INVITED AND CONTRIBUTED PRESENTATIONS

Vitamin D: how much is enough?

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Clinical vitamin D deficiency leading to rickets and osteomalacia may occur when serum 25-hydroxyvitamin D (25(OH)D) is lower than 25 nmol/l. More subtle consequences of vitamin D deficiency such as secondary hyperparathyroidism, bone loss and increased risk for fractures, and muscle weakness may occur when serum 25(OH)D is lower than 50 nmol/l (vitamin D insufficiency) or even with higher levels up to 75 nmol/l, as claimed by some investigators. Regular sunshine exposure is efficient to prevent clinical vitamin D deficiency in temperate climates. In order to increase serum 25(OH)D over 50 nmol/l all year long , an increase in sunshine exposure and vitamin D supplementation in winter or all year long is necessary. A vitamin D dose of 400 IU/day may be sufficient in older adults. However, immobilised older persons or residents of nursing homes, and other risk groups such as non-western immigrants will need more, up to 800 IU/day. With this dose and adequate compliance, serum 25(OH)D will be higher than 50 nmol/l in more than 90% of the subjects. When the required level is set at 75 nmol/l, for which the evidence is marginal, much higher doses are needed, because the increase of serum 25(OH)D with vitamin D supplementation is non-linear. Vitamin D status may also be improved by consumption of fatty fish such as herring or mackerel, or by the addition to dairy products or other foods.
In conclusion, serum 25(OH)D should be higher than 50 nmol/l all year long and this can be obtained by sunshine exposure and/or vitamin D supplementation with a dose of 400-800 IU/day.

IS2
Osteoblasts, Wnt signalling and the effects of glucocorticoids

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Glucocorticoids (GC) are widely used for their potent immunomodulatory actions, but diabetes, obesity and osteoporosis are serious side effects of long-term exogenous GC therapy. The catabolic effects of supraphysiological levels of GC on bone are in direct contrast to anabolic actions of endogenous GC at physiological levels.
Using a transgenic model in which a GC-inactivating enzyme, 11ß-hydroxysteroid-dehydrogenase type 2 (11ßHSD2) has been targeted exclusively to mature osteoblasts and osteocytes (Col2.3-11ßHSD2-tg mice) we have demonstrated that endogenous GC act through the osteoblast/osteocyte to direct mesenchymal lineage commitment away from adipogenesis towards osteoblastogenesis.
We have further demonstrated that endogenous GC play a role in the development and maintenance of bone, and in the MMP-14 mediated removal of calvarial cartilage through GC-activated Wnt signaling pathways. Of note, GC appear to regulate osteoblastic Wnt expression in a concentration-dependent biphasic mode, which may in part explain the apparently paradox anabolic and catabolic effects of GC on bone.
We further observed that endogenous GC, through the osteoblast, promote inflammation in a mouse model of auto-immune arthritis, a finding that challenges our traditional concept of the ubiquitous anti-inflammatory actions of GC.
Treatment of Col2.3-11ßHSD2-tg mice with pharmacological doses of GC revealed that the bone loss effects of exogenous GC are mediated via the osteoblast.
Taken together, endogenous and exogenous GC often act in contrasting ways, depending on their concentrations and the target cell. In bone, osteoblasts are the primary target for both the catabolic and anabolic effects of endogenous and exogenous GC.

IS3

Osteoporosis in older persons: senescence vs. disease

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Senescence is interpreted as a common pattern of impairment that predisposes to disease. In the case of bone senescence, cellular and structural changes start early in life and are mostly independent of environmental, dietary or hormonal factors. The typical cellular features of bone senescence include a reduction in osteoblast differentiation and function, decreasing number of bone marrow mesenchymal stem cells, increasing marrow adipogenesis and high levels of osteoblast and osteocyte apoptosis. In contrast, osteoporosis is an age-related disease secondary to alterations in genetic, dietary and hormonal factors. In fact, bone senescence and osteoporosis are highly interconnected. It is well known that bone senescence increases the susceptibility to osteoporosis and fractures when risk factors for osteoporosis are present. However, the molecular trigger for bone senescence, which may link aging and osteoporosis, remains elusive. In this session, the potential molecular triggers for bone senescence will be discussed. In addition, the links between senescence and osteoporosis will be presented in a "bench to bedside" manner. Finally, potential therapeutic targets for both, osteoporosis and bone senescence, will be discussed.

OR1

SOST and DKK-1 are downregulated in the spines and joints of mice suffering ankylosing spondylitis creating a permissive environment for excessive bone formation

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Ankylosing spondylitis (AS) is a form of spondylarthritis targeting the spine and pelvis that progresses from the initial inflammation to uncontrolled bone formation in the affected joints frequently resulting in complete joint fusion. To date very little is known about the progression from the initial inflammatory stages of the disease to this uncontrolled bone formation and no therapies are available that can slow this progression.
The proteoglycan-induced spondylitis model (PGISp) is a well-characterised mouse model of AS. Disease is induced by injections with a human proteoglycan extract resulting in intervertebral disc destruction which is then followed by ectopic cartilage formation that eventually ossifies resulting in ankylosis.
We investigated the role of two inhibitors of bone formation which act through the Wnt pathway, DKK-1 and SOST, and expression levels of both genes were significantly down-regulated in the spine and knee joints of PGISp mice with advanced disease.
We then undertook an array study comparing knee joints from control and PGISp mice that had maximally decreased levels of SOST and DKK-1. 2600 genes were significantly differentially expressed between control and PGISp mice with 125 genes upregulated >2-fold and 18 downregulated >2-fold. Interesting candidates included genes involved in inflammation, connective tissue matrix, immune-regulation and genes specifically involved in bone regulation including other members of the Wnt pathway.
This study identifies a number of genes that may play a role in the uncontrolled bone formation seen in AS and highlights the Wnt pathway as a likely key mediator of disease progression.

IS4

Osteocytes – the hunt for the mechanostat

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Mechanical loading is perhaps the most important physiologic factor regulating bone mass and shape. Age related bone loss and consequent osteoporosis have been attributed, at least in part, to the decrease in mechanical usage of the skeleton. Conversely it has been demonstrated that mechanical overloading results in enhanced bone formation, thus a detailed understanding of the biochemical processes governing load regulated bone formation could lead to the identification of molecular targets for the prevention and treatment of diseases such as osteoporosis. The hunt for the mechanostat has shown that osteocytes are key mediators of load induced bone remodeling. However, owing to their inaccessible location the molecular mechanisms by which mechanical forces are translated into anabolic signals remain poorly understood. In vivo studies have attempted to identify load regulated genes expressed by osteocytes, however, their interpretation is limited by the fact that they report the average effect of tens of thousands of cells in whole bone cell fractions. Furthermore, depending on their location within the diverse micro-architectures of bone these cells are exposed to varying magnitudes of mechanical strain. As part of the presentation, a novel framework to study osteocyte function in an in vivo model of bone adaptation will be presented addressing the above mentioned limitation, also illustrating the use of novel imaging and modeling approaches for a better understanding of the mechanostat theory. In the future, this might allow more in-depth investigations of the role of the osteocyte network in bone homeostasis.

IS5

Obesity and fractures

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Body mass index is a major determinant of bone mineral density (BMD) and obesity is widely believed to protect against fracture. However, we recently reported a high prevalence of obesity in postmenopausal women presenting to a fracture liaison service with low trauma fracture. Overall, 27.7% of those aged 75 years or less were obese and over 80% had normal femoral neck BMD (T-score > -1), whilst osteoporosis (T-score > -2.5) was present in fewer than 2%.
In order to investigate further risk factors for fracture in obese women we have examined two large databases, the Study of Osteoporotic Fractures (SOF) and the GLobal study of Osteoporosis in Women (GLOW). BMD T-scores in the spine, femoral neck and total hip were significantly lower in the women with fracture than in those without. Fractures of the upper arm, lower leg and ankle were significantly more common in obese women whilst fractures of the hip, pelvis and wrist were significantly less common. 50.2% of obese women with fracture had a previous history of fracture after age 50 years compared to 33.1% of obese women without fracture ($p<0.0001$). Neuromuscular function tended to be worse in obese women with fractures.
We conclude that fractures in obese postmenopausal women contribute substantially to the overall fracture burden in the postmenopausal population. Although BMD is often normal in these women, it is significantly lower than in obese women without fractures and a previous fracture history is more common indicating that these fractures have some of the characteristics of osteoporotic fractures. Our findings have important public health implications in view of the rising incidence of obesity and emphasise the need to develop effective preventive strategies.

IS6

Genetic studies in osteoporosis

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The era of the genomewide association study (GWAS) has delivered a rich harvest of genes for osteoporosis researchers. Currently there are 27 genes or genetic loci reported which pass internationally accepted levels of statistical confidence that they represent true positive findings. Using a novel study design costing about 20% of the cost of the previous studies, the Australian Osteoporosis Genetics Consortium study has identified a further 6 genes to add to this list, and replicated 21 of the known genes. Whilst the first genes to be identified were 'the usual suspects' such as *RANK*, *RANKL*, *OPG*, *LRP5* and other key Wnt pathway members, more recent genes have been more novel, with little or no prior evidence of their involvement in musculoskeletal pathology or biology, raising welcome challenges for the functional biology community. More genes are being identified, principally through meta-analysis by large international consortia, but opportunities still exist for gene-identification using less expensive cleverer designs. Such approaches include gene-mapping in different populations, admixture mapping, and delving deeper into the existing gwas data (deep replication). There is considerable interest at this time in human genetics as to the relative role of common and rare genetic variants in common human diseases, and the bone disease field is no exception to this, with a long record of research in rare variants of extreme effect. More genes and better localization of genetic effects is likely to flow from research into rare variants of moderate effect found in the general population.

OR2

Modulation of PYY regulates bone formation – a signal from gut to bone?

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Hypothalamic neuropeptide Y (NPY) or Y2 receptors expression has a strong, negative relationship to bone anabolism. In addition to the central components, NPY system could also regulate bone homeostasis through osteoblastic Y1 receptors in the periphery. Peptide YY (PYY), a NPY receptor ligand released from the gastrointestinal system, was inversely correlated with BMD. Given the critical role of diet in mineral homeostasis, and recent findings regarding serotonin action in bone, we hypothesize that PYY may be a link from gut to skeletal system.

Bone cell activity and structure of distal femur were examined in two mouse models – PYY knockout ($PYY^{-/-}$) model and a model with tamoxifen-inducible PYY over-expression (PYYtg) in the periphery where ubiquitous gene *ROSA26* is expressed.

$PYY^{-/-}$ exhibited greater cancellous bone volume ($p<0.005$) and trabecular number ($p<0.001$) in distal femur, accompanied by greater mineral apposition rate (MAR) ($p<0.005$) with no change in osteoclast surface. Endocortical MAR of $PYY^{-/-}$ was also greater ($p<0.05$).

In PYYtg, bone cells activities were the opposite of those observed in $PYY^{-/-}$. PYYtg had reduced MAR ($p<0.005$) and increased osteoclast number ($p<0.001$) and surface ($p<0.005$) with lower trabecular number ($p<0.05$) and a trend towards reduced cancellous bone volume ($p=0.07$). Endocortical MAR was also reduced in PYYtg ($p<0.05$).

The contrasting effect of the knockout and transgenic mouse models indicates that peripherally produced PYY regulates bone mass through suppression of osteoblast activity in cancellous and cortical bone. Greater understanding of the role of PYY signalling in bone may reveal novel links between gut and bone homeostasis.

IS7

Inflammatory mediators: triggers of the bone anabolic-catabolic switch

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Inflammatory mediators are elevated in many bone diseases including osteoporosis, osteoarthritis, rheumatoid arthritis (RA) and multiple myeloma (MM). Indeed, systemic osteoporosis is a common complication in both RA and MM. The roles of inflammatory mediators in these diseases have been studied largely from the point of view of their role in the inflammation and the ensuing bone and cartilage destruction, and anti-inflammatory therapies are known to target these actions. There is now good evidence that inflammatory conditions associated with increased bone destruction are also associated with defective bone formation. It is also evident that at least some inflammatory mediators, directly or indirectly, may both trigger osteoclastic bone resorption and inhibit or delay bone formation. For example, it has been shown that TNFα induces expression of the Wnt inhibitor, DKK1, in a mouse model of inflammatory arthritis and that this was at least in part responsible for the bone destruction phenotype.(1) Recent work has also highlighted a pathological role for TWEAK in inflammatory arthritis.(2) TWEAK stimulates RANKL production by osteoblasts, and in MM, circulating TWEAK levels correlate with those of bone destruction (β-crosslaps).(3) We have recently published evidence that both TNFα and TWEAK induce the expression of the negative regulator of bone formation, sclerostin,(4) suggesting that inflammatory-mediated inhibition of bone formation may converge in inhibition of the Wnt pathway..

1. Diarra et al 2007 Nat Med 13:156
2. Perper et al 2006 J Immunol 177:2610
3. Williams et al 2010 Br J Haematol (in press)
4. Vincent et al 2009 J Bone Miner Res 24:1434

IS8

Osteomacs versus inflammatory macrophages in bone biology

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Macrophage is a generic name that encompasses a large number of heterogeneous cell sub-populations that have undergone local environment-directed maturation. They can be broadly classified into inflammatory or resident macrophages. Inflammatory macrophages have known roles in diseases that have associated bone pathology, including rheumatoid arthritis and osteoporosis. The potential role(s) of resident macrophages in bone biology have until recently been overlooked. We have identified a population of resident osteal tissue macrophages (osteomacs) that are intercalated within bone lining tissues. Immunohistochemical analysis has characterized osteomacs during physiological bone growth as $F4/80^{+}Mac3^{+}Mac2^{-}TRAP^{-}$ cells that show variable expression of Ly6C. This phenotype, combined with their tissue distribution, confirmed that osteomacs are resident, not inflammatory macrophages and that they are not osteoclasts or immediate osteoclast precursors. Functionally, osteomacs enhance osteoblast mineralization *in vitro,* encapsulate osteoblast bone forming surfaces (ObS) and are required for maintenance of mature osteoblasts *in vivo*. Inflammatory Mac-2^{+} macrophages where absent in bone tissues during normal skeletal growth. A tibial bone injury model was used to characterize macrophage populations during bone healing. At least two macrophage populations were identified during bone healing, osteomacs and $F4/80^{+}Mac2^{hi}TRAP^{-}$ inflammatory macrophages, and the latter demonstrated no affinity for bone formation sites. *In vivo* macrophage depletion experiments supported that osteomacs as opposed to inflammatory macrophages were critical for optimal bone healing. Understanding the specific contributions of these different macrophage populations to physiologic and pathological bone events is essential to harnessing the therapeutic potential of osteomacs.

IS9

Unraveling the endosteal hematopoietic stem cell niche - induction of mobilization

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In the bone marrow (BM), the most quiescent and potent hematopoietic stem cells (HSC) reside in specific niches near osteoblast-lineage cells at the endosteum. These endosteal niches provide factors that regulate HSC quiescence and self-renewal. The critical importance of osteoprogenitors in supporting HSC has recently been demonstrated by deleting the Dicer-1 gene specifically in osteoprogenitors, which results in ineffective hematopoiesis with pan-leukopenia, myelodysplasia and secondary leukaemia. To further investigate the dynamic regulation of these endosteal niches, we took advantage of the therapeutic mobilization of HSC in response to granulocyte colony-stimulating factor (G-CSF) or cyclophosphamide. Both G-CSF and cyclophosphamide rapidly ablates endosteal osteoblasts leading to suppression of endosteal bone formation and decreased expression of cytokines regulating HSC function. Importantly, G-CSF administration led to the ablation of trophic BM macrophages that support osteoblasts at the endosteum (osteomacs). Osteomac loss occurred concomitantly to osteoblast suppression and HSC mobilization, suggesting that loss of osteomacs could disrupt endosteal niches. Indeed we found that *in vivo* depletion of macrophages, in either macrophage Fas-induced apoptosis (Mafia) transgenic mice or by administration of clodronate-loaded liposomes to wild-type mice, recapitulated the loss of endosteal osteoblasts, inhibition of HSC trophic cytokine expression at the endosteum, and HSC mobilization into the blood observed during G-CSF administration. Together these results establish that BM macrophages are pivotal in maintaining osteoblasts and endosteal niches for HSC and that loss of these macrophages leads to collapse of endosteal niches and egress of HSC from the BM.

IS10

RANKL and its signaling as therapeutic targets for osteolytic bone diseases

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Bone loss induced by excessive osteoclast formation is the major mechanism underlying many bone diseases, including osteoporosis, Paget's disease, tumor metastasis to bone and aseptic loosening of orthopaedic implants. Despite current treatment options that include bisphosphonates, calcium, vitamin D, and hormone replacement therapy, osteoporosis-related fractures result in significant morbidity and mortality. Development of novel therapy is vital for improved therapeutic strategies against osteolytic bone diseases. Current approach aims to target osteoclast differentiation and activation via the modulation of receptor activator of nuclear factor-kappaB ligand (RANKL) and its key signaling pathway of NF-κB. These strategies include the generation of monoclonal antibodies to RANKL, RANKL peptides/mutants and NF-κB inhibitors. We have generated RANKL mutants that inhibit wild-type RANKL-induced osteoclastogenesis and bone resorption via the interference of ligand trimerization. Using site directed mutagenesis, we have defined that methionine at position 199 (M199) is an essential residue for RANKL function. In addition, we have established several assays that allow robust screening for inhibitors that attenuate RANKL-induced signaling pathways and osteoclast formation and function. We have identified parthenolide and parthenolide analogs that inhibit key RANK-mediated NF-κB signaling pathways; including p65 nuclear translocation, IkB-α degradation and NF-κB luciferase reporter gene activities. These NF-κB inhibitors suppressed RANKL-induced osteoclastogenesis, bone resorption and inflammation-induced bone destruction in mouse models. Unraveling the structure and function of RANKL and its key signaling pathway of NF-κB, and identifying novel approaches to modulate RANKL activity in osteoclasts, will be important in developing new therapeutic strategies for the treatment of osteolytic bone diseases.

OUTSTANDING ABSTRACT - CLINICAL

OR3

A dedicated intervention program reduces refracture rates in patients with incident non-vertebral osteoporotic fracture: Results of a 4-year prospective controlled study (OMEN)

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Aims: The risk of refracture significantly increases following an osteoporotic fracture. This analysis measured the 4-year-effect of a coordinated intervention program on refracture rates in patients with osteoporotic fractures.

Methods: Within the framework of a prospective controlled cohort study, we established a preventative clinical care program to identify, investigate and treat patients presenting with an osteoporotic fragility fracture. Patients 45+yrs with a recent minimal trauma fracture (MTF) were identified and referred to the MTF program. Patients were assessed for clinical risk factors, BMD, prevalent fractures and secondary causes of osteoporosis. If a diagnosis of osteoporosis was made, patients were commenced on appropriate therapy and followed-up every 6-12 months. Patients with a MTF not attending the program formed the concurrent control group, undergoing a telephone interview at study completion.

Results: During 5/2005-10/2009, there were 246 patients in the MTF and 157 in the control group. Groups were balanced for anthropometric, socio-economic and clinical risk factors at baseline. Ten patients (4.1%) in the MTF group suffered a further fragility fracture within 37.7 months. In the control group, 31 patients (19.1%) re-fractured within 35.2 months (HR 5.3; 95%CI 2.61-10.7 $p<0.01$). In women, the HR of refracture was 6.6 (95%CI 2.98-14.4, $p<0.01$). Median times to refracture were 23 (MTF) and 13 months (controls; $p<0.01$).

Conclusion: A dedicated post-fracture program greatly reduces the risk of further fracture. The results from this prospective controlled study prove that active identification and management of patients with osteoporotic fracture prevent further fractures and hence disability associated with osteoporosis.

OR4

Calcium supplementation and the risks of atherosclerotic vascular disease in postmenopausal women

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Aims of the study: Concern has been expressed that calcium supplementation, a key public health intervention for preventing osteoporotic fracture in older women, may increase the risk of atherosclerotic vascular disease (ASVD). The risk was evaluated by examination of verified data on ASVD hospitalisation and mortality from a 5-year RCT of calcium carbonate and 4·5-year post-trial follow-up.

Methods: Complete hospital admission and mortality data were obtained from the Western Australian Data Linkage Service (WADLS), which provides 100% ascertainment and ICD coding of all events in Western Australia, for patients recruited to the 5-year, randomised double-blinded placebo controlled trial (Calcium Intake Fracture Outcome Study, CAIFOS). 1,460 female participants aged 75·1 ± 2·7 years were recruited and randomised to receive 1,200 mg of calcium carbonate daily or an identical placebo.

Results: In the 5-year *intention-to-treat* analysis 104 participants (31·4/1000 person years) in the calcium supplementation group and 103 (30·9/1000 person/years) in the placebo group sustained either hospitalisation or death from ASVD; age-adjusted ITT HR 1·005 95% CI 0·766-1·320, all covariate-adjusted ITT HR 0·938 95% CI 0·690-1·275. At 9·5 years, 195 participants (33·9/1000 person years) in the calcium supplementation group and 200 participants (34·5/1000 person years) in the placebo group sustained hospitalisation or death from ASVD; age adjusted ITT HR 0·975 95% CI 0·800-1·187, all covariate adjusted ITT HR 0·919 95% CI 0·737-1·146.

Conclusion: This trial provides compelling evidence that calcium supplementation of 1,200 mg daily does not significantly increase the risk of atherosclerotic vascular disease in elderly women.

OR5

Depletion of osteoclastic-like giant cells by denosumab alters the differentiation of stromal-like tumour cells in giant cell tumour of bone

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Giant cell tumour of bone (GCT) is a primary osteolytic tumour characterised by abundant osteoclast-like giant cells interspersed amongst undifferentiated mononuclear tumour stromal cells. We and others have previously shown that the tumour-stromal component expresses several key growth factors including RANKL, which is crucial for osteoclastogenesis in GCT. Aim: Here we report the therapeutic potential of the fully human monoclonal anti-RANKL antibody (Denosumab) on tumour progression and histogenesis in patients presenting with GCT. Methods: The radiological, histological and cytochemical features of GCT were established pre-operatively. Three biopsy confirmed cases were treated with subcutaneous denosumab (120 mg/month) and tumour response monitored by radiology. Following surgical resection, histological and morphological parameters were assessed. Results: We find that denosumab treatment switched the histolopathological features of GCT from osteolytic to osteogenic. While the rich neovascularisation characteristic of GCT was still maintained, a drastic reduction (>90%) of giant cells and increase in new osteoid surrounded by undifferentiated tumour stromal cells and monocytes were observed in all cases. Consistent with this osteogenic shift, comparative analysis of pre- and post-treatment *ex vivo* cell cultures showed a dramatic replacement of multinuclear, tartrate-resistant acid phosphatase/vitronectin receptor-positive, bone-resorbing giant cells with mononuclear stromal tumour cells intermixed with alkaline phosphatase-positive osteoblastic cells which supported bone nodule formation. Conclusion: These findings demonstrate the therapeutic potential of densoumab on GCT and suggest that depletion of RANKL-differentiated giant cells switches the differentiation of stromal-like tumour cells towards osteogenesis, thus highlighting the intricate cellular cross-talk that exists during GCT histogenesis.

OR6

Changing pattern of age- and sex-specific hip fracture incidence, 1990-3 to 2006-7: Geelong Osteoporosis Study

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Aim: Based on hospital separations, hip fracture incidence appears to be declining in Australia. The aim of this study was to investigate changes in hip fracture incidence in the Barwon Statistical Division over a period spanning from 1990 to 2007, using fracture ascertainment from radiological records.

Methods: Comprehensive hip fracture data were obtained from radiology reports for a geographically distinct region, the Barwon Statistical Division, and compared for the two periods 1990-3 and 2006-7. Duplicate reports and pathological fracture cases were excluded. During the study period, the population increased by 19%. For ages 55+ there was a 53% increase (57% men and 49% women); corresponding figures for ages 85+ were 133% (176% and 116%).

Results: Between 1990-3 and 2006-7 the absolute number of hip fractures per year increased by 86% in men and by 19% in women. Mean age-specific hip fracture incidence rates decreased for women aged 55-64, 65-74, and 75-84 years (RR 0.30 (95%CI 0.13-0.71) p=0.003; 0.62 (0.41-0.92) p=0.02; 0.58 (0.46-0.72) p<0.001, respectively). Non-significant decreases were observed for women aged 45-54 and 85+ and for men aged 65-74 and 75-84 years. Age- and sex-specific rates are tabulated.

Conclusions: Hip fracture incidence rates have decreased over time in older women, the relative effect being greatest for ages 55-64 years. Although this may reflect improved efficacy and increased uptake of anti-fracture drug treatments in this age-group, other factors such as cohort effects or other environmental influences cannot be excluded.

Table. Hip fracture number and rates for 1990-3 and 2006-7.

Age (yr)	Men				Women			
	No. cases		Rate (per 1000 p-yr)		No. cases		Rate (per 1000 p-yr)	
	1990-3	2006-7	1990-3	2006-7	1990-3	2006-7	1990-3	2006-7
15-24	1	3	0.02	0.09	3	2	0.06	0.06
25-34	3	0	0.06	0.00	1	1	0.02	0.03
35-44	2	5	0.04	0.14	1	1	0.02	0.03
45-54	4	9	0.12	0.25	5	4	0.15	0.11
55-64	6	10	0.21	0.34	22	7	0.76	0.23
65-74	34	19	1.54	0.97	71	35	2.62	1.61
75-84	60	66	5.69	5.04	201	125	12.4	7.13
85+	32	64	19.9	18.4	129	169	26.6	24.3

OR7

Evidence of Gene-Gene Interaction Determinants of Fracture Susceptibility

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The risk of fracture is determined by multiple genes, but the genetic variants identified by GWAS so far explained little genetic variance of fracture liability. This study sought to test the hypothesis that interactions of multiple loci influence the susceptibility to fracture in an individual. We studied 74 single nucleotide polymorphims (SNPs) in 34 genes and fracture in 603 men and 974 women aged 60+ who had participated in the Dubbo Osteoporosis Epidemiology Study. The SNPs were selected because they have been shown to be associated with fracture or bone mineral density (BMD) in a previous GWAS. Fracture was ascertained by radiological reports during the follow-up period (1989 and 2007). Multifactor dimensional reduction (MDR) analysis was used to identify effects of gene-gene interaction on fracture risk. Interaction effects were assessed for all possible *n*-SNP combinations, where $n = 2$, 3, or 4. MDR analysis identified 4-SNP interaction involving *ESR1*, *SMPDL3A*, *OPG*, and *SP7* genes, with the lowest prediction error (34%; $P = 0.0001$) and maximum cross-validation consistency (50%). Moreover, 3-SNP interactions involving 2 SNPs within the *ESR1* gene and one SNP within the *RPS6K5* gene were also found with prediction error of 37% ($P < 0.0001$) and cross-validation consistency of 60%. The interactions among SNPs did not follow simple dominant, recessive or additive models for any alleles. These results have demonstrated the presence of gene-gene interaction effects on fracture susceptibility. The incorporation of gene-gene interactions may improve the accuracy of prognosis of fracture for an individual.

