disease, overall mortality, and various cancers by amounts consistent with published data on the prevalence of these diseases in PHPT. Since no evidence exists that parathyroidectomy reduces body weight, our findings suggest that parathyroidectomy to reduce the risk of cardiovascular or neoplastic disease is not justified.

P51

ANTI-EPILEPTIC DRUG USERS HAVE AN INCREASED RELATIVE PROPORTION OF ABDOMINAL FAT AS DETERMINED BY DUAL ENERGY X-RAY ABSORPTIOMETRY (DEXA): A USAGE-DISCORDANT FEMALE TWIN AND MATCHED SISTER STUDY

S Petty[1,2], L Paton[1], S Kantor[1], TJ O'Brien[1,2] & JD Wark[1]
Department of Medicine[1], Neuroscience[2], University of Melbourne, Melbourne, VIC

Antiepileptic medication (AED) usage is associated with a change in total body weight. Our aim was to investigate possible differences in the distribution of body fat with discordance for AED using a matched design.

9 monozygous, 7 dizygous twin and 3 sister pairs discordant for >12 months of any AED use with a mean (SD) age of 37.8 (16.0) were included. Body composition was acquired by DEXA including the abdominal region extending from the superior surface of the 2nd to the inferior surface of the 4th lumbar vertebra and laterally to the inner aspect of the ribcage. Abdominal fat was expressed a percentage: of the abdominal region (Afat%); of total trunk fat (AfatTT%); of total body fat (AfatTB%).

There was no significant within-pair percentage difference (WIPD) in height, weight, total body fat and lean mass. There was a WIPD (AED user vs. non-user) in AfatTB% (17.9%, p=0.038) and in Afat% (25.4%, p=0.079). Pairs with more than 2 years of AED use (n=16) had a WIPD in AfatTT% (13.5%, p=0.038), AfatTB% (21.8%, p=0.030) and in Afat% (13.5%, p=0.057). In pairs discordant for use of an inducer (n=17) there was a WIPD in AfatTB% (17.5%, p=0.04) and a trend in Afat% (29.4%, p=0.092). The WIPD in current valproate users (n=7) was similar to that of non-valproate AED users (n=12) for all measures.

AED users have altered body fat distribution compared to non-users, with an increase in the relative proportion of abdominal fat that is independent of differences in body weight.

P52

BONE DENSITY, FRACTURES AND THE DEFINITION OF OSTEOPOROSIS

BEC Nordin
Royal Adelaide Hospital and Institute of Medical and Veterinary Science, Adelaide, SA

Some 60-90% of postmenopausal fractures occur in women with BMD T-scores above -2.5 [1] and we asked whether this current cut-off value for diagnosing osteoporosis was appropriate.

We measured BMD at 3 forearm sites and at lumbar spine, femoral neck and total hip with the Norland XR-36 QuickScan in a consecutive series of 444 postmenopausal women, of whom 278 (age 59.0 (SD 8.2)) had no prevalent adult fracture and 166 (age 62.5 (9.5)) had one or more such fractures.

Only 34 (20%) of the fracture cases had mean T-scores below -2.5 but 140 (84%) had mean T-scores below -1. The proportion of cases with fracture rose from 0.15 at T-scores >0 (Group V) to 0.70 at T-scores below -3 (Group I) (Table). The mean values in Groups I-III were all significantly higher than in Groups IV and V but the difference between Groups IV and V was not significant (ANOVA with Tukey test).

Group	I	II	III	IV	V
Mean T-score	<-3	-3 to <-2	-2 to <-1	-1 to 0	>0
Proportion of cases with fracture (SE)	0.70 (.10)	0.48 (.045)	0.40 (.038)	0.22 (0.044)	0.15 (.053)

Conclusions:
Fracture risk appears to be a significant negative linear (not exponential) function of BMD at T-scores <-1 but perhaps not at T-scores >-1. T-scores between -1 and -2.5 carry a much higher relative risk of fracture than is generally realised. We suggest that the accepted cut-off for the diagnosis of osteoporosis is too low and that fracture risk may not be a multiplicative function of bone density.

[1] Stone KL, et al. J Bone Miner Res 2003;18:1947-1954.

P53

LONGITUDINAL STUDY OF BONE-RELATED BIOCHEMICAL VARIABLES ACROSS THE MENOPAUSE

BEC Nordin[1,2,4], JM Wishart[2], PM Clifton[3], R McArthur[3], F Scopacasa[2], AG Need[1,2,4], HA Morris [2,4], PD O'Loughlin[1] & M Horowitz[2]
[1] Institute of Medical and Veterinary Science; [2] Royal Adelaide Hospital; [3]CSIRO; [4]Hanson Institute, Adelaide, SA

Cross-sectional observations have shown major menopausal changes in calcium-related variables which needed to be validated by a longitudinal study.

We recruited 104 premenopausal volunteers over the age of 44 for measurement of radiocalcium absorption (α), serum FSH and oestradiol, and collection of blood and fasting urines for freezing. We followed each subject annually by questionnaire and FSH assay until she had had amenorrhoea and a raised FSH level for at least one year, when α was remeasured and further blood and urine samples collected and frozen. When 34 women had passed the menopause, we measured 12 variables in paired thawed blood samples and 6 in paired urine samples.

Total and ionised serum calcium, alkaline phosphatase, Ca/Cr and crosslinks rose and α and TmCa fell across menopause (all $P<0.001$) but 1,25D, 25D and PTH did not change. There were significant correlations between the first and second measured values of all variables with r-values ranging from 0.40 ($P<0.05$) for Ca/Cr to 0.53 for ionised calcium, 0.56 for PTH, and 0.67 for α (all $P<0.001$).

We conclude that the menopausal falls in TmCa without change in 1,25D and TmCa without change in PTH are probably due to the loss of direct effects of estrogen on these end organs. The tracking of most of the variables across the menopause is particularly remarkable.

P54

THE MINERAL MASS AND EXTERNAL DIMENSIONS OF THE FEMORAL NECK ARE DISSOCIATED BECAUSE OF THE DIFFERING BEHAVIOUR OF THE PERIOSTEAL AND ENDOCORTICAL ENVELOPES

R Zebaze, G Maalouf* & E Seeman
Department of Endocrinology, Austin and Repatriation Medical Centre, University of Melbourne, VIC
** Orthopaedic Department, Babmand University Hospital, Beirut, Lebanon*

Femoral neck apparent volumetric bone mineral density (FN vBMD) is assumed to be independent of the external volume of the FN. This assumption is valid provided that increases in the external dimensions of the FN and the bone mineral content (BMC) within its periosteal envelope are proportional. In 697 women aged 20 - 87 years, assessed using DXA, FN BMC correlated with FN volume before ($r = 0.22$, $p < 0.001$), but not after, adjustment for age, height and weight. FN BMC did not increase as FN volume increased so a 1 SD higher FN volume was associated with a 0.67 SD lower apparent vBMD. Height, weight and age had differing effects on FN BMC and FN volume, that was reflected in vBMD. For example, a 1 SD greater height was associated with a 0.15 SD higher FN BMC, but 0.32 SD higher FN volume and so a 0.13 SD lower FN vBMD. Ex vivo data, derived using QCT and direct measurement on 26 postmortem FN specimens were confirmed the dissociation between bone mineral mass and its external dimensions; BMC and FN volume did not correlate ($r = - 0.11$) and vBMD decreased with increasing FN volume ($r = - 0.65$; $p < 0.001$). In tubular bones like the femoral neck, BMC and external volume are independent. Apparent vBMD is size dependent and is lower in bigger bones and higher in smaller bones. This dissociation between bone mineral mass and its external volume may be the reflect of different mechanisms regulating and co-regulating cellular activity on the periosteal and endosteal surfaces to optimize whole bone's tissue mass, size, its tissue mineral density, size, geometry and architecture to regulate strain.

P55

A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, MULTI-CENTRE EVALUATION OF THE EFFICACY AND SAFETY OF ZOLEDRONATE (4MG THREE MONTHLY), ADMINISTERED INTRAVENOUSLY, TO PATIENTS WITH β-THALASSAEMIA-ASSOCIATED OSTEOPAENIA: 12 MONTH INTERIM ANALYSIS

CP Gilfillan[1], BJ Strauss[2], C Rodda[3], D Bowden[4], A-M Kean[5], M Obaid[5] & B Crawford[5]
[1] Prince Henry's Institute of Medical Research, Departments of [3] Paediatrics, [2] Medicine and [4] Medical Therapy Unit, Monash Medical Centre, Melbourne, VIC and [5] Royal Prince Alfred Hospital, Sydney, NSW

Beta-thalassaemia major is associated with low bone mass and fractures. We embarked upon a 2 year randomized controlled trial of i.v. zoledronic acid (4mg 3 monthly) or placebo in the treatment of β-thalassaemia associated osteopaenia. We recruited 25 subjects from 2 centres with t-score < -1.0 at at least one site, and 23 subjects completed one year of the study (17M, 6 F). Treatment groups did not differ significantly with respect to BMD, age, height, weight and BMI at baseline. Bone mineral density was assessed at baseline and at 12 months by Lunar DPX at the lumbar spine, femoral neck, total hip and total body. Average lumbar spine BMD at 12 months in the treatment group was 0.099 g/cm2 (11.2%) greater than the 12 month mean for the placebo group adjusting for baseline BMD.(95% CI: 0.053 to 0.145 g/cm2; p-value <0.001). Average total body BMD at 12 months in the treatment group was 0.559 SD units greater than the 12 month mean for the placebo group adjusting for baseline BMD.(95% CI: 0.365 to 0.753 g/cm2; p-value <0.001). Similar increases were seen at all skeletal sites measured. Plasma ALP fell 45% by 12 months (p=0.004) and urinary deoxypyridinoline /creatinine ratio fell 47% by 3 months (NS). There were no adverse events attributed to the treatment. We conclude that i.v. zoledronic acid (4mg 3 monthly) suppresses bone turnover and dramatically increases bone mineral density in β-thalassaemia associated osteopaenia.

P56

FRAGILITY FRACTURES IN FRANKSTON: THE FAILURE OF MANAGEMENT OF OSTEOPOROSIS IN OUTER METROPOLITAN MELBOURNE

K Quick, S Varadarajan, D Dutta, S Morris & C Gilfillan
Frankston Hospital, Peninsula Health Care Network, VIC

We undertook a retrospective audit of patients sustaining a low-trauma fracture over the age of 40 admitted to Frankston Hospital over a 6 month period in 2003. Files on 323 patients were reviewed of which 70 (22%) were male (mean age 76)

and 253 (78%) were female (mean age 79). 157 (49%) had hip fractures whereas only 11 (3%) had spinal fractures. The other most common fractures were wrist, humerus and ankle, with an over representation of wrist fractures in females. One hundred and seventeen (36%) had a previous fracture history documented, with 25 (8%) having a previous hip fracture. Only 7% were on calcium and 2% on vitamin D prior to admission and only 10% were on any other specific osteoporosis therapy. The most common was alendronate at 8%. Only 9 (3%) patients had any form of osteoporosis therapy commenced in hospital. A DEXA scan was ordered in only 2 patients. Sixty-five (20%) patients had a serum calcium estimation, no patient had their vitamin D status or thyroid function checked, no male patient had their testosterone level checked. We conclude that patients with low-trauma fractures are presently almost universally denied investigation and treatment of osteoporosis during their hospital stay. We are currently reviewing these patients 12 months after discharge to see if investigation and treatment has been initiated whilst outpatients. We are also developing an in-hospital protocol to address this treatment gap.

P57

BALANCE, GAIT AND STRENGTH CHANGES IN 45-78 YEAR OLD HEALTHY WOMEN: A FOLLOW UP STUDY

N El Haber[1,2], K Hill[2], A-M Cassano[1,2], LM Paton[1], R MacInnis[3], JL Hopper[3] & JD Wark[1]
[1] *Department of Medicine, Royal Melbourne Hospital, University of Melbourne, Parkville, VIC*
[2] *National Ageing Research Institute, Parkville, VIC*
[3] *Centre for Genetic Epidemiology, University of Melbourne, Parkville, VIC*

Changes in balance, gait and strength function were assessed over a follow-up period of two years (2.56 years $\pm$ 0.66) in 123 healthy women aged 45-78 years at baseline. Static and dynamic measures of balance [Lord's Balance Test (LBT), Step Test (ST), Chattecx Balance System (CBS)], lower-extremity muscle strength, activity level assessment and gait analysis were performed at both visits.

Mean score performances changed over time. There was a significant decrease in right hip abductor muscle strength (mean score visit 1, mean score visit 2; 10.32, 9.38), gait analysis (26.26, 23.65), activity performance (80.95, 79.66) and increased sway on the LBT (50.46, 60.20). The sample was divided into two groups by age (cut-off 57yo). In both groups there were significant changes over time with increases in sway (LBT) and significant decreases in activity. In the older group (57+ years) there was a significant change in ankle dorsiflexion resulting in increased muscle weakness and an increase in postural sway (CBS) by visit 2. Expressed as the change per year there was a decrease in most measures, with weakening in muscle strength (-0.63 kg per year), slower velocity (-0.25 m/sec per year), increase in postural sway (+3.84 mm per year) and decrease in activity level (-0.54 units per year).

These findings indicate that postural sway, muscle strength, activity level and gait performance decline over time with increasing age. Hence, strengthening exercises may be beneficial to reverse these effects.

P58

THE EFFECT OF AN INPATIENT FRACTURE PROTOCOL ON PRESCRIPTION OF OSTEOPOROSIS THERAPY

S Warr, T Greenaway, E Francis & G Jones
Departments of Endocrinology and Rheumatology, Royal Hobart Hospital, Hobart, TAS

Effective therapies for the treatment of osteoporosis have been available in Australia for a number of years. Despite being subsidised by the Government for those who fracture, there are numerous reports indicating very low uptake rates in those admitted to hospital with fracture. The aim of this audit was to assess the impact of a fracture protocol on inpatient prescriptions of osteoporosis therapy. The fracture protocol was arrived at by consensus and was based on recommendations from the fracture prevention summit which included orthogeriatric referral as well as specific advice on the commencement in hospital of calcium, vitamin D, SERMs and bisphosphonates. We studied subjects who were treated for fractured neck of femur at Royal Hobart Hospital from March 2002 to March 2004 and included 170 prior to the start of the protocol and 100 after. As compared to the baseline period, subjects after the introduction of the protocol had higher rates of in hospital prescription for any treatment (54 v 31%, $p<0.01$), calcium (50 v 25%, $p<0.01$), vitamin D (47 v 28%, $p<0.01$) and oral bisphosphonates (23 v 5%, $p<0.01$) but not SERMs as expected (1 v 1%, $p=0.70$). Additional factors affecting decision to start treatment included female sex ($p=0.03$) but not in hospital death or age. In conclusion, a structural approach to changing hospital policy from the top down is effective at substantially increasing the usage of effective therapy after fractured neck of femur. Further research to identify additional reasons for non-treatment is now a priority.

