

ABSTRACTS

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their presentation**

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INVITED SPEAKERS

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CHOLESTEROL EFFECTS ON OSTEOCLAST APOPTOSIS

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Low cholesterol levels increase *in vitro* osteoclast apoptosis, while high levels increase osteoclast lifespan. This was documented by withdrawing cellular cholesterol with methyl beta cyclodextrin (MBCD) or high density lipoproteins (HDL), which dose-dependently disrupted the actin cytoskeleton and increased nuclear fragmentation, membrane blebbing and caspase 3 activation by 4- to 10-fold. Conversely, increased exogenous cholesterol availability, provided by low-density lipoprotein (LDL), increased osteoclast survival. Furthermore, osteoclasts from LDL receptor deficient mice (LDLR^{-/-}) were smaller and had a shorter lifespan than wild type controls. This phenotype was “rescued” by chemical delivery of cholesterol (from cholesterol-saturated MBCD) during osteoclast differentiation. The high sensitivity to ambient cholesterol seems to be due to lack of feedback regulation of the endogenous HMG-CoA reductase, which is very low in osteoclasts, and unlike in fibroblasts (NIH/3T3), osteoblasts (MB1.8) or liver cells (HEPG2), is not induced by lowering cellular cholesterol levels. Osteoclasts also express very low levels of the ubiquitous cholesterol transporting protein caveolin 1 present in membrane rafts. This inverse relationship between cholesterol and osteoclast lifespan and activity could play a role in the observed link between osteoporosis and atherosclerosis, since the osteoclast appears to rely on its external environs to control intracellular cholesterol levels.

**OSTEOBLASTS AND OSTEOCLASTS – A TOUCHING COUPLET
– HOW WAS ODF/RANKL DISCOVERED AND IDENTIFIED? –**

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It is well established that osteoblasts are primarily involved in bone formation and osteoclasts are in bone resorption. The concerted action of these two types of cells is important for maintaining bone mass constant. Osteoclasts, which are present only in bone, are derived from hematopoietic cells of the monocyte-macrophage lineage, and osteoblasts are derived from bone marrow stromal cells.

The idea that osteoblastic cells intervene in the process of osteoclast formation and activation under the primary stimulus of bone-resorbing hormones and local factors was first brought about by Rodan and Martin¹⁾ in 1981, which was based on the facts that osteoblasts, but not osteoclasts, have receptors for such bone-resorbing factors, and that their relative potencies in osteoblastic cells resemble those in promoting bone resorption. In 1988, we developed a co-culture system of mouse osteoblasts/stromal cells and hematopoietic cells (spleen cells or bone marrow cells), in which osteoclasts were formed in the presence of M-CSF in response to bone-resorbing factors such as $1\alpha,25(\text{OH})_2\text{D}_3$, PTH and IL-11²⁾. Cell-to-cell contact between osteoclasts/stromal cells and hematopoietic cells was critical for osteoclast development²⁾. The target cells of bone-resorbing factors for inducing osteoclast formation in the co-cultures were osteoblasts/stromal cells, but not osteoclast progenitors, since osteoblasts prepared from VDR knockout mice and PTH receptor knockout mice similarly failed to induce osteoclast formation in co-cultures with normal spleen cells in response to $1\alpha,25(\text{OH})_2\text{D}_3$ and PTH³⁾. IL-6, IL-11, OSM and LIF, all of which utilize gp130 as a signal transducer, all acted on osteoblastic cells to induce osteoclast formation from its progenitors in co-cultures. From these results, we proposed a hypothesis that osteoblastic cells induce a membrane-associated factor (osteoclast differentiation factor, ODF) in response to several bone-resorbing hormones and cytokines (1992)³⁾.

In 1997, we purified and molecularly cloned osteoclastogenesis-inhibitory factor (OCIF)^{4,5)}, which was identical to osteoprotegerin (OPG) reported by Amgen. OPG/OCIF was a secreted member of the tumor necrosis factor (TNF) receptor family and inhibited osteoclastogenesis by interrupting the cell-to-cell interaction between osteoblasts/stromal cells and osteoclast progenitors⁵⁾. By expression cloning using specific binding to OPG/OCIF, we finally cloned a ligand for OPG/OCIF from a complementary DNA library of mouse stromal ST2 cells treated with $1\alpha,25(\text{OH})_2\text{D}_3$ and dexamethasone⁶⁾. The protein cloned was found to be a member of the membrane-associated TNF ligand family and induced osteoclast formation from spleen cells in the presence of M-CSF even in the absence of osteoblasts/stromal cells⁶⁾. OPG/OCIF completely abolished the osteoclast formation induced by ODF and M-CSF⁶⁾.

The long-sought ligand mediating an essential signal to osteoclast progenitors for their differentiation into osteoclasts was indeed ODF⁷⁾. ODF was found to be identical to TRANCE/RANKL, which enhances T-cell growth and dendritic cell function. We should emphasize that the idea of the presence of the decoy receptor (OPG/OCIF) was not taken in mind in our original concept proposed in 1992. The molecular cloning of ODF/RANKL would not have been successful without the discovery of OPG/OCIF. In my talk, we will show the inside story how ODF/RANKL was discovered and identified.

References:

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PROSTAGLANDINS – STILL CRAZY AFTER ALL THESE YEARS

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We have known that prostaglandins, particularly PGE₂, are potent agonists in bone for more than 30 years. However it has been difficult to fully understand their action, perhaps because prostaglandins, particularly PGE₂, can both stimulate and inhibit bone resorption and formation. Recent identification of multiple receptors for PGE₂ and studies of mice in whom these receptors have been deleted, as well as the use of selective agonists and antagonists has brought new excitement and knowledge to this field. Among PGE₂ receptors, the EP2 and EP4 receptors, which increase camp, are clearly involved in the stimulation of bone resorption and formation by PGE₂. EP4 appears to play a greater role in the resorption mediated through osteoblast-osteoclast interactions, while EP2 may be important in enhancing osteoclast production from hematopoietic precursors. Endogenous production of PGE₂ in cells of the osteoblastic lineage, largely by the inducible cyclooxygenase (COX-2), plays an important role in modulating the response to a wide variety of other regulators including PTH, vitamin D, growth factors and cytokines. These factors have both prostaglandin dependent and independent effects on bone cells, supporting the concept that prostaglandins may act to enhance or facilitate the skeletal effects of other agonists. Prostaglandins can also auto-amplify their own effects through induction of COX-2. Studies using knockouts and COX-2 inhibitors indicate that endogenous prostaglandin synthesis is important in inflammatory bone loss, fracture healing and in the response to mechanical loading. There is evidence that prostaglandins play a role in bone loss after ovariectomy in mice. Thus it may be possible to develop targeted and selective agonists and antagonists which have useful therapeutic effects on bone.

PTHrP AND SKELETAL DEVELOPMENT

Henry Kronenberg

When Jack Martin's group discovered the gene encoding parathyroid hormone-related protein (PTHrP), the findings raised the important question of the normal roles of PTHrP in health and disease. Many groups have subsequently shown that PTHrP is made in most organs throughout life, where it usually serves a variety of paracrine functions. A common theme is that PTHrP signaling often regulates cellular proliferation, differentiation, and death and usually works through the PTH/PTHrP receptor, a receptor that responds identically to amino-terminal fragments of both PTH and PTHrP. When either PTHrP or the PTH/PTHrP receptor genes are ablated in mice, the resultant fetuses die before or at the time of birth and exhibit multiple abnormalities. One of the most striking involves the development of growth plates in bones that form by endochondral bone formation. The important roles of PTHrP in bone development can be viewed as a paradigm of the actions of PTHrP in many tissues.

Chondrocytes in the growth plate follow a characteristic program in which they proliferate several times while making a matrix rich in type II collagen. These chondrocytes then stop proliferating and hypertrophy. The hypertrophic chondrocytes produce a matrix rich in type X collagen, mineralize the matrix, and subsequently undergo apoptosis. Then osteoclasts, blood vessels and osteoblasts replace the cartilage-produced matrix with bone. In fetal life, PTHrP is synthesized primarily by perichondrial cells and early chondrocytes at the ends of long bones. The PTHrP then acts on the PTH/PTHrP receptor on chondrocytes to keep chondrocytes proliferating. This results in the formation of characteristic columns of proliferating chondrocytes and delay in differentiation of hypertrophic chondrocytes until the chondrocytes are sufficiently distant from the source of PTHrP. When the chondrocytes stop dividing they express large amounts of another paracrine factor, Indian hedgehog (Ihh). Ihh has three distinct actions: it stimulates chondrocyte proliferation, it acts on adjacent perichondrial cells to direct them to become osteoblasts, and it stimulates the production of PTHrP. This last action completes a negative feedback loop: PTHrP keeps chondrocytes proliferating and, therefore, not synthesizing Ihh. When chondrocytes have moved far enough away from the source of PTHrP stop proliferating and make Ihh, which then signals back to the end of the bone to assure adequate PTHrP production.

How PTHrP acts to keep chondrocytes proliferating and delays their differentiation is poorly understood. Through a series of studies of genetically engineered mice, we have found that the PTH/PTHrP receptor activates two distinct G protein-mediated pathways in the growth plate. G_s activation leads to the continued proliferation of chondrocytes. Somewhat surprisingly, activation of the G_q pathway by PTHrP acts in an opposite fashion, to hasten differentiation of chondrocytes. The G_s effect dominates in normal growth plates. How this signaling affects proliferation is a topic of ongoing experimentation. Studies by Helen MacLean have shown that one crucial action of PTHrP is to suppress the production of p57 mRNA and protein. p57 is an inhibitor of the cell cycle kinases (cdk's) that drive the cell cycle. In many bones, ablation of the p57 gene reverses many of the abnormalities in bones missing PTHrP. Thus, part of PTHrP's action involves targeting of a crucial regulator of the cell cycle.

COLLAGEN TURNOVER AND BONE

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Removal and deposition of collagen are critical processes during embryogenesis and adult remodeling of the skeleton. To assess the role of collagenases (MMPs) in these processes, we introduced, by homologous recombination in ES cells, a null mutation into the mouse MMP-13 gene and obtained germline transmission. MMP-13 is normally expressed predominantly in the distal growth plates and centers of ossification in developing embryos and by osteoblasts/stromal cells in adult bone. The mutation results in deletion of the critical, catalytic, Zn-binding domain and, if expressed, an inactive enzyme. In MMP-13 ^{-/-} embryos and young adults there is increased width of growth plates, predominantly in hypertrophic zones with increased expression of markers of chondrocyte differentiation. Responses to PTH and IL-1 α in newborn calvarial organ cultures were decreased in MMP-13 ^{-/-} vs. MMP-13 ^{+/+} mice. As the MMP-13 ^{-/-} mice age, they show increased trabecular bone mass (DEXA, microCT, static/dynamic histomorphometry) decreased resorption but increased bone formation compared with MMP-13 ^{+/+} mice. We used bone marrow cultures (BMC) of cells from 1 month-old mice. In early BMC, osteoblast proliferation and expression of alkaline phosphatase were increased in BMC from MMP-13 ^{-/-} vs. MMP-13 ^{+/+} mice and potentiated by ascorbic acid and/or PTH. Thus, disruption of MMP-13 function results not only in abnormal endochondral ossification during development but in abnormal adult bone remodeling as well with increased deposition of trabecular bone. Although MMP-13 is expressed in osteoblasts/related cells and not in osteoclasts, bone resorption is decreased in the MMP-13 ^{-/-} mice. MMP-13-mediated degradation of collagen must therefore be an important regulator (directly/indirectly) of chondrocyte, osteoblast and osteoclast function.

THE SEED AND THE SOIL

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When tumor cells metastasize to the bone marrow, they reside in an environment which is different from that of the primary site, or other soft tissue metastatic sites. It is now clear that in the case of human breast cancer cells, this is associated with a change in their phenotype. The best characterized change is the expression of the tumor peptide PTH-rP, first identified by Jack Martin and his colleagues, whose expression is increased in the bone marrow microenvironment, but there are also other changes in phenotype that have been described including increased expression of PTH receptors and altered expression of estrogen receptor isoforms. This change in phenotype has important functional consequences in the bone microenvironment. Increased expression of PTH-rP by the breast cancer cells can be stimulated by TGF β , which is produced in active form as bone resorbs. This leads in turn to a further increase in PTH-rP and even greater bone destruction. This is the basis of the "vicious cycle" hypothesis, and makes bone unique as a site for breast cancer metastasis. Other bone-derived growth factors such IGF-1 also play an important role as stimulators of tumor cell proliferation in this microenvironment. These cellular interactions in the bone marrow microenvironment are also present in other cancers with a proclivity to grow in bone. For example, in osteoblastic metastases which are frequently associated with prostate cancer, inhibition of bone resorption by bisphosphonates causes decreased metastatic growth of the tumor cells in bone, and in myeloma, complete inhibition of bone resorption by the osteoclast inhibitor RANK.Fc leads not only to reduction in osteolysis, but also in tumor burden. There are several other important implications of these observations. Other common metastatic sites such as liver, lung and brain also likely have unique tumor-specific characteristics which select for or alter the behavior of tumor cells that metastasize to these sites. Secondly, if the vicious cycle maybe achieved is interrupted, for example by drugs which inhibit osteoclastic bone resorption, not only will bone loss be reduced but also tumor burden in bone. This important concept has now been defined and confirmed experimentally. Interruption of the vicious cycle by osteoclast inhibitors, by antagonists to PTH-rP, or by inhibitors of TGF β activity. These approaches all reduce not only bone lesions associated with cancer metastasis, but also the accumulation of tumor cells in the bone marrow microenvironment.

This concept of the bone microenvironment in cancer is simply all extension of the "Seed and Soil" hypothesis proposed over 100 years ago by Stephen Paget. Since the molecular mechanisms responsible for making some soils sympathetic to certain cancer cells is now being clarified and understood, this is leading to new ideas not only for how patients with cancer-induced bone disease may be treated, but also how tumor cells modify their phenotype in different environments.

PARATHYROID HORMONE (PTH): FROM HORMONE TO DRUG

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Rediscovery is a recurring theme in medicine. PTH is a vivid example. Reticence to change established beliefs is also common, a truism that applies to PTH. I shall review the slow to be accepted evidence indicating that exogenously administered PTH elicits a strong bone formation-enhancing effect in animals and people.

Particularly interesting (to me) is the profound importance of the mode of administration on the pharmacological effect. One single, transient (<3-4 hours) elevation of plasma PTH (sc injection) per 24-hour period, when repeated daily, elicits a strong bone anabolic effect. Frequent repeated injections or continuous infusion (exogenous PTH or endogenous PTH in hyperparathyroidism) tips the balance toward catabolism. No satisfying explanation exists for the crucial importance of the pharmacokinetic profile although there are clues. Findings in animals and people demonstrate that the new bone formed in response to PTH is of normal quality and is organized to improve biomechanical properties leading to decreased fragility and a lower risk of fracture. Safety of once-daily PTH has been established in animals and people with the exception that two near-lifetime investigations in rats have shown that PTH can cause osteosarcomas in this species. The neoplastic process appears to be a consequence of the profound pharmacodynamic effects of PTH in the rat, effects not observed in monkeys or human beings. Recombinant human PTH (1-34) was approved by the FDA in Nov. 2002 for the treatment of severe osteoporosis in women and to increase BMD in men. It will be important to compare the long-term outcomes (efficacy and safety) in the treatment of osteoporosis with PTH or resorption inhibitors. Options for the treatment of osteoporosis have been uniquely broadened with PTH as the first, truly anabolic therapy.

BISPHOSPHONATES: FROM SOAP SUDS TO A DRUG**H. Fleisch: Av. Désertes 5, CH-1009 Pully, Switzerland.**

This presentation will deal with the history of the development of the bisphosphonates from its start in the early sixties to today. Emphasis will be laid on the parts played by such factors as logical deduction, serendipity, untimely discoveries, conventional wisdom, role of industry and of course luck.

The story started with the discovery by our group in the early sixties that biological fluids as plasma and urine contained an inhibitory activity of calcium phosphate precipitation. Part of this activity was then identified as inorganic pyrophosphate which had not been identified before in these fluids. This is the simplest of the polyphosphates, compounds which had been extensively used in industry as antiscaling additives in washing powders, water and oil brines because of their property of inhibiting calcium carbonate formation. Furthermore pyrophosphate also inhibited calcium phosphate dissolution, both effects being explained by the strong adsorption onto the surface of calcium phosphate. These results opened the possibility that pyrophosphate might modulate both formation and dissolution of calcium phosphate in the body, through the modulation of its concentration by pyrophosphatases. This was supported by the finding that pyrophosphate could inhibit ectopic calcification in animals when injected. However, no effect was present when given orally, or on bone resorption, possibly because of hydrolysis. Therefore polyphosphates found a therapeutic use only in skeletal scintigraphy when linked to ^{99m}Tc .

This restricted use prompted us to search analogs with a similar physicochemical activity, but which would resist enzymatic hydrolysis and would therefore not be broken down metabolically. The bisphosphonates fulfilled these conditions. They are compounds synthesized first in 1865 and characterized by a P-C-P bond instead of the P-O-P bond of pyrophosphate and were used industrially. By substitution of the hydrogens on the C atom it is possible to synthesize a variety of different bisphosphonates, each with its distinct physical-chemical, biological, therapeutical and toxicological characteristics.

The first description of their action on bone was published by our group in 1968/69. The bisphosphonates were found to have indeed similar physicochemical effects to pyrophosphate. However, *in vivo* they blocked various ectopic calcification models in the animal both when given parenterally and orally, and inhibited strongly bone resorption, as described in numerous animal models. Their potency in this respect varies greatly, the newest compounds being 10'000 times more active than the first ones described.

In contrast to the inhibitory effect on mineralization, which is due to a physical chemical inhibition of crystal growth, it was soon realized that the antiresorbing effect was not physical chemical, as initially postulated, but cell mediated through the osteoclasts. These are fewer because of a decreased formation and a shorter survival due to apoptosis, and are less active. Recent evidence indicates that not all bisphosphonates act by the same mechanism. Thus, while the compounds containing a nitrogen atom work by inhibiting the isoprenylation of GTP-binding proteins, secondary to the inhibition of the mevalonate pathway, etidronate, clodronate and tiludronate are incorporated into ATP-containing molecules and may then act by a different mechanism.

The study of the effect in humans has a long and tortuous history. The first human investigations started in diseases with ectopic mineralization in the late sixties with an uncertain effect. At the same time etidronate was found to be active in inhibiting bone destruction in Paget's disease. The first investigations on tumor bone disease, especially tumoral hypercalcemia, date back to the early eighties. But it is only in the nineties that osteoporosis was successfully investigated.

The availability of these compounds to the practicing clinician started only in the nineties that is about 25 years after our first report of their efficacy *in vivo*. Today about 10 bisphosphonates are available for one or another indication all around of the world, the bisphosphonates being the most used drug in metabolic and tumoral bone disease. The reason for this long delay will be discussed.

BACK TO THE BEGINNING: PYROPHOSPHATE REVISITED
G Russell

**VITAMIN D THERAPY AND OSTEOPOROSIS
–D HORMONE ANALOGS AND BONE EFFECTS–**

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Not only the extent but also the direction of effects on bone differs depending on the dosage of D hormone *in vivo*. D hormone stimulates bone resorption at toxic doses, but inhibits it at the pharmacological window of doses. In toxic doses, D hormone probably stimulates production of RANKL in bone which then causes production and activation of osteoclasts as amply shown in pertinent systems *in vitro*. At pharmacological doses *in vivo*, D hormone was shown in mice not to produce changes in the expression of RANKL in bone. On the contrary, it suppressed the pool size of preosteoclasts expanded by estrogen withdrawal, and the osteoclast function super-induced in OPG⁻KO mice. The latter finding indicates that one of the site of action of D hormone is downstream of RANK. In *in vitro* system, D hormone was shown to suppress osteoclastogenesis in the presence of MCSF and sRANKL. This was paralleled by the suppression of c-Fos and Fra-1 protein.

Therefore, D hormone has at least two attributes for their function, the bone anabolic (the bone effect) and the bone catabolic effect (the calcemic effect). The bone anabolic effect was observed in murine systems in the absence of changes in the serum levels of PTH, calcium and phosphate, and was not reproduced by fortification of nutritional supply of vitamin D and calcium. The bone anabolic effect was confirmed in clinical studies as well.

We could demonstrate that relative potency regarding these attributes differ depending on the analogs: some are more potent in the calcemic, and others in the bone effect. Thus, it is possible to broaden the therapeutic window by developing compounds that have much higher potency in the bone effect and less in the calcemic effect. Needless to say that such compounds are another candidate of the remedy for osteoporosis.

BRAIN MATTERS TO BONE MORE THAN THOUGHT**Gerard Karsenty****Baylor College of Medicine, Houston, Texas, USA**

Our laboratory has a long-standing interest in the genetic control of skeletal physiology. One of the functions we have been studying is bone remodeling with a particular emphasis on the control of osteoblast function. Two sets of observations led us to search for hormones regulating bone formation. One observation came from our own work. In the course of the analysis of a mouse model of inducible osteoblast ablation we were surprised that the recovery of these animals was both precise and quick. This indicated that osteoblasts could deposit bone matrix at a high rate and then when bone mass was restored they could decrease their output. We chose as one of the many possible explanations of this phenomenon to hypothesize that bone formation was under the regulation of hormones, as bone resorption is. The second observation that led us to search for hormones regulating bone formation comes from the clinical literature. Without ignoring all the evidence demonstrating that gonadal failure increases bone resorption we postulated that the fact that osteoporosis follows gonadal failure and that obesity protects from it were not two observations but a single one. This observation itself may be the clinical translation of the following endocrine principle: body weight, reproduction and bone mass are regulated by the same hormones. Two aspects of this hypothesis have to be emphasized. It can be tested *in vivo* through the combined use of genetic and histology. Moreover, given the hypothalamic nature of the control of body weight and of reproduction if this hypothesis is true it will lead to show that bone mass is also under hypothalamic control. This notion should not be surprising *a priori* as most homeostatic function, and bone remodeling is one of them, are controlled centrally.

Because of the hypothesis linking the control of body weight, reproduction and bone mass we became interested in leptin. Accordingly, leptin is a hormone made by the adipocytes which control (negatively) appetite and favors the onset of puberty following its binding to a signal transducing receptor present on hypothalamic neurons. Leptin-deficient (*ob/ob*) and leptin receptor-deficient mice (*db/db*) have, as already known phenotype, an obesity and a hypogonadism both of hypothalamic origin. To our surprise when we performed a histologic analysis of *ob/ob* and *db/db* mice we noted that they have a 40% increase in their bone mass. This phenotype was truly unusual as those mutant mice are the only known animal models in which there is coexistence of high bone mass and hypogonadism. Further study of this pathway showed that it was the absence of leptin signaling not the weight that caused these phenotypic abnormalities. Consistent with what is known about the biology of leptin infusion of leptin and with the initial hypothesis in the third ventricle of leptin-deficient mice rescued their high bone mass phenotype without any detectable leptin in blood. This experiment demonstrated unambiguously the existence of a central control of bone formation.

Following this series of observations we searched for a neuronal pathway in the hypothalamus that controls bone formation. Chemical lesioning identified neurons that are antiosteogenic i.e. their lesioning increases bone mass and is necessary for leptin antiosteogenic action. More importantly, we could dissociate anorexigenic and antiosteogenic function of leptin. To search for a peripheral mediator of leptin antiosteogenic function we performed a parabiosis experiment between two *ob/ob* mice followed by leptin ICV infusion in one mouse of each pair. The mouse receiving leptin lost bone while the controlateral mouse never did, suggesting that the mediator of leptin action is of neuronal nature. Using both clinical observations and observations made in leptin signaling deficient mice we could demonstrate that the sympathetic nervous system is a peripheral mediator of leptin that controls more efficiently bone mass than body weight.

OSTEOPOROSIS GENETICS – OF MICE AND MEN

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Osteoporosis is a major public health problem in all communities affecting particularly its aging and older members; both men as well as women and results in increased risk of fracture from relatively minor injuries and thus major health care costs, significant long-term morbidity and even mortality. Although fractures of the spine, forearm and proximal femur are the most common, almost all bones are subject to such fractures. Studies in twins and in families have shown strong heritability for osteoporosis risk. This has been assessed as quantitative traits such as bone mineral density and quantitative ultrasound and as actual fracture, such as wrist fracture, outcomes. Various studies have assessed this genetic component as strong, explaining from 50 to 80% of variance in measurable traits. Three interdependent questions, each of which lead to further questions, arise from these findings: Can this heritability be attributed to causative variations in a small number of key genes? How could such gene variants be identified? How could such knowledge impact on osteoporosis prevention and treatment?

Some studies point out that “familial” clustering of traits could be explained by shared genes, by shared environments or by a combination or interaction of genes and environment. Environmental factors seem to be of modest relevance, since most family-based studies have failed to identify environmental factors with sufficient sharing and of sufficient effect size to explain more than a small proportion of the observed “heritability”. In several studies, heritability of bone mass and geometry, as measured as bone density at different sites and by different techniques, could be explained by some shared and some site-specific genes. Inherently this indicates that more than 2 - 3 genes must be involved and some studies suggest many, perhaps up to 30, genes explain the majority of the heritable effect and indeed many gene polymorphisms have been associated with bone density, other bone parameters and in some cases with osteoporotic fracture risk. However some recent family-based studies suggest that one major gene may explain much of any shared bone trait within families. These complex trait segregation analysis studies and some small pedigree studies of extreme bone traits support the concept that single gene loci may contribute a large part of heritability of bone phenotypes within a family. Overall these studies support the concept that clinically useful genetic loci could be identified and as such have lead to various approaches to gene searching for osteoporosis “susceptibility” genes. Consideration of what might be the “best” approach relates to what particular bone phenotype might be studied, bone density, ultrasound, geometry, turnover or even fracture outcomes. The choice will depend on the underlying aims, as discussed below.

Given that a few genes could contribute significantly to various bone phenotypes of clinical relevance, the question is then what approach may be the “best” to identify such genes. Some argue that animal models are the best as any trait identified can be dissected with greater speed and molecular accuracy than would be possible in human studies. Others argue that various family-based, including twin or sib-pair based, studies offer the greater potential to identify genetic loci of direct relevance to human disease and health. The advantages and limitations of these approaches, which will be the focus of the remainder of this presentation, depend upon what might be the aim of the identification of these genes, eg clinical risk assessment, tailoring of therapy or identification of targets for new therapies. Each of these is a valid aim but lead inherently to different approaches. If the fundamental aim is to identify high (or low) risk individuals or to tailor therapy for an individual, then gene association studies of family-based studies or outcomes in large clinical trials make the most sense. These sorts of studies essentially pursue the “candidate” gene approach of examining the association of polymorphisms of or SNPs in genes known to involved in bone biology and homeostasis with the clinical outcomes. On the other hand, if the fundamental aim is to identify new targets for novel therapies or to further basic understanding, then the most rational approach would appear to involve “genome-wide” scans, arguably best done in extended human pedigrees, or trait segregation phenomenon in animal models. Similarly analysis of gene expression under different conditions in animal models and sometimes human tissues, using microarray approaches, provide another unique opportunity to identify genes not previously realised to be critically involved in bone and calcium homeostasis. The “candidate” approach can clarify a suspected role of a known gene or genes and determine the size of a clinical effect. However only the exploratory, genome-wide or microarray, approaches can lead to new, deeper understanding of the basic biology underpinning bone phenotypic traits.

The candidate gene approach has identified a range of genes already know to be involved in bone and calcium homeostasis as having effects on measurable traits such as bone density and more interestingly on fracture risk independent of such quantitative traits, i.e. independent of bone density. The first gene so implicated, although the

initial report was subsequently noted to be flawed, was that for the vitamin D receptor. Further studies support a modest effect and perhaps not surprisingly some of these studies suggest effects on calcium requirements and responses. The other gene most widely reported and supported in effects on bone density and even on fracture outcomes is that of the collagen 1A1 gene. As for the VDR gene the polymorphisms do not affect the coding region but may effect the regulation of the transcription of the gene. These data now implicate polymorphisms or SNPs in upwards of 30 different genes; each with relatively small clinical effect size. Hence it would seem that these genetic markers might not yield major value for identification of a high risk group. However in some population samples, the combination of two or more of these polymorphisms has appeared to identify a smaller subgroup of individuals at substantially increased fracture risk. It remains to be seen how useful these combinations of genetic markers may be in assessment of absolute fracture risk, which is still a rather inexact science in osteoporosis.

The genome-wide scan approach has been used effectively in some unusual human pedigrees with high bone density and others with osteoporosis. These studies have identified a cell surface receptor that is part of the Wnt-frizzled signalling pathway. The original human data has been reproduced in animal models. Other human pedigrees with high bone density have led to the identification of other genes of less clear function. Crossbreeding studies of inbred mouse strains have implicated a large number of chromosomal loci in bone and calcium homeostasis. An issue with these mouse models is whether they really relate to human conditions. However many studies, in which mutations in homologous mouse genes match human clinical disorders, suggest that mouse models do offer insights into human biology. Moreover the synteny between human and mouse chromosomes allows mouse genome studies to identify human chromosomal regions as potential locations for relevant human genes. Mouse transgenesis and knockout studies have also identified unexpected changes in bone biology and as such have identified novel anabolic pathways augmented with microarray studies to identify involved pathways. Thus, perhaps most importantly, these studies have lead to new insights into normal physiological pathways that may contribute to osteoporosis susceptibility or resistance.

In summary, there are strong heritable factors contributing to each individual's liability to osteoporotic fracture. Candidate gene studies in human clinical samples continue to provide important insights into the probable effects of known genes and may lead to better risk assessment and tailoring of therapy while genome-wide scan and expression microarray approaches in human and animal models can identify new targets that may lead to novel therapies in the future. There is no single perfect approach rather the complimentary use of these two types of approaches seems to provide the best chance for the most rapid advances.

COLLAGENASE GENES AND REGULATION**N Partridge****Robert Wood Johnson Medical School, Piscataway, NJ 08854 USA**

Apart from producing osteoclast-activating factors such as RANKL and interleukin -6, the osteoblast appears to also have a direct role in matrix degradation. For instance, parathyroid hormone (PTH) induces collagenase-3 gene transcription in osteoblastic cells through a cAMP-dependent pathway requiring *de novo* protein synthesis. Thus, this is a secondary effect that involves the induction and activation of specific transcription factors acting on this gene. We identified the PTH-response elements as being the activator protein-1 (AP-1) and the core binding factor a1 (Cbfa1) binding sites in the collagenase-3 promoter. We have demonstrated a PTH-dependent cooperative interaction between the sites and the proteins binding to them. Supporting our earlier work, the PKA pathway was shown to be the only pathway regulating the collagenase-3 promoter as a mediator of PTH action. The importance of this pathway was demonstrated by the fact that PTH stimulates the transactivation of activation domain-3 in Cbfa1 through its PKA site. PTH regulates both transcription factors through this pathway, either by increasing their expression or altering their phosphorylation. We have shown that Cbfa1 and Fos/Jun associate both *in vitro* and *in vivo*. This does not require binding to DNA nor phosphorylation of Cbfa1. Recent work has shown that PTH causes increased acetylation of histone H4 bound to the collagenase-3 promoter in the PTH-response element. This occurs as early as 5 minutes after PTH treatment of UMR 106-01 cells and indicates modification of the nucleosomal structure in the promoter region of this gene. Our hypothesis is that PTH causes phosphorylation of Cbfa1, recruiting other proteins such as modifiers of the nucleosome, and that later, Cbfa1 interacts with Fos/Jun recruiting co-activators and the general transcription factors.

VITAMIN D: A MAN FOR ALL SEASONS

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Vitamin D has been recognized first as a true vitamin at the beginning of the 20th century but was immediately an exception to the vitamin rule as it could also be synthesized in the skin under the influence of ultraviolet light. The clinical picture of vitamin D deficiency was already known in the 16th as demonstrated by several monographs. Its vitamin status further evolved into a hormone status by the detection of (1) its further metabolic activation into 1,25(OH)₂D₃ and (2) its action as a ligand for the vitamin D receptor, VDR, a major nuclear transcription factor. Surprisingly VDR is expressed not only in tissues involved in calcium and bone homeostasis but also in numerous, in fact nearly all cells and tissues. This raised the question of a possible role outside the strict calcium/bone metabolism.

The creation of VDR KO mice confirmed the essential role of vitamin D in calcium homeostasis as such animals have the typical picture of human vitamin D resistant rickets type 2, or true hormone resistance, characterized by hypocalcemia, rickets and growth delay, secondary hyperparathyroidism and increased 1,25(OH)₂D₃. This phenotype cannot be rescued by large excess of 1,25(OH)₂D₃, whereas a high calcium diet with either lactose or high fat content can normalize serum and bone homeostasis as well as parathyroid size and secretion. This demonstrated that the primary target tissue was the intestine and not bone, parathyroid or kidney. To identify the genes involved we studied the intestinal calcium transporting genes and demonstrated that a new class of epithelial calcium channels (TRP) are crucial. Indeed, comparison of duodenal gene expression patterns in Tokyo and Leuven VDR null mice indicated that among the candidate calcium transporting genes, only CaT1 and EcaC1 are severely impaired in the absence of a functional VDR. This implies a regulatory role for the epithelial calcium channels in active calcium absorption, or a more complex regulation driven by the interaction between calcium influx and calbindin-D_{9K}. The activation of the CaT1 and EcaC1 channels may indeed require the immediate intracellular uptake of each calcium molecule delivered through the channel by calbindin-D_{9K}, with subsequent mobilization of calcium towards the basolateral membrane.

Deletion of the 25-hydroxyvitamin D-1 α -hydroxylase also produces the typical features of vitamin D deficiency or resistant rickets with disorganization and widening of the columnar alignment of hypertrophic chondrocytes, resulting in increased width of the growth plate and accumulation of osteoid (unmineralized matrix) in trabecular and cortical bone. This clearly demonstrates that 1,25(OH)₂D₃ is the key metabolite responsible for the parent vitamin action and that the other metabolites are redundant for bone mineralization. This is in line with the results observed in 24-hydroxylase KO mice which develop a high perinatal mortality due to hypercalcemia. 24,25(OH)₂D thus is primarily responsible for regulating vitamin D catabolism. This does not exclude a role for one of the vitamin D metabolites in growth plate or bone metabolism during stress situations (e.g. fracture repair).

The extracellular effects of vitamin D have been clearly demonstrated by the skin phenotype of all VDR-KO mice as well as by human true vitamin D resistance. The abnormality can be described as the inability of the hair follicle to produce more than one generation of hair. This abnormality cannot be corrected by a high calcium/lactose/fat diet and is also not observed during even severe vitamin D deficiency, arguing for a direct ligand independent action of the VDR. The precise genes involved in this alopecia are still not identified. The role of vitamin D for the skin is further demonstrated by the ability of keratinocytes to (1) produce 1,25(OH)₂D₃ itself from 7-dehydrocholesterol (2) respond to this autocrine factor by inhibition of proliferation and induction of differentiation.

The role of the vitamin endocrine system in the regulation of immune system was first suggested by the presence of VDR in nearly all immune cells and the capability of monocytic cells/macrophages to produce 1,25(OH)₂D₃ by an authentic 1 α -hydroxylase. VDR-KO mice have indeed major abnormalities of macrophages but this phenotype can be corrected by a calcium rescue diet.

The vitamin D hormone can also block cell differentiation at the G1 level and this is achieved in nearly all proliferating cells. The exact "primum movens" genes involved are not yet identified and numerous candidate genes have been proposed. This has been extended by recent μ Array experiments demonstrating the crucial role of the E2F family of transcription factors.

Vitamin D binds to several critical proteins: (1) the VDR which has a large binding pocket which can accommodate multiple steroidal as well as nonsteroidal analogs. A few thousand vitamin D analogs have now already been synthesized and the properties of some of them warrant their description as selective vitamin D receptor modulators. The second (2) major binding protein for all vitamin D metabolites is a close member of the albumin/alfafetoprotein family, the plasma vitamin D binding protein (DBP). The crystal structure and its binding pocket for 25-OHD as well as its interface with actin have now been resolved. Moreover DBP binds to a LDL-type of receptor, megalin, which is responsible for the cellular uptake of the DBP 25-OHD complex at the luminal site of the nephron.

The clinical aspects of vitamin D are numerous. Simple vitamin D deficiency was recognized at the beginning of the 20th century as an endemic disease in infants, causing rickets. At the end of that century the same deficiency was recognized as a major contributing factor to senile osteoporosis.

Moreover serum vitamin D metabolites are useful indicators for many metabolic bone disorders. Finally vitamin D is the most widely used drug in the world and some selective vitamin D analogs might well be used for a variety of diseases.

NEW INSIGHTS INTO OSTEOGENESIS IMPERFECTA**Francis H. Glorieux, M.D., Ph.D.****Shriners Hospital for Children, and McGill University
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Osteogenesis Imperfecta is a heritable disorder characterized by bone fragility caused by alterations in bone quantity and quality, which may present with a wide range of severity. The term osteogenesis imperfecta (OI) has been used to describe the clinical features of this disorder since the nineteenth century, long before any underlying gene mutations were described. About 70% of all individuals clinically classified as OI possess a mutation in either one of the two genes encoding type I collagen (COL1A1 and COL1A2). In those individuals with mutations, the majority involves point mutations perturbing a glycine codon. Other mutations reflect deletions or insertions giving rise to frameshift, splicing error or the generation of a stop codon. All attempts at correlating location and type of amino acid substitution with disease severity have been unable to delineate a precise model for distinguishing clinical phenotypes on the basis of mutations. Thus the classification of OI remains a clinical one. The most widely used was proposed by Silence. Four types are distinguished: mild (I), lethal (II), progressively deforming (III), with a fourth (IV) group made of all the patients who do not correspond to the first three types. It is thus by definition heterogeneous, and within that group three new forms have been recently identified, a type V with distinctive hypertrophic callus formation and calcification of the interosseous membrane of the forearm, a type VI with evidence of a matrix mineralization defect in the absence of osteomalacia/rickets, and a type VII with rhizomelia and recessive inheritance. In the latter form, linkage studies in a large pedigree have positioned the disease locus on chromosome 3p22-24.1, a region in which no collagen or other bone protein gene has yet been described. Bone histomorphometric studies of the various forms of OI have helped to establish that bone fragility stems from: decreased bone mass, disturbed tissue organization and altered bone geometry (size and shape). Except for type V, increased turnover rate is the rule in OI, a probable consequence of the repair activity needed to replace weak tissue. Osteoblast productivity is inherently decreased inducing an increase in activation frequency. In addition to these basic abnormalities, disuse bone loss often further compounds the decrease in bone mass. At a time when gene-based therapy is still a remote perspective, the observed bone abnormalities provide justification for the use of bisphosphonates in order to reduce osteoclast mediated bone resorption. Cyclical intravenous pamidronate administration reduces bone pain, and increases both bone size and density. Except for the well documented acute phase reaction at first exposure to the drug, no negative effects on growth, modeling or fracture repair have been observed. Targeted decreased resorption during modeling of cortical bone allows for an increase in cortical width. These effects clinically translate into a decrease in fracture incidence and an increase in the level of ambulation. Bone changes also allow for more efficacious corrective surgery, and better occupational and physiotherapy programs. However long-term bisphosphonates therapy will suppress bone turnover to levels lower than those in healthy children, and the consequence of chronically low bone turnover in children with OI are unknown at the present time.

GENETICALLY MODIFIED ANIMAL MODELS AS A TOOL FOR STUDYING BONE AND MINERAL METABOLISM

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Genetic modification of animals, particularly mice, is an extremely powerful experimental tool which is beginning to contribute substantially to our understanding of mineral metabolism and the biology of bone. The task of identifying all genes in the human or mouse genome, substantial though it is, remains but a first step in our understanding of the function of these genes. One powerful mechanism of understanding a gene under investigation is to modify the expression or structure of the protein in an animal model and measure the effect of this alteration on changes in bone structure, function or metabolism. It is clear from reviewing the literature that the number of genetically modified animals in this field is growing extremely rapidly making this review of necessity somewhat selective.