OR8

Frequent walking is associated with an increased fracture risk in middle aged and older adults: A national, population-based prospective study (AusDiab)

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Current physical activity (PA) guidelines recommend that older adults accumulate ≥2.5 hrs/week of moderate aerobic PA, or at least 20 min/d of vigorous PA 3 d/week and resistance training 2-3/week, as well as reduce sedentary behaviour (prolonged sitting). This study examined whether: 1) adults who meet current PA guidelines are at reduced risk of fracture; 2) fracture risk varies by PA frequency and intensity; and 3) prolonged TV viewing time is associated with an increased fracture risk. This national, population-based prospective study with a 5-yr follow-up included 2780 postmenopausal women and 2129 men aged ≥50 years. Incident non-traumatic clinical fractures were self-reported. Overall, 307 (6.3%) participants sustained ≥1 fracture (women 9.3%; men 2.3%). After adjusting for age, BMI and physical function, women accumulating ≥2.5 hrs/week were not protected against fractures [OR (95% CI), 1.06 (0.81, 1.39)]. When walking and mod+vig PA time (hr/week) or frequency (≥10 min continuous walking or mod-vig PA) were entered separately into the model, each 1-hour increment in weekly walking time or walking frequency was associated with a significant 5-6% increased risk of fracture. When walking time and frequency were entered together in the model, only walking frequency remained significant [OR 1.06 (1.01, 1.10)]. Similar results were observed in men, and after adjusting for fracture history, 25OHD, smoking and alcohol. TV viewing time was not related to fractures. In conclusion, adults who adhered to the current PA guidelines were not protected against fragility fractures, and more frequent walking was associated with an increased fracture risk.

OUTSTANDING ABSTRACT - BASIC

OR9

Enhanced osteoclast formation in Oncostatin M receptor knockout (OSMR KO) mice in response to Parathyroid Hormone (PTH)

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Signalling through gp130 contributes to the osteoclastogenic effect of Parathyroid Hormone (PTH). PTH also increases Oncostatin M receptor (OSMR) expression in osteoblasts.

To determine whether OSMR is required for PTH anabolic action, 6 week old male OSMR knockout (OSMR KO) mice and wild-type (WT) littermates were treated with 30µg/kg/day hPTH(1-34) for 3 weeks. In WT mice, PTH increased trabecular bone volume (BV/TV) by 40% ($p<0.05$ vs saline), and trabecular thickness by 17% ($p=0.001$). In contrast, PTH treatment of OSMR KO mice reduced BV/TV by 22% ($p<0.05$ vs. saline) and trabecular number by 27% ($p=0.002$). Osteoblast surface, osteoid surface and mineral apposition rate were increased to the same extent by PTH treatment in WT and OSMR KO mice. However, osteoclast surface in OSMR KO mice was doubled by PTH ($p=0.003$ vs. saline), an effect not observed in WT. This was surprising because OSM stimulates RANKL expression and osteoclast formation through OSMR.

When osteoclast precursors were cultured with OSMR KO osteoblasts, >3-fold more osteoclasts were formed in response to PTH than when cultured with WT osteoblasts. Furthermore, even though the cAMP response to PTH was identical in WT and OSMR KO osteoblasts, PTH-induced RANKL expression was 5-fold greater in OSMR KO osteoblasts, and this level of RANKL expression was retained up to 24 hours after PTH exposure.

These results indicate that despite the known pro-osteoclastic and pro-RANKL influence of OSM, OSMR signalling suppresses PTH action in osteoblasts to enhance RANKL expression and osteoclast formation, indicating significant cross-talk between these pathways.

OR10

Trabecular bone volume is increased in a novel mouse model expressing an osteoblast-specific CYP27B1 transgene

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We have demonstrated skeletal synthesis of 1,25D by osteoblasts, osteocytes and osteoclasts by virtue of their expression of the 25-hydroxyvitamin D 1alpha-hydroxylase (CYP27B1). While locally produced 1,25D in bone may mediate anabolic and/or catabolic activities within the skeleton, in osteoblasts, it is our hypothesis that synthesis of 1,25D in bone performs autocrine functions that support bone remodelling. We have constructed a plasmid, in which transcription of the human CYP27B1 sequence is driven by the human *osteocalcin* promoter (hOcn-CYP27B1). Transient transfection of the hOcn-CYP27B1 construct into HOS cells treated with 25D (50nM), results in higher 1,25D production (63.5±14.8 pM) than control vector transfected HOS cells (7.6±2.1 pM), or transfected kidney 293T cells (3.5±0.7 pM). This construct was recently used to generate transgenic mice, of which two lines (OSC1 and OSC3) are undergoing detailed characterisation. Expression of human CYP27B1 mRNA in both mouse lines, as measured by qRT-PCR, is restricted to bone tissue where it is expressed at high levels (>50-fold higher than any other tissue). OSC mice maintain normal serum calcium and 1,25D levels. Seven week old male OSC1 mice demonstrate a 12.4% increase in BV/TV of the distal femoral metaphysis (n=6/gp, $p<0.05$). This was associated with a 10.8% increase in trabecular number ($p<0.05$) with trabecular thickness unchanged. Dynamic histomorphometry and osteocyte measurements will further clarify the mechanism of increased trabecular bone volume in OSC mice. Our data support the concept of the skeleton as an intracrine organ for vitamin D, with locally synthesized 1,25D exerting important actions for bone remodelling.

OR11

Neuropeptide Y, Y6 receptor (Y6R) - a novel regulator of bone mass and energy homeostasis

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Neuropeptide Y is a central neuro-regulator of bone. Hypothalamic Y2R and osteoblastic Y1R are reported to be critical in the regulation of bone homeostasis. However, to date, the role of Y6R is poorly defined. We generated Y6 null mice ($Y6R^{-/-}$) and characterised their energy and bone homeostatic phenotype.

Y6R deficiency significantly reduced body weight (11%) through reduced lean (8.6%) and fat mass (32.5%), associated with increased in energy expenditure and an elevated respiratory quotient.

In bone, $Y6R^{-/-}$ displayed significantly reduced whole body and femoral BMD (mg/cm^2) and BMC (mg) (femur BMD: 47.1±1.1 vs. 57.9±2.4, $p<0.01$; femur BMC: 18.6±0.1 vs. 23.7±1.0, $p<0.05$).

µCT analyses indicted that cancellous BV/TV (%) was significantly decreased in $Y6R^{-/-}$ compared to wt (7.3±0.5 vs. 10.1±0.7, $p<0.01$), with a significant loss of trabecular number (mm^{-1}) (1.4±0.1 vs. 1.8±0.1, $p<0.01$) albeit no change in trabecular thickness (µm) (wt 55.6.±1 vs. $Y6R^{-/-}$ 53.5±1, ns). A significant loss of cortical thickness (µm) was found in $Y6R^{-/-}$ (18.4±1.6 vs. 21.3±6.2, $p<0.01$) accompanied by significant reduction in periosteal surface (mm) (wt: 5.5±0.1 vs. $Y6R^{-/-}$ 5.1±0.1, $p<0.05$) with no change in endosteal (mm) surface (wt: 4.1±0.1 vs. $Y6R^{-/-}$ 4.0±0.1, ns). However, bone changes were still evident in fat-fed $Y6R^{-/-}$, despite similar body weight to fat-fed wild type controls.

These results suggest an important role of the Y6R in energy and bone homeostasis. The reduction bone mass evident in $Y6R^{-/-}$ mice is in contrast to other NPY-mediated models, indicating the potential for a counter regulatory role of Y6 receptor within the NPY system.

OR12

Homozygous deletion of the *Sost* gene results in enhanced healing and increased callus formation in healing fractures

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Sclerostin, transcribed by the *Sost* gene, is expressed by osteocytes and antagonizes Wnt/β-catenin signaling, negatively regulating osteoblast differentiation and hence bone formation. The complete deletion of *Sost* in mice results in extensively high bone mass. We sought to examine bone repair in the absence of *Sost* in these mice, hypothesizing they would show enhanced repair.
Homozygous-null mice (*Sost*-/-,) were compared to wildtype (*Sost*+/+, WT) littermates after externally fixed tibial closed fractures with harvests at 2 weeks (cartilage callus) and 4 weeks (hard callus).
At 2 weeks there was a 79% decrease in cartilage content in the *Sost*-/- calluses ($p<0.05$ vs WT), consistent with a trend to increased union rate. QCT revealed increases of 25% and 31% in callus BMD in *Sost*-/- at 2 and 4 weeks respectively ($p<0.01$ vs WT). µCT showed 51% and 44% increases in callus 3D BV/TV at 2 and 4 weeks respectively ($p<0.01$ vs WT). Histologically the original cortex was excluded revealing increases in BV/TV of 120% and 41% at 2 and 4 weeks ($p<0.01$ vs WT). Interestingly there was a 61% decrease in callus area in *Sost*-/- at 4 weeks ($p<0.05$ vs WT).
During early fracture repair *Sost*-/- mice showed enhanced cartilage removal and advanced hard callus union, along with increased bone content, density, volume and BV/TV. During hard callus remodelling *Sost* -/- mice continued to have increased bone density and BV/TV but reduced callus area. These results suggest enhanced endochondral ossification to union resulting in a smaller, denser hard callus in *Sost*-/- mice.

OR13

The antipsychotic clozapine, but not its derivative quetiapine, induces microarchitectural changes in bone

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Atypical antipsychotic drugs, such as clozapine and quetiapine, are commonly used in treatment of schizophrenia, which affects >1% of the world population; schizophrenia and its treatments have also been associated with an increased risk of fracture. We previously showed that clozapine decreases osteoblast proliferation and differentiation in vitro, and decreases BMD and bone volume in growing rats. The skeletal effects of quetiapine, a chemical derivative of clozapine, are unknown. Here we investigated the differences between quetiapine and clozapine on bone. 4-6 week old male rats (n=6/group) received daily clozapine or quetiapine for 42 days (10mg/kg/day).
DXA showed >30% reduced bone mineral content after clozapine, but not quetiapine, treatment. In clozapine treated animals, µCT analysis of tibial trabecular bone showed 30% reduction in bone volume (ANOVA, P=0.0316) and 23% reduction in trabecular number (P=0.0137) but quetiapine treatment caused no significant changes in trabecular bone. In cortical bone, we found further changes with clozapine treatment: 14% reduction in cross sectional area (P=0.0046), and 24% reduction in bone perimeter (P=0.0313); quetiapine treatment changed neither parameter. In vitro, 10µM clozapine treatment reduced proliferation of osteoblast-like cells by 80%, but a quetiapine concentration 5 times greater was required to achieve similar effects.
These data suggest that quetiapine causes less skeletal toxicity than clozapine in vivo. Long-term quetiapine administration may therefore pose less risk than clozapine to skeletal health.

OR14

Skeletal cell apoptosis in methotrexate cancer chemotherapy-induced bone loss

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Cancer chemotherapy often causes bone defects such as growth arrest, bone loss and fractures. Using chemotherapy models in young rats treated with the commonly used anti-metabolite methotrexate (MTX) (5 once daily injections at 0.75mg/kg), we investigated how skeletal cell damage contributes to the bone defects. MTX significantly induced growth plate chondrocyte apoptosis and reduced collagen-2 expression, leading to thinner growth plate and primary spongiosa, suggesting depressed endochondral bone formation. In the metaphysis, there was significant apoptosis of osteoblasts and osteocytes, accompanied by a reduced bone volume. Consistent with the reduced bone formation potential, isolated marrow stromal cells had a smaller pool of osteoprogenitors, a decreased mineralisation potential but an enhanced adipogenic differentiation. Also contributing to bone loss was an increased osteoclast density on trabecular bone and increased osteoclastogenesis in the marrow. Consistently, there was increased expression of osteoclastogenic cytokines (RANKL, TNF-α, IL-1β, and IL6) in the bone. Interestingly, indicative of a potential role of osteocyte apoptosis in the local osteoclast recruitment, more apoptotic osteocytes were found localised closely with $TRAP^+$ osteoclasts in the bone. Cultured MLO-Y4 osteocytic cells treated with MTX underwent apoptosis and expressed a higher level of IL-6, and the conditioned medium supported osteoclastogenesis from normal mouse marrow cells. Our *in vivo* and *in vitro* data indicate that MTX chemotherapy directly damages skeletal cells and their precursors, suppressing endochondral bone formation, reducing osteogenic potential of bone marrow stromal progenitor cells, and increasing osteoclastic bone resorption, and that osteocyte apoptosis appears to be associated with the increased osteoclastic recruitment.

IS12

Could ephrins be therapeutic targets in osteoporosis?

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Ephrin ligands and their Eph tyrosine kinase receptors are local mediators of cell function through largely contact-dependent processes in development and in maturity. Their effects are achieved by forward signalling through receptor or reverse signalling through ligand, or a combination of both. Reverse signalling from osteoblast-derived EphB4 through ephrinB2 ligand in the osteoclast lineage has been shown to inhibit osteoclast formation, and osteoblast EphB4 has been suggested to favour bone formation. Production of ephrinB2 mRNA and protein are rapidly increased by PTH and PTHrP in osteoblasts *in vitro* and bone *in vivo*. Both a synthetic peptide antagonist of ephrinB2/EphB4 receptor interaction and recombinant soluble extracellular domain of EphB4 (sEphB4), which is an antagonist of both forward and reverse EphB4 signalling, were able to inhibit mineralization of osteoblasts in culture and the expression of several osteoblast genes involved late in osteoblast differentiation. These antagonists also block the enhanced osteoblast differentiation that results from pharmacological inhibition of ROCK, a downstream effector of RhoA signalling. The findings are consistent with ephrinB2/EphB4 within the osteoblast lineage having a paracrine role in osteoblast differentiation and bone formation, perhaps predominantly by reverse signalling through ephrinB2. Such local regulation might contribute to control of osteoblast differentiation and bone formation at remodelling sites. It might be possible to target the pathway through ephrinB2 reverse signalling to enhance formation and inhibit resorption, but the involvement of ephrin/Eph interaction in so many different organ functions, including especially vasculogenesis, presents major challenges.

IS13

Osteoporosis: increased resorption or failed anabolism? New approaches to therapeutic management

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Age-related bone loss results from an increase in remodelling rate together with reduced bone formation at the level of the individual Basic Multicellular Unit (BMU). These changes in bone remodelling result in structural damage in both cortical and trabecular bone and may also contribute to age-related changes in bone material composition. The increase in remodelling rate is driven by increased osteoclastogenesis and increased osteoclast activity, whereas the deficit in bone formation at the cellular level results mainly from impaired osteoblast function.

Drugs that inhibit osteoclasts, for example bisphosphonates, are effective in preventing bone loss and fracture risk but produce only relatively modest changes in bone mass. In contrast, anabolic agents such as the parathyroid hormone peptides lead to large increases in trabecular bone mass but have variable and even negative effects in cortical bone depending on the skeletal site. Whereas reduction in hip fracture has been demonstrated after treatment with anti-resorptive therapy, there are no corresponding data for parathyroid hormone peptides. Overall the magnitude of fracture reduction in the spine and non-vertebral sites (other than hip) appears to be comparable for both types of agent, despite their different mode of action.

Newer approaches in clinical development include anti-sclerostin antibodies and cathepsin K inhibitors. In animals, inhibition of sclerostin both inhibits bone resorption and stimulates formation, providing the potential for more complete prevention or reversal of age-related changes in bone than existing therapies. Cathepsin K inhibits the action of individual osteoclasts but not osteoclast formation, so that tissue level bone formation may be less suppressed than with other anti-resorptive drugs. Whether these approaches will improve current rates of fracture reduction, particularly at non-vertebral sites, remains to be established.

OR15

Strontium ranelate-induced osteogenic effects in human osteoblasts via a sclerostin decrease and a canonical Wnt signaling increase

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Strontium ranelate is a treatment for osteoporosis that has been shown to reduce fracture risk associated with increased bone apposition rates and decreased bone resorption. We have previously shown that strontium increases the replication, differentiation and survival of primary human osteoblasts (HOBs), at least in part, through the calcium sensing receptor (1). In the current study we have investigated the effect of strontium on the canonical Wnt signaling pathway. This pathway is known to be activated via AKT and also by Wnt-induced Frizzled/LRP complex formation in the plasma membrane, the formation of which is inhibited by sclerostin. Using Western blot analysis, we demonstrated that treatment of monolayer HOBs with strontium resulted in phosphorylation of AKT at Thr^{308} and Ser^{473}, GSK-3β at Ser^{9}, β-catenin at Ser^{552}, the stabilization of β-catenin in the cytosol and its subsequent translocation to the nucleus. Wortmannin was shown to abolish strontium-induced phosphorylation of AKT at Thr^{308} implicating PI3-kinase as an upstream effector in this pathway. To investigate the role of strontium in mature HOBs, HOBs were differentiated in organized multilayer cultures. The presence of strontium during mineralization significantly increased both the rate and degree of mineralization, measured by ARS staining at 7 and 14 days post-treatment ($p<0.05$-0.001). Under these conditions, strontium also dose-dependently decreased the level of sclerostin expression, measured by Western blot analysis at 7 and 14 days post-treatment ($p<0.001$). We propose that these two discrete pathways that promote canonical Wnt signalling are responsible, at least in part, for the osteogenic properties of strontium ranelate in vivo.

1. Brennan et al. Brit J Pharmacol 157:1291, 2009.

IS14

Bone imaging - the future

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With recent advances in molecular medicine and disease treatment there is a strong need for quantitative imaging of bone structure in the context of bone quality assessment. A number of new microstructural imaging modalities have been put forward recently allowing quantification with high precision and accuracy. Although biomedical imaging technology is now readily available, few attempts have been made to expand the capabilities of these systems by adding quantitative analysis tools and by exploring structure function relationships in a hierarchical fashion over the different length scales. Nevertheless, such quantitative endpoints have become an important factor for success in basic research and the development of novel therapeutic strategies in biomedicine and clinical practice. Computed tomography is key to these developments being an approach to image and quantify trabecular bone in three dimensions and providing multi-scale biological imaging capabilities with isotropic resolutions ranging from a few millimeters down to one hundred nanometers. As part of the presentation, new strategies for advanced hierarchical quantification of bone and their structure function relationship will be presented. The focus will be on hierarchical micro- and nano-imaging as well as image-guided biomechanics. The bone imaging approach of the future will help improve predictions of bone failure, clarify the pathophysiology of skeletal diseases, and define the response to therapy. We expect such an approach to improve our understanding of structure function relationships in bone and with that to also allow improved quality control and more successful outcomes in studies dealing with the pharmacological treatment of bone.

IS15

Cortical bone decay: beyond density and thickness

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Research in osteoporosis was focused on vertebral fractures and the role of trabecular bone loss in the pathogenesis of bone fragility. This paradoxical given that non-vertebral fractures account for ~80% of all fractures, occur at predominantly cortical sites, escalate with age and produce 90% of the morbidity and mortality associated with fractures. Recent reports show that most bone loss with age is cortical not trabecular as commonly believed and occurs by intracortical resorption which increases the number, size and coalescence of intracortical cavities, particularly adjacent to the marrow leaving chaotically connected 'trabecularized' cortical remnants and a thin more porous but still compact appearing cortex. Bone loss occurring during the process of production of cortical remnants represent ~ 50% of total bone loss occurring during ageing. Furthermore, ignoring cortical remnants results in 3 to 4 fold underestimation of cortical and trabecular bone loss and decay. Altogether these findings suggest that accounting for cortical remnants may help better assess the effects of ageing, diseases and treatment on bone.

This presentation will discuss non-invasive systems and methods to non-invasively quantify cortical remnants from peripheral quantitative computed tomographic (pQCT) images. These methods can be used in clinical and research settings. I will then demonstrate that accounting for these remnants when assessing cortical bone decay may allow identification of individuals at risk for fracture above and beyond commonly used parameters such as BMD and cortical thickness.

IS16

Clinical bone imaging: beyond BMD...

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Current clinical bone imaging generally reports bone mineral density (BMD, g cm^{-2}) and vertebra shape analysis derived by dual energy X-ray absorptiometry (DXA); with BMD having predictive accuracy of bone failure load between 40-75%; the remaining portion explained by the term '*bone quality*', describing factors including shape, structure, mineralisation, porosity etc. There is increasing interest therefore in developing and utilising clinical bone imaging techniques that are additionally dependent upon bone quality. A number of DXA-based numerical analysis techniques have been described including hip strength analysis (HSA) that provides a combination of cross-sectional area and cross-sectional moment of inertia, and 3-D X-ray absorptiometry based upon bi-planar acquisitions.

A measurement of true volumetric bone density (g cm^{-3}) and bone structure may be derived using quantitative computed tomography (QCT); peripheral quantitative computed tomography (pQCT) is typically performed at the distal radius and distal tibia, with a radiation dose comparable to DXA. Magnetic resonance imaging (MRI) may also provide a measure of volumetric bone density and structure by subtracting the recorded marrow image.

Quantitative ultrasound (QUS) measurements of velocity and attenuation cannot to date be performed at primary osteoporotic fracture sites and are not generally reported as images; they are however inherently dependent upon both material and structural properties of bone.

Finite element analysis (FEA) is inherently sensitive to the geometry and material distribution of bone with studies utilising both CT and DXA clinical bone imaging to predict the mechanical integrity of the proximal femur.

So where do we go from here?

OR16

Bone mineral density assessed via DXA and microarchitecture assessed via micro-CT: predicting whole vertebral body strength

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Strong relationships exist between areal bone mineral density (aBMD) derived from dual energy X-ray absorptiometry (DXA) and bone strength. However, the predictive validity of aBMD for osteoporotic vertebral fractures remains suboptimal. Rather than assessing aBMD from commonly used posterior-anterior (PA) projections, the diagnostic sensitivity of DXA may be improved by assessing aBMD from lateral projections. Nowadays, X-ray microcomputed tomography (micro-CT) allows three-dimensional structural characterization of entire bone segments, non-destructively and at high resolution. The aim of this study was to measure aBMD by lateral-projection DXA and bone volume (BV) by micro-CT, and to assess their respective capability to predict vertebral strength determined experimentally. Eight human cadaver spines (age at death 78±10 years) immersed in a water bath were scanned by DXA in PA and lateral projections, and aBMD for L2 vertebrae was determined. The L2 vertebrae were then dissected and entirely scanned by micro-CT (18µm pixel size). BV was calculated over the micro-CT trabecular bone volume of the entire vertebrae. The vertebrae were then mechanically tested in uniaxial compression to determine ultimate load. aBMD by lateral-projection DXA and BV by micro-CT were predictive of ultimate load (r^2=0.89, $p<0.01$, and r^2=0.89, $p<0.01$). aBMD by lateral-projection DXA was significantly related to BV (r^2=0.63, $p<0.05$). Conversely, aBMD by PA-projection DXA was not significantly related to ultimate load (r^2=0.37, p=0.15), and to BV (r^2=0.23, p=0.27). These findings highlight the capability of aBMD assessed using lateral-projection DXA to predict vertebral strength, and provide the basis for further exploring the clinical application of lateral-projection DXA analysis.

OR17

Prognosis of fracture risk by quantitative ultrasound measurement and bone mineral density

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We sought to determine whether the combined use of calcaneal QUS and BMD measurements could improve the accuracy in fracture risk prediction, and to develop a nomogram based on the predictive model.
The study was designed as a population-based prospective investigation, which involved 407 women and 421 men aged 62-89 year, who had been followed for a median of 13 years during the period of 1994-2009. BMD was measured at femoral neck by DXA using GE Lunar DPX-L densitometer and BUA was measured at the calcaneus using CUBA sonometer.
During the follow-up period, 18% men (n=77) and 38% women (n = 154) had sustained a fragility fracture. Each standard deviation decrease in BUA was associated with a hazard ratio [HR] of fracture 1.86 (95%CI, 1.57-2.27) in women and 1.50 (95% CI, 1.19-1.94) in men. After adjustment for BMD, BUA remained significantly associated with fracture risk in women and men with reduced magnitude (HR 1.57, 95%CI, 1.26-1.95 in women; HR 1.32, 95% CI, 1.03-1.69 in men). Reclassification analysis also yielded a total net reclassification improvement (NRI) of 9.7% (p = 0.02) and 3% (p = 0.69) for women and men respectively. Overall, 21% of women and 33% of men were reclassified into a different risk category. Based on the estimated parameters of the final model, two nomograms were constructed for individualizing fracture risk prediction.
These results suggest that combination of QUS and BMD in form of a nomogram can enhance the accuracy of categorizing individuals according to their risk of fracture.

OR18

Atypical femoral fractures are associated with bisphosphonate use

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Aims: The association between bisphosphonate use and subtrochanteric ("atypical") femur fractures remains controversial.
Methods: We reviewed 152 non-hip femoral fractures (152 patients, f=132) admitted to Concord Hospital between 6/2003-5/2008. An orthopedic surgeon reviewed all fracture radiographs twice in random sequence (Cohen's κ=0.8), identifying atypical fractures as a lateral transverse fracture line within cortical thickening and a contra-lateral beak.
Results: Twenty fractures were classified as atypical. Of these, 17 fractures were sustained by patients on current oral bisphosphonates (15 alendronate; 2 risedronate; mean treatment duration 5.1 and 3yrs). Of the remaining 132 patients, 2 were taking alendronate and 1 was on risedronate (mean treatment duration 3.5 and 1yr). The risk of atypical vs. typical fracture in non-bisphosphonate users was increased 37.4-fold in bisphosphonate users (95%CI 12.9-119, P<0.001).
Atypical fractures were 97% specific to bisphosphonate users. Risk factors for atypical fractures included prevalent low-energy fractures (OR3.2, 95%CI 2.1–17.1), glucocorticoids >6 months (OR5.2, 95%CI 1.3–31), RA (OR16.5, 95% 1.4–142.3) and 25OHD levels <16ng/mL (OR3.5, 95%CI 1.7–18.7).
Based on the centre's catchment population, the mean annual incidence of atypical femur fractures was 0.23/10,000 (1.66/10,000 >65years). Using dispensing data and wide to narrow indices for the catchment locality, the mean annual incidence of atypical fractures was 11-33/10,000 alendronate users, and 2.5-7.4/10,000 risedronate users.
Conclusion: There is an apparent association between oral bisphosphonate use and atypical subtrochanteric femur fractures. The absolute frequency of these fractures is very low and does not outweigh the beneficial effects of bisphosphonates in patients with osteoporosis.