P59

IN VIVO PRECISION OF DXA-DERIVED HIP STRUCTURAL ANALYSIS VARIABLES UNDER CLINICAL CONDITIONS: COMPARISON WITH CONVENTIONAL BMD USING IDENTICAL DXA SCANS

BCC Khoo[1,2], TJ Beck[3] & RI Price[1]

[1] Medical Technology & Physics, Sir Charles Gairdner Hospital; [2] School of Surgery & Pathology, The University of WA, Perth, WA; [3] The Johns Hopkins University, Baltimore, MD, USA

DXA-derived BMD is widely used to diagnose osteoporosis. Osteoporosis arises from poor bone-mass and micro-architecture, and reduces bone mechanical strength. However, BMD does not provide direct information on strength, which may be better derived from structural-geometry. Hip Structural Analysis provides a mechanical interpretation of DXA, using its mass profiles to compute cross-sectional structural-geometry, particularly at the narrowest cross-section of the femoral neck (NN), including deriving cross-sectional area (CSA) and section modulus (Z), surrogates for compressive and bending strength, respectively. This is the first report of short-term "head-to-head" clinical precision of HSA, compared with "conventional" BMD.

Paired hip DXA scans taken <91 days apart, derived from 2 large multi-centre osteoporosis trials (utilising multiple DXA machines) were analysed for HSA at NN and short-term precisions expressed as "percent-coefficient-of-variation", below.

Variable (scan-pairs)	QDR 1000 (n=233)	QDR 1500 (n=50)	QDR 2000 (n=524)	QDR 4500 (n=129)	Lunar DPX (n=119)	*Norland XR26* (n=31)
Convent. BMD	2.3	2.1	2.3	2.5	3.4	1.9
HSA						
CSA	3.6	2.8	2.8	3.0	7.9	2.6
Bone Width	2.6	2.4	2.2	2.1	3.3	2.9
Endocort. Diam.	2.8	2.7	2.4	2.6	3.4	3.4
Cort. Thickness	3.1	2.5	2.8	3.2	6.5	2.9
Z	5.7	4.1	3.4	4.0	10.1	5.0

Poorer HSA precision of some DXA systems arose from inadequate inclusion of shaft in the DXA region-of-interest, rendering (crucial) computation of shaft and femoral-neck axes inaccurate.

It is concluded that precision of the bending-strength variable, Z, is significantly poorer than for convention BMD, and HSA demands significantly more attention paid to limb repositioning and scanning region-of-interest.

P60

CONVERSION OF LUNAR SPINE BMD TO HOLOGIC UNITS AT NON STANDARD VERTEBRAL REGIONS OF INTEREST

B White[1], M Hooper[1], M Henry[2], G Nicholson[2] & N Pocock[3]

[1] Department of Endocrinology and Metabolism, Concord Hospital, Concord, NSW

[2] Department of Clinical and Biomedical Sciences, University of Melbourne, Barwon Health, Geelong, VIC

[3] Nuclear Medicine Dept, St Vincent's Hospital , Sydney, NSW

The interpretation of DXA BMD requires comparison to appropriate reference ranges. To standardize DXA results from different brands of densitometers, the ANZBMS has recommended that population derived reference data from the Geelong Osteoporosis Study (GOS) be adopted as the Australian reference range for DXA interpretation. The GOS data for the lumbar spine (L2–L4), and proximal femur sites, acquired on a Lunar DPXL, were previously converted using published equations[1] to equivalent units for the other major brands of densitometer used in Australia (Hologic and Norland). The reference ranges of individual vertebrae and other combinations of vertebrae (e.g. L1-L4) could not however initially be provided as appropriate conversion equations were not available.

To achieve suitable conversion equations for non-standard vertebral regions of interest, 100 subjects were scanned on both a Lunar Prodigy and an Hologic QDR 4000 scanner on the same day. The data from the various individual vertebrae, and non-standard vertebral regions, were subsequently compared using regression analysis. The resulting equation for L2-L4 was compared to the corresponding published conversion equation[1] and found to be not statistically different. Subsequently the appropriate equations for individual vertebrae, and non-standard vertebral regions, were used to convert the GOS data into Hologic units.

In conclusion the study has provided Lunar to Hologic conversion equations for individual vertebrae, and non-standard vertebral regions, which will allow the utilization of the corresponding GOS reference ranges at centres using Hologic scanners.

1. Genant HK et al. Universal standardization for Dual X-ray Absorptiometry: Patient and phantom cross calibration results. JBMR 1994; 9(10): 1503-1514.

P61

LIMITED RESIDUAL BENEFITS OF CALCIUM SUPPLEMENTATION ON BONE MASS ACCRUAL IN PREPUBERTAL CHILDREN ONE YEAR AFTER THE CESSATION OF TREATMENT

S Iuliano-Burns, A Evans, X-F Wang, S Matthews & E Seeman
Department of Endocrinology, Austin Health, Heidelberg, VIC

Only one study in girls using milk-based calcium reported residual benefits to bone mass and size after supplementation. To test the hypothesis that calcium from milk minerals, but not calcium carbonate, will result in permanent changes to BMC and bone dimensions, 106 pre-pubertal children (5-11 yrs), randomly assigned to 800 mg/d calcium from milk minerals (MM), calcium carbonate (CAL), or a placebo (PLA), for 9 months, were followed up one year later. Bone dimensions & BMC were assessed yearly using DXA. Dietary calcium was measured bi-yearly using 3-day food diaries. Anthropometry and hours of weight bearing exercise were recorded. Differences between groups were determined using ANCOVA, adjusting for baseline values and changes in limb lengths. Data was analysed using Statview (version 4.51).

Despite randomisation, the PLA group was older and larger than the CAL group ($p < 0.05$). The MM & CAL groups accrued more BMC at the pelvis, and the MM group more BMC at the tibia than the PLA group (26% v 20-22%, $p < 0.05$). For total gains (yrs 1 + 2) there was a tendency for greater BMC accrual at the pelvis and tibia in the MM than PLA group (43% v 38-40%). A dose response for supplementation was observed in the MM group for appendicular BMC accrual and gains in periosteal widths ($r = 0.4$-0.6, $p < 0.05$).

Cautious speculation can be made about the long-term benefits of MM on bone mass and dimensions, which may be elucidated on follow up of participants after maturity.

P62

TRENDS IN PREVALENCE OF OSTEOPOROSIS IN SOUTH AUSTRALIA AND EFFECT ON QUALITY OF LIFE

T Gill[1], A Taylor[1], P Phillips[2], G Leach[3] & P Kelly[3]
[1] Department of Human Services, Adelaide, SA, [2] The Queen Elizabeth Hospital, Adelaide, SA, [3] Arthritis SA, Adelaide, SA

Aims:
Osteoporosis is increasingly being recognised as a disease which has a significant impact on the health care system. This paper presents the self-reported osteoporosis prevalence, in South Australia since 1995 using the South Australian Health Omnibus Survey (HOS), and the impact of osteoporosis on quality of life as measured by the Short Form 36 (SF-36).

Method:
HOS is an annual, face to face survey, of people aged 15 years or older living in both metropolitan Adelaide and country South Australia. Data is weighted (by gender, age, possibility of selection in the household and the geographic region), to the most recent census data ensuring that the results are representative of the South Australian population. Response rates are generally over 70%. In 2002, quality of life of those with various conditions, including self reported osteoporosis was also examined using the SF-36.

Results:
Self reported prevalence of osteoporosis has ranged from 3.2% in 1995 to 4.7% in 2003. A chi-square test for trend indicates that the increasing prevalence of self reported osteoporosis in South Australia is significant ($\chi^2=18.39$, $p<0.05$). In 2002, when quality of life was examined, the self reported prevalence of osteoporosis of people aged 15 years and over was 4.5% (n=136). These respondents scored statistically significantly lower than people who did not have osteoporosis on all eight dimensions of the SF-36.

Conclusion:
The prevalence of self-reported osteoporosis is increasing in South Australia and self reported osteoporosis is associated with a significant decrease in quality of life.

P63

BONE STIFFNESS REDUCTION ASSOCIATED WITH REMODELING OF ADULT EQUINE P1 BONE MEASURED BY ULTRASOUND SPEED

Z Bechor, S Toulson, B Kehoe, EJ Mackie & HMS Davies
Faculty of Veterinary Science, The University of Melbourne, VIC

Aims:
Turning exercise caused a transient reduction in ultrasound speed (SOS) at a specific site of the first phalanx (P1) of adult horses (Bechor et al, 2004). This pilot study was designed to test whether the SOS change was associated with modelling or remodelling by counting the number of osteoclasts associated with SOS reduction following round yard exercise in adult horses.

Methods:
When the SOS ("Equs", Sunlight, Tel Aviv, Israel) showed a clear reduction at the P1 dorsomedial site, the mares were euthanized, and bone samples from the P1 cortical site processed for cryostat sectioning and staining for the presence of tartrate resistant acid phosphatase.

Results:
The SOS reduced on the outside limb of horse 1 (3945 to 3789m/s outside, vs. 3968 to3881 m/s inside) and the inside limb of horse 2, probably due to a lesion on the outside limb of the second horse, (3729 to 3593m/s inside vs 3840 to 3841m/s outside). The average number of osteoclasts/field in the P1 dorsomedial cortex at all 3 levels of sectioning correlated well with the higher loaded limb, 5.3 vs 3 in horse 1 and 11.16 vs 8.5 in horse 2.

Conclusions:
Turning exercise stimulates a remodeling response in the dorsomedial cortex of P1. Bone stiffness changes associated with this remodelling can be measured by SOS.

References:
Z.Bechor, Jonathan Merritt and H.M.S Davies (2004) Site and Timing of Bone Response to Turning Exercise in the Distal Forelimb of Adult Horse. ANZBMS submitted.

P64

SITE AND TIMING OF BONE RESPONSE TO TURNING EXERCISE IN THE DISTAL FORELIMB OF ADULT HORSES

Z Bechor, J Merritt & HMS Davies
Faculty of Veterinary Science, The University of Melbourne, VIC

Aims:
To identify sites on distal forelimbs bones affected by turning exercise and accessible to ultrasound speed (SOS) measurements because of the frequency of fractures in this region in racehorses during turning.

Methods:
Six horses were exercised in a 16m diameter round yard at 5 to 7 m/s. Two Thoroughbred geldings, were exercised once for 5 minutes. SOS (Sunlight Omnisense, Tel Aviv, Israel) was measured pre exercise and daily for 9 days post exercise at 15 sites around and along the third metacarpal bone (MC3) and at 5 sites around the first phalanx (P1). Four mares were exercised twice per day with 6 hours gap, for 5 minutes over 6 - 9 days. SOS was measured before exercise and at the end of exercise sessions, on each of MC3 and P1.

Results:
There were no changes in SOS for the ex racehorse gelding, but SOS decreased from 4229 m/s pre exercise to 3609 m/s 72 hours post exercise in the untrained gelding's outside limb on the dorsomedial aspect of P1. The SOS at this site remained low for at least 24 hours and then returned to the pre exercise value on day 5 post-exercise. From pre-exercise values, all mares showed a reduction in SOS post-exercise (mean 402*8 m/s) at the same dorsomedial site on P1 in the outside limb and not at any other site.

Conclusions:
A specific site on the dorsomedial aspect of P1 showed a consistent and transient drop in SOS following turning exercise.

P65

EVALUATION OF A CARE PATHWAY TO IMPROVE THE DIAGNOSIS AND TREATMENT OF OSTEOPOROSIS IN PATIENTS WITH LOW TRAUMA FRACTURE

J Whitham[1], C Gibb[2], P Kelly[1] & J Pink[3]
[1] *Osteoporosis SA, Adelaide, SA*
[2] *Department of Orthopaedics and Trauma, The Queen Elizabeth Hospital, Adelaide, SA*
[3] *Department of Pharmacy, The Queen Elizabeth Hospital, Adelaide, SA*

Aims:
This study aims to evaluate the efficacy of an osteoporosis care pathway that was intended to increase rates of identification and treatment for osteoporosis in patients sustaining a low trauma fracture. The present study seeks to determine the rates of follow-up by a General Practitioner and subsequent rates of investigation and treatment for osteoporosis. It also aims to examine the incidence and source of patient osteoporosis education.

Methods:
Patients aged ≥45 years admitted to the Department of Orthopaedics and Trauma at The Queen Elizabeth Hospital, Adelaide, South Australia, with a low trauma fracture between May and December 2003 will be approached to participate in the study. Patients will complete a questionnaire that asks about medication use, follow up visits to a GP, whether any osteoporosis investigations have been suggested or carried out, falls/fracture history, osteoporosis risk factors and whether they have received any osteoporosis education since sustaining their fracture. The outcome of patients who were captured in the pathway will be compared to those whose fracture occurred prior to the implementation of the pathway.

Results/Conclusions:
This evaluation is part of a larger project that is funded by the Department of Human Services that aims to develop a best-practice osteoporosis care pathway. Study progress to date and future directions will be discussed.

†This project is supported by the steering committee of the Osteoporosis SA Refracture Prevention Pathway Project: Nick Fazzalari, Anna Fergusson, Linda Ferris, Tiffany Gill, Trevor Hearn, Pauline Kelly, Laura Laslett, Alice McLennan, Julian McNeil, Pat Phillips, Malcolm Smith, Anne Taylor, Vanessa Wells, Valerie Williams.

P66

DECREASED FEMORAL NECK BONE DENSITY WITH INCREASED NECK SECTION MODULUS, A DECADE FOLLOWING ATTAINMENT OF PEAK BONE DENSITY; A PROSPECTIVE HIP STRUCTURAL ANALYSIS STUDY

RI Price[1], NK Briffa[2], TJ Beck[4], AL Mein[2], BCC Khoo[1], RL Prince[3] & SS Dhaliwal[1]
[1] *Medical Technology & Physics, Sir Charles Gairdner Hospital;* [2] *School of Physiotherapy, Curtin University;* [3] *School of Medicine & Pharmacology, The University of WA, Perth, WA,* [4] *School of Medicine, The Johns Hopkins University, Baltimore MD, USA*

Unlike areal BMD (aBMD), Hip Structural Analysis (HSA; also DXA-derived), describes bone-structural geometry, in particular Section Modulus (Z; bending strength surrogate), and complements BMD. Maximising premenopausal BMD helps prevent fractures. Calcium intake increases BMD, but with uncertainty about the long-term benefit of calcium-supplementation around attainment of peak aBMD (PaBMD). This study examined changes in HSA and aBMD in women, a decade following attainment of PaBMD, with/without calcium-supplementation for first 2yr.