Construction of Genetically Modified Animal Models

There are four basic approaches to genetic modification in animals.

1. Introducing a transgene with its own regulatory control regions to cause over-expression of the desired protein.
2. Deletion of genetic sequences to inactivate the function of a target gene.
3. Introduction of modified sequences to modify gene function.
4. Combinations of the above.

Over-expression of target genes was the first approach used in the production of transgenic animals. Target genes use one of hundreds of promoters now available which can either be constructed or obtained off the shelf. These promoters may provide tissue specificity, time specificity or the ability to be regulated. The target gene is usually introduced into the construct as modified cDNA. The presence of one or more introns are used to improve levels of expression.

A number of examples of over-expressing transgenic mice have been generated in the bone field. The tartrate-resistant acid phosphatase (TRAP) promoter has been used to produce transgenic mouse lines that overexpress *c-fos*. This over-expression resulted in a phenotype of increased osteoclast number and activity that resembled a number of features consistent with Paget's disease. The 2.3kb type 1a1 collagen promoter has been used to over-express the transcription factor *Cfba1* (*runx2*) specifically in osteoblasts resulting in severe osteopenia and multiple fractures. The osteocalcin promoter has been used in a number of studies to over-express target genes in mature mineralising osteoblasts. These include insulin-like growth factor I (IGF I) and human osteoblast stimulating factor-1 (OSF-1). In the growth plate, the collagen II promoter has been widely used to drive the expression of transgenes in proliferating chondrocytes, for example causing over-expression of *cbfa1*.

The strength of over-expression experiments is that with good design, appropriate attention to experimental models and some degree of luck, high levels of expression can be obtained and marked effects on animal physiology can be studied. The disadvantage of this approach is its unpredictability. The target DNA is randomly inserted into the genome. Regulation can be unpredictable and occasionally effects are caused by the disruption of endogenous genes rather than by the expression of the introduced gene. These transgenic animal models are relatively easy to produce and relatively inexpensive. They have contributed substantially to our understanding of bone and mineral metabolism.

Knockout Animal Models

The introduction of embryonic stem cells has enabled targeted deletion of genes, exons or smaller target sequences within genes to be investigated. Deletion may involve insertion of non-functional sequences to disrupt protein expression or function. These knockout models are more predictable and reproducible than the over-expression models. Universal knockouts have been used to identify important functions for many genes, including roles for PTHrP and *Ihh* in chondrocyte development and the estrogen receptors in bone. However, the surprise in using these models has sometimes been the lack of an obvious phenotype after the deletion of a supposedly essential gene. For example, mutations in FGF receptor family members cause skeletal dysplasias, and FGF2 is abundantly

expressed in chondrocytes; however, FGF2-null mice have normal bone development. It is often necessary to delete two or three genes simultaneously to disrupt a particularly important pathway with multiple levels of redundancy. The power of these models is that they can uncover unexpected actions of the target gene. For example global deletion of the gene that encodes calcitonin, a potent inhibitor of bone resorption and calcitonin gene-related peptide resulted in a surprising phenotype with knockout mice having greater trabecular bone volume and increased bone formation compared to controls. Furthermore, the knockout mice were able to maintain bone mass following ovariectomy. Knockout models are significantly more difficult to produce than the over-expressing transgenic animals and require particular expertise in handling the stem cells.

Insertion of Modified Target Sequences

The ability to change a single base, an exon or a gene or a portion of a gene has allowed this field to expand very significantly. Using homologous recombination and targeted insertion sequences can be removed from the endogenous gene and replaced with sequences of any design. This has allowed the study of point mutations and other subtle changes in gene activity, such as knock-in of activating mutations in the FGFR3.

Homologous recombination technology has also allowed the introduction of small functional genetic sequences. One example of this type of approach is the use of the Cre-lox system. Insertion of 32 base pair sequences flanking target DNA can be performed using standard gene targeting technology. These sequences are then targets for the enzyme Cre which removes the DNA between the lox sites and re-ligates the target DNA. Thus deletion of particular gene sequence is dependant on expression of Cre. Cre expression can be regulated in tissue and time specific ways as described in Section 1. The Cre-lox model has been successfully used to specifically delete the type I IGF receptor in mature osteoblasts using Cre expression driven by the osteocalcin promoter. Examples of studies focussing on cartilage-specific gene functions include chondrocyte-specific knockout of the Sox9 and PTH/PTHrP receptor genes using collagen II to drive Cre expression. The targeted regulation of genetic function will revolutionise our approach to the understanding of physiology of known genes. Time and energy is required to produce such Cre-lox hybrid systems and the amount of effort should not be underestimated.

Genetic targets within the bone and mineral field are ubiquitous. These targets genes can be described as follows:

1. Structural genes within bone and cartilage cells.
2. Regulatory genes within bone.
3. Widely expressed regulatory genes.
4. Genes encoding hormones or other distant regulatory molecules.

For those involved in this type of experimental biology the field is extensive, expanding and intellectually challenging. The responses from those watching from outside can include bewilderment, concern about the lack of appropriate controls, and worry about the length of time any experiment can take to come to its conclusion. There is no doubt that there is significant scope for improving the level of rigour within this relatively new experimental field. Control of animal breeding, use of appropriate control lines, and reproducibility remain issues to be resolved in this fast moving field.

IMMUNE SYSTEM AND BONE

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In determining the fundamental mechanisms of bone formation and resorption, most attention has been paid to the role of 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 and GM-CSF act directly upon osteoclast precursors to inhibit osteoclast formation, whilst IL-6, IL-7 and IL-17 act to stimulate this process.

The primary role of activated T lymphocytes in inflammatory diseases such as arthritis to influence bone destruction through their production of soluble RANKL has recently been demonstrated. Such a mechanism is also likely in the pathology of periodontal disease.

The capacity of naïve T cells to influence bone remodeling is now emerging. Using genetically altered mice, in which targeted cellular populations can be specifically addressed *ex vivo*, we have demonstrated that IL-12 and IL-18 can inhibit osteoclast formation as a result of their actions upon T cells. Histomorphometric analyses of IL-12, IL-18, and IL-12+IL-18 null mice reveal that these interleukins do indeed perturb normal bone architecture. Further, the actions of IL-18 were not restricted to impacting upon osteoclast formation, but also modulate osteoblast function.

More recently we have extended our analyses of factors acting through T cells to identify that IL-4 can inhibit osteoclast formation directly or as a consequence of action through T cells. This latter activity has been unnoticed possibly due to the direct action of IL-4 upon osteoclast formation.

Future studies are aimed to decipher the complex and overlapping effects of the many immune and hemopoietic cytokines, as well as determining the role that immune cells play in normal bone remodelling and in pathological conditions.

**HIV/AIDS AND BONE
P Ross (USA)**

BREAST CANCER: BAD TO THE BONE!

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Breast cancer shows an amazing predilection for metastasis to bone. Interestingly, approximately 80% of patients with breast cancer also develop bone metastases at some point during the course of their disease. The osteolysis that accompanies bone metastasis is a major cause of morbidity, often causing severe intractable bone pain, susceptibility to fractures, hypercalcemia and nerve compression syndromes, resulting in significant decline in the quality of life of these patients. An overwhelming majority of evidence in the literature demonstrates that the primary mechanism responsible for bone destruction in metastatic breast cancer is due to stimulation of osteoclastic bone resorption. Breast cancer cells produce many known stimulators of bone resorption with significant research effort focused on the role of parathyroid hormone related protein (PTHrP).

Although there are other cross sectional studies showing that PTHrP positive breast cancers are more likely to develop bone metastasis (Bundred *et al.* 1992); (Grill *et al.* 1991), the question remains somewhat controversial. A recent (and to date the only) prospective clinical study has questioned the primary role of PTHrP in this process (Henderson *et al.* 2001). The Henderson study suggested that women with PTHrP-positive primary breast cancers have a more favorable outcome, with fewer bone metastases. These important observations question the proposed role of PTHrP in defining metastatic potential, and implicate factors other than PTHrP in the process of bone metastasis. These observations highlight the importance of understanding the mechanism(s) by which these breast cancer cells grow as tumors in bone. We have been exploring the hypothesis that tumor-derived paracrine factors, distinct from PTHrP, mediate the local increase in osteolytic bone destruction that is the hallmark of breast cancer metastasis to bone.

Using *in vivo* selection (Fidler 1990) to identify populations of human breast cancer cells with an enhanced osteolytic phenotype *in vivo*, we have developed an excellent cell model of osteolytic breast cancer, MDA-MET cells (Bendre *et al.* 2002). As in the human disease, PTHrP expression in MDA-MET cells does not appear to correlate with bone destructive potential.

To identify differences in gene expression between MDA-MET and the parental MDA-231 cells we compared the gene expression profile in both cell lines using microarrays (Bendre *et al.* 2002). Using a functional annotation approach to determine the significance and relevance of these differentially expressed genes in MDA-MET versus MDA-231 breast cancer cells focused attention on categories of potentially important genes. Interleukin 8 (IL-8), heparanase and syndecan-1 were identified as differentially regulated. Based on the functional annotation approach, IL-8 was expressed in the most categories and considered the most likely candidate to be associated with the aggressive osteolytic phenotype of MDA-MET cells. The increased expression of IL-8 by MDA-MET was confirmed by both RT-PCR and ELISA.

The marked over expression of IL-8 by bone-seeking breast cancer cells was intriguing and prompted further investigation of the role of IL-8. Our initial hypothesis was that IL-8 regulates the expression of RANKL, the factor absolutely required for osteoclast differentiation. This idea was tested *in vitro*. IL-8 treatment (10 ng/ml) markedly induced the expression of RANKL mRNA and cell associated protein expression in MC3T3-E1 cells, with no change in levels of OPG mRNA. IL-8 was able to stimulate human osteoclast precursors, derived from peripheral blood mononuclear cells in the presence of M-CSF, to become bone resorbing TRAP positive multinucleated osteoclasts, expressing alpha v beta 3 integrin, in the absence of exogenous RANKL. In addition, IL-8 was also able to directly stimulate the formation of TRAP positive multinucleated osteoclasts in the presence of neutralizing concentrations (200ng/ml) of RANK-Fc. The direct effect of IL-8 on osteoclast precursors was associated with the expression of the IL-8 specific receptor CXCR1 on the surface of osteoclasts and their precursors.

These and other emerging data suggest that the existing dogma regarding the role of PTHrP in bone metastasis needs to be refined. It now appears that PTHrP may play a role later in the metastatic cascade. The ability of IL-8 to stimulate osteoclastogenesis via both RANKL-dependent and independent mechanisms suggests an important role in the process of osteoclast formation and function. Ideally, this information will provide a means to rationally design new therapeutic approaches to attenuate the ectopic expression of IL-8 by tumor cells that may provide novel opportunities to control tumor progression. For the moment, it appears more likely than not that the osteotropism of human breast cancer is not just about PTHrP.

BONE GROWTH TRAJECTORY IN YOUTH: ORIGIN OF FRAGILITY FRACTURES IN ADULT LIFE

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The mechanical strength of bone depends of several structural elements, which follow distinct development trajectories from the origin of intrauterine life to the end of the skeletal growth process, i.e. when peak bone mass is attained by the end of the second decade. The size of the bone, the amount of bony tissue within the periosteal envelope and its space distribution, i.e. the micro –and macro-architecture, and the degree of mineralization of the organic matrix appear to be the most important structural elements, which determine the resistance to mechanical loading. During development the increase in areal bone mineral density (aBMD) is essentially due to a rise in bone size, which results in a proportional augmentation in the amount of mineralised tissue within the periosteal envelope. In contrast, the volumetric (v) BMD increases very little from infancy to the end of the growth period. In either gender the main bone strength determinants follow a trajectory similar to that recorded for standing height. In healthy girls longitudinal examination at 4.5 years interval, before and during pubertal maturation, of lumbar spine development shows that the Z-scores of aBMD, BMC, vBMD, as estimated by calculating BMAD, as well as vertebral body width and height are highly correlated, with correlation coefficients ranging from 0.70 to 0.82, as compared to 0.85 for standing height. In the lumbar spine, the gender difference observed when peak bone mass is attained consists mainly in a greater vertebral body diameter in the frontal plane of males as compared to females. This gender related structural differentiation, which does not attenuate with ageing, is certainly an important macro-architectural determinant of the difference in fragility vertebral fracture incidence observed between female and male subjects in later life. It probably also plays an important role in the vertebral fracture risk within each gender, as demonstrated in postmenopausal women with equally low trabecular vBMD, those with vertebral fracture displaying a smaller vertebral body cross-sectional area. The gender difference in either aBMD or BMC observed at the level of the radial or femoral diaphysis once peak bone mass is attained appears to be also essentially due to a greater gain in bone size in males than females during pubertal maturation. The large interindividual variability in peak bone mass of healthy young adults, which is several times larger than the variability in standing height, is the outcome of the broad range of the trajectories followed by the various bone components during development. Peak bone mass is considered as important as postmenopausal or age-dependent bone loss in the risk of fragility fractures. Information from epidemiological studies suggest that an increase by 10 %, i.e. an increase by about one standard deviation (SD) in peak bone mass would reduce the fracture risk by 50 %. This quantitative relationship prompted the interest for exploring ways to influence positively the trajectory of bone development. Prospective randomised controlled trials suggest that increasing the calcium intake or enhancing mechanical loading could shift the age-bone mineral mass trajectory upward. Prepuberty appears to be an opportune time to obtain substantial benefit from increasing either calcium intake or physical activity. Further studies should demonstrate that the changes observed remain substantial at the end of the second decade and thus translate into a greater peak bone mass. In this long-term evaluation of the consequence of modifying the environment, it will be of critical importance to assess whether any positive change in densitometric or morphometric bone variables observed when peak bone mass is attained will confer a greater resistance to mechanical loading. Finally, any influence of nutritional or mechanical factors on bone mineral mass accrual will have to consider their possible effect on the timing of pubertal maturation, which in turn can affect the risk of adult osteoporosis.

FRAGILITIES WEAPON - ESTROGEN DEFICIENCY
-ESTROGEN DEFICIENCY IS THE PRIMARY CAUSE OF BONE LOSS IN BOTH AGING WOMEN
AND CONTRIBUTES SUBSTANTIALLY TO BONE LOSS IN AGING MEN-
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Women undergo two phases of bone loss – an early accelerated phase and a late slow phase – but men undergo only the late slow phase. The early accelerated phase begins at menopause, can be prevented by estrogen replacement, and clearly is caused by estrogen (E) deficiency. This phase is thought to be the result of loss of suppressive action of E on bone turnover acting on bone cells.

The late phase of slow bone loss involves equivalent amount of cancellous and cortical bone and continues indefinitely. In contrast to the early phase in which serum PTH is partially suppressed, this phase is associated with progressive increases in serum PTH. Recent studies, however, have shown that the increased bone resorption and secondary hyperparathyroidism can be normalized by either large dosages of calcium supplements or by E replacement. There now is convincing evidence that E acts directly on the gut to increase calcium absorption and on renal tubular function to enhance renal calcium conservation. The loss of these functions would lead to external calcium wasting, secondary hyperparathyroidism, and, unless there are huge intakes of dietary calcium, to bone loss.

Because elderly men have low circulating levels of bioavailable testosterone and E and because recent observational and interventional studies have established that E is more important than testosterone in maintaining bone mass in men, a relative E deficiency appears to contribute to involutional bone loss in aging men. In population studies from Rochester, MN, our group has shown that bone loss and markers of bone resorption do not increase in men until a critical threshold of below ~30 pg/ml serum total E. This level is reached by about half of men by age 70, but all postmenopausal women are below this level.

Thus, E deficiency is the main determinant of age-related bone loss in both women and men and combined deficiency with testosterone produces the greatest degree of bone loss.

WHO TO TREAT

John A Kanis

The aim of treatment of osteoporosis is to prevent fractures, but in whom is treatment worthwhile? At the one extreme, all individuals might be treated, but at a high financial cost. Moreover, it is unacceptable to expose individuals to treatments who will never fracture since all treatments carry risks as well as benefits. At the other extreme, treatment might be given only to individuals at very high risk, for example, elderly women with established osteoporosis. This strategy is cost-effective, but has a low impact on the societal burden of fracture, since it neglects the many individuals who will fracture in the future. A middle course between these extremes is required, but demands the optimisation of the methodology to predict fracture so that interventions are directed to those most at need and to avoid unnecessary treatment. Since the determinants of osteoporotic fracture are multifactorial, risk prediction will always be imperfect. To date, assessment of bone mineral density (BMD) has been the corner stone for the diagnosis of osteoporosis and the WHO definition of osteoporosis is widely accepted as an intervention threshold for drug development and in practice guidelines. The recommended diagnostic test is BMD at the proximal femur by dual energy X-ray absorptiometry and the same BMD threshold for osteoporosis for diagnosis (a T-score for BMD of -2.5 SD or less in young health women) can be used in both men and women.

Although hip fracture prediction with BMD alone is at least as good as blood pressure readings to predict stroke. Like blood pressure tests, the test has high specificity, but its sensitivity (detection rate) for fracture outcome is low over most reasonable assumptions. When the WHO thresholds are used, the majority of fractures will occur in those individuals characterised to be at low risk. The predictive value of BMD can be enhanced by the use of other factors such as biochemical indices of bone resorption and clinical risk factors. Clinical risk factors that contribute to fracture risk independently of BMD include age, previous fragility fracture, premature menopause, a family history of hip fracture neuromuscular incompetence and the prolonged use of corticosteroids. The presence of such factors increases fracture risk over and above that which can be explained on the basis of BMD. Therefore, diagnostic thresholds differ from intervention thresholds. Intervention thresholds should be based on the absolute risk of fracture. In the absence of validated population screening strategies, a case finding strategy is recommended based on the finding of risk factors and the computation of fracture probability.

Because of the many techniques available for fracture risk assessment, the ten year probability of fracture is the desirable parameter to determine intervention thresholds. The setting of intervention thresholds is ultimately dependent on health economic considerations. When BMD is used as a test alone, an intervention threshold of -2.5 SD is cost-effective. In the presence of other independent risk factors less stringent criteria are appropriate so that intervention can be directed to individuals where hip fracture probability ranges from 2% to 10% (depending on age). These thresholds, derived from Sweden, require modification in different countries to take account of different costs and risks that vary markedly in different regions of the world.

PTH – ADVANCES IN TREATMENT**P.D. Delmas, INSERM Research Unit 403 and Claude Bernard University of Lyon, Lyon, France**

Three clinical data obtained in a variety of species indicate that daily injection of parathyroid hormone has a major anabolic effect on bone, leading to a marked improvement of skeletal strength. This beneficial effect has been now confirmed in clinical trial.

In a controlled clinical trial, teriparatide injection (rDNA origin) [teriparatide; parathyroid hormone (1-34) (PTH)], was shown to significantly reduce both vertebral and nonvertebral fragility fractures by 65-69% and 53-54% respectively, in women with one or more vertebral fractures (median 19 month teriparatide exposure). Teriparatide was also associated with statistically significant increases in bone mineral density in the lumbar spine (9.7-13.7%) and total hip (2.6-3.6%), and total body bone mineral content (1.3-2.3%). Of the original study population, 77% or 1262 women volunteered for an 18-month observational study to determine the effects of cessation of PTH treatment on recurring vertebral fractures. All women remained on calcium (1000mg/day) and 400-1200IU/day of vitamin D. After discontinuation of PTH, investigators were allowed to put women on other approved osteoporosis treatments (54% of patients). Because the use of treatments was balanced across groups, the analyses were performed without adjusting for use, type, or duration. Vertebral fracture incidence was assessed from lateral spinal radiographs in 1043 women with original study endpoint and 18 month follow-up radiographs. During the observational study, the risk of a new vertebral fracture was significantly higher for women from the original placebo group compared to women from the original pooled PTH treatment groups [19.5% vs. 11.2% ($P < 0.001$)], consistent with previous observations. The incidence of new or worsened back pain was also higher for women from the placebo group compared to the rhPTH(1-34) treatment [19.3% vs. 12.9% ($P = 0.002$)]. In the original placebo group, the risk of a new vertebral fracture during the observation period was significantly higher for women who had fractured during the treatment period than for those who had not fractured [44.7% vs. 15.8% ($p < 0.001$, Fisher's exact test)]. In contrast, in the original pooled PTH group, the incidence of new fractures during the observation period was similar for women who had and had not fractured during the treatment period [13.3% vs. 11.3% ($P = 0.453$)]. The odds ratio for fracture during the observation period for women who did and did not fracture during treatment was significantly different between treatment groups ($P = 0.043$, Breslow-Day). During the 18-month follow-up period, new nonvertebral fractures were reported in 99 women. Of these new fractures, 49 women were determined to have had nonvertebral fragility fractures (3.9%): placebo, $n = 23$ (5.6%); PTH20, $n = 14$ (3.2%); PTH40, $n = 12$ (2.9%). Although each teriparatide group was not significantly different from placebo ($P = 0.094$ and $P = 0.059$ for PTH20 and PTH40, respectively), the difference in fractures was significant when the teriparatide-treated patients were combined versus placebo ($P = 0.032$). From baseline of the original study through the 18-month follow-up visit (median 39 months), 89 women reported fractures (7.1%): placebo, $n = 44$ (10.6%); PTH20, $n = 24$ (5.5%); PTH40, $n = 21$, 5.1%. This was statistically significant for each dose compared with placebo (PTH20, $P = 0.006$; PTH40, $P = 0.003$). A Kaplan-Meier analysis of time from the original baseline to first nonvertebral fragility fracture demonstrated the persistence of a statistically significant teriparatide effect, relative risk 0.52 and 0.48 for PTH20 and PTH40, respectively ($P < 0.01$). In summary, the positive effects of teriparatide injection (rDNA origin) in reducing the cumulative proportion of women with vertebral and nonvertebral fragility fractures compared with placebo continued to be significant 18 months after discontinuation of treatment.

A study recently published performed in osteoporotic men treated with the same compound suggest that teriparatide has similar effect on the skeleton as what has been found in women.

In conclusion, teriparatide is an attractive alternative for the treatment of patients with severe osteoporosis, and opens new possibility for sequential therapies with antiresorptive treatments.

MECHANISM(S) OF PTH ACTIONS IN BONE: A SYSTEMS BIOLOGY APPROACH
J Onyia (USA)

MECHANISM OF ACTION OF STRONTIUM RANELATE ON BONE: PRECLINICAL STUDIES

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Promising *in vivo* data obtained in rats and monkeys led to clinical trials of strontium ranelate (SR) in osteoporosis, revealing it as a novel approach to fracture prevention. Several studies in rats showed that treatment with SR improves bone resistance and is effective in preventing the trabecular bone loss of estrogen deficiency, as well as reducing the ovariectomy (OVX)-induced increase in osteoclast number and other histomorphometric parameters of increased bone resorption. Further features of these studies were that SR treatment resulted in biochemical and static indices of bone formation remaining unchanged in OVX animals, and that no toxic effects or mineralisation defects were observed at any dose of the drug. In monkeys also, SR treatment for 6 months resulted in dose-dependent decreases in histomorphometric indices of bone resorption while indices of bone formation were maintained. The conclusion from the *in vivo* preclinical data was that SR offered an approach to resorption inhibition that could be achieved while maintaining bone formation, thus leading to improvement of bone resistance. With the aim of achieving a better understanding of these effects, studies were carried out to assess the distribution of strontium (Sr) in bone after treatment of monkeys. These showed that Sr was dose-dependently incorporated into the mineral substance of compact and cancellous bone, mainly by ionic exchange on the hydroxyapatite surface, and in each case being 3- to 4-fold higher in new than in old bone. *In vitro* studies have yet to provide further insights into the cellular mechanisms of action of SR on bone. Stimulation by SR of the replication of pre-osteoblastic cells and collagen synthesis in rat calvaria culture and in isolated cells culture has been observed at 1 mM. *In vitro* experiments showed that strontium ranelate reduced the osteoclastic differentiation in a chicken bone marrow macrophage model. These results were not confirmed in other differentiation models and therefore, further studies are required. On the other hand, treatment of freshly isolated or *in vitro*-generated osteoclasts with Sr (0.1 to 24 mM) resulted in up to 60% inhibition of resorption. Taken together with the studies of Sr distribution in monkey bone, such inhibition of osteoclast activity might contribute to the observed *in vivo* inhibition of resorption. The cellular mechanisms of action of SR warrant further investigation.

STRONTIUM RANELATE: REVIEW OF THE EFFICACY AND SAFETY OF A NEW AGENT FOR PREVENTION OF SPINE AND NONSPINE FRACTURES IN OSTEOPOROSIS.

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Strontium Ranelate (SR) reduces bone resorption and, in *in vitro* and animal studies, there is evidence of increased bone formation. Two randomised, double-blind, placebo controlled, phase 3, multicentre trials were carried out in 75 centres in 12 countries to evaluate the anti-fracture (Fx) efficacy of this drug. The first study, SOTI, enrolled 1649 postmenopausal women, age 69.7(7.3) [mean (SD)] with lumbar BMD T-score: - 3.6 (1.2); 87.5% of patients had a prevalent vertebral fx (2.2 prevalent vertebral Fx/patient). Subjects were randomly assigned to oral SR 2 g/day or Placebo (P). All received daily calcium/vitamin D. Analysis was by intent-to-treat. In the first year, the number of women sustaining vertebral Fx was 44 (6.4%) SR v 85 (12.2%) Plac; RR [95% CI] = 0.51 [0.36 - 0.74], $P < 0.001$. Over 3 years, the figures were 139 (20.9%) SR v 222 (32.8%) Plac, RR = 0.59 [0.48 - 0.73], $p < 0.001$. Bone specific alkaline phosphatase increased and serum CTX decreased compared with controls. Lumbar BMD increased by +11.4% in SR group v -1.3% in P group, $p < 0.001$. There were no specific adverse events, particularly no upper gastro-intestinal events. In the second study, TROPOS, the anti-non-vertebral fracture efficacy study, enrolled 5091 women [age: 76.8 (5.0); femoral neck BMD T-score: -3.1 (0.6)]; 38.6% had at least one prevalent non-vertebral fracture. In the treated group, the reduction in the risk for a first non-vertebral fracture during the 3 years was 16% (RR=0.84 95% CI [0.71;1.00] $p \leq 0.05$) in the intent-to-treat population. A 41% ($p=0.025$) reduction in risk of hip fracture was observed in the per-protocol population. SR is a new effective and safe orally active drug which reduces the risk of vertebral and non-vertebral fractures in women with osteoporosis.

RISEDRONATE – ARCHITECTURAL CHANGES
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Risedronate is a third generation bisphosphonate that has been found in Phase III trials to reduce the risk of vertebral and non-vertebral fractures in women with postmenopausal osteoporosis treated for three years. The increase in bone mineral density only explains about 28% of the effect on fracture risk reduction (1). Furthermore, the reduction in fracture risk occurs within the first six months of therapy, long before the complete evolution of the increase in bone mineral density. The decrease in bone resorption markers is maximal within 3 months of starting therapy and the change in the bone resorption markers explain 66% of the fracture risk reduction (2). This decrease in bone resorption markers is reflected in the changes in bone turnover observed using bone histomorphometry on iliac crest biopsies from the above studies; there was a 47% decrease in activation frequency and a 58% decrease in mineralising surfaces (3). These biopsies have been examined by 3-D microCT and risedronate prevented the deterioration in connectivity over the period of the study (4). Experiments over 18 months in minipigs who had ovariectomy and then were treated with risedronate or placebo confirms that risedronate preserves the connectivity (marrow star volume and trabecular number) in the vertebra of these animals (5). Thus, risedronate reduces the risk of fracture by increasing bone mineral density and preserving the microarchitecture of bone.

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ALENDRONATE**Ian R Reid, Department of Medicine, University of Auckland**

While alendronate is the most widely used therapy for osteoporosis at the present time, it should be remembered that only 10 years ago it was medication still in phase 3 clinical trials. Those trials demonstrated that alendronate increased bone density throughout the skeleton to an extent comparable to HRT, then the most commonly used agent for managing osteoporosis. More importantly, they demonstrated prevention of vertebral fractures and reduced height loss in osteoporotic women taking alendronate. Following close behind the publication of the phase 3 trials was that of the Fracture Intervention Trial, which was truly a landmark in the therapeutics of osteoporosis since it demonstrated reduction in the incidence of each of the major classes of clinical fracture (vertebral, forearm and hip). Subsequent analyses demonstrated confirmed anti-fracture efficacy in osteoporotic women with or without baseline fractures, and indicated that they resulted in fewer days affected by disability and fewer days in bed. Thus, the myth that the management of osteoporosis had no impact on real-world events was exploded forever. The anti-fracture efficacy of alendronate has now been demonstrated in other studies and the trend towards fewer fractures is evident within a few months of the initiation of the therapy. This has raised interesting questions regarding the mechanism of action of bisphosphonates, and suggested that substantial increases in bone density are not a requirement for a reduction of fracture risk. Possibly the reduction of bone resorption produced by these drugs is itself a direct contributor to fracture prevention.

The positive effects of alendronate on bone density and its anti-fracture efficacy have now been demonstrated in a number of other contexts also. Bone loss is prevented in normal postmenopausal women with alendronate. Similar effects are seen in non-white women and in men. The osteoporosis resulting from use of glucocorticoid drugs is also responsive to alendronate and in these patients and in osteoporotic men, fracture prevention has been demonstrated.

Alendronate entered the marketplace without a detailed understanding of its mechanism of action. This is now available. Alendronate, like other bisphosphonates, is taken up with high avidity onto bone surfaces as a result of its strong negative charge and the strong positive charge of the bone surface. It is subsequently ingested by osteoclasts as they resorb bone. It blocks the enzyme farnesyl pyrophosphate synthase, a critical enzyme in the pathway leading to the synthesis of cholesterol. This action potentially gives it the capacity to be widely toxic, but its very specific targeting to bone surfaces, confers on it a very high specificity which results in a low incidence of side effects.

The binding of bisphosphonates to bone surfaces is so high that once deposited, they remain there almost indefinitely, and are incorporated into the crystal structure of newly forming bone. This gives it a very long duration of action and has opened up the possibility of using bisphosphonates at intervals much greater than daily dosing. It is now clearly established that alendronate given at weekly intervals has the same effects on markers of bone turnover and bone density as the same average dose given daily. It is likely that longer inter-dose intervals will also be effective.

The anti-resorptive actions of alendronate have proven useful in conditions other than osteoporosis. In Paget's disease it dramatically reduces biochemical evidence of disease activity, results in healing of lytic lesions in bone and its use is associated with the deposition of normal lamellar bone where previously the disorganized woven bone characteristic of Paget's disease was present.

Despite the large numbers of patients studied in clinical trials over periods of up to 10 years there are still significant gaps in our knowledge of alendronate. How long does the reduction in fracture numbers last? The 10 year follow up of the original phase 3 trial suggests that the number of fractures remains stably reduced over this period of time, though the relatively small number of individuals providing data to 10 years and the lack of a control group over the entire period makes this data suggestive rather than definitive. Because of the accumulation of alendronate in bone over time, can or should the dose be reduced or discontinued after a certain period of time? These are critical issues since there are now patients who have been receiving alendronate for more than 5 years and they and their physicians need to consider this matter each time a prescription is renewed. It is clear that when alendronate is discontinued, there is some increase in markers of bone resorption but even after several years off long-term alendronate, markers have not returned to baseline levels. Similarly, there is some decline in bone density, but this is rather slower than has been seen after the discontinuation of HRT.

Despite these remaining questions, alendronate has transformed the landscape of osteoporosis, has prevented fractures in many millions of patients and has forcefully made the point that skeletal decay does not have to be accepted as an inevitable consequence of growing older.

ESTROGEN THERAPY – A DEATH OF AN ICON?

C Christiansen

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Since the early 80's hormone replacement therapy (HRT) has been extensively used to prevent and treat postmenopausal osteoporosis based on its marked inhibitory effect on bone resorption. Numerous studies, inclusive the WHI trial, demonstrated the efficacy of this treatment in the prevention of osteoporotic fractures. However, the cardiovascular benefits of HRT has been less well understood. Several animal and observational studies indicated marked lipid-lowering and anti-inflammatory effects and improvement of endothelium-dependent responses yet the recent reports from the HERS and the WHI trials, failed to provide confirming evidence for this preventive effect. On the contrary, the results indicated a increased risk for coronary heart disease and stroke, especially in the early period of the treatment. In addition, there was a small, but significant increase in the incidence of breast cancer. These adverse effects could not be outbalanced by the favorable effects in terms of preventing osteoporotic fractures and colon cancer. These findings evoked a general confusion among scientist and practitioners that resulted in a more cautious consideration of this treatment, especially for preventive purposes. It is to be pointed out that several investigators raised concerns as to the adequacy of the study design and whether subjects truly represented the target population. Major concerns were the relatively advanced age and late initiation of HRT. Thus, it remains to be clarified whether early initiation of HRT could provide the expected beneficial effects. Another, important point to be addressed is the influence of gestagens with which estrogens are combined during HRT. Several observations indicate that some of the frequently used gestagens have counteracting effects on lipids and other effects. In addition, little is known about how lifestyle factors influence gestagen metabolism and how these interactions affect the overall efficacy of HRT. One treatment arm of the WHI trial that receives estrogen replacement therapy is expected to provide some clues in this regard.

This presentation intends to give an overview of the current status of the role of HRT and point out some of the remaining questions that require clarification before we totally give up on the hopes and expectations related to the importance of HRT in the prevention and treatment of menopause-related disease.

RALOXIFENE – NEW INSIGHTS INTO BONE, BREAST AND HEART

**Pierre D. DELMAS, M.D., Ph. D., Professor of Medicine, Claude Bernard University and Director,
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Selective oestrogen receptor modulators (SERMs) are a class of compounds that have been developed with the aim to retain the beneficial effects of hormone replacement therapy (HRT) without some of its side effects. Tamoxifen, which is widely used in the treatment of breast cancer, is the first compound of this class of agents. It has oestrogen agonist action on the skeleton and on lipoprotein metabolism in postmenopausal women. However, because its long term use is associated with an increased risk of endometrial cancer, tamoxifen cannot be used in healthy postmenopausal women. Raloxifene, a benzothiophene non steroidal molecule which has been extensively studied in phase II and III trials is now available widely. The results of a multicentric phase III placebo-controlled trial performed in 602 early postmenopausal women in 11 European centres showed that raloxifene, given orally at the daily dose of 30 mg, 60 mg or 150 mg, prevents early postmenopausal bone loss. This effect was accompanied by a dose related decrease in markers of bone turnover (urinary CTX for bone resorption, serum bone alkaline phosphatase and osteocalcin for bone formation) that fell within 3 to 6 months within the premenopausal range. Serum total and LDL-cholesterol decreased significantly under raloxifene, while HDL-cholesterol and plasma triglycerides did not change significantly. The treatment was well tolerated and adverse event incidence did not differ between groups, including breast pain and vaginal bleeding. Endometrial thickness assessed every 6 months by transvaginal ultrasonography did not change in either group throughout the study. Results from the MORE study, performed in 7 500 osteoporotic women, indicates a marked reduction (-30% to -50%) in vertebral fracture rate with raloxifene 60 or 120 mg/day for 3 years both in patients with and without prevalent vertebral fractures. Thus, in patients with osteoporosis based on their bone mineral density measurement, raloxifene 60 mg/d reduces by 50% the risk of the first vertebral fracture. So far, no significant reduction of non vertebral fractures has been demonstrated except in a subgroup of patients at high risk characterized by severe vertebral fractures at baseline. The MORE study has been extended into a double blind, placebo controlled design where the investigator is allowed to prescribe another antiresorptive agent (except HRT). The preliminary analysis after the first year of extension, i.e. after 4 years of raloxifene treatment, indicates that the reduction of vertebral fractures is maintained. The year by year analysis indicates a marked and significant reduction (more than 60%) at the first year of treatment, and a significant reduction during the 4th year alone, demonstrating an early and sustained efficacy of raloxifene on vertebral fractures. The increase in spine bone mineral density is modest at 3 years and triggers new hypothesis for the mechanism responsible for fracture reduction that will be discussed. That study has also provided valuable information on the safety profile of the drug : the only serious adverse event is an increase of venous thrombo embolism (comparable to that reported with hormone replacement therapy), and the rate of new breast cancer was decreased by 60% during 4 years of therapy. Raloxifene has no effect on cardiovascular morbidity and mortality in the overall population of osteoporotic women but appears to decrease it in women at high risk of cardiovascular disease; an interesting observation that will be tested prospectively in the RUTH study. In summary, raloxifene represent an interesting alternative for the prevention and for the treatment of postmenopausal osteoporosis. The ability of Raloxifene to decrease the risk of breast cancer has been confirmed in the 4th year of the trial, (63% reduction, 84% decrease of estrogen receptor positive cases). Balancing the effects of Raloxifene on major health outcomes in postmenopausal women allows to better define its role in the management of postmenopausal women.

PAGET'S DISEASE OF BONE: CAN WE IMPROVE LONGTERM OUTCOMES?

M Hooper

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Paget's Disease of Bone is the second most common metabolic bone disease. The availability of potent bisphosphonates and realization that complications are more likely to progress without effective treatment has changed treatment philosophy.

In our Paget's Clinic of over 500 patients our strategy is to treat continuously, those with active disease involving strategic sites, irrespective of symptoms or biochemical indices, with the goal of both normalisation of biochemical indices and normalisation or marked reduction of scintographic activity. Our experience has shown that this approach is effective and safe.

In 150 consecutive patients treated with alendronate continuously the alkaline phosphatase was normal in 31% at baseline, in 93% at 6 months, 92% at 12 months, 98% at 18 months and 97% at 24 months (mean pretreatment value 278 ± 324 , range 64 - 2179 U/l). Persistent scintographic activity remained in many patients after more than 18-24 months of continuous alendronate despite normalisation of biochemistry. In 28% of patients who had evaluable scans before and after 12 months of continuous therapy normalisation of scintogram occurred. Prior pamidronate-resistance did not influence the response. Normalisation of disease activity was followed by prolonged remission. Secondary hyperparathyroidism was observed in a third of patients. No evidence of osteomalacia in Pagetic or normal bone was seen.

Our experience is that the potent bisphosphonates are highly effective in the goal of normalisation of disease activity. The PRISM trial of intensive versus symptomatic treatment may confirm that this approach to treatment will improve long-term clinical outcome.

PANCREAS – A NEW PLAYER IN BONE PHYSIOLOGY
J Cornish, Department of Medicine, University of Auckland, New Zealand

Until 10 years ago the pancreas was not known to be involved in bone physiology, other than as a source of insulin. However, in the last few years a number of new hormones that have been isolated from the secretory granules of the beta pancreatic cells have been found to be active on bone cells. These hormones include, amylin, adrenomedullin and preptin, and they circulate at picomolar concentrations or greater. The hormones from the pancreatic beta cell are hypersecreted in obesity and could partly explain the higher bone mass found in this condition. Body weight impacts on both bone turnover and bone density and is therefore a prominent risk factor for vertebral and hip fractures and, in many studies, has a greater impact than age. The effect of body weight is likely to be contributed to by both fat mass and lean mass although fat mass has been more consistently demonstrated to be important, especially in postmenopausal women. Insulin is a potential regulator of osteoblast growth as these cells have insulin receptors as well as IGF-1 receptors to mediate insulin's effects. Insulin stimulates proliferation of osteoblasts in vitro and increases indices of bone formation when administered locally over bone in vivo. Circulating insulin levels have been found to be directly related to BMD. The hyperinsulinemia of obesity arises from resistance to the hypoglycaemic effects of insulin, the extent of this resistance being directly related to circulating insulin levels. On the other hand, insulin sensitivity has been found to have an inverse relationship to BMD and bone density tends to be reduced in insulin deficiency. In hyperinsulinemic patients there is androgen and oestrogen overproduction in the ovary and reduced production of sex hormone-binding globulin in the liver. These phenomena result in increased free concentrations of sex hormones, resulting in reduced osteoclast activity and possibly increased osteoblast activity, leading to increased bone mass. These indirect mechanisms complement the direct effects of insulin on the osteoblast.