OR19

Treatment with interleukin-6 receptor antibodies inhibits breast cancer growth in a murine model of bone metastasis

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Aim: High circulating interleukin-6 (IL-6) levels are associated with poor cancer prognosis. We demonstrated that increasing or decreasing bone resorption results in corresponding changes of tumour IL-6 expression and tumour progression, indicating that IL-6 may sustain cancer growth in bone (Zheng *et al.,* 2009). Here we investigated the effect of disrupting IL-6 receptor (IL-6R) signalling on cancer growth in a murine xenograft model, using an anti-human [Tocilizumab=(Tmab)] or an anti-mouse (MR16-1) IL-6R antibody.
Methods: Five-week-old BALB/c nu/nu female mice were inoculated intra-tibially with 50,000 cells of the human breast cancer cell line, MDA-MB-231. Tibiae were X-rayed on d10, d17 and d21 (sacrifice), followed by analysis of tumour proliferation, apoptosis, osteoclast activity, histology and histomorphometry.
Results: Mice received Tmab or MR16-1 at 20, 50 or 100mg/kg i.p./3 days, or vehicle. Inhibitory effects on cancer growth in bone were most pronounced at 50mg/kg/3d for Tmab, and 100mg/kg/3d for MR16-1. At the latter doses, both Tmab and MR16-1 reduced X-ray osteolysis, histological tumour area and tumour proliferation at all time points ($p \leq 0.066$). MR16-1 inhibits endogenous IL-6 signalling in the host cell whereas Tmab not only blocks tumour-induced IL-6 activity but also affects tumour-derived IL-6, which acts on the murine IL-6R as well. Of note, we observed similar effects on tumour progression in bone for treatment with Tmab and MR16-1, suggesting that IL-6 affects tumour progression regardless of species origin.
Conclusion: Our data indicate that IL-6 plays an important role in bone metastatic growth and may be a potential treatment target in breast cancer.

OR20

Micro-CT and biomechanical analysis of the leptin receptor-deficient db/db mouse tibia

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Leptin, a major hormonal product of the adipocyte regulates appetite, reproductive function through its hypothalamic receptors. The leptin receptor has been found in several cell types including osteoblasts and chondrocytes. Previously we have shown leptin to be an anabolic bone factor in vitro, stimulating osteoblast proliferation and inhibiting osteoclastogenesis. Leptin increases bone mass and reduces bone fragility when administered peripherally but can also indirectly reduce bone mass when administered into the central nervous system. Furthermore, data from animal models deficient in either leptin (ob/ob) or its receptor (db/db) have been contradictory.
We compared the bone phenotype of leptin receptor-deficient (db/db) and wild-type (WT) mice using Micro-CT (Skyscan 1172 scanner) analysis of the proximal tibiae. Db/db mice had reduced percent trabecular bone volume (13.0±1.62% in WT vs 6.01±0.601% in db/db, p=0.002) and cortical bone volume (0.411±0.0215mm^3 vs 0.316±0.00353mm^3, p=0.0014), trabecular thickness (0.0484±0.00107mm vs 0.0451±0.000929mm, p=0.041) and trabecular number (2.68±0.319mm^{-1} vs 1.343±0.1478mm^{-1}, p=0.0034). Additionally, the material properties of db/db cortical bone were determined by three-point bending and at the nanoscale by nano-indentation, showing decreased bone strength (13.3±0.280N vs 7.99±0.984N, p=0.0074), and material stiffness (28.5±0.280GPa vs 25.8±0.281GPa, p<0.0001).
These results demonstrate that bone mass is reduced in the absence of leptin signalling, indicating that leptin acts in vivo as a bone anabolic factor. This concurs with the in vitro and peripheral leptin administration results and together with the fact that leptin is produced peripherally by fat tissue and bone marrow adipocytes, suggest that leptin's direct effects on bone cells over-ride the central effects.

OR21

Calcium supplementation does not rescue the programmed adult bone deficits associated with perinatal growth restriction

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Low birth weight programs adult diseases. We have reported that offspring born small, resulting from uteroplacental insufficiency (UPI), have shorter femurs, lower BMC and bone strength as adults. We determined the effects of calcium supplementation on growth restricted offspring.
Bilateral uterine vessel ligation (Restricted) or sham surgery (Control) was performed on gestational day 18 (term=22 days) in rats inducing UPI and growth restriction. At 2 months pups were allocated to diet groups: 1-constant normal calcium, 2-variable normal calcium, 3-constant high calcium, 4-variable high calcium. Diet groups 1 and 3 consumed their diets constantly. Groups 2 and 4, rats consumed one diet for 5 days, switching to a low calcium diet for the next 5 days. At post-mortem (6 months), dual energy xray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT) were performed on the femur.
Male and female Restricted offspring were born 14% lighter than Controls; females remained smaller at 6 months ($p<0.05$). Restricted males and females had reduced trabecular and cortical BMC, regardless of diet ($p<0.05$). Trabecular BMD was lower in Restricted females ($p<0.05$). Consuming constant high calcium increased cortical BMC in Restricted males and both female groups ($p<0.05$). Stress strain index of bone strength was lower in Restricted offspring, regardless of diet. DXA results matched pQCT.
Being born small programs reduced adult femur length, dimensions and strength. High constant calcium increases adult cortical BMD in low birth-weight offspring and normal-weight females but did not rescue the programmed bone deficit.

OR22

Comparison of the FRISK Score to the FRAX (UK) Algorithm and Garvan Nomogram: Geelong Osteoporosis Study

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The FRAX algorithm and Garvan nomogram calculate absolute fracture risk with factors including BMD measured at the proximal femur. By contrast, the FRISK score[1] uses BMD at the spine and proximal femur. The aim of this study was to compare the algorithms.
An age-stratified random population-based sample of women was recruited from the Barwon Statistical Division during 1994-7(n=594; age 60+yr). Risk factors used in the Garvan, FRISK and FRAX algorithms were measured: spine and femoral neck BMD, falls, prior fracture, weight, height, parental fracture, smoking, medication, secondary osteoporosis, and alcohol consumption. Absolute fracture risk for each algorithm was calculated. Subjects were followed for 10yr and fractures recorded. Area under the receiver operating characteristic curves (AUC), optimal sensitivity and specificity (%) were calculated.
There was no significant difference in AUC using the Garvan(AUC: 0.70, 95%CI: 0.65-0.75; Sens 64.2, Spec 66.2), FRAX(AUC: 0.68, 95%CI: 0.63-0.73; Sens 60.8, Spec 65.6) and FRISK(AUC: 0.66, 95%CI: 0.60-0.71; Sens 59.2, Spec 64.8) algorithms. In those with low spine BMD (T-score 1SD< less than femoral neck, n=77), the FRISK score tended towards a higher sensitivity (FRISK: Sens 85.7, Spec 71.4; FRAX: Sens 71.4, Spec 68.3, Garvan: Sens 78.6, Spec 68.3). Nevertheless, there was no difference in the AUCs.
The FRISK score is comparable with the FRAX algorithm and Garvan nomogram. However FRAX uses more risk factors and does not include falls. The Garvan nomogram includes BMD at the femoral neck only. The FRISK score is more sensitive to fracture in women with low spine BMD.
References:
1) Henry MJ, Pasco JA, Sanders KM, Nicholson GC, Kotowicz MA. Fracture Risk Score (FRISK Score). 2006 Radiology 241(1):190-6

OR23

Choline Kinase Beta is a critical regulator of bone homeostasis

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The maintenance of bone homeostasis requires a tight balance between bone formation and bone resorption by osteoblasts and osteoclasts. The molecular mechanism underlying the fundamental activities of these cells still remains largely unclear. In search of novel molecules that potentially play an important role in bone homeostasis we screened a number of ENU-induced mutant mouse lines. We identify Choline kinase beta, a kinase that phosphorylates the first reaction in the biosynthesis of phosphatidylcholine, as a novel candidate regulator of bone homeostasis. Choline kinase beta mutant mice exhibit: 1) an osteoporotic phenotype as evidenced by microCT and histological assessment, 2) increased bone fragility with poor mechanical properties and 3) elevated osteoclast numbers *in vivo* and *in vitro*. Osteoclasts from choline kinase beta mice display an increased sensitivity to RANKL in culture and exhibit increased resorptive activity as compared to those of littermate controls. Real-time RT-PCR analysis showed an increase in osteoclast marker gene expression both *in vitro* and *ex vivo*. Taken together, our data documents for the first time that choline kinase beta plays a critical role in bone homeostasis mainly via the regulation of osteoclast formation and activity.

OR24

The association between oral contraceptive use, bone mass and fractures in both young and older women

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The association between hormonal contraceptive use and BMD remains controversial. The aim was to describe the associations between current and ever use of oral contraceptives (OC), bone mass and fractures in two population-based samples of young and older women. Data were collected from women aged 26-36 years (n=687) in the Childhood Determinants of Adult Health (CDAH) study and aged 50-80 years (n= 491) in the Tasmanian Older Adult Cohort (TASOAC) study. Bone mass was measured by quantitative ultrasound (QUS) at heel, and DXA at spine, total hip and body. Women were categorized as current OC users (young women), ever users (older women), and duration of use for users (< 5 years, 5-10 years and >10 years). In young women, current OC use was significantly associated with higher broadband ultrasound attenuation (BUA, β=0.21), speed of sound (SOS, β=0.23) and quantitative ultrasound index (QUI, β=0.23) (all, $P<0.05$). The associations remained significant after adjustment for confounders except for SOS. In older women, ever use of OC was associated with a higher BMD at the total body (6%, $P<0.001$) and spine (4%, $P=0.05$) but not hip after adjustment. There was also a significant association between duration of OC use and total body and spine BMD, most notable for those with more than 10 years use. Consistent reductions in spinal fractures were observed but these only reached significance for 5 to 10 years use. We concluded that OC use is consistently associated with higher bone mass in both young and older women in these population-based samples.

OR25

Apomab, a fully human agonistic DR5 monoclonal antibody, inhibits tumour growth and osteolysis in murine models of breast cancer development and progression

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Apomab is a fully human agonistic DR5 monoclonal antibody that triggers apoptosis through activation of the extrinsic apoptotic signalling pathway. In this study we assessed the cytotoxic affect and signaling of Apomab *in vitro* and evaluating its antitumour activity in murine models of breast cancer development and progression at both the orthotopic site and in bone. MB-231-TXSA breast cancer cells, tagged with a triple reporter gene construct (NES-HSV-tk/GFP/Luc), were transplanted directly into the mammary gland or into the tibial marrow cavity of nude mice. Apomab was administered early, postcancer cell transplantation, or after tumours progressed to an advanced stage. Tumour burden was monitored progressively using bioluminescence imaging, and the development of breast cancer–induced osteolysis was measured using microcomputed tomography and histology. *In vitro*, Apomab treatment induced apoptosis in a panel of breast cancer cell lines but was without effect on normal human primary osteoblasts, fibroblasts, or mammary epithelial cells. This was associated with processing and activation of caspases 8, 10, 9 and 3, over time which was concomitant with activation of the Bcl2 family protein Bid and cleavage of the apoptosis target proteins PARP. *In vivo*, Apomab exerted remarkable tumour suppressive activity leading to complete regression of well-advanced mammary tumours with no evidence of recurrence. All animals transplanted with breast cancer cells directly into their tibiae developed large osteolytic lesions that eroded the cortical bone. In contrast, treatment with Apomab following an early treatment protocol inhibited both intraosseous and extraosseous tumour growth and prevented breast cancer–induced osteolysis. In a delayed treatment protocol, Apomab treatment resulted in the complete regression of advanced tibial tumours with progressive restoration of both trabecular and cortical bone leading to full resolution of osteolytic lesions. These results suggest that Apomab represents a potent immunotherapeutic agent with strong activity against the development and progression of breast cancer and highlights the need to clinically evaluate Apomab in patients with primary and metastatic disease.

IS17

Basic aspects of OA

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Although the initiating event of Osteoarthritis (OA) has yet to be identified, the notion that early OA is characterised only by the degeneration of the articular cartilage has recently been reconsidered, as subchondral bone (SCB) alterations were also found to be involved in the early phase of the disease process. Data suggest that SCB is a driving force behind the cartilage degradation observed in OA. Studies performed in animal models of OA have revealed, at an early stage of the disease, a thinning of the SCB plate indicating bone resorption, and, at a later stage, a SCB formation process resulting in sclerosis of the tissue. Data will be presented from our recent study using a non-trauma rat model of OA to characterise longitudinal SCB architectural change in association with cartilage degradation. SCB changes have been associated with local osteoblast metabolism involving abnormal activation of biochemical pathways. Further, there is *in vitro* evidence that SCB osteoblasts are capable of influencing chondrocyte metabolism more directly, leading to abnormal remodelling of OA cartilage. Interestingly, many of the candidate susceptibility genes for OA, identified by genetic screening approaches, have bone-related functions, which further suggests the involvement of bone in OA. There is now evidence from animal models that anti-resorptive agents that inhibit SCB remodelling, also prevent the SCB changes and loss of cartilage seen in OA, and reduce joint damage. In conclusion, structural and compositional changes seen in OA SCB, brought about by altered bone remodelling, are a likely contributor to the breakdown of the articular cartilage in the joint.

IS18

Bone remodelling in inflammatory arthritis

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The term "inflammatory arthritis" encompasses a myriad of degenerative joint diseases that are characterized by the presence of local inflammation within the joint. Factors produced by cells present within the inflammatory infiltrate contribute to a perturbation in the balance between bone formation and bone resorption. This leads to either net bone loss as observed in rheumatoid arthritis (RA), or net bone gain as observed in spondylarthropathies (SpAs). In RA-affected joints the local cytokine milieu favours osteoclast differentiation and bone erosion with an increase in expression of the osteoclast differentiation factor, receptor activator of nuclear factor-kappa B ligand (RANKL) compared to expression of its decoy receptor osteoprotegerin (OPG). Formation of properly mineralized bone by osteoblasts is impaired at sites of bone erosion in RA, further contributing to bone loss at these sites. Recently, inhibition of the Wnt signalling pathway in RA-affected joints due to increased expression of Wnt signalling antagonists, including members of the Dickkopf and secreted frizzled-related protein families, has emerged as a mechanism that not only contributes to inhibition of bone formation but also promotes bone resorption via modulation of RANKL:OPG expression favouring osteoclastogenesis. In contrast to RA, inflammation in SpAs results in periosteal bone formation. Local decreases in expression of the Wnt signalling antagonist sclerostin in osteocytes has been identified as one mechanism that may promote bone formation in SpAs. The differences in the modulation of bone remodelling in RA compared with the SpAs, clearly demonstrates that the site of inflammation, and the cell types, cytokines and factors present at this site, dictates the impact of inflammatory arthritis on bone.

IS19
Clinical aspects of OA

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It has long been hypothesised that subchondral bone plays a key role in the initiation of osteoarthritis. Evidence in humans has been lacking until recent years when newer modalities such as MRI and DXA have been applied to study this in more depth. This presentation will cover the independent roles of bone size, bone density and bone marrow lesions in osteoarthritis including their relationship to pain, subsequent cartilage damage and joint replacement. In addition, it will look at whether cartilage damage precedes bone changes. The implications of these results for the development of new therapies will also be discussed.

OR26
Macrophage migration inhibitory factor (MIF) promotes osteoclastogenesis and arthritic bone erosion.

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In rheumatoid arthritis (RA), MIF promotes leukocyte recruitment and activation but its role in bone erosion is unclear. We sought to investigate the effect of MIF on bone erosion in an *in vivo* model and *in vitro* osteoclastogenesis assays.
K/BxN serum transfer arthritis was induced in $MIF^{-/-}$ and WT mice and characterised by scoring clinically, histologically, by TRAP staining (to identify osteoclasts) and real time RT-PCR analysis. Osteoclastogenesis assays were performed using bone marrow macrophages (BMM) or RAW 264.7 cells cultured with CSF-1 and RANKL. RAW 264.7 cells stably transfected with an NFκB-luciferase reporter were also used to examine RANKL-induced NFκB activity.
Compared to WT mice, $MIF^{-/-}$ mice had significantly lower clinical scores, and histological synovitis and bone damage. Significantly reduced $TRAP^{+}$ osteoclast numbers, and OC-STAMP and DC-STAMP mRNA expression was found in $MIF^{-/-}$ joints. These findings were paralleled by formation of significantly fewer $TRAP^{+}$ osteoclasts, and significantly fewer large osteoclasts (nuclear number >5), in RANKL/CSF-1 stimulated cultures of $MIF^{-/-}$ BMM compared to WT. RANKL induction of osteoclastogenesis in BMM precursors was accompanied by significant dose-dependent increases in MIF expression. In RAW cells, RANKL-induced NFκB reporter luciferase activation was significantly abrogated by anti-MIF monoclonal antibody neutralization.
In summary, MIF deficiency reduced osteoclastogenesis *in vivo* and *in vitro*, and reduced NFκB activation and expression of OC-STAMP and DC-STAMP . These findings suggest therapeutic MIF neutralization as a potential strategy in RA bone erosion and potentially other forms of bone loss.

OR27

Leukemia Inhibitory Factor inhibits resorption of mineralised cartilage during growth and stimulates bone formation in remodelling

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Leukemia inhibitory factor (LIF) has been reported to stimulate bone formation *in vivo* and to regulate osteoclast size in neonate mice. To identify unique roles of LIF in bone, LIF knockout (KO) mice were studied from birth until late adulthood. Neonate LIF KO mice demonstrated very low trabecular bone volume (BV/TV), associated with many "giant" osteoclasts adjacent to the growth plate, as previously reported (Bozec, Nature 2008). At 6 weeks of age however, while the osteoclast phenotype immediately below the growth plate remained, BV/TV of LIF KO and wild type littermates (WT) were not significantly different. Histomorphometry of 12 week old tibiae and vertebrae demonstrated that while the size and number of osteoclasts resorbing calcified cartilage (chondroclasts) was still significantly higher in male and female LIF KO mice than in WT, the size and number of osteoclasts on bone surfaces were not significantly altered. This points to distinct LIF-dependent pathways controlling osteoclast/chondroclast size and differentiation at the growth plate that do not have the same influence on bone surfaces.
Although calcified cartilage destruction was enhanced in LIF KO mice, BV/TV adjacent to the growth plate (where new trabeculae form), by microCT and histomorphometry, was significantly greater in LIF KO tibiae, femora and vertebrae compared to WT. In contrast, at regions of lamellar bone remodelling, BV/TV was significantly lower in LIF KO mice, and this was associated with a significant reduction in osteoblast surface, osteoid surface, osteoid thickness and mineral appositional rate. The deficit of osteoblast activity in this region indicates that LIF is critical for normal bone formation in the process of remodelling.

IS20

Basic aspects of vitamin D

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Vitamin D contributes to the maintenance of calcium, and phosphate homeostasis as well as exerting a wider range of biological activities including regulation of cellular differentiation and proliferation. The endocrine action of vitamin D acts through its renal metabolism, producing 1,25 dihydroxyvitamin D (1,25D) in the circulation for which the intestine is the major responsive organ controlling absorption of calcium and phosphate. 1,25D is also synthesised in a wide range of tissues including bone cells where it is investigated as an autocrine or paracrine agent. Vitamin D insufficiency in the elderly increases the risk of hip fracture due to osteoporosis. A major question is the cellular and molecular mechanisms by which depleted levels of vitamin D produce osteoporosis. Rodent studies with low vitamin D diets demonstrate that serum 25-hydroxyvitamin D (25D) levels between 20 and 80 nmo/L result in trabecular and cortical bone loss without any evidence of osteomalacia. This bone loss is due to increased bone resorption with increased expression of the *RankL* gene in bone and increased osteoclastogenesis. No relationship is evident between bone volume and either serum 1,25D or parathyroid hormone in these animals. 25D is metabolised to 1,25D by each of the major bone cell types which is essential for anabolic bone cell activities particularly related inhibition of proliferation and promotion of cell maturation. These preclinical data suggest that optimal bone health is achieved through the supply of adequate dietary calcium and vitamin D, sufficient for metabolism to 1,25D by bone cells

IS21

Vitamin D: classical and non-classical functions

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Vitamin D is hydroxylated in liver and kidney into 1,25-dihydroxyvitamin D, the active metabolite. This binds to the nuclear vitamin D receptor leading to the activation of many genes in many organs. In addition, some non-genomic actions occur through a putative membrane receptor. The classical function of vitamin D is bone mineralization mainly through the active absorption of calcium in the intestine. In addition, the active metabolite stimulates longitudinal bone growth, and decreases parathyroid function. Last decades, many non-classical functions of vitamin D have been discovered such as stimulation of insulin release and insulin sensitivity, stimulation of the immune response and decrease of auto-immune reactivity, decrease the function of the renine-angiotensin system, and decrease proliferation and stimulate differentiation of different cells. Through these non-classical functions vitamin D has been claimed to decrease the incidence of auto-immune disease, decrease rate of respiratory infections, improve insulin resistence, and decrease the incidence of several types of cancer. Non-classical effects of vitamin D may occur through extrarenal hydroxylation of 25-hydroxyvitamin D into 1,25-dihydroxyvitamin D and this may occur in many organs. Clinical significance of these non-classical functions has to be proven by double-blind clinical trials.

IS22

Vitamin D supplements do not mitigate the effect of calcium supplements on cardiovascular events

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Calcium and vitamin D (CaD) supplements are widely used in osteoporosis management in older people. Recently, the Auckland Calcium Study reported increased rates of the composite endpoint of MI, stroke, or sudden death in women allocated to calcium (without vitamin D). These results were not definitive but highlighted a need for further research.

We have now completed a meta-analysis of placebo-controlled trials of calcium (without vitamin D). Patient-level were data available for 5 studies (N=8151), and trial-level data for 11 studies (N=11,921). The HR for MI in trials with patient-level data was 1.31(1.02-1.67) and in those with trial level data the RR was 1.27(1.01-1.59).

WHI reported no effect of CaD on cardiovascular events in their placebo-controlled RCT. However, 54% of participants in WHI CaD took non-protocol calcium supplements, which potentially may have masked adverse effects of CaD. Therefore we reanalyzed WHI CaD by non-protocol calcium use at randomization. In 16,718 women not taking non-protocol calcium, CaD increased cardiovascular events by 13-22%, whereas in women taking non-protocol calcium, CaD did not alter cardiovascular risk.

We updated the meta-analysis with these data from WHI. There were complete trial-level data for 28,072 participants, and 1384 individuals had an incident MI or stroke. With calcium/CaD, the RR for MI was 1.24(1.07-1.45), stroke 1.15(1.00-1.32), and MI/stroke 1.15(1.03-1.27). In 3 trials of CaD vs placebo, the RR with CaD for MI was 1.21(1.01-1.44), stroke 1.20(1.00-1.43), and MI/stroke 1.16(1.02-1.32).

In summary, calcium supplements used with or without vitamin D increase the risk of cardiovascular events.

IS23

Changes in muscle strength three-months after annual 500,000 IU dose of cholecalciferol: Further analysis from the Vital D Study

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The Vital D RCT investigated the efficacy of a single annual 500,000IU dose cholecalciferol in the prevention of falls and fractures in older women. An unexpected increased rate of falls (15%) and fractures (26%) in the vitamin D group compared to the placebo group has recently been reported. Post-hoc analysis demonstrated increased risk in the 3-month period after dosing. Muscle functioning assessments performed at baseline and 3-months post-dose in a subgroup of Vital D participants may inform the mechanism(s) of this adverse outcome.

From the 2256 participants randomised and followed for 3 to 5 years, a subgroup of 125 underwent annual assessments of muscle strength, balance, gait, vision, heel ultrasound and biochemistry. This analysis investigates change from baseline to the 3-month post-dose assessments in 2007 for 97 substudy participants.

After adjusting for age and baseline hip flexion strength, a quadratic relationship was observed between change in hip flexion strength and percent change in serum 25-hydroxyvitamin D (25D) (25D changes, p=0.017).

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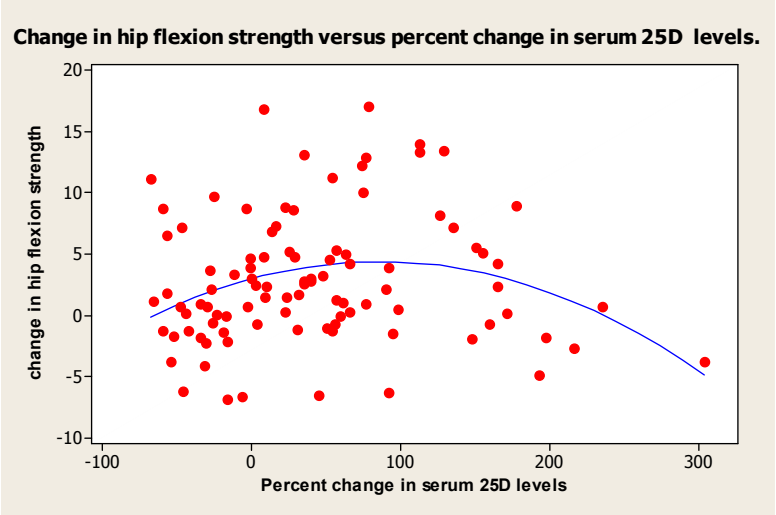

Increases in 25D of less than ~100% were associated with progressively increased hip flexion strength whereas greater increases were associated with decreasing strength.