Sixty-two women, mean(SD) age 27.8(1.0) yr were recalled and re-measured for aBMD and HSA, 9.4(0.9) yr following baseline recruitment measurements at 18yr into a two-yr randomised calcium-supplementation trial (Henderson NK etal, JBMR 1995). Recalled group was divided into “Control” (n=30) and “Calcium” (n=32). aBMD was measured at femoral neck (FN), and HSA at “narrow neck” (NN) of FN, using identical DXA scans.

Over the 9.4yr, FN aBMD decreased 3.72[-5.18,-2.26]% (Mean [95% CI]), ($p<0.001$) in the combined group, and in each subgroup. However, NN Z increased 6.44[1.76,11.12]%, ($p<0.01$) in combined group, and also in “Calcium” (7.94[3.50,12.39]%, [$p<0.01$]), but not “Control” subgroup. There were no changes in NN Cross-sectional Area (CSA), an HSA bone-mass index, but NN width increased 2.76[0.48,5.04]%, ($p<0.025$) in combined group.

It is concluded that FN aBMD fell in the decade following attainment of PaBMD, but neck Z (and therefore likely bone strength) increased in the calcium-supplemented subgroup, due to redistribution of bone further from the bending axis, rather than increasing bone mass. Assessment of changes in bone strength using aBMD may mislead, and could be clarified by HSA.

P67

A LONGITUDINAL STUDY OF EXERCISE EFFECTS ON BONE MASS AND STRUCTURE IN PRE- AND EARLY-PUBERTAL FEMALE NOVICE DANCERS AND CONTROLS

BL Matthews[1], KL Bennell[1], HA McKay[2], KM Khan[2] & JD Wark[3]

[1] *Centre for Health, Exercise and Sports Medicine, University of Melbourne, Melbourne, VIC*

[2] *Human Kinetics, University of British Columbia, Vancouver, BC, Canada*

[3] *Medicine, University of Melbourne, Melbourne, VIC*

The ideal maturational stage whereby osteogenic exercise effects may be maximized remains unclear. Ballet dancing provides a model of site-specific mechanical loading. We performed a longitudinal study comparing the absolute change and rate of change in bone mass (BMC) and bone structure in the same girls at Tanner breast stage I (TI) and at Tanner stage II (TII). Seventeen ballet dancers, mean (SD) age 9.6 (0.8) years, and 13 controls 9.5 (0.5) years, were assessed at baseline, 12, 18 and 30 months. BMC was determined by DXA for the lumbar spine, and proximal femur The Hip Structural Analysis program assessed cross-sectional area and section modulus at the narrowest section of the femoral neck and femoral shaft regionsBody size, exercise levels, hip muscle strength and calcium intake were also assessed. At baseline, dancers attended classical ballet classes for mean (SD) 5.4 (1.5) years. Weekly hours of dance classes did not differ between TI (3.7 (1.6)) and TII (4.4 (2.0)). There was no difference in age, body size, hip strength, or calcium intake between groups at any time-point. When controlling for baseline or change in bone area, height and weight, there were no significant differences between groups for change in bone parameters at either Tanner stage. This suggests that there was no significant effect of dancing on bone accrual during TI and TII. Differences in bone mineral accrual and bone geometric adaptations to dancing may not reach significance until the later tanner stages (III-V).

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"DANCING FOR BONE HEALTH": A 3-YEAR LONGITUDINAL STUDY OF EXERCISE EFFECTS ON BONE MINERAL ACCRUAL ACROSS PUBERTY IN FEMALES

BL Matthews[1], KL Bennell[1], HA McKay[2], KM Khan[2], ADG Baxter-Jones[3], RL Mirwald[3] & JD Wark[4]

[1] *Centre for Health, Exercise and Sports Medicine, University of Melbourne, Melbourne, VIC*

[2] *Human Kinetics, University of British Columbia, Vancouver, BC, Canada*

[3] *Kinesiology, University of Saskatchewan, Saskatoon, SK, Canada*

[4] *Medicine, University of Melbourne, Melbourne, VIC*

Weight-bearing exercise during growth enhances peak bone mass. However, the window of opportunity for optimizing positive effects of exercise on peak bone mass remains to be fully defined. We assessed the effects of ballet dancing (a model of site-specific, targeted exercise) on bone mineral accrual in female novice dancers and controls for 3-years across puberty. We recruited 82 ballet dancers and 61 controls aged 8-11 years at baseline. DXA was used to measure total body (TB) bone mineral content (BMC) and soft tissue composition, annually, and proximal femur and lumbar spine (LS) BMC semi-annually. Anthropometry, exercise levels and calcium intake were also measured semi-annually. Maturational age was determined from age at peak height velocity. A multilevel regression model was used to determine independent effects of body size, body composition, maturation, exercise levels and calcium intake at each measurement occasion. At baseline, dancers had attended dance classes for 1-9 (mean 5.3) years. During the study, total weekly dance hours ranged from 1-16. Dancers were significantly lighter with less fat mass than controls and consumed more calcium. When controlling for body size, body composition, maturation, and exercise dancers had significantly greater BMC at the TB, lower limbs, femoral neck (FN) and LS than controls. Excepting the FN region, these differences became apparent at 1-year post-PHV, or the peripubertal years. At the FN the dancers had 4% ($p<0.05$) greater BMC than controls in prepuberty and maintained this advantage throughout the pubertal years. Results provide evidence for site-specific, and maturity-specific effects of mechanical loading on bone.

P69

COMPARISON OF LOWER LIMB STRENGTH, BALANCE, GAIT STABILITY VALUES BETWEEN ASIAN AUSTRALIAN AND CAUCASIAN AUSTRALIAN WOMEN

TN Hutabarat[1,2,3], N El Haber[1,2], EL Bond[1], K Hill[2], LM Paton[1] & JD Wark[1]

[1] *Department of Medicine, Royal Melbourne Hospital, University of Melbourne, Parkville, VIC*

[2] *National Ageing Research Institute, Parkville, VIC*

[3] *Faculty of Medicine, University of Indonesia, Indonesia*

Balance and muscle strength are important contributors to falls- and fracture-risk. We sought to document and compare lower limb strength, balance and gait stability measures between Asian Australian women (AAW) and Caucasian Australian women (CCA).

Thirty-five AAW mean (SD) age of 53.3 (5.6), range 45 – 72 years, were match hierarchically (age, height, weight) to CAW. The AAW had a significantly lower mean weight (-12kg), therefore all data were adjusted for age-height-weight. All women completed the Lord's Balance Test, static and dynamic posturography tests (Chattecx Balance System) with and without a cognitive task. Muscle strength was assessed for knee extensors [(isometric, concentric, eccentric) (Kinematic-Communicator (Kin-Com)] and ankle dorsiflexor strength (Nicholas Manual Muscle Tester (MMT)). Mobility and gait was assessed using the stride analyzer (CSA).

AAW had consistently higher sway with both the Lord's Balance Test (27 – 32%) and on the Chattecx system (20 – 41%) compared to CAW ($P < 0.05$). AAW had a lower stride velocity (-7.4%) and higher double support duration (28%), ($P < 0.05$). AAW had lower concentric knee strength (-8.1%, $P = 0.03$) and performed worse on the step test (left and right legs) (-20 - -23%, $P<0.001$). Muscle strength (MMT) was lower in AAW for both the left (-30%) and right (-28%) knees, $P < 0.001$. AAW had higher left ankle (19%) and right hip (17%) strength.

AAW had consistently worse sway and gait performance ability than CAW. This study will help to develop the risk profile for falls and fracture in this population.

P70

PRECISION AND ACCURACY OF AUTOMATED VERSUS MANUAL HIP STRENGTH ANALYSIS IN YOUNG ADULTS

CR Ong[1,2], HG Ahlborg[2], TV Nguyen[2] & NA Pocock[1]
[1] *St Vincent's Hospital, Sydney, NSW*
[2] *Garvan Institute of Medical Research, Sydney, NSW*

Hip geometry and bone mineral density (BMD) are independent predictors of hip fracture risk. The predictive value of hip strength analysis (HSA) is dependent on its reproducibility and accuracy. The traditional manual HSA is however time consuming and operator-dependent. The current study examined the reproducibility and accuracy of automated HSA (Lunar Prodigy Software) compared to manual analysis.

Thirty healthy women aged 20 to 35 years were studied. Each woman had two dual energy x-ray absorptiometry scans performed 6 months apart. At each time point, HSA was performed using both manual and automated analysis.

There was no significant difference in the coefficients of variation for manual and automatic analysis for BMD (1.4 vs 1.4%), hip axis length (HAL 1.01 vs 0.81%), cross-sectional moment of inertia (CSMI 6.6 vs 7.1%), cross-sectional area (CSA 4 vs 4%), femoral neck diameter (FND 2 vs 2%) and neck shaft angle (NSA 1.63 vs 1.73%). There was also no significant difference in the absolute values of any of the parameters between manual and automatic analysis. The coefficient of concordance between the two modes of analysis was 0.99 for BMD, HAL, FND, and NSA, and 0.97 for CSMI and CSA.

These data indicate that the absolute values and reproducibility of the manual and automated HSA were comparable, and the concordance was high. The use of automated HSA would save time and reduce operator-dependent variability in younger subjects.

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CONCORDANCE OF AUTOMATED AND MANUAL HIP GEOMETRY ESTIMATION IN OLDER ADULTS

CR Ong[1,2], TV Nguyen[2] & NA Pocock[1]
[1] *St Vincent's Hospital, Sydney, NSW*
[2] *Garvan Institute of Medical Research, Sydney, NSW*

Hip strength analysis (HSA) may potentially aid in the assessment of osteoporotic fracture risk. The predictive value of HSA is dependent on its precision and accuracy. Manual HSA may be more reliable than automated analysis in older individuals where low bone mineral density (BMD) may impact adversely on bone edge detection and automatic region of interest placement. This study examined the concordance of automated (Lunar Prodigy Software) versus manual HSA in young and old individuals.

Two groups were compared: women aged over 70 with no exclusion criteria (n=30) and healthy women aged between 20 and 35 (n=30). Various parameters of hip geometry were measured from the dual energy x-ray absorptiometry scans using

manual and automated HSA. The concordance between the two modes of analysis was assessed by the coefficient of concordance (cC).

The cCs in the older versus the younger women were: BMD (0.99 vs 0.99), hip axis length (HAL 0.84 vs 0.99), cross-sectional moment of inertia (CSMI 0.98 vs 0.97), cross-sectional area (CSA 0.96 vs 0.97), femoral neck diameter (FND 0.99 vs 0.99) and neck shaft angle (NSA 0.85 vs 0.99). The 'limit of agreement' analysis revealed a greater between-mode variability in CSMI and HAL in the older group.

These data indicate that there was a high concordance between the automated and manual HSA in the older population. However, in some parameters, the concordance was lower in the older subjects, suggesting that automatic HSA may be clinically less reliable in the aged.

P72

COMPARABILITY OF NORMATIVE PAEDIATRIC BONE DENSITY DATA

J Briody[1], M Henry[2], A Goulding[3], G Jones[4], R Howman-Giles[1] & N Pocock[5]
[1] *Children's Hospital at Westmead, Westmead, NSW*
[2] *Geelong Hospital, Geelong, VIC*
[3] *University of Otago Medical School, Dunedin, New Zealand*
[4] *Menzies Centre for Population Health Research, Hobart, TAS*
[5] *St Vincent's Hospital, Darlinghurst, NSW*

Aim:
To determine the comparability of three paediatric normative bone mineral density (BMD) data-sets.

Methods:
Reference data sets from three centres[1,3,4] were compared using regression techniques. The Tasmanian data contained 321 subjects (Males=215, age: 7-17 yrs), NZ had 200 subjects (Males=100, age: 3-19 yrs) and NSW had 315 subjects (Males=157, age: 0-20 yrs).

At the NSW and NZ centres[1,3], a Lunar densitometer was used for PA spine (L2-L4) and femoral neck (FN) BMD measurements. The Tasmanian centre[4] used a Hologic QDR 2000.

No differences were seen between centres in FN BMD. In female children, there was no difference in L2-L4 BMD between Tasmania and NSW, but both were statistically lower compared to NZ data (mean difference=0.04g/cm²). In males, Tasmania and NZ L2-L4 data were not significantly different. NSW data was however significantly lower compared to NZ and Tasmania L2-L4 (mean difference = 0.05g/cm^2).

Conclusion:
Although FN BMD agreed between the 3 centres, there were differences (variable agreement) in L2-L4. Cross calibration of the DXA scanners could resolve some of these differences. As the disagreement was not only machine based, closer examination of patient demographics, fracture history, scanning and analysis technique may help clarify the reason for differences between the centres.

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INTER MANUFACTURER CONVERSION OF L2-L4 BONE DENSITY IN THE PAEDIATRIC POPULATION

M Thompson[1], J Briody[1], R Howman-Giles[1] & N Pocock[2]
[1] *Children's Hospital at Westmead, Westmead, NSW*
[2] *St Vincent's Hospital, Darlinghurst, NSW*

Currently, the relatively small amount of paediatric bone mineral density (BMD) reference data available has been collected in different populations, and on different types/brands of densitometers. In order to standardize the reference range used, existing data could be amalgamated, or one reference database could be adopted. Either choice requires that BMD measurements be able to be converted between machines from different manufacturers.

Aim:
To test the validity of using published adult conversion equations with paediatric L2-L4 BMD values.

Method:

Using data from children measured on both Norland and Lunar DPX scanners L2-L4 BMD was converted[1] to Norland units (NcBMD) and the converted results compared to the measured Norland BMD (NmBMD).

Results:
There were 17 children (5-17 yrs), with a median time between measurements of 126 days (range: 6-216 days). The ranges of BMD values (g/cm²) were 0.48-1.08 (NmBMD), 0.59-1.22 (Lunar) and converted values (NcBMD) were 0.46-1.09.