Amylin, a 37-amino acid peptide hormone is co-secreted with insulin from the beta pancreatic cell and has evolutionary links to insulin. Amylin directly stimulates osteoblast proliferation in vitro and in vivo, as well as having a calcitonin-like action on the osteoclast. Thus, its daily systemic administration over a four-week period has been associated with a 70% increase in trabecular bone volume of the tibia in adult mice.

Adrenomedullin, a potent vasodilator, also belongs to the amylin-calcitonin family of molecules. It is a 52-amino acid peptide, which is produced in several tissues including adrenal medulla, vascular beds and pancreatic islets. Like amylin, adrenomedullin is a peptide hormone that directly stimulates osteoblast proliferation in vitro and in vivo. When the adrenomedullin fragment (27-52) was administered systemically to adult mice, the adrenomedullin-treated animals had a 50% increase in trabecular bone volume of the tibia. The putative receptors for adrenomedullin are present on osteoblasts and beta pancreatic cells.

A further recently discovered beta cell hormone, preptin, is a 34-amino acid peptide hormone also isolated from the secretory granules. Interestingly preptin corresponds to Asp⁶⁹ - Leu¹⁰² of proIGF-II. Preptin, like the other beta-cell hormones, dose-dependently stimulates the proliferation of primary fetal rat osteoblasts and osteoblast-like cell lines at periphsiological concentrations ($>10^{-11}$ M). When preptin was administered to adult mice by unilateral local hemicalvarial injection, there were increases in dynamic histomorphometric indices of bone formation and bone area. These findings may be clinically relevant to the rare condition of high bone mass associated with chronic hepatitis C, in which the patients secrete high levels of proIGF-II.

The mitogenic effect of these peptide hormones on osteoblasts appears to be dependent upon signalling via p42/44 MAP kinases, inducing dose-dependent phosphorylation, as assessed by immunoblotting. The proliferative effects of the peptides on primary osteoblasts were blocked when the cells were pre-treated with either of the MAP kinase inhibitors PD-98059 or U-0126. In addition, these bone-active factors protect primary osteoblast from apoptosis induced by serum deprivation, as assessed using a modified TUNEL assay.

Amylin, adrenomedullin, preptin are all known to be involved in the modulation of pancreatic function and glucose metabolism, and acute hyperinsulinemia has been shown to be associated with increased circulating levels of these hormones in patients with type 2 diabetes mellitus. The association of fat mass with the secretion of bone-active hormones from the pancreatic beta cell, likely acting in concert, are complemented with the secretion of bone-active hormones from the adipocyte. Clearly, the functions of these two cells are closely linked because there is evidence that leptin, adiponectin, and resistin all have substantial (probably indirect) effects on insulin secretion. These hormones that regulate nutritional status and also impact on bone metabolism, will be an important area of research in the coming years and should give us a much greater understanding of the normal regulation of bone mass, potentially leading to novel mechanisms for its therapeutic manipulation.

IDIOPATHIC HYPERPHOSPHATASIA – GENOTYPE & PHENOTYPE**Tim Cundy University of Auckland, New Zealand-**

Idiopathic Hyperphosphatasia (IH) is a rare autosomal recessive bone disease (MIM 239000). Affected children are normal at birth, but develop progressive long bone deformities, fractures, vertebral collapse, short stature, skull enlargement and deafness, with striking radiological changes. However, considerable phenotypic variation exists: some patients present in infancy, others are not recognized until later childhood. The earlier the diagnosis is made, the more severe the phenotype. Deformity can progress rapidly during the pubertal growth spurt, so even 'mild' phenotypes can end up with severe deformity, shortening life expectancy. Both plasma alkaline phosphatase activity and the excretion of type I collagen breakdown products are very high –indicating that IH is a disorder of dysregulated bone turnover. Two recent publications have implicated deletion or mutation of the gene *TNFRSF11B* in the aetiology of IH. *TNFRSF11B* encodes osteoprotegerin (OPG) an osteoblast-derived peptide that binds to RANK-L, inhibiting the RANK-L/RANK interaction that stimulates osteoclastogenesis. Deficient, or inactive OPG would allow unfettered osteoclastogenesis, and very high bone turnover would result.

Through the International Hyperphosphatasia Collaborative Group our group has identified 11 subjects with a clinical diagnosis of IH, from 9 families. In 8 subjects from 6 families we have identified homozygous mutations in *TNFRSF11B*, and been able to relate the mutations to the putative effect on OPG, and to phenotype. The *TNFRSF11B* gene has five exons: exon1 encodes a signal peptide, exons 2-3 the ligand (RANK-L) binding region, and exons 4-5 heparin-binding and dimerization domains. The ligand-binding region, crucial for OPG activity, consists of 4 cysteine-rich domains, each with 4 (or 6) cysteine residues forming 2 (or 3) disulphide bonds. Major deletions in the gene result in a severe phenotype, with presentation before the age of 18 months, delayed walking and short stature (<3rd centile). Mutations of the cysteine residues in the ligand-binding domain produce a similarly severe phenotype, presumably because ligand binding is disrupted. Non-cysteine mutations in this region result in a milder phenotype, with later diagnosis (~5 yrs), no delay in walking, and characteristic x-ray appearances. The mildest phenotype seen– with normal stature and minimal deformity, resulted from a deletion/insertion mutation in exon 5. Fractures of the long bones were more common in subjects with milder phenotypes. Mutations in the *TNFRSF11B* gene account for the majority of cases of IH – and the phenotypic variability in the syndrome is in concordance with the predicted effects of the mutations on OPG function.

ORAL PRESENTATIONS

O01

POLYALANINE DELETION POLYMORPHISM IN *RUNX2* IS ASSOCIATED WITH AN INCREASED RISK OF FRACTURE AND DECREASED SERUM OSTEOCALCIN LEVELS

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Runt related transcription factor 2 (*RUNX2*) is the key regulator of osteoblast differentiation. Several variations within *RUNX2* have been associated with significant changes in BMD, which is a major risk factor for fracture. In this study we report that an 18bp deletion (17Ala->11Ala) within the polyalanine track of *RUNX2* is significantly association with fracture. Carriers of the 11Ala allele were found to be nearly twice as likely to have fracture (Odds ratio 1.9, 95% CI 1.1 to 3.2). A previously identified single nucleotide polymorphism within the polyalanine track (termed the A allele) was also investigated in the study. It was found that the A allele was significantly underrepresented in Colles' and ankle fractures.

To further characterize the deletion polymorphism the relationship between bone parameters and the 11 Ala allele was investigated. 78 osteoarthritis (OA) subjects were examined who had multiple determinations of 45 serum parameters including serum osteocalcin. In osteoblasts, *RUNX2* regulates the expression of osteocalcin, and we found that the 11Ala allele was significantly associated with reduced osteocalcin serum levels in the OA patients (p=0.01). This finding did not hold for other parameters measured. These findings suggest that the 11Ala allele is a biologically relevant polymorphism in that it influences serum osteocalcin and confers enhanced fracture risk.

O02

11ALA AND A ALLELES OF *RUNX2* ASSOCIATED WITH BMD IN SCOTTISH WOMEN; INTERACTION OF *RUNX2* ALLELES WITH WEIGHT

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We previously reported the association of the *RUNX2* A allele with increased bone mineral density (BMD) and protection against a common form of osteoporotic fracture within a Geelong population. To further decipher the role of the *RUNX2* A allele we genotyped 992 women from a Scottish cohort. The A allele was associated with higher femoral neck (FN) BMD (p=0.035) within a postmenopausal subgroup of the population (n=312). When the postmenopausal group was segregated into thin/normal (BMI ≤ 25 kg/m²) and overweight/obese (BMI > 25 kg/m²), within the BMI > 25 kg/m² group (n=140) the *RUNX2* A allele showed a stronger effect on FN BMD, with the A allele accounting for 6.8% of the variance of FN BMD. Significant differences in FN BMD were detected in both A allele carriers (GA and AA genotypes) and non-A allele carriers (GG genotype) when comparing thin/normal women to overweight/obese women.

The 11Ala *RUNX2* deletion allele was significantly associated with decreased lumbar spine (LS) BMD (p=0.018) within the BMI > 25 kg/m² group (n=546) of the whole group. The 11Ala allele was significantly associated with increased levels of pyridinoline (p=0.014) and deoxypyridinoline (p=0.038) in the HRT treated subgroup of the population (n=492). Glutamine variants and an alanine insertion were identified within the group. These data suggest that the *RUNX2* 11Ala and A alleles exert differing affects on BMD showing preference for different skeletal sites.

O03

INHIBITORS OF THE MEVALONATE PATHWAY SENSITISE OSTEOSARCOMA CELLS TO APO2L/TRAIL-INDUCED CELL DEATH.

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We have previously shown that the nitrogen-containing bisphosphonate, zoledronic acid, (ZOL) sensitises human osteogenic sarcoma (OS) cells to Apo2L/TRAIL-induced apoptosis by inhibiting downstream enzymes of the mevalonate pathway; a biosynthetic pathway responsible for the production of cholesterol and isoprenoid lipids, particularly farnesyl-and geranyl-pyrophosphates. The aim of this study was to assess if similar effects could be observed with other inhibitors of the mevalonate pathway including statins (lovastatin and mevastatin) and the transferase inhibitor (GGTI). We found that like ZOL, statins and GGTI reduced cell number in a dose dependent manner, however combination treatment with Apo2L/TRAIL resulted in a significantly greater induction of cell death than for either agent alone. Importantly, OS cells that are normally resistant to Apo2L/TRAIL, are sensitised by these agents to the apoptotic effects of Apo2L/TRAIL. Neither statins alone, GGTI alone, nor in combination with Apo2L/TRAIL affected normal human bone cells under equivalent conditions. Addition of geranylgeraniol, an intermediate of the mevalonate pathway, blocked the ability of these agents to sensitise OS cells to Apo2L/TRAIL-induced cell death suggesting that geranylgeranylated proteins may be involved in regulating Apo2L/TRAIL-mediated cell death in the combined treatment. Although the mechanisms of this synergistic activity are not yet clear they could be due to differences in the proapoptotic signalling of the different agents. By understanding the pathways activated by these agents and exploiting their use, there is exciting potential for their use to manage osteosarcomas clinically.

O04

REGULATION OF GENE EXPRESSION BY THE CYP27B1 PROMOTER IN THE KIDNEY

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The enzyme 25-hydroxyvitamin D-1 α - hydroxylase (CYP27B1), is required for the synthesis of 1 α ,25-dihydroxyvitamin D, which is important for maintaining plasma calcium levels and other cellular functions. Although the kidney is considered to be the major producer of circulating 1,25D, little is known of the molecular regulation of CYP27B1 expression in this tissue. To study this regulation in vivo, we established a transgenic mouse model that expresses luciferase under the control of the 1.5 kb CYP27B1 promoter. To study the effect of development, 3 animals were sacrificed at 2, 4, 8, 12 and 64 weeks of age. The effects of dietary calcium and vitamin D status were studied by feeding the animals diets containing varying levels of dietary calcium (0%, 0.05% and 1.4%) and a 0.1% calcium, vitamin D deplete diet. Endogenous CYP27B1 mRNA levels were measured using Real-Time PCR. The promoter-directed luciferase activity and the CYP27B1 mRNA levels were highest in the 2 week old animals and were reduced by at least 80% at 6 weeks of age (p<0.01). In the dietary study, both the luciferase activity and CYP27B1 mRNA levels were highest in the animals fed the 0% Ca diet and were reduced with increasing amounts of dietary calcium in a dose responsive manner. When mice were fed the vitamin D deficient, 0.1% calcium diet, marked up-regulation of both the luciferase activity and the CYP27B1 mRNA levels were detected. Luciferase activity and CYP27B1 mRNA expression were also detected in various other tissues, which were unaffected by variation in dietary calcium, vitamin D status or maturity. These findings indicate that the regulation of gene expression in the kidney, associated with increasing maturity and variation of dietary calcium and vitamin D status, occurs through the 1.5 kb CYP27B1 promoter.

O05

TELEVISION WATCHING, TYPE AND LEVEL OF PHYSICAL ACTIVITY AND UPPER LIMB FRACTURE RISK IN CHILDREN

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The aim of this population based case-control study was to examine the association between television, computer and video viewing, type and level of physical activity and upper limb fracture risk in children 9-16 years.

A total of 321 fracture cases and 321 randomly selected individually matched controls were studied. Television, computer and video viewing, types and levels of physical activity were determined by interview-administered questionnaire. Bone strength was assessed by metacarpal morphometry and dual energy X-ray absorptiometry (DXA).

In general, sports participation increased upper limb fracture risk in boys and decreased fracture risk in girls. Gender-specific risk estimates were significantly different for total, contact, non-contact and high-risk sports participation as well as four individual sports (soccer, cricket, surfing and swimming). In multivariate analysis, time spent television, computer and video viewing was associated with increased wrist and forearm fracture risk (OR 1.58/category, 95% CI 1.14-2.20) while time involved in light physical activity participation decreased risk (OR 0.81/category, 95% CI 0.65-1.00). High-risk sports participation was the best predictor of both hand (OR 1.43/sport, 95% CI: 1.08-1.88) and upper arm fractures (OR 2.55/sport, 95% CI 1.15-5.65). Further adjustment for bone density and metacarpal morphometry did not alter these associations.

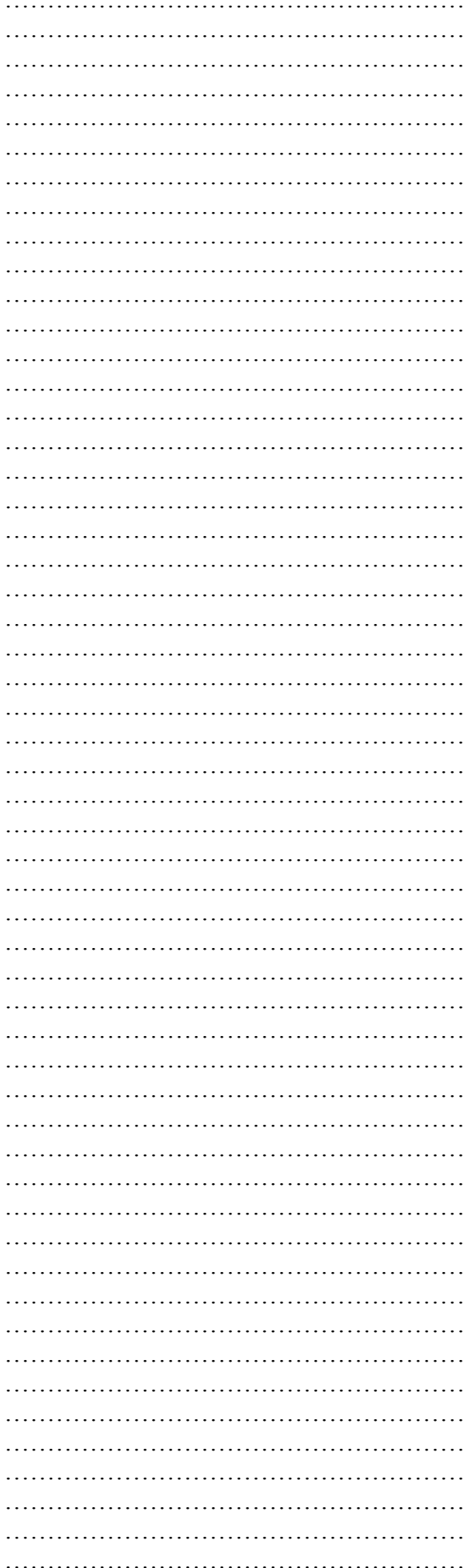
In conclusion, there is gender discordance with regard to sports participation and fracture risk in children which is likely to reflect different approaches to sport particularly given that light physical activity is protective. Importantly, television, computer and video viewing is an independent risk factor for wrist and forearm fractures. The mechanism is unclear but television viewing is most likely to be acting as either a cause or a marker of behavioural and psychosocial disturbance in children or may lead to changes in bone quality not detected by DXA or metacarpal morphometry.

O06

STRUCTURAL BASIS FOR DIFFERENCES IN FEMORAL NECK FRAGILITY IN CHINESE AND CAUCASIANS

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We hypothesized that relatively greater periosteal apposition during growth and aging in Chinese accounts for the lower hip fracture rates reported in epidemiological studies. We measured FN dimensions and bone mass, estimated endocortical diameter, cortical thickness, section modulus, and buckling ratio (subperiosteal radius/cortical thickness) in 738 Chinese (490 females) and 1181 Caucasians (788 females) aged 18 to 93 years. In young adult women, after adjusting for racial differences in height and weight, FN axis length and diameter remained 4-8% lower in Chinese, while cortical thickness and vBMD were no different by race; growth produced racial difference in FN geometry; the *same* cortical thickness was distributed further from the FN neutral axis conferring greater bending strength in Caucasians than Chinese. From young (~30yrs) to old age (~70yrs) FN periosteal diameter (height and weight adjusted) increased less in Chinese than Caucasian men (1.0% vs. 9.1%), but increased similarly in Chinese and Caucasian women (4.6% vs. 3.3%). Endocortical diameter also increased less in Chinese than Caucasian men (2.6% vs. 12.5%), but similarly in Chinese and Caucasian women (8.5% vs. 6.5%). Net bone loss expressed as the decline in vBMD was less in Chinese than Caucasian men (9.9% vs. 18.4%) and in Chinese than Caucasian women (16.7% vs. 21.7%). These changes produced 4.9-5.6% higher vBMD and 6.9-8.7% lower buckling ratio in elderly Chinese than Caucasians in both sexes. Despite the smaller FN diameter and lower bending strength, the relatively thicker cortex and higher vBMD in elderly Chinese resulted in a lower risk of structural failure by local buckling than Caucasians. These structural differences in Chinese and Caucasians are likely to be established during both growth and aging. expression in the kidney, associated with increasing maturity and variation of dietary calcium and vitamin D status, occurs through the 1.5 kb CYP27B1 promoter.



O07

MYELOMA CELLS CONTRIBUTE DIRECTLY TO THE POOL OF RANKL (TNFSF11) IN THE BONE MICROENVIRONMENT

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Multiple myeloma is a malignant plasma cell neoplasm, frequently associated with osteolytic bone destruction. Osteoclast activity is regulated by the coordinated expression of RANKL (TNFSF11), its receptor RANK (TNFRSF11A) and decoy receptor OPG (TNFRSF11B). There are clear discrepancies in the literature as to whether myeloma cells contribute directly to the pool of TNFSF11 in the bone microenvironment (see Pearse *et al.*¹; Croucher *et al.*²; Giuliani *et al.*³ and Roux *et al.*⁴).

TNFSF11 (RANKL) mRNA transcripts can be detected by *in situ* hybridization on bone marrow biopsies, rich in myeloma cells. We have extracted RNA from CD138-purified cells (plasma cells) isolated from bone marrow biopsies taken from multiple myeloma patients. mRNA transcripts encoding TNFSF11 (either isoform-1 membrane form, isoform-2 secreted form or both) were detected in 17 of the 19 samples examined by RT-PCR employing isoform-specific primers. Similarly, we detect mRNA transcripts encoding TNFSF11 in the five human myeloma cell lines we examined (H929, LP-1, OPM-2, RPMI 8226 and U-266). Immunohistochemistry experiments on bone marrow biopsies rich in myeloma cells, and indirect immunofluorescence staining of the human myeloma cell lines provide evidence that these mRNA transcripts are translated to protein. Finally we present *in vitro* data that multiple myeloma cells can induce differentiation and maturation of osteoclast progenitors into functional giant multinucleated osteoclasts. We conclude myeloma cells in the bone microenvironment not only stimulate increased production of RANKL (TNFSF11) by the stroma (see Pearse *et al.*¹), but that they also contribute directly to the pool of RANKL. These observations lead to the possibility that myeloma cells can directly stimulate osteoclasts bypassing the classic stroma-osteoclast route.

Pearse *et al.*, 2001 *Proc Natl Acad Sci USA*. 98(20):11581-6.
Croucher *et al.*, 2001 *Blood* 98(13):3534-40.
Giuliani *et al.*, 2001 *Blood* 98(13):3527-33.
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O08

BIPHASIC EFFECTS OF GM-CSF ON OSTEOCLASTOGENESIS FROM HUMAN CORD BLOOD.

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

GM-CSF supported sRANKL-induced osteoclastogenesis in the absence of M-CSF (20% of M-CSF/sRANKL control). The effect of GM-CSF treatment in the presence of M-CSF was divergent, depending on the timing and duration of exposure. Short-term treatment (2-48h) stimulated cell proliferation and enhanced OC formation and resorption of dentine (up to 100%) independent of sRANKL. Long-term treatment (>3d) in the presence of sRANKL, however, inhibited OC generation by 96% with formation of CD1a⁺ DC. Inhibition was accompanied by a 6.5-fold decrease in c-Fos expression, a transcription factor essential for OC formation. Delayed addition of GM-CSF had no effect on osteoclastogenesis but did result in the formation of multiple DC clusters. These results demonstrate that GM-CSF can partially compensate for M-CSF in osteoclastogenesis. Furthermore, short-term treatment with GM-CSF potentiates OC differentiation by causing further proliferation of the OC precursor pool. Continued exposure diverts differentiation of early precursors towards DC via down-regulation of c-Fos. GM-CSF does not inhibit mature OC function.

O09

STUDIES ON THE MECHANISM OF INHIBITION OF OSTEOCLAST DIFFERENTIATION BY PAR-2

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Protease-activated receptor-2 (PAR-2), which is expressed by osteoblasts, is activated specifically by a small number of tissue proteases or by a specific PAR-2-activating peptide (RAP). We have previously shown that RAP inhibits osteoclast differentiation induced by parathyroid hormone (PTH) or 1,25 dihydroxyvitamin D3 (1,25D3) in mouse bone marrow cultures. RAP also inhibits induction of various mediators of osteoclast differentiation by osteolytic factors in bone marrow or osteoblast cultures. Here we show that RAP inhibits osteoclast differentiation induced by interleukin-11 (IL-11). RAP did not inhibit PTH-, 1,25D3- or IL-11-induced osteoclast differentiation in cultures generated from PAR-2 knockout mice, confirming that the response to RAP in wildtype cultures is specifically mediated by PAR-2. Since cAMP production is an important mediator of responses to PTH, the effect of RAP on cAMP levels was investigated. RAP was shown to down-regulate cAMP levels in bone marrow or osteoblast cultures treated with PTH, although it had no effect on cAMP levels in control cultures. Since we have previously shown that cells of the osteoclastogenic RAW 264.7 cell line express PAR-2, we investigated whether RAP could inhibit RANKL-induced osteoclastogenesis in these cells. RAP was found to have no effect on osteoclast differentiation in cultures treated with RANKL, suggesting that osteoclast precursors are not the target cells for the inhibitory effects of PAR-2 activators on osteoclast differentiation. These observations indicate that activation of PAR-2 leads to inhibition of osteoclast differentiation induced by multiple signalling pathways, and that the cellular targets of PAR-2 activators are likely to be cells of the osteoblast lineage.

O10

ANALYSIS OF THE L-AMINOACID BINDING SITE ON THE CALCIUM-SENSING RECEPTOR BY SITE-DIRECTED MUTAGENESIS

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Ca²⁺ ions act as the physiological agonists of the human calcium-sensing receptor (CaR). In addition, L-amino acids act as allosteric activators of the cloned CaR (1) and a study in our laboratory on chimeric receptors has implicated the N-terminal Venus FlyTrap (VFT) domain as the site of amino acid binding. Furthermore, a recent site-directed mutagenesis study has implicated a triple serine motif SSS169-171 in the VFT domain in amino acid action (2). These findings indicate that it may be possible to engineer a CaR that retains normal or near normal Ca²⁺ sensitivity but is unable to respond to amino acids. To this end, we have initiated a detailed study of the impact of conservative mutations of residues that form the predicted amino acid binding site based on the crystal structure of the homologous rat metabotropic glutamate receptor-1. HEK293 cells were transfected (lipofectamine-2000) with mutants synthesized by site-directed mutagenesis of the wild-type CaR in pcDNA3.1 using the QuickChange method (Stratagene). CaR-dependent responses were detected by aequorin luminescence as described previously (3). The following binding site mutations exhibited normal or near-normal Ca²⁺ sensing: S147T, S169A, S169T, S171A and S171T. The following mutations exhibited impaired Ca²⁺ sensing: S147A, S170A and Y218F. The mutation S170T, on the other hand, exhibited enhanced Ca²⁺ sensitivity. Amino acids restored Ca²⁺ sensitivity in the cases of S147A and Y218F but not S170A. S170T restored Ca²⁺ sensitivity in the case of S147A. Characterization of other mutants and double mutants is in progress. The development of a CaR mutant that exhibits normal Ca²⁺ sensitivity but fails to respond to L-amino acids seems feasible.

1. Conigrave AD, Quinn SJ, Brown EM (2000) PNAS 97:4814-4819. 2. Z. Zhang, W. Qiu, S.J. Quinn, A.D. Conigrave, E.M. Brown M. Bai (2002) J. Biol. Chem. 277, 33727-33735. 3. Y.M. Tan, J. Cardinal, A.H. Franks, H-C. Mun, N. Lewis, L.B. Harris, J.B. Prins, A.D. Conigrave (2003) J. Clin. Endocrinol. Metab. 88, 605-610

O11

THE SPECIFIC NFATC-BLOCKERS, 3,5-BISTRIFLUORO-METHYL PYRAZOLES (BTPs), ARE POTENT INHIBITORS OF HUMAN OSTEOCLAST DIFFERENTIATION.

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Nuclear factor of activated T cells cytoplasmic-1 (NFATc1) has recently been shown to have a critical role in osteoclast (OC) differentiation [1,2]. The novel low MW, immunosuppressive agents, BTPs, block the activation-dependent nuclear translocation of NFATc by maintaining it in the cytosol in a phosphorylated state. Unlike cyclosporin-A and tacolimus, the BTPs do not block NFATc by inhibiting calcineurin phosphatase activity.

Using gene array, we found that NFATc1 expression was upregulated during RANKL-induced OC generation and confirmed this using real time PCR and Western blot. The latter showed increased expression of NFATc1 at 7d and 14d, particularly proteins of 93 kD and 112 kDa corresponding to NFATc1/A isoform. In a model employing human CFU-GM as precursors, we investigated the effects of BTP19 [3] on OC differentiation. BTP19 produced a concentration-dependent inhibition of OC generation with a significant effect (-32%) at 10 nM and 70% inhibition at 1µM. Resorption was inhibited by 90% at 1µM BTP. Pretreatment of precursors with M-CSF ± BTP19 for 24h followed by 14d treatment with sRANKL and M-CSF ± BTP19 resulted in a more potent and sensitive inhibition of resorption with close to 100% inhibition with BTP19 1µM. The inhibitory effect required exposure to BTP19 for at least 3d at the beginning of the culture and 7-10d exposure was required for maximum effect.

The BTPs are potential antiresorptive therapies for osteoporosis, particularly in patients requiring immunosuppression therapy.

[1] Ishida N et al 2002 J Biol Chem 277:41147; [2] Takayanagi H et al 2002 Dev Cell 3:889;[3] Djuric SW et al 2000 J Med Chem 43:2975.

O12

ACCELERATED MICRO-STRUCTURAL CHANGE AT LOW BONE VOLUME

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While bone mass is the major determinant of bone strength this mass based paradigm does not fully account for the contribution of the bone microstructure to mechanical efficiency. Geometric models of cancellous bone structure have been formulated based on stylised representations of the trabecular elements. The relationships between bone volume and bone surface of cancellous bone are complex and reflect the modulating effect on the cancellous bone structure of bone remodeling at the trabecular surfaces. Using the plate model of cancellous bone structure the interrelationships between parameters of cancellous bone structure have been studied.

Two hundred and eighty histological sections of cancellous bone from 8 skeletal sites were analysed. The structural parameters of cancellous bone (BV/TV, BS/TV, BS/BV, Tb.Th, Tb.Sp, Tb.N and TBPf) were obtained. The data were classified into two groups by BV/TV, low BV/TV (<15%) and high BV/TV (>= 15%).

This study shows that change to cancellous bone structure is bone volume dependent in a non-linear manner. When BV/TV decreases from 10% to 5%, Tb.Th decreases by 27µm and Tb.Sp increases by 764µm, whereas when BV/TV decreases from 30% to 25%, Tb.Th decreases by 12µm and Tb.Sp increases by 68µm. This suggests that the structural integrity of the cancellous bone may become rapidly compromised when bone volume falls below a critical value.

The complex relationships between bone mass and bone structure, identified in this study, are often overlooked in the mass based paradigm of bone strength. Histomorphometric descriptors of cancellous bone structure highlight the potential for accelerated deterioration of the structure with low BV/TV, which may lead to increased risk of fracture. From a clinical viewpoint estimation of an individuals fracture risk is constrained by available non-invasive techniques. Therefore, there is a need to better correlate measurement of bone mass with structural parameters of cancellous bone.

O13

INTERLEUKIN-3 MODULATION OF HUMAN OSTEOCLASTOGENESIS

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Interleukin-3 (IL-3) is a macrophage-inducing cytokine that can also induce other haematopoietic lineages. The actions of IL-3 on osteoclastogenesis remain unclear. *In vitro* studies in mouse and human models report that IL-3 can either stimulate or inhibit osteoclast (OC) formation and resorptive capacity. To investigate the role of IL-3 in osteoclastogenesis we have used human assays employing precursors from either adult peripheral blood mononuclear cells (PBMC), CD14⁺ isolated from PBMC, or CFU-GM colonies derived from umbilical cord blood mononuclear cells.

Gene array analysis of human CD14⁺ treated with M-CSF with and without sRANKL showed down-regulation of the α -chain of the IL-3 receptor in the presence of sRANKL. A maximal 5.7-fold down-regulation was confirmed by Real-Time PCR analysis after 4d treatment with sRANKL.

PBMC or CFU-GM precursors were cultured on dentine slices in the presence of M-CSF (25ng/ml) and sRANKL (125ng/ml) for 21 and 7d respectively. Co-treatment of PBMC with IL-3 and sRANKL/M-CSF dose-dependently inhibited OC number and resorption (IC₅₀ 0.3ng/ml) with 85% inhibition of both at 1ng/mL at 21d. Co-treatment of CFU-GM with IL-3 and sRANKL/M-CSF dose-dependently inhibited OC number and resorption (IC₅₀ 1ng/ml) with 70% inhibition of both at 100ng/mL at 7d. Pre-treatment of CFU-GM for 7d with IL-3 (10ng/mL) and M-CSF followed by sRANKL/M-CSF for 7d produced OC with 73% larger plan area and correspondingly larger but shallower resorption lacunae.

Our findings demonstrate that IL-3 can modulate human osteoclastogenesis in two ways. In the presence of sRANKL, IL-3 is a potent inhibitor of OC differentiation, despite the fact that sRANKL down-regulates the IL-3 receptor. Pre-treatment of precursors with IL-3, prior to sRANKL exposure, leads to the generation of much larger OC that form shallow resorption lacunae. The biological roles of IL-3 in OC differentiation and of these large OC remain to be determined.

O14

VITAMIN D METABOLISM IN THE KIDNEY: REGULATION BY PTH AND CALCITONIN

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25-Hydroxyvitamin D (25D) is metabolised by the renal enzymes 25D-1 α -hydroxylase (CYP27B1) and 25D-24-hydroxylase (CYP24) and we now report on the regulation of circulating 1,25-dihydroxyvitamin D (1,25D) by factors modulating the kidney mRNA levels of these enzymes. Serum 1,25D levels were assayed and kidney CYP27B1 and CYP24 mRNA levels were quantified by real-time RT-PCR in three separate studies using female Sprague-Dawley rats. Rats were either (1) aged 3 weeks to 2 years-old, (2) adults fed dietary calcium (Ca) concentration ranging from between 0.05% and 1%, or (3) vitamin D-replete (D+) and vitamin D-deplete (D-) adults fed 1% Ca, followed by either a 0.1% Ca (LC) or 1% Ca (HC) diet for 3 months. Circulating 1,25D levels decreased with age and as a result of increased dietary calcium concentration. In both these studies, the reductions in serum 1,25D levels were associated with decreases in CYP27B1 mRNA levels (Age R²=0.72; Diet Ca R²=0.51) and increases in CYP24 mRNA levels (Age R²=0.71; Diet Ca R²=0.49). CYP27B1 and CYP24 were equally major determinants of the levels of serum 1,25D (Age mult R²=0.88; Diet Ca mult R²=0.70). Stimulation of CYP27B1 by PTH occurred only in the hypocalcemic D-/LC animals, increasing CYP27B1 mRNA levels by 280-fold (p<0.001). In these animals, kidney CYP24 mRNA levels were markedly reduced when compared to the D+/LC animals (p<0.01). A positive association was found between kidney CYP24 mRNA and calcitonin levels both in the age study (R²=0.69) and in the dietary calcium and vitamin D study (R²=0.80). These results suggest that the regulation of circulating levels of 1,25D involves both the synthesis and the breakdown of vitamin D in the kidney. The regulation of vitamin D metabolism, by PTH during hypocalcemia and possibly calcitonin during normocalcemia suggests the control mechanism for the supply of 1,25D from the kidney is complex.

O15

A LARGE SCALE GENOME-WIDE SCREEN OF TWINS FOR LOCI THAT REGULATE CALCANEAL QUANTITATIVE ULTRASOUND. SG Wilson, T Andrew, PW Reed, J Lichter and TD Spector. Endocrinology & Diabetes, Sir Charles Gairdner Hospital, Nedlands, Australia. Twin & Genetic Epidemiology Research Unit, St Thomas' Hospital, London, UK. Sequenom Inc., San Diego, USA.

Quantitative ultrasound (QUS) of the calcaneus provides a measure of osteoporotic fracture risk. Substantial heritability has been shown for QUS parameters - broadband ultrasound attenuation (BUA: 0.48-0.58) and velocity of sound (VOS: 0.19). This suggests that there are genetic effects, which contribute to variance in these phenotypes. We performed a genome screen of a large cohort of dizygous twin pairs to identify regions of the genome that contain quantitative trait loci (QTL) that regulate these QUS parameters.

Female twins aged 18-75 years were recruited from the St Thomas' Hospital, UK Twin Registry. Anthropometric and general medical/lifestyle questionnaire data were collected at interview. QUS was measured at the calcaneus (CubaClinical). DNA from 1067 twin pairs was used in genome scans of 737 markers from the ABI Prism & Genethon linkage mapping sets. Genotyping was performed using PCR and electrophoresis on an ABI 377. Linkage analysis used SOLAR.

The maximum linkage defined was for BUA at 2q33-37 (LOD = 2.1-4.8). For VOS, a maximum LOD of 2.1-3.5 was found at 4q12-22. Weaker linkage was found for BUA and VOS to twelve additional genomic regions.

We have previously shown that 2q33-36 may be an important region for genes influencing bone mineral density (1). This new data showing linkage of BUA to that region, adds further weight to the relevance of the area for studies of genes that influence bone density and trabecular micro-architecture. Additional regions, likely to be important for genes that influence heel ultrasound parameters and fracture risk, were also identified.

Wilson SG, Reed PW, et al., 2003, Am J Hum Genet 72:144-155.

A rudimentary analysis of a subset of individuals from this cohort was reported at: The Second International Workshop on the Genetics of Bone Metabolism and Disease. Davos, 15-18 Feb 2003.

O16

A NOVEL FUNCTION OF CD69 IN INHIBITING OSTEOCLAST FORMATION

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CD69 belongs to the NK cell receptor group of the C-type lectin super-family, including OCIL that shares 36% homology with CD69 in their C-lectin domain. Previously, we have shown that OCIL inhibits osteoclastogenesis (1). Recently, we have also observed that OCIL exhibits carbohydrate binding specificity for high molecular weight sulfated glycans, λ-carrageenan, fucoidan and dextran sulfate and that recognition of carbohydrates by OCIL may be involved in OCIL's inhibitory action in osteoclast formation (2). In this study we aimed to determine the function of CD69 in osteoclast formation as well as compare the carbohydrate binding specificity of CD69 with OCIL.

The extracellular domain of CD69 was expressed as recombinant protein in *E. coli*. Bone marrow macrophage precursors were treated with M-CSF and RANKL for 7 days, in the presence or absence of either recombinant CD69 or OCIL. Recombinant CD69 inhibited osteoclast formation with a similar potency to OCIL.

In order to determine carbohydrate binding specificity of CD69, we used an ELISA based assay where sugars were covalently linked to free amine groups. Binding of recombinant CD69 was detected using an antibody against the recombinant tag. CD69 showed binding to the high molecular weight sulfated glycans λ-carrageenan, fucoidan and dextran sulfate, similar to OCIL.

In summary, the C-lectin domain of both CD69 and OCIL acted to inhibit osteoclast formation and showed similar sugar binding specificity. This may indicate a common role of C-type lectin-like domains in the inhibition of osteoclastogenesis.

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O17

SUPPRESSION OF OSTEOCLAST FORMATION BY GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF).

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The standard model of osteoclastogenesis uses M-CSF and RANKL to differentiate osteoclasts from peripheral blood mononuclear cells (PBMCs). Using real time PCR, we found that RANKL profoundly up-regulates the GM-CSF receptor in this model. This suggests that GM-CSF receptor might represent a target for regulation, whereby osteoclast precursors integrate RANKL and GM-CSF signals to either promote or inhibit differentiation. Exogenous GM-CSF was added to the *in vitro* osteoclastogenesis model to test these alternative hypotheses. Cells were examined through time, with TRAP staining, morphology and gene array analysis. Continuous exposure to GM-CSF totally represses osteoclast differentiation; and resulted in an alternative cell phenotype induced by GM-CSF in the presence of M-CSF and RANKL when compared to the alternative treatments.

A 19,000 gene microarray was used to compare GM-CSF+RANKL+M-CSF treated cells against osteoclasts. Microarray analysis showed GM-CSF mediated repression of osteoclast differentiation was concurrent with suppression of osteoclast marker genes: cathepsin K; osteoclast specific H⁺ ATPase; surface marker, CD68; and transcription factors we have shown are up-regulated in osteoclasts, such as NFATc1. The inhibition by GM-CSF of known osteoclast markers indicates an alternate GM-CSF dependent differentiation pathway. Real-time PCR analysis of 7 regulated genes validated the array data (FBP, GABP α , GABP β , ILF3, Kox31, NFATc1 and SCYA2). These data support the hypothesis that GM-CSF receptor up-regulation by RANKL sensitises the cell for inhibition of differentiation. In the cytokine milieu of the bone marrow, the ratio of GM-CSF and RANKL may be an important determinant of osteoclast differentiation.

O18

FGF23 AND PHEX EXPRESSION IN RESPONSE TO PHOSPHATE AND MINERALISATION IN BONE CELLS

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Mutations in *FGF23* (*Fibroblast Growth Factor 23*) and *PHEX* (*Phosphate-regulating gene with Homologies to Endopeptidases on the X chromosome*) have been associated with renal phosphate wasting disorders. There is evidence that PHEX is involved in the development and mineralisation of bone and teeth, and indications that FGF23 is involved in bone growth and development. Little is known, however, of the mechanism by which this occurs, or how the two gene products may interact.