This novel finding progresses our understanding of a possible mechanism underlying the occurrence of increased falls following annual high-dose vitamin D supplementation.

OR28

The effects of a nine month RCT of vitamin D supplementation (150,000 IU every 3 months) on bone density and muscle strength in older postmenopausal women

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Aim: Calcium and Vitamin D supplementation has been shown to reduce falls (Prince et al. Arch Intern Med 2008;168:103-8) and fractures in elderly women. Because of problems of compliance we trialled the effects of an administered 3-month oral vitamin D dose on falls propensity.

Methods: 686 ambulant women not selected for a previous fall were randomized to receive either a vitamin D3 150,000 IU every 3 months (n=353) or an identical placebo (n=333). All were advised to increase their calcium intake by diet. Falls data were collected using a validated diary every 3 months and muscle strength measured by a hand held dynamometer.

Results: Mean age was 76.7 ± 4.1 years, calcium intake at baseline and 9 months was 863.6 ± 412.2 and 855.1 ± 356.7 mg/day, respectively. The falls rate in the vitamin D group was 102/353 (30.6%) similar to the falls rate in the placebo group 89/333 (26.7%). Muscle strength at baseline was similar in the two groups (vitamin D 19.9 ± 4.9, placebo 19.9 ± 5.2) and there was no effect of vitamin D compared to placebo at 9 months.

Conclusion: Vitamin D administered in this way had neither beneficial nor adverse effects on falls or grip strength in these subjects. The possible reasons for this include lack of calcium supplementation, the dosage and frequency of vitamin D or the lower falls propensity compared to our previous study in which women sustaining a previous fall and had a higher falls rate (62.9% /yr) were studied.

POSTER PRESENTATIONS

P1

Vitamin D deficiency promotes human prostate cancer growth in a murine model of bone metastasis

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Aim: Prostate cancer frequently metastasises to the skeleton where the bone microenvironment plays a pivotal role in supporting metastatic cancer cell growth. Vitamin D (vitD) deficiency has recently been shown to enhance breast cancer growth in an osteolytic bone metastasis model (Ooi *et al.*, 2010). In this study, we investigated the effect vitD deficiency on prostate cancer cell growth in bone and subcutaneous tissue.

Methods: Three-week–old male nude mice were either weaned onto a vitD-free diet or kept on normal chow, the vitD-free diet leading to severe hypovitaminosis-D within 6 weeks. We then injected 50,000 cells of the prostate cancer cell line PC-3 intra-tibially or subcutaneously into vitD-deficient and vitD-replete mice. Animals were monitored for osteolysis on d21, 28 and 35, and tibiae were analysed by micro-CT and histology at endpoint (d35). Osteoprotegerin (OPG, 3mg/kg/3days) was co-administered in a subset of mice to determine the contribution of the bone microenvironment to tumour growth.

Results: Inoculation of PC-3 cells into tibiae caused predominantly osteolytic lesions with minor osteosclerosis. At endpoint, all outcomes (osteolytic lesion area, total tumour and sclerosis area, tumour mitotic activity) were significantly increased in vitD-deficient c/t vitD-replete mice ($p<0.05$). Co-treatment with OPG completely prevented osteolysis, significantly reduced total tumour area, sclerosis area, and tumour mitotic activity, and increased cell apoptosis in both vitD-deficient and replete mice. The growth of subcutaneously implanted tumours was similar in vitD-deficient and replete mice.

Conclusion: Modulation of the bone microenvironment secondary to vitD deficiency plays a critical role in stimulating tumour growth.

P2

The role of tumour derived interleukin 6 in a murine model of breast cancer bone metastasis

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Aim: High circulating interleukin-6 (IL-6) levels have been associated with disease progression / poor clinical outcomes in metastatic breast cancer patients. We found that increasing or decreasing bone resorption results in corresponding changes in tumour IL-6 expression and tumour proliferation, indicating that IL-6 expression by cancer cells may play a role in sustaining breast cancer growth in bone (Zheng *et al.*, 2009). However, it is unclear whether tumour-derived IL-6 affects cancer cell behavior *in–vitro* and *in–vivo*.

Methods: IL-6 expression was silenced in MDA-MB-231 cells via a lentiviral-based expression system driving the production of short hairpin RNA. Knock-down efficacy was 80% as assessed by real-time RT-PCR (mRNA) and ELISA (secreted protein). Control and IL-6 knock-down MDA-MB-231 cells (50000/injection) were then implanted intra-tibially into 4-week-old BALB/c nu/nu female mice (n=7) kept on a low (0.1%) calcium diet to induce high bone turnover (Zheng *et al.*, 2007 & 2008). Tumour growth was monitored using X-ray on d10, d17 and d21, and bones were analysed by histology and histomorphometry following sacrifice (d21).

Results: *In–vitro* characterization of control *vs.* knock-down MDA-MB-231 cells demonstrated that silencing of IL-6 expression significantly reduces invasiveness without affecting proliferation. Compared to controls, IL-6 knock-down resulted in significantly smaller osteolytic lesions on all timepoints ($p<0.05$), and significantly reduced total tumour area on d21 ($p<0.05$). Growth of subcutaneously implanted tumours was similar in animals injected with either cells.

Conclusion: We conclude that tumour-derived IL-6 has an important role in the biology of metastatic breast cancer and may be a potential therapeutic target.

P3

Anticancer efficacy of Apo2L/TRAIL is retained in the presence of high and biologically active concentrations of osteoprotegerin *in vivo*

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Osteoprotegerin (OPG) is a secreted member of the TNF receptor superfamily, which binds to the ligand for receptor activator of nuclear factor kB (RANKL) and inhibits bone resorption. OPG can also bind and inhibit the activity of the TNF-related apoptosis inducing ligand (Apo2L/TRAIL), raising the possibility that the anticancer efficacy of soluble Apo2L/TRAIL may be abrogated in the bone microenvironment where OPG expression is high. In this study we used a murine model of breast cancer growth in bone to evaluate the efficacy of recombinant soluble Apo2L/TRAIL against intratibial tumours, which were engineered to overexpress native full-length human OPG. *In vitro,* OPG-overexpressing breast cancer cells were protected from Apo2L/TRAIL-induced apoptosis, an effect that was reversed with the addition of soluble RANKL or neutralizing antibodies to OPG. *In vivo,* mice injected intratibially with cells containing the empty vector developed large osteolytic lesions. In contrast, OPG overexpression preserved the integrity of bone and prevented breast cancer–induced bone destruction. This effect was primarily due to the complete absence of osteoclasts in tibiae of mice inoculated with OPG transfected cells, confirming the biological activity of the transfected OPG *in vivo*. Despite the secretion of supra-physiological levels of OPG, treatment with Apo2L/TRAIL resulted in strong growth inhibition of both empty vector and OPG overexpressing intratibial tumours. While Apo2L/TRAIL-induced apoptosis may be abrogated *in vitro* by OPG overexpression, the *in vivo* anticancer efficacy of recombinant soluble Apo2L/TRAIL is retained in the bone microenvironment even in the face of biologically active OPG at supra-physiological concentrations.

P4

Muscle progenitors make a major contribution to open fracture repair

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Muscle can accommodate bone formation and we hypothesized that this may be facilitated by endogenous inducible osteoprogenitors. We aimed to test the contribution of myogenic cells in mouse models of open and closed fracture repair.

To track cells of the myogenic lineage, we employed the *MyoD*-cre$^+$:Z/AP$^+$ double transgenic mouse line. In these mice, muscle specific Cre/*loxP* recombination leads to a targeted and permanent expression of a heat-resistant *human alkaline phosphatase* (*hAP*) reporter. To examine the contribution of these cells to fracture repair, reporter$^+$ cells were measured in closed and open tibial fractures and tracked at 1, 2, and 3 week time points.

Staining in control non-fractured *MyoD*-cre$^+$:Z/AP$^+$ tibiae showed no native contribution of myogenic cells to bone. In closed tibial fractures where the periosteum was largely undamaged, reporter$^+$ cells were not seen. In open tibial fractures where the periosteum was circumferentially stripped, there was a high (>50%) contribution by reporter$^+$ cells to the healing callus. In serial sections, these cells were surrounded by collagen type-I or type-II staining matrix supporting the concept that these cells had assumed an osteogenic or chondrogenic phenotype. Control fractures from *MyoD*-cre$^-$:Z/AP$^+$ littermates showed no staining, indicating the *hAP* staining was specific for the transgene.

Understanding the cellular contribution to bone repair in different orthopaedic scenarios is critical to further advancements in the field. We demonstrate for the first time that muscle progenitors play a significant role in bone repair. These findings are important in the context of developing new treatments for severe fractures.

P5

A comparison of lumbar intervertebral disc height in mature and adolescent sheep following injection of a matrix degrading enzyme

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Introduction and aims: Lumbar intervertebral discs in adolescent subjects may have a greater regenerative capacity than more mature discs. Using a validated radiological measure of disc integrity (disc height index (DHI)) this study examined the influence of age on the natural capacity of the disc to regenerate following experimental disc degeneration.
Methods: In a validated animal model of human disc degeneration, lumbar discs of 30 sheep aged 12 weeks (n=15) and 3 years (n=15) were injected with Chondroitinase ABC (cABC). Saline- and non-injected discs served as controls. The progression of degeneration and possible spontaneous regeneration of discs was monitored radiologically up to 12 weeks after injection.
Results: DHI in adult sheep decreased significantly from baseline after 4 weeks in all treatments (cABC -29%, Saline -19%, Control -15%, $P<0.01$). After 12 weeks there was some recovery in the control discs and the saline-injected remained relatively the same, but the disc height of the cABC-injected discs showed progressive degeneration (-32%, $P<0.01$). In lambs the cABC-injected discs lost disc height after 4 weeks (-28%, $P<0.01$) and then recovered slightly after 12 weeks (-25%, $P<0.01$) while saline- and non-injected levels at both 4 weeks (Saline -4%, Control -4%) and 12 weeks (Saline -7%, Control -11%) did not change. DHI of cABC-injected discs decreased similarly in adults and lambs after 4 weeks, but an increase (implying regeneration) was observed in the lambs only after 12 weeks. This was less apparent in the saline- and non-injected discs.
Conclusions: The degenerative effect of cABC in adult and adolescent sheep is consistent with previous experience with this model but the developing discs of lambs have a greater regenerative capacity. This may have implications for potential treatment options involving biological-based therapies but further investigation is required.

P6

Inhibition of osteoclastogenesis in patients with traumatic brain injury: a possible contributor towards increased callus size and enhanced fracture healing

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Background: Patients with a severe traumatic brain injury (TBI) and associated fractures experience enhanced fracture healing with hypertrophic callus formation. The mechanism of this phenomenon is still unknown. However, osteogenic humoral factors have been suggested to be released from the injured brain into systemic circulation after TBI and enhance fracture healing. Although the effects of the suggested factors on osteoblasts have been reported, their effects on osteoclasts remain unstudied. Here, we investigate the effects of serum from TBI patients on osteoclastogenesis.
Methods: Serum was collected from patients with long-bone fractures with or without TBI at 24 hours post injury. The expression of osteoclast-influencing cytokines (TNF-α, IL-4, and IL-10) by activated lymphocytes was measured by incubating lymphocytes in the presence of 1.5% serum for 24 hours. Additionally, human primary osteoblasts where incubated in the presence of 2% serum for five days. The resulting conditioned medium was used to culture peripheral blood monocytes, and measure their differentiation towards osteoclasts.
Results: TBI patient serum increased the lymphocytes expression of the anti-osteoclastic cytokines (IL-4 and IL-10) but simultaneously decreased the pro-osteoclastic cytokine TNF-α ($p<0.05$). TBI patient serum also decreased TRAP expression and degree of multinucleation in the developing osteoclasts.
Conclusion: These results suggest that TBI patient serum inhibits osteoclastogenesis indirectly through the modulation of cytokines produced by immune cells, and the modulation of osteoblastic influence on osteoclast precursors.

P7

Osteomacs are pivotal for optimal intramembranous bone formation during bone healing

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Osteal tissues contain a resident macrophage population (osteomacs) that are intimately associated with physiological osteoblast bone forming surfaces. A tibial injury model was employed to investigate the role of osteomacs during *in vivo* bone formation. Osteomacs were abundant during all stages of bone repair where they coincided anatomically and kinetically with the recruitment, condensation and maturation of osteoblasts participating in intramembranous ossification. The functional contribution of osteomacs to bone formation was tested using the bone injury model combined with osteomac/macrophage depletion via the Macrophage-Fas-Induced Apoptosis transgenic mouse. Local macrophage depletion resulted in a striking reduction in F4/80$^+$ osteomacs and CT1$^+$ osteoblasts throughout injury healing. Quantitative micro-CT analysis confirmed significantly impaired mineralized woven bone deposition at 7 ($p=0.036$) and 9 ($p=0.001$) days post surgery. Delayed depletion of osteomac/macrophages indicated that failure in bone formation was not due to loss of early macrophage-mediated inflammatory events. Osteomac/macrophage depletion using clodronate liposomes also compromised bone healing. Osteoclasts are also susceptible in these depletion strategies, however osteoprotegerin treatment during bone healing had no effect on bone formation or osteomac distribution. The differentiation, proliferation and survival of macrophages are dependent on M-CSF, and its administration during bone healing resulted in a significant increase in injury site-associated F4/80$^+$ osteomacs ($p=0.002$) and new CT1$^+$ matrix deposition ($p=0.026$). Micro-CT analysis confirmed active mineralization of this woven bone matrix. Overall these results confirm that osteomacs are pivotal in supporting osteoblast mediated intramembranous bone formation and are a significant step towards establishing osteomacs as novel cellular participants in bone dynamics.

P8

Local expression of sclerostin protein is reduced adjacent to the stress fracture following ulnar loading

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Stress fractures are debilitating injuries that affect athletes and are caused by fatigue loading. However, the exact mechanisms that initiate and co-ordinate the repair of stress fractures are not completely understood. Osteocytes are terminally differentiated osteoblasts embedded in bone matrix and have been proposed as a mechano-sensor that detects bone damage and initiates a subsequent remodelling response. We hypothesized that a local decrease in sclerostin and increase in caspase-3 protein expression would be an early signalling event in the remodelling response to the stress fracture. Early phase of stress fracture repair is examined by creating a stress fracture in the right ulna of mature female wistar rats using cyclic end-loading. Rats were euthanized 1, 4 and 7 days after loading (n=5/group). Standard histological staining was used to examine stress fracture morphology and immunohistochemistry to detect the localization of these proteins along the stress fracture line. Unloaded ulnae from the control group of animals served as controls. The labelling index of sclerostin protein was significantly lower in osteocytes adjacent to the stress fracture region when compared to controls at all three time points ($P<0.001$). Additionally, the labelling index of caspase-3 was significantly elevated in the region of stress fractures at all time points compared with controls ($P<0.001$). These data reinforce the importance of osteocyte apoptosis in the healing of fatigue damage in bone, and demonstrate that local expression of sclerostin is a key signalling event for the required new bone formation.

P9

Hypophosphataemic osteomalacia in two patients on adefovir dipivoxil

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Background: Fanconi's syndrome results from generalised renal tubular toxicity and, due to phosphate wasting can cause hypophosphataemic osteomalacia. Large clinical trials advocated the safety of adefovir dipivoxil at a daily dose of 10mg, the standard dose given to patients with hepatitis B. We diagnosed Fanconi's syndrome in conjunction with severe osteomalacia in two hepatitis B-positive patients on standard-dose adefovir therapy.
Results: The first patient was a 40 year-old male with a five month history of bone pain involving his knees, ankles and ribs. He demonstrated hypophosphataemia, urinary phosphate wasting and aminoaciduria. These abnormalities resolved within weeks of discontinuation of adefovir dipivoxil and supplementation with elemental phosphate, calcium carbonate and cholecalciferol.
The second patient was a 53 year-old female with a six month history of lethargy, cachexia and generalised bone pain. She had hypo-phosphataemia, hypocalcaemia, metabolic acidosis and severe vitamin D deficiency but initially no urinary phosphate wasting. Four months of high dose cholecalciferol supplementation unmasked her Fanconi's syndrome including significant urinary phosphate wasting. The patient improved within weeks of discontinuation of adefovir and supplementation with elemental phosphate, calcium carbonate and calcitriol.
Conclusions: In spite of large clinical trials advocating the safety of adefovir dipivoxil at 10mg daily, long-term use of this agent may be nephrotoxic and in rare cases, cause Fanconi's syndrome and severe hypophosphataemic osteo-malacia. Clinicians prescribing this drug should be aware of this potential complication.

P10

Proteomic assessment of cell surface proteins of periodontal cell subsets

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Stem-cell based therapy is one of the most promising strategies for the replacement of damaged tissues caused by periodontal diseases. To isolate periodontal ligament stem/stroma cells (PDLSCs) from a heterogeneous population, a major challenge is the identification of cell surface markers that are uniquely expressed by PDLSCs that give rise to bone, cementum and periodontal ligament or Sharpe's fibres. Another resident population, the epithelial cell rests of Malassez (ERM) are the only odontogenic epithelial cells in the periodontal ligament. However, their exact function is still unknown, although there are a number of different theories such as supporting the homeostasis of periodontal ligament, maintaining periodontal ligament space and contributing to cementum formation and repair. The aim of this study was to identify unique cell surface proteins of human PDLSCs compared with human gingival fibroblasts (HGFs), as a non-mesenchymal stem cell population control and to isolate and characterise ERM cells. Two-Dimensional Fluorescence Difference Gel Electrophoresis (2D-DIGE) following live cell CyDye labelling showed some similarities between PDLSCs and HGFs surface proteome, however, PDLSCs exhibited some unique cell surface proteins. Mass spectrometry was used to identify these unique proteins. Iimmunocytochemical and flow cytometric analysis showed that ERM cells are positive for epithelial cell markers (cytokeratin 8, E cadherin, Epithelial Membrane Protein 1) but do express some mesenchymal cell associated markers (CD44, CD29, HSP90), and lack the hematopoietic cell markers (CD14, CD45) and the endothelial cell marker, CD31. Preliminary studies also suggested that ERM cells may have the potential to form mineral *in vitro*. Taken together, unique profiling of cell surface proteins may act as biomarkers to distinguish between PDLSCs and ERM cells that may play important roles in the maintenance and repair of adult periodontium.

P11

The first case report of hypophosphataemic osteomalacia secondary to deferasirox therapy and clinical audit

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A 28-year-old woman with transfusion-dependent beta-thalassemia major was referred for low bone mineral density (BMD). Routine BMD monitoring with DXA revealed a progressive decline in the preceding 3 years, with a 16%, 28% and 34% loss in her total body, lumbar spine and femoral neck BMD respectively. The patient was well and denied current bone pain although a year earlier, had transient hip pain following a snow-boarding accident. Her medical history included renal calculi, hepatitis C and diet-controlled diabetes. Current medications were vitamin D3 1000 IU daily and the iron-chelating therapy deferasirox 1g daily. Biochemical testing revealed a low serum phosphate (0.39 mmol/L) with normal renal function, serum calcium, magnesium, 25OH vitamin D and PTH. Alkaline phosphatase was 2-3 times the upper limit of normal. Tubular reabsorption of phosphate (TRP) was abnormal at 47.2% in the setting of low serum phosphate. Diffuse demineralisation was reported on thoracolumbar X-ray. A bone scan demonstrated healing fractures of the right superior and inferior pubic rami and two older rib fractures. Given a case report[1] of proximal tubular injury and acute renal insufficiency with deferasirox therapy, a urine metabolic screen was performed and demonstrated generalised mild aminoaciduria. Following cessation of deferasirox, rapid normalisation of the serum phosphate, TRP and aminoaciduria occurred and significant improvement in BMD was seen four months later. We report the clinical monitoring of this patient following the necessary reintroduction of deferasirox at reduced doses. Subsequent screening of 86 patients with beta-thalassemia major on deferasirox is planned.

1. Rafat *et al.,* Am J Kidney Dis, 2009

P12

Can craniosynostosis give a leg up to fighting other bone diseases?

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Craniosynostosis is a congenital disease affecting 1 in 2500 live births, where premature suture fusion causes cranial deformities in young children. The current treatment for this condition involves costly and invasive surgery and could benefit greatly from the development of non-surgical adjunctive therapies. Our aim is to develop therapies for the treatment of craniosynostosis that could also be applied to other bone pathologies. Using microarray technology, we have identified a number of genes differentially regulated between patent and prematurely fused human sutures (Coussens *et al.* (2007) BMC Genomics 12(8):458). We have also found these genes are expressed during murine endochondrial ossification and are currently exploring their roles in long bone biology. Using RT-qPCR, *in situ* hybridisation and immunohistochemistry we have shown that *Rbp4*, *Cors26* and *Anxa3*, and their protein products, are expressed during embryonic hind limb development. Expression of Cors26 was restricted to chondrocytic cells, although the secreted protein persisted into the fringes of the mineralizing zone. Expression of Anxa3 was restricted to a subpopulation of cells found only in the mineralizing bone matrix, while expression of Rbp4 within bone tissue was found to be restricted to certain subpopulations of chondrocytic cells that faced joint surfaces. We are currently conducting lentiviral-based trials into the overexpression and silencing of these genes and the subsequent affect on osteogenesis in mouse bone marrow stromal cells.

P13

Higher expression of ITAM-related osteoclastogenesis co-stimulatory factors in is associated with peri-implant osteolysis tissues

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Peri-implant osteolysis is thought to be caused by an inflammatory response to prosthetic wear debris that results in an increase in osteoclast activity. It has been shown that, besides the crucial RANK/RANKL/OPG axis, osteoclastogenesis also requires co-stimulation of immunoreceptor tyrosine-based activation motif (ITAM)-associated receptors like osteoclast-associated receptor (OSCAR) and triggering receptor of myeloid cells-2 (TREM2)[1]. This study aimed to investigate the levels of these ITAM-associated receptors and their respective associated adaptor molecules, Fc receptor-gamma (FcRγ) and DNAx activation protein-12kDa (DAP12) in human peri-implant tissues.

The expression of OSCAR, TREM2, FcRγ and DAP12 was detected in human tissues using immunohistochemistry. Peri-implant tissues were obtained from patients undergoing revision surgery following peri-implant loosening (n=13) and tissues from osteoarthritic patients undergoing primary joint replacement (n=12) were used as controls. Cathepsin K and tartrate-acid phophotase (TRAP) was detected as markers of osteoclast-like cells. Expression of the ITAM-associated molecules was scored using 5-scale semiquantitative analysis (SQA) grading system.

There was significantly higher expression of OSCAR (p=0.020), TREM2 (p=0.021) and their related adaptor molecules, FcRγ (p=0.002) and DAP12 (p=0.001) peri-implant tissues than osteoarthritic tissues. Expression of TREM2 and OSCAR was more exclusive to the multinucleated cells, while DAP12 and FcRγ expression were also detected by monocyte-like cells.

High expression of ITAM-associated factors in peri-implant osteolysis tissues suggests that ITAM signaling is involved in the increased osteoclast actively that causes osteolysis and implant loosening. Regulation of the molecules may provide therapeutical approach for attenuating peri-implant osteolysis.

1. Koga T Nature (2004)

P14

Bone cell abnormalities *in vitro* from a patient with a rare bone condition

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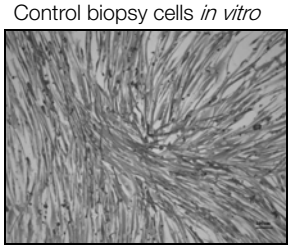
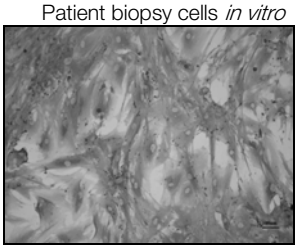
Case report: A 22y old male Caucasian student complained of pain in knees and ankles – becoming more constant after 2.5 years of migratory joint pain after viral infection. There was no joint swelling however C-reactive protein (CRP) was elevated, complement was low and an IgG paraprotein was identified. There was no evidence of known viral/parasitic infections. Recent back pain was associated with loss of lordosis and multiple spine fractures. X-rays and DEXA showed osteoporosis (T score spine <5) but metaphyseal and epiphyseal sclerosis, increased uptake in these areas on bone scan and raised tartrate-resistent acid phosphatase (TRACP). Bone marrow trephine showed absence of lamellar collagen in some areas of osteoid. Bone cells were grown from biopsies taken with informed consent from the distal femur of this patient and from a control 19y subject undergoing elective knee surgery. When established in culture, morphological differences between control and patient's cells became evident, including relative disorganization of the patient's cells (images below). The control cells were similar in appearance to the human osteoblast cells regularly used by us. Preliminary studies showed 50-100 fold lower proliferation rates, assessed by thymidine incorporation, in the patient's cells compared to control. Furthermore, the patient's cells exhibited blunted or paradoxical responses to a variety of bone active agents, including benzoylbenzoyl-ATP (BzATP), and 1α,25 dihydroxyvitamin D. These studies indicate a primary problem in the patient's osteoblasts, but the nature of this problem remains to be elucidated.

Control biopsy cells *in vitro*



Patient biopsy cells *in vitro*



P15

Longitudinal studies of bone health during antiepileptic drug (AED) therapy

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Background: Antiepileptic drugs (AEDs) have been implicated as a cause of bone disease since the 1960s. However, there are limited longitudinal data available to assess the long term effects of AED therapy on bone health.
Objective: To conduct a longitudinal cohort study investigating long term effects of AED therapy on bone measures in twin/sibling pairs.
Methods: Twin/sibling pairs discordant for AED treatment with baseline and follow-up measurements (2 or more years apart) of dual energy X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT) were included. Areal bone mineral density (BMD) was measured at the lumbar spine (LS), total hip (TH), femoral neck (FN), and total forearm (FA). Data were adjusted for age, height and weight. Annualized rates of change in bone measurements and their mean within-pair differences (MWPD) were calculated.
Results: In 19 AED-discordant twin/sibling pairs, the follow-up median interval was 2.4 years (IQR= 1.00) and did not differ between AED users and non-users. There was a significant difference in the rate of decline in TH BMD with a MWPD of -0.81% per year (SD: 1.381, p= 0.024) in AED users vs non-users. No other significant difference was found in the rate of change in bone mineral measures.
Conclusion: There was an accelerated rate of decline in hip BMD in AED users which may help explain their reported increase in fracture risk. The sample size was relatively small and recruitment is ongoing. Further longitudinal investigation including balance and pQCT measurements will provide additional insight into AED-associated bone disease.