Although L2-L4 NmBMD and NcBMD were well correlated (r^2=0.99, p<0.001), there was a small but significant difference between the two data sets (difference = 0.01±0.02, p = 0.03). A Bland and Altman analysis showed no trend with BMD or time.

Conclusions:
The small L2-L4 difference between NmBMD and NcBMD is within the measurement error in an individual. These results indicate the published L2-L4 BMD Lunar to Norland conversion equations, potentially could be used in children in the range examined. Application to the entire range of paediatric BMD would require further validation.

1. Genant HK et al. JBMR. 1994. 9:10: 1503-14.

P74

SAFETY AND EFFICACY OF IMI CHOLECALCIFEROL FOR VITAMIN D DEFICIENCY:
TH Diamond, P Rohl & M Meerkin
Departments of Endocrinology. St George Hospital Campus and Mayne Laverty Pathology, Sydney, NSW

Currently there are no effective high dose vitamin D preparations to treat vitamin D deficiency in Australia.

A prospective study was undertaken in 36 men and women (mean age 65.7 yrs) with vitamin D deficiency to assess the safety and efficacy of a single therapeutic dose of intramuscular cholecalciferol (vitamin D3) 600,000 IU (15 mg) ('Arachitol', Solvay Pharma, India Ltd). Serum (s) calcium, creatinine, 25OHD3 and PTH measurements, as well as early morning 2-hour urinary (u) calcium and creatinine specimen were collected at baseline and after 4 and 12 months of therapy. Values are expressed as a mean ± 1 SEM.

There was 1 (3%) patient with severe (<12 nmol/L) and 12 (33%) with moderate (12-25 nmol/L) vitamin D deficiency. Seventeen patients (47%) had secondary hyperparathyroidism.

Variable	Baseline	4 months	12 months	% Change (12 months)
(s) calcium (2.2-2.65 mmol/L)	2.39 ± 0.02	2.39 ± 0.02	2.44 ± 0.02*	+2
(s) 25OHD3 (>50 nmol/L)	31.5 ± 1.4	113 ± 6***	73 ± 2***	+153
(s) creatinine (<0.11 mmol/L)	0.08 ± 0.004	0.07 ± 0.004	0.08 ± 0.004	0
(s) PTH (<7.5 pmol/L)	7.4 ± 0.07	5.8 ± 0.5**	5.2 ± 0.05**	-30
(u) Ca/Creat (<0.30)	0.24 ± 0.04	0.28 ± 0.05*	0.37 ± 0.05***	+47

P values: * = <0.05; **= <0.01 and ***= <0.001

As demonstrated above, significant increases in serum 25OHD3 were seen at 4 months (range 37-191 nmol/L), which continued even after 12 months of therapy. Mild hypercalcaemia (serum calcium =2.66 mmol/L) was seen in only 1 patient. While mild (Uca/creat 0.30-0.60) and moderate hypercalciuria (Uca/creat >0.60) occurred in 12 (28%) and 5 (14%) patients respectively, at least half presented with significant hypercalciuria prior to commencing therapy.

Once yearly IMI cholecalciferol 600,000 IU appears a safe and effective therapy for vitamin D deficiency.

P75

OSTEOPOROSIS AND SPINAL FRACTURES IN MEN WITH PROSTATE CANCER: RISK FACTORS AND THE EFFECTS OF ANDROGEN DEPRIVATION THERAPY

TH Diamond, J Bucci, JH Kersley, P Aslan, WB Lynch & C Bryant
Departments of Endocrinology, Radiation Oncology, Urology and Radiology. St George Hospital Campus, University of New South Wales, Sydney, NSW

A retrospective study was undertaken to determine the risk factors for osteoporosis and spinal fractures in men with prostate cancer receiving androgen deprivation therapy.

We evaluated 87 consecutive men with prostate cancer receiving androgen deprivation therapy referred for osteoporosis. Their data comprised of lateral thoraco-lumbar radiographs, bone densitometry, serum biochemistry and a detailed assessment of osteoporotic risk factors. A multivariate regression analysis was used to determine the major risk factors for osteoporosis and spinal fractures.

There were 38 (44%) men aged 74.5 years who had radiographic evidence of spinal fractures. They had an initial mean PSA value of 52.8 ng/mL and had received androgen deprivation therapy for a mean of 39.6 months (95% confidence interval, 28.7 to 50.4 months). Their mean spinal (quantitative computed tomography t-score = -4.2) and femoral neck bone mineral densities (dual energy x-ray absorptiometry t-score = -2.1) were significantly lower than men without spinal fractures ($P<0.001$ for all measurements). In the regression analysis, the duration of androgen deprivation therapy ($P=0.002$), serum 25 hydroxyvitamin D levels ($P=0.003$), and a history of alcohol excess (defined as more than 4 standard drinks per day) ($P=0.04$) were the main determinants of spinal fractures.

Prolonged androgen deprivation therapy, low serum 25 hydroxyvitamin D levels and a history of alcohol excess are important risk factors for osteoporosis and spinal fractures in men with prostate cancer.

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VITAMIN D AND PARATHYROID HORMONE LEVELS PREDICT FALLS RISK IN OLDER WOMEN

ML Read, GC Nicholson, MA Kotowicz, JA Pasco & KM Sanders
The University of Melbourne, Dept Clinical and Biomedical Sciences: Barwon Health, Geelong, VIC

Vitamin D deficiency is associated with increased risk of falls and fracture. The aim of this study was to determine whether vitamin D deficiency/insufficiency and associated secondary hyperparathyroidism predict the results of various falls risk assessment tests.

Community-living women aged 70+ years (n=72, median age 75.5) were assessed using the Gait Rite electronic walkway system (Gait Mat) and the 'Timed Up and Go' test. The period of double support during a normal 'stride' determined by Gait Mat is positively related to falls risk. Postural sway was tested using a 'Balance Master'. Lower limb muscle strength was measured using a manual muscle tester (Nicholas MMT). Serum PTH and 25-OH vitamin D (VitD) levels were measured using Incstar assays.

Mean (±SD) VitD was 48 ± 13 nmol/L with 61% of women in the insufficient range (<50 nmol/L). Median PTH was 4.95 (1.5-15.2) pmol/L with 50% having elevated levels (>5.0 pmol/L). Both VitD and log PTH were predictive of the Gait Mat tests (R^2 adj 5.5%, p=0.03; R^2 adj 12%, p=0.002 respectively). Log PTH remained predictive when adjusted for age (R^2 adj 20%, p=0.02). VitD was predictive of the 'Timed Up and Go' test (R^2 adj 8.1%, p=0.009). Hip flexion muscle strength was predicted by log PTH (R^2 adj 4.9%, p=0.035). VitD and log PTH were not associated with the Balance Master tests.

These results indicate that in older women lower VitD and elevated PTH are associated with increased risk of falling as measured by falls risk assessment tests.

P77

QUANTIFICATION OF METAPHYSEAL REMODELLING IN CHILDREN TREATED WITH BISPHOSPHONATES

DG Little, K Ward & CT Cowell
Children's Hospital at Westmead, Sydney, NSW

There has been recent concern that bisphosphonate treatment in children can have an adverse effect on metaphyseal remodelling. We created a normal database from 439 radiographs of the distal femur, defined as metaphyseal index (MI). This allowed us to quantify the inwasting remodelling process in children on bisphosphonates.

From the radiographs, a measurement of the distal femoral growth plate width (GPW) was recorded. The femoral width at an interval of 0.5GPW proximal to the distal femoral growth plate was also recorded (0.5W). MI was defined as a ratio of 0.5W / GPW.

We found MI to be constant with minimal variability with age, averaging 0.55 for boys and 0.57 for girls. This small difference was significant ($p<0.01$). We charted the MI for a patient reported in the literature with "drug induced osteopetrosis". His MI at age 7¾ was at the upper limit of normal, but post pamidronate treatment of 2800 mg over 4 years was 9 SD above the mean. Of 19 bisphosphonate treated patients from our Institution the mean value was 0.5 SD above the normal mean. 3 patients had MI outside 2 SD, and all were within 3 SD of the mean.

Metaphyseal remodelling in the distal femur is constant, with slight variation between sexes, resulting in a similar shape of the distal femur throughout childhood. While patients given very large doses of bisphosphonates outside the clinical range display disordered metaphyseal remodelling, clinically relevant doses do not necessarily disturb this process, while the beneficial clinical effect is maintained.

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BONE TURNOVER MARKERS IN ELDERLY MEN AND THEIR RELATIONSHIPS TO PROSPECTIVE CHANGE IN BONE MINERAL DENSITY

TV Nguyen[1], C Meier[2], JR Center[1], JA Eisman[1] & M Seibel[2]
[1] Bone and Mineral Research Program, Garvan Institute of Medical Research, [2] Bone Research Program, ANZAC Research Institute, The University of Sydney, Sydney, NSW

Whether bone turnover markers can be used to monitor change in bone mineral density (BMD) in an individual is uncertain. This study was designed to address three specific questions: is there an association between bone turnover markers (BTMs) and BMD within an individual; is there a relationship between baseline measurements of BTM and subsequent change in BMD; and is there a causal relationship between changes in BTMs and changes in BMD.

Serum C-terminal telopeptide of type I collagen (sICTP), N-terminal propeptide of type I collagen (sPINP) and femoral neck (FN) BMD were measured in 101 men aged 70±4.1 years (mean±SD) at 3 different time points with a median follow-up of 6 years. Within-subject mixed-effects analysis indicated that sPINP decreased by 0.7%/year ($p=0.028$) while sICTP increased by 1.7%/year ($p<0.001$). FN BMD decreased by 0.4%/year ($p<0.01$). In relation to the questions posed, (i) within a subject, the increase in sICTP, but not in sPINP, was significantly associated with the decrease in FNBMD ($p=0.022$); (ii) baseline measurements of either sPINP or sICTP were not significantly associated with subsequent change in FNBMD; and (iii) changes in sPINP and sICTP between visit 2 and visit 1 were not significant predictors of subsequent change in femoral neck BMD between visit 3 and visit 2.

These results suggest that in elderly men, measurements of sPINP or sICTP were not predictive of the rate of change in BMD within an individual. The predictive value of these markers in monitoring bone loss in untreated elderly men seems limited.

P79

EFFECTS OF BISPHOSPHONATES ON HIP FRACTURE RISK REDUCTION: A BAYESIAN ANALYSIS OF CLINICAL TRIALS

ND Nguyen, JA Eisman & TV Nguyen
Garvan Institute of Medical Research, St Vincent's Hospital, Sydney, NSW

The evidence of anti-hip fracture efficacy of alendronate and risedronate is conflicting despite 7 randomized clinical trials (RCTs) have been conducted since 1995. Meta-analysis for each drug also yielded no statistically significant effect.

The discrepancy in results is partly related to "conventional" statistical analyses of RCTs, including meta-analysis, and p-values derived from those analyses that do not take into account pre-existing data about therapeutic effect. To address this issue and to assess the effects of bisphosphonates on hip fracture risk, the Bayesian concept of probability was utilized to systematically review past RCT data.

A previous RCT involved alendronate treatment showed that the RR of hip fracture was 0.22 (95% CI: 0.02-2.13, $p=0.90$). By incorporating these prior data to the 3 subsequent RCTs, the average posterior RR of hip fracture was estimated at 0.60 (95% credible interval (CrI): 0.39-0.92). Moreover, the probability that alendronate reduces hip fracture risk by at least 30% ($RR<0.7$) was 76%. A similar analysis of 3 risedronate trials yielded an RR of 0.61 (95% CrI: 0.45-0.85), with the probability

that risedronate reduces hip fracture risk by at least 30% being 80%. Furthermore, the probability that alendronate and risedronate reduce hip fracture by at least 50% (ie, RR<0.5) was 23% and 12%, respectively.

These Bayesian analyses indicated that alendronate or risedronate treatment reduces hip fracture risk at a clinically significant and public health relevant magnitude.

P80

IMPACT OF WITHIN-SUBJECT VARIABILITY ON THE ASSESSMENT OF CHANGE IN BONE TURNOVER MARKERS

TV Nguyen[1], C Meier[2], JR Center[1], JA Eisman[1] & M Seibel[2]
[1] Bone and Mineral Research Program, Garvan Institute of Medical Research, [2] Bone Research Program, ANZAC Research Institute, The University of Sydney, Sydney, NSW

The validity and reliability of the use of bone turnover markers (BTMs) in monitoring bone mineral density (BMD) change and predicting fracture risk is highly dependent on the intra-subject variability. This study examined the long-term intra-subject biological variability and analytical imprecision of serum C-terminal telopeptide of type I collagen (sICTP) and N-terminal propeptide of type I collagen (sPINP) and their impacts on the assessment of BMD change for an individual.

In a group of 101 community-dwelling healthy elderly men aged 70 ± 4.1 years (mean ± SD), sPICP and sICTP were measured [in duplicate] at three different time points with a median follow-up of 6 years. The intra-subject coefficient of variation (CV) for sPINP was 19%, and sICTP, 21%. The analytical CV for sICTP and sPINP was 3.5% and 2.6%, respectively. The index of individuality for sICTP (0.86) was higher than sPINP (0.67). Subjects with extreme baseline values tend to experience more pronounced change in subsequent visits, consistent with the "regression-toward-the-mean" phenomenon. For a subject whose baseline sPINP measurement of 2SD below the population mean, an observed increase of 30 µg/L reflects a true change of only 12.2 µg/L. For an individual undergoing treatment, there needs to be an observed increase of 17 µg/L before one can be 80% certain that the true increase of 10 µg/L has occurred.

The utility of bone turnover markers such as sPINP and sICTP in the monitoring of change in bone density is limited due to the relatively high intrasubject biological variability.

P81

LONG-TERM BONE LOSS IN MEN AND WOMEN: EFFECTS OF QUADRICEPS STRENGTH AND BODY WEIGHT

TV Nguyen, ND Nguyen, JR Center & JA Eisman
Garvan Institute of Medical Reseach, St Vincent's Hospital, Sydney, NSW

This study was designed to estimate the long-term rate of change in FNBMD and its risk factors in the elderly population. Femoral neck BMD was measred in 1382 men and women in the Dubbo Osteoporosis Epidemiology Study, who had been followed since 1989. At biennial visit, quadriceps strength (QS), body weight and lifestyle factors were also measured. The relationships between FNBMD, weight and quadriceps strength across visits were analyzed for *each* individual in a mixed-effects model.