PHEX and *FGF23* mRNA expression were detected at low levels in a human osteoblast-like bone cell line. Changes in mRNA expression were measured by quantitative real-time RT-PCR. Mineralisation of the bone cells, treated with β -glycerophosphate (β GP), increased almost 2-fold from confluence to 20 days post-confluence, as measured by Alizarin Red-S staining. As bone cell mineralisation increased, *PHEX* mRNA expression levels decreased by 44%, whereas *FGF23* mRNA expression increased 7.5-fold. Treatment with 10⁻⁸ M dexamethasone increased mineralisation over treatment with β GP 1.3-fold at 10 days post-confluence, with a corresponding increase in *FGF23* mRNA expression. In bone cells treated with 0-3 mM extracellular phosphate, *FGF23* mRNA expression increased with increasing phosphate concentration.

The change in expression of *FGF23* and *PHEX* mRNA detected in mineralising bone cells indicates that these genes may play a role in the mineralisation of bone cells. The increased *FGF23* mRNA expression in response to increasing extracellular phosphate is consistent with the involvement of this gene in phosphate regulation.

O19

DISTINCT REGULATION OF BONE AND MUSCLE MAINTENANCE DURING HINDLIMB SUSPENSION BY A CONCENTRIC RESISTANCE EXERCISE REGIMEN.

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Exposure to microgravity causes dramatic loss of muscle and bone mass. In normal gravity, resistance exercise has been used to increase muscle and bone mass. We tested a novel form of resistance exercise training (RT) using only concentric force production to offset the loss of musculoskeletal mass during 2 weeks of hindlimb suspension (HS). Male, S-D rats (6-months old; 4/group) were operantly conditioned to perform RT, and then randomly assigned to groups of sedentary control (CON) or HS, +/- RT (CONRT and HSRT, respectively). RT groups engaged in 2 sets of ~21 repetitions with 100-300 Newtons of concentric force, 3 days/week for 2 weeks, +/- suspension. BMD and architecture were analyzed by pQCT and microCT. HS significantly (p<0.05) reduced volumetric BMD of trabecular bone in the tibia, as well as increased trabecular spacing, and decreased trabecular number, thickness and bone volume (BV/TV) in HS rats compared to CON. HSRT restored trabecular BMD, trabecular spacing, number, thickness, and BV/TV to levels indistinguishable from CON rats (p<0.05). Further, the trabecular thickness of HS was decreased compared to HSRT. Surprisingly, this concentric RT did not prevent loss of muscle mass during the 2 week HS period. Both soleus muscle weight and muscle weight to body weight ratio in control rats were significantly greater (p <0.05) than both HS and HSRT. Neither were gastrocnemius weights and weight ratios maintained by HSRT. Together, these data demonstrate that this concentric force production exercise regimen was able to maintain bone mass, but not muscle mass. These data suggest that distinct thresholds of training force exist, and/or the type of exercise regimen (concentric versus eccentric) utilized may distinctly regulate bone and muscle maintenance.

O20

THE MICROPHthalmia TRANSCRIPTION FACTOR: INTEGRATION OF SIGNALING, TRANSCRIPTION AND THE CELL CYCLE DURING OSTEOCLAST DIFFERENTIATION

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The microphthalmia transcription factor (MITF) is a bHLH-zip transcription factor that regulates terminal differentiation of osteoclasts. MITF regulates genes like tartrate resistant acid phosphatase (TRAP) and cathepsin K that are necessary for osteoclast functions. Approximately 23 spontaneous and induced alleles of MITF have been characterized and these provide a unique set of tool to study the function of this gene in osteoclast differentiation.

MITF is expressed in distinct cell lineages, for example melanocytes and osteoclasts, and regulates different sets of genes in the distinct cell types. We have used a combination of genetics, molecular biology and biochemistry to determine how MITF differentially regulates genes expression in osteoclasts.

Our results have revealed two mechanisms by which MITF achieves osteoclast-specific gene regulation. First, MITF requires the ets-factor PU.1 as a partner for regulating specific target genes. PU.1 expression is limited to hematopoietic lineages, including macrophages and osteoclasts. MITF and PU.1 interact genetically, physically and functionally to regulate target genes in osteoclasts. Second, MITF is a direct target for Receptor-activator of NF-KB ligand (RANKL) signaling in the osteoclasts. RANKL persistently activates the p38 MAP kinase pathway which in turn leads to persistent phosphorylation of MITF at conserved serine 307 and persistent activation of target genes.

Recently using p27/p21 double null mice, we have demonstrated a role for these cyclin inhibitors in cell cycle withdrawal and differentiation of osteoclasts. RANKL induces expression of p21 and p27, and p21/p27 double mutant mice develop osteopetrosis and show impairment of osteoclast terminal differentiation, including reduction in the expression of MITF target genes like TRAP and cathepsin K. Attempts are underway to determine if p27 and p21 directly regulate MITF activity during osteoclast differentiation.

O21

V-ATPASE ACCESSORY SUBUNIT ATP6S1 MUTANT IMPAIRS OSTEOCLASTIC BONE RESORPTION

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The vacuolar H⁺-adenosine triphosphatases belong to a unique class of ATPases responsible for the acidification of osteoclast during bone resorption. The core structure of V-ATPases consists of V₁ domain, which is responsible for hydrolysis of ATP, and V₀ domain which functions as a proton translocation unit across the membrane. Here we reported the identification of a V-ATPase accessory subunit ATP6S1 in osteoclasts. Using bioluminescence resonance energy transfer (BRET) assay, we showed that ATP6S1 interacts with subunit a, c, c' but not d of V₀ domain. By confocal analyses, ATP6S1 was partially co-localized with PH-dependent lysotracker and transferrin receptor. On the other hand, over expression of ATP6S1 in osteoclast precursor Raw cells increased the uptake of transferrin. Most importantly, osteoclasts with the C-terminal truncated mutant of ATP6S1 (Δ aa437-463) generated from a stably transfected Raw cell line display a significant defect of bone resorption in vitro. Compared with osteoclasts with EYFP vehicle expression, ATP6S1 mutant osteoclasts were small in size and had dramatic reduction of bone resorption pits as evidenced by scanning electron microscopic assessment. In short, our data showed that V-ATPase accessory subunit ATP6S1 is critical for the assembly of V-ATPase complex necessary for osteoclastic bone resorption.

O22

12-O-TETRADECANOYLPHORBOL-13-ACETATE (TPA) INHIBITS OSTEOCLASTOGENESIS BY SUPPRESSING RANKL-INDUCED NF- κ B ACTIVATION

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The protein kinase C (PKC) pathway has been suggested to be an important regulator of osteoclastic bone resorption. The role of PKC in RANKL-induced osteoclastogenesis is however not clear. In this study, we determined the effects of 12-O-tetradecanoylphorbol-13-acetate (TPA), a PKC activator, on osteoclastogenesis and studied the role of PKC activation in RANKL signaling in osteoclastic precursor cell line, RAW264.7 cells. We found that TPA inhibits RANKL-induced RAW264.7 cell differentiation into osteoclasts. Time course analysis showed that the inhibitory effect of TPA on RANKL-induced osteoclastogenesis occurs predominantly at the early stage of osteoclast differentiation. To investigate the molecular mechanisms of TPA suppression on osteoclastogenesis, we have examined the effects of PKC activation by TPA on RANKL-induced NF- κ B and c-Jun N-terminal kinase (JNK) signal transduction pathways. Using nuclear translocation and reporter gene assays, we showed that TPA alone has little effect on NF- κ B activation in RAW cells, but suppresses the RANKL-induced NF- κ B activation in a dose-dependent fashion. Supershift studies revealed that the RANKL-induced DNA binding of NF- κ B complexes consists of C-Rel, NF- κ B1 (p50), and RelA (p65). In addition, we showed that TPA induces the activation of JNK in RAW cells, but has little effect on RANKL-induced activation of JNK. Given NF- κ B activation is obligatory for osteoclast differentiation, our studies imply that inhibition of osteoclastogenesis by TPA is, at least in part, due to the suppression of RANKL-induced activation of NF- κ B during the early stage of osteoclastogenesis. Selective modulation of RANKL signaling pathways by may have important therapeutic implications for the treatment of bone diseases associated with enhanced bone resorption.

O23

PTHrP REGULATES CHONDROCYTE PROLIFERATION THROUGH P57^{KIP2}-DEPENDENT PATHWAYS

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PTHrP is a major regulator of chondrocyte proliferation during bone development. We are investigating whether the negative cell cycle regulator p57 is a downstream mediator of PTHrP's actions in chondrocytes. We have previously shown that in mice lacking PTHrP, chondrocytes have premature exit of proliferation, and accelerated differentiation. We also demonstrated that mice lacking both PTHrP and p57 show partial rescue of the early cell cycle exit, with normalization of the PTHrP-null phenotype occurring in a bone-specific manner. We have now characterised the effects of acute PTH treatment (used *in vitro* as an analogue of PTHrP) on chondrocyte proliferation in metatarsal (MT) cultures. MTs were isolated from the hind paws of E15.5 embryos, and cultured for 2 days in serum-free α -MEM. MTs (3/grp) were treated with 10⁻⁷ M rat PTH(1-34), or vehicle, for 24 hrs, and were labelled with 1 mg/ml BrdU for the final 4 hrs of culture. MTs were paraffin-embedded and sectioned for analysis.

PTH treatment of MTs significantly increased chondrocyte proliferation rate (14.2% vs 20.6% BrdU positive cells); and delayed exit of proliferation, seen by an enlargement of the proliferative domain and a reduction in size of the central, hypertrophic region of the MT. *In situ* hybridisation showed that *collagen II* expression in the proliferative chondrocytes was unaffected by PTH treatment; however, *p57* expression was specifically decreased in both the proliferative and the pre-hypertrophic chondrocytes of MTs treated with PTH. *Collagen X* expression was also down-regulated by PTH, indicating a general suppression of hypertrophic differentiation by PTH treatment. These data further support the hypothesis that the proliferative effects of PTHrP on chondrocytes are mediated in part via suppression of *p57* expression, leading to maintenance of chondrocytes in the cell cycle.

O24

AMYLIN DEFICIENCY RESULTS IN BONE LOSS IN ADULT MICE

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Amylin is a 37 amino acid protein that acts on bone to stimulate bone formation and inhibit bone resorption. This study investigates the effects of amylin deficiency on bone using the amylin global deletion transgenic model (amylin KO). (Preliminary results were presented at the 2002 ANZBMS ASM.) Femora from 6 male and 6 female control and amylin KO mice were collected at 4, 6 and 26 weeks of age. Distal femora were prepared for quantitative histomorphometry using established resin embedding techniques. Trabecular bone volume (BV/TV), trabecular thickness (TbTh) and trabecular number (TbN) were calculated in the epiphysis and metaphysis using a Quantimet 500 image analysis system. Amylin deficiency in male and female mice at 26 weeks of age resulted in decreased BV/TV ($P<0.05$) in the metaphysis compared with controls. This was associated with a decrease in TbN (Females $P<0.05$, Males $P<0.001$) and increased osteoclast surface (Female control: 1.22±0.65, Female KO: 2.15±0.88, Male Control: 0.23±0.15, Male KO: 0.81±0.4), although the latter difference was not significant. Despite a compensatory increase in TbTh in male amylin KO mice ($P<0.05$), this was not sufficient to prevent bone loss. In contrast epiphyseal BV/TV, TbN and TbTh were unaffected by amylin deficiency in males and females at 26 weeks of age. Amylin KO male and female mice were able to accrue BV/TV in the metaphysis during growth at 4 and 6 weeks of age at the same rate as controls. However, male amylin KO mice had increased TbTh ($P<0.001$; 4 and 6 wks) and decreased TbN ($P<0.05$; 4 wks) compared to controls suggesting an increase in bone turnover with amylin deficiency. In conclusion, amylin deficiency in mice results in trabecular bone loss in the metaphysis due to increased bone resorption.

025

IMPAIRED BONE ACCRUAL IN MATURING ANDROGEN DEFICIENT (*hpg*) MICE NORMALISES WITH REPLACEMENT BY A NON-AROMATISABLE ANDROGEN

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To further elucidate the role of androgens in the regulation of trabecular bone structure and turnover during growth, normal (wt) and androgen-deficient (*hpg*) mice were analysed by bone histomorphometry at 3 and 9 weeks of age. Subgroups of mice were treated with testosterone (T) or dihydrotestosterone (DHT) (s.c. silastic implants) for full androgen replacement from 3 weeks, and tissues were collected at 9 weeks of age.

At 3 weeks of age, there was no difference in trabecular bone volume (BV/TV) between wt (3.1± 0.4%) and untreated *hpg* mice (3.4± 0.4%), confirming that androgens do not determine pre-pubertal trabecular bone mass. By 9 weeks of age, however, BV/TV had increased in wt, while untreated *hpg* mice had significantly lost BV/ TV (5.1± 0.8 vs. 0.8± 0.2; p< 0.05 vs. wt at age 3 and 9 weeks). Already at 3 weeks of age, osteoid volume and thickness, and osteoblast surface were all significantly lower in *hpg* than in wt mice (ObS/BS: 29.1± 2.9 vs. 40.8± 1.3), while osteoclast surface was significantly higher in *hpg* than in wt mice (32.3± 3.3 vs. 23.2± 1.6). Notably, BV/TV in 9 week old *hpg* mice was equivalent to levels in 9 week old wt mice orchidectomised at 3 weeks of age (0.5± 0.2%).

In *hpg* mice, both T and the non-aromatisable androgen DHT maintained BV/TV (9.3±1.2 & 10.3±1.1%, respectively) by reducing both bone formation and bone resorption.

In conclusion, prepubertal *hpg* mice have structurally normal bones, but impaired bone turnover, leading to pronounced bone loss during maturation. Treatment with T and DHT normalised bone turnover and bone structure, indicating that androgens are able to maintain normal bone metabolism and accrual without aromatisation.

026

GENDER DIFFERENCE IN THE NEUROREGULATION OF BONE MASS: EVIDENCE FROM THE NEUROPEPTIDE Y Y2/Y4 DOUBLE KNOCKOUT MOUSE.

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Neuropeptide Y (NPY) Y2 receptor (Y2) regulates cancellous bone formation in the mouse by a central mechanism. Elevated circulating levels of pancreatic polypeptide, the major ligand for NPY Y4 receptor (Y4), implicated the Y4 in the Y2 knockout (KO) bone phenotype.

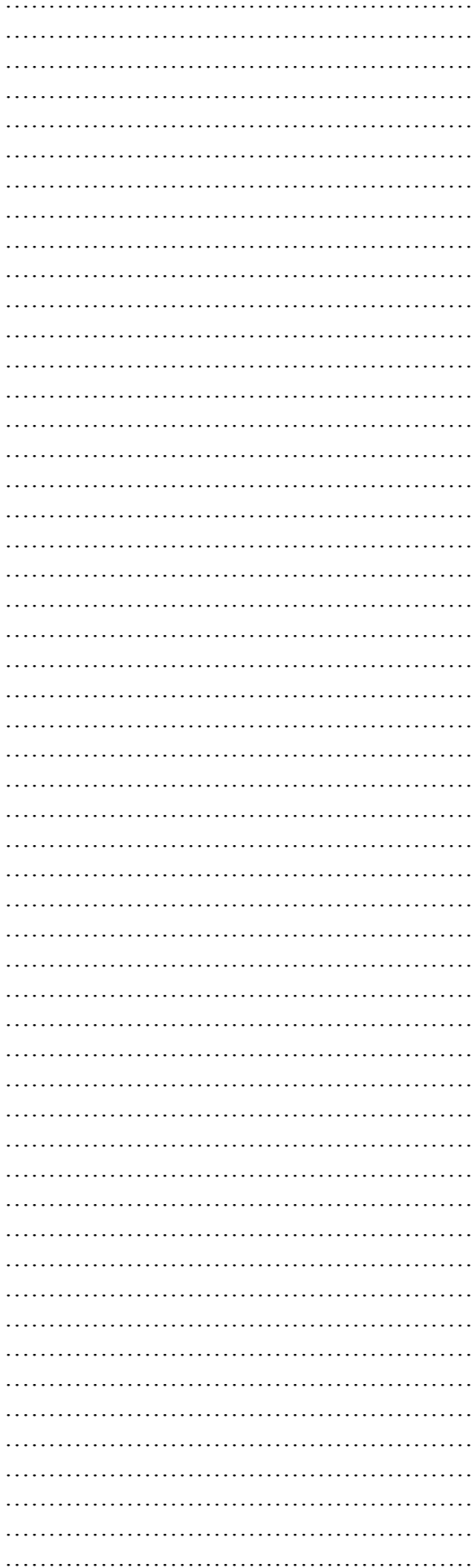
This study compared the effect of NPY Y2 KO, Y4 KO and Y2/Y4 double KO on bone mass in male and female mice. Femora from mature knockout and wildtype mice were examined. Values are mean (SEM).

Y4 KO in male mice did not affect cancellous or cortical bone mass. BV/TV [%] in Y4 KO and wildtype mice were 8.2 (1.2) and 6.6 (1.4). Both these values were lower than in Y2 KO mice 12.4 (1.7). Cortical area [mm²] in Y4 knockout 1.1 (0.1) did not differ from wildtype 1.2 (0.1) or Y2 KO mice 1.1 (0.1).

Y2/Y4 KO in male mice produced a synergistic increase in cancellous bone. BV/TV in Y2/Y4 KO mice 17.4 (2.1) was elevated compared to Y4 KO 8.2 (1.2) and Y2 KO 12.4 (1.7), (p< 0.06). In contrast, cortical area was reduced in Y2/Y4 KO 0.92 (0.03) compared to Y4 KO 1.12 (0.04) and Y2 KO 1.09 (0.05).

Interestingly, in female Y2/Y4 KO mice, there was no synergistic increase in BV/TV, with Y2/Y4 mice 10.6 (0.6) not different from Y2 KO females 10.8 (2.1), but with both greater than wildtype 5.9 (0.3). Moreover, there were no sex differences in Y2/Y4, with cortical area 0.91 (0.2), femoral length [mm] 16.0 (0.1) and mid-femoral circumference [mm] 5.2 (0.1) similar in wildtype and both male and female Y2/Y4 KO mice 0.92 (0.03), 16.0 (0.1) and 5.2 (0.1), despite being present in Y2KO and Y4KO.

Thus, although the Y4 pathway does not appear to independently regulate bone mass, there is an interaction in males between the Y2 and Y4 pathways to synergistically reduce cancellous bone volume and increase cortical bone mass. These data further suggest an interaction between the control of bone mass by sex hormones and the NPY pathways.



O27

CHARACTERISATION OF AN INACTIVATING MUTATION IN OSTEOPROTEGERIN

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Previously we have reported our genetic study of a family with Familial Idiopathic Hyperphosphatasia. We identified a mutation in the gene for osteoprotegerin, the deletion of an aspartate residue at position 182. Recombinant wildtype (wtOPG) and mutant (OPGΔ182) OPG were produced in HEK293 cells. Unlike wtOPG, OPGΔ182 was unable to inhibit bone resorption in neonatal murine calvarial organ cultures (1). Subsequently, we tested the peptides in a murine bone marrow osteoclastogenesis system - wtOPG inhibited osteoclast formation (p<0.0001); whereas OPGΔ182 did not.

We have investigated the RANKL binding ability of wtOPG and OPGΔ182. Using both a rRANKL-GST pulldown system and the surface plasmon resonance technology of BIAcore™, we have demonstrated OPGΔ182 cannot bind with RANKL to the same extent as wtOPG. A kinetic study using BIAcore™ has revealed the RANKL binding affinity of OPGΔ182 is much lower than wtOPG (Kd: OPG; 6.24nM; OPGΔ182; 27.0nM).

OPGΔ182 is hyperglycosylated (1). We have determined glycosylation is important for the function of full-length OPG. Cells expressing wtOPG were treated with tunicamycin, a glycosylation inhibitor. This non-glycosylated OPG could not inhibit bone resorption when tested in murine neonatal calvarial organ culture. Using site-directed mutagenesis we are elucidating the sites of hyperglycosylation in OPGΔ182. Initial results indicate both sites at residues N178 and N183 are hyperglycosylated in OPGΔ182.

Our study highlights the effects of a single amino acid deletion on the structure and function of OPG. Also, it confirms the crucial role of OPG in normal bone physiology in humans.

(1) Cundy et al. Hum Mol Genet 2002; 11(18):2119-2127

O28

ANABOLIC EFFECTS OF LACTOFERRIN AND RELATED MOLECULES ON BONE

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Lactoferrin (Lf), an 80-kDa glycoprotein that belongs to the transferrin family, binds 2 atoms of iron per molecule of Lf. Lf is found in high concentrations in human colostrum and milk, and in other body fluids secreted from glandular epithelia. Lf circulates at 2-7 µg/ml and has anti-microbial and anti-cancer activities as well as roles in the regulation of iron metabolism and embryonic development. We have recently discovered that human and bovine Lf are anabolic to bone. Lf potently stimulates proliferation of cultured osteoblastic cells of rodent and human origin. Lf strongly inhibits osteoclastogenesis but does not affect the bone-resorbing activity of mature osteoclasts. Unilateral local hemicalvarial injection of Lf to adult mice results in substantial increases in the dynamic histomorphometric indices of bone formation and bone area.

We have also found that Lf acts on osteoblast differentiation and apoptosis. Lf significantly increases the number of mineralized bone nodules in long-term osteoblast cultures. It also inhibits apoptosis induced by serum withdrawal in primary rat osteoblasts.

We have assessed the importance of iron delivery by Lf in its proliferative effect on osteoblasts. If the Lf is stripped of iron and re-loaded with chromium or manganese ions, the osteogenic activity is similar to the iron-loaded molecule, implying that the iron itself is not crucial for Lf mitogenic activity in osteoblasts. Further, transferrin, an important iron transporter, is not a potent mitogen on osteoblasts. Lactoferricin, a short N-terminal peptide of Lf that is iron-free, has only a modest osteogenic effect. Taken together, these data demonstrate that iron transport is not required for the potent anabolic effects of Lf on bone.

O29

INHIBITION OF CHAPERONIN HSP90 STIMULATES OSTEOCLASTOGENESIS IN VITRO AND INCREASES BONE DESTRUCTION BY INVADING BREAST CANCER CELLS.

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17-allylaminogeldanamycin (17AAG) is an Hsp90 inhibitor that impairs tumour growth in various mouse models and is undergoing clinical trials. However, 17AAG effects on tumour metastases in bone have not been investigated. We studied this with a nude mouse model employing intracardiac inoculation by MDA-MB-231 human breast cancer cells, which results in direct seeding of tumour cells in bone. In mice treated with 17AAG (70mg/kg/day) the size and incidence of osteolytic lesions caused by the tumour cells (observed by Faxitron x-ray analysis) were greatly increased compared to controls. 17AAG treatment of mice without tumour challenge did not detectably affect femoral bone microarchitecture

To investigate whether 17AAG increases tumour-associated bone destruction by stimulating osteoclast formation we studied its effects in vitro. 17AAG enhanced osteoclast formation 2.5- to 4-fold in bone marrow/osteoblast co-cultures (stimulated by 1,25 dihydroxyvitamin D3 and prostaglandin E2) and RANKL stimulated bone marrow or RAW264.7 cells. Other Hsp90 inhibitors, radicicol and herbimycin A showed similar effects on osteoclast formation though they and 17AAG had little effect on osteoclast survival or bone marrow macrophage survival and proliferation.

Our data suggest Hsp90 inhibitors powerfully stimulate osteoclast formation by action on osteoclast progenitors. This may underlie the increased osteolysis in our in vivo model. 17AAG may not affect normal bone turnover due to regulation by homeostatic mechanisms, regulation which may be undermined by tumour invasion.

Some of the work in this abstract has been submitted to IBMS/IBMR Osaka meeting

O30

MONOCYTIC CELLS INDUCE AN OSTEOCLASTOGENIC PHENOTYPE IN HUMAN OSTEOBLASTS

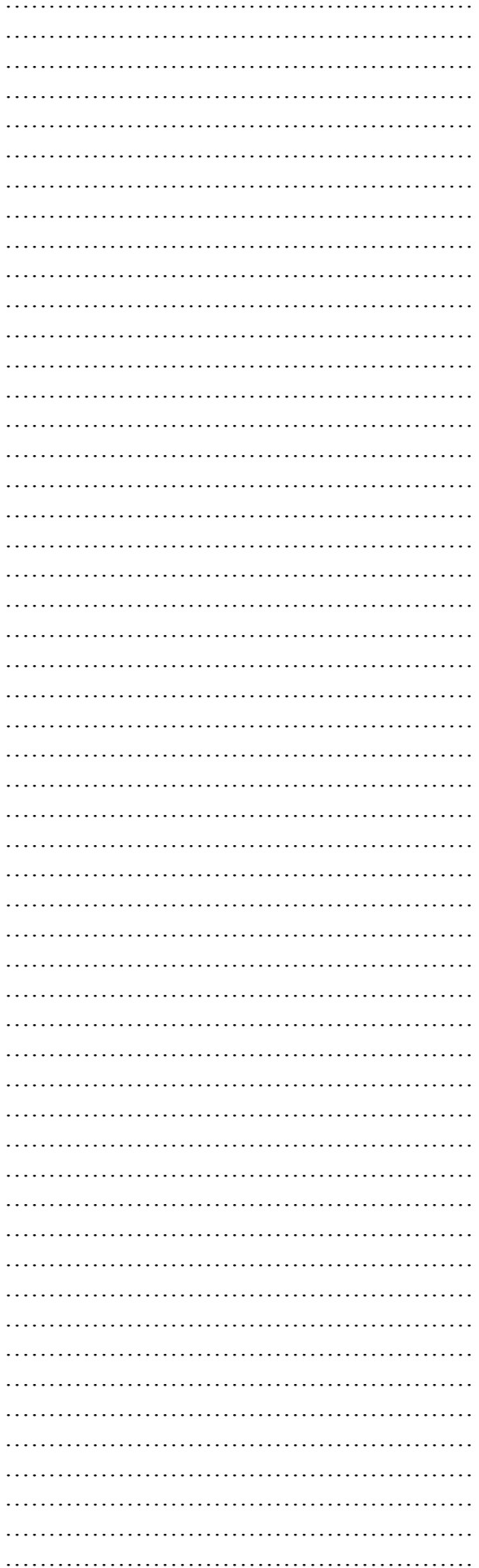
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Normal human bone-derived cells (NHBC) are a mixed population of osteoblast-like cells, defined in terms of their osteogenic phenotype using the pre-osteoblast cell surface marker STRO-1 and the mature osteoblast marker, alkaline phosphatase (AP). We have used these markers to examine the dual functionality of the human osteoblastic stromal cell in both forming bone and supporting osteoclastogenesis. We have previously established a positive relationship between STRO-1 expression and RANKL expression in response to 1,25(OH)₂vitaminD₃ (vitD₃), suggesting that immature osteoblasts are more responsive to pro-osteoclastic stimuli than mature osteoblasts and thus immature osteoblasts likely participate in osteoclast (OC) formation.⁽¹⁾

We have now used the above model to show that both freshly isolated and M-CSF-treated CD14⁺ peripheral blood mononuclear cells (PBMC) induce an increase in the proportion of STRO-1⁺ NHBC and specifically induce the proliferation of these cells. We have also found that co-culture of CD14⁺ PBMC with NHBC induced the release of the pro-osteoclastic factors IL-6, TNF- α and PGE₂, and an increase in the expression of RANKL mRNA. The phenotypic effects of PBMC on NHBC could be mimicked to varying degrees by the addition of recombinant TNF- α , IL-6 or IL-1, and could be partially reversed by the addition of indomethacin. Together, our results suggest that CD14⁺ monocytic cells induce a pro-osteoclastogenic phenotype in stromal osteoblasts, which in turn support the formation of osteoclasts.

Atkins GJ, et al. J Bone Miner Res, In Press.



O31

VENOUS SAMPLING FOR FGF-23 CONFIRMS THE DIAGNOSIS OF AND LOCATES THE RESPONSIBLE TUMOR FOR TUMOR-INDUCED OSTEOMALACIA (TIO)

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TIO is a paraneoplastic disease that causes severe muscle weakness. Although TIO can be cured by resection of the responsible tumor, it is sometimes very difficult to find the causative one. We have cloned FGF-23 as a responsible factor for TIO. We have also shown that serum FGF-23 was high in a patient with TIO and rapidly decreased after resection of the tumor. However, because serum FGF-23 is high in most patients with X-linked hypophosphatemic rickets/osteomalacia that has similar clinical features to TIO, high FGF-23 alone cannot confirm the diagnosis of TIO. In order to establish a diagnostic method of TIO, we tried venous sampling for FGF-23. A 37-year-old man presented typical features of hypophosphatemic osteomalacia. He had noticed the tumor in the right inguinal lesion for 17 years. Although TIO was strongly suspected, it was not certain whether this tumor was responsible for his disease. We obtained venous blood from all major veins in the body and measured FGF-23 by previously described ELISA. There was about 3-fold step-up of serum FGF-23 level between right femoral vein (79 pg/ml) and right common iliac vein (219 pg/ml), a 25% step-down after the merge of both common iliac veins (168 pg/ml), and a further decrease beyond junction of both renal veins (79 pg/ml). After removal of the tumor, serum FGF-23 rapidly decreased to undetectable level with a half-life of 22 minutes. These results suggest that endogenous FGF-23 production is suppressed and the responsible tumors for TIO are the only sources of FGF-23 in patients with TIO. Thus, venous sampling for FGF-23 confirms the diagnosis of and helps to locate the responsible tumor for TIO.

O32

INCREASED EXPRESSION OF RANKL AND IL-6 IN HUMAN OSTEOPOROTIC FEMORAL FRACTURES

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Natural menopause is associated with a rapid decline in estrogen, and has recently been associated with changes to the pro-inflammatory cytokine IL-6. However, the mechanism of post-menopausal and age related bone loss remains controversial, as does the gene expression of pro-resorptive molecules in the osteoporotic (OP) bone microenvironment. The aim of this study was to investigate, in control and OP individuals, the expression patterns of bone-related genes, including the recently identified osteoclastogenic molecules, RANKL and RANK, and IL-6. Cancellous bone cores from the human proximal femur were obtained from 22 autopsy cases (11 females, 11 males; median age 71.5 years) with no evidence of skeletal pathology, and 14 patients (9 females, 5 males; median age 81.5 years) undergoing surgery due to sub-capital OP hip fracture. Total RNA was extracted, and semi-quantitative RT-PCR was performed, with normalisation of results according to the expression of GAPDH. Non-parametric statistics were used and the data reported as the median [25th and 75th percentiles]. Striking differences in the gene expression pattern of key osteoclastogenic factors have emerged. OP bone showed significantly elevated RANKL (1.6 [1.3-3.4] > 1.0 [0.6-1.5]; p<0.05), RANK (2.9 [2.0-2.8] > 1.6 [0.5-1.8]; p<0.009) and IL-6 mRNA expression (3.9 [1.8-6.2] > 0.8 [0.7-1.8]; p<0.003). Moreover, RANKL mRNA expression was significantly age dependent in OP females (r=0.68, p<0.02), suggesting a link between RANKL expression and estrogen deficiency. Importantly, IL-6 mRNA levels associated strongly with RANKL mRNA levels in the fracture group (r=0.77, p<0.001), suggesting that IL-6 could act as an upstream positive regulator of RANKL expression in OP bone, or could work with RANKL to increase osteoclast resorption. Collectively, our data suggest that the changes in OP bone that predispose it to fracture are associated with an increase in expression of RANKL and IL-6 mRNA, consistent with the hypothesis that altered levels of pro-osteoclastogenic and inflammatory factors contribute to the deterioration of bone in osteoporosis.

O33

TRABECULAR BONE ARCHITECTURE IN PRIMARY HIP OSTEOARTHRITIS

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Epidemiological studies suggest that there is a negative association between primary osteoarthritis (OA) and osteoporosis. Recently, elevated BMD has been shown to associate with a subsequent accelerated joint space narrowing rate.¹ The aim of this study was to examine trabecular bone architecture in primary hip OA, and to investigate a relationship between trabecular structure in OA and the ratio of RANKL/OPG mRNA expression. Femoral heads, intertrochanteric (IT) tube saws, and iliac crest biopsies, were obtained from 28 patients with primary OA at total hip arthroplasty surgery (9 males, 19 females, mean age 72 [60-83] years). Proximal femurs and iliac crest wedge samples from 69 non-OA controls were taken at autopsy ($n=37$, aged <50 years, 20 males, 17 females, 27 [10-49] years; $n=32$, aged >50 years, 9 males, 23 females, 73 [57-95] years). Undecalcified bone histology was prepared to histomorphometrically determine trabecular bone volume (BV/TV[%]), at three proximal femoral regions and at the iliac crest, and static indices of bone turnover, at the IT region. For 12 OA and 11 control cases, total RNA was isolated from half of each IT bone sample for semi-quantitative RT-PCR analysis of RANKL and OPG mRNA. At all four skeletal regions, both the controls aged <50 years and the OA group had mean BV/TV values greater than the controls aged >50 years, consistent with the generalised maintenance of bone volume in OA. The combination of histomorphometric and molecular analyses at the IT region, *molecular histomorphometry*, revealed strong associations between the RANKL/OPG mRNA ratio with BV/TV, erosion surface, and osteoid surface, in the control bone samples.² Intriguingly, these associations were not found in OA bone, suggesting that bone turnover may be regulated differently in OA. If primary OA is caused or exacerbated by altered bone structure, a *molecular histomorphometry* approach may identify ways to modify or prevent the bone changes and thus delay joint degeneration. ¹Goker *et al.* (2000) *J Rheum* 27:735-8; ²Fazzalari *et al.* (2001) *JBMR* 16:1015-27.

O34

CONTRIBUTION OF THE VITAMIN D RECEPTOR AND COLLAGEN I ALPHA 1 GENES TO HIP FRACTURE RISK

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The risk of hip fracture risk is partly determined by genetic factors. Polymorphisms of the vitamin D receptor (VDR) and collagen 1 α 1 (COLIA1) genes have been associated with bone mineral density (BMD), and with fracture risk. It is not clear whether the two genes have additive effects on hip fracture risk. This study examines the contribution of VDR and COLIA1 genotypes to the liability to hip fracture in postmenopausal women.

Genotypes of the VDR (*TT*, *Tt*, *tt*) and COLIA1 (*SS*, *Ss*, *ss*) genes were determined in 677 (69 hip fracture and 608 non-fracture) women of Caucasian background, aged 70 \pm 7 (mean \pm SD), who were participants in the Dubbo Osteoporosis Epidemiology Study, Australia. Women with the *tt* genotype was significantly higher in the hip fracture group (26%) compared to those in the non-hip fracture group (14.4%; $p = 0.03$). Moreover, women with the *ss* genotype were over-represented in the hip fracture (10%) compared to those in the non-fracture group (4.4%; $p = 0.04$). After adjusted for age and femoral neck BMD in a multiple logistic regression model, women with *tt* genotype had a 2.6-fold (95% CI: 1.2 to 5.3), and women with *ss* genotype were associated with a 3.8-fold (95% CI: 1.3 to 10.8) increase risk of hip fracture. Approximately 33% of the liability to hip fracture was attributable to the presence of the *tt* genotype of the VDR gene and *ss* genotype of the COLIA1 gene.

Caucasian women with VDR *tt* genotype and COLIA1 *ss* genotype were associated with increased hip fracture risk, and the association was independent of BMD and age.

O35

HOW MANY FRACTURES IN MEN ARE ATTRIBUTABLE TO LOW BONE DENSITY

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In the elderly population, approximately one third of osteoporotic fractures occur in men. Among many risk factors for fracture, low bone mineral density (BMD) is considered an important determinant. However, it is not known how many fractures are attributable to low BMD. This study was designed to estimate the proportion of fractures attributable to low BMD in a sample of 821 men (average age: 70 yr, range: 60 to 92 yr) who have been participating in the Dubbo Osteoporosis Epidemiology Study since mid-1989.

Approximately 11% and 21% of the men had femoral neck BMD T-scores<-2.5 and T-scores<-2.0, respectively. During the 13-year follow-up period, 118 men sustained a symptomatic low trauma fracture, including femoral neck (n=26), vertebrae (n=43), and distal radius and humerus (n=12). Approximately 23% and 34% of those with fracture had femoral neck BMD T-scores<-2.5 and T-scores<-2.0, respectively. The risk of fracture increased 3-fold (95% confidence interval [CI]: 1.8 - 5.0) among those with T-scores<-2.5, and 2.2-fold (95% CI: 1.4 - 3.4) among those with T-scores<-2.0. Between 18% and 20% of all fractures in elderly men were attributable to low BMD. The BMD-attributable risk was 38% for hip fractures, 41% for symptomatic vertebral fractures, and 34% for distal radius and humerus fractures.

In these older men, low BMD was a strong predictor of fracture risk, but still accounted for only a modest proportion of fractures. The majority of fractures occurred in men with BMD T-scores>-2.5. The data imply that current cut-off values of BMD for the diagnosis of osteoporosis in men are inappropriate.

O36

CORTICAL BONE INSTABILITY OF HIP FRACTURE CASES COMPARED TO AGE AND SEX MATCHED CONTROLS

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This *ex vivo* study tested the hypothesis that femoral neck fracture cases were more vulnerable to failure due to cortical bone instability (local buckling), than age and sex matched controls. The biopsies were from (n = 21, F) subjects that had suffered an intracapsular hip fracture. The control material (n = 29, F) was from post-mortem subjects. Serial Peripheral Quantitative Computed Tomography (pQCT) 1mm thick cross-sectional images using the Densiscan 1000 pQCT clinical forearm densitometer were obtained, and matched for location along the neck. At this site the thickness of the cortical bone is not uniform and is asymmetric, the pQCT images were therefore divided in to eight regions: Superior, Superoanterior, Anterior, Inferoanterior, Inferior, Inferoposterior, Posterior and Superoposterior. The local buckling ratio was derived from each region's mean distance from the cross-section's centroid divided by each region's mean thickness. The local buckling ratio for the mid cross-section was the mean of the regions' ratios.

The results show that the fracture cases are more vulnerable to failure by local buckling than the controls (Cases: median 14.12, interquartile range(IQR):11.9 - 16.4; Controls: median 10.63, IQR: 8.7-14.3; p < 0.004)

This study suggests that biomechanical analysis of the distribution of bone within the femoral neck, using Computed Tomography, may offer a marked improvement in the ability to discriminate patients with an increased risk of intracapsular fracture.

O37

CORTICAL BONE EFFECTS ASSESSED BY HIP STRUCTURAL ANALYSIS: A CO-TWIN CALCIUM INTERVENTION

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A high dietary calcium intake is widely recommended for children to enhance peak bone mass. The mechanism(s) of calcium effects on bone during growth are uncertain and influences on both remodelling and modelling have been proposed. We conducted a randomized, placebo-controlled trial of calcium supplementation (1200mg daily) in female twins aged (mean ± SD) 10.4 ± 1.4 years at baseline. Bone properties were evaluated by Hip Structural Analysis (HSA) of proximal femur densitometry scans in 33 twin pairs (18 monozygotic, 15 dizygotic) up to 18 months intervention. HSA parameters were measured 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. Height and weight did not differ between groups at any time point. By 18 months at the FS site, there were significant differences in percent change from baseline as follows comparing placebo vs calcium-treated twins, respectively: areal bone mineral density (ABMD) (+15.8% vs +19.9%, p = 0.019), subperiosteal width (+9.7% vs +7.1%, p = 0.038), endocortical diameter (+7.7% vs +2.5%, p = 0.015), average cortical thickness (+17.2% vs +22.8%, p = 0.015) and average buckling ratio (-5.1% vs -11.6%, p = 0.014). No consistent or major effects were seen at the NN and IT sites. Therefore, observed effects were limited to cortical bone at the femoral shaft where there was inhibition of modelling at both endosteal and periosteal surfaces, resulting in narrowing of the femoral shaft and a thicker cortex. Our findings compliment the sparse body of literature that, importantly, investigates the geometric adaptation of growing bone to lifestyle factors.