P16

Dietary Emu Oil supplementation may suppress chemotherapy-induced formation of bone-resorptive cells osteoclasts and prevent bone loss

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Cancer chemotherapy is known to cause bone defects (osteoporosis and fracture) in cancer patients and survivors. Currently no supplementary treatments are clinically available that can be used to protect the bone from being damaged by chemotherapy. Using rat models, we have previously shown that chemotherapy drugs can increase expression of pro-inflammatory cytokines, enhance formation of bone-resorptive cells osteoclasts and cause bone loss. In this study, using a rat model of acute chemotherapy with one-bolus injected dose of 5-Fluorouracil (5-FU), we examined potential protective effects of dietary supplementation with emu oil, which is known to possess a potent anti-inflammatory property and has been widely used by the Australian aboriginals to treat arthritis and joint pains.
Adult female Dark Agouti rats were given 1ml/day of emu oil or water by oral gavage. After 5 days pre-treatment, rats were given one bolus subcutaneous injection of 5-FU (150mg/kg) and continued to receive oral emu oil or water gavage treatment. Third, fourth, fifth and sixth day after 5-FU treatment, bone specimens were collected. Histological staining (H&E) reveal no significant morphological changes in the growth plate. 5-FU caused a significant reduction in the metaphyseal bone volume. It significantly increased the densities of $TRAP^+$ bone resorbing cells namely chondroclasts (along the growth plate cartilage and metaphyseal bone transitional zone) and osteoclasts (on metaphyseal trabecular bone surface), and elevated the number of osteoclasts formed ex vivo from cultured bone marrow cells of treated rats compared to the control. Supplementary treatment with Emu Oil prevented this 5-FU-induced bone loss and the induction of chondroclasts and osteoclasts. In line with the histological and ex vivo osteoclastogenesis findings, gene expression studies using quantitative Real Time RT-PCR confirmed the inhibitory effect of emu oil on osteoclasts as emu oil treatment suppressed 5-FU-induced expression of osteoclastogenic markers, namely receptor activator of nuclear factor kappa B (RANK) and osteoclast associated receptor (OSCAR). Therefore, the described ability of emu oil to suppress the chemotherapy-induced osteoclast formation and to preserve the trabecular bone volume suggests that supplementary treatment of Emu Oil may be potentially useful in protecting bone and in preventing 5-FU chemotherapy-induced bone loss.

P17

Errors in self reported myocardial infarction in calcium intervention studies

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Aims of the study: Concern has been expressed that calcium supplementation, may increase the risk of myocardial infarction (MI). To evaluate the risk further an examination of self reported and verified myocardial infarction hospitalisation and mortality data from a 5-year RCT of calcium carbonate was undertaken.
Methods: The participants were 1,460 postmenopausal women, recruited from the general population and randomised to receive 1,200 mg of calcium carbonate daily or an identical placebo for 5 years. Self reported adverse events were recorded by the patient and entered into a database devised to classify self reported data. These data were then compared to verified hospital admission and mortality data from the Western Australian Data Linkage System (WADLS).
Results: During the 5-year RCT 38 individuals self reported MI (21 calcium vs. 17 placebo groups) of which only 68% were verified. Of patients misreporting MI (10) 70% was in the calcium group compared to 30% in the placebo group. In the calcium group 16% of the self reported MI had intestinal disorders as the adjudicated discharge diagnosis versus none in the placebo group. Furthermore, in the calcium group there were approximately twice the hospital admissions for acute abdominal pain compared to the placebo group (29 and 16), P = 0.049.
Conclusion: These data show that calcium supplementation increases the risk of verified acute intestinal disorders. Furthermore it identifies misclassification of myocardial infarction events by patients as the basis for the apparent increase in the risks of myocardial infarction in some studies.

P18

Patient recruitment strategies for RCT's

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Aim: Patient recruitment for population based research studies is getting increasingly difficult. We compared 4 recruitment techniques for an RCT of vitamin D supplementation.
Methods Women over 70 were recruited from the ambulant population to receive either vitamin D3 150,000 IU by mouth every 3 months or an identical placebo for the nine months. The recruitment techniques trialled were contacting GP practice patients, individuals on the electoral roll, individuals living in life style villages and local newspaper advertisements.
Results 198 recruitment letters were sent to GP's 7 of whom expressed interest and 5 of whom were able to provide the addresses of 776 patients who were sent a letter. 236 (30%) replied of whom 199 (25%) were eligible to attend a clinic visit. Electoral roll letters were sent to 10500 individuals of whom 1862 (17%) replied and 637 (6%) were eligible to attend a clinic visit. Recruitment flyers were sent to 4 life style villages comprising 237 residents, 8 replies were received, 7 were eligible. I local newspaper advertisement resulted in 1 eligible patient replying.
Conclusion: The principal problem with the use of GP practice lists was the poor response rate from the GP's perhaps linked to the low payment available to cover their costs ($10 per patient). The electoral role had a lower response rate and eligibility to the unsolicited letter but because of the larger pool available was more successful. Local newspaper and lifestyle village recruitment methods were not successful.

P19

Persistence to oral osteoporosis therapy in Australia

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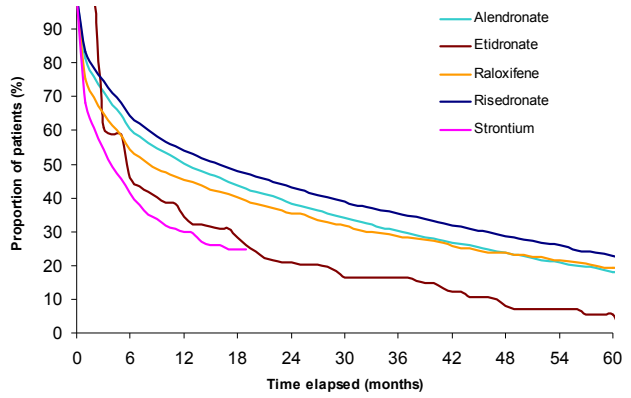
Persistence to osteoporosis therapy in Australia was last described in 2006. The aim of this study was to provide an updated analysis which includes strontium (PBS listed in April 2007). Men and women from a random 10% sample of patients in the Medicare Australia database were included in this retrospective analysis if they initiated alendronate, risedronate, etidronate, strontium or raloxifene between 1 June 2003 and 30 September 2008. Initiation was defined as a 12-month pre-period without osteoporosis medication. Patients were deemed non-persistent if there were three consecutive calendar months in the follow-up period (up to end 2008) without a filled prescription. Kaplan-Meier curves were generated based on persistence to the first medication taken and also to "osteoporosis therapy" (i.e. allowing for medication changes). Data from 35,143 patients (77% female, mean age = 72) starting therapy with alendronate (57%), risedronate (34%), raloxifene (4%), strontium (4%) or etidronate (1%) were analysed. Cessation of all treatments was greatest in the first 12 months with a relatively steady decline thereafter (Figure 1). Persistence to risedronate, alendronate and raloxifene was similar. The lowest level of persistence was seen with strontium (30% at 1 year). Persistence to "osteoporosis therapy" was 5-10% higher than persistence to the first medication indicating a small proportion of patients switch to an alternative medication. This analysis shows that persistence to osteoporosis therapy in Australia remains a significant problem despite the seriousness of the condition. Medication changes are uncommon and the introduction of strontium has not improved persistence.

Figure 1: Kaplan-Meier curves: persistence to initiation medication



Note: Strontium was PBS listed on 1 April 2007 which limits the duration of follow-up

P20

Geographical variation in bone density in South Australia

Wormald JL and Schultz CG

Normal ranges for BMD have assumed age & race as a variable but not rural and metropolitan addresses. We compared anthropometric data, age and the T & Z scores of patients scanned in metropolitan Adelaide and rural South Australia over an 8 year period.

Results of the first scan (to minimise the effect of treatment) of the lumbar spine (L2-L4) and total femur of each patient from a metropolitan GE-Lunar Prodigy and mobile GE-Lunar DPX-MD were analysed.

Data were stratified into Australian Bureau of Statistics statistical subdivisions based on patient postcode and comparisons between rural and metropolitan patients made using a Student's t-test.

	Female		Male	
	Rural			
	Spine	Femur	Spine	Femur
T score	-0.59±1.7*	-0.57±1.3	-0.42±1.9*	-0.74±1.2*
Z score	0.37±1.6*	0.27±1.1*	-0.07±1.9	-0.02±1.1*
Height	157.50±17.4*	157.49±17.2*	170.32±13.2*	170.20±13.3*
Weight	71.28±16.6*	71.43±16.6*	82.24±15.6*	81.95±15.8*
Age	65.28±11.2*	62.95±11.4*	66.53±12.2*	67.17±12.1*
Total patients	13155	13963	1428	1631
	Metropolitan			
	Spine	Femur	Spine	Femur
T score	-0.64±1.6	-0.55±1.2	-0.57±1.9	-0.87±1.3
Z score	0.26±1.5	0.21±1.0	-0.17±1.9	-0.20±1.2
Height	159.95±7.3	160.00±7.0	170.99±9.9	171.85±7.9
Weight	69.99±14.9	70.37±14.8	78.71±16.2	80.23±14.6
Age	59.24±12.4	59.77±11.6	59.16±18.5	62.04±15.4
Total patients	12583	13093	1683	1737

*$p < 0.05$

Rural patients are shorter and heavier but at most sites (apart from the female femur T score and the male spine Z score) had significantly higher BMD.

One might expect that in an older population the BMD would be lower. However in our rural patients the BMD is higher, and we speculate that this may be related to differences in diet, work patterns & sun exposure.

P21

Socioeconomic status and bone health in older men: The CHAMP study

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Aims: The relationship between socioeconomic status (SES) and bone health is controversial. The objective of this study was to investigate associations of SES with bone health in community-dwelling men aged 70years+ who participated in the baseline phase of the Concord Health in Ageing Men Project (CHAMP).

Methods: The Australian Socioeconomic Index 2006 (AUSEI06) based on the Australian and New Zealand Standard Classification of Occupations was used to determine SES in 1705 men. Bone mineral density (BMD) and bone mineral content (BMC) were determined by DEXA. Bone-related biochemical and hormonal parameters, including markers of bone turnover, parathyroid hormone and vitamin D were measured in all men.

Results: General linear models adjusted for age, weight, height, and bone area revealed no significant differences across crude AUSEI06 score quintiles for BMC at any skeletal site or for any of the bone-related biochemical measures. However, multivariate regression models revealed that in Australian-born men, marital status was a predictor of higher lumbar BMC (β=0.07, p=0.002), higher total body BMC (β=0.05, p=0.03) and lower urinary NTX-I levels (β=-0.08, p=0.03), while living alone was associated with lower BMC at the lumbar spine (β=-0.05, p=0.04) and higher urinary NTX-I levels (β=-0.07, p=0.04). Marital status was also a predictor of higher total body BMC (β=0.14, p=0.003) in immigrants from southeastern and eastern Europe.

Conclusions: Although crude SES scores were not significantly associated with bone health in older Australian men, marital status, living circumstances and acculturation were predictors of bone health in both Australia-born men and European immigrants.

P22

A cultural models approach to osteoporosis prevention and treatment

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The study aimed to explore barriers to treatment and prevention of osteoporosis. One objective was to identify cultural models of osteoporosis shared by community dwelling older women and their implications for social marketing campaigns. Cultural models are mental constructs about specific domains in everyday life, such as health and illness, which are shared within a community.

Case studies were developed using in-depth interviews with four women aged 75+ years who had recently experienced fracture. Cultural models were identified by applying a 'constant comparative' method of analysis of audio-transcripts.

Seven response domains were identified. Perceptions shared among participants across these domains included: osteoporosis is, to some degree, self-inflicted; osteoporosis is not a serious disease; osteoporosis happens to other people; a balanced lifestyle is sufficient to prevent and manage osteoporosis; having a diagnosis of osteoporosis is not sufficient to motivate behavioural change; and confusion about the visibility of a largely symptomless disease. There were (contradictory) beliefs about the cause/s of osteoporosis. Together, these domains make up a cultural model of 'low salience': that osteoporosis has low prominence, particularly when ranked against such threats to health as arthritis, cancer and heart disease. Social marketing messages were inconsistent, some promoting the consumption of functional foods and supplements for overall good health and others using fear-based strategies to encourage behavioural change.

Cultural cues embedded in marketing messages may be internalised and motivating in unintended ways. Our study highlights the value in identifying and understanding cultural models, to better inform development of health messages on osteoporosis.

P23

Development of a Model of Care for Osteoporosis in Western Australia utilising Health Networks

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Osteoporotic fractures represent a significant and escalating personal and societal burden. Although effective treatments are available, optimal management and effective primary and secondary prevention are infrequently achieved, due to inadequate uptake of best-practice management, poor adherence to clinical guidelines, and health system inefficiencies. To address these issues, Health Networks WA is developing a Model of Care for Osteoporosis. The goal of a Model of Care is to describe how the right care should be delivered at the right time, by the right team, in the right place. Strategies will be developed for State-wide reform in delivery of health services for Western Australians with osteoporosis and those at risk of developing the condition to meet the following objectives:

- Consistent evidence-based care will be delivered across Western Australia.
- The level of care will be appropriate to the complexity of the individual patient.
- Health promotion, disease prevention and treatment will be delivered to the appropriate people at the appropriate time.
- The role of the patient/carer in the management of osteoporosis will be recognized.
- A multidisciplinary team will provide coordinated and integrated care across the continuum of primary, secondary and tertiary services.
- There will be adequate workforce capacity to meet demand.
- The location of care will be appropriate to the complexity of the patient to decrease inappropriate use of tertiary facilities.
- Communication systems will enable adequate sharing of information between health professionals and patients.
- Resources will be adequate and coordinated across the continuum of care.

P24

The utility of bone health to predict mortality in older women

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Low bone mineral density (BMD) and bone loss predict mortality in older women (1, 2). As Medicare subsidises BMD tests for all people aged ≥ 70 yr, we investigated the utility of bone measurements to identify older women at increased risk of mortality.

In a prospective study, 169 women, aged 70-79 yr, were followed up biennially from 1994. BMD was measured using dual energy X-ray absorptiometry. Weight and mobility were recorded at 6 yr follow-up and mortality until Dec 2006 was confirmed using the deaths registry. Bone measurements were converted to standard deviations (SD) from the mean for the sample. Using Cox proportional hazard models, hazard ratios (HR) for mortality were estimated. Associations are expressed as HR and 95% CI (HR, 95% CI) for each SD decrease in bone measurement.

There were 25 deaths in total. Six yr BMD at any site did not predict mortality. Analysis of bone loss at the total body and femoral neck showed a trend towards an association (1.47, 1.01-2.14, p=0.05 and 1.39, 0.99-1.96, p=0.06, respectively) that did not remain after adjusting for change in weight and mobility. After adjustments, bone loss at the mid forearm (MF) decreased mortality risk (0.62; 0.42-0.92, p=0.02) and there was a similar trend with bone loss at the ultradistal forearm (0.74, 0.50-1.07, p=0.11).

These results indicate that an increase in bone at the MF may predict premature death in older women. However, reasons for this unexpected finding need to be identified before care practices for the elderly are modified.

1. Browner WS. Non-trauma mortality in elderly women with low bone mineral density. The Lancet. 1991;338:355.
2. Nguyen ND, Center JR, Eisman JA, Nguyen TV. Bone Loss, Weight Loss, and Weight Fluctuation Predict Mortality Risk in Elderly Men and Women. J Bone Miner Res. 2007;22(8):1147-54.

P25

Targeted exercise against osteoporosis: a systematic review and meta-analysis of exercise RCTs for optimising bone strength throughout life

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Exercise is regarded as an important strategy to improve bone density, but its effects of whole bone strength remain unclear. This meta-analysis aimed to provide an update on the current state of knowledge regarding the effects of long-term supervised exercise (≥6-months) on estimates of lower extremity bone strength from childhood to older age. We identified 10 RCTs, which assessed the effects of exercise on bone strength using (p)QCT, MRI or DXA HSA. Our analysis did not find any significant exercise effects among pre- and early- pubertal girls (standardised mean difference, -0.01 (95% CI -0.18 to 0.17)) or boys (0.10 (-0.04 to 0.25)), adolescent girls (0.21 (-0.53 to 0.97)) or boys (0.10 (-0.75 to 0.95)), premenopausal women (0.00 (-0.43 to 0.44)), or postmenopausal women (0.00 (-0.15 to 0.15)). This is likely due to the heterogeneity among the studies; some trials were short in duration, small in sample size, and the recommended weekly training doses varied considerably. Nevertheless, per protocol analyses of individual trials in children and adolescents indicated that programmes incorporating regular weight-bearing exercise can result in 1-8% improvements in bone strength at the loaded sites. In premenopausal women with high exercise compliance, improvements ranging from 0.5 to 2.5% have been reported. In conclusion, the findings from our meta-analysis of the limited number of RCTs available indicate that exercise does not significantly enhance bone strength in children or adults. However, there is some evidence for a beneficial effect of diverse weight-bearing impact activities on bone strength during the pre-pubertal period.

P26

Inverse association between hip fracture and social disadvantage: Geelong Osteoporosis Study

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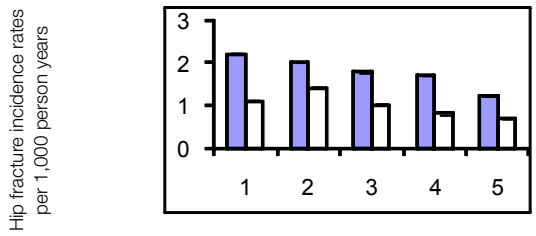
Aims: Hip fractures pose a significant public burden on society. Adding to this burden is the inverse association between socioeconomic status (SES) and most causes of morbidity. However the association between quintiles of area-based SES and incident hip fractures has not been examined in Australia. In a comprehensive register of hip fractures for the entire Barwon Statistical Division (BSD), we assessed the association between SES and hip fractures over a two-year period.
Methods: Incident hip fractures during 2006-7 were identified using a computerised keyword search of radiological reports from all the radiological centres serving the BSD. Radiology reports were cross-referenced with hospital medical records. Pathological fractures were excluded (n=9). SES was determined by cross-referencing residential addresses with Australian Bureau of Statistics census data, and categorized in quintiles based upon the BSD reference range. Hip fractures in each quintile and within each age strata for the entire BSD region were compared using chi-square test of independence, and rates defined as fractures per 1,000 person years (p-y).
Results: During 2006-7, 519 hip fractures (343 female) were sustained. An inverse association was observed between SES and fracture incidence (Figure), with differences observed between SES quintiles for both females (**p=0.005, solid fill) and males (*p=0.007).
Conclusion: The inverse association between incident hip fractures and quintiles of area-based SES provides unequivocal evidence that those of greater social disadvantage should be a specific target population for intervention to reduce the burden of hip fracture.



P27

Bone mineral density is positively associated with knee cartilage volume in healthy, asymptomatic adult females

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The association between osteoporosis and osteoarthritis(OA) is controversial. Whilst previous studies have shown BMD and cartilage volume to be positively associated, the relationship between site-specific measures of BMD and other knee structures is unknown. We examined the associations between BMD at eight skeletal sites, and the outcome of properties of knee structure in asymptomatic young to middle-aged females without any clinical signs of OA.

Magnetic resonance imaging of the knee was performed on 142 asymptomatic females(aged 35-49yr). BMD was measured at the spine, hip, total body and forearms by dual energy x-ray absorptiometry, and at the calcaneus by quantitative ultrasound. BMD was tested for an association with cartilage volume, defects, and bone marrow lesions(BMLs). Models were adjusted for age, bone area and BMI.

Medial cartilage volume was positively associated with BMD at the total body, femoral neck, and Ward's triangle(all $p<0.04$); with non-significant associations in the same direction at the spine($p=0.06$) and trochanter($p=0.08$). Associations were similar in the lateral compartment. Medial cartilage defects were associated with BMD at the spine($p=0.04$), with non-significant associations at the total body and femoral neck(both $p=0.10$). BMD was not associated with lateral cartilage defects or BMLs. No associations were observed with calcaneus BMD.

Site-specific BMD is associated with cartilage volume in asymptomatic young to middle-aged adults. Cartilage defects, an earlier stage of disease process than loss of volume, showed a trend of association. These data suggest that the association between BMD and knee structures may vary at different stages in the pathogenesis of OA.

P28

Clinical utility of combined femoral neck and lumbar spine bone mineral density measurements in the individualized prognosis of fracture

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The present study sought to exam the additional contribution of LSBMD to the prediction of fracture.

The Dubbo Osteoporosis Epidemiology Study was designed as a community-based prospective study, with 1358 women and 858 men aged 60+ years as at 1989. Baseline measurements included FNBMD and LSBMD, and a history of fractures and falls. The Cox's proportional hazards model was used.

During the follow-up period, 426 women and 149 men sustained a low-trauma fracture. There were 77 first hip fractures in women and 26 in men. LSBMD was not a significant and independent predictor in the presence of FNBMD. The area under the ROC (AUC) of the model including FNBMD, age, prior fracture and falls was 0.75; when LSBMD was added to the model the AUC was still 0.75. For hip fracture, the AUC value of the model with FNBMD and the model combined FNBMD and LSBMD were the same of 0.85. When the lower value of either LSBMD or FNBMD was used in the model, these results remained unchanged. Further re-classification analysis revealed that while the use of lower LSBMD did not improve the AUC over and above that of FNBMD, value of LSBMD was useful in ruling-out fracture (i.e., increase specificity by ~17% to 38%).

In conclusion, although lumbar spine BMD may not be an independent predictor of fracture risk, its use in conjunction with femoral neck BMD could be useful for ruling out fracture cases. This "ruling-out" utility can be very valuable in the individualization of fracture prognosis.

P29

Relationship between low BMD and fracture in mortality risk: an 18-year prospective study from Dubbo Osteoporosis Epidemiology Study

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Osteoporotic fractures and low bone mineral density (BMD) increase mortality risk. It is unclear whether low BMD has a direct effect on mortality or whether its contribution is through increased fracture risk. This study examined BMD, fracture and mortality risk in women and men participating in the Dubbo Osteoporosis Epidemiology Study (April 1989-May2007).
Fracture and mortality data recorded from 1164 women and 819 men. Co-morbidities, BMD, muscle strength collected 2-yearly. Cox proportional hazards models used to determine mortality risk.
There were 359 fractures in women and 129 in men, 435 deaths in women and 386 in men over 15 (IQR: 9-17) and 14 years (IQR: 7-16). Fracture event independently predicted mortality risk in both genders in multivariate models [HRs, women 1.85 (1.48, 2.31) and men 2.30 (1.70, 3.11)]. Low BMD predicted mortality risk in both genders [HRs, women 1.17 (1.01, 1.37) and men 1.22 (1.07, 1.38)], independent of age and co-morbidities. However, when fracture was included in this model, in women there was an interaction between fracture and BMD, such that BMD was only significant in those with osteoporosis, and in men, the effect of BMD was attenuated.
Osteoporotic fracture increased mortality risk independent of BMD and co-morbidities in both genders. Low BMD was associated with mortality, but its effect was modified by fracture, particularly in women. In men BMD was not consistently a mortality predictor in the presence of fracture suggesting that its effect maybe through increasing fracture events. This suggests a complex relationship between BMD and mortality.

P30

Osteoporosis medication and reduced mortality risk in elderly women and men: an 18-year prospective study from the Dubbo Osteoporosis Epidemiology Study

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Antiresorptive treatment reduces re-fracture but its role in mortality is unclear. This study examined the effect of osteoporosis treatment [bisphosphonates (BP), hormone therapy (HT) and calcium ± vitamin D only (CaD)] on mortality risk.
Fracture and mortality data were collected from 1223 women and 898 men aged 60+ from the Dubbo Osteoporosis Epidemiology Study (April 1989-May 2007). Co-morbidities and medication obtained 2-yearly. Bone loss was the difference between first and last BMDs, and for fractures, was measured post-fracture. Cox proportional hazards models used to determine mortality risk.
There were 325 (BP, n=106; HT, n=77, CaD, n=142) women and 37 men (BP, n=15; CaD, n=22) on treatment. In multivariate analyses, mortality risk was reduced for BP [HR: 0.3 (0.2, 0.6)] but not for HT [HR: 0.8 (0.4, 1.8)] and CaD [HR: 0.99 (0.76, 1.31)] in women compared with non-treated individuals. In men, mortality risk was non-significantly lower for BP [HR 0.48 (0.11, 1.98)] and CaD [HR 0.82 (0.39, 1.74)].
The relationship between bone loss and BP was explored in 65 subjects on BPs, and 1285 non-treated individuals with at least two BMDs. Bone loss was not significantly lower for BP than non-treated (-0.44g/cm^2 vs -0.63g/cm^2; p= 0.37. Notably, the effect size of BP on mortality risk was increased by 20% in the presence of bone loss. This effect was driven by those on both BPs and CaD where bone loss was significantly less and fewer deaths occurred.
Osteoporosis therapy appears to reduce mortality risk, possibly through a reduction in bone loss.

P31

Determinants of bone mass in smokers: A study in smoking-discordant twin pairs

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We investigated the effects of smoking, a major risk for fracture, on bone in same-sex, smoking-discordant twin pairs [13 male and 56 female; age 40-76 (mean±SD 53±8.9) years]. Within-pair differences (WPD; smoking twin - non-smoking twin) were calculated. Stepwise forward regression (adjusted R2 [adjR2]) was used to identify predictors of WPD in bone mineral density/bone mineral content (BMD/BMC).
Smokers and non-smokers did not differ in lifestyle factors or height (premenopausal and postmenopausal female, male subgroups). Height-weight-adjusted total hip (TH) BMD and whole body (WB) BMC were lower in all smoker sub-groups. Among females, serum DHEAS was higher ($p<0.02$) and 25–hydroxyvitamin D lower ($p<0.05$) in smokers; lean mass (LM) was the strongest predictor of WPD in BMD/BMC at all sites (adjR2 12–41%; $p<0.005$). LM was the strongest predictor of WB BMC in males; fat mass (FM) or FM + PTH was the strongest predictor of lumbar spine (LS); and leptin for TH. In females, LM predicted 26–60% of variation in BMC/BMD WPD pre-menopause, with FSH included in a second model; FM was not an independent predictor. Hormones had stronger associations with BMD post menopause: FSH was the strongest predictor of LS (32%); estradiol (40%)/estradiol + LM (59%) for TH; LM (39%)/LM + FSH (52%) for WB.
BMD determinants differed in male and female smokers. Post menopause, LM, estradiol and FSH were key BMD/BMC predictors with LM and FSH being important pre menopause. FM and possibly leptin and PTH were significant influences in male smokers.