On average FNBMD decreased by 0.57±1.28% and 0.30±1.05% (mean±SD) per year for women and men, respectively. However, the rate of decline accelerated with advancing age in women such that by the age of 80 yr, their rate of loss was equivalent to that in men (0.69±1.18%). The rate of change in BMD for *a given individual* was negatively correlated with both change in weight and change in QS, such that increasing weight and QS was associated with lesser bone loss. These two factors accounted for 13% and 12% of the variance of bone loss in men and women, respectively. There was no significant association between dietary calcium intake, smoking or alcohol use and the loss in FNBMD.

These results indicate that the rate of loss in FNBMD is lower than previously reported and that change in QS and change in body weight were significant determinant of bone loss. Enhancement of physical fitness may have a protective effect against bone loss in the elderly.

P82

SMOKING AS A MAJOR OSTEOPOROSIS RISK FACTOR AMONG IRANIAN AUSTRALIAN WOMEN

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

While the prevalence and risk factors of osteoporosis in Caucasian populations have been well documented, such a profile has not been studied in ethnic Australian population. This study was designed to estimate the prevalence and risk factors for osteoporosis among Iranian women in Sydney.

Ninety women aged 35+ years were recruited via a media campaign and community invitation. All subjects completed a set of questionnaires from which data on socio-demographic, clinical characteristics and lifestyle factors were obtained. Smoking was assessed as past and current cigarette intake. Bone mineral density (BMD) was measured at the lumbar spine (LS) and femoral neck (FN) using DXA (GE Lunar Corp, WI, USA), and was expressed in g/cm^2 as well as T-score.

Approximately 37% of women were considered obese (body mass index $\geq 30 kg/m^2$). IN multiple regression analysis, advancing age, lower BMI and smoking were independently associated with lower LS and FN BMD; with the 3 factors explaining up to 38% of variance in FN BMD. LS and FN BMD in smokers was 7% lower than that in non-smokers, and the smoking effect was more pronounced in obese women. In post-menopausal women, the prevalence of osteoporosis (T-score $\leq$ -2.5) was 26%. Smoking was associated with a 5-fold (95%CI: 1.8-15.6) increased risk of having osteoporosis, independent of age and BMI.

These data, for the first time, indicate that the prevalence of osteoporosis in the Iranian women is comparable to Caucasian women, and more importantly, cigarette smoking is a major modifiable lifestyle risk factor of osteoporosis in this population.

P83

A CAUSAL MODEL ANALYSIS AND OSTEOPOROSIS PREVENTION

A Baheiraei[1, 2], JE Ritchie[2], JA Eisman[1] & TV Nguyen[1]
[1] Bone and Mineral Research Program, Garvan Institute of Medical Research, St Vincent's Hospital, University of New South Wales, [2] School of Public Health and Community Medicine, University of New South Wales, Sydney, NSW

Lack of osteoporosis knowledge and mistaken health beliefs could influence osteoporosis preventive behaviours. This study explored the relationships between osteoporosis knowledge and health beliefs among Iranian Australian population.

A community-based sample of 160 men and women aged 35+ years was studied. Subjects completed 3 sets of questionnaire: Osteoporosis Knowledge Test, Osteoporosis Health Belief Scale, and The Osteoporosis Self-Efficacy Scale. The knowledge score was 13.8 ± 3.8 out of a maximal score of 24 in women, which was significantly higher ($p<0.05$) than in men. For health beliefs, out of the maximal score of 30 the susceptibility score was averaged at 17, seriousness: 19, benefits of exercise: 24, benefits of calcium intake: 23, barriers to exercise: 14, barriers to calcium intake: 13 and health motivation: 23. There was no significant difference in these scores between men and women. In women there were positive correlations between osteoporosis knowledge scores and health belief scores ($r = 0.31$), such that knowledgeable women were more likely to be aware of the benefits of exercise and were more confidence in taking preventive measures.

Thus, women and men of Iranian background demonstrated limited knowledge related to osteoporosis and risk factors associated with the disease. However, most recognised the benefits of exercise and calcium. These findings should encourage researchers and educators to develop or enhance existing educational programs to adequately gear to the needs and capabilities of the different ethnic populations.

P84

THE CLINICAL EFFICACY OF THREE-DIMENSIONAL RECONSTRUCTIONS OF TWO-DIMENSIONAL MR IMAGES: EVALUATION IN A SIMULATED TIBIAL PLATEAU DEPRESSION FRACTURE MODEL

AP Kurmis[1] & JP Slavotinek[2]
[1] Department of Orthopaedics, Division of Surgery, Repatriation General Hospital, Daws Road, Daw Park, SA
[2] Department of Medical Imaging, Flinders Medical Centre, Flinders Drive, Bedford Park, SA

Aims:
To explore the potential clinical utility of a three-dimensional (3-D) MR-based technique in the assessment of displaced, multi-fragment, bony trauma. Images of a simulated, depressed tibial fracture were used to assess the accuracy of measurement of bony fragment volume, depression and fracture gap width.

Methods:
An isolated tibial plateau injury was created in a disarticulated ovine tibia by surgically inducing two oblique condylar fractures. An MR-opaque acrylic spacer was used to provide a precisely maintained fracture gap and allow progressive known fragment depression. At each of ten steps of progressive bilateral fragment depression the model was imaged using a 2-D intermediate-T2-weighted turbo spin-echo MR sequence. Three-dimensional image reconstruction was performed using the Velocity 2 Professional software package and measurements of fracture fragment volume, depression and fracture gap width were made. Respective image percentage measurement error (PME) values were calculated.

Results:
Assessment of fragment volume, mean depression and fracture gap width measurement revealed mean PME's of 2.35%, 2.71% (+0.31mm) and 3.98% (+0.12mm) respectively.

Conclusion:
The findings of this study suggest that MRI, using 3-D reconstruction from conventional 2-D slices, may provide a non-invasive alternative to CT for the secondary assessment of displaced tibial plateau depression fractures. Such a technique provides accurate geometric bony information, relevant to surgical management, that complements the high-quality soft tissue detail provided by conventional MR imaging.

P86

BONE MASS AND ESTROGEN RECEPTOR STATUS IN WOMEN WITH BREAST CANCER

KM Sanders, B Ekanayake, GC Nicholson, MJ Henry, MA Kotowicz, JA Pasco & DA Watters
The University of Melbourne, Department of Clinical and Biomedical Sciences, Barwon Health, Geelong, VIC

Several prospective studies have reported an increased risk of breast cancer in women with high bone density although it remains uncertain if hormonal factors exert a greater influence on the risk of estrogen receptor positive breast tumours (ER+). This case-control study investigated if the relationship between BMD and breast cancer risk was strengthened when the analysis was restricted to women with ER+ tumours. Women with recently diagnosed breast cancer (102 days, 12-395: n=103; age 55 years, 33-83) had DEXA scans (Lunar DPX-L) and anthropometrical measurements. ER+ status was established from breast tumour specimens (n=70). Controls were 185 women randomly selected from the electoral roll, and without a diagnosis of cancer (age 57 years, 30-86). There was no difference in age, weight, BMI or menopausal status although cases were taller (p=0.001) and more likely to have a waist-hip ratio greater than 0.8 (p=0.008).

Cases were twice as likely to have femoral neck (FN) BMD in the highest tertile (age-adj. OR 2.2, p=0.02). When adjusted for height and waist-hip ratio this relationship was no longer significant (p=0.2). Age-adjusted BMD at L2-L4 did not differ between cases and controls (p=0.4). When the analysis was restricted to ER+ cases, BMD at L2-L4 and FN were significant predictors (OR; L2-L4: 2.5, p=0.02, FN: 2.3, p=0.02) but were not significant after adjustment for height and waist-hip ratio (OR; L2-L4: 1.7, p=0.2, FN: 1.7, p=0.2).

These results do not suggest that hormonal factors may be more strongly associated with ER+ breast tumours.

P87

BARRIERS TO EFFECTIVE MANAGEMENT OF OSTEOPOROSIS IN MODERATE AND MINIMAL TRAUMA FRACTURES: A PROSPECTIVE STUDY

D Bliuc, CR Ong, JA Eisman & JR Center
Bone and Mineral Program, Garvan Institute of Medical Research, St Vincent's Hospital, University of NSW, Sydney, NSW

Osteoporosis is suboptimally managed even in high-risk people with prior fractures. There is also evidence that individuals with moderate trauma fracture have lower bone density and are at higher risk of subsequent fracture. This study aimed to define factors influencing management of those at risk for osteoporosis and risk profiles of individuals with minimal and moderate trauma fractures. Consecutive fracture patients (n=218) from the outpatient fracture clinic (St Vincent's Hospital Sydney) were interviewed. Fracture risk factors, prior investigation and treatment for osteoporosis were collected and participants contacted after 3 months to ascertain follow-up.

Osteoporotic risk factors including family history, dietary calcium and conditions associated with bone loss were similar between low and moderate trauma groups and between sexes. Even though half of participants had had prior fractures, only 37% had a bone density scan and 14% were on adequate treatment. There was minimal (5%) increase in investigation and treatment rates after 3 months, and less in moderate trauma group and males. Independent predictors for being investigated were age $\geq$ 50, prior fracture and female gender, while predictors for treatment were age $\geq$ 50 and being investigated.

This study has confirmed low rates of investigation and treatment even in those with prior fracture and especially in those < 50 and in males. People with moderate and minimal trauma fractures had similar risk factors supporting the concept that people with moderate trauma fractures are at higher subsequent fracture risk. This study highlights the need for further exploration of barriers to osteoporosis management.

P88

BMD-INDEPENDENT CONTRIBUTION OF HIP STRENGTH INDICES TO HIP FRACTURE RISK IN ELDERLY MEN AND WOMEN

HG Ahlborg[1,3], ND Nguyen[1], TV Nguyen[1], JR Center[1,2] & JA Eisman[1,2]
[1] Bone and Mineral Research Program, Garvan Institute of Medical Research, [2] St Vincent's Hospital, University of NSW, Sydney, NSW and [3] Department of Orthopaedics, Malmö University Hospital, Malmö, Sweden

This study was designed to characterize the association between hip strength indices and hip fracture risk in relation to bone mineral density (BMD, g/cm^2) in an elderly population.

From the prospective, population-based Dubbo Osteoporosis Epidemiology Study, 71 women and 25 men aged 60 and above, who had sustained a hip fracture during the study period of 1989-2003, were randomly matched (1:2) for age with 142 women and 50 men who has not had any fractures. BMD were measured at the femoral neck by DXA (Lunar DPX-L) and hip strength indices such as femoral neck diameter (FND), cross-sectional moment of inertia (CSMI) and section modulus (Z) were estimated using hip strength analysis software.

In women, after adjustment for BMD, smaller FND, lower CSMI or Z were each significantly associated with hip fracture risk with odds ratio being between 1.6 and 2.3. Using the results in women as a prior distribution, it was estimated that the BMD-adjusted odds ratio for FND, CSMI or Z were also each significantly associated with hip fracture risk in men, with odds ratio being between 1.5 and 2.3. BMD alone accounted for 32% and 16% of the variance of fracture liability, in women and men respectively. The addition of FND, CSMI or Z to the model increased the respective variance proportion to 34% and 19%.

These data suggest that smaller FND, lower CSMI or Z are independent risk factors for hip fracture, however the BMD-independent contribution of these measures was modest in elderly men and women.

P89

DESIGN AND PERFORMANCE TESTING OF A NEW BONE-EQUIVALENT CORTICAL SHELL PHANTOM: COMPARATIVE MEASUREMENTS OF ITS STRUCTURAL GEOMETRY DERIVED FROM DXA AND pQCT

BCC Khoo [1,2], TJ Beck [3], B Turk[1] & RI Price[1]
[1] Medical Technology & Physics, Sir Charles Gairdner Hospital; [2] School of Surgery & Pathology, The University of WA, Perth, WA, [3] Johns Hopkins University, Baltimore, MD, USA

DXA-derived Hip Structural Analysis (HSA), describes bone-structural geometry, including Section Modulus (Z; bending-strength index), and is complementary to DXA areal BMD (aBMD) which infers bone strength less directly. While conventional (vendor-provided) phantoms calibrate DXA machines for aBMD and assess long-term in-vitro reproducibility and accuracy, analogous phantoms for calibrating structural geometry are lacking. This study describes a conveniently fabricated radiologically bone-equivalent material; application to constructing a novel "femoral-neck" phantom with "cortical" shells and "cancellous" core; comparison of phantom structural geometries derived from DXA and pQCT.

Powdered calcium-sulphate cement (CSC), vacuum water-mixed and cured, was chosen for its; hydroxyapatite-like DXA photon-attenuation properties; density monotonically related to added water-mass; homogeneous bubble-free composition; mass and aBMD temporal stability (CV%=0.03%, n=4 specimens over 49d). Using CSC designed for aBMD=1.04g/cm^2,

(for plate-thickness 10mm), a cylindrical phantom with cortical shells 0.5, 1.0, 2.0, 4.0mm thicknesses; internal diameter 26mm; acrylic-based surrounding soft-tissue and removable "cancellous" core, was constructed. Phantom was scanned by DXA (QDR1000W) and pQCT (Stratec XCT2000, pixel-size 0.15mm). Selected cortical structural-geometric variables, derived from calculated geometry; pQCT mass-projections, and DXA HSA, are:

Variable Method	Calc. Geom.	pQCT	DXA HSA
Cortical Thickness (mm)			
"Thin cortex"	0.50	0.50	0.63
"Thick cortex"	4.00	4.07	3.37
Bone Width (mm)			
"Thin"	27.00	27.34	26.47
"Thick"	34.00	34.43	34.07
Section Modulus (mm^3)			
"Thin"	271	316	331
"Thick"	2539	2241	2240

In conclusion, dimensions of this novel cortical-shell phantom are accurately rendered by pQCT, width by DXA HSA, and though Z derived radiologically from pQCT and DXA agree, there is discrepancy with calculations.

P90

DEPRESSION AND BONE MINERAL DENSITY IN A COMMUNITY SAMPLE: GEELONG OSTEOPOROSIS STUDY

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

Bone mineral density (BMD) is reduced in women with a history of current or previous depressive disorders in some but not all studies. Data generated by the Geelong Osteoporosis Study (GOS) has shown a prevalence of 15% depression among adult women in the region, with a rate of 21% among perimenopausal women[1]. This study aimed to investigate the association between self-reported depression and BMD in an age-stratified community sample of perimenopausal women enrolled in the GOS. Symptoms of past and present depression for the 12mo period July 2000-1 were assessed by self-report questionnaire based on DSM-IV criteria. Women in the perimenopausal group who had undergone a BMD total hip assessment within the 12mo period following the depression assessment were included in the analysis, resulting in a sample of 78 women aged 45-60yr.