O38

HUMAN LACTATION: CHANGES IN HIP BONE GEOMETRY BUT NOT BONE DENSITY DEPEND ON CALCIUM INTAKE

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Full human lactation (lac) delivers 5-8 mmol/d of milk Ca. Maternal Ca homeostasis theoretically taps; (i) increased dietary Ca (+/- incr. Ca gut absorption efficiency [Fa; ~0.25-0.3]); (ii) decreased urinary Ca excretion; (iii) bone mass loss. We and others showed (ii) & (iii) are relevant; maternal DXA areal (a) BMD decreases (eg; 0-6% in hip) at peak lac (~6 mo postpartum [pp]), with postweaning (pw) recovery (1), but dietary Ca affects neither bone loss nor Fa (eg 2). Unlike aBMD, Hip Structural Analysis (HSA; 3) describes bone structural geometry, including *section modulus* (Z; a strength index). This study applied HSA to human lactation.

From 100 pregnant women at 36 wk, randomised to Ca (25mmol/d) and studied through lac to 12mo pw, those N=48 with Fa measured (2xstable Ca isotopes; 2) at peak lac were divided into tertiles of "Abs Ca" = {(Dietary Ca +/- 25mmol Ca x [supplement tablet compliance]) x Fa} (tertile means 6.6, 11.8 & 15.8 mmol/d). % changes (Δ%; = peak lac minus 2wk pp) in BMC, aBMD & HSA, at the femoral neck (FN), intertrochanter (IT) and shaft (Sh) were compared between tertiles. %Δ for 6-12 mo pw minus peak lac (N=30) were also analysed by tertiles of "Abs Ca".

Main findings; (i) Δ%"narrow neck" Z=6.3% (p<0.01) by peak lac, with NS between tertiles & NS recovery pw; (ii) Δ%ITZ unchanged at peak lac but increased by pw (1.9-5.3%, p<0.05); (iii) middle-tertile ShZ decreased (2.4%, p<0.05) at peak lac (flanking tertiles NS), tending towards recovery, pw. Bimodal response of ShZ arises from a *thresholded* incr. in Sh bone width, *only* at high Ca (1.4%, p<0.05), but a *linear* increase in Sh endosteal diameter with Ca (1.2 – 3.9%, p<0.05). (iv) aBMD fell by peak lac at all sites except troch., with part recovery by pw. No Ca-dep. differences in Δ%aBMD. **Conclusion:** lactation-induced changes in Z, but not aBMD, are Ca-intake dependent. ITZ *increase* by postweaning suggests possible site-selective long-term biomechanical bone protection. 1. Kent GN *etal* 1993 OI S1:44-7; 2. Kent GN *etal* 1991 CTI 48:293-5; 3. Beck TJ *etal* 2000 JBMR 15:2297-2304

O39

A NEW INDICATOR OF SPINAL STRUCTURAL FAILURE. Zebaze RMD¹, G Maalouf², E Seeman¹. 1. Austin and Repatriation Medical Centre, University of Melbourne; 2 Saint George University Hospital, Beyrouth, Lebanon.

A method for reliable assessment of spinal osteoporosis is urgently needed before credible comparisons across epidemiological studies can be made.

Current studies of osteoporotic fractures in the spine focus on the shape of the vertebral body to define 'fracture'. However, it is the spinal column that functions as a single anatomical unit. True fracture (ie structural failure) should lead to irregularity of the curvature of the whole spine while anatomical variants should not. We captured the spinal curvature irregularity in a single continuous variable (SCI). A critical value was determined to define a new notion of spinal fragility fracture (SFF) in contrast to fracture due to other causes. BMD and vertebral heights were measured using dual x-ray absorptiometry in 697 Lebanese women aged 20 to 87. Deformities were assessed according to quantitative vertebral morphometry criteria using cut-offs of 15, 20, 30%, 3SD and 4SD. The SCI increased steeply after (but not before) 50 years of age and was correlated with height loss (r=0.23), BMD (r=0.18), and the number of deformities (0.35-0.58). The prevalence of SFF was 0.8% in women under 50 years of age and increased exponentially, reaching 17% after age 80 (r=0.97). Women with SFF had 3 to 9 times more deformity than those with none, lower BMD (-0.67 SD) and reduced height (-0.4 SD). Women with SFF but no deformity had reduced BMD and height but not women with deformity and no SFF. SCI is easy to compute. It is a sensitive and specific indicator of spinal fragility and structural failure and should be extremely useful in the investigation of spinal osteoporosis.

O40

A STUDY OF PROPOSED DOWN STREAM EFFECTS OF LRP5 IN BENIGN HUMAN OSTEOPETROSIS J Bollerslev^{1,2} and T Ueland¹. ¹Section of Endocrinology, National University Hospital, Oslo, Norway and ²School of Medicine and Pharmacology, University of Western Australia, Perth, WA.

Osteopetrosis is a heterogeneous group of inherited metabolic disorders characterised by reduced osteoclast number or function, leading to impaired bone resorption with increased bone mass. Benign osteopetrosis is inherited in the autosomal dominant way (ADO). At least two different forms of ADO have been described, both showing diffuse, symmetrical osteosclerosis, however with well defined clinical, biochemical and histological differences. We have recently related the two disorders to mutations in the LRP5 gene for ADO I [1] and to the CICN7 gene in ADO II [2]. Whereas ADO II resembles other human osteopetroses with multiple large, multinucleated osteoclasts, ADO I is osteoclastopenic like murine mutations characterised by developmental defects in the bone resorptive lineage.

In this study, biochemical markers of bone turn-over including markers of proposed down stream effects of LRP5 was investigated at baseline and following stimulation with thyroid hormone in patients with ADO I (n=8) and ADO II (n=9). Compared with normal controls (n=10), both types showed unremarkable levels of osteocalcin and NTx, whereas serum levels of TGFβ1 were significantly increased and Fibronectin levels decreased in both types. Serum level of OPG was slightly increased in ADO I compared to controls, however highly significantly increased compared to ADO II.

While a causative role for the mutation in the first propeller of the LRP5 gene is indicated in ADO I, the functional significance as related to disturbed bone resorption is still not well understood.

Van Wesenbeeck L, Cleiren E, Gram J, Beals RK, Benichou O, Scopelliti D, Key L, Renton T, Bartels C, Gong Y, Warman ML, De Vernejoul MC, Bollerslev J, Van Hul W (2003). Am J Hum Genet; 72: 763-71.

Cleiren E, Benichou O, Van Hul E, Gram J, Bollerslev J, Singer FS, Beaverson K, Aledo A, Whyte MP, Yoneyama T, deVernejoul M-C, Van Hul W (2001). Hum Mol Gen; 10: 2861-7.

O41

WHEN IS AN ANKLE FRACTURE AN OSTEOPOROTIC FRACTURE? JR Center, TV Nguyen, KP Chang, JA Eisman. Bone and Mineral Research Program, Garvan Institute of Medical Research, Sydney, NSW Australia.

Ankle fracture is not considered an osteoporotic fracture. However, a history of a low trauma fracture is a major risk factor for a subsequent fracture. The aim of this study was to examine whether a history of ankle fracture was a risk factor for future fracture. Data from 821 men and 1286 women aged 60+ years, who were participants of the Dubbo Osteoporosis Epidemiology Study were analysed. During 13 years of follow-up (April 1989 to May 2002), 30 women and 16 men had sustained an ankle fracture and 431 women and 152 men had sustained a non-ankle fracture.

	No fracture	Non-ankle fracture	Ankle fracture
Women			
Age (yrs)	70 ± 6	73 ± 8 ^b	74 ± 7 ^b
BMD (g/cm ²)	0.81 ± 0.12	0.71 ± 0.13 ^b	0.77 ± 0.09 ^{b,c}
RR ^a (95% CI)	1	2.9 (2.4, 3.5)	0.9 (0.3, 2.4)
Men			
Age	70 ± 6	73 ± 7 ^b	69 ± 6 ^c
BMD	0.93 ± 0.15	0.83 ± 0.16 ^b	0.95 ± 0.17 ^c
RR	1	3.6 (2.4, 5.2)	6.9 (3.4, 14.2)

^aRelative risk for subsequent fracture; ^bp<0.05 for comparison with non fracture; ^cp<0.05 for comparison with non-ankle fracture.

Thus women and men with ankle fractures behaved differently. Analysis of the type of fracture classified as "ankle" may shed some light on these differences. In women, ankle fractures were not a risk for future fractures, yet women with ankle fractures were older and had lower BMD than those without fracture. Although the men resembled those who had not sustained a fracture, an ankle fracture was a predictor of future fracture.

O42

EXERCISE REGIMES IN PRE- AND EARLY PUBERTAL BOYS: LIMITATIONS OF LONG TERM EXERCISE INTERVENTIONS

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We have previously reported that 8.5 months of moderate impact exercise combined with 400 mg/d of increased dietary calcium resulted in a greater osteogenic effect at the loaded sites than either intervention alone in both boys and girls.^{1,2} Greatest effects were evident in those children with lowest levels of participation in organised sport. The questions asked were: i) Did an additional 8 months of exercise increase bone mass? ii) Did calcium supplementation in phase 1 influence the effect of exercise in phase 2? In phase 1, boys aged 9.0 (1.1) yrs (mean (SD)) of Tanner stages 1 or 2 were randomly assigned to either moderate impact exercise (with or without calcium) or control (with or without calcium). In phase 2, 71 of the original 89 boys continued with their randomised assigned exercise allocation in the following school year without the additional calcium: 29 exercisers (14 calcium and 15 placebo in phase 1) and 38 controls (14 calcium and 24 placebo in phase 1) completed at least 1 session per week (mean 1.7 and 1.4 sessions per week respectively). Regional BMC, anthropometry, sexual maturity, dietary calcium intake using a 24 hour recall and FFQ and physical activity levels were assessed at the beginning and end of the first year and the end of the second year. ANOVA was used to determine the effect of exercise. Effect modification by calcium supplementation in phase 1 on the effect of exercise in phase 2 was examined by the inclusion of an interaction term in the ANOVA. A greater proportion of the controls participated in high impact sports than the exercise group (67% vs 40%). In phase 2, there were no differences between the groups, nor was there any effect modification by previous calcium supplementation. In this subgroup there was no evidence of an exercise or calcium effect in phase 1. In summary, there are several factors that appear to have affected the success of this program, including compliance, levels of incidental exercise and difficulties associated with conducting long-term exercise interventions. In conclusion, these results have implications for the delivery of public health programs aimed at improving bone health in children through exercise. ¹Iuliano et al JBMR 2003;18:1,156-62 ² Bass et al JBMR 2002; 17:1,S294

O43

INTRAVENOUS PAMIDRONATE OR ORAL ALENDRONATE FOR THE TREATMENT OF PAGET'S DISEASE OF BONE: RESULTS OF A RANDOMISED CONTROLLED TRIAL.

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Second and third generation bisphosphonates are the treatment of choice for Paget's disease of bone. These drugs are known to be more effective than calcitonin and etidronate, but there have been no randomised controlled trials comparing the efficacy of different second or third generation bisphosphonates.

We conducted a 2 year, randomised, open label trial comparing intravenous pamidronate with oral alendronate in 72 subjects with Paget's disease. Randomisation was stratified according to baseline plasma alkaline phosphatase (pAP) and previous pamidronate treatment. Assigned treatments were pamidronate 60 mg every 3 months or alendronate 40mg daily in 3 month blocks, continued until full biochemical or until a clear plateau effect was observed. At 1 year, non-responders to pamidronate were crossed over to alendronate treatment.

Of subjects who completed the trial and were randomised to alendronate, 97% achieved full biochemical remission compared to 59% for pamidronate (P<0.001). In previously untreated patients, alendronate resulted in full remission in 100% of subjects compared with 85% of pamidronate-treated subjects (P=0.23). In patients who previously had been treated with pamidronate, alendronate treatment resulted in complete remission in 92% of cases, and pamidronate in only 17% (P<0.001).

We conclude that, in the doses used in this study, alendronate has superior efficacy to pamidronate in both previously untreated and APD treated patients with Paget's disease of bone.

O44

EFFECTS OF HABITUAL CALCIUM INTAKE AND PHYSICAL ACTIVITY ON BONE MASS IN ELDERLY WOMEN. A Devine, IM Dick, SS Dhaliwal, J Bollerslev, RL Prince. School of Medicine & Pharmacology, UWA; Dept. Endocrinology and Diabetes, SCGH

In a population-based sample of 1499 elderly women, using a cross sectional study design, we examined the relation between calcium consumption (CC), physical activity (PA) and bone mass. These women were on average 75±3y and weighed 69±12kg. The outcome variables were hip bone mass (BMD) measured using DXA (Hologic 4500A) (n=1115) and heel bone mass (SOS) measured by quantitative ultrasound (QUS, Lunar Achilles) (n=1435). The independent variables were CC (mg/day) measured by a validated habitual food frequency questionnaire and PA (kJ/day) measured by a validated activity questionnaire assessing hours of activity per week.

Quintiles of PA and CC on bone mass were examined using oneway ANOVA to define groups. The population was dichotomised according to whether CC and PA were above or below 1100 mg/day and 80 kJ/day (equivalent to 2 hours walking per week) respectively. High PA was associated with increased BMD and SOS after adjustment for weight [Mean (95%CI)] total hip BMD (mg/cm²) 794 (784-804) vs 821 (813-829); SOS (m/s) 1509 (1507-1511) vs 1515 (1513-1516)]. After adjustment for weight, high CC was associated with increased BMD at all hip sites, except the intertrochanteric site [Mean (95%CI) total hip (mg/cm²) 807 (800-814) vs 827 (813-842)]. No difference was observed for QUS parameters. Entering both CC and PA into an ANOVA model indicated that the effects on total hip BMD were independent. The effect of high CC and PA were additive. [Mean (95%CI) low CC low PA 794 (782-805); low CC high PA 816 (807-825); high CC low PA 798 (773-823); high CC high PA (825-860)]. Thus elderly women who consume more than the RDI for calcium have a higher hip BMD but not QUS. Activity equivalent to 2 hours walking per week or greater improves both BMD and QUS regardless of CC. The combination of high PA and CC significantly increases hip and heel bone mass.

O45

BETA-ADRENERGIC BLOCKERS REDUCE THE RISK OF FRACTURE PARTLY BY INCREASING BONE MINERAL DENSITY: GEELONG OSTEOPOROSIS STUDY.

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Animal data suggests that bone formation is under β -adrenergic control and that β -blockers stimulate bone formation and/or inhibit bone resorption. β -blockers used in the management of cardiovascular disease may also reduce bone fragility. We evaluated β -blocker exposure, bone mineral density (BMD) and fracture risk in a population-based, case-control study. β -blocker use and concomitant therapy, including thiazide exposure, were documented by questionnaire for 569 women (age 69.5 ± 9.5 yr) with radiologically-confirmed incident fractures (69 hip, 119 vertebral, 107 Colles' and 318 others) and 775 controls (age 69.2 ± 11.1 yr) without incident fracture.

There were 59 β -blocker users among the fracture cases and 112 among controls (age 72.7 ± 7.6 vs 70.2 ± 10.3 yr, $P=0.08$). β -blocker use was associated with a higher BMD at the total hip (0.19 SD, $P=0.03$) and ultradistal-forearm (0.18 SD, $P=0.04$) after adjustment for age, anthropometry and thiazide use. Odds ratio (OR) for fracture associated with β -blocker use was 0.68 (95%CI 0.49-0.96). Adjusting for age, weight, concurrent medications and lifestyle factors had no effect on the OR. BMD-adjusted OR were 0.76 (0.54-1.06) for the spine, 0.78 (0.55-1.11) for the total hip, 0.72 (0.50-1.02) for the whole body, 0.72 (0.51-1.03) for the ultradistal-forearm, and 0.69 (0.49-0.98) for the mid-forearm.

β -blockers use is associated with a reduction in fracture risk and higher BMD.

O46

DOES HIGH INTENSITY RESISTANCE TRAINING MAINTAIN BONE MASS DURING MODERATE WEIGHT LOSS IN OLDER ADULTS WITH TYPE 2 DIABETES?

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Weight loss improves glycaemic control in persons with type 2 diabetes, but it has also been associated with bone loss in non diabetic adults. While the influence of type 2 diabetes on BMD is equivocal, bone mass is frequently reduced in patients with poor glycaemic control. The aim of this study was to examine the effects of moderate weight loss, with and without high intensity resistance training (RT), on bone mass, body composition and hormonal parameters in overweight ($BMI \geq 27$ kg/m²), sedentary older adults aged 67.4 (5.1) yrs with established type 2 diabetes. All participants (n=36) followed a moderate weight loss program and were randomised to either RT [3/wk@75-85% of 1 RM] (RT+WL, n=19) or placebo exercise [stretching, 3/wk] (WL, n=17) for 6 months. A third non-randomised group served as controls. TB and L2-4 BMC, fat mass (FM) and lean mass (LM) were assessed by DXA at baseline and 6 months. HbA_{1c}, IGF-1, testosterone, estradiol, SHBG and insulin were assessed at baseline, 3 and 6 months. After 6 months, the RT+WL and WL groups had similar reductions in weight (-2.7% vs -3.3%) and FM (-7.4% vs -5.6%) ($p<0.01$), whereas LM increased in the RT+WL relative to WL group (0.9% vs -0.9%, ANCOVA interaction $p<0.05$). TBBMC remained unchanged in the RT+WL group ($0.2 \pm 1.9\%$) but decreased in the WL group ($-1.5 \pm 1.8\%$) (interaction, $p<0.05$). Similar though non-significant results were detected for L2-4 BMC (RT+WL -0.2% vs WL -1.1%). BMC did not change in controls (TB -0.2%; L2-4 -0.6%). These results remained after adjusting for age, height, weight, gender or use of oral hypoglycemic medication. With the exception of HbA_{1c} which decreased in the RT+WL relative to WL group after 3 and 6 months ($p<0.05$), there were no within or between group differences for any of the hormonal parameters. Changes in FM were correlated with changes in L2-4 BMC ($r=0.78$, $p<0.01$) in the WL but not RT+WL group. In conclusion, these results indicate that resistance training should be recommended to older adults with type 2 diabetes during weight loss to maintain both lean mass and bone mass.

O47

CAN WE AFFORD TO TREAT WOMEN WITH OSTEOPOROSIS WITHOUT BASELINE FRACTURES?: GEELONG OSTEOPOROSIS STUDY (GOS)

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This analysis estimates the number of women needed to treat (NNT) and the cost per averted fracture (Fx) using drugs in all women 50 years and over with osteoporosis (OP) compared with treating women with both OP and a previous Fx (incurred after 50 years of age). The cost of therapy for each fracture averted was estimated by assuming treatment cost \$2.00 (Aust D) daily with an immediate 50% reduction in the fracture rate. The proportion of women with OP (T score <-2.5) and Fx since 50 years in the random sample of the Barwon region was used to estimate the burden in the Australian population. The proportion of Fxs averted was estimated using the prevalence of these two risk factors in the 587 women with BMD measured around the time of Fx (median 57 days).

Among women aged 50 to 59 years, only 21% of all Fx occurred in those with OP. If the 9% of the 50-59 y.o population with OP received drug therapy, the NNT to prevent one Fx is 123. This equates to a cost of \$92,000 per averted Fx. If therapy was restricted to OP women with a previous Fx only 1% of this population group would be treated and the NNT reduces to 33 women. By contrast, the presence of both OP and prior Fx in older women has little effect on the cost per averted Fx (\$23,000 vs \$20,000) and the NNT (32 vs 27) as a majority of this older population has OP (58%) and almost half of all Fx occur in women with both OP and prior Fx (43%).

In 'younger' women therapy is cost effective only in those with osteoporosis and Fx. In young women with OP alone, many must be treated to avert one event because of the low absolute risk of Fx. In older women with higher absolute Fx risk, drug therapy is worthwhile in those with osteoporosis alone as well as those with osteoporosis and prior Fx(s).

O48

AUTOLOGOUS CHONDROCYTE THERAPY FOR CARTILAGE INJURY IN RABBITS: A NEW APPROACH OF BIOTHERAPEUTIC REGENERATION

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Osteochondral injury is a clinically relevant orthopaedic condition that is therapeutically irreversible within current treatment parameters. All contemporary therapies have shown no significant long-term durability of induced regenerative tissue. Here we propose that combination autologous chondrocyte therapy may be effective for the restoration of osteochondral lesions. Using a rabbit model of osteochondral injury, we used concomitant treatment of TGFβ3-saturated collagen sponge and Autologous Chondrocyte Implantation (ACI) to stimulate regeneration in osteochondral defects. New Zealand White rabbits were divided into groups (n = 8) treated with TGFβ3-sponge, ACI alone, ACI combined with TGFβ3-sponge, and no treatment (control) at different time points. Collagen membrane and collagen sponge were characterized by SEM and LM to detail their functional microstructure. Samples were scored using a semi-quantitative scoring system of ICRS with modification. The results showed that untreated defects are limited to an eruption of inferior fibrous tissue. Interestingly, ACI groups demonstrated the ability to regenerate osteochondral injury independently, with clearly differentiable osteochondral architecture and high chondrocyte biosynthesis after 6 weeks. TGFβ3-sponge groups induced hypertrabecular new bone formation with poor osteochondral architecture. Combination treatment produced regeneration tissue comprised of hypertrabecular bone and hypercellular cartilage that remodeled over time to produce osteochondral tissue with improved architecture but thinner and less biosynthetically active articular cartilage. In short, our data suggested that long-term stability of induced regeneration tissue may be achieved by the biotherapeutic approach of cell and growth factor therapy.

O49

THE EFFECT OF AGE ON CALCIUM ABSORPTION IN POSTMENOPAUSAL WOMEN

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It is generally thought that calcium absorption falls with age due to a decline in serum 1,25D levels secondary to declining renal function. We measured fractional Ca⁴⁵ absorption (α)^[1], serum 1,25D and 25D, and serum creatinine in 266 untreated postmenopausal women aged 40-94 with normal spine Xrays (n=186) or only one wedged vertebra (n=80) and serum creatinines \leq 0.12 mmol/L. Mean values (SD) analysed by age groups were:

Age (yrs)	40-55	56-65	66-75	76-94
n	48	112	81	25
α (fx/hr)	0.70 (.26)	0.70 (.26)	0.66 (.26)	0.48(.23)
1,25D (pmol/L)	103 (37)	121 (39)	115 (36)	110 (34)
25D (nmol/L)	61 (27)	62 (27)	63 (21)	52 (24)

In the whole set, α fell significantly with age (P=0.010) only because it was significantly lower in those over than in those up to age 75 (P<0.001). Serum 1,25D was no different between those up to and over 75 years (P=0.48) but serum 25D was lower in those over than up to 75, though this was not quite significant (P=0.051). In the whole set, α was significantly related to serum 1,25D (P<0.001) but not to serum 25D (P=0.17) though the two vitamin D metabolites were significantly internally related (P<0.001). In subjects over 75 years, mean α was lower than expected for the serum 1,25D (P<0.001)

We conclude that, in apparently healthy postmenopausal women, calcium absorption does not fall until after age 75. This fall does not reflect a fall in plasma 1,25D and is probably due to a primary defect in intestinal calcium transport, but an independent role for serum 25D cannot be excluded. [1] Nordin *et al.* 1998 J Nucl Med 39:108-113.

O50

DOES CALCIUM FROM MILK MINERALS ENHANCE BONE MASS ACCRUAL AND ALTER BONE DIMENSIONS DURING GROWTH BETTER THAN CALCIUM CARBONATE?

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Only one study in girls, using calcium derived from a milk extract, reported a residual benefit to bone mass and bone size following cessation of supplementation¹. To test the hypothesis that calcium from milk minerals, but not calcium carbonate will enhance bone mineral content (BMC) accrual and alter bone dimensions, 75 pre-pubertal children were randomly assigned to receive either 800 mg/d calcium from milk minerals, calcium carbonate, or a placebo, for 9 months, in a double blind fashion. Bone dimensions & BMC were assessed pre- and post-supplementation using DXA. Dietary calcium intake was measured pre- and post-intervention using 3-day weighed food diaries. Anthropometry and hours of weight bearing exercise were recorded pre- and post-intervention. Differences between groups were determined using ANCOVA, adjusting for baseline values. Data was analysed using Statview (version 4.51).

Despite randomisation the placebo group was older and larger than the two calcium groups. The calcium carbonate group accrued more BMC (%) at the ulna-radius (20 ± 1 v 15 ± 2 , $p < 0.05$) and showed a tendency to accrue more bone (%) at the humerus than the placebo group (16 ± 2 v 12 ± 2). The milk mineral group accrued more BMC (%) at the tibia-fibula than the placebo group (27 ± 2 v 22 ± 2 , $p < 0.07$). No differences were reported between groups for % BMC gain at the femur. There was a non-significant trend towards a greater gain (%) in periosteal width (%) in the milk mineral group compared to the placebo group (7 ± 2 v 4 ± 3). The effect of both forms of calcium was evident at the peripheral than distal appendicular skeleton. However, no differences were reported between the two forms of calcium. The effect of calcium supplementation on bone size, and the long-term benefits of calcium supplementation remain to be determined. ¹. Bonjour *et al.* J Clin. Invest. 1997, 99:1287-94

O51

CALCIUM SUPPLEMENTATION INCREASES BONE DENSITY IN PERIPUBERTAL GIRLS IN A DOUBLE-BLIND, PLACEBO-CONTROLLED, RANDOMISED TRIAL.

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We proposed that calcium supplementation during the peripubertal years may substantially increase the rate of bone mass accrual at this critical time. We recruited 94 healthy girls 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=46) (Caltrate, Whitehall) or placebo (n=48), for a mean follow-up of 33.7 ± 13.0 months. Baseline and 6 monthly follow-up data included height, weight, sitting height, urinary calcium, creatinine, sodium and pyridinoline, and completion of food frequency and exercise questionnaires. Bone age and DXA total body and lumbar spine BMD and BMC were performed annually (Lunar DPX). Compliance was 85.5% overall. The main study results are summarized in the following table.

	treatment			P-value
	placebo	calcium	effect	
total body BMD				
baseline (g/cm ²)	0.9165	0.9286		0.465
absolute change/year	0.0267	0.0314	0.0048	0.023
relative change/year	2.7%	3.1%	0.4%	0.032
lumbar spine BMD				
baseline (g/cm ²)	0.8187	0.8344		0.565
absolute change/year	0.0481	0.0566	0.0085	0.045
relative change/year	5.0%	5.8%	0.8%	0.048

In summary, calcium supplementation (1.2g/day) in peripubertal girls leads to a linear increase in bone density at lumbar spine and total body over time and on extrapolation, ongoing calcium supplementation may lead to a clinically significant increase in peak bone mass.

O52

ZOLEDRONIC ACID INCREASES TOTAL BONE VOLUME IN OP-1 MEDIATED BONE FORMATION IN A SEGMENTAL RAT FEMORAL DEFECT MODEL

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Osteogenic Protein 1 (OP-1 = BMP-7) and other BMPs up-regulate osteoblast differentiation and bone production. However, BMPs also directly up-regulate osteoclasts through BMP receptors and indirectly via osteoblasts through RANK / RANKL. In some clinical situations this may produce high bone turnover, which could limit the volume of callus produced. Premature resorption of the calcified matrix scaffold may also be undesirable. We hypothesised that if the osteoclastic upregulation is modulated by Zoledronic acid (ZA), the combination of OP-1 and ZA should produce increased callus over OP-1 alone.

A rat 6mm critical size defect model was utilized. Groups consisted of collagen carrier alone, carrier plus ZA, OP-1 alone and OP-1 with ZA either given locally, systemically at surgery or systemically 2 weeks post surgery. Doses were OP-1 50 micrograms, local ZA 0.05 mg/kg, and systemic ZA 0.1 mg/kg. QCT analysis of the defect was followed by histomorphometry.

Carrier alone and carrier ZA groups had not united by 8 weeks. Radiological union occurred in all OP-1 groups. Addition of ZA increased BMC in the 6mm defect by 48-100% (p<0.05) and callus volume by 40-74% (p<0.05). MAR and Oc.N were not significantly affected by addition of ZA. BV/TV in the 6mm defect, was increased by 64% and 70% respectively for systemically given ZA groups (p<0.05) but not for the local ZA group.

Zoledronic acid significantly increased the amount of OP-1 mediated callus production in a femoral critical size defect in rats. Modulation of the high bone turnover state induced by pharmacotherapeutic doses of OP-1 may optimise the amount and density of callus produced, which could be of clinical benefit in obtaining initial bone repair.

O53

FRACTURE RISK SCORE: GEELONG OSTEOPOROSIS STUDY

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Recognised risk factors for fracture include low BMD, previous fracture, age, propensity to fall and body weight. The use of BMD to predict fracture is complicated when site-specific BMD values are discordant. Since the clinical aim is to prevent any fracture, assessment of fracture risk based on multiple site BMD and other risk factors would be clinically useful and was the aim of this study.

BMD was measured at the proximal femur and spine for women (35yr+) who sustained a low trauma fracture (n = 492, median age 69yr, IQR 58-76yr) during a 2 year ascertainment period and for a random population-based sample of women recruited concurrently who had not sustained a fracture during this time (n = 730, mean age ± SD, 56 ± 14yr). Falls in the previous year were scored [(1) never or rarely (2) a few times (3) several times (4) regularly] and the number of self-reported fractures in adult life were recorded. Discriminant analysis derived the best equation to classify those expected to fracture (Sens 68%, Spec 72%). A positive score forecasts fracture:

RISK SCORE = 2.773 – 2.803 BMD_{SPINE} – 2.612 BMD_{TOTALHIP} +0.627 falls score +0.404 previous fractures +0.062 body mass index

Risk scores for the cases were +0.5 ± 1.1 (mean±SD) and controls –0.5 ± 1.0. A 1SD decrease in BMD at the PA Spine, increases the fracture risk score by 0.379 and is equivalent to decreasing the total hip BMD by 1.1 SD, increasing the body mass index by 6.2 kg/m², increasing number of previous fractures by 0.9 or increasing Falls Score by 0.6. Among women who fall a few times a year or have sustained one previous fracture, a total hip T-score = -1 and a PA spine T-score = -2.5, or the reverse, has a similar risk of fracture as a non-faller with a total hip and PA spine T-score both equal to –2.5. The fracture risk equation should assist treatment decisions.

O54

ZOLEDRONIC ACID PREVENTS BONE LOSS AT 3 MONTHS IN LIVER TRANSPLANT PATIENTS: AN INTERIM ANALYSIS OF A RANDOMISED CONTROLLED TRIAL

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Osteoporosis and fracture are frequent complications of liver transplantation, occurring in a population already exhibiting low bone density. Earlier studies have shown the greatest bone loss occurring within 3 months after transplantation (1). We are conducting a Phase II, 12 month, randomised placebo-controlled trial of zoledronic acid 4 mg, given within 7 days after liver transplantation, and again at months 1, 3, 6 and 9 post-transplant. All patients received calcium carbonate 600 mg and ergocalciferol 1000U daily. We present here the result of the interim analysis of the primary end-point, the 3 month BMD of hip and lumbar spine (LS) (measured on a Lunar Prodigy densitometer). The BMD data (g/cm²) was analysed by repeated measures ANOVA. Data is mean ± SEM.

Of the 54 patients recruited in this study, 45 (33 males, 12 females, aged 48.6 ± 1.4 yrs) have completed the 3 month data collection. The predominant underlying liver disease was viral hepatitis. The 2 treatment groups (n=24, 21) were well balanced with respect to age, sex, underlying disease, baseline BMD and baseline 25 vit D levels. For the whole group, baseline BMD levels (and mean T scores) in males and females, respectively, were: LS 1.12 ± 0.03 g/cm² (-0.9 SD); 1.01 ± 0.04 g/cm² (-1.6 SD); neck of femur (NOF) 0.95 ± 0.03 g/cm² (-0.8 SD); 0.81 ± 0.04 g/cm² (-1.3 SD); total hip 1.00 ± 0.02 g/cm² (-0.6 SD); 0.85 ± 0.04 g/cm² (-1.2 SD). At all sites the placebo group lost bone density at 3 months, ranging from 2.8% in the LS to 8.0% in the trochanter. This loss was not seen at any site in patients treated with zoledronic acid. The difference between the 2 groups was highly significant for all hip parameters (total hip and Ward's p<0.0001, NOF p=0.002; trochanter p=0.02) and close to significance in LS (p=0.09). We conclude that intravenous administration of zoledronic acid at the time of transplant and 1 month post-transplant prevents the acute bone loss associated with liver transplantation.

McDonald et al. 1991 *Hepatology*; 14:613-9.

POSTER PRESENTATIONS

P01

TRANSCRIPTION FACTORS INVOLVED IN OSTEOCLASTOGENESIS

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Surprisingly, very few transcription factors have been implicated in osteoclast differentiation. Newly discovered upregulated transcription factors are obviously important in understanding osteoclast differentiation. However, we speculate that repression of transcription factor genes may also be necessary for osteoclast differentiation. In particular, we have looked for differential regulation of transcription factors between the two alternative states: macrophage and osteoclast. Two recent papers verified the role of NFATc1 in osteoclastogenesis [1,2]; NFATc1 is required for the production of TRAP positive multinucleated osteoclast like cells (OCLs). We show here that NFATc1 and other transcription factors are strongly induced by RANKL in human osteoclast like cells. NFATc1 regulation steadily increases across time with a peak of 26 fold at three weeks post treatment with macrophage colony stimulating factor (M-CSF) and RANKL. Along with NFATc1 we have observed significant up-regulation of four other transcription factors: GA-binding protein α and β (GABP), early response growth factor 1 (EGR-1), and FUSE binding protein (FBP). The dynamics of regulation of ERG-1 and GABP α and β were identical to that of NFATc1. However FBP regulation peaks earlier and is of great magnitude. These data show that NFATc1 is not the only strongly regulated transcription factor in osteoclast differentiation and is later induced than FBP.

1 Ishida N, Hayashi K, Hoshijima M, Ogawa T, Koga S, Miyatake Y, Kumegawa M, Kimura T, Takeya T. 2002. J Biol Chem 277:41147

2 Takayanagi H, Kim S, Koga T, Nishina H, Isshiki M, Yoshida H, Saiura A, Isobe M, Yokochi T, Inoue J, Wagner EF, Mak TW, Kodama T, Taniguchi T. 2002. Dev Cell 3:889

P02

MULTIPLE TISSUE-SPECIFIC PROMOTERS CONTROL EXPRESSION OF THE MURINE TARTRATE RESISTANT ACID PHOSPHATASE GENE

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Tartrate Resistant Acid Phosphatase (TRAP) is highly expressed in osteoclasts and in a subset of tissue macrophages and dendritic cells. We have identified two novel murine TRAP mRNA transcripts that differ in their 5'-untranslated region (5'-UTR) sequences but align with the known murine TRAP mRNA from the start of the protein coding sequence at the first base of Exon 2. The novel 5'UTRs represent alternative first exons located upstream of the known 5'UTR. A similar genomic structure exists for the human TRAP gene with partial conservation of the exon and promoter sequences. Expression of the most distal 5'UTR (Exon 1A) is restricted to adult bone and spleen tissue. Exon 1B is expressed primarily in tissues containing TRAP-positive non-haematopoietic cells. The known TRAP 5'UTR (Exon 1C) is expressed in tissues characteristic of myeloid cell expression. In addition the Exon 1C promoter sequence is shown to comprise distinct transcription start regions, with an osteoclast-specific transcription initiation site identified downstream of a TATA-like element. Macrophages are shown to initiate transcription of the Exon 1C transcript from a purine-rich region located upstream of the osteoclast-specific transcription start point. The distinct expression patterns for each of the TRAP 5'UTRs suggest that TRAP mRNA expression is regulated by the use of four alternative tissue and cell restricted promoters. This study highlights the molecular basis of osteoclast-restricted murine TRAP gene expression.

P03

INFUSION OF CALCIUM-SENSING RECEPTOR-ACTIVE AMINO ACID ELEVATES RAT URINARY CALCIUM EXCRETION

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Increases in dietary protein intake have been previously shown to elevate urinary calcium excretion in humans, although the mechanisms that underlie this effect have not been elucidated [1]. Recently, it has been shown that some L-amino acids including aromatics and aliphatics are allosteric activators of the human calcium-sensing receptor (CaR) [2]. In the present study, we examined the effects of infusion of the CaR-active amino acid L-Phenylalanine (Phe) and its inactive D-isomer on urinary calcium excretion in female Wistar rats. 8 week old rats were infused with one of the following: vehicle, 200 mM L- or D-Phe at 5 mL/h. Blood and urine samples were collected at regular intervals. The calcium levels and osmolality of serum and urine were measured, and the glomerular filtration rate was determined by creatinine clearance. Plasma and urinary D- and L-Phe concentrations were determined by HPLC. The results demonstrated an increase in urinary calcium excretion in response to an elevation in plasma L-Phe from around 0.044 ± 0.009 mM to 2.2 ± 0.24 mM. Concurrently, urinary calcium excretion increased from 0.17 ± 0.1 $\mu\text{mol}/\text{min}$ to 0.34 ± 0.15 $\mu\text{mol}/\text{min}$ ($n = 5$). D-Phe was less effective than L-Phe. Preliminary data suggest that Phe also suppressed urinary osmolality. The results are consistent with the hypothesis that renal CaRs are responsible for the elevation in urinary calcium excretion that is induced by high dietary protein.

[1]Johnson NE, Alcantara EN, Linkswiler H (1970) Effect of level of protein intake on urinary and fecal calcium and calcium retention of young adult males. *J Nutr.* 100: 1425-1430.

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

P04

CONCENTRATION DEPENDENT ACTIVATION OF THE CALCIUM-SENSING RECEPTOR IN HUMAN PARATHYROID CELLS BY L-AMINO ACIDS

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We have previously demonstrated that the human calcium receptor (CaR) is allosterically activated by L-amino acids when expressed in HEK293 cells (1) and that active amino acids enhanced the Ca^{2+} sensitivity of the CaR in fura-2 loaded human parathyroid cells. We have now undertaken an analysis of the concentration dependence of various L-amino acids on CaR-induced Ca^{2+} mobilization in fura-2 loaded normal human parathyroid cells in the presence of various extracellular Ca^{2+} concentrations from 1.0 to 2.0 mM, encompassing the normal physiological and pathophysiological ranges. The order of potency was similar at all three Ca^{2+} concentrations tested: L-His = L-Phe = L-Trp > L-Glu = L-Ala > L-Arg > L-Leu. The EC_{50} values for the most potent L-amino acids (L-His, L-Phe and L-Trp) were approximately 0.1 mM at an extracellular Ca^{2+} concentration of 2.0 mM and 0.2 mM at an extracellular Ca^{2+} concentration of 1.5 mM. At an extracellular Ca^{2+} concentration of 1.0 mM, however, the responses were substantially reduced indicating the presence of a Ca^{2+} threshold for amino acid dependent effects. Consistent with these data, plasma-like amino acid mixtures and fold concentrations of this mixture enhanced the Ca^{2+} sensitivity of the CaR-dependent responses. Given that the total amino acid concentration in serum normally varies between about 2.5 – 5 mM, the data are consistent with the view that CaRs on the surfaces of normal human parathyroid cells are modulated physiologically by L-amino acids.

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

P05

THE ROLE OF MICROPHthalmia TRANSCRIPTION FACTOR IN OSTEOCLAST GENE REGULATION

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The Microphthalmia Transcription Factor (Mitf) is a key regulator of the expression of genes that are important for the activity of bone resorbing osteoclasts. Mutations in Mitf including the *Mitf-mi* dominant negative form result in the development of osteopetrosis in mice. Different isoforms of Mitf that have variant 5' exons resulting from different promoter usage, including Mitf-A and Mitf-M, are differentially expressed in a tissue specific manner. We hypothesise that Mitf plays a general role in the osteoclast differentiation program. To identify novel target genes of Mitf regulation, Mitf activity in cells has been altered and RNA from these cells can be analysed by a cDNA microarray approach.