P32

Table tennis activity is positively associated with bone mineral measures in older Asian Australian women and men

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Background: Exercise is associated with higher peak bone mass, preservation of bone mineral density (BMD) in older people and may reduce fracture risk.
Aim: To investigate the associations of regular table tennis (TT) activity in elderly Asian Australians with bone mineral measures by comparing body composition and BMD.
Methods: 112 healthy Asian descent women and men, aged 50-85y who were regular TT players (>1 hour/week for over 1 year) and non-players (no regular recreational activity) were recruited in 2006-2010. Dual-energy X-ray absorptiometry (DXA) was performed to measure total body (TB) BMC, fat mass (FM), lean mass (LM), % body fat (%BF), lumbar spine (LS), total hip (TH), and femoral neck (FN) BMD.
Results:

DXA parameters adjusted for age, weight, and height	Male players (N=33) vs male non-players (N=19)		Female players (N=31) vs female non-players (N=29)	
	Mean difference (%)	p	Mean difference (%)	p
TB BMC (g)	171.2 (8.1)	0.003	122.4 (7.8)	0.005
FM (g)	-1722.7 (-10.1)	0.004	-425.8 (-2.2)	0.282
LM (g)	1707.5 (3.5)	0.021	272.9 (0.8)	0.471
%BF	-2.717 (-10.8)	0.002	-0.596 (-1.8)	0.408
LS BMD (g/cm^2)	0.071 (7.0)	0.129	0.074 (8.8)	0.015
TH BMD (g/cm^2)	0.057 (6.2)	0.025	0.066 (9.1)	0.001
FN BMD (g/cm^2)	0.078 (10.4)	0.001	0.064 (9.9)	<0.001

TT players had higher adjusted bone mineral measures than non-players, while male players had higher LM and lower FM and %BF than male non-players.
Conclusion: TT activity in older people is associated with favourable differences in bone mass and soft tissue composition.

P33

Pleiotropic genetic effects contribute to the correlation between bone mineral density and quantitative ultrasound measurements

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Both bone mineral density (BMD) and quantitative ultrasound (QUS) parameters of bone are under genetic influence. Whilst the two are correlated, whether this is due to genetic or environmental influences is not known.
This study involved 622 individuals aged 18+ from 33 multigenerational families recruited from the Dubbo Osteoporosis Genetics Study. BMD at the femoral neck (FNBMD), lumbar spine (LSBMD) and total body (TBBMD) was measured by DXA (GE-Lunar Corp). Speed of sound (SOS) at the distal radius (DRSOS), midshaft of tibia (MTSOS) and proximal phalanges (PPSOS) was measured by Sunlight Omnisense. Heritability (h^2) of these parameters was estimated using the variance components model implemented in SOLAR, using age, sex and anthropometric variables as covariates. The genetic correlation, a measure of pleiotropy, between each of the traits was estimated using bivariate analysis.
Heritability was higher for BMD [FNBMD: 0.42 (standard error: 0.09), LSBMD: 0.59 (0.07), TBBMD: 0.54 (0.14)] than for QUS parameters [DRSOS: 0.21 (0.09), MTSOS: 0.39 (0.12), PPSOS: 0.23 (0.12)]. A strong genetic correlation between BMD measurements was found [FNBMD-LSBMD: 0.55 (0.10), FNBMD-TBBMD: 0.77 (0.06), and LSBMD-TBBMD: 0.81 (0.09)]. Whilst a genetic correlation between DRSOS and FNBMD (0.52 (0.22)) and LSBMD (0.40 (0.17)) was found, no statistically significant correlations were found in other QUS measurements (0.08 to 0.40; $p>0.05$). Furthermore, a modest genetic correlation was observed between total fat mass and LSBMD: 0.38 (0.17).
The presence of genetic correlation provides the rationale for multivariate analyses to identify novel genetic loci with pleiotropic effects on these bone traits.

P34

BMD in a cohort of women a decade apart: Geelong Osteoporosis Study

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Treatment regimes for osteoporosis have changed over the past decade. Publicity campaigns suggest that a healthy lifestyle may improve the quality of bone. The aim of this study was to assess how BMD in the population in general has changed over the past decade.
The GOS recruited an age-stratified random population-based sample of women (n=1,494, 20-96yr) from the years 1994-7 and has followed them biennially until the 10yr follow-up (2004-8, participation 82%). An additional group of 20yr olds was recruited (n=221) in 2004-8. BMD was measured at the spine, femoral neck, whole body and ultradistal forearm using a Lunar densitometer. Regression techniques assessed any temporal shift in BMD within pre- (<35yr) and post- (≥35yr) peak BMD groups after adjusting for age, weight and height.
Pre- Peak BMD
BMD was increased for 2004-8 at the whole body (1.0%, p=0.04) and at the forearm (9.1%, p<0.01) in obese women only. No significant difference was found at the spine, femoral neck or in normal weight individuals at the forearm.
Post- Peak BMD
As a cohort adjusted BMD was increased at the 2004-8 visit, at the spine (by 2.8%, p<0.01), whole body (1.5%, p<0.01) and forearm (2.0%, p=0.01) but not the femoral neck.

Mild increases in BMD independent of weight and height suggest an improvement in bone health among older women and might contribute to the recently reported reduction in hip fractures. Geelong Osteoporosis Study will soon commence their 15yr follow up recruitment and further assessment of BMD over time will be informative.

P35

The neglected fracture sites: non-hip and non-vertebral fractures

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While hip and vertebral fractures receive the most attention in the literature and are the targeted sites for fracture prevention, it is the non-hip non-vertebral sites that are responsible for the largest absolute number of fractures. In this study we compared characteristics of non-hip non-vertebral fractures (NHNVF) to those who sustained a hip fracture (HF) or vertebral fracture (VF).
Men and women who sustained a fracture were identified from radiological reports of the practices that service the Barwon Statistical Division, surrounding Geelong. NHNVF sites included the ribs, pelvis, humerus, forearm, wrist, upper leg and lower leg (*men,* n=115 and *women*, n=243) and for comparison, HF (n= 41 and n=65) and VF (n=57 and n=116) after a minimal trauma event. Age, weight, and height were recorded. BMD was measured at the spine, femoral neck, total body and ultradistal forearm sites.

	VF	HF	NHNVF
age (yr)	*men* 70.0 (59.5-81.0) *women* 71.0(64.3-76.8)	78.0 (66.0-83.0) 78.0 (73.5-85.0)	60.0 (41.0-74.0) 68.3 (56.5-75.0)
weight (kg)	*men* 77.9 ± 1.9 [ab] *women* 63.7 ± 1.1 [a]	70.6 ± 2.2 [a] 59.5 ± 1.4 [a]	82.9 ± 1.3 [b] 69.7 ± 0.9 [b]
height (cm)	*men* 171.2 ± 1.0 [a] *women* 156.6 ± 0.6 [a]	171.8 ± 1.2 [a] 157.4 ± 0.8 [b]	175.5 ± 0.8 [b] 159.2 ± 0.4 [b]
BMD spine	*men* 1.186 ± 0.024 [a] *women* 0.918 ± 0.016 [a]	1.163 ± 0.029 [a] 1.036 ± 0.021 [b]	1.218 ± 0.017 [a] 1.015 ± 0.011 [b]
femoral neck	*men* 0.911 ± 0.018 [b] *women* 0.762 ± 0.012 [a]	0.854 ± 0.022 [a] 0.761 ± 0.017 [a]	0.909 ± 0.012 [b] 0.783 ± 0.008 [a]
total body	*men* 1.181 ± 0.012 [b] *women* 0.992 ± 0.008 [a]	1.121 ± 0.014 [a] 1.035 ± 0.012 [b]	1.169 ± 0.008 [b] 1.024 ± 0.006 [b]
ultradistal forearm	*men* 0.383 ± 0.008 [b] *women* 0.247 ± 0.005 [a]	0.353 ± 0.011 [a] 0.267 ± 0.007 [b]	0.372 ± 0.006 [ab] 0.256 ± 0.004 [ab]

median (IQR) or mean ± SE, alike superscripts = not significantly different, BMD adjusted for age, weight and height,

Those sustaining a NHNVF appear to be younger, heavier than those sustaining a HF and taller than those sustaining a VF but have comparable BMD at most sites. This data suggest that risk factors for NHNVF may be different to those for HF and VF. The high proportion of NHNVF sustained in the community is a neglected clinical issue and targeting osteoporosis assessment to those only with a lighter frame or reduced height may overlook those at risk of these fractures.

P36

Association between beta-blocker use and bone loss: the Dubbo Osteoporosis Epidemiology Study

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Aim: To examine the association between BB use and bone loss in elderly men and women.

Materials and methods: The study was undertaken as part of the on-going Dubbo Osteoporosis Epidemiology Study, which involved 1403 participants (492 men), whose bone health has been continuously monitored since 1989. Individuals with at least three BMD measurements (GE-LUNAR Corp, Madison, WI) were included in the analysis. Use of beta-blockers was ascertained by direct interview and verification of medication records. The association between BB use and bone loss was analyzed by a mixed-effects model, with adjustment for potential effects of participants' characteristics.

Results: Eighty six (17%) men and 148 (16%) women had been on BB before the first BMD measurement. BB users had higher lumbar spine BMD and greater body weight than non-users. In men, BB use was not significantly associated with bone loss at the femoral neck (-0.44 versus -0.14 %/year, *P*=0.60) and lumbar spine (0.35 versus 0.36 %/year, *P*=0.92). In women, BB use was significantly associated with a greater increase BMD at the lumbar spine (0.34 versus 0.17 %/year, *P*=0.03), but not at femoral neck (-0.60 versus -0.62 %/year, *P*=0.69). The associations did not change after adjusting for age, body weight, and height.

Conclusion: These data suggest that beta blockers use was significantly associated with increased BMD at the lumbar spine in women, but not in men, and that the protective effect of beta-blockers on fracture susceptibility may be mediated through reduced bone loss.

P37

Relationship between dietary selenium intake and bone mineral density across multiple anatomical sites in adult women

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Background: The role of reactive oxygen species (ROS) in the aetiology of multiple disease states is well documented. However, the link between antioxidant intake and osteoporosis is unclear. In particular, the relationship between dietary selenium intake and bone mineral density (BMD) remains uncertain and has rarely been investigated. Grain based foods are major sources of dietary selenium with the content being largely dependent on the selenium content of soil.

Aim: This study aimed to investigate whether dietary selenium intake is associated with BMD in adult females.

Method: Selenium intake was assessed for 556 women drawn from an age-stratified randomly selected sample of 1494 women participating in the Geelong Osteoporosis Study. Using a detailed history semi – quantitative food frequency questionnaire and two nutrition composition databases (FSANZ 1996; 1999; 2001 and the USDA data bank 1999; 2001; 2002) average selenium content of 179 food items as well as 180 additional foods was calculated. Women taking oral multivitamins (n=32) were excluded from the analysis. Bone mineral density (BMD) at the lumbar spine, total hip, forearm, and of the total body was measured via dual energy x-ray (Lunar DPX-L). Using regression analysis the relationship between BMD and selenium intake was determined after adjusting for age, height and weight in 524 women (median age 51 years)(range 20-88 years).

Results: Median intake of selenium was 72ug / day (range 18-230). There was a significant association between dietary intake of selenium and bone mineral density (BMD) at the total hip (partial correlation for selenium = $r_{s=}$ 0.017, p=0.003), mid forearm (r_s =0.010, p=0.025), ultradistal forearm (r_s=0.008, p=0.040) and for the total body (r_s =0.010, p=0.019). There was a non-significant association between BMD and selenium intake at the spine (r_s= 0.010, p=0.068).

Conclusion: Selenium intake was associated with increased BMD at multiple sites in this sample of adult females. The results may aid recommendations for daily intakes of selenium as a potentially beneficial antioxidant contributing to optimal BMD in women.

P38

Effects of vitamin D deficiency and high parathyroid hormone on mortality risk in elderly men

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The aim of study was to examine the inter-relationship between vitamin D and PTH levels on the risk of mortality in men.

The study (part of the Dubbo Osteoporosis Epidemiology Study) involved 413 men aged 60+ years, whose had been followed-up for 14yrs. Mortality was ascertained. Vitamin D and PTH were measured at baseline. Vitamin D status was classified in 3 groups: deficient (25(OH)D<50nmol/L), insufficient (50-75nmol/L) and sufficient (≥75nmol/L). Cox's proportional hazards model was used for analysis.

During the follow-up period, 200 men died. The prevalence of vitamin D deficiency was 17.5% in the deceased and 8.0% in survivors. Men who died had lower baseline 25(OH)D levels than those who survived (70.9±22.9 vs. 77.3±23.0nmol/L, P=0.005), such that each SD decreased in 25(OH)D was associated with a 33% increase in the risk of mortality (RR=1.33, 95%CI:1.12–1.57). Higher serum levels of PTH were associated with an increased risk of mortality (+0.5pmol/L, 1.16; 1.02-1.32). After adjusting for age and PTH, the association between 25(OH)D and mortality remained statistically significant (1.22; 1.02-1.46). Men with vitamin D deficiency and high levels of PTH were 1.63 (1.01-2.66) fold more likely to die from those with adequate vitamin D and low levels of PTH. Approximately 7% and 4% of mortality risk was attributable to vitamin D deficiency and vitamin D deficiency with high PTH, respectively.

The independent effects of vitamin D and PTH on mortality risk observed in this study suggests that men with vitamin D deficiency and hyperparathyroidism are at increased risk of all-cause mortality.

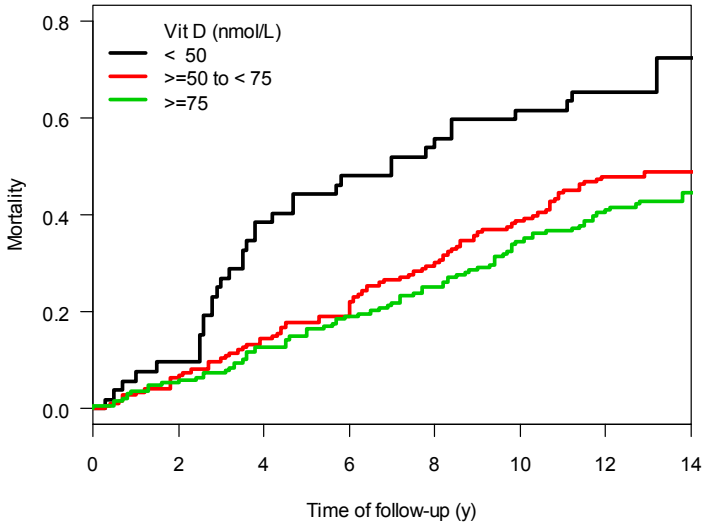

Figure: The association between vitamin D and mortality in men

P39

Explaining the "missing heritability" of osteoporosis: contributions of *ESR1* and *LRP4* polymorphisms to fracture susceptibility in families

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Genetic factors account for up to 50% of fracture liability, but variants identified by GWAS have explained less than 5% of the genetic variance in fracture. We hypothesize that part of the "missing heritability" can be explained by considering the genetic effects within families. The study involved 147 individuals (age: 18 – 80) in 53 families as part of the on-going Dubbo Osteoporosis Genetics Study (DOGS). Seventy-four SNPs in 34 genes were genotyped. Bone mineral density (BMD) at the femoral neck was measured by DXA (GE-Lunar). Fracture was ascertained by X-ray report. The association between SNPs and fracture risk was performed by the Genomewide Association Analyses with Family. Four genes (*SPTBN1, PRDM12, RPS6K5,* and *CHD20*) were associated with BMD and the genes explained 6% of the variance in BMD. From 1990 to 2009, 13 individuals (9.7%) had sustained fracture. Two SNPs in the *ESR1* gene (rs4870044, rs712219) and 3 SNPs in the *LRP4* gene (rs10838635, rs7935346, and rs7121418) were significantly associated with fracture risk (relative risk: 1.73 – 1.87), independent of age, BMD, and gender. The area under the ROC curve (AUC) was 0.77 for a model with age, BMD and gender as predictors; incorporation of the variants into the model increased the AUC to 0.93 ($P < 0.001$). The results are consistent with the hypothesis that a large part of "missing heritability" can be explained by effects of genes within families. These results also indicate that genetic factors can enhance the prognosis of fracture over and above clinical risk factors.

P40

Contribution of Genetic Profiling to Individualized Prognosis of Fracture

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Fracture risk is determined by multiple genes, each with a modest effect. However, it is contentious whether a combination of genes can help the prognosis of fracture. This study was built on the Dubbo Osteoporosis Epidemiology Study including 858 men and 1358 women aged 60+. Fracture was ascertained by radiological reports. Bone mineral density at the femoral neck was measured by DXA (GE-Lunar). Using the actual clinical data, 50 genes were simulated with allele frequencies ranging from 0.01 to 0.60, and relative risk from 1.01 to 3.0. Three models were developed to predict fracture: (I) clinical risk factors only (age, BMD, prior fracture, and falls); (II) genes only; and (III) clinical risk factors and 50 genes. The area under the ROC curve (AUC) was used to assess the degree of discrimination; and reclassification analysis to assess the incremental prognostic value of model with genes. During the follow-up period, 17% men and 31% women had sustained a fracture. Compared to those with <10 risk genotypes, those with 10-16 risk genotypes had their odds of fracture increased by 5.47 (3.03 – 9.90). Those with >16 risk genotypes had the highest risk of fracture (OR 43.6; 95% CI 22.8 – 83.2). The incorporation of genes into the clinical risk factors model increased the AUC from 0.77 to 0.88, and improved the accuracy of fracture classification by 45%, with most (41%) was the improvement in specificity. These results suggest that genetic profiling could enhance the predictive accuracy of fracture prognosis, and help identify high-risk individuals for intervention to appropriate management of osteoporosis.

P41

Low-trauma fractures signal increased risk of hip fracture in frail older people

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This study aimed to investigate risk of subsequent hip fracture after low-trauma fracture in very frail older people. We assessed 2005 institutionalized older people in Sydney, and then followed them up for all fractures for 2 years and hip fractures for at least five years. The study analysed 1412 (mean age 86.2 ± 7.0 years, female 77%) residents without total hip replacement and compared risk of subsequent hip fracture among 1294 who did not fracture (Group 1) to 118 who suffered a non-hip fracture (Group 2) in the first year. Follow-up for subsequent hip fracture started at one year from baseline for Group 1 or at the time of the initial fracture for Groups 2. Group 1 were more likely to be older, female, have a previous fracture, be more mobile and to have lower broadband ultrasound attenuation (BUA) and weight compared to Group 2. During a mean follow-up of 3.1 ± 2.1 years, 150 and 23 subsequent hip fractures occurred in Groups 1 and 2 respectively, giving incidence rate (per person-year) of 3.7% and 6.9% respectively. According to Kaplan-Meier curves, Group 2 had an increased risk for about 2.5 years compared to Group 1. For the first 2.5 years, HR for Group 2 vs. Group 1 was 2.65 (95% CI: 1.62-4.33; P<0.001) after adjusting for age, sex, residence type, BUA, fracture history, weight, lower leg length, immobility, cognitive function and medications. In conclusion, frail older people with a newly acquired fracture are at increased risk of subsequent hip fracture.

P42

Bisphosphonates and glucocorticoid osteoporosis in men: results of a randomized controlled trial comparing zoledronic acid with risedronate

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We studied 265 men, aged 18 – 83 years, in a randomized, double-blind, 1-year study comparing effects of Zoledronic acid (ZOL) *vs.* Risedronate (RIS) in patients commencing glucocorticoid treatment at dose of ≥7.5 mg/day of prednisone or equivalent (prevention arm, *n*=88) or continuing long-term glucocorticoid treatment (treatment arm, *n*=177).
Patients received ZOL 5 mg infusion or RIS 5 mg oral daily, with calcium (1000 mg) and vitamin D (400-1200 IU). Primary endpoint: difference in lumbar spine (LS) bone mineral density (BMD) at Month 12. Secondary endpoints: changes in BMD at total hip (TH) and femoral neck, bone turnover markers (β-CTx and PINP), and overall safety.
In treatment arm, ZOL increased LS BMD by 4.7% *vs.* 3.3% for RIS and TH BMD by 1.8% *vs.* 0.2%, respectively. In prevention arm, both treatments prevented bone loss. LS BMD changes were 2.5% *vs.* -0.2% for ZOL *vs.* RIS and TH BMD changes were 1.1% *vs.* -0.4%, respectively. Treatment differences for ZOL *vs.* RIS was significant at LS in treatment (p<0.025) and prevention (p<0.0025) arms and at TH in treatment (p=0.0004) and prevention (p<0.025) arms. In treatment arm, ZOL significantly reduced serum β-CTx and PINP *vs.* RIS at all time-points. In prevention arm, ZOL significantly reduced β-CTx at all time-points, and PINP at Month 3 (p=0.0297). Both treatments were well tolerated in men, albeit with a higher incidence of influenza-like illness and pyrexia post-infusion with ZOL.
Treatment with ZOL preserves or greatly increases BMD within 1 year than daily RIS in men receiving glucocorticoid therapy.

P43

Intravenous zoledronate use in adult delayed non-union fracture healing

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Multiple surgical procedures are often required in patients with compound lower leg fractures with the result of either non-union or malunion with significant shortening and angulation. We examined the effect of pulsed intravenous Zoledronate, Calcium and Vitamin D supplements in such patients to assist bone healing. We included 7 patients who had malunion or non-union with shortening/angulation who required tibial revision surgery. Six males, age range between 42 – 68 years were included and 1 female aged 39. All subjects had undergone corrective revision surgery at least 2 – 3 years after their original compound fracture. All patients had been treated with an external fixator for deformity correction and lengthening but had failed to heal within 6 months of the revision surgery. All patients had biochemical/hormonal profile performed; technetium bone scans to assess ongoing bone resorbtion. All patients except the 39 year old female had an active bone scan indicating ongoing bone resorbtion and were therefore treated 6 weekly on 3 – 4 occasions with pulsed intravenous Zoledronate and prior and post treatment with Calcium and Vitamin D. The dose of Zoledronate 0.0125mg per Kg for the first dose and 0.025mg per Kg for subsequent dose and repeat doses were given on evidence of radiological healing. In all cases the fracture deficit healed allowing early removal of the Taylor spatial frame. There were no adverse effects and no continuation of non-union. In conclusion, it appears that pulsed low dose intravenous Zoledronate with Calcium and Vitamin D may aid healing of non-union fractures.

P44

Zoledronic acid improves Health-related quality of life in patients with hip fracture: Results of HORIZON-RFT

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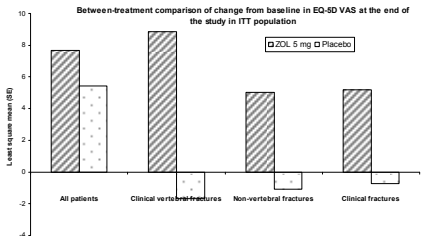
In HORIZON-RFT, zoledronic acid (ZOL) 5 mg infusion significantly reduced the rate of new clinical fractures and all-cause mortality *vs.* placebo (Lyles *et al. N Engl J Med.* 2007). A pre-defined exploratory objective: to analyze the benefits of ZOL *vs.* placebo on Health-related quality of life (HRQoL) using EuroQol instrument in selected countries.

In this double-blind, placebo-controlled trial 2127 patients were randomized to single infusion of ZOL (n=1065) or placebo (n=1062) within 90 days after surgical repair of low-trauma hip fracture, followed by annual infusions till 3 years (median follow-up time: 1.9 years). HRQoL was measured using EQ-5D-Visual Analogue Scale (VAS) and -utility scores at end of the study. Analysis of covariance model included baseline EQ-5D status, region and treatment as explanatory variables.

At baseline, patients (mean age: 75 years; 24% men, 76% women) were well-matched between treatment groups with mean EQ-5D VAS of 65.82 for ZOL and 65.70 for placebo. At the end of study, mean change from baseline in EQ-5D VAS was greater for ZOL *vs.* placebo in all patients and in subgroups of patients experiencing clinical vertebral fractures, non-vertebral fractures, and clinical fractures with treatment difference significantly in favor of ZOL (Figure). EQ-5D utility scores were comparable for ZOL and placebo but more patients on placebo consistently had extreme difficulty in mobility (1.74% for ZOL *vs.* 2.13% for placebo; p=0.6238), self-care (4.92% *vs.* 6.69%; p=0.1013) and usual activities (10.28% *vs.* 12.91%; p=0.0775).

Treatment with ZOL significantly improved overall quality of life in patients with low-trauma hip fracture.



P45

Subtrochanteric fractures: results from the HORIZON-Recurrent Fracture Trial

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Aim: To identify the risk factors for subtrochanteric fractures in osteoporotic patients treated in HORIZON-Recurrent Fracture Trial (RFT).

Methods: In this randomized, double-blind, placebo-controlled trial the efficacy of once-yearly i.v. infusion of zoledronic acid (ZOL) 5 mg was assessed in 2127 patients aged ≥50 years who had undergone surgical repair of a low-trauma hip fracture in the preceding 90 days. We conducted a post-hoc analysis of the baseline and post-treatment characteristics of patients with subtrochanteric fractures *vs* incident hip fracture.

Results: 106/2127 (5.0%) patients had sustained subtrochanteric fractures (ZOL, *n*=50; placebo, *n*=56) at baseline. The mean age, age distribution, sex distribution and BMI were similar between groups. Femoral neck and total hip BMD, and distribution of femoral neck BMD were similar between groups. There were no clinically relevant differences in concomitant medications and comorbidities between groups. Health status as measured by EQ-5D (total score and all domains) between groups was also similar (Table). The post-treatment hip fracture rate in overall study population was 2.0% (*n*=23) in the ZOL group and 3.5% (*n*=33) in the placebo group, a nonsignificant 30% risk reduction with ZOL. The number of patients with a recurrent subtrochanteric hip fracture was too few to be able to draw any conclusions.