In this sample, 14 women were identified as depressed. There was no difference in age, smoking, oestrogen use or unadjusted BMD between the depressed and non-depressed women (p=0.15 and 0.57, respectively), but the depressed women tended to be heavier (79.7 $\pm$ 14.7 vs 71.9 $\pm$ 14.5 kg, p=0.072). Age- and weight-adjusted BMD for the depressed women was 7.8% lower than for the non-depressed (mean $\pm$ SE = 0.95 $\pm$ 0.034 vs 1.03 $\pm$ 0.016 g/cm^2, P=0.045).

These results suggest that, despite a tendency towards increased body weight, depression is associated with lower BMD in perimenopausal women.

[1]Jacka FN et al. Nutritional Neuroscience 2004 (in press)

P91

CLINICAL EXPERIENCE WITH ZOLEDRONIC ACID IN OSTEOPOROSIS

D Hughes[1], B White[1], B Crawford[1,2,3] & M Hooper[1,2,3]
Endocrinology, Concord[1] and RPA[2] Hospitals and The University of Sydney[3], NSW

Intravenous (IV) zoledronic acid (ZA:Zometa, Novartis Pharmaceuticals) therapy is used in the management of osteoporosis (Reid et al. 2002). In this study we report our experience with ZA in routine clinical practice in regard to effect on bone mineral density (BMD) and bone markers in osteoporosis. We reviewed the records of patients who had been given IV ZA (4mg) as a single annual dose. Lumbar spine (LS) and femoral neck (FN) BMD, serum bone specific ALP (bALP; Ostase) and urinary deoxypyridinoline/creatinine ratio (dpd/cr) were measured at baseline and the 12 month infusion. Results were available for 55 patients (36 women and 14 men), with a mean age 68 years (range, 19-92 years). Baseline mean BMD

scores ± SD were: LS 0.87 ± 0.12 g/cm^2 (N=48); FN 0.69 ± 0.12 g/cm^2 (N=53). The analyses used paired t tests. Results are mean ± SEM. LS BMD increased significantly (3.8 ± 0.7%) at 12 months (P<0.0001); femoral neck BMD also increased significantly (2.0 ± 0.5%) at 12 months (P=0.0001). The level of bALP, a marker of bone formation, decreased significantly (14.4 ± 1.8 ug/L to 10.5 ± 0.7 ug/L, P=0.02, N=23), a median decrease of 18%. The level of dpd/cr, a marker of bone resorption, was similar at baseline and 12 months (P=0.07, N=44). However, of the patients with dpd/cr levels at baseline >7.5 mmol/mmol (N=16), 88% had a significant reduction at 12 months (P=0.0004). A similar effect was seen with baseline bALP >13.0 ug/L. An annual infusion of zoledronic acid appears to be an effective treatment for osteoporosis.

Reid, IR et al. NEJM 2002; 346:653-661

P92

β-BLOCKERS REDUCE BONE RESORPTION MARKER IN PERIMENOPAUSAL WOMEN: GEELONG OSTEOPOROSIS STUDY

JA Pasco[1], MJ Henry[1], HG Schneider[2], GC Nicholson[1] & MA Kotowicz[1]
[1] The University of Melbourne, Department of Clinical and Biomedical Sciences: Barwon Health, Geelong, VIC
[2] Alfred Pathology Services, Prahran, VIC

Our group has previously reported that β-blocker therapy is associated with reduced fracture risk and higher BMD[1]. In order to investigate the mechanism of the protective effect of β-blockers we evaluated β-blocker exposure in association with serum levels of biochemical markers of bone turnover, C-telopeptide (CTx) and bone-specific alkaline phosphatase (BSAP), and rates of bone loss. β-blocker use, concomitant therapy and lifestyle were documented by questionnaire for 197 women aged 50-59 yr, 175 of whom had changes in whole body BMD monitored over a 2-yr period. CTx was measured using an electrochemiluminescence immunoassay (precision 8.5%); BSAP was calculated from total alkaline phosphatase measured colorimetrically after BSAP precipitation (precision 11.3%).

Twenty-four β-blocker users were identified at baseline. After controlling for concomitant use of hormone therapy, CTx levels were 6.7% lower among β-blocker users (P=0.02). No association was detected between BSAP and β-blocker use. Analysis of 15 β-blocker users and 152 non-users identified 2-years post-baseline showed that adjusted rates of bone loss were -0.001±0.007 g/cm^2/2yr for the users and –0.004±0.002 g/cm^2/2yr for non-users, but this difference was not significant (P=0.7). Levels of CTx but not BSAP were predictors of adjusted rates of bone loss (P=0.008 and P>0.05, respectively).

These results indicate that β-blockers might suppress bone resorption with relative preservation of bone formation. A study with greater power will be required to determine whether β-blockers are associated with lower rates of bone loss.

[1]Pasco JA, et al. J Bone Miner Res 2004;19:19-24.

P93

FRACTURE RISK ASSOCIATED WITH BONE MINERAL DENSITY: A PROSPECTIVE STUDY

JA Pasco[1], MJ Henry[1], EN Merriman[1], GC Nicholson[1], E Seeman[2] & MA Kotowicz[1]
The University of Melbourne, [1] Department of Clinical and Biomedical Sciences: Barwon Health, Geelong, [2] Department of Medicine, Austin Medical Centre, Heidelberg,VIC

The purpose of this study was to quantify fracture risk associated with areal bone mineral density (BMD). This prospective analysis follows 628 postmenopausal women (median age 74.0 yr, range 60-94) who had BMD assessments 1994-7 as part of the Geelong Osteoporosis Study. According to WHO criteria, 37.1% had normal BMD at the total hip, 48.2% were osteopenic and 14.8% osteoporotic. Subjects were followed until the end of 2002, or until sustaining a fracture, death, or migration from the study region. Post-baseline fractures were ascertained radiologically.

During the study period 66 women died, 25 left the region, 127 sustained at least one fracture and 410 remained fracture-free, alive and residing in the region, generating 3220 person years of follow-up. After 5 years, the proportion of fractures occurring in each category of BMD was 17.0% normal, 54.2% osteopenia and 28.8% osteoporosis. Survival plots (Kaplan-Meier) show successively reduced probability of remaining fracture-free for normal, osteopenic and osteoporotic women (see figure). Using an age-adjusted Cox proportional hazards model and normal BMD as the referent group, the relative risk (RR) for fracture was 2.7 (95%CI 1.6-4.7) for women with osteopenia and 4.2 (2.3-7.7) for women with osteoporosis (both p<0.001).

The categories of decreasing BMD define increasing risk of fracture, independent of age. Postmenopausal women with osteoporosis are at greatest risk for fracture but contribute less than a third of the fracture burden.

P94

TOLERABILITY AND RENAL SAFETY OF INTRAVENOUS ZOLEDRONIC ACID IN BENIGN METABOLIC BONE DISEASE

D Hughes[1], K Sommer[1], J Hetherington[2], B Crawford[1,2,3] & M Hooper[1,2,3]
Endocrinology, [1] Concord and [2] RPA Hospitals and The University of Sydney[3], NSW

Intravenous (IV) bisphosphonate therapy may be followed by symptoms associated with an acute phase reaction and a recent report has associated IV zoledronic acid (ZA: Zometa, Novartis Pharmaceuticals) with renal impairment in cancer patients[1]. We have treated 256 patients, 19-95 years of age, F:M 2:1, with benign bone disease in routine clinical practice with IV ZA (4 mg) either as a single annual infusion for osteoporosis (193) or three monthly for other benign bone disease (63). Clinical tolerability data was collected by telephone interview 7–14 days post infusion on a standardised form for 171 patients. The majority of patients (67%) had been treated previously with an oral bisphosphonate (15%) or by IV pamidronate (53%). 52% had no symptoms. 22% had mild symptoms. At least one severe symptom was reported by 28% of patients. Severe symptoms tended to occur more frequently in patients not previously treated with bisphosphonates. Generalised bone and muscle aches and pain occurred in 38% patients (16% mild, 22% severe), fever in 16% (7%, 9%), headache in 15% (9%, 6%), chills/shakes in 12% (5%, 7%), nausea/vomiting in 9% (6%, 3%), breathlessness in 2% (1%, 1%) and chest pain in 2% (0%, 2%). Paired t test analyses of serum creatinine concentration, measured at baseline and three months for patients with Paget's disease (N=44) and 12 months for osteoporosis patients (N=95) revealed no significant differences in their means. These data demonstrate the high tolerability and renal safety of ZA.

[1] Chang JT, et al. NEMJ 2003; 394:1676-1679

P95

LIFETIME PARTICIPATION IN IMPACT SPORTS IS AN IMPORTANT DETERMINANT OF BONE SIZE AND STRENGTH, BUT NOT BMD, IN OLDER MEN

S Kukuljan, RM Daly, SL Bass & C Nowson
School of Exercise and Nutrition Sciences, Deakin University, Melbourne, VIC

It is uncertain whether participation in impact sports during different stages of life affects bone strength in old age. Thus, we asked, does participation in impact sports during adolescence (13-18 yrs) and/or adulthood (19-49 yrs) enhance bone strength in men aged 50+ yrs? We assessed DXA FN and L2-4 BMD (n=159), and femoral mid-shaft total (ToAr) and cortical area (CoAr) and the polar moment of inertia (Ip) by QCT (n=105). Current (50+ yrs) and past hours of sports participation were assessed by questionnaire, which was used to calculate an osteogenic loading index (OI) for each participant at each period. A greater lifetime (13-50+ yrs) and adult (19-49 yrs) OI was associated with greater femoral ToAr, CoAr and Ip ($p<0.05$). No associated was found with DXA BMD. Subjects were then categorised into a high (H) or low/non-impact (L) group according to their OI scores in adolescence and adulthood. Four groups were formed to reflect impact categories during these periods: LL (n=28), LH (n=7), HL (n=40) and HH (n=32). Compared to the LL group, ToAr and Ip were 7-14% ($p=0.05$) higher in the HH and 11-21% ($p<0.01$) higher in the LH group of older men. No differences were detected between LL and HL groups. We conclude that: 1) regular impact loading in men led to an increase in bone strength due to an increase in bone size and not BMD, and 2) continued participation in weight-bearing exercise throughout life is important for reducing the risk of low bone strength in old age.

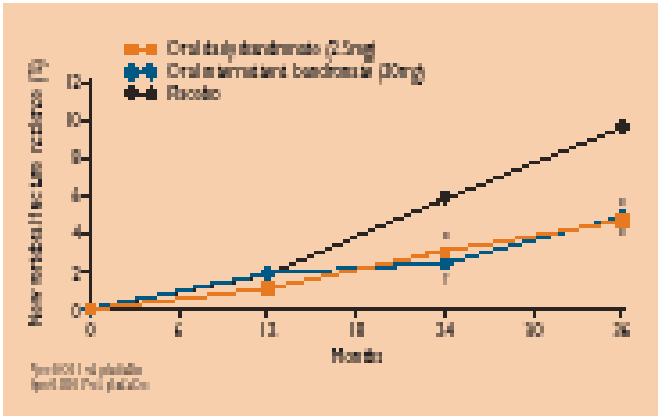
P96

A NEW DOSING CONCEPT FOR BISPHOSPHONATE THERAPY: RATIONALE AND DESIGN OF THE MONTHLY ORAL IBANDRONATE IN LADIES (MOBILE) STUDY

PR Ebeling[1], RK Will[2], J Graham[3], AG Need[4], RR Recker[5], JY Reginster[2] & B Bonvoisin[7]
[1] *Royal Melbourne Hospital, Melbourne, VIC,* [2]*Royal Perth Hospital, Perth, WA,* [3] *Ashford Medical Centre, Adelaide, SA,* [4] *Royal Adelaide Hospital, Adelaide, SA,* [5]*Creighton University, Omaha, Nebraska, USA,* [6]*University of Liège, Liège, Belgium,* [7] *F. Hoffmann-La Roche Ltd, Basel, Switzerland*

Ibandronate (Bonviva®) is a potent, nitrogen-containing bisphosphonate with proven antifracture efficacy in postmenopausal osteoporosis (PMO) when administered daily or intermittently with an extended between-dose interval of >2 months (vertebral fracture risk reduction: 62% and 50%, respectively see Figure 1).

Figure 1



There is a strong rationale to support the clinical utility of a once monthly oral ibandronate dosing regimen in PMO, including reducing the need for fasting and maximising GI tolerance.

The Monthly Oral iBandronate In LadiEs (MOBILE) study is a randomised, double-blind, parallel-group, multinational study in 1609 postmenopausal women (lumbar T score <-2.5 to ≥5.0) that is comparing the efficacy and safety of the proven oral daily ibandronate regimen (2.5 mg daily) with three different oral monthly regimens.

- 100mg on a single day
- 100mg as separate 50mg doses on two consecutive days
- 150mg on a single day.

MOBILE is a non-inferiority study, with measures of bone mineral density (BMD) as the primary and secondary endpoints. Bone turnover markers (incl. CTX), adverse events and safety labs are also assessed.

- Vertebral fracture efficacy will be inferred if MOBILE shows non-inferiority of the oral monthly regimens to the oral daily regimen with proven antifracture efficacy for lumbar spine BMD change.

The availability of a once monthly oral bisphosphonate regimen is likely to enhance patient convenience and compliance and thus improve long-term therapeutic outcomes.

P97

MUSCLE-BONE RELATIONSHIP IN ABLE-BODIED AND IMMOBILISED INDIVIDUALS

P Eser[1], S Bass[1], R Daly[1] & H Schiessl[2]
[1] *School of Exercise and Nutrition Sciences, Deakin University, Burwood, VIC*
[2] *Stratec Medical, Pforzheim, Germany*

We have reported that in spinal cord injured subjects (SCI) BMC and muscle CSA in the paralysed legs were reduced by 30-50%. It is unknown, however, whether the muscle-bone relationship found in able-bodied (AB) subjects persists after immobilisation. The aim of this study was to investigate the relationship between muscle CSA and bone strength within and between groups (AB and SCI). Diaphyses and distal epiphyses of the femur and tibia were measured by pQCT in 24 healthy AB and 54 SCI subjects. At the epiphyses, AB and SCI showed a significant linear relationship between muscle CSA (measured at the diaphysis) and BMC (Figure). At the femur epiphysis the intercept for the regression line was significantly different between the two groups ($p<0.001$), but not the slope. This indicates that in the SCI group the decrement in bone was comparatively greater than in muscle. At the tibial epiphysis, the slope was significantly less in the SCI compared to the AB group ($p<0.001$), implying that muscle had a minimal role in preserving bone. In contrast, at the diaphyses of the femur and tibia, significant linear relationships were found between muscle CSA and the stress strain index in both groups, with slope and intercepts not differing between groups. In conclusion, at the diaphyses the muscle bone relationship evident in AB subjects is maintained in SCI subjects. In contrast, at the epiphyses deficit in bone mass exceeded the corresponding deficit in muscle leading to a discordant muscle-bone relationship between AB and SCI subjects.