The macrophage cell line, RAW264.7, has the potential to differentiate into multinucleated and bone resorbing osteoclast-like cells (OCL). Stably transfected cell lines have been prepared that overexpress the Mitf-A, Mitf-M and *Mitf-mi* forms of the transcription factor.

The expression of these isoforms has been established by immunocytochemical staining and western blotting and their activity has been confirmed by the analysis of the effect on the TRAP and cathepsin K promoters. Mitf-A and Mitf-M have been found to differentially regulate the TRAP promoter and the cathepsin K promoter. Over-expression of *Mitf-mi* was shown to alter the phenotype of RAW264.7-derived OCL after differentiation with RANKL. The extension of these studies will permit the characterisation of new targets of Mitf activity in osteoclasts.

P06

GENERATION OF AN ANDROGEN RECEPTOR LOXP MOUSE LINE FOR OSTEOBLAST SPECIFIC GENE KNOCKOUT

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Evidence suggests that androgens stimulate osteoblastic bone formation directly via the androgen receptor (AR). We plan to study the direct role of androgen action on osteoblast development and function by using the Cre/loxP site-specific recombination system to inactivate the AR specifically in osteoblasts.

We have cloned and characterised the exon 3 region of the AR gene from a 129SV/J mouse genomic library. DNA from this clone was used in the design and generation of an AR-loxP targeting construct. We believe that deletion of exon 3 will result in the permanent inactivation of the AR as this exon encodes the 2nd zinc finger of the DNA binding domain and plays a critical role in stabilising receptor-DNA interaction and receptor dimerisation. The AR-loxP targeting construct has been sequenced and the loxP sites were demonstrated to be functional in Cre recombinase expressing bacteria. The construct was electroporated into 129SV/J mouse embryonic stem (ES) cells and 200 neomycin resistant ES cell colonies were selected and screened for homologous recombination events by PCR analysis. 16 positive ES cell clones were identified and one was microinjected into C57Bl6 mouse blastocysts. Male chimeric offspring were backcrossed to female C57Bl6 mice and resulting agouti female mice genotyped by PCR to confirm germline transmission.

Our collaborators (Prof B. Kream, University of Connecticut Health Centre, USA) have generated and characterised a *Coll1a1-Cre* transgenic mouse line, in which Cre recombinase expression is driven by the rat alpha 1(I)-collagen promoter. We have obtained this line and plan to breed it with the AR-loxP line to generate an osteoblast-specific knockout of the AR gene.

P07

**IN VITRO ANALYSIS OF A BIOACTIVE BONE IMPLANT -
RELEASING HEPARIN-LIKE MOLECULES FROM PHBV AND
ITS EFFECT ON OSTEOGENESIS.**

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Given the growing fracture rate in Australia¹, and the inability of current orthopaedic technologies to adequately meet these demands, a need exists to develop more suitable implants that are biodegradable and osteoinductive. This study is the first to describe the attachment of a novel osteoinductive/osteoconductive heparin-like molecule (HS) to a biodegradable implant material, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) - BiopolTM (PHBV) in order to create such an implant surface.

Novel methods to attach HS to PHBV were developed and characterised using XPS, AFM, SEM and water contact angle. Release kinetic profiles of HS from the newly functionalised PHBV was examined using radiolabelled HS. The murine preosteoblast cell line (MC3T3-E1 S14) was cultured on these surfaces and resultant differences in cell proliferation (BrdU), differentiation and maturation (calcium content and real-time PCR for bone markers) rates compared to non-functionalised PHBV and tissue culture plastic.

PHBV treatment altered both the surface morphology and amount/strength of HS attachment. This in turn affected cell behaviour, with more mature phenotypes correlating to roughened surfaces and faster HS release rates. This illustrates that a desired cell response to a biomaterial can be achieved by varying the attachment method of bioactive compound to a polymer surface. Therefore, improved orthopaedic implants can be developed using these principles.

[Sanders et al. 1999, MJA; 170:467-470](#)

P08

**STUDIES ON THE PROCESS OF ENDOCHONDRAL
OSSIFICATION IN EQUINE ARTICULAR-EPIPHYSEAL
GROWTH CARTILAGE**

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Osteochondrosis is a disease of horses and humans which results from a failure in endochondral ossification in articular-epiphyseal growth cartilage. We aim to document the cellular and molecular processes involved in normal endochondral ossification in horses, as a prelude to investigation of how these processes are altered in osteochondrosis. Samples of equine articular-epiphyseal growth cartilage were collected from normal joints of growing horses at different ages (1 – 24 months old). Demineralised cryosections were stained for the presence of apoptotic cells using the TUNEL method and immunohistochemistry was applied to detect the expression of the extracellular matrix protein tenascin-C. Apoptotic cells were recognised in the resting zone and lower hypertrophic zone of articular-epiphyseal growth cartilage and the proportion of apoptotic cells at the bone-cartilage interface decreased with increasing skeletal maturity. Tenascin-C expression was detected in the resting zone and extending into the proliferative zone, but the intensity of staining decreased with age. These observations provide important baseline information for our future studies on the pathophysiology of osteochondrosis, and suggest that tenascin-C may be important in maintenance of the chondrocytes in the resting zone of articular cartilage in growing horses.

P09

IDENTIFICATION OF RANKL REGULATED GENES IN PERIPHERAL BLOOD MONONUCLEAR CELL DERIVED OSTEOCLASTS

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Treatment of adherent Peripheral Blood Mononuclear Cells (PBMC) with M-CSF and RANKL stimulates the formation of multinucleate, bone resorbing osteoclast-like cells, while treatment with M-CSF alone results in the formation of macrophages. To date, much that has been learned concerning the osteoclast phenotype has been derived from a few osteoclast markers. These include calcitonin receptor, cathepsin K, an osteoclast specific subunit of the proton pump, and tartrate resistant acid phosphatase (TRAP). We used the default macrophage pathway as counterpoint to the differentiation of osteoclasts so that meaningful array experiments could be used to identify novel genes that are regulated during osteoclast differentiation.

Initial cDNA expression arrays identified many target genes regulated by RANKL including numerous cytokines and cytokine receptors, such as RANTES and IL2RA, integrins, such as VE-cadherin, and proteases such as MMP 9. Real-time PCR was used to verify the regulation of CD44, CSF2R, Calmodulin 1, IL3RA, MMP 7, 8, 9, CD27 and Cyclin-dependent Kinase 7.

These data provide opportunity to better understand osteoclast differentiation as well as expansion of the number of known osteoclast related genes.

P10

PERIPHERAL QUANTITATIVE COMPUTED TOMOGRAPHY (pQCT) IN ELDERLY WOMEN: IN VIVO PRECISION.

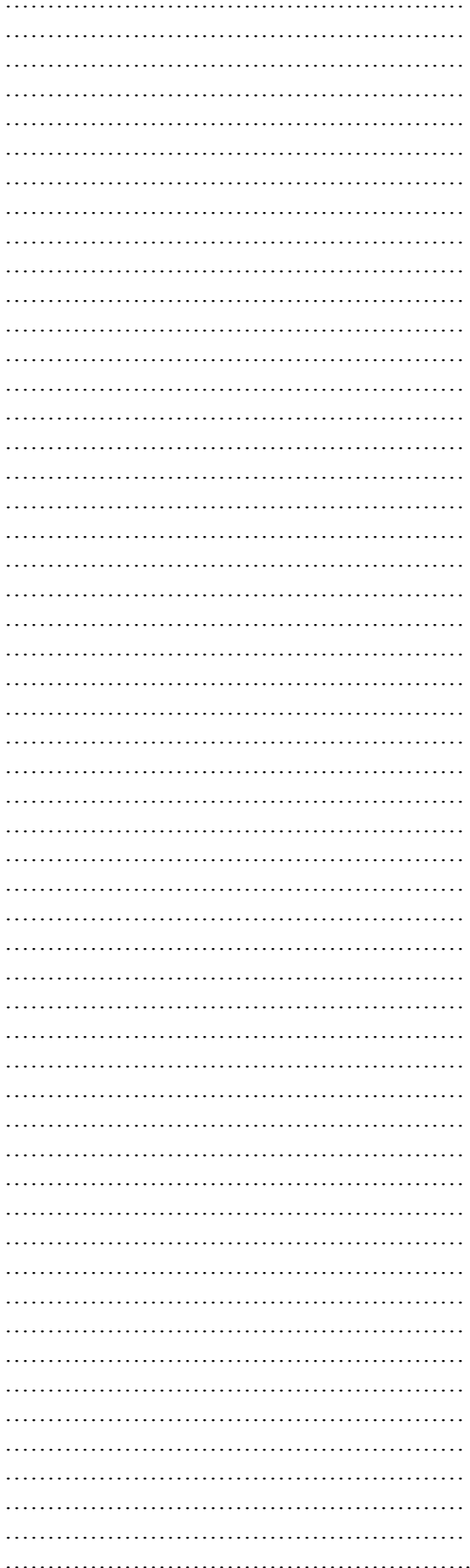
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Peripheral Quantitative Computed Tomography (pQCT) has the advantage of being able to determine both trabecular and cortical bone mineral density (BMD) and it is known that disease processes and medical therapies affect compartments of bone in different ways. In vitro precision is well described, but in vivo precision needs to be further clarified prior to using pQCT to assess patients in longitudinal trials and clinical practice.

We evaluated the in vivo precision of pQCT in elderly women aged between 77 and 85 using the Stratec XCT2000 scanner. Scans were performed twice at the non-dominant distal radius (n=47) and left distal tibia (n=48). Subjects were completely re-positioned between scans. The limb was held with a cushioned holder and the subject instructed to keep as still as possible during the scan. A resolution of 0.15mm pixel size and 1mm CT slice was used. Radiation dosimetry was determined to be less than 0.6 µSv for all 4 scans. Iterative (IT) and concentric peel (CP) algorithms were used to calculate the total, cortical and trabecular BMD at each site.

The in vivo coefficient of variation (CV) values for the total BMD at the distal radius was 3.6% and 1.3% at the distal tibia. Trabecular BMD CV using the CP algorithm was 3.3% for the distal radius and 2.2% for the distal tibia. Trabecular BMD CV using the IT algorithm was 3.7% for the distal radius and 1.7% for the distal tibia. Cortical BMD CV using CP was 5.4 % at the distal radius and 2.7% at the distal tibia. Cortical BMD CV using the IT method was 8.4% at the distal radius and 9.7% at the distal tibia.

We conclude from this study that pQCT is a precise method for evaluating both trabecular and total BMD in vivo and holds promise in research and clinical applications.



P11

IS BACK EXTENSOR STRENGTH RELATED TO THORACIC POSTURE IN ELDERLY WOMEN?

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Hyperkyphotic posture has been suggested to be a clinical indicator of poor health outcome and predictor of new vertebral fracture. Previous studies reported negative associations between back extensor strength (BES), tested in prone, and thoracic kyphosis (Cobb angle) in estrogen deficient (mean age 54)¹ and, osteoporotic women (mean age 65)². The aim of this study was to examine the association between maximal isometric BES, tested in a functional upright position, and thoracic kyphosis in a group of elderly females.

Extensor strength of 100 community living women (mean age 71±6[sd]) was measured in standing using an instrumented back strength assessment device. Thoracic kyphosis was defined using 1) the modified Cobb angle measured from T4 to T11 off erect lateral thoracic x-rays and, 2) back surface curvature of the thoracic region, from the vertebral prominence to T12, using video rasterstereography. Regression analyses were used to test the overall relationship between BES and the two indices of thoracic kyphosis. The subjects were allocated into tertiles according to the extensor torque and ANOVA was used to determine associations between the tertiles of BES and the two indices of kyphosis. Age was used as the covariate in the analyses, as it was found to be associated to BES, unlike height, weight and BMI.

There were no overall linear relationship between BES and Cobb angle (r=-0.04; p=0.67) and, BES and back surface curvature (r=0.001; p=0.99). No significant differences in thoracic Cobb angle (p=0.91) and the back surface curvature (p=0.54) between the tertiles of BES were noted. In conclusion, this study found no association between BES and erect thoracic spinal curvature in elderly females.

Sinaki, M., Itoi, E., Rogers, J., Bergstralh, E., Wahner, H. (1996), *Am J of Phys Med and Rehabil*, 75(5) 370-4. (2) Sinaki, M., Wollan, P., Scott, R., Gelczer, R. (1996) *Mayo Clinic Proc* 71(10) 951-6.

P12

THE EFFECT OF ANTICONVULSANT MEDICATION ON BONE MINERAL DENSITY AND OSTEOPOROSIS - A TWIN AND SISTER STUDY.

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Studies of the risk that long-term use of anti-epileptic drugs (AEDs) on bone mineral density (BMD) and the risk of osteoporosis and fractures are limited by inadequate controls, use of cross sectional design, and lack of adjustment for other BMD determinants. A pilot study was performed using the *Twin and Sister Longitudinal Bone Health Study* to investigate this issue further.

Participants with epilepsy or AED use were identified from the database. Bone mineral density (BMD, g/cm²) was measured at the lumbar spine (LS), total hip (TH), femoral neck (FN) and 1/3 distal radius (FA). Total body bone mineral content (TB BMC, kg), fat mass (kg) and lean mass (kg) were calculated. Results were expressed as the within-pair percentage difference, relative to the non-user.

Thirteen pairs (8 MZ and 5 DZ pairs) and 4 sibling pairs (< 3 years age difference) were assessed (mean 37.4, range 18 - 63 years). Pairs were discordant for AED use, with one having >12 months AED use (user) and the other no exposure (non-user), except for 1 pair discordant for dose. Five pairs (29.4%) had two-four follow-up assessments, providing a total of 27-paired measures. There were significant differences in BMD at the TH (-5.0%, P = 0.009) and FN (-6.1%, P = 0.01). There was no difference in LS and FA BMD, TB BMC, weight, or lean and fat mass, but there was difference in height (-1.7%, P = 0.01). Using a univariate general linear model analysis to account for different numbers of repeated assessments, no differences attained statistical significance, but there was a trend for a difference in TH BMD (P=0.068). 5/17 AED users vs. 1/17 non-users had a TH z-score < -1.5 indicating increased risk of fracture for age.

Preliminary findings using a matched twin-sister approach suggest that patients using AEDs may have lower BMD at the hip, but further numbers are required before a definitive conclusion can be made.

P13

DRUG INTERVENTION AMONG MEN REFERRED FOR BONE DENSITOMETRY. S Kom, JA Pasco, MJ Henry, GC Nicholson, MA Kotowicz. The University of Melbourne, Department of Clinical and Biomedical Sciences: Barwon Health, Geelong Australia.

There is limited information concerning the management of low bone mass and fracture in men. We assessed 100 men (aged 22-85 yr, median 61.9) with longitudinal bone mass data, referred for bone densitometry at The Geelong Hospital between 1991-9 (median time to follow up, 1.2yr, IQR 1.1-1.9). Until 1998 the centre was the sole provider of bone densitometry for the region, serving a male population of 108,606. Drug exposure and reasons for scan were abstracted from patient records. T-scores were determined from bone density measurements at the PA-spine L2-4 (Lunar DPX-L). Differences in age and t-scores for each intervention and reason for requesting scan were tested using ANOVA and Tukey's test for multiple comparisons.

<i>Medication</i>	<i>n</i>	<i>Mean age ±SD</i>	<i>Mean T-score ±SD</i>
<i>None</i>	22	59.9 ± 15.6	-1.52 ± 1.44
<i>Calcium/calcitriol</i>	32	57.8 ± 14.1	-1.81 ± 1.56
<i>Testosterone</i>	23	53.9 ± 18.2	-1.66 ± 1.49
<i>Bisphosphonate</i>	22	62.7 ± 12.0	-2.90 ± 1.81

Indications for referral were glucocorticoid use (n=41), fracture (n=35) and others (n=23) (eg thyroid disease, hypogonadism). Drug use was independent of age (p=0.2). Men prescribed bisphosphonates had the greatest bone deficits with t-scores significantly lower than those prescribed testosterone or no medications (p<0.05). Compared with non-users, a smaller than expected proportion of glucocorticoid users were prescribed testosterone (3/41) with greater than expected prescribed no medication (14/41) (χ^2 , p=0.002). Among glucocorticoid users, treated men tended to have greater bone deficits than untreated (t-score -2.13 vs -1.39, p=0.1).

Fifteen percent of men diagnosed with osteoporosis (t-score <-2.5SD) remain untreated, despite bone densitometry assessment. Men with the most severe bone deficits are treated with a bisphosphonate.

P14

REFINING THE ASSESSMENT OF BALANCE, MUSCLE STRENGTH AND GAIT MEASURES IN WOMEN TO PREDICT FALLS-RELATED HIP FRACTURE RISK.

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Many testing procedures of balance and other related measures are available for clinicians to assess falls-related fracture risk. Fracture risk is determined by the interaction of several factors including low bone mineral density, poor postural stability, muscle weakness, physical inactivity and falls. Assessment of balance, muscle strength and physical activity is therefore important in the prevention of hip fractures. The aims of this study were to identify age effects in test performance and to refine a test battery for the assessment of balance and related measures.

A range of validated tests assessing gait, body sway, activity levels and muscle strength were performed on 352 women aged 21-82, mean(SD) 50.5(13.3) years. Significant age effects were evident between young and older groups [21-50 yo, N=172; 51-82 yo, N=180] on all of the measures of balance, gait and muscle strength (p<0.05). A factor analysis of 24 measures from the Chattecx Balance System, stride analyser, Lord's balance test, step test, and the Human Activity Profile (HAP) questionnaire, resulted in the extraction of 6 factors. This accounted for 70.8% of the variance in global balance. These factors were: 1) muscle strength (ankle, knee, hip), 2) Chattecx perturbation measures (stable and antero-posterior), 3) Lord' balance test, 4) HAP, 5) step test, 6) Chattecx perturbation (medio-lateral). We found no difference in the factor analysis by age (21-50 vs.51-82 years).

Session duration can be shortened from 90 to 40 minutes by data reduction methods involving factor analysis. This could have significant clinical application, providing a refined balance assessment for prediction of hip fracture risk and targeting of preventive interventions.

P15

THE UTILITY OF AN ULTRASONIC MOTION TRACKER FOR THE ASSESSMENT OF FALLS RISK.

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The Sway Meter (SM) has been used successfully to discriminate between fallers and non-fallers and to predict fracture risk in the elderly. However, this method can only assess sway displacement about the lumbar region. Ultrasonic motion trackers can track displacements of several body parts simultaneously, and have been used to examine balance control strategies in healthy and neurological subjects. The aim of this study was to compare postural sway derived from an ultrasonic motion tracker (V-Scope) with the SM.

Fifty-three subjects were assessed using the SM and VS simultaneously. The subjects were tested in six conditions: standing with eyes open (EO) and closed (EC) on a firm surface; standing on a compliant foam with eyes open (FEO) and closed (FEC) and; standing in semi-tandem stance with eyes open (STEO) and closed (STEC). Spearman rank correlation was used to assess the correlation of total sway (mm) of all six test conditions derived from the two methods. Results of the analyses with average bias (VS minus SM), and 95% confidence intervals (CI) are summarised in the table.

Condition	Spearman Rho	Average Bias	95% CI
EO	0.67*	99.6	93.9 – 105.4
EC	0.71*	106.2	100.9 – 111.4
FEO	0.73*	87.9	76.6 – 99.1
FEC	0.79*	107.8	93.4 – 122.3
STEO	0.53*	161.5	144.8 – 178.3
STEC	0.82*	188.8	166.6 – 211.0

* p <0.0001

High concordance was noted for total sway derived from both methods. The VS has the added advantage of having the potential to monitor simultaneous displacements of several body parts, which can provide information regarding balance strategies and be used in formulating balance training programmes for falls prevention.

P16

EFFECT OF WATER LEVEL ON AP SPINE PHANTOM BMD AS MEASURED BY THE DPXL AND PRODIGY.

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The quality control of DXA scanners involves regular scanning of a suitable phantom. Lunar recommends the use of an aluminium spine bar phantom immersed in 15cm water for both the DPXL and Prodigy instruments. We examined how variations in H₂O depth may effect the phantom BMD.

An aluminium spine phantom was scanned on both Lunar Prodigy and Lunar DPXL machines at multiple water depths between 10 and 20cm at 1cm increments. Scans were performed three times at each water level using Medium 750 and 3000 modes for the DPXL and both the thin and standard modes for the Prodigy. The average L1-L4 BMD values were used for analysis.

The DPXL, in both modes, demonstrated a slight variability of BMD of approximately 1-2% between 11 and 20cm. With the water level below 11cm testing was not accurate. The Prodigy thin mode demonstrated a significant positive correlation with water depth (r=0.956, p<0.0001). In the Prodigy standard mode there was a curvilinear decrease in BMD with increasing water depth between 11 and 15cm. Above 15cm in H₂O depth the phantom BMD results appeared stable.

In conclusion reliable DXA quality control using phantoms is crucial in order that machine drift be detected. Variations in water depth may influence the results and phantom scan protocols should be modified to ensure that the effects of variations in water depth are minimised.

P17

LONG TERM IMPACT OF PERCEIVED HEALTH ON FUTURE FALLS AND FRACTURE IN HEALTHY ELDERLY WOMEN.

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The aim of the present study was to identify the influence of self-reported mental and physical function on the prevalence of falls, fear of falling (FoF) and fracture in elderly women over a 3-year period.

1290 women were recruited from a population based sample of females ≥70 yrs and followed for 3 years. SF-36 was administered to this group at baseline and scores for mental (MCS) and physical (PCS) health calculated. Incident clinical fractures were confirmed by x-ray. Questionnaires relating to i) falls ii) FoF, iii) household and outdoor activity restriction due to FoF, iv) walking aid use and v) lack of physical activity were administered at baseline and 3 years.

Logistic Regression Analysis, with adjustments for age, weight, and respective baseline co-variates, demonstrated that poor MCS and PCS had a long-term impact on future falls (R.R 0.98: 95%CI 0.96-0.99) (R.R 0.98: 95%CI 0.97-0.99), increased FoF (R.R 0.98: 95%CI 0.96-0.99) (R.R 0.97: 95%CI 0.96-0.98), restriction of outdoor activity due to FoF (R.R 0.98: 95%CI 0.97-1.0) (R.R 0.95: 95%CI 0.93-0.96) and increased use of a walking aid (R.R 0.97: 95%CI 0.94-0.99) (R.R 0.93: 95%CI 0.91-0.93). Furthermore, poor PCS had a long-term impact on restriction of household activity due to a FoF (R.R 0.96: 95%CI 0.94-0.97) and a reduced level of participation in physical activity (R.R 0.96: 95%CI 0.94-0.97). MCS and PCS had no long-term impact on fracture outcome.

These results support current literature that suggests poor self-rated health is a significant predictor of future falling episodes. Examination of the long-term impact of self rated mental and physical health may provide information as to the severity and chronic nature of the individual's perception of their health and highlight their potential risk for fall related factors.

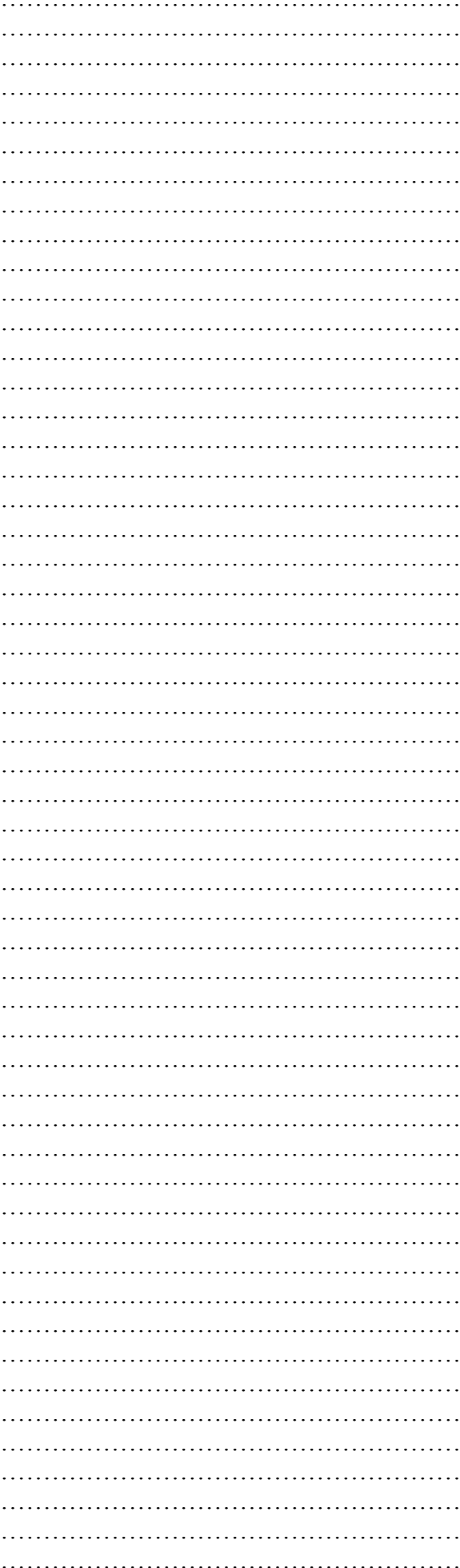
P18

BONE FORMATION IS HIGHLY COUPLED WITH BONE RESORPTION IN OSTEOPROTEGERIN-DEFICIENT MICE

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The discovery of RANKL elucidates the mechanism of osteoclast differentiation and function regulated by osteoblasts. Osteoprotegerin (OPG), a soluble decoy receptor of receptor activator of NF-κB ligand (RANKL), inhibits both differentiation and function of osteoclasts. Deficiency of OPG in mice induces severe osteoporosis caused by enhanced bone resorption, although bone formation was also elevated in the deficient mice. Here we examined whether bone formation is coupled with bone resorption in OPG deficient (OPG^{-/-}) mice, using risedronate, an inhibitor of bone resorption. Histomorphometric analysis showed that elevated bone formation-related parameters in OPG^{-/-} mice were sharply decreased with the suppression of bone resorption by daily injection of risedronate for 30 days. OPG^{-/-} mice also showed high serum alkaline phosphatase activity, which was decreased to the level lower than that of the wild-type mice by the treatment with risedronate. The serum levels of soluble RANKL were markedly elevated in OPG^{-/-} mice, but unaffected by the treatment. When bone formation induced by bone morphogenetic protein-2 implantation was compared between OPG^{-/-} and wild-type mice, the ectopic bone formation in OPG^{-/-} mice was similar to that in wild type mice but the ectopic bone resorption was accelerated in OPG^{-/-} mice. These results suggest that bone formation is accurately coupled with bone resorption at the local sites in OPG^{-/-} mice, and the serum RANKL level does not reflect this coupling.



P19

ASSOCIATION BETWEEN CYP27B1 GENE POLYMORPHISM AND LUMBAR SPINE BONE MINERAL DENSITY IN POSTMENOPAUSAL WOMEN.

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The gene *CYP27B1* encodes the mitochondrial enzyme 1-alpha-hydroxylase that converts 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D. We hypothesised that *CYP27B1* polymorphisms might be associated with bone density or calcitropic hormone metabolism in postmenopausal women. A cohort of 123 healthy Caucasian women aged 45-68 y and not using hormone replacement therapy were recruited through media advertisements. The subjects were genotyped for a *CYP27B1* polymorphism within intron 6 (+2838 T/C) by restriction digest of PCR products generated with modified primers. The following measurements were performed: areal bone mineral density at lumbar spine, femoral neck and forearm; serum calcium, PTH, 25-hydroxyvitamin D, and 1,25-dihydroxyvitamin D. The mean lumbar spine BMD Z-score in women with the *CYP27B1* genotype TT was -0.05±0.94 compared with 0.53±1.05 in women with the CC genotype (*p*=0.05). *CYP27B1* genotype was an independent predictor of lumbar spine BMD after accounting for age, height and weight in a general linear model (*p*=0.01). *CYP27B1* genotype was also associated with serum intact PTH concentrations. Women with *CYP27B1* genotype TT had mean serum PTH concentration 0.25±0.30 pg/mL compared with 0.15±0.08 pg/mL in women with the CC genotype (*p*=0.04). *CYP27B1* genotype was not associated with bone density at the femoral neck or distal forearm, nor with serum concentrations of 25-hydroxyvitamin D or 1,25-dihydroxyvitamin D. We conclude that *CYP27B1* is an important novel candidate for study in both osteoporosis and hyperparathyroidism.

P20

PROXIMAL FEMUR MALE REFERENCE RANGES IN LUNAR AND NORLAND DENSITOMETERS

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We have previously reported significant differences in manufactures' reference ranges for females. Differences may also occur in male reference ranges. We assessed the femoral neck reference ranges in Lunar DPXL (Version 1.35) and Norland Excel (Version 2.3.1) instruments in 30 male subjects scanned on both machines. We compared femoral neck BMD as well as T and Z scores in 30 males measured on the same day.

	BMD (g/cm ²) mean (SD)	SBMD mean	T score mean (SD)	Absolute T score	Z score mean (SD)
Lunar	0.926 (0.22)	846	-1.05 (1.73)	1.68 (1.11)	-0.48 (1.32)
Norland	0.879 (0.23)	872	-1.31 (1.92)	1.83 (1.25)	0.20 (1.59)

Absolute femoral neck BMD using both instruments was highly correlated *r*= 0.98. There was no significant difference when femoral neck BMD was expressed as sBMD. In addition there were no significant differences in T scores between the two instruments. Using paired T tests significant differences are present between Z scores (mean difference= 0.69, *p*<0.0001).

In conclusion the male femoral neck young normal mean and SD, and consequently T scores, used by the two manufacturers are similar (Lunar mean sBMD 1016, SD 98; Norland mean sBMD 1006, SD 125) but they differ in their age matched reference ranges (Z scores). This difference potentially may lead to unnecessary testing in some individuals to exclude underlying causes of increased bone loss. Standardisation of reference ranges in males should be a high priority.

P21

BONE-DENSITY-INDEPENDENT ASSOCIATION OF QUANTITATIVE ULTRASOUND MEASUREMENTS AND FRACTURE RISK

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Quantitative ultrasound measurements (QUS) of bone, that may reflect micro-architectural aspects of bone, have been shown to be associated with fracture. However, it is not clear whether this association is independent of bone mineral density (BMD). This study was designed to examine the contributions of cortical QUS and BMD measurements to the prediction of fracture risk in postmenopausal Caucasian women.

Speed of sound (SOS) at the distal radius, tibia, and phalanx (Sunlight Omnisense) and BMD at the lumbar spine and femoral neck (GE Lunar) were measured in 549 women, aged 63.2 ± 12.3 yr (mean ± SD; range: 49 – 88 yr), including low trauma 77 fracture cases. Lower SOS at the distal radius, tibia and phalanx, which were correlated with each other, were associated with increased risk of fracture. Independent predictors of fracture risk (in multivariate analysis) were: distal radius SOS (OR: 1.8 per SD; 95% CI: 1.3 – 2.4), femoral neck BMD (OR per SD: 1.9; 95% CI: 1.4 – 2.4) and age (OR per 5-yr: 1.2; 95% CI: 1.0 – 1.5). Approximately 30% of the women had distal radius SOS T-scores < -2.5; however, only 6.6% of women had both BMD and SOS T-scores < -2.5. Among the 77 fracture cases, only 14 (18.2%) had both BMD and QUS T-scores below -2.5.

In these postmenopausal women, speed of sound at the distal radius was associated with fracture risk, independent of BMD and age. The combination of QUS and BMD measurements may improve the accuracy of identification of women who likely sustain a low trauma fracture.

P22

CHILDREN WHO HABITUALLY AVOID DRINKING COW'S MILK BREAK A LOT OF BONES AT A YOUNG AGE

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This study was undertaken to compare the fracture rates of a group of Dunedin children habitually avoiding cow's milk with the fracture rates of children living in the same community. The lifetime fracture histories of 50 milk-avoiders (30 girls and 20 boys) aged 3-13 years were compared with those observed in a birth cohort of over 1000 children from the same city¹. A goodness of fit Chi square test was used to test the observed frequencies in these groups. The milk-avoiders did not use calcium-rich food substitutes appropriately and had low (mean (SD) dietary calcium intakes (434 (226) mg/day) and volumetric bone mineral density (33% radius -0.72 (1.2), lumbar spine -0.72 (1.3) z scores). Many were overweight (22 of 50 having a body mass index > 85th percentile for age). Significantly more milk-avoiding subjects than expected had sustained fractures (16 observed versus 6 expected in the birth cohort, $\chi^2 = 31.0$, $P < 0.001$, $df = 5$). Milk-avoiders also experienced more fractures in total than the birth cohort population (22 observed versus 8 expected, $\chi^2 = 33.6$, $P < 0.001$, $df = 5$) with the majority of these fractures (18 of the 22) being associated with only slight trauma. All fractures in milk-avoiders occurred prepubertally, 19 being recorded before the age of 7 years. The distal forearm was the most common skeletal site affected (12 fracture events among 9 subjects). We conclude that young milk-avoiders who use few substitute calcium-rich foods are fracture-prone. Families of milk-avoiders need to be advised of the association of this habit with fragility bone fractures. (Grant support: Fonterra and Health Research Council of New Zealand)

1. Jones IE *et al* (2002) *Osteoporosis International* 13:990-995.

P23

1,25-DIHYDROXYVITAMIN D3 ENHANCES HUMAN OSTEOBLAST RESPONSIVENESS TO ESTRADIOL IN VITRO.

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Estrogen is important in the regulation of bone mass. Decreases in circulating E₂ concentrations are associated with bone loss due to an uncoupling of bone formation and resorption in association with increased bone turnover. Tissue response to estrogen is mediated by two estrogen receptor (ER) subtypes, ER α and ER β , both of which are present in human osteoblasts. The possibility that 1,25-dihydroxyvitamin D (1,25D) might enhance human osteoblast responsiveness to 17 β -estradiol (E₂) was investigated. Semiquantitative RT PCR showed that ER α but not ER β expression was increased in SV40 transformed human osteoblast-like bone cells (SV40HBC). In functional studies, cell proliferation, assessed by thymidine incorporation, was significantly stimulated by E₂ (10⁻⁸ M) only with 1,25D pre-treatment. Pre-treatment with 1,25D (10⁻⁹ M) significantly enhanced E₂-induced (10⁻⁹ and 10⁻⁸ M) alkaline phosphatase activity. Pre-treatment with 1,25D also significantly stimulated E₂-induced (10⁻¹⁰ and 10⁻⁸ M) increases in extracellular matrix synthesis as measured by trans ³⁵S incorporation. E₂ (10⁻¹¹ and 10⁻⁹ M) significantly protected cells from doxorubicin-induced apoptosis. The protective effect, however, was not increased in cells pre-treated with 1,25D. The data, consistent with previous studies in rodent osteoblasts, indicate that many estrogen responses in human osteoblasts are enhanced in the presence of 1,25D, possibly through its effect to increase ER α expression. The anti-apoptotic effect of E₂, known to be mediated by a non-classical pathway, is not enhanced by 1,25D. Vitamin D deficiency may adversely impact on maintenance of bone mass by estrogens.

P24

MICROARRAY ANALYSIS OF OSTEOCLAST-LIKE CELLS DIFFERENTIATED WITH LIPOPOLYSACCHARIDE.

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Osteoclasts are large multinucleated hematopoietic cells that act to resorb bone. The differentiation of osteoclast progenitor cells into mature osteoclasts *in vivo*, is tightly regulated by bone-forming osteoblasts through the action of M-CSF and RANKL. The substitution of RANKL with bacterial lipopolysaccharide (LPS) has been shown to induce the differentiation of osteoclast progenitor cells *in vitro* into osteoclast-like cells (OCL). Our aim in this study was to identify novel genes regulated by LPS during this process. We have used RIKEN 40,000 element mouse cDNA microarrays to establish the gene expression profile of LPS-differentiated mouse bone marrow OCL (LPS-OCL) and compared this with mouse co-culture derived osteoclast-like cells (OCL), and mouse bone marrow-derived macrophages (BMM). We identified gene clusters that are: (i) up-regulated in OCL compared to BMM and LPS-OCL; (ii) LPS-inducible genes; (iii) down-regulated by LPS relative to BMM and OCL; (iv) shared by OCL and LPS-OCL. Amongst these genes are known osteoclast marker genes as well as novel genes that have not also been associated with the three different cell populations. Shared osteoclast marker genes with lower expression in LPS-OCL implies that LPS-OCLs represent immature osteoclasts under pathological conditions. Co-culture derived osteoclast may represent more mature osteoclasts. We are characterising differentially expressed genes in OCL and LPS-OCL to identify novel regulators of osteoclast differentiation and function.

P25

THE ROLE OF PROTEASE-ACTIVATED RECEPTOR-1 IN BONE HEALING

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Protease-activated receptor (PAR)-1 is a G-protein-coupled receptor which is specifically activated by the coagulation protease, thrombin. PAR-1 is expressed by osteoblasts *in vitro* and *in vivo*, and thrombin stimulates proliferation of cultured osteoblasts. The aim of the current study was to investigate the role of PAR-1 in bone healing using PAR-1-null mice. Holes were drilled transversely through the diaphysis of both tibiae of PAR-1-null and wild-type mice (12 weeks old). Tibiae were collected at different times and processed for histological examination and histomorphometry. In wild-type mice, cells had migrated into the drill site within 3 days of drilling, and new trabecular woven bone had been deposited along the adjacent periosteal surface by day 5 and in the drill site by day 7. Three days after drilling, 50 ± 7 % fewer cells had invaded the drill site in PAR-1-null mice than in wild-type mice. More osteoclasts (306 ± 59 %) were observed in the drill site of PAR-1-null mice than in wild-type mice 7 days after drilling. The area of new mineralised bone was less in the drill site (28 ± 3 %) and on the adjacent periosteal surface (43 ± 5 %) in PAR-1-null mice than in wild-type mice at day 9. From day 14, no obvious differences in the healing bone could be seen between PAR-1-null and wild-type tibiae. *In vitro*, thrombin caused a 3-fold increased in proliferation of calvarial osteoblasts derived from wild-type mice, but did not stimulate proliferation of osteoblasts from PAR-1-null mice. The results demonstrate that responses to thrombin mediated by PAR-1 play a role in early bone healing. It is likely that thrombin in the drill site influences cell proliferation and migration through activation of PAR-1.

P26

SINGLE NUCLEOTIDE POLYMORPHISM ANALYSIS OF THE RUNX2 GENE AND P2 PROMOTER.

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Runt related gene 2 (*RUNX2*)/ core binding factor alpha (*CBFA1*) is a key transcription factor in osteoblast differentiation¹. The aim of this study was detection of polymorphism within the *RUNX2* gene and proximal promoter, in order to test the hypothesis that *RUNX2* is genetically associated with BMD. We previously demonstrated a silent single nucleotide polymorphism in exon 1 contributes to adult bone mineral density (BMD): an effect that may be explained by linkage disequilibrium to nearby functional variants². Two *RUNX2* promoters have been identified (P1 and P2), with the P2 promoter immediately upstream of exon 1. In order to identify SNP related to BMD, we sequenced the P2 promoter from individuals at the extremes of the BMD distribution. Five SNPs and one indel were found within a 3kb region (SNP quoted bp from the proposed ATG exon 1 start site): -2691bp (G>T); -2650bp (G>A); -2415bp (G>A); -1171bp (indel); -1080bp (A>G); -1025bp (T>C). All SNP were highly correlated with the previously reported allele in exon 1. Little is known of the control of *RUNX2* promoters in humans. Unlike the P1 promoter (95kb upstream), the P2 promoter has no TATA box and has a high GC content. Our data demonstrate that the P2 promoter of *RUNX2* contains numerous SNP. Collectively these SNP may influence promoter function.

¹ Ducy P, Zhang R, Geoffroy V, Ridall A, Karsenty G. (1997). *Cell*. 89:747-754.

² Vaughan T, Pascoe J, Kotowicz M, Nicholson G, Morrison N. (2002). *Journal of Bone and Mineral Research*. 17(8):1527.