Conclusions: Subtrochanteric fractures are not uncommon and do occur in bisphosphonate-naive patients, though it failed to show factors that would identify those at greater risk for subtrochanteric fracture. The incidence of subtrochanteric fractures after ZOL treatment was rare and too small to draw any meaningful conclusion.

P46

Measures of body fat are associated with prevalent vertebral deformities in older women but not men

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Aim: To describe the relationship between fatness and anterior wedge deformities.

Methods: A population-based cross-sectional study of older adults (N=1007). Measures of body fat included weight, BMI, waist-hip ratio (WHR), waist circumference, trunk fat (%) and total fat mass. Anterior wedging of T4-L4 was determined by morphometric dual-emission X-ray absorptiometry (MXA) and used as a continuous variable. We used mixed models and adjusted for age, hip and spine BMD.

Results: Median age of participants was 61.3 years (range 50-80); median BMI was 27.1 (range 17.6 - 52.9); 52.1% were female. Prevalence of anterior wedge deformities (≤20% reduction in anterior height) was 36.6% in women and 47.4% in men (thoracic spine); 1.4% in women and 5.3% in men (lumbar spine). As body fat increased, the ratio of anterior to posterior vertebral heights decreased, indicating worsening vertebral deformity. In women in the thoracic region, this association was present for weight (Sβ 0.006, p=0.001), BMI (Sβ 0.007, p<0.001), trunk fat (%) (Sβ 0.004, p=0.006), waist circumference (Sβ 0.005, p=0.003), and total body fat mass (adjusted for lean mass) (Sβ 0.005, p=0.012); but not waist-hip ratio or total lean mass (adjusted for fat mass). Associations in the thoracic region in men or the lumbar region in men or women were not significant.

Conclusions: Obesity may be deleterious for thoracic anterior wedge deformities in women but not men. The associations with waist circumference and trunk fat suggest a direct effect from increased skeletal loading of the thoracic spine.

P47

Zoledronic acid (ZOL) prevents bone loss in postmenopausal women with low bone mineral density (BMD): The HORIZON prevention study

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Aim: This study evaluated the efficacy and safety of two.regimens of intravenous (IV) ZOL in preventing bone loss in postmenopausal women with low BMD.

Method: In this 2-year, double-blind, placebo-controlled study, 581 participants, aged ≥45 with low BMD (T-score <-1.0 and >-2.5 at lumbar spine [LS] and >-2.5 at femoral neck), were randomly assigned to receive either ZOL 5 mg IV at baseline and Month 12 (ZOL12), ZOL 5 mg IV only at baseline and placebo at Month 12 (ZOL24), or placebo at baseline and Month 12 (PBO). The primary efficacy endpoint was the percentage change in LS-BMD from baseline to Month 24.

Results: Baseline characteristics of the 3 treatment groups were comparable. At Month 24, ZOL12 and ZOL24 regimens significantly increased LS-BMD versus PBO (mean percentage change [SD]: 5.31 [3.269] and 4.55 [3.665] vs. -1.19 [3.889]; both p<0.0001). Both ZOL regimens were superior over PBO in increasing BMD at LS at Month 12 and at total hip, femoral neck and trochanter at Months 12 and 24 (all p<0.0001). Both ZOL regimens significantly reduced βCTX, P1NP and BSAP versus PBO at Months 12 and 24, (all p<0.0001). These reductions during 2-year were greater with ZOL12 than ZOL24 regimen (all p<0.001). Adverse events (AEs) and serious AEs were similar among treatment groups.

Conclusions: Both ZOL regimens increased bone density and reduced bone turnover in postmenopausal women with low BMD and were well tolerated. Intravenous ZOL appears to be an effective strategy to prevent bone loss in postmenopausal women.

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Effect of Denosumab on the incidence of hip, new vertebral, and nonvertebral fractures over 3 years among postmenopausal women with higher fracture risk: a subgroup analysis from the FREEDOM Study

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The FREEDOM study showed that denosumab significantly reduced risk of new vertebral, nonvertebral, and hip fractures by 68%, 20%, and 40%, respectively. However, subjects in FREEDOM had less severe osteoporosis than subjects in previous trials. Therefore, reduction in fracture incidence by denosumab was re-assessed in higher risk subgroups.

Subjects were identified as higher risk for fracture if they met ≥2 pre-specified criteria: >70 years old; baseline T-score ≤-3.0 at lumbar spine, total hip, or femoral neck; and prevalent vertebral fracture at baseline. Additional clinically relevant criteria (post hoc) were used to identify subjects at higher risk for fracture at the hip (≥75 years old) or vertebrae (≥2 prevalent vertebral fractures, moderate/severe prevalent vertebral fractures, or both).

Of 7808 subjects analyzed, 45% were considered higher risk for fracture. The pre-specified and post hoc criteria correctly identified subjects with increased fracture risk. In the former group, denosumab significantly reduced the risk of hip and new vertebral fractures, but not that of nonvertebral fractures. Using the post hoc criteria, the greatest risk reduction was seen for hip fractures in women ≥75 years old. Risk reduction in hip fractures also was seen in women with a femoral neck BMD T-score ≤-2.5 and in women at high risk for new vertebral fractures.

The anti-fracture efficacy of denosumab in the higher risk subgroups was consistent with the risk reductions for the overall population. The reduction in hip fracture risk observed in women ≥75 years old could be of particular clinical relevance.

Table 1. Fracture Incidence and Fracture Risk Reduction With Denosumab Treatment Over 3 Years in Subjects at Higher Risk of Fracture by the Prespecified Criteria[a]

Fracture	Fracture Incidence in Overall Placebo Group (N = 3906)	Fracture Incidence in Higher Risk Placebo Group (N = 1752)	Fracture Incidence in Higher Risk Denosumab Group (N = 1761)	Percent Risk Reduction With Denosumab Treatment in Higher Risk Group (95% CI)	p-value
Hip fracture	1.2%	2.1%	1.1%	48% (9%, 71%)	0.0208
New vertebral fracture	7.2%	10.0%	3.5%	65% (53%, 74%)	< 0.0001
Nonvertebral fracture	8.0%	9.3%	8.3%	12% (-11%, 30%)	0.2901

[a]Subjects with ≥ 2 of the 3 prespecified risk factors: (1) age > 70 years, (2) baseline BMD T-score ≤ -3.0 at lumbar spine, total hip, or femoral neck, and (3) prevalent vertebral fracture at baseline.

Table 2. Fracture Incidence and Fracture Risk Reduction With Denosumab Treatment Over 3 Years in Subjects at Higher Risk of Fracture at Specific Skeletal Sites by Post Hoc Clinical Criteria

Fracture	Fracture Incidence in Overall Placebo Group[a]	Fracture Incidence in Higher Risk Placebo Group	Fracture Incidence in Higher Risk Denosumab Group	Percent Risk Reduction With Denosumab Treatment in Higher Risk Group (95% CI)	p-value
Hip fracture[b]	1.2%	2.3%[b]	0.9%[b]	62% (22%, 82%)	0.0065
Hip fracture[c]	1.2%	2.8%[c]	1.4%[c]	47% (8%, 70%)	0.0227
New vertebral fracture[d]	7.2%	16.6%[d]	7.5%[d]	55% (31%, 71%)	0.0002

[a]Number of subjects in overall placebo group N=3906; [b]Subjects ≥75 years old (higher risk placebo N=1236; higher risk DMAb N=1235); [c]Subjects with baseline femoral neck BMD T-score ≤ -2.5 (higher risk placebo N=1406; higher risk DMAb N=1384); [d]Subjects with ≥2 prevalent vertebral fractures, moderate or severe vertebral fracture, or both (higher risk placebo N= 372; higher risk DMAb N=387)

P49

Characterization of and risk factors for acute phase reactions following zoledronic acid infusion

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Aim: The occurrence of flu-like symptoms in patients receiving high-dose oral or iv amino-bisphosphonates (referred as the acute phase reaction [APR]) has been recognized for more than 20 years. This analysis compared the APRs and the potential risk factors of these following annual injections of zoledronic acid (ZOL) 5mg *vs* placebo over 3 years in 7765 postmenopausal osteoporotic women in the HORIZON-PFT study.

Methods: Adverse events (AEs) were collected using the MEDRA dictionary, and all AEs occurring within 3 days of the first infusion were compared between the groups.

Results: We found >30 AE terms and clustered into 5 groups that were significantly more common with ZOL(Table).Overall, 42.5% of ZOL-treated patients had an APR after the first infusion, *vs* 11.7% in placebo. All APRs had their peak onset within 1 day of infusion, and very few began after Day 3. Median duration of all APR components was 3 days (interquartile range: 2–5 days), except for eye symptoms, with median duration of 5 days. The APRs are mild or moderate in 90%, and led to drug discontinuation in 0.8% patients. Most putative risk factors had only a small impact on APR incidence. Stepwise regression showed that APR was more common in non-Japanese Asians, younger patients and those using NSAIDs, and less common in smokers, diabetics, those with previous bisphosphonate use and the Latin Americans (OR 0.26, *vs*. Europe).

Conclusion: The components of the APR are more diverse than those recognized earlier; however, is seldom a major issue following ZOL infusion.

Symptom Cluster	ZOL (%)	Placebo (%)
Fever	20.3%	2.5%
Musculoskeletal (pain and joint swelling)	19.9%	4.7%
Gastrointestinal (abdominal pain, vomiting and diarrhea)	7.8%	2.1%
Eye Inflammation	0.6%	0.1%
Others (non-specific systemic symptoms[fatigue, malaise] and significant increase in the frequency of nasopharyngitis and peripheral edema	22.1%	5.8%
Acute Phase Reaction (i.e. any of the above)	**42.5%**	**11.7%**

Each symptom cluster was more common in the ZOL group, $P<0.0001$

P50

Dairy Phospholipid for Bone Health

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Phospholipids (PL) are naturally occurring calcium-binding molecules.

In this study, we used PL-rich diets to examine effects on bone microstructure. Four groups of ten 2y old rats were fed one of the gelatine-formulated PL diets (tabled below) or blank gelatine (control) daily for eight weeks. After sacrifice, tibiae were removed and examined by micro-computed-tomography (SkyScan).

Phospholipid diet profiles (mg/kg/day)

	Soy-PS	Dairy-PS	Mixed Dairy PL
Phosphatidyl serine (PS)	34	34	31
Phosphatidyl ethanolamine (PE)		0.9	89
Phosphatidyl inositol (PI)		2.0	22
Phosphatidyl choline (PC)			59
Sphingomyelin (SM)			53

Two of the diets contained mainly PS - one from dairy, the other soy. Thus, any differences could be due either to the fatty acid composition, or to other minor phospholipid components. The third test diet contained a variety of other dairy phospholipids in addition to PS.
The findings demonstrate significant differences in the bones of dairy versus soy PS diet-fed animals; with increases in weight-adjusted measurements of trabecular bone surface ($p=0.045$) and parameters of trabecular connectivity (connectivity $p=0.030$; connectivity density $p=0.025$ and Euler number $p=0.034$). In the mixed PL diet-fed animals, there were significant increases in cortical bone parameters compared to control diet-fed animals. Bone surface ($p=0.020$), bone volume ($p=0.049$), mean total cross-sectional bone area ($p=0.049$) and mean total cross-sectional bone perimeter ($p=0.022$) are increased.
These findings indicate that animals on dairy phospholipid-rich diets after 8 weeks of feeding had increases in both trabecular and cortical bone parameters indicating these diets have positive effects on bone health.

P51

Low treatment rates of vertebral fractures in a large New Zealand District Health Board

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Background: Vertebral Fractures are a strong predictor of future fracture risk; they are inadequately managed. This study looks at the treatment rates on discharge in patients diagnosed with vertebral fractures during a hospital admission.
Methods: This study reports on chart reviews of patients over 65 admitted with Vertebral Fractures between July 2006 and June 2007. We collected data on 163 patients with a mean age of 81.5 years, Two-thirds were female.
Results: One third had treatment with a combination of Bisphosphonates, calcium and Vitamin D. 27% had no treatment. Patients receiving no treatment were more likely to be male. Treatment rates in subgroups showed that 52 % on steroid were on full treatment, 32% in the new vertebral fracture group and 46% in the previous non-vertebral fracture group.
Conclusion: The rates of treatment with anti-resorptive agents after a vertebral fracture were low, even in sub-groups with high fracture risk. This was independent of age or discharge destination. There was expected variability depending on the service looking after the patient.

P52

Audit of bone protection treatment in patients aged 65 yrs presenting to North Shore Hospital with fractures.

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Background: Fractures in the Elderly are undertreated despite clear evidence for treatment benefit.
Methods: We report an audit of chart reviews of patients over 65 admitted to North Shore Hospital (Auckland, NZ) between December 2008 and May 2009 with significant fracture. 329 patients were evaluated --- 80 males (mean age 78.7) and 249 females (mean age 81.1).
Results: Overall 43% were on Bisphosphonates (142 patients). 111 met local criteria for Bisphosphonate (BP) funding but were not on treatment. 71 did not qualify and 5 had side-effects with Bisphosphonates in past. 153 were on Vitamin D, 165 on Calcium and 129 (39%) on triple therapy (BP, calcium and Vitamin D).
When analysed by speciality, treatment with BP was 80% in Aged Care, 16% in Orthopaedics, 8% in Emergency Department, 57% in General Medicine and 0% (0/9 patients) in General Surgery.
Conclusion: Fractures in the Elderly remain undertreated especially in surgical wards. The % treated in General Medicine was disappointingly low.

P53

Paracetamol or ibuprofen alleviates post-dose symptoms related to intravenous infusion of zoledronic acid

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Once-yearly intravenous zoledronic acid 5 mg is a safe and effective treatment for postmenopausal osteoporosis. Administration is sometimes associated with transient, influenza-like, post-dose symptoms. In an international, multicentre, randomized, double-blind, double-dummy, parallel-group study, bisphosphonate-naïve postmenopausal women with osteopenia (n=481) were randomized to receive zoledronic acid plus acetaminophen/paracetamol (n=135), ibuprofen (n=137) or placebo (n=137), or placebo plus placebo (n=72). Acetaminophen/paracetamol and ibuprofen were administered every 6 hours for 3 days beginning 4 hours post-infusion. The proportion of patients with increased oral temperature (≥1°C and above 37.5°C), the primary efficacy endpoint, and with worsening post-infusion symptom scores over 3 days was significantly lower in patients receiving ibuprofen (36.8%, 48.5%) or acetaminophen/paracetamol (37.3%, 46.3%) versus those receiving placebo (63.5%, 75.9% respectively) (all $p<0.0001$). Overall incidence of adverse events was comparable for patients receiving acetaminophen/paracetamol or ibuprofen.
In conclusion, oral acetaminophen/paracetamol or ibuprofen effectively managed the transient flu-like symptoms associated with zoledronic acid 5 mg.

P54

The effects of a two year RCT of whey protein supplementation on bone density and urinary calcium excretion in older postmenopausal women

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Aims of study: Epidemiological studies including our own (JBMR 2009;24:1827-1834) and randomized trials in frail women after hip fracture have suggested that increased protein intake may reduce bone loss in elderly women. To evaluate this a 2-year protein supplementation RCT was performed.

Methods: 219 healthy ambulant women aged 70-80 years were randomized to either a high protein (n=109) or low protein drink (n=110) both of which consisted of a 250 ml drink providing 600 mg of calcium and 3.3 kJ/ml of energy. The high protein drink had 30 g of whey protein and the low protein drink had 1.7 g protein. DXA total hip BMD and 24h urinary calcium excretion was measured at baseline and year 2.

Results: Baseline protein intake was 76.2±18.0 g/day (1.14±0.33 g/kg body weight/day). There was a significant decrease in total hip BMD at year 2 in both the protein (-11±3 mg/ cm^2) and placebo (-8±2 mg/ cm^2) groups but there was no between group difference. At baseline 24h urine calcium did not differ in the two groups but there was a significant increase over the two years in the protein group (0.53±1.62 mmol/day) compared to placebo (-0.07±1.57 mmol/day, P=0.14).

Conclusion: Although the high protein drink produced the expected increase in urine calcium there was no detrimental or beneficial effect on hip bone mass. In these healthy ambulant women with baseline protein intake above current Estimated Average Requirement of 0.75g/kg body weight/day, extra protein was not a critical beneficial or deleterious regulator of their bone mass.

P55

Identification of osteoporosis through a retrospective and prospective audit in patients following an acute fracture

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The identification of people with a low bone density following fracture is often neglected. In an attempt to address this we initially, retrospectively, selected males and females with a recent fracture, aged 45 to 74 years, from an acute orthopaedic outpatient list. These patients were telephoned and offered a bone density scan (BMD). Over a one month period 164 patients were identified, and 16 were scanned, a low scan rate total of 9.75%. From the BMD results 43.75% had normal values, 31.25% had osteopenia and 25% were osteoporotic. Only three patients were already on treatment for osteoporosis. To increase the BMD scan rate we invited patients prospectively by letter to contact us to arrange a BMD appointment. This was done in partnership with the same orthopaedic department for acute outpatient fractures. Over a two month period 56 patients with acute fractures, excluding ankle, fingers and toes, have been contacted. To date 14 patients have been scanned, 50% with a normal BMD, 42.85% have osteopenia and 7.15% have osteoporosis. Daily identification of patients continues with more BMD appointments booked. This prospective audit will conclude in June 2010.

P56

Zoledronic acid reduces the increased risk conferred by further fractures

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Aim: In HORIZON-Pivotal Fracture Trial (PFT), once-yearly infusion of zoledronic acid (ZOL) 5 mg reduced the risk of morphometric vertebral (by 70%), hip (by 41%), and all clinical fractures (by 33%) during 3 years1. As with all treatments, fracture risk is reduced but not abolished. Nevertheless, treatment slows progression of fragility or partly reverses it. As fractures beget fractures, we tested the hypothesis that ZOL will reduce the increased risk conferred by fractures occurring in the subset of individuals who sustained fractures despite therapy.

Methods: In a pre-planned analysis, we examined the effect of ZOL in preventing recurrence of all clinical fractures and a second morphometric vertebral fracture in 7765 postmenopausal women with osteoporosis randomized to an annual i.v. infusion of ZOL 5 mg (n=3889) or placebo (n=3876) during 3 years. Clinical fractures were reported by all patients to the investigator every 3 months. Lateral spine x-rays were done at baseline and yearly in stratum 1 (patients not receiving other antifracture therapy) and at baseline and end of study in stratum 2 (patients receiving other antifracture therapy). Recurrence of clinical fractures was evaluated by using multivariate proportional hazards regression model in all intent-to-treat patients stratifying by the use of other antifracture therapies. Multiple morphometric vertebral fractures were evaluated using logistic regression adjusting for treatment and number of baseline fractures (stratum 1 and 2 separately).

Results: In ZOL-treated patients, 36 (11.7%) of the 308 (7.95%) sustaining a clinical fracture had ≥2 subsequent fractures. While in placebo-treated patients, 94 (20.6%) of the 456 (11.81%) sustaining a clinical fracture experienced ≥2 subsequent fractures. This corresponded to 38% risk reduction (95% CI: 28, 46) of multiple fractures (p<0.0001) with ZOL. The risk reduction of ≥2 morphometric vertebral fractures with ZOL was 89% (95% CI: 77, 95) in stratum 1 (Figure 1) and 61% (95% CI: -23, 88) in stratum 2 (NS).

Conclusion: Once-yearly, ZOL 5 mg reduces the worsening fragility accompanying a fragility fracture.

References: Black DM, et al. N Engl J Med. 2007; 356:1809–22.

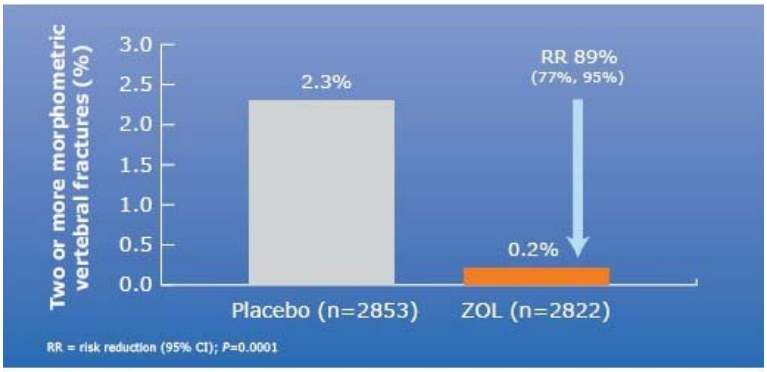

Figure 1. *Reduction in the risk of experiencing two or more morphometric vertebral fractures in postmenopausal women treated with ZOL over 3 years (Stratum 1)*

P57

Relationship between baseline remodelling intensity and changes in HR-pQCT parameters at the radius in postmenopausal women treated with denosumab or alendronate

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Denosumab (DMAb) suppresses remodelling more than alendronate (ALN)[1]. As remodelling intensity is a major determinant of structural decay, we hypothesized that DMAb produces greater morphological changes than ALN, particularly in women with high remodelling. Postmenopausal women aged 50–70 years with low BMD at the spine or total hip were randomized to placebo, DMAb (60 mg sc twice yearly) or ALN (70 mg oral/week). All received calcium (≥500 mg/d) and vitamin D (≥400 IU/d). Radial total, trabecular and cortical volumetric BMD (vBMD) and cortical thickness were assessed by HR-pQCT (XtremeCT®, Scanco Medical) at 0, 6 and 12 months. Serum CTX (sCTX) was measured at baseline and throughout the study. The relationship between baseline sCTX and morphology was assessed by ANCOVA. Within one week, sCTX decreased by 87% in the DMAb group (n=81) and 52% in the ALN group (n=80). Near maximal reduction in sCTX was seen at 1 month with DMAb (89%) and ALN (75%). Total, trabecular and cortical vBMD and cortical thickness decreased in placebo. In subjects with baseline sCTX > median (0.736 ng/mL), increases in total, cortical and trabecular vBMD were greater with DMAb than with ALN. Changes in subjects with baseline sCTX ≤0.736 ng/mL were smaller and differed less between treatments. DMAb increased distal radial vBMD in subjects with higher baseline remodelling, ALN prevented the decrease observed in placebo. Greater remodelling suppression results in increases in structural parameters that may contribute to a reduction in fracture risk, particularly in individuals with high bone remodelling.

1. Kendler et al. J Bone Miner Res 2010;25:72-81.

This study was sponsored by Amgen Inc.

P58

The antipsychotic clozapine, but not its derivative quetiapine, induces microarchitectural changes in bone

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Atypical antipsychotic drugs, such as clozapine and quetiapine, are commonly used in treatment of schizophrenia, which affects >1% of the world population; schizophrenia and its treatments have also been associated with an increased risk of fracture. We previously showed that clozapine decreases osteoblast proliferation and differentiation in vitro, and decreases BMD and bone volume in growing rats. The skeletal effects of quetiapine, a chemical derivative of clozapine, are unknown. Here we investigated the differences between quetiapine and clozapine on bone. 4-6 week old male rats (n=6/group) received daily clozapine or quetiapine for 42 days (10mg/kg/day).

DXA showed >30% reduced bone mineral content after clozapine, but not quetiapine, treatment. In clozapine treated animals, μCT analysis of tibial trabecular bone showed 30% reduction in bone volume (ANOVA, P=0.0316) and 23% reduction in trabecular number (P=0.0137) but quetiapine treatment caused no significant changes in trabecular bone. In cortical bone, we found further changes with clozapine treatment: 14% reduction in cross sectional area (P=0.0046), and 24% reduction in bone perimeter (P=0.0313); quetiapine treatment changed neither parameter. In vitro, 10μM clozapine treatment reduced proliferation of osteoblast-like cells by 80%, but a quetiapine concentration 5 times greater was required to achieve similar effects.

These data suggest that quetiapine causes less skeletal toxicity than clozapine in vivo. Long-term quetiapine administration may therefore pose less risk than clozapine to skeletal health.

P59

Reduced osteocyte density and morphological changes of canaliculi in trabecular bone of osteoporotic sheep

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Aim: Osteocytes regulate the function of osteoclasts and osteoblasts and therefore likely play a role in the bone loss associated with osteoporosis [1]. This study compares the density of osteocytes and morphology of the canalicular network in trabecular bone of osteoporotic sheep with normal sheep.

Methods: Osteoporosis was induced in nine ewes by chronic corticosteroid injection, ovariectomy and low calcium diet [2]. Six age-matched ewes were used as controls. Three months after withdrawal of corticosteroid administration all animals were humanely killed and their spines were collected for histology using DAPI and Holmes' silver impregnation method [3].

Results: The density of osteocytes in the osteoporotic sheep was over 30% lower than the control sheep ($p<0.05$). The number of empty lacunae in the osteoporotic sheep was over 35% greater ($p<0.05$). The canaliculi were shorter and not connected to those of the neighboring cells. The staining intensity was weaker in the osteoporotic group than in the controls.

Conclusion: Reduced osteocyte density and greater number of empty lacunae in osteoporotic sheep suggest that the function and activity of osteocytes may be impaired by the osteoporosis induction. The weaker silver staining of canaliculi in the osteoporotic bone is a result of decreased osteopontin presence. Fewer osteocytes and decreased connectivity may impair intracellular communication between osteoblasts and osteoclasts and consequently bone remodeling in this osteoporotic model.

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P60

Osteoporotic characteristics in vertebral trabecular bone of osteoporotic sheep remain after withdrawal of corticosteroid administration

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Aim: Osteoporosis has been induced in sheep by a combined treatment of ovariectomy, steroid administration and a calcium restricted diet 1. Before any therapeutic intervention can be tested in this model it is desirable to terminate steroid administration. This study was undertaken to characterise the morphology of vertebrae in normal and osteoporotic sheep following cessation of steroid administration.

Methods: Osteoporosis was induced in nine ewes by chronic corticosteroid injection, ovariectomy and low calcium diet. Bone mineral density (BMD) of the lumbar spine (LS) and body weight were assessed at regular intervals. After five and a half months corticosteroid treatment was withdrawn systematically over one month. Six ewes were used as controls. Three months later all animals were euthanised and the LS were collected for histomorphometric analysis using micro-CT.