P98

ANDROGEN RECEPTOR CAG REPEAT POLYMORPHISM AND BONE DENSITY: A FAMILY AND CASE-CONTROL ASSOCIATION STUDY IN MEN WITH PRIMARY OSTEOPOROSIS

Z Hussein[1], JA Ellis[2], A Lamantia[2], K Greenland[3], S Yeung[1], ZYH Wong[2], SB Harrap[2], JD Zajac[4] & PR Ebeling[1]
[1] Depts. Diabetes and Endocrinology, Royal Melbourne Hospital, [2] Physiology, University of Melbourne, [3] Centre for Hormone Research, Murdoch Children's Research Institute, Royal Children's Hospital, [4] Medicine, Austin and Repatriation Hospital, VIC

Androgens are important modulators of bone mass in men. CAG trinucleotide repeat length in exon 1 of the AR gene is inversely associated with its transcriptional activity and may affect bone metabolism.

We therefore investigated the association between the AR CAG repeat length, by automated DNA sequencing of exon 1 of the AR gene, and BMD in men with primary osteoporosis, their first-degree relatives and in normal men. Lumbar spine (LS), proximal femur and total body BMD was measured by DXA.

Forty-nine pedigrees were recruited and 38 sib-pairs were available for linkage analysis. From the family studies, hereditability was 0.74 (0.11) at the lumbar spine and 0.56 (0.17) at the femoral neck. Standardised bone density (Z-score) were mean z-LS = -1.35; and mean z-FN = -0.91. The CAG repeat length effect was equivalent to 0.3 SDs per 5 base pair difference in CAG repeat length at the lumbar spine, but not at the femoral neck. Two association studies were also performed. The first was a case-controlled study of 81 men with primary osteoporosis and spinal fractures (mean age 55.6 years, mean LS T-score -2.53 and mean FN T-score -2.93) and 25 normal men (mean age 44.7 years, mean LS T-score 0.46 and mean FN T-score -0.14). Median AR CAG repeat length for the osteoporotic group was 20 (SD 3.2) versus 21.0 (SD 2.4) for the control group. Each group was also dichotomised with regards to short (<22) or long (≥22) repeat lengths. The second study comprised 157 unrelated men (mean age 54.3 years, mean LS T-score -1.60, mean FN T-score -2.11 and median CAG repeat length of 20) who were subdivided according to genotypes of different AR CAG repeat lengths (range 6-29). BMD was compared using one-way ANOVA. No associations were seen between CAG repeat length and BMD in either study.

Hereditability of BMD in families of men with spinal fractures is high and greater for the spine than proximal femur. CAG repeat length in the androgen receptor has a small effect on spinal, but not femoral neck BMD. Other genetic factors are more likely to be more important.

P99

DECREASED AREAL AND VOLUMETRIC BONE MINERAL DENSITY (BMD) IN MEN WITH PRIMARY OSTEOPOROSIS AND THEIR FIRST-DEGREE MALE RELATIVES: EVIDENCE FOR A FAMILIAL EFFECT ON BONE ACQUISITION

B Erbas[1], S Ristevski[2], C Poon[2], S Yeung[2] & PR Ebeling[2,3]
[1] Department of Public Health, University of Melbourne, [2] Department of Diabetes and Endocrinology, Royal Melbourne Hospital, [3] Department of Medicine, University of Melbourne, Parkville, VIC

Introduction and Aim:
Gender- and site-specificity of the inheritance of BMD exists. We determined familial effects on areal and volumetric bone mineral density in relatives of men with primary osteoporosis.

Methods:
A cross-sectional study of areal and volumetric bone mineral density and body composition in 121 men with spinal fractures due to primary osteoporosis, aged 22 to 88 years, and in 73 FDMR, aged 18 to 84 years, and 66 normal men, aged 21 to 80 yrs.

Results:
Areal BMD at the spine and femoral neck was lowest in the men with osteoporosis and bone volumes at the third lumbar vertebra (V_{L3}) and femoral neck (V_{FN}) tended to be lower in men with osteoporosis than in controls (p=0.08 and p=0.09, respectively). Deficits in age- and height- corrected areal BMD and volumetric BMD between controls and men with osteoporosis were 23.7% and 23.4%, respectively, at the spine and 25.1% and 21.7%, respectively, at the femoral neck. FDMR were divided into two groups according to T-score for femoral neck areal BMD: low BMD (T-score ≤ -2) and normal (T-score > -2). 47% of FDMR had low BMD and were older, thinner and shorter than controls. Deficits in age- and height- corrected areal BMD and volumetric BMD between controls and first-degree male relatives was 10.7% and 10.2%, respectively, at the spine and 10.5% and 7.1%, respectively, at the femoral neck. Height- and age-corrected third lumbar vertebral and femoral neck volumes were not reduced in first-degree male relatives compared with controls. Multiple linear regression analysis revealed age, height and lean mass were the most important predictors of BMD in first-degree male relatives. Together they explained 20 to 30% of the variance in areal and volumetric BMD.

Conclusion:

A deficit in bone acquisition may underlie the pathogenesis of idiopathic osteoporosis in men. This is likely, at least in part, to be determined by genetic factors.

P100

SURGEON / PHYSICIAN JOINT MANAGEMENT OF FRACTURED HIP INPATIENT MANAGEMENT REVIEW

J Thwaites[1], F Mann[2], A Rothwell[4], C Frampton[3] N Gilchrist[1]
[1] The Princess Margaret Hospital, [2] Christchurch Hospital, [3] Christchurch School of Medicine, [4] Department of Orthopaedics, Christchurch Hospital, New Zealand

Shared medical management of acute fracture patients is routine practice at our centre. Between December 02 and June 03, 150 neck of femur fracture inpatient's management was reviewed. Median age was 83 years 115, (70%) were female and 35 were male. The median time to operation was 43 hours, 25% received a hemi-arthroplasty, 60% canulated hip screws, 10% total hip replacement. The median length of stay which included acute and rehabilitation period was 23 days. The acute length of stay was 8 days. Mortality was low, occurring in 1 patient only in this group. Of those admitted from home (98) 88% returned home. 43 patients were admitted from rest home care and 93% returned back to rest home care. All patients who came from 24 hour hospital care (6) or dementia care (3) returned to their place of origin.

By discharge 82% of people were on calcium and vitamin D and only 10% had the addition of bisphosphonates.

In conclusion, the length of stay remains comparable to other units. Almost all people are able to be returned to their original place of domicile. Pleasingly the mortality appears to be very low and there is much better treatment of underlying osteoporosis. Combined management of orthopaedic patients appears to be effective.

P101

EFFECTIVENESS OF THE CLINICAL PATHWAY FOR FRACTURED NECK OF FEMUR

Fractured Neck of Femur Study Group - NL Gilchrist[1], J McKie[1], J Thwaites[1], K Wilson[1], J Gapes[1], D Jones[1], S Newman[1], P Larking[2] & N Gibson[2]
[1] Canterbury District Health Board, [2] ACC, New Zealand

The purpose of this fractured neck of femur pathway is to enable it to be instituted across acute rehabilitative and community phases within the CDHB. Effectiveness will be measured against established organisation expected outcomes, outlined by the ACC, guidelines from the UK Audit Commission, Health Round Table and Elder Care Canterbury. The purpose is to measure best patient outcomes, reduce fall strategy, osteoporosis treatment, community rehabilitation and reduction of stay. Data will be collected and compiled in a dedicated data base. Treatment in Accident & Emergency, venous thrombus embolism prophylaxis, infection, pressure sores and nutritional supplementation after fracture. Reasons for delay to theatre, management of urinary retention, dementia delirium, post-operative mobilization, medical and surgical complications. Surgical management including type of anaesthesia. Multi disciplinary team assessments both pre and post-op and at discharge will be collected. Once discharged, ongoing data will be collected by the General Practitioner including ongoing pain, the need for community support, additional medication etc. The Otago Exercise Pilot Programme will also be collecting data on fall occurrence, treatment modalities, muscle strength and balance.

It is anticipated that this prospective pathway if successful, will be adopted as a nationwide generic clinical pathway for inpatient management of patients 65 years and over who have sustained a fractured neck of femur.

P102

ESTABLISHED OSTEOPOROSIS PATIENTS - DO BONE REPORTS AND RECOMMENDATIONS ALTER PATIENT MANAGEMENT?

N Gilchrist[1], A Heard[2], P Maguire[2], R March[2], P Reilly[2]
[1] CDHB, [2] CGM Research Trust, Christchurch, New Zealand

We contacted over 800 patients who had BMD, T<-3.0 with and without pre-existing fracture. Information on treatment, fracture status and medication eligibility was obtained. Of those with a T<-3.0 and fracture, 51% were on alendronate, calcium and vitamin D, 27% calcium and vitamin D and a much smaller percentage on either etidronate or rocaltrol or HRT. Those with T<-3.0, without fracture, 36% were on calcium and vitamin D alone, 33% were on alendronate, calcium and vitamin D, 30% were on cyclical etidronate. Rocaltrol was 11% and HRT, 10%. Unable to be contacted (n=130). General

Practitioners (144) were contacted regarding treatment eligibility. On alendronate were 9%, 28% were started on alendronate, the rest were either on additional treatment or unable to tolerate alendronate.

150 people were contacted about their questionnaire fracture details. Of the 116 that returned replies there was a 30% new fracture pick up rate. Individual patients were contacted who had a T<-3.0 and fracture. Only a third were returned. 50% were already on either alendronate or etidronate but approximately one third were not on a bisphosphonate.

From this data we have identified that there is still a large percentage of patients that are not on evidence based treatment, fracture history as ascertained by questionnaire can be misleading and some patients are on treatment for which the efficacy of fracture reduction is lacking.

P103

ANTIOXIDANT SUPPLEMENTS AND MARKERS OF BONE TURNOVER IN NON-SMOKING WOMEN: GEELONG OSTEOPOROSIS STUDY

LK Wilkinson[1], JA Pasco[1], MJ Henry[1], HG Schneider[2], GC Nicholson[1] & MA Kotowicz[1]
[1] The University of Melbourne, Department of Clinical and Biomedical Sciences: Barwon Health, Geelong, and [2] Alfred Pathology Service, Prahran,VIC

While several epidemiological studies have reported a positive relationship between the dietary intake of antioxidants and bone mineral density (BMD), none has demonstrated an effect of supplemental antioxidants on bone turnover. We evaluated the association between the use of vitamins E and C supplements and serum levels of biochemical markers of bone turnover, serum C-telopeptide (CTx) and bone-specific alkaline phosphatase (BSAP), and whole body BMD. From 939 randomly-selected women enrolled in the Geelong Osteoporosis Study, 616 were included for analysis (median age 70.2 yr, range 45-89). Exclusion criteria: incomplete data (74), current smokers (89), taking multivitamins (36), HRT (97) and indeterminate menopause status (27).

Thirty-one were currently taking supplemental vitamin E (n=17) and/or C (n=19). There were no differences in age, weight, calcium intake or activity levels between supplement users and non-users. Multivariate models for predicting bone turnover markers included adjustments for age and menopause status, BMD was adjusted for age and weight. Adjusted CTx values were significantly lower in supplement users than non-users (p=0.04). No significant differences were detected for adjusted BSAP or BMD.

Adjusted means (95% confidence interval)

	Antioxidant supplement		P value
	Users	Non-users	
CTx (pg/mL)	352 (266, 465)	455 (393, 526)	0.04
BSAP (U/L)	27.5 (22.4, 33.8)	25.2 (24.0, 26.4)	0.4
BMD (g/cm^2)	1.064 (1.035, 1.093)	1.064 (1.057, 1.070)	1.0

Small numbers may have limited the power to detect differences less than 5.7% for BSAP and 9.6% for BMD. The results suggest that vitamin E and/or C supplements may suppress bone resorption.

P104

PROTEIN CONSUMPTION IS AN IMPORTANT DETERMINANT OF LOWER LIMB BONE MASS IN ELDERLY WOMEN

A Devine[1,2,3,4], RL Prince[1,2,3], FMA Islam[1,3], SS Dhaliwal[3,5] & IM Dick[1,2,3]
[1] School of Medicine and Pharmacology, University of Western Australia, [2] Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, [3] Western Australian Institute of Medical Research, [4] School of Biomedical and Sports Sciences, Edith Cowan University, [5] School of Public Health, Curtin University of Technology, Perth, WA

The effect of protein intake on bone remains controversial.

Using a cross sectional study design a population-based sample of 1497 elderly women mean age 75±3y had measurements of hip and heel bone mass measured using DXA (Hologic 4500A) (n=1127) and quantitative ultrasound (QUS, Lunar Achilles) (n=1438) respectively. Protein consumption was measured by a validated habitual FFQ.

Subjects consumed on average 80±28 g of protein/day or 1.20±0.46 g of protein per kg of body weight. Only 12%`consumed less than the recommendation for protein. In multiple regression analysis protein intake was a positively associated with BMD and BUA after adjustment for co-variates. Division of protein consumption into tertiles best described

the dose response effect. Subjects in the Low PTN tertile had significantly lower hip BMD (2.6% P < 0.05) and BUA (1.2% P < 0.05) compared to those with Medium or High PTN intake. Tertiles of PTN intake were also expressed as a function of body weight. Those in the Medium tertile (0.97-1.3 g protein per kg of bodyweight) had significantly greater total hip (2.5%) and femoral neck (2.9%) BMD compared to those in the Low tertile. BMD at the total hip and femoral neck were not different between those with High (>1.3 g protein per kg of bodyweight) and Low (<0.97 g protein per kg of bodyweight) intakes of PTN.

These data support the concept that the RDI for protein intake should be increased in elderly women but that the recommendation should be related to body weight.