P27

ENHANCING OSTEOGENESIS USING HEPARAN SULFATES

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Although there is strong evidence that heparin-binding growth factors (HBGF) contribute to bone repair¹, little is known about how they coordinate osteogenesis. Heparan sulfates (HS) play a key role in holding and presenting HBGFs to progenitor cells, and thus coordinating the bone repair process. This study examined the *in vitro* and *in vivo* effects of HS on bone formation.

In vitro, pre-osteoblast cells (MC3T3-E1) were assessed for changes in cell proliferation (BrdU), differentiation (alkaline phosphatase) and mineralisation (calcium accumulation and von Kossa staining) following the addition of HS. In the absence of serum, low concentrations (3µg/ml) of HS increased cell proliferation by 75%, whilst high concentrations (25µg/ml) had no effect on proliferation when compared to controls. Similarly, low-dose HS also increased calcium deposition within the extracellular matrix. The potential of HS was further investigated in a rat midfemoral closed-fracture model, 2 and 5 weeks post-surgery. Two doses of HS (5µg & 50µg) were examined, falling within the effective HS dose observed *in vitro*. At 2 weeks, CT scanning showed that 5µg of HS had increased BMC/BMD by 10% compared to saline controls, whilst BV/TV derived from von Kossa stained sections increased by 20% over saline (p<0.05). High dose HS (50 µg) had no effect, supporting our *in vitro* findings that high doses of HS may inhibit proliferation. No differences were observed for the 5 week groups.

This study illustrates that HS may have the potential to increase fractures healing rates, presumably by acting with HBGFs present within the fracture site. We are yet to determine an appropriate delivery vehicle for HS and how it affects progenitor cells, as evidenced by the lack of difference at 5 weeks.

Berrada, S *et al* (1994) J.Biomat,Sci.Pol.Edn, Vol 6:2 pp. 211-222

P28

TRABECULAR ROD STRENGTH SUGGESTS A GREATER RISK OF VERTEBRAL FRACTURE FOR FEMALES.

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The strength of cancellous bone depends on volume fraction, architecture and tissue material properties. Volume fraction of vertebral bodies is lower than many other anatomical sites and as such architecture plays a major role in vertebral body strength. In this study the strength of trabecular rods was assessed by calculating a load to buckling index using rod thickness and length measurements from SEM anaglyphs of T12 and L1 vertebral bodies. Eight men and seven women were chosen to represent each decade of adult life (20-80 years).

Whilst thickness and length measurements are essentially normally distributed, the load to buckling distribution is severely skewed to the left in a log normal pattern. When males and females are analysed separately it was found that the modal peak of rod strength was less for females than for males (peak values of 16 versus 20 respectively). The log normal distribution is illustrative of a fundamental architectural economy of design which serves to maximise vertebral body strength for a minimal bone mass. However, the skewed shape of the load to buckling distribution indicates that, in an abnormal loading where higher forces are experienced (eg in a fall), a large proportion of the rods would be put at risk of fracture. Female rods have a lower safety margin because their strength is shifted closer to a putative fracture threshold and so for the same abnormal force females are more likely to fracture a significant proportion of their rods.

The log normal skew of the load to buckling index illustrates the relationship between rod dimensions and their strength. In this study the load to buckling index for thoraco-lumbar vertebral trabecular rods quantifies a greater risk of vertebral fracture for females.

P29

GENES FOR OSTEOCLAST PRECURSOR CELL DETERMINATION: OSTEOCLAST OR MACROPHAGE

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Peripheral blood mononuclear cells (PBMCs) exposed to M-CSF differentiate into macrophage cells whereas M-CSF and RANKL treatment results in osteoclast cells. Differential Display (DD) analysis was used to identify genes induced in either osteoclasts or macrophages. DD identified 29 candidate genes differentially expressed between PBMC precursor cells, macrophage cells and osteoclast cells. These include ADO21, ERV-L, ODC-AZ, HLM3, TRIM9, IFN α β R, TLR8, EP2, SF3b s2, PRO1859, ribosomal protein L41 and a number of uncharacterized sequences. Further analysis with real time PCR validated the differential expression of a novel transcription repressor (HLM3) up-regulated 6.0 fold in macrophages compared with osteoclasts. HLM3 contains a Kruppel-associated box (KRAB), which is a potent transcriptional repressor domain. Toll-like receptor 8 (TLR8) expression was up-regulated in osteoclasts compared with macrophages. TLR(1-9) stimulation of murine osteoclast precursor cells by microbial products has been reported to inhibit osteoclast differentiation, with stimulation of TLRs on mature multinucleated murine osteoclasts promoting cell survival. EP2 (Epididymis-specific 2) was up-regulated in osteoclasts and has significant homology to the β -defensin family. β -defensins have been shown to increase expression in response to elevated Ca²⁺ and activate ion channel activity in biomembranes. ERV-L (Endogenous retroviral-like) was found to be up-regulated in osteoclasts. Several genes were not regulated between osteoclast and macrophage cell types, including a gene SF3b s2, and TRIM9. These provide useful controls for gene expression studies.

P30

THE ROLE OF COX-2 IN WOVEN BONE ADAPTATION

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Woven bone is a transient tissue that acts to rapidly provide and restore strength when bone is damaged, under physical trauma or undergoing growth. This study aimed to investigate whether remodelling of woven bone induced by 4-point-bending is prolonged by treatment with DFU, a specific COX-2 inhibitor. The right tibia of 104 female Wistar rats received a single bout of 4-point-bending in vivo for 300 cycles as haversine waves at a frequency of 2Hz and a magnitude of 65N. Turner et al. [1] have previously shown that this high strain loading regime induces woven bone formation at the periosteal surface 5-9 days post-loading and peaks after about 15 days. Following daily injections of vehicle or DFU, commencing 7 days after loading, rats were sacrificed weekly at 2, 3, 4 and 5 weeks post-loading to encompass one complete remodelling period. Tibiae were harvested, embedded in polymethylmethacrylate and sectioned for histomorphometric analysis. Initial measurements of the woven bone area (Wo.B.Ar, μm^2) have indicated a strong trend with time, such that there was a significant increase in area from week 3 to week 5 ($P < 0.05$). Wo.B.Ar markedly increased from 0.059 mm^2 and 0.055 mm^2 at week 3 to 0.152 mm^2 and 0.165 mm^2 at week 5 in the DFU[0.02mg/kg/d]- and DFU[2.0mg/kg/d]-treated rats, respectively. This suggests increased formation or decreased remodelling of woven bone during this period. This early result may indicate that COX-2 inhibition may stop or delay the reduction or consolidation of woven bone, increasing woven bone area parameters 5 weeks post-loading. Further analyses of these data are underway, which should increase our understanding of the involvement of COX-2 in woven-bone adaptive events.

1. Turner, C., T. Woltman, and D. Belongia, *Structural Changes in Rat Bone Subjected to Long-Term, in Vivo Mechanical Loading*. Bone, 1992. 13: p. 417-422.

P31

SMOKING AND BONE MINERAL MEASURES.

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A reduction in measures of bone mineral density (BMD) and an increase in fracture risk are widely reported in smokers. We sought to compare bone mineral measures and rates of bone loss in healthy female smokers (n= 134) and non-smokers (n = 246) age 40 years and above.

Lumbar spine (LS), total hip (TH), femoral neck (FN), total forearm (FA) BMD and total body (TB) bone mineral content (BMC) were measured (Hologic QDR 1000W). Subjects' self-reported the history of their smoking habits, and cumulative smoking was expressed as pack years (years of smoking x cigarettes per day ÷ 20).

The mean (SD) age, height and weight of smokers and non-smokers were: 50.8 (8.5) vs. 53.8 (9.9) years (p=0.001), 1.62 (0.06) vs. 1.60 (0.07)m (p=0.007) and 65.2 (12.7) vs. 68.3 (14.5)kg (p=0.029), respectively. The smokers mean (SD) number of pack-years was 4.4 (9.3). Smokers were on average 3.1 kg lighter than non-smokers due to a difference in total fat mass. BMD adjusted for age, height and fat mass was significantly lower in smokers at multiple sites: by 4.2% at the TH (p=0.003), 3.6% at the FN (p=0.02), 2.8% at the FA (p=0.003), and 4.4% for the TB BMC (p=0.001). There were significant differences in annual rates of change in BMD between smokers and non-smokers, respectively [TH: -0.9% vs. -0.5% (p=0.028); FA: -1.2% vs. -0.8% (p=0.01)].

These data further characterize the adverse effects of smoking on bone health. Smoking cessation may provide a major opportunity to reduce osteoporotic fracture risk. However, direct evidence of the skeletal benefits of quitting smoking is needed.

P32

RECEPTOR ACTIVATOR NFκB LIGAND (RANKL) AND OSTEOPROTEGERIN (OPG) IN PERIODONTITIS. Tania N. Crotti, Malcolm D. Smith, Helen Weedon, David R Haynes. Department of Pathology, University of Adelaide. SA. 5005, Australia.

This study investigated the expression of key mediators that regulate differentiation of osteoclasts, RANKL, and its natural inhibitor, OPG, in periodontitis. We aimed to compare the levels of the RANKL and OPG in the granulomatous tissue adjacent to areas of alveolar bone loss from patients with periodontitis to that present in tissue from patients without periodontitis. In addition, we determine the types of cells expressing these factors in these tissues and to demonstrate the expression of the osteoclastic markers, RANK and tartrate resistant acid phosphatase (TRAP), in periodontitis. Frozen biopsy specimens were analysed using specific monoclonal antibodies and were evaluated by semi-quantitative analysis and digital image analysis to compare levels of RANKL and OPG protein expression. Double labelling of frozen sections with antibodies to different cell lineage specific markers were used to determined the types of cells expressing these proteins. In situ hybridisation was used to detect cells expressing RANK mRNA. Semi-quantitative image analysis demonstrated that significantly higher levels of RANKL protein (p<0.05) was expressed in the periodontitis tissue. Conversely, OPG protein was significantly lower (p<0.05) in the periodontitis tissues. RANKL protein was associated with lymphocytes and macrophages. OPG protein was associated with endothelial cells in both tissues. Many leucocytes expressing RANK mRNA and tartrate resistant acid phosphatase were observed in periodontitis tissues. The change in the levels of these key regulators of osteoclast differentiation may play a major role in the bone loss seen in periodontitis.

P33

THE EFFECTS OF THE CALCIUM-SENSING RECEPTOR GENE POLYMORPHISM A986S ON BONE AND CALCIUM HANDLING IN A LARGE COHORT OF ELDERLY WOMEN.

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Genetic variation in factors involved in calcium homeostasis may contribute to effects on bone mass and osteoporotic fractures. We hypothesised that the A986S polymorphism in the calcium sensing receptor gene (CASR), which results in an Alanine for Serine substitution, may result in variations in the calcium set-point, with consequent effects on bone mass and fracture in elderly women. The association of A986S with variation in calcium homeostasis, markers of bone formation and resorption and bone mass was studied in 1332 ambulatory Australian women. Bone mass was evaluated by quantitative ultrasound measurements (QUS Lunar Achilles) of the heel and DXA (Hologic 4500A) of the hip. Prevalent and 36 month incident fracture data were collected. Of the population, 24% was heterozygous and 1% homozygous for the S allele. No effect of the absence compared to the presence of the S allele was found on albumin adjusted calcium concentration (mean±SD) mmol/L 2.26±0.11 vs 2.28±0.13, renal calcium creatinine ratio (mmol/mol) 259±202 vs 295±207 or PTH (pmol/L) 4.0±1.7 vs 3.9±1.5. No effect was seen on osteocalcin (µg/L) 5.0±3.2 vs 5.8±3.9 or the deoxypyridinoline creatinine ratio (µmol/mol) 28.9±10.1 vs 30.7±12.1. The A986S was not associated with bone mass (DXA total hip BMD g/cm² 0.81 ± 0.12 vs 0.81 ± 0.12) or QUS (SOS m/sec, 1512±25 vs 1513±26). No association with prevalent or incident fractures was observed. A difference in calcium levels of 0.05 pM, DXA bone density of 24 mg and fracture rate 1.3 fold could have been detected at a power of 0.8. It is therefore unlikely that the A986S polymorphism is of any clinical significance in this age group.

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GLUCOCORTCOIDS INFLUENCE ON OSTEOCLAST GENERATION & ACTIVITY

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Glucocorticoids are widely used in the treatment of patients with chronic noninfectious inflammatory diseases. Pharmacological doses of glucocorticoids cause bone loss but the mechanisms underlying this are not fully understood. Glucocorticoids predominantly affect osteoblast proliferation and life span. Much of the bone loss is due to reduced bone formation but there is also an element of increase osteoclastic activity. We previously reported that, using a murine myeloid osteoclast precursor cell line RAW264.7, incubated with soluble RANK-Ligand (RANKL) there was no change in early osteoclast generation with dexamethasone, except at the highest dose, but there was an increase in osteoclast lifespan in the presence of dexamethasone. The current studies used human peripheral blood mononuclear cell cultured on whale dentine and induced to differentiate to osteoclasts by RANK-L and human M-CSF. Osteoclast activity was measured by pit area. In the earlier phase of osteoclast generation from 0-16d, dexamethasone at 10⁻⁸M increased pit area by 2.5fold while lower concentrations had no effect. At the highest dexamethasone concentration (10⁻⁷M), pit area was reduced (as seen with RAW264.7 cells). In contrast to the RAW264.7 cell studies, in separate studies using 2 different donors, no evidence of increased life span of osteoclasts was obtained in the presence of dexamethasone, RANK-L and h-MCSF. In addition to direct effects of dexamethasone on osteoclast precursors, osteoprotegerin expression by human osteoblasts measured by real time RT-PCR was dose-dependently reduced by dexamethasone. These data provide some explanation at a cellular and molecular level for the observed increase in resorption seen in patients treated with glucocorticoids.

P35

DIFFERENTIATION POTENTIAL OF A MOUSE BONE MARROW STROMAL CELL LINE

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Kusa O cells are a mouse bone marrow stromal line that have the capacity for multilineage differentiation and to support osteoclast formation. We have subcloned these cells to obtain genetically similar clones that could be used in the study of osteoblast differentiation.

Four subclones were selected for characterization. One that neither differentiated into osteoblasts nor adipocytes, a second that differentiated into osteoblasts but not adipocytes and two that differentiated into both osteoblasts and adipocytes. The non-mineralizing clone had no detectable alkaline phosphatase activity at 21 days. Alkaline phosphatase activity and mRNA in the three mineralizing clones was comparable with the parent cells after 21 days in osteoblast differentiating medium. PTH receptor-1 mRNA and activity increased in the four subclones and parent cells with differentiation. mRNA for osteopontin and bone sialoprotein, were similarly expressed in the parent cells and subclones while mRNA for the transcription factors, Runx2 and osterix were detectable in both parent and subclone cells. Interestingly PPAR α mRNA expression did not correlate with cell line potential to differentiate into adipocytes. Indian hedgehog mRNA and its receptor (patched) mRNA levels both increased with differentiation while mRNA levels of the Wnt pathway components, β -catenin and dickkopf 1, also increased with differentiation. We have focussed on characterizing these clones from the osteoblast perspective but they may be useful for studying both osteoblast and adipocyte differentiation and well as their transdifferentiation.

P36

WHAT A VERTEBRAL FRACTURE?

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As there is no gold standard to distinguish anatomical variation from vertebral fracture (VF), group differences in the VF prevalence may reflect differences in methodology, not differences in fragility. A quantitative definition of VF was developed based on (i) a new method of defining threshold values and (ii) the requirement for two abnormal height ratios which reduces the chance of false positives ten fold. BMD and vertebral heights were measured using dual X-ray absorptiometry (Lunar Expert-XL) in 697 Lebanese women (age 20-89 years). VF prevalence ranged from 7 to 70 percent using published cut-offs defining 'fracture'. At 3 SD, the prevalence of deformities was 70-80% in older women(aged 50 and over) and women with lower BMD, but ~65% in younger women (aged 20-49) and women with normal BMD; 4 SD or 15% cut offs produced similar high sensitivity and poor specificity. The 20% cut off produced a 40-50% prevalence in older women and women with low BMD, a 25-35% prevalence in young women and women with normal BMD. The 30% cut off produced a low prevalence which decreased with age. The new classification produced prevalence figures of 3.3% in younger and 14% in older women, 20, 10, and 7% in the lower, middle and upper BMD tertials respectively. 'Deformities' detected in younger women were not associated with height loss or low BMD. Subtracting the prevalence of deformities in young from older women resulted in a decrease the disparity from 10 to 2 fold between methods. Current morphometric criteria for VF produce prevalence figures which are unrelated to age or low BMD and are likely to capture anatomical variation rather than structural failure. A standardized definition of deformities is needed before credible comparison across epidemiological studies can be made.

P37

COMPARISONS OF BONE MASS AND AREA IN MATCHED OBESE AND NON-OBESE CHILDREN AND ADOLESCENTS

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To determine whether obese and lean children differ in bone area or bone mineral content (BMC) we studied 54 pairs (29 girls, 25 boys) of obese (BMI > 95 centile for age) and non-obese (BMI < 85 centile for age) Caucasian children matched for gender, chronological age, height and Tanner stage of pubertal development. In each gender we compared bone area (cm²) and BMC (g) in the obese and non-obese subjects using measurements obtained from Lunar DPX-L dual energy x-ray absorptiometry scans of the total body, spine, hip, and radius. Obese subjects carried significantly greater body weight (boys 47% and girls 38% more) and had larger skeletons (8% greater total bone area in both genders) than their non-obese controls. Regionally, bone area was also greater in the neck of femur (5%) and hip trochanter (21%) in obese boys and at the 33% radius site (7%) in obese girls. Obese boys had higher BMC in the whole skeleton (13%), hip trochanter (24%) and 33% radius (13%) sites than controls, while obese girls had greater BMC in the total body (13%) lumbar spine (9%), ultradistal radius (14%) and 33% radius (13%) sites. Obese girls, but not boys, had higher volumetric spinal values than controls (12%) but volumetric values were not higher at the wrist and hip sites in obese subjects. The greater bone size and mineral content of these obese children cannot be attributed to greater height or earlier pubertal development. However, their adaptive bone changes were small in magnitude relative to the excess weight they carried. This mismatch of bone to weight could explain the susceptibility of obese children and adolescents to fracture.

(Grant support: Health Research Council of New Zealand.)

P38

DO ALTERATIONS IN KNEE BONE SIZE AND OTHER FACTORS MEDIATE THE GENETIC RISK OF KNEE OSTEOARTHRITIS?

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A genetic contribution to knee osteoarthritis is well documented. However, the mechanism of this is unclear. The objective of this population-based case control study was to describe the differences in body composition, muscle strength, pain, cartilage volume and bone size between offspring of those with severe knee osteoarthritis (OA) and age and sex matched controls without this history.

We interviewed 372 male and female adults aged between 26 and 61. Of these, 50% had at least one parent who had severe knee OA requiring knee replacement and 50% were age and sex matched randomly selected controls. Articular cartilage volume and bone size were determined at the patella, medial and lateral tibia sites by quantitative MRI. Anthropometric factors were measured while knee injury, knee pain and occupation were assessed by questionnaire.

Offspring were significantly different to controls in body weight (+3.2 kg, 95% CI 0.2, 6.1), lower limb muscle strength (-8.4 kg, 95% CI -14,-2), knee pain (47% v 23%, p<0.001) and medial tibial bone area (17.6 v 17.0 cm², p=0.01). There were no significant differences in other lifestyle factors and no significant reduction in knee cartilage volume at any site (lateral tibial +92ul, p=0.07, medial tibial +57ul, p=0.22, patella -2ul, p=0.99). In multivariate conditional logistic regression, muscle strength, knee pain and tibial bone area (but not body weight) remained significant predictors of offspring status.

In conclusion, there was no difference in cartilage volume between offspring of subjects with severe knee OA and controls. However, offspring weighed more, had weaker lower limb muscles, more knee pain and larger tibial bone area than controls provocatively suggesting that all these factors play a role in the genetic regulation and development of knee osteoarthritis.

P39

THE ASSOCIATION BETWEEN QUANTITATIVE ULTRASOUND AND ESTROGEN RECEPTOR STATUS IN BREAST CANCER PATIENTS

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Bone mass may serve as a marker for cumulative oestrogen exposure, which is an established risk factor for breast cancer. Several international studies have supported this theory in observing an increased incidence of breast cancer in women with higher bone mass.

We used quantitative ultrasound (QUS) of the calcaneus as a measure of bone quality in a case-control study of women within the Barwon Region. Cases include 64 women (median age = 55 yrs, range = 32-82 yrs) recently diagnosed with breast cancer with a median time between diagnosis and bone measurements of 4 months. Controls include 94 women (median age = 57.5 yrs, range 30-88 yrs) randomly selected from the electoral roll, without prior diagnosis of cancer. Data collected included quantitative ultrasound of the calcaneus by Lunar Achilles Insight ultrasonometer, bone mineral density (BMD) by DEXA (Lunar DPX-L). Estrogen receptor status in cancer cases was established from tumour tissue specimens.

Our results showed that positive estrogen receptor status in breast cancer cases is associated with a higher broadband ultrasound attenuation (BUA) and speed of sound (SOS), and therefore stiffness index (SI) after adjusting for age and weight (p < 0.058, p < 0.023 and p < 0.024 respectively). Progesterone receptor status was not associated with these indices. In case-control analyses there was a trend towards higher BUA in breast cancer cases (p < 0.16), while SOS was significantly lower (p < 0.008) after adjusting for age and weight. BMD at the spine and hip did not differ between cases and controls (p< 0.326, p < 0.607, respectively).

These results suggest a complex relationship between estrogen, tumour estrogen receptor status and bone in breast cancer patients and warrants further investigation.

P40

GENETIC AND ENVIRONMENTAL CONTRIBUTIONS TO HIP STRENGTH: A TWIN STUDY

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A new technique [hip structural analysis (HSA)] allows the mechanical properties of bone to be estimated from densitometric scans of the proximal femur. The aim of this paper is to investigate variation in the biomechanical measures derived using HSA for genetic and environmental components in young females.

Proximal femur scans from 83 female twin pairs (43 monozygotic, 40 dizygotic) were analysed. HSA provided measures of BMD (g/cm³), cross-sectional area (CSA, cm²), cross-sectional moment of inertia (CSMI, cm⁴), width (cm), section modulus (SM,cm³), average cortical thickness (CT, cm) and buckling ratio (BR) at the narrowest region of the femoral neck (NN), intertrochanteric (IT), and femoral shaft (FS) regions. Data were adjusted using developmental age [DA; chronological age adjusted for the difference between subjects' age at menarche and the population mean age of menarche (12.9 years)]. The mean(SD) age and DA was 12.9 (2.8) years and 12.8 (2.9) years, respectively. Falconer's estimate of heritability [h = 2*(rMZ-rDZ)], was used to estimate the genetic contribution to variance.

At the NN, DA- adjusted CSMI, width, SM, and BR were moderately to highly heritable (h range: 0.43 – 0.79). Adjustment for DA-height-weight increased the estimate of h (h range: 0.79 – 0.94). DA-adjusted measures of BMD, CSMI, width, SM, and BR were moderately to heritable at the IT region (h range: 0.62 – 0.67). The DA-adjusted FS BMD, CSA, CT and BR, were moderately to highly heritable (0.51 – 0.70). Adjustment for DA-height-weight increased h for BMD, CSA, and CT (h range: 0.66 – 0.85).

This analysis of the mechanical properties of the proximal femur derived by HSA has demonstrated for the first time a genetic influence on putative determinants of hip strength that are not captured by BMD and hip geometry (hip axis length).

P41

DISCORDANCE IN LUMBAR SPINE DXA REFERENCE RANGES IN MALES

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We have previously demonstrated significant differences in the female reference ranges used by the various DXA manufacturers. Similar differences may occur in male reference ranges. We assessed the reference ranges in Lunar DPXL (Version 1.35) and Norland Excel (Version 2.3.1) instruments in 30 male subjects scanned on both instruments. We compared lumbar spine (L2-L4) BMD, as well as T and Z scores, in 30 males measured on the same day.

	BMD (g/cm ²) mean (SD)	sBMD mean	T score mean (SD)	Absolute T score	Z score mean (SD)
Lunar	1.201 (0.22)	1143	-0.30 (1.85)	1.59 (0.94)	-0.03 (1.76)
Norland	1.078 (0.23)	1161	-0.19 (1.36)	1.14 (0.74)	0.32 (1.31)

Absolute BMD measured by the two instruments was highly correlated (r = 0.98, p<0.0001). As expected the Lunar absolute BMD was higher than the Norland (average difference 0.121g/cm²) but there was no significant difference in sBMD. There were significant differences in lumbar spine T scores when expressed as absolute units (average difference 0.45, p<0.0001). In the subgroup of subjects in the clinically significant range (T< -2.0) the differences between absolute T scores were more marked (n= 7, average difference 0.95, p< 0.002).

In conclusion there are significant differences in the male lumbar spine reference ranges used by different DXA manufacturers which potentially may effect clinical decision making. Standardisation of reference ranges in males is urgently required.

P42

A COMPARISON OF BONE MINERAL DENSITY IN ASIAN AUSTRALIAN AND CAUCASIAN AUSTRALIAN WOMEN.

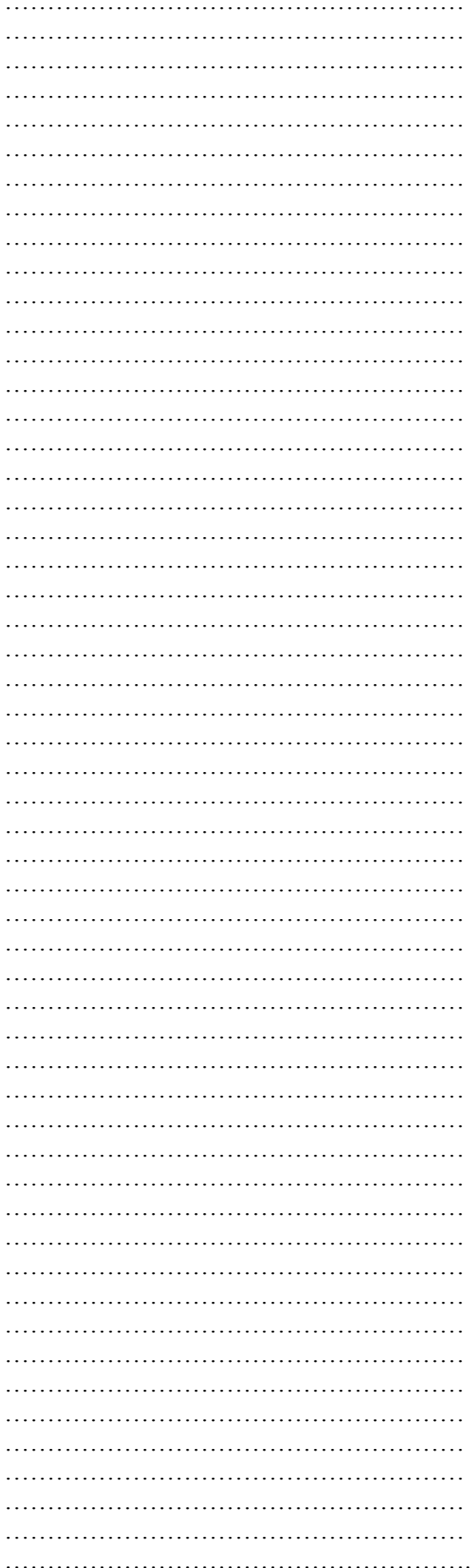
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There is marked heterogeneity in bone mineral density (BMD) and fracture epidemiology between Asian and Caucasian populations. Currently, interpretation of bone mineral density (BMD) values of Asian Australians (AA), who comprise 8% of our population, usually relies on Caucasian reference data. We compared bone mineral measures and soft tissue composition of AA women and Caucasian Australian women (CA) to determine if it is appropriate to use Caucasian reference data for AA.

80 healthy volunteer AA of Chinese ethnicity had lumbar spine (LS), forearm (FA), total hip (TH) femoral neck (FN) and whole body (WB) BMD measured (Hologic QDR 1000W). The 80 AA were matched for age, height and weight with CA, from cross-sectional data on 1300 CA. Comparisons were made using paired t-tests for LS, FA, TH, FN, and WB BMD, bone mineral content (BMC), area, fat and lean mass.

There was no significant within-pair difference in age, height or weight. A significant within-pair difference (p<0.05) was found in WB lean mass, and FA, TH, FN and WB area. No significant within-pair difference was found in LS, TH, FN or WB BMD. A significant difference (p<0.05) was found, however, when AA and CA LS BMD were compared with age-matched only CA normative LS BMD data.

After controlling for anthropometric differences, there appears to be no difference in AA and CA BMD, but differences in size and lean mass remain. Further research is needed to establish normative BMD and fracture risk data for AA. These data will improve the understanding and assist management of bone health in Asian Australians. Our observations also suggest the need to develop an appropriate reference range, using anthropometric adjustments, for CA in the extreme percentiles of height and weight.



P43

BONE CELL BIOLOGY IN AN ANIMAL MODEL OF IMPAIRED BONE MATRIX TURNOVER.

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Mucopolysaccharidosis type VI (MPS VI) is characterised by a deficiency in N-acetylgalactosamine 4-sulphatase. As a result glycosaminoglycans accumulate within lysosomes of connective tissues and the skeletal system is a major site of pathology. The pathology has been analysed in the MPS VI cat, which displays severe osteopenia due to a reduction in the bone formation rate [1]. To further characterise the pathology, the ability of MPS VI osteoblasts to produce and elaborate bone matrix was analysed.

Cultures were established from trabecular bone of normal and MPS VI cats. Similar amounts of type I collagen and alkaline phosphatase (ALP) were produced by normal and MPS VI cultures although a potentiation of ALP in day 5 MPS VI cultures was observed, suggesting a lag in osteoblast maturation. MPS VI cell proliferation was also significantly elevated (1.5 fold), in agreement with a departure in MPS VI, from the normal processes controlling the timely sequence of osteoblast maturation. Furthermore, MPS VI bone cells showed a 1.9 and 1.75 fold-decreased trend in total proteoglycan synthesis and turnover rates. Results strongly support the involvement of osteoblasts in MPS VI skeletal pathology and suggest that it may partly arise from the reduced ability of these cells to mature and elaborate a functional bone matrix.

[1] Byers S, Nutall JD, Crawley AC, Hopwood JJ, Smith K and Fazzalari NL (1997). Bone 21: 425.

P44

LEPTIN INHIBITS OSTEOCLASTOGENESIS IN VITRO VIA LYMPHOCYTES

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Lymphocytes (Ly) produce a range of cytokines that can influence osteoclast (OC) formation and activity. Previously, using PBMC as OC precursors, we showed that leptin inhibits human OC formation *in vitro*, acting via the Ly fraction. In the current study we have investigated which Ly populations mediate the leptin effect.

Total human Ly and CD4+, CD8+ and CD19+ Ly were prepared by negative selection (96-98% purity). Human cord blood-derived CFU-GM cells, expanded for 10 d in methylcellulose containing GM-CSF, IL-3 and SCF, were used as OC progenitors. CFU-GM cells (10⁴ cells/well), which are Ly-free, were cultured for a further 12 days on dentine slices with RANKL and M-CSF to form OC.

When added with leptin (10⁻⁷ M) in the CFU-GM model, CD8+ cells suppressed OC formation (80% inhibition) while CD8+ cells had no effect when added without leptin. The addition of CD4+ cells (>10⁴ cells/well) abrogated OC formation indicating that CD4+ Ly can inhibit OC formation independently. Leptin caused induction of GM-CSF mRNA and OPG mRNA expression in Ly isolated from PBMC and both GM-CSF and OPG, in the CFU-GM model, caused inhibition of OC formation (IC₅₀ of 0.2 ng/ml and 8 ng/ml, respectively). 2D proteomic analyses of leptin-induced phosphoprotein changes in non-adherent PBMC (>95% Ly) and adherent monocyte/macrophages showed marked differences; STAT1 and 3, leptin receptor (ObRb) and src-family members were phosphorylated. Results have been corroborated by flow cytometry.

In conclusion, lymphocytes are potent regulators of osteoclastogenesis and leptin, a regulatory cytokine that affects bone turnover in both *in vitro* and *in vivo* models, appears to act by cytokine release from CD8+ Ly. Other osteotropic factors must now be reassessed to determine the role of Ly in normal bone turnover and in metabolic bone diseases.

P45

BIOCOMPATABILITY OF TANTALUM FOR OSTEOBLASTS

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The metal tantalum (Ta) is increasingly used for a variety of orthopaedic applications. However, there is no information on the interaction of human osteoblasts with this material. Therefore, the aim of this study was to investigate the ability of Ta to support the growth and function of normal human osteoblast-like cells (NHBC). Since microtexture appears to be a key parameter affecting cellular response, polished Ta discs were compared with chemical vapour deposited discs, which gives a textured surface. Cell responses to Ta were compared with responses to other common orthopaedic metals, titanium and cobalt-chromium alloy and with cell growth on tissue culture plastic.

No consistent differences, that could be attributed to the different metal substrates or to the surface texture, were found in the following parameters. The rate and extent of attachment of NHBC to each substrate was similar, as was cell morphology, determined by confocal microscopy. Cell proliferation, measured over 7 days by labelling NHBC with the fluorescent cell dye, CFSE, showed that neither the absolute cell numbers, nor the number of cell divisions, was significantly different between Ta and the other substrates. Using RT/PCR, no consistent, substrate-dependent differences were seen in the expression of mRNA species corresponding to the pro-osteoclastic (RANKL, OPG, IL-11, TNF α) and the osteogenic (CBFA1, Col-1, BSP and OCN) activity of osteoblasts, or in secreted OPG protein levels. No substrate-dependent differences were seen in the extent of *in vitro* mineralisation by NHBC.

These results indicate that Ta is a good substrate for the attachment, growth and differentiated function of human osteoblasts.

P46

THEIR FUTURE IN THE BALANCE – A STUDY OF YOUNG TWINS.

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Extensive research has established balance function and muscle strength as important and measurable contributors to fall- and fracture-risk in the elderly. Twin studies have provided preliminary evidence to suggest a significant genetic contribution to balance function in females over the age of 45. The aim of the current study was to investigate genetic contributions to balance and muscle strength that might operate during childhood and adolescence in a population of female twins, between 8 and 21 years of age.

Forty-three (20 monozygous (MZ) and 23 dizygous (DZ)) pairs aged between 8 and 21 years, completed tests of balance and strength function. Tests included a one-legged stance test, the Lord's Balance Test, static and dynamic posturography tests using a moving platform (the Chattecx Balance System). Test conditions included: single-limb stance, eyes closed, cognitive task and varied base of support. Muscle strength was assessed for knee extensors both for isometric and isokinetic measures of strength using the Kinematic-Communicator (Kin-Com). Ankle dorsiflexor strength was assessed using the Nicholas Manual Muscle Tester (MMT).

After adjustment for age, there were no significant within-pair correlations for the Lord's Balance Test, under a variety of conditions. Height and age-adjusted measures of balance (Chattecx System), identified significant intrapair correlations for both MZ (r=0.59) and DZ (r=0.76). There were no differences (Fisher z-test) between MZ and DZ intrapair correlations, except for stable anterior-posterior sway amplitude (P=0.001). All measures of strength were found to be well-correlated among MZ pairs, with a significant difference between zygosity groups identified (P<0.05). Heritability of muscle strength was estimated to account for up to 78% of the variance of muscle strength.

From this preliminary investigation of muscle strength and balance in young females genetics were estimated to contribute substantially to the variation in muscle strength, but not balance function. Development of more sensitive test procedures and measurements may be required to detect developmental changes.

P47

cDNA EXPRESSION ARRAY ANALYSIS OF DIFFERENTIAL GENE EXPRESSION IN OSTEOBLASTS

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Thrombin has been shown to inhibit serum deprivation-induced osteoblast apoptosis by a pathway independent of the main thrombin receptor, protease-activated receptor-1 (PAR-1). The aim of this study was to identify genes which are differentially expressed by PAR-1 null osteoblasts in response to thrombin. Calvarial osteoblasts isolated from PAR-1 null mice were either serum deprived (control) or serum deprived with the addition of 100nM thrombin (thrombin-treated) for 36 hours. RNA was extracted and used as template for \square ³²P-dATP-labelled cDNA probe synthesis. Control and thrombin-treated probes were each hybridised to an Atlas Mouse 1.2 cDNA Expression Array (Clontech). Images of the control and thrombin-treated autoradiographs were imported into AtlasImage 2.01 (Clontech) for analysis. Of the 1178 genes spotted onto the array, 386 genes were expressed. Of these, 105 were not regulated, 137 genes were up regulated and 144 were down regulated in response to thrombin. Expressed genes included markers of osteogenesis such as RUNX2, ALX4 and osteopontin. Differentially expressed genes represented a number of functional groups including those encoding intracellular signalling molecules, growth factors and cytokines, apoptosis-related factors, proteases, constituents of the extracellular matrix, transcription factors and cell cycle regulators. We are currently using real-time PCR to accurately quantitate the expression of a number of these genes including the IGF binding proteins, MCSF and protein kinase B. Thrombin treatment of primary PAR-1 null osteoblasts results in significant changes in gene expression. We are currently using the information derived from the cDNA expression arrays to identify the signalling pathway involved in thrombin's inhibition of osteoblast apoptosis.

P48

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

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Cross-sectional associations between lean mass (LM) and bone mineral density (BMD) have been widely reported and accepted. The genetic and environmental basis of these associations remains controversial. We studied the extent to which genetic and environmental factors explained LM-BMD associations in young female twins aged 8-17 years.

Data from 162 (88 monozygous and 74 dizygous) twin pairs with a mean (SD) age of 12.58 (2.33) years were included in this analysis. Age- and age-height-adjusted lumbar spine, total hip and total body bone mineral measures were moderately/highly heritable in young twins (Falconer estimate (h) = 0.71 – 0.82) and (h = 0.60 – 0.82), respectively. Age-height-adjusted LM had an heritability estimate of 0.62 in young twins. Analysis of age- and age-height-adjusted cross-trait correlations (LM-BMD) within-individuals (WI) and within MZ and DZ pairs revealed that in young twins, within-pair shared environment plus additive genetic (AG) factors (partly related to height) explained a moderate/high LM-BMD correlation (WI r = 0.55 – 0.87) and (WI r = 0.41 – 0.71) for age- and age-height adjusted data, respectively.

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

P49

EARLY, SOFT TISSUE FRACTURE CALLUS CONTRACTS AND RELAXES *IN VITRO*

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Wound contraction is an essential process in early soft tissue healing, yet this process has not been demonstrated in fracture repair. Fracture callus consists of several cell types, many of which may have the potential to contract.

The aims of this study were to (a) determine whether early fracture calluses contract or relax *in vitro* and (b) identify α -smooth muscle actin in calluses.

The 6th rib of adult male rats was fractured under anaesthesia and the ensuing callus was examined 7-9 days later. Force generated by calluses was measured using a sensitive strain gauge. Calluses contracted in normal Krebs-Henseleit solution (pH = 7.4) at 25°C in the 5 calluses examined. The force response was phasic in 2 calluses and tonic in 3 others. Solution having 64 mM [K⁺] relaxed 3 calluses, and abolished the contraction in 2 others. Solutions having no added Ca²⁺ resulted in contraction, relaxation or no change in the forces produced by the calluses. Under acidic conditions (pH = 5.4) 3 calluses contracted in solutions having high [K⁺], while the other 2 did not contract. Western blot and immunohistochemical studies identified α -smooth muscle actin in whole callus and in the majority of osteoprogenitor cells of fibrous callus respectively.

Our results show that (i) callus is capable of contracting and relaxing (ii) callus shows different contraction and relaxation responses to typical smooth muscle and (iii) cells in soft tissue callus contain extensive α -smooth muscle actin, which underlines the observed contraction of callus.