P105

LOW BODY WEIGHT IS ASSOCIATED WITH INCREASED INCIDENT FRACTURE RISK IN OLDER WOMEN

R Naheed[1,2], A Devine[1,2,3,4], I Dick[1,2,3] & RL Prince[1,2,3]
[1] School of Medicine and Pharmacology, University of Western Australia, [2] Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, [3] Western Australian Institute of Medical Research, [4] Edith Cowan University, Perth, WA

Low body weight is considered to be a determinant of fracture risk but it is not certain if this is independent of bone mineral density. As part of a five-year study, we examined the association of low body mass index (BMI), age at baseline and hip BMD with the risk of incident osteoporotic fracture.

1499 women mean age 75 were recruited from the whole population. Study exclusions were bone active treatment, calcium supplementation or diseases that may have prevented the subject completing the 5 year study. Of the responders, 18% were eligible and agreed to participate. Hip BMD (cv 1.0%) was measured using an Hologic 4500A. Incident X-ray verified osteoporotic fractures were ascertained 4 monthly. Study outcomes were examined using the Cox proportional hazard model to calculate hazards ratios (HR).

Low BMI, low BMD and increasing baseline age were all related to an increased risk of fracture. A 1 year increase in age was associated with a 9.8% (P<0.001) increase in 5 year fracture risk. A 1 SD reduction in BMD was associated with an increased risk of incident fracture (HR 2.64: 1.86-3.73). Patients in lowest tertile of BMI (<24.9) compared to the highest tertile (>28.6) had an increased risk of fracture (HR 1.45: 1.01-2.10). After adjustment for BMD and age, a low BMI was no longer associated with increased fracture risk (HR 0.86: 0.56-1.30).

Therefore, a low body mass index is associated with increased risk of fracture due to its association with low BMD.

P106

ENDOGENOUS ESTRADIOL IS ASSOCIATED WITH RENAL CALCIUM AND PHOSPHATE HANDLING IN OLDER POSTMENOPAUSAL WOMEN

IM Dick[1,2,3], A Devine[1,2,3,4] & RL Prince[1,2,3]
[1] School of Medicine and Pharmacology, University of Western Australia, [2] Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, [3] Western Australian Institute of Medical Research, [4] School of Biomedical and Sports Sciences, Edith Cowan University, Perth, WA

Postmenopausal endogenous estrogen concentrations are an important determinant of preservation of bone mass and reduced fracture in elderly women. Evidence exists that estrogen acts directly on the kidney to regulate renal calcium and phosphate handling. However it is not known if this is true at the low endogenous estradiol concentrations present in older postmenopausal women.

We have used a cross sectional population of ambulant elderly women to determine the association of endogenous estradiol with urine calcium and phosphorus excretion. The subjects were 293 postmenopausal women over 70 years. Filtered calcium and phosphate load, PTH, estradiol and SHBG were measured. The free estradiol concentration (FE) was calculated from a previously described formulae.

Reduced renal calcium excretion was associated with a high plasma estradiol concentration (r^2 = 0.023, P =0.01) and a high FE (r^2 = 0.045, P=0.001). The estradiol and FE effect on renal calcium excretion remained significant after adjusting for filtered calcium load and serum PTH. A reduced renal phosphate threshold was associated with a high FE (r^2 = 0.023, p =0.010). The effect remained significant after adjustment for serum PTH. Across the range of the FE observed in the study

patients the effect on reducing renal calcium excretion and increasing renal phosphate excretion was of the same order of magnitude as the effect of PTH.

This study indicates that estradiol regulates renal calcium and phosphate handling in elderly postmenopausal women and that the effect is of a similar magnitude to the well-recognized effect of PTH on these physiologically regulated parameters

P107

PREVALENT OSTEOPOROTIC FRACTURE IS ASSOCIATED WITH INCREASED INCIDENT OSTEOPOROTIC FRACTURE RISK IN OLDER WOMEN

A Devine[1,2,3,4], I Dick[1,2,3], R Naheed[1,2] & RL Prince[1,2,3]
[1] School of Medicine and Pharmacology, University of Western Australia, [2] Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, [3] Western Australian Institute of Medical Research, [4] Edith Cowan University, Perth, WA

In epidemiological studies a history of previous fracture is considered to be a determinant of future fracture risk. It is not certain if this is independent of bone mineral density.

We have examined the association of prevalent osteoporotic fracture independently and in association with BMD on the risk of 5 year incident osteoporotic fracture.

1499 women mean age 75 were recruited from the whole population. BMD was measured using an Hologic 4500A. Fractures since the age of 50 were ascertained at baseline and coded as none, 1, or >1 prevalent fractures. Incident fractures, defined as all fractures, except those of phalanges or skull, verified by x-ray, were recorded. Hazards ratio and 95% confidence intervals (HR) of the outcomes were examined using the Cox proportional hazard model.

20.2% and 7.7% of patients had sustained 1 or >1 prevalent fracture respectively after age 50. 235 individuals (16.1%) sustained 296 fractures during the study. During the study, 4.6% of patients died and 11.3% were lost to follow up. The 5 year incident fracture risk was associated with >1 prevalent fracture (HR: 1.99: 1.26-3.15) but not 1 prevalent fracture (HR 1.27 0.88-1.85). A 1 SD lower BMD was associated with an increase risk of incident fracture risk (HR 2.64: 1.86-3.73). After adjustment for BMD and age, >1 prevalent fracture remained an increased incident fracture risk (HR 1.79: 1.13-2.84).

Therefore, multiple prevalent fractures, but not one, significantly increase the risk of fracture independently of BMD.

P108

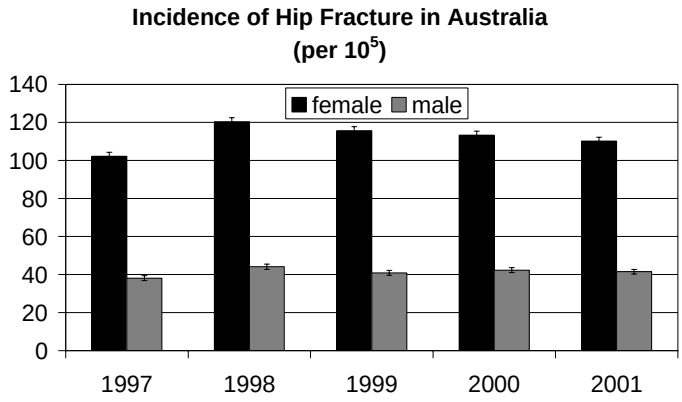
INCIDENCE OF HIP FRACTURES RESULTING IN SURGERY IN AUSTRALIA

VM Wells[1], A Esterman[2] & TC Hearn[1]
[1] Department of Orthopaedics, Repatriation General Hospital and Flinders University and [2] Flinders Centre for Epidemiology & Biostatistics, Flinders Medical Centre, Adelaide, SA

Aims:
Osteoporosis is a common metabolic bone disease associated with an increased risk of fracture. Hip fracture is the most serious osteoporosis related fracture and there is an exponential increase in hip fractures after 50 years of age. This poses a significant public health problem due to associated morbidity, mortality, disability and cost. It is therefore necessary to evaluate the incidence of hip fracture surgery in the context of changing population demographics in Australia to plan for future hospital admissions.

Methods:
The numbers of hip fractures resulting in surgery in Australia between 1997 and 2001, stratified for age and gender, were obtained from the Australian Institute of Health and Welfare. The incidences were calculated and tested for changes over time using Poisson regression.

Results:
In Australia, in 1997 there were 9,088 treated hip fractures in women and 3,318 in men. By 2001, hip fractures increased by 24.4% to 11,306 for women and by 27.4% to 4,230 for men: an overall increase of 25.2%. The age-standardized incidences for men and women are shown in the figure. In comparison to 1997, our reference year, there is no evidence of a change in hip fracture incidence.



Conclusion:
Despite the availability of preventative measures such as anti-osteoporosis medication, hip protectors, falls prevention programs, and community education, the overall incidence of hip fractures in Australia did not decrease.

P109

A NON-CLINICAL TRIAL ASSESSMENT OF MEDICATIONS USED IN THE TREATMENT OF OSTEOPOROSIS

S Korn[1], JA Pasco[2], MJ Henry[2], GC Nicholson[2] & MA Kotowicz[2]
[1] School of Health Sciences, Deakin University, Burwood and [2] Department of Clinical and Biomedical Sciences: Barwon Health, The University of Melbourne, Geelong, VIC

The information currently available about the efficacy of medications used in the treatment of osteoporosis is derived from clinical trials. Randomised clinical trials are used to determine the efficacy of osteoporosis but the entry criteria and general environment of these trials do not usually reflect routine clinical practice. The aim of this observational, community-based study was to compare different medications used in the treatment of osteoporosis in a non-clinical trial setting, using rate of change in BMD over time as an end-point.

Subjects were 518 women (aged 60+ yr) being monitored by bone densitometry between 1991-99 at the Geelong Hospital Medical imaging service , excluding clinical trial patients. Medication used was abstracted from questionnaires returned from referring doctors (80%, n=412) The median duration of medication use was 1.3yr (IQR 0.9-2.0). BMD at PA-spine and hip sites was obtained from bone density records. Women using a medication for osteoporosis demonstrated a significant increase in BMD at the PA-spine compared to women using no medication (p=0.03). Both hormone therapy (HT) and bisphosphonates produced a significant increase in BMD compared to no medication (mean annual change 2.1% for HT, 2.3% for bisphosphonates) (p=<0.05). A significant change in BMD was not observed at the hip (p=0.9).

This study demonstrates that in this community setting, medications used stabilised bone mass. As the changes in BMD were comparable to that seen in clinical trials, it would be expected that this would translate to the decreased fracture incidence observed in clinical trials.

P110

RATIONALE FOR INTERMITTENT INTRAVENOUS IBANDRONATE INJECTIONS IN POSTMENOPAUSAL OSTEOPOROSIS

PN Sambrook[1], RL Prince[2], MJ Hooper[8], JA Eisman[4], P Miller[5], RR Recker[6], S Adami[7], B Bonvoisin[8], RC Schimmer[8]
[1] Royal North Shore Hospital, St Leonards, NSW; [2] Sir Charles Gairdner Hospital, Nedlands, WA; [3] Concord Hospital, Concord, NSW; [4] Garvan Institute of Medical Research, Sydney, NSW; [5] 1CCBR, Lakewood, Colorado, USA; [6] Creighton University, Omaha, Nebraska, USA; [7] University of Verona, Verona, Italy; [8] Hoffmann-La Roche Ltd, Basel, Switzerland

Intravenous (i.v.) bisphosphonates must currently be given as prolonged infusions, requiring specialised resources and skilled personnel.

Rapid i.v. injections with extended between-dose intervals could provide a convenient alternative to current bisphosphonates in convenience, upper GI safety and treatment compliance.

Ibandronate (Bonviva®) is a highly potent, nitrogen-containing bisphosphonate of proven fracture efficacy and no indicators of renal toxicity, which can be administered as a convenient rapid i.v. injection (15-30 seconds) with extended dose intervals.

3 monthly i.v. ibandronate regimens have been evaluated in approximately 3,500 women with PMO. A 2mg i.v. ibandronate injection regimen provided highly significant and clinically meaningful BMD increases and bone turnover marker decreases. As these are similar to oral bisphosphonates of proven fracture efficacy, including oral daily ibandronate (vertebral fracture risk reduction: 62% [p=0.0001]), these data highlight possible substantial antifracture efficacy with optimal i.v. doses of ibandronate.

The Dosing IntraVenous Injection Administration (DIVA) study is a 2-year, randomised, double-blind, parallel-group (2mg and 3mg i.v.), phase III, non-inferiority study to an oral daily ibandronate (2.5mg) regimen with proven antifracture efficacy. A total of 1,395 postmenopausal women with osteoporosis (mean lumbar spine [L2–L4] BMD T-score <–2.5 and ≥–5) have been randomised as follows (see Figure 1).

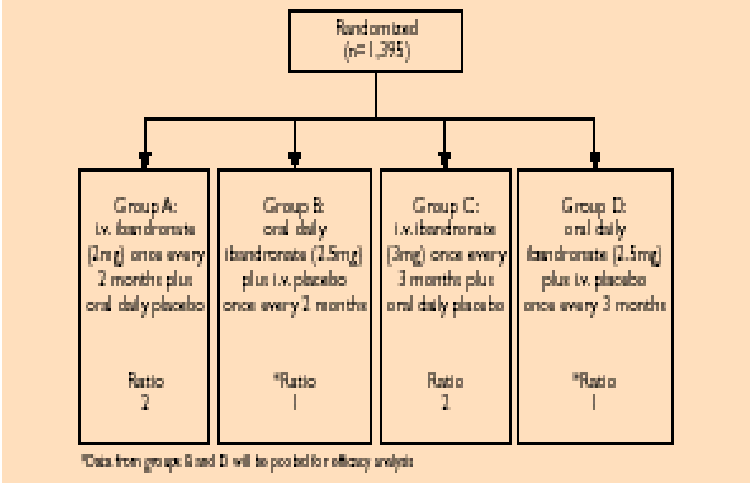


Figure 1. DIVA study design

The widely used and accepted non-inferiority analysis of lumbar spine BMD for demonstrating the therapeutic equivalence of alternative dosing regimens of the same agent is used to infer likely antifracture efficacy for the IV regimen. Other BMD parameters, bone turnover, lab and safety endpoints will also be assessed.

P111

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

MH Zheng, C Willers, L Kirilak, J Xu, D Wood & A Shimmin
School of Pathology and Surgery, Unit of Orthopaedics, University of Western Australia, Nedlands, WA

Conventional treatment regimes for articular cartilage injury only manage biomechanically inferior tissue comprised mainly of fibrocartilage. The development of autologous chondrocyte implantation has seen improvements in patients outcomes over conventional therapy, but complications associated with periosteum has led to the search for alternative scaffolds for the seeding of autologous chondrocytes. We have conducted an objective assessment of matrix-induced autologous chondrocyte implantation (MACI) patients by histological examination. Seven biopsies were analysed at 48 hours, 21 days, 6, 8, and 12 months postoperatively. Scanning electron microscopy and RT-PCR confirmed ACI-Maix collagen membrane efficiently integrates chondrocytes into its matrix and maintains the chondrolineage phenotype (aggrecan and collagen II expression). Results of sequential histology and collagen II staining show that MACI induces the regeneration of cartilage-like tissue as early as 21 days, with hyaline-like cartilage formed at 6 months. In summary, we have shown that MACI is a reliable paradigm for the regeneration of articular cartilage.

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