Contraction of early, soft tissue fracture callus observed *in vitro* may promote bony apposition *in vivo* and thus facilitate fracture repair.

P50

EFFECTS OF BONE DENSITY FEEDBACK AND SMALL GROUP EDUCATION ON OSTEOPOROSIS KNOWLEDGE AND SELF-EFFICACY IN PREMENOPAUSAL WOMEN.

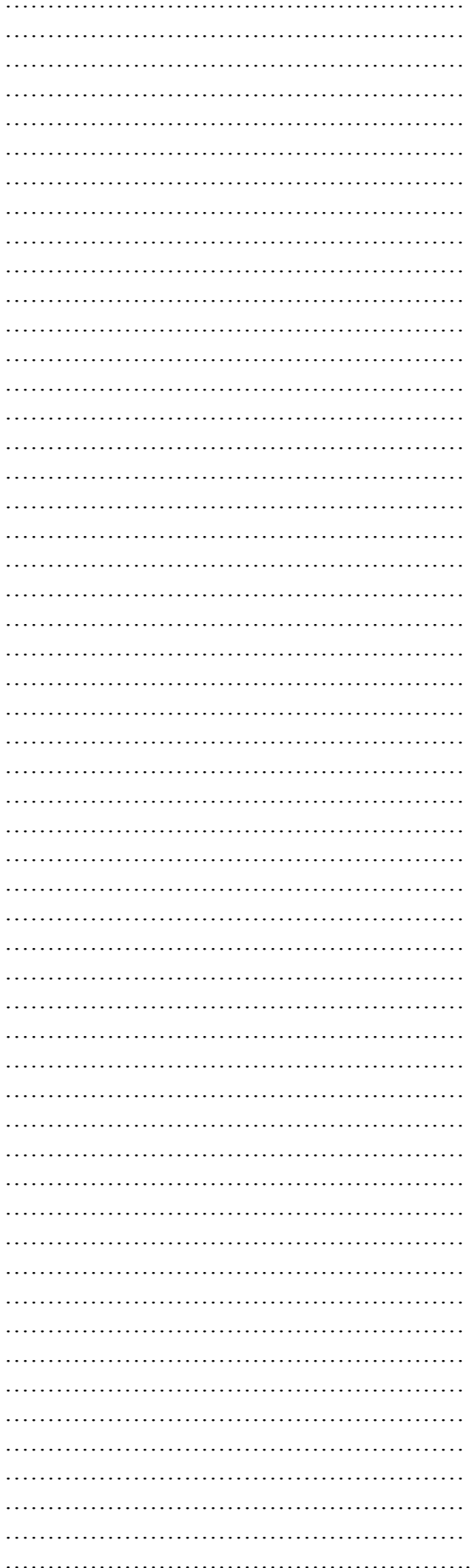
TM Winzenberg, B Oldenburg, S Frenin, G Jones. Menzies Centre for Health Research, Hobart.

Osteoporosis knowledge and osteoporosis self-efficacy are important contributors to exercise and calcium intake behaviours.(1) Low levels of osteoporosis knowledge and self-efficacy have commonly been reported (2-6), yet there is little information available about how to best increase these factors. Two potential interventions are bone density (BMD) feedback and the Osteoporosis Prevention and Self-management Course (OPSMC), which is a small group educational intervention currently delivered in the community setting by Osteoporosis Australia. The aim of this population-based randomised controlled trial was to assess the effect of BMD feedback and a small group educational intervention on osteoporosis knowledge and self-efficacy in premenopausal women.

We studied 468 healthy, non-pregnant women aged 25-44 years who were randomly selected from the electoral roll. Subjects were randomly assigned to receive either an osteoporosis information leaflet or the OPSMC, and also received written feedback regarding their BMD results.

There was an increase in osteoporosis knowledge across all intervention groups. In multivariate analysis, those receiving the OPSMC had a greater increase in both short and long-term osteoporosis knowledge, compared to those receiving the leaflet. The magnitude of the increase declined over time. In contrast, low BMD was associated with an increase in long-term, but not short-term osteoporosis knowledge. Changes in osteoporosis self-efficacy were not associated with either low BMD or receiving the OPSMC but were negatively associated with both number of children and hours of employment.

In conclusion, both the OPSMC and bone density feedback increased osteoporosis knowledge but not self-efficacy over two years. Further research is needed to determine whether increases in osteoporosis knowledge leads to changes in BMD and fracture risk. Women with children who are also in the workforce decreased osteoporosis self-efficacy over two years, suggesting that this group should be a specific target for interventional strategies.



P51

DETERMINANTS OF BONE MINERAL AT DIFFERENT LEVELS OF TOTAL HIP AREAL BONE MINERAL DENSITY

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Determinants of BMD in individuals with low bone mass are of particular clinical interest and have been little reported.

We examined determinants of BMD in 213 pairs of female twins (106 monozygotic, 107 dizygotic) aged more than 45 years (mean age 56 years), divided by tertiles of within-pair average total hip (TH) BMD Z-score. Tertile 1 (T1) was the lowest and T3 the highest tertile. Within-pair differences in risk factors were compared with within-pair percent differences in lumbar spine (LS) and TH BMD and with total body bone mineral content (TBBM), each adjusted for height or height and weight as appropriate.

In the pooled data, lean mass (LM) and fat mass (FM) were significant determinants of height-adjusted LS and TH BMD and TBBM. Cigarette use (in total pack-years) was a significant determinant of height-weight-adjusted LS and TH BMD and TBBM. Current sporting activity was associated significantly ($R^2 = 0.028$, $p=0.02$) and calcium intake marginally ($R^2 = 0.017$, $P = 0.07$) with height-weight-adjusted TBBM. In the analysis by tertiles, values for the determinants did not differ by tertile of TH BMD. LM remained strongly associated with all bone measures in all tertiles. In contrast, the association of FM with LS and TH BMD was non-significant or marginal in T3 ($R^2 = 0.028$, $p = 0.18$; $R^2 = 0.006$, $p = 0.05$, respectively), but persisted in T1 and T2. Current calcium intake was associated with height-weight-adjusted LS BMD ($R^2 = 0.075$, $p = 0.026$) and TBBM ($R^2 = 0.099$, $p = 0.01$) in T1 only.

We conclude that (i) LM was associated strongly with bone mass at all levels of BMD, (ii) the association of FM with bone mass was diminished at high BMD, (iii) calcium intake was associated with bone mass only in subjects with low BMD and (iv) smoking was an adverse factor at all levels of BMD. The findings support efforts to increase calcium intake in individuals with low BMD.

P52

CHARACTERISTICS AND LIFESTYLES OF VITAMIN D-REPLETE WOMEN. JA Pasco, MJ Henry, GC Nicholson, MA Kotowicz. The University of Melbourne, Department of Clinical and Biomedical Sciences: Barwon Health, Geelong, Australia.

There is evidence that vitamin D provides a range of health benefits. We have previously reported highest serum 25OHD levels of 77 nmol/L (95%CI 74-81) among young women (20-39 yr) during summer¹, which is substantially less than levels over 200 nmol/L, reported as maximal in response to sunlight exposure². It may be that occult hypovitaminosis D is compromising our health. We aimed to determine the characteristics and lifestyles of the most vitamin D-replete women enrolled in the Geelong Osteoporosis Study (latitude 38-39S). Serum 25OHD was measured using RIA in 861 women (median age 46 yr, range 20-92).

Age-adjusted z-scores for 25OHD were calculated for women presenting in summer (Nov-Apr) and winter (May-Oct). The groups were pooled and z-scores ranked. Women with z-scores in the top 10% (n=85) were categorised as having high vitamin D status; a corresponding number at the lower end of the spectrum were categorised as having low vitamin D. Age-adjusted odds ratio (OR) for high vitamin D status was positively associated with the following (all $p<0.05$):

	High 25OHD n(%)	OR (95% CI)
BMI ≤ 30	77 (91%)	3.26 (1.31 - 8.10)
Physically active	72 (63%)	3.33 (1.46 - 7.59)
Sunseekers	34 (76%)	2.97 (1.32 - 6.69)
Alcohol drinkers	6 (60%)	4.32 (1.06-17.62)
Living with partner	62 (61%)	2.65 (1.34 - 5.22)

Trends ($p=0.05-0.1$) were observed for android fat distribution, regular walkers, vitamin D supplement users and tea drinkers. No association was found for body surface area, employment status, low dietary calcium, coffee drinkers or smokers. Although causality was not determined in this study, most of the factors associated with high vitamin D status reflect healthy, active lifestyle and body habitus.

¹Pasco JA et al 2001 MJA 175:401; ²Vieth R 1999 Am J Clin Nutr 69:842.

P53

EFFECT OF MAGNIFICATION ON VOLUMETRIC BMD MEASUREMENTS

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Magnification errors associated with fan beam DXA scanners effect area and BMC, and potentially volumetric BMD (vBMD). We compared pencil beam to fan beam DXA scanners to examine the effect of magnification on vBMD.

A total of 71 subjects (66 females, 5 males); age= 62+/11yrs were scanned on the same day using a Lunar Expert followed by a Lunar DPXL densitometer. L2-L4 vBMD was calculated using the method of Carter et al, (vBMD= BMC/AParea^{3/2}). Data from 55 subjects were available to calculate L3 vBMD by the method of Kroger (vBMD= (4BMD)/(□Width)). Femoral neck (FN) vBMD was calculated as vBMD= 4hBMC/□(AParea)², where h is the width of the FN ROI box. The coefficient of concordance between vBMD measured by the Expert and DPXL were as follows: L2-L4: 0.943; L3 (Kroger): 0.691; L3 (Carter): 0.918; and FN: 0.897. The limits of agreement are as follows:

	DPX (mean, SD)	Expert (mean, SD)	Difference (Limits of agreement)
L2-L4 vBMD	0.163 (0.028)	0.160 (0.030)	-0.003 (-0.022, +0.016)
L3vBMD (Kroger)	0.328 (0.070)	0.344 (0.078)	0.015 (-0.098, +0.128)
L3vBMD (Carter)	0.294 (0.057)	0.289 (0.057)	-0.005 (-0.050, +0.040)
FN vBMD	0.329 (0.051)	0.323 (0.049)	-0.006 (-0.049, +0.037)

These results suggest that despite high concordance between the two instruments, magnification errors in fan beam DXA may effect the measurement of volumetric BMD, particularly when using the method of Kroger et al.

P54

DOES SLEEP DISTURBANCE OR URINARY INCONTINENCE PREDICT FALLS IN ELDERLY WOMEN?

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This cross-sectional study aims to determine whether sleep disturbance or urinary incontinence (UI) predicts falls in elderly women. If so, effective treatment, which is currently available, has the potential to reduce those at risk.

782 female subjects, mean (SD) age 79 (3) years were recruited from within an on-going population-based cohort study (CAIFOS). Data were collected via self-completed questionnaires and all variables were dichotomous in nature. Prevalence among non-fallers and fallers and the p-value for comparison between groups, corrected for age, is indicated in the table below.

<u>Variable</u>	<u>Non-fallers</u>	<u>Fallers</u>	<u>p-value</u>
n	507	275	
Abnormal daytime sleepiness	5.7%	12.4%	0.002
Trouble falling asleep	15.2%	18.9%	0.21
Trouble staying asleep	20.7%	21.8%	0.72
Trouble waking up in morning	3.0%	7.6%	0.005
Trouble waking up too early	16.2%	24.0%	0.012
Urge urinary incontinence	30.8%	46.5%	<0.001
Stress urinary incontinence	69.0%	70.2%	0.80

In multiple logistic regression analysis, after adjusting for age, urge UI (OR 1.79; 95% CI 1.31, 2.44), abnormal daytime sleepiness (OR 2.04; 95% CI 1.2, 3.46) and trouble with waking up in the morning (OR 2.12; 95% CI 1.05, 4.28) were identified as independent predictors of falls. In conclusion, strategies to manage urge incontinence, daytime sleepiness and trouble waking up in the morning could reduce the risk of falling in elderly women.

P55

IN VITRO DOWN-REGULATION OF OSTEOPONTIN IN HUMAN OSTEOCLAST PRECURSOR CELLS BY ADENOVIRAL GENE TRANSFER INHIBITS OSTEOCLAST DIFFERENTIATION AND RESORPTION.

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To investigate the role of osteopontin (OPN) in osteoclast formation and resorption we used an OPN antisense adenoviral vector (AdOPN-AS) to inhibit OPN expression in osteoclast precursors derived from human CFU-GM.

A control adenoviral vector containing the β -galactosidase gene (AdLacZ) was used to demonstrate that high efficiency of infection could be achieved and that this had little or no effect on osteoclast differentiation and function. Precursors exposed to sRANKL before infection with AdOPN-AS showed normal osteoclast formation as compared to the AdLacZ control. Resorption was reduced early in the culture, but normalised as the osteoclasts matured. However mature osteoclasts infected with AdOPN-AS did not survive as well as the AdlacZ control osteoclasts. This suggested OPN down-regulation had an effect on the ability of more immature osteoclasts to resorb and impaired the survival ability of mature osteoclasts. Precursors that were exposed to sRANKL simultaneously with infection with the AdOPN-AS were inhibited in their ability to form osteoclasts. The osteoclasts formed were reduced in number and mean cell plan area and had a reduced ability to resorb as compared to AdLacZ infected cells.

Human osteoclast precursors can be efficiently infected with adenoviral constructs and this technique can be used to modulate their gene expression. Inhibition of sRANKL-induced OPN up-regulation in osteoclast precursors impairs osteoclast generation and function provided that the inhibition occurs early in the differentiation process.

P56

THE ROLE OF GROWTH HORMONE IN THE CONTROL OF OSTEOGENESIS

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The development of bone from mesenchymal progenitors is a highly regulated process, passing through distinct developmental stages and characterised by the sequential expression of factors vital to osteogenesis. It is known that growth hormone (GH) mediates some development of ossified tissues in vivo, as is shown by a reduced bone mineral concentration in individuals with a GH receptor knockout (GHR KO) phenotype, but its direct effects on osteoblastic differentiation at a cellular level are poorly understood. This study utilised a GHR KO mouse model to examine the osteogenic development of mesenchymal progenitors in the absence of GH.

Mesenchymal progenitor cells were removed from the bone marrow of both GHR KO and wild type (WT) mice, and were cultured for 14 days in a maintenance media designed to isolate osteogenic progenitors. The cells were then replated into 12 well plates at a density of 100 000 cells per well, in media containing L-glutamine, dexamethasone, ascorbic acid and β -glycerol phosphate to induce osteogenic differentiation. Cells were phenotypically analysed using phase contrast microscopy, and osteogenic marker expression was analysed using real time PCR on mRNA transcripts at 5-day intervals over a period of 20 days. Clear differences in size and cell shape were observed between WT and KO cell cultures from day 10 onwards; KO cells showing a clear reduction in the cell elongation and process extension observed in WT cells. Real time PCR results showed a 10-day lag in osteogenic marker gene expression patterns, supporting the idea that GH may indeed have a directly mediated affect on the development of osteogenic tissue on a cellular level.

GH is known to be responsible for controlling developmental pathways, but the extent of GHs influence on the development of osteogenic tissue is less clearly understood. Our results suggest that GH itself mediates a direct effect on the process of osteogenesis at a cellular level, and that the absence of GH signalling leads to a reduced expression of osteogenic factors. This, in turn, results in a reduced rate of the osteogenic development of mesenchymal progenitors in osteogenic culture. A more detailed knowledge of the influence of GH on the process of osteogenic development would undoubtedly help provide a greater understanding of the overall regulation of osteogenesis, and has the capacity to lead to the development of novel ways to treat injury and disease in ossified tissue.

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IDENTIFICATION OF A PARATHYROID HORMONE IN THE FISH, *FUGU RUBRIPES*

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A parathyroid hormone (PTH) gene has been isolated from *Fugu rubripes* and the eighty amino acids of the protein coding region determined. The N-terminal 34 residues of fugu PTH (fPTH) share 15 residues with other known PTH sequences and is most closely related to chicken PTH(1-88) (cPTH). In the amino acid sequence of fPTH after the first 34 amino acids there is no significant homology to either human PTH (hPTH) or cPTH, indicating weak evolutionary pressure to conserve the C-terminus of the PTH molecule. The potency of fPTH(1-34) in promoting cyclic adenosine monophosphate (cAMP) formation in UMR106.01 cells is consistently less than hPTH(1-34), human parathyroid hormone-related protein(1-34) (hPTHrP) and fugu parathyroid hormone-related protein (1-34) (fPTHrP) but the maximum amplitude of response was significantly greater than that achieved with the highest concentrations of hPTH or human or fugu PTHrP. The cAMP studies indicate that fPTH(1-34) acts through the PTH/PTHrP receptor, (PTH1R). But fPTH(1-34) is not recognized immunologically by a number of antisera raised to the N-terminus of hPTHrP or hPTH. Therefore fPTH is a member of the PTH family with only N-terminal homology to other family members, but with the least homology with any PTH so far sequenced.

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IS THE EFFICACY OF ANTIRESORPTIVE OSTEOPOROSIS TREATMENT DEPENDANT ON BONE TURNOVER?

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For better insight into the condition of bone turnover of osteoporotics at the time of diagnosis before treatment, we systematically took transiliacal bone biopsies from 178 patients. Undecalcified bone histology was done by G Delling (University of Hamburg, Germany). In 110 cases (61.7%) a low turnover state of bone metabolism was observed, in 44 cases (24.7%) we found a high turnover state. 20 biopsies (11.2%) could not be classified. In 3 biopsies (1.7%) mastocytosis was found, 1 case (0.5%) revealed a lymphoma of the bone marrow.

When considering treatment, we analyzed the literature for data mentioning efficiency in the high or low turnover condition. We found considerable differences:

HRT over 3 years conserved 0.033gm/qcm BMD in women between 50 and 60 years of age, but only 0.006 gm/qcm between 70 and 80 (1). Calcitonin (one year) induced a 22 % gain in BMC in women with high turnover, but no gain in low turnover (2). Alendronate (two years) increased spine BMD by 7.9 % at high turnover, but only by 3.1 % at low turnover (3). New spine fractures were more frequent if the increase in BMD was smaller (4). If raloxifene given over 3 years induced a distinct fall in osteocalcin (<-8.9 ug/l), fracture risk decreased to 37 % compared with placebo, but the risk did not decrease if OC stayed high (5).

We conclude that the turnover status should be part of the differential therapeutic considerations (antiresorptive vs osteoanabolic treatment).

- (1) Quigley MET et al (1987) Am J Obstet Gyn 156: 1516;
- (2) Civitelli R et al (1988) JCI 82: 1268;
- (3) Gonelli S et al (1999) CTI 65: 359;
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- (5) Bjarnason NH et al (2001) OI 12: 922.

P59

DETERMINANTS OF URINARY PYRIDINIUM CROSS-LINKS IN POSTMENOPAUSAL WOMEN

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Since negative calcium balance may increase bone resorption to maintain the plasma ionised calcium we examined the relationships between radiocalcium absorption, fasting obligatory calcium and the excretion of a biochemical marker of bone resorption, urinary pyridinoline (PYD) in 266 untreated postmenopausal women attending our osteoporosis clinics. All had lateral spine Xrays performed and all attended fasting for blood and urine collection after which they had their hourly fractional calcium absorption (α) and serum 1,25-dihydroxyvitamin D measured [1]. PYD was measured by HPLC after overnight hydrolysis. Urine calcium and PYD were expressed relative to creatinine (Ca/Cr and PYD/Cr).

Ca/Cr and α were not significantly related. Multiple linear regression showed that PYD/Cr was related positively to Ca/Cr (P<0.001) and inversely to α (P<0.001) as shown in the following equation:

$$PYD/Cr = 91.9 + 46.8 Ca/Cr - 28.2 \alpha \quad (R = 0.32; P < 0.001)$$

The number of spinal compression fractures (score of 1 for a wedge and 2 for a crush fracture) was related positively to PYD/Cr (P<0.001) and inversely to α (P=0.028). Those with scores greater than 1 had lower α than the others (0.59,0.21SD vs 0.67,0.26; P=0.017) but their serum 1,25D values did not differ significantly (111,37 vs 119,36). From the regression of α on 1,25D we estimate that the difference in 1,25D can account for 21% of the difference in α between the two groups. We conclude that both decreased calcium absorption and increased urine calcium increase bone resorption.

1. Nordin BEC et al, J Nuclear Med 1998;39:108-113

P60

DESIGN OF SHORT-TERM PRECISION STUDIES IN BONE DENSITOMETRY: SAMPLE SIZE DETERMINATION.

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Current bone densitometry literature recommends that the combination of numbers of subjects and repeat measurements per subject in the design of short-term precision study as: Number of subjects X (Number of repeat scans - 1) = 30, to ensure statistical validity. The aim of a precision study is to determine the Least Significant Change (LSC). It is the smallest difference between serial measurements above which it can be concluded that the change is statistically significant. However there is an error on this estimate.

We conducted a short-term precision study of 72 unselected females (Age: 50.5±6.5y) representative of the population of patients visiting our bone densitometry facility. These subjects were scanned twice at the spine and hip using the Hologic QDR2000. Differences between total spine BMD repeat measurements were normally distributed (0 ± 0.01578). Simulation studies were then performed to quantify the variability in LSC values at the 80% and 95% level of confidence. An analytical solution was also developed which agreed very closely with the simulation approach. The sample size table for various levels of precision (95% CI) for total spine BMD LSC is:

Confidence	LSC 95% CI	No. subjects	No. repeat scans
95%	± 5 mg	73	2
95%	± 5 mg	37	3
95%	± 8 mg	29	2
95%	± 8 mg	15	3

In practice, for a precision study of about 30 subjects scanned twice the total spine BMD LSC 95% CI is about ± 8 mg.

In addition to statistical validity requirements, variability in LSC values should be considered when designing precision studies.

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STRUCTURAL AND BIOMECHANICAL BASIS FOR DIFFERENCES IN VERTEBRAL FRAGILITY IN CHINESE AND CAUCASIANS

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We hypothesized that the structural abnormalities predisposed to vertebral fracture are similar in Chinese and Caucasians, accounting for the similar vertebral fracture rates between races. We studied 687 healthy Chinese (449 females) and 1088 healthy Caucasians (738 females) aged 18 to 92 yrs. Vertebral body (VB) cross-sectional area (CSA) and volumetric BMD (vBMD, excluded posterior elements) were measured using dual x-ray absorptiometry by postero-anterior and lateral scanning. We calculated VB stress (load/CSA) and FRI (load/strength) during bending forward. In young adulthood, VB stress did not differ by race in either sex because the lower load (10-14%) in Chinese was distributed on a proportionally lower CSA (13-14%) than in Caucasians. However, vBMD was 9-13% higher in Chinese than Caucasians, conferring 12-19% lower FRI in Chinese men and women. Ageing was associated with increased CSA in both Chinese and Caucasian men and women. However, racial differences in periosteal expansion were minimal, increasing by 8.7% and 11.8% in elderly Chinese and Caucasian men, and increasing by 8.6% and 5.7% in elderly Chinese and Caucasian women (both no significant different to each other). VB stress decreased similarly in Chinese and Caucasian men (13.3% vs 13.7%) but decreased more in Chinese than Caucasian women (10.0% vs 5.5%, $p < 0.01$). Net decline in vBMD was greater in elderly Chinese than Caucasian women (33% vs 27%, $p < 0.01$) but similar in Chinese and Caucasian men (11% vs 12%). These structural changes were captured by FRI; a similar proportion of elderly Chinese and Caucasians men (5% vs 6%) and women (25% vs 29%) had the $FRI \geq 1$. The results are consistent with the notion that vertebral fractures occurs more commonly in women than in men but similar proportions of Chinese and Caucasians (of either sex) sustain fractures.

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BONE MINERAL DENSITY IN PATIENTS AGED 10-20 YEARS WITH MAJOR THALASSEMIA IN TEHRAN-IRAN

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The purposes of this study were to evaluate the bone mineral density (BMD) of 208 thalassemic patients aged 10-20 years in Tehran-Iran and identify potential risk factors. BMD of lumbar spine and femur was determined by dual-energy X-ray absorptiometry (Lunar). Height, weight, body mass index, growth indices, pubertal development, calcium intake were determined. Blood samples were taken for assessment of ferritin, serum calcium, phosphate, alkaline phosphatase, parathyroid hormone (PTH) and vitamin D. BMD z scores between -1 and -2.5 identified as osteopenia and z scores less than -2.5 considered as osteoporotic. Osteoporosis was seen in 50.2% of lumbar spine, 10.1% of femur and 8% of both regions. No differences were seen in lumbar spine and femur osteoporosis in boys (lumbar=46.4%, femur=11.2%) and girls (lumbar=54.7%, femur=10.8%). Osteopenia was 39.5% in lumbar spine and 37.5% in femur. The age of osteoporotic patients identified in lumbar spine was significantly higher ($p=0.001$). Years on Desferrioxamine were significantly higher in patients with normal BMD comparing with osteoporotic patients in lumbar spine and the reverse held true in femur ($p= 0.03$). Growth indices including height for age, weight for age and weight for height percentiles had significant correlation with BMD of lumbar spine ($p= 0.001$). Patients with vitamin D deficiency (36.1%) had lower lumbar BMD values ($p<0.05$). PTH showed a significant correlation with lumbar BMD ($r=0.195, p=0.009$). The data confirm significant reductions in BMD in Iranian thalassemic patients like other nations worldwide. Although further research is needed, we recommend the use of known preventive methods such as hormone therapy, calcium and vitamin D supplementation and early treatment of osteoporosis and osteopenia in thalassemic patients.

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SKELETAL MUSCLE MASS AND BONE MASS IN SPINAL CORD INJURED MALES

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Skeletal muscle mass has an important metabolic and structural role in maintaining bone health. Spinal cord injured (SCI) persons lose skeletal muscle and bone mass as a result of their environmentally enforced immobilisation. The purpose of this study was to investigate the association between skeletal muscle mass and bone mass in SCI men. Twenty SCI men were matched for age, height and weight with 20 able-bodied control men. DXA scanning (Lunar DPX-L) was undertaken to measure total body and regional lean tissue mass (LTM), bone mineral content (BMC) and bone mineral density (BMD) in all participants. Total body skeletal muscle mass (SM_{est}) was estimated from the regional DXA measures of appendicular lean soft tissue (ALST) using the method of Kim *et al.* (2002)¹. Total body and leg measures of LTM, BMC, BMD and the SM_{est} were all substantially lower in the SCI group (P < 0.001). Analysis of covariance revealed that SM_{est} was approximately 4.8 kg less in the SCI group than in the controls for the same BMC value. While skeletal muscle mass was found to correlate with bone mass in both groups, the SCI participants had significantly reduced level of both skeletal and muscle mass when compared with their controls. Preservation of bone mass, especially in the upper limbs of SCI persons, is vital as the upper limbs are responsible for movement and activities of daily living. Further research is required to ascertain the most effective measure(s) for preserving bone mass in SCI persons. Maintaining skeletal muscle mass may provide one way of achieving this. (Grant Support: The Lamar Trust, Christchurch.)

1. Kim, J., Wang, Z., Heymsfield, S., *et al* (2002). *Am J Clin Nutr*; 76:378-83

P64

BODY MASS INDEX, AGE AND BONE MINERAL DENSITY IN 557 POST MENOPAUSAL WOMEN IN CHINA.

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To research the relationship between body mass index (BMI) and bone mineral density (BMD) in postmenopausal women at different ages.

Bone mineral density on the lumbar spine(L2-4) and the femoral neck (FN) of 557 postmenopausal women measured with dual energy x-ray absorptiometry (DXA) and BMI was calculated. The women were divided into 3 groups according to BMI(Group 1 = BMI ≤ 20 kg/m², Group 2 = 25 ≥ BMI ≤ 20 kg/m², Group 3 = BMI >25 kg/m²), and on the basis of their ages they were also divided into 3 groups (50 ~ 59, 60 ~ 69 and 70 ~ 78 years). The relationship between BMI and BMD was examined for each age group.

Compared to the BMI Group 3 those in the Group 1 had significantly lower lumbar spine and femoral neck BMD, at all age groups. The percentage difference in lumbar spine and femoral neck BMD between Groups 1 and Group 3 were: -10.8% and -3% for 50 – 59 year olds, -10.7% and -9.4% for 60-69 year olds and -8.6 and -8.2% for 70-79 year olds, respectively.

Bone mineral density at each site was related directly to BMI and inversely to age. This cross-sectional study suggested that high BMI does not prevent the age-related decline in BMD.

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DIFFERENCES IN INDICATIONS FOR REFERRAL FOR BONE DENSITOMETRY BETWEEN THE SEXES

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We have previously shown that men are under-represented in referrals for bone densitometry.¹ Age-adjusted rate ratio of referrals for women:men is 8.6 (95%CI 7.8-9.6) and only 2.6% of men with fracture are referred for assessment. The aim of this study was to compare the indications, both clinical and non-clinical, for referral for bone densitometry between the sexes.

In this study all men (n=393) and women (n=4591) referred for bone densitometry in the Geelong region (1991-8) were identified and their bone density results and indications for referral were documented. Sixty-eight indications were identified and categorised into 8 groups then into clinical and non-clinical indications. The subgroups included screening, fracture, amenorrhoea/hypogonadism, thyroid disease, glucocorticoid therapy, rheumatoid arthritis, co-morbidities, and other. Using the Chi square test, differences in reasons for referral were compared between the sexes.

Referred men were older than women (median age (range) 65 (15-92) vs 57 (6-92) yr, p<0.05). There was a greater than expected proportion of men referred because of glucocorticoid exposure and fewer for screening; females showed the opposite trend (men 43% vs 18%; women 10% vs 63%, p<0.001). In a comparison of clinical vs non-clinical indications, men showed a greater than expected proportion referred for clinical and women a greater than expected proportion for non-clinical indications (men 77% vs 23%; women 33% vs 67%, p<0.001).

This data is subject to potential bias as some of the indications for referral are rebatable by Medicare and this may have influenced the stated reason for referral. This data suggests that men are referred because of risk factors whereas there is a predominance of referrals among women for perimemopausal screening.

¹Kotowicz MA et al 2001 Bone 28:S237

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EFFECTS OF ED-71 ON BONE MASS AND STRENGTH AND BONE METABOLISM IN OVARECTOMIZED MICE.

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Objective This study is to compare the effects of a synthtic 2 β -(3-Hydroxypropoxy)-calcitriol(ED-71) with 17 β -Estradiol(E2) on bone mass, bone strength and bone metabolism.

Methods Bone mineral density(BMD), bone mineral content(BMC), bone strength and bone histomorphometric parameters were measured .40 female Kunming mice, weighing an average of 35g, were randomly divided into 4 groups. After 6 weeks treatment of ED-71 at 0.4 μ g/Kg/week and E2 at 30 μ g/Kg/day in OVX mice.

Results: Compared with OVX mice femoral BMD and BMC increased respectively 3.8%, 5.9% and 3.2%, 5.7%; maximum load of femur increased respectively 18.7% and 16%;trabecular bone volume of lumbar vertebra, increased respectively 10.6% and 16.1 and serum alkalinephosphatase decreased respectively 58% and 37%..

Conclusions: ED-71 significantly increased BMD, BMC and bone strength and significantly inhibited bone turnover in OVX mice. Also ED-71 could not induce uterus proliferation.

[Key words] 2 β -(3-Hydroxypropoxy)-calcitriol(ED-71) Bone metabolism Ovariectomized mice.

P67

BONE MINERAL DENSITY IN JAPANESE HIP FRACTURE PATIENTS YOUNGER THAN 75 YEARS OLD.

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Bone mineral density (BMD) of the lumbar spine and femoral neck in hip fracture patients of Japanese women, who were younger than 75 years old were measured, and compared with those of nonfractured controls. The BMD measurements with dual energy X-ray absorptiometry (DEXA) were undertaken in 49 consecutive hip fracture patients and 131 nonfractured osteoporosis patients. Twenty fractured and 102 nonfractured patients were younger than 75 years old. All the fractured patients were operatively treated, and the BMD was measured an average of 14 days after injury. The BMD compared with the young adult reference range (T-score) was used for the comparison. In the fractured patients, the T-score of the lumbar spine was significantly higher, and the T-score of the femoral neck was significantly lower than those of the nonfractured patients. The value obtained by dividing the T-score of femoral neck by that of lumbar spine was defined as the F/L ratio. The average F/L ratio starts to decrease at about 70 years old, and it becomes 1.0 between the age 75 to 79. The results of this study demonstrated that the percentage of the patients whose F/L ratio was less than 1.0 were 55% in fractured patients and 26.5% in nonfractured patients. Moreover, the patients whose F/L ratio was less than 1.0 and femoral neck T-score was less than 0.7 in the fractured group was significantly more than those in the nonfractured group (40.0%, 11.8%, respectively. $p=0.0053$). These data suggests that small F/L ratio can be a risk factor of femoral neck fracture. To find the high-risk patients from relatively young age group, the F/L ratio measurement at the first examination is recommended.

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PERSISTENCE AT 1 YEAR WITH THE ACTNOW™ PATIENT SUPPORT SERVICE

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This service commenced in February 2001, aiming to provide information and support to osteoporotic patients receiving risedronate¹. The main objective is to address the recognised low treatment adherence levels with bisphosphonates through ongoing patient education on osteoporosis and its treatment.

Enrolled patients receive regular mailed information and newsletters. They are also contacted by phone by trained nurses at 0, 4, 10, 26, 52 and 78 weeks, and invited to discuss treatment or disease specific issues. Patient demographics, treatment history and risedronate adherence data were obtained following patient consent. Patients enrolled for ≥ 12 months were also asked to complete a brief mailed questionnaire.

5751 patients have been enrolled, 96.7% female. 2354 (41%) patients had received previous osteoporotic treatment. 1736 (30.2%) patients have been enrolled for ≥ 12 months, and 1239 (71.4%) patients reported they were still taking risedronate at 12 months. Response rate to the survey was 60% (n=735). 90% rated the programme as “very good” or “excellent”; 78% strongly agreed that the programme had increased their knowledge of osteoporosis and 93% reported that ActNow™ had helped them understand the importance of taking risedronate correctly.

The ActNow™ patient support service provides information and regular contact by trained nurses that may enhance persistence of treatment for osteoporosis, leading to better patient outcomes.

- 1. Actonel - Aventis Pharma, Australia.

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OSTEOPOROSIS KNOWLEDGE AND SELF-EFFICACY AMONG IRANIAN AUSTRALIAN MEN AND WOMEN

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Osteoporosis is one of the major quality of life threatening diseases for both women and men, regardless of ethnicity. Little information on prevention and treatments is available to Australians with ethnic background, who may be at risk for the disease. The aim of this study is to assess knowledge of osteoporosis risk factors and self-efficacy among Iranian Australians in Sydney.

Seventy participants (46 women and 24 men), aged between 35 and 70 years, have been randomly recruited for this on-going study. Each participant completed the Osteoporosis Knowledge Test¹ (24 questions) which assesses general knowledge as well as the roles of calcium and exercise in the prevention of osteoporosis; and the Osteoporosis Self-Efficacy Scale, which assesses the level of confidence (by visual analogue scale, range: 0 for totally lack of confidence, to 100 for total confidence) to undertake osteoporosis prevention behaviors.

On the osteoporosis knowledge test, the average proportion of correct answers was 61±13% (mean±SD); range: 33 to 87%) in women, which was significantly higher (p<0.001) than in men (43±17%; range: 12 to 71%). On the osteoporosis self-efficacy, both the calcium (49±4) and exercise scores (54±10) in women were significantly lower (p<0.001) than in men (63±13 for calcium and 61±20 for exercise).

These data reveal that in Iranian Australians, only half had adequate knowledge of osteoporosis, and a high frequency of incorrect assumptions about prevention behaviors, particularly in men.

1.Kim, K., Horan, M., & Gendler, P.(1991).Osteoporosis knowledge test and osteoporosis self-efficacy scale. Grand Valley State University.

P70

DOES MEASURING THE WRIST HELP? CORRELATION OF CHANGE BETWEEN DEXA MEASUREMENTS AT THE SPINE, FEMORAL NECK AND DISTAL WRIST IN FOLLOW-UP SCANS.

A Doube, Midland Osteoporosis Group

There is often discordance between spine and hip DEXA measurements on follow-up scans. Current convention regards the spine as the relevant correct indicator of change with hip influenced by technical factors to make it less reliable. The wrist has not been advocated for routine use but it has greater precision than either spine or femoral neck, is sensitive to bone loss postmenopause and has measurable response to treatment.

I have reviewed the results of 500 patients who had all 3 sites measured on 2 occasions using the same machine and software[Norland XR 36]. Scans were performed in a standard clinical manner.

Full data was available for 496 patients. The average increase/decrease in bone density was; spine 5.4/5.05 ; femoral neck 5.44/5.75 ; distal wrist 5.22/4.24.

Scattergrams show a closely similar distribution of change in spine vs femoral neck, spine vs wrist and femoral neck vs wrist. A correlation matrix is significant between all 3 set at p < 0.001. Regression analysis indicates both hip and wrist predict spine values.

The wrist offers another site to measure change in bone density and performs at least as well as the femoral neck.

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BILATERAL FEMORAL HEAD OSTEONECROSIS FOLLOWING SEPTIC SHOCK AND MULTIORGAN FAILURE.

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A 66 year old woman had a life-threatening illness consisting of septic shock secondary to *Escherichia coli* urosepsis, complicated by severe multiorgan failure requiring prolonged treatment and hospitalisation. Five months later she developed left groin pain. MR imaging showed bilateral osteonecrosis of the femoral heads with a pathological fracture on the left requiring hip joint replacement. Two months later she fractured her right neck of femur requiring right hip joint replacement. The histological findings confirmed the diagnosis of osteonecrosis. No other cause for the osteonecrosis was identified. We propose that she suffered an ischaemic insult to her femoral heads bilaterally, on the basis of systemic ischaemia secondary to the severe sepsis through a combination of hypotension, low cardiac output, and blood flow diversion. To our knowledge femoral osteonecrosis has not previously been described following septic shock.

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THE ASSESSMENT OF SECONDARY HYPERPARATHYROIDISM AND RENAL OSTEODYSTROPHY PREVALENCE & TREATMENT RESULTS WITH ORAL ROCALTROL PULSE AMONG HEMODIALYSIS PATIENTS

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Statement of Purpose: Renal osteodystrophy is one of the most important complications of ESRD (end stage renal disease). Prevention and precise treatment of renal osteodystrophy decrease associated mortality and morbidity. Prescription of calcium, low phosphate diet and rocaltrol are essential in these patients. The aim of this study is I) Identification of renal osteodystrophy and secondary hyperparathyroidism prevalence in ESRD patients was covered by Semnan-Fatemieh haemodialysis centre and II) Detect the oral rocaltrol pulse response on secondary hyperparathyroidism and renal osteodystrophy in some of these patients.

Statement of Methods: In first step, 31 patients with ESRD checked for serum calcium, Phosphor, Alkaline Phosphatase and iPTH (IRMA) and bone deformity in bone x-ray from 2001-2002. In second step, 12 patients categorized in three groups according to iPTH level and treated with 1, 2, 4 microgram oral-rocaltrol pulse, three times a week for 6 months respectively, then Ca, P, alkaline phosphatase, iPTH levels and bone graphy compared with before treatment results.

Summery of Results: From 31 patients with age average 52 ± 17.7 and haemodialysis duration average 36 ± 223 months, 3.5 percent had Ca level equal or higher than 10.4 mg/dl, 77.3 percent had P level equal or higher than 4.5 mg/dl, 80.6 percent had iPTH level higher than 120 pg/ml and 72.4 percent had alkaline phosphatase level higher than 175 lu/l. Abnormality in bone graphy reported in 64.5 percent of patients that osteopenia was universal.

No meaningful correlation observed between haemodialysis duration with serum Ca, P and alkaline phosphatase level, but this correlation observed between iPTH level and haemodialysis duration (P value < 0.04).

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