Results: BMD in the LS of the osteoporotic sheep was 25% lower than the control sheep at the end point. Body weight of osteoporotic sheep was reduced in the first month of the corticosteroid withdrawal period but returned to baseline level thereafter. Trabecular bone volume of lumbar vertebrae in the osteoporotic sheep was 27% lower than controls, and showed a heterogeneous structure.

Conclusion: Osteoporotic characteristics remain in the vertebra after ceasing corticosteroid administration providing an opportunity to evaluate potential systemic or local treatments in vivo under realistic physiological conditions. Despite being relatively denser the microstructural variation of vertebrae trabecular bone in sheep is similar to humans demonstrating a further advantage of this animal model.

Reference

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P61

In vivo imaging of subchondral bone using micro-CT in a rodent model of osteoarthritis

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Aim: To non-invasively track subchondral bone changes using *in vivo* micro-CT in a rodent model of monosodium iodoacetate (MIA) induced osteoarthritis (OA).

Methods: Twelve rats were injected intraarticularly with 0.2 mg MIA in the right knee joint and sterile saline in the left (control). The knees were scanned *in vivo* by high-resolution micro-CT at 2, 6, and 10 weeks after injection. Serum cartilage oligomeric matrix protein (COMP) and C-telopeptide of type I collagen (CTX I) were measured to assess cartilage and bone turnover rate, respectively. Tibial subchondral bone histomorphometry was determined from the axial micro-CT tomographs. End-point histological changes in the tibia were observed.

Results: MIA-injected knees showed subchondral bone sclerosis with significantly higher bone volume and trabecular thickness at 6 and 10 weeks compared to controls ($p<0.05$). Medial side of tibia had higher bone volume than lateral side. Subchondral bone sclerosis and cysts observed from micro-CT images at 10 weeks correlated with histology. MIA-injected knees had cartilage lesions at 10 weeks whereas controls had no cartilage damage. Serum COMP and CTX I levels were higher at baseline and 2 weeks, relative to the levels at 10 weeks ($p<0.05$).

Conclusion: This study demonstrates that *in vivo* micro-CT enables non-invasive tracking of subchondral bone changes in this rodent model of OA. Serum COMP and CTX I are markers of cartilage and bone turnover rate, which are associated with cartilage and subchondral bone changes. *In vivo* micro-CT will be applied to monitor the efficacy of OA disease-modifying drugs in this animal model.

P62

Fatty acid composition of osteoarthritic, osteoporotic and control femoral heads

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Introduction: Osteoarthritis (OA) and osteoporosis (OP) are prevalent musculoskeletal diseases with the incidence increasing as society ages. While OA is linked to obesity, OP individuals have low body mass index. Bone forming osteoblasts have a common mesenchymal stem-cell precursor with adipocytes. It is therefore reasonable to expect differences in fatty acid profiles of individuals with these diseases. While omega-3 fatty acids have been linked to reduced severity of OA, the omega-6 fatty acids are precursors to the pro-inflammatory eicosanoids.

Aim: To compare the fatty acid profile of osteoarthritic, osteoporotic and control femoral heads.

Methods: Femoral heads were obtained at hip arthroplasty surgery from 14 hip OA patients (8 Female, 6 Male) and 26 fractured neck of femur (OP) patients (19 Female, 7 Male). Controls (Ctrl) were collected from autopsy cases. Trabecular subchondral bone from the principal compressive region was sampled from the femoral heads for gas chromatography analysis.

Results: The concentration of 57 fatty acids, including n-3, n-6 and n-9, was analysed. Significant differences between disease status and/or gender are shown in Table 1. OA bone, from both males and females, had higher concentrations of n-6 (in agreement with previous studies) and n-3 fatty acids (in contrast to previous studies) than OP femoral heads, which may be a reflection of the larger dataset here.

Conclusion: This work has expanded current knowledge on the fatty acid composition of OA and OP bone. The increased levels of n-6 fatty acids in OA bone may be associated with joint inflammation in this disease.

Table 1. Fatty acid profile of OA, OP and Ctrl bone

Fatty Acid	OP OA Ctrl (M, F)	P value
16:2 (n-3)	OP<OA<Ctrl	P=0.017
20:5 (n-3) Eicosapentaenoic (EPA)	OP(F)<OP(M) OA(F) nsd OA(M) OP(F)<OA(F) OP(M)<OA(M)	P<0.001 P<0.001 P=0.012
22:6 (n-3) Docosahexaenoic(DHA)	OA>OP/Ctrl	P=0.001
14:1	OP<OA/Ctrl	P<0.001
20:2 (n-6) Eicosadienoic	OP<OA/Ctrl	P<0.005
20:4 (n-6) Arachidonic	OA>OP/Ctrl	P<0.001
20:3 (n-6) Dihomogamma linoleic	OA>OP/Ctrl	P<0.001
20:4 (n-6) Arachidonic	OA>OP/Ctrl	P<0.001

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Peri-osteocytic osteolysis with no decline in osteocyte-lacunae density occurs in association with bone volume loss due to estrogen depletion

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Although the effect of estrogen depletion is well known to cause rapid bone loss due to mobilisation of calcium from bone stores by increased osteoclastic activity, it is less clear whether there are also changes to osteocyte density with or without peri-osteocytic osteolysis. Thus, short-term ovariectomy (OVX) in mice was performed specifically to investigate the effects on osteocytes and their lacunae. Ten week old female mice (n=20) were assigned to ovariectomy (OVX) or Sham-OVX groups. Tibiae from each animal were scanned by in vivo micro-CT 3 days prior to their assigned operation, and 14 days after operation, at which time animals were killed for further analyses. Femora and tibiae were prepared for dynamic histomorphometry and osteocyte-lacunae measurements. Serum crosslaps and alkaline phosphatase levels were modestly increased in OVX animals compared to Sham-OVX animals, indicating that resorption and formation processes were both increased. Tibial metaphyseal BV/TV was reduced by 10% when compared to Sham-OVX animals ($P<0.05$), which was due to a 20% decline in trabecular number ($P<0.001$). OVX animals demonstrated a 15% and 16% increase in trabecular and cortical bone lacunae size occupied by osteocytes respectively compared to Sham-OVX mice ($P<0.05$). However, preliminary data indicates that the density of occupied lacunae did not change. Therefore, short-term OVX induces bone loss and a concomitant increase in peri-osteocytic osteolysis, without an apparent loss of osteocytes, suggesting that mobilisation of calcium from lacunae regions contributes to maintaining serum calcium levels. The mechanism by which this occurs is currently under investigation.

P65

Measurement of *ex vivo* subregional vertebral bone mineral density using lateral projection dual energy X-ray absorptiometry (DXA): validation with peripheral quantitative computed tomography (pQCT)

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Although a strong relationship exists between areal bone mineral density (aBMD) derived from dual energy X-ray absorptiometry (DXA) and bone strength, the predictive validity of aBMD for osteoporotic vertebral fractures remains sub-optimal. The diagnostic sensitivity of DXA may be improved by assessing aBMD within vertebral subregions, rather than relying on an estimate derived from the total area of the vertebra. The aim of this study was to validate a method of measuring subregional vertebral aBMD, *in vitro,* using lateral-projection DXA against subregional volumetric BMD (vBMD) measured with peripheral quantitative computed tomography (pQCT). A mixed set of 49 lumbar and thoracic vertebrae from 25 donors were scanned using lateral-projection DXA and pQCT. aBMD and apparent vBMD were measured in 7 vertebral regions (1 total area and 6 subregions) from the lateral DXA scan. vBMD was calculated in anatomically equivalent regions from pQCT scan data, using a customised software program designed to increase efficiency of the analysis process. Significant differences in densitometric parameters between subregions were observed by DXA and pQCT ($p<0.01$). Subregional vBMD derived from pQCT was explained by a significant proportion of the variance in DXA-derived aBMD (R^2=0.51-0.67, $p<0.05$) and apparent vBMD (R^2=0.64-0.75, $p<0.05$). These results confirm the validity of measuring aBMD in vertebral subregions using lateral-projection DXA. The clinical significance should now be explored.

P66

Regional analysis of trabecular bone microdamage in the human lumbar vertebra

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Microdamage (MDX) accumulation in the human lumbar spine may be contributing to increased fracture and degeneration. Our aim was to quantitate MDX present regionally in lumbar vertebrae 2 (L2) and determine its relationship to age, bone structural and resorption indices. L2 vertebrae were obtained from 15 human cadaveric spines (8 males, 7 females; age 62±20 [mean±SD] years, age range:16-87 years). A sagittal slice from each vertebra was *en bloc*-stained in basic fuchsin, resin-embedded, and cut into 9 equal regions (region 1: superio-anterior). This paper reports on the anterior and central regions of the L2 vertebra. Trabecular MDX and histomorphometric analyses were undertaken using a Leica Quantimet Image Analyser and bright-field microscope (10X objective). The central region of the L2 vertebra (region 5) had the lowest bone volume fraction (BV/TV[%]), and this minimum was exacerbated by the decrease in BV/TV with advancing age. This region also showed a non-linear increase in microcrack density with age. A negative association between eroded bone surface and microcrack density was observed in the central region 5, the region with the lowest BV/TV, highest microcrack density and microcrack length. Interestingly, resorption density was significantly lower in region 4 compared to anterior regions 1 and 7. The increased MDX in region 5 of low bone turnover may be associated with altered bone mineralisation. Further analysis of regions (2,3,8,9) within L2 vertebrae, together with assessment of disc degenerative changes and comparisons with mechanical data, will provide a more holistic understanding of the degenerative human lumbar spine.

P67

Increased density of hypermineralised osteocyte lacunae and microdamage accumulation in fragility hip fracture patients

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Hypermineralised osteocyte lacunae are a known feature of the aging human skeleton; however, no data are available for fragility fracture. Therefore, the study aim was to determine the extent of hypermineralised osteocyte lacunae in relation to bone architecture, mineralisation and accumulated microdamage for fragility hip fracture patients compared to non-fracture controls. Intertrochanteric bone cores were obtained from patients at surgery for non-traumatic femoral neck fracture (FNF:10F,4M,65-94y) and from cadaveric controls (CTL:5F,13M,60-84y). All bone samples were resin-embedded for quantitative backscattered electron imaging (qBEI) of the degree of mineralisation. Using a custom image-processing algorithm for qBEI images, hypermineralised and total lacunar densities were quantified. A subset of FNF (4F,4M,65-94y) and CTL (4F,8M,64-84y) cases were initially micro-CT imaged for 3D trabecular architecture, and *en bloc*-stained in basic fuchsin before resin-embedding for histomorphometry. Bone tissue mineralisation (wt%Ca), lacunar and total lacunar density were not different between FNF and CTL. Strikingly, hypermineralised lacunar density (HL.Dn[#/mm^2]: 28.9[23.7-42.0]vs10.2[4.8-32.5], $p<0.02$; median[quartiles]) and percent hypermineralised lacunae (HL/TL[%]: 9.5[8.0-15.6]vs5.2[1.7-13.7], $p<0.04$) were significantly higher in FNF. FNF was associated with trabecular architectural insufficiency (reduced BV/TV:$p<0.02$,Tb.N:$p<0.01$,DA:$p<0.04$; increased Tb.Sp:$p<0.02$). Microcrack density (Cr.Dn:$p<0.002$) and diffuse microdamage (DxV/BV:$p<0.02$) were increased in FNF, coupled to elevated bone resorption (ES/BS:$p<0.01$,Rs.Dn:$p<0.0001$). In conclusion, these study data suggest that in addition to architectural decay, fragility hip fracture is associated with an increased density of hypermineralised lacunae and an accumulated microdamage burden. Unlike empty osteocyte lacunae, hypermineralised lacunae do not permit extracellular fluid circulation, and therefore their presence may contribute to microdamage accumulation by inhibiting damage detection and repair signalling.

P68

Combined high dietary calcium and vitamin D are necessary to improve cortical thickness to resist bone failure in senescent animals

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We have previously reported that vitamin D depletion in rats causes osteopenia. However, the interaction between dietary vitamin D and calcium in determining serum 25 hydroxyvitamin D (25D) levels and changes to bone structure and strength remains unclear. Hence, nine-month-old female Sprague-Dawley rats (n=5-6/grp) were pair-fed a semi-synthetic diet containing varying levels of vitamin D_3 (D) (0, 2, 12 and 20 IU/day) and either low (0.1%, LCa) or high (1%, HCa) dietary calcium until 15 months-old. Animals were then killed for bone cellular, structural and mechanical strength (tibial 3-point bending) analyses and biochemical and tissue gene expression analyses. Firstly, both dietary calcium and vitamin D were positive determinants of serum 25D levels, suggesting that HCa diet protects serum 25D levels even when animals were fed 0 vitamin D, most likely due to reduced renal 1,25 dihydroxyvitamin D (1,25D) synthesis. The mid-shaft cortical bone volume of both femora and tibiae were greatest in animals fed either 12 or 20IU/day vitamin D and HCa. Importantly, serum 25D levels, which ranged from 22 (±2.9) to 161 (±38.8) nmol/L, positively associated with both femoral (R^2=0.23, $p<0.05$) and tibial (R^2=0.22, $p<0.01$) cortical bone volume and were strongly associated with cortical thickness in the sagittal (loaded) axis (R^2=0.3, $P<0.001$). Sagittal cortical thickness is the primary determinant of tibial strength (ultimate load to failure, (R^2=0.20, $p<0.01$). Thus, a diet containing high levels of both vitamin D and calcium are required to raise serum 25D levels and attain optimal bone structure and strength.

P69

BMD, trabecular architecture and bone turnover following estrogen deficiency in an ovine model of osteoporosis

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Osteoporosis is a skeletal bone disease characterised by an imbalance between bone resorption and formation, resulting in bone loss and deterioration of the trabecular microarchitecture leading to an increased risk of fracture. This study sought to determine some of the molecular mechanism by which estrogen deficiency ultimately results in changes in bone mineral density and architecture.

Twenty skeletally mature (4+years) sheep were divided into two groups, OVX (n=10) and control (n=10). Half of each group were sacrificed 12 and 31 months post-OVX. Dual energy x-ray absorptiometry and micro computed tomography were used to assess the bone mineral density (BMD) and trabecular architecture, respectively, in the proximal femur. Real time RT-PCR of RNA extracted from bone samples was used to determine mRNA expression of a series of bone turnover markers.

Significant decreases in BMD in the femoral neck of OVX animals were measured over time. There were also significant changes in the trabecular architecture as a result of reduced bone volume fraction, trabecular thickness, trabecular number and an increase in trabecular separation. Increased mRNA expression of RANKL/OPG ratio, osteocalcin, osteopontin and type 1 collagen from RNA extracted from the OVX groups indicated increased bone turnover following estrogen deficiency.

This study found that 12 and 31 months of estrogen deficiency in sheep produces significant changes in mRNA expression of a series of bone turnover markers which correlates with changes in BMD and trabecular architecture. These changes are consistent with those seen in other animal models and also in humans, thus supporting the use of the ovariectomised sheep to model postmenopausal osteoporosis.

P70

Historically active postmenopausal women have greater trabecular bone volume and thickness than inactive women

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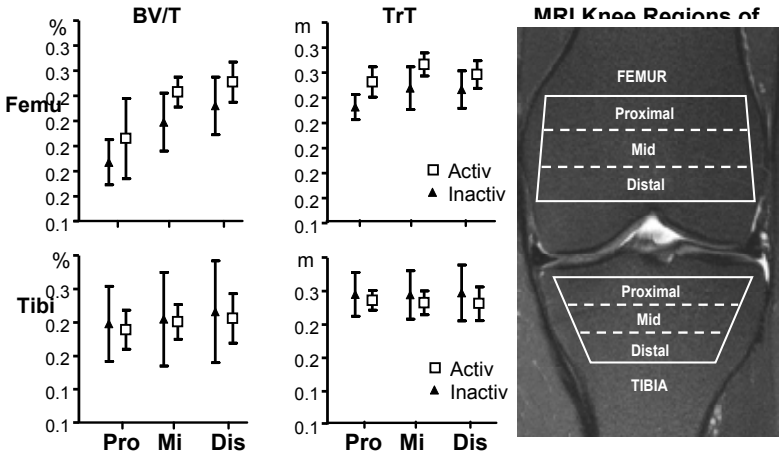
Weight-bearing exercise can enhance cortical bone geometry in older adults, but its effects on trabecular bone microarchitecture are not known. This study compared trabecular microarchitecture in historically active and inactive postmenopausal women (n=20) aged 60-76 years. Trabecular bone volume fraction (BV/TV), trabecular number (TrN), separation (TrSp) and thickness (TrTh) were assessed using 3T MRI (resolution 0.195x0.195, 1mm slice thickness) at the femur and tibia. Each site was divided into three ROIs to evaluate the region-specific effects of loading (Figure). Age and height were similar in the two historically contrasting loading groups, but weight was lower in the active women ($p<0.05$). Age, height and weight did not correlate with any trabecular parameters. There were no significant group-by-region interactions at either site, but in all women there was a trend for BV/TV to decrease from the distal to proximal femoral region. This was due to a decrease in TrN (not TrTh). There were no regional differences at the tibia for BV/TV. When we compared trabecular microarchitecture between the active and inactive women, there was a trend for BV/TV to be greater in the active women in the mid (9.5%, $p=0.05$) and proximal (7.2%, $p=0.14$] femoral regions. This was due to an increase in TbTh (mid 6.9% and proximal 7.2%, both $p<0.05$). Adjusting for differences in weight tended to attenuate these results. In conclusion, this pilot study highlights that trabecular bone volume is increased in historically active women, which appears to be due to an increased trabecular thickness rather than number.



Values represent means with 95%

P71

Quantifying intracortical porosity producing cortical remnants identifies individuals at risk for fracture beyond existing parameters

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Background: Most age-related bone loss occurs by intracortical remodelling of cortex adjacent to the marrow which produces coalescence of pores leaving a transitional zone (TZ) of trabecular-appearing cortical remnants. We therefore hypothesized that quantifying this TZ improves identification of individuals with fracture more effectively than current methods.
Methods: We studied 20 women with prevalent forearm fracture and 47 age, height, and weight matched controls. Images were collected at the distal radius using HRpQCT (Scanco). Total, cortical and trabecular volumetric density (Ct.D, Tr.D, and vBMD) were measured. Thicknesses of the compact-appearing cortex (Th.CC) and TZ (Th.TZ) and the proportion of the total (CC + TZ) cortical thickness occupied by the CC and the TZ (%CC and %TZ) were measured. Porosity (Po) was measured in each zone.
Results: The %CC was lower in cases than controls (70.91±1.03 vs 72.85±0.40; p=0.03) mostly due to an increase in the %TZ in cases (15.59±1.1 vs 12.82±0.43 %; p=0.01). While Th.CC was reduced in absolute terms (664.1±39 vs 710.3±28 μm) in fracture cases compared to controls, this was not significant. Porosity in the TZ (48.32±2.93 vs 42.02±1.48%; p=0.03) not CC (2.4±0.52 vs 2.89±0.43%, NS) was higher in cases than controls
Differences in Ct.D, vBMD and Tr.D between cases and controls were smaller and/or non-significant respectively 262.5±12.79 vs 288.54±9.5 (p=0.12), 853.6±14.59 vs 858.94 (NS) and 111.8 ±7.84 vs 132.2±5.55 (p=0.04)
Conclusion: Assessment of the cortical decay of the transitional zone composed of cortical remnants may allow identification of individuals at risk for fracture beyond currently existing parameters.

P72

Defining a region's surface/volume ratio identifies its susceptibility to being remodelled and decayed better than bone density measurements

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Background: Bone's surface/volume ratio determines its susceptibility to being remodelled and liability to decay. We therefore hypothesized that an in vivo estimate of this geometric property better predicts fracture risk than 'density' measurements.
Methods: We measured total volumetric bone mineral density (TvBMD), cortical vBMD (CtvBMD), and trabecular vBMD (TrvBMD) in women with forearm fracture and age, height and weight matched controls using HR-pQCT images of the distal radius (23 cases, 50 controls), tibia (in 32 cases, 53 controls) and fibula (32 cases, 52 controls). In addition, we processed these images using a new software (Strax1.0) to estimate the fraction of bone volume within the subperiosteal envelope accessible to remodelled (%BV). Results expressed as mean ± SEE notation
Results: At the distal radius, %BV was 18.5% lower in cases than controls (22.48±1.17 vs 27.61±0.96; p=0.002) . Whilst densities were lower in cases than controls, the differences were modest, 4.5% for CtvBMD (847.9±13.12 vs 858.3±11.4; NS), 10% for TvBMD (261.69±12.9 vs 291.0±9.68; p=0.08), and 16.6% for TrvBMD (-16.6%)(109.8±8.14 vs 131.84±5.51; p=0.028).
The odds ratios for fracture were 5.68 (95% CI: 1.49-21.57; p<0.05) for %BV, 1.54 (95% CI: 0.39-6.1; NS) for Ct vBMD, 2.9 (95% CI: 0.92-9.14; NS) for Tr.vBMD, and 2.03 (95% CI: 0.73-5.64; NS) and TvBMD. Results were similar at the tibia and fibula.
Conclusion: Bone fragility is not fully captured by 'density'; *in vivo* identification the structural configuration of bone accessible to being remodeled and so decayed is likely to improve identification of individuals at risk for fracture.

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Creation of a 3D finite element analysis model of the proximal femur incorporating cortical, trabecular and medullary compartments derived from a single 2D DXA scan image

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Finite Element Analysis (FEA) of the proximal femur provides an accurate assessment of fracture load. Although previous studies have generally used CT scans to create 3D FEA models, a technique that may be applied to conventional 2D DXA scan images has been recently reported [1]. The aim of this study is to further develop this technique by incorporating separate cortical, trabecular and medullary compartments.

A 3D template has been created from a pQCT scan of an excised proximal femur; enabling separate segmentation of cortical, trabecular and medullary cavity compartments. A sequence of spatial transformations are applied to match various features of the individual's DXA scan image, specifically a) shaft and neck axis alignments, b) exterior (periosteal) and interior (endosteal) cortical surfaces, and c) extent of trabecular bone. Within the resultant 3D FEA model, medullary cavity voxels are assigned the material properties of marrow and voxels between the periosteal and endosteal surfaces are assigned to be bone. Trabecular compartment voxels normal to each 2D DXA pixel are assigned a volumetric density based upon the corresponding DXA image derived bone mineral content and cortical thickness. It is envisaged that this multi-compartment 3D FEA model with provide even further improvement in prediction of fracture load of the proximal femur compared to conventional BMD.

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P74

A new approach to modelling bone remodelling: Smooth Particle Hydrodynamics

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Knowledge of age-related changes in cortical pore structure in the femur is critical to understanding the mechanisms that lead to catastrophic hip failure. In the neck of the femur about 70% of the strength is contributed by the cortical bone which is the most highly stressed part of the structure and is the site where catastrophic failure is almost certainly initiated. A deeper understanding of changes at this anatomical site is essential if an understanding of the mechanisms by which hips weaken and become vulnerable to fracture is to be gained. The aim of this study was to (i) examine a hypothesis that local strain fields are modified by the presence of Haversian canals and that low strains induced by this can lead to bone resorption, and (ii) use a meshless Lagrangian particle-based computational modelling approach 'Smooth Particle Hydrodynamics' (SPH) to capture bone remodelling features efficiently. The key findings from this study were firstly that bone remodelling due to strain changes at the osteon level generated pore merging and that this increased with age. Secondly, SPH was shown to be effective at modelling the changes in pore sizes and shapes as they evolved over time. In two regions of the bone the sequential modelling results showed the development of what could be interpreted as resorption lacunae by the merging of Haversian canals. This process was clearly associated with regions of locally reduced strain.

P75

Synchrotron-radiation micro-ct of cortical bone reveals new data on osteocyte lacunar volume and spacing

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In a study of femoral cortical bone from a 20 year old male, carried out at the Advanced Photon Source in Chicago, tomographic reconstructions were created from x-ray data sets taken using synchrotron radiation of 26.4 keV and with isotropic voxels 1.47μm on a side. This paper demonstrates that it is possible to segment the data to isolate both the osteocyte lacunae and the Haversian canals in the bone as well as identifying osteon boundaries. From this information a wealth of data relating to bone structure becomes available. The data was used to map the spatial positions of the osteocyte lacunae, relative to the Haversian canals and of the osteon boundaries. The dimensions and volume of the osteocyte lacunae were measured for approximately 10,000 lacunae. When averaged over the 11 osteons measured, osteocyte densities varied from $2x10^4$ per mm^3 close to the Haversian canals to about $4x10^4$ per mm^3 at 80% of osteon radius. The osteocyte-to-osteocyte nearest-neighbour distances varied from 10 μm to 40 μm with a peak at 23μm and an approximately normal distribution. The distribution of lacunar long-axis length was also approximately normal with a small positive skew and the peak value was 8 μm with a range from 3 μm to 20 μm. The most significant finding from this study was that the distribution of the measured volumes of osteocyte lacunae had two distinct peaks, one at 200 μm^3 and a second at 330μm^3.

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Are changes in muscle size related to changes in bone material and structural properties at the axial and appendicular skeleton in older men? An 18-month study

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Since bones adapt to their prevailing loads, bone loss should follow any decline in muscle during aging. This study investigated whether muscle size and bone material and structural properties change in a uniform manner at different skeletal sites during ageing, and whether changes in muscle and bone are related. This study included 89 men aged 61±8 years (±SD) followed for 18-months. Mid-femur, mid-tibia and L1-L3 (psoas+erector spinae) muscle CSA, and bone geometry, vBMD and strength were measured by QCT. Body weight increased by 0.9±2.6kg ($p<0.01$) over 18-months, but there were similar significant 0.8-1.2% reductions in muscle CSA at all sites ($p<0.05$). Bone strength decreased similarly (1.0-1.6%, $p<0.001$) at the mid-femur and mid-tibia, but not the distal tibia (-0.4%) and increased at L1-L3 (2.5%, $p<0.001$). At both mid-shafts this was due to a greater relative loss in cortical vBMD (0.9-1.2%, $p<0.001$) than cortical area (both -0.4%, $p<0.01$), which decreased due to endosteal resorption. Neither L1-L3 TotAr or trabecular vBMD changed, but total vBMD increased, indicating there was an increase in cortical density. Multiple regression analysis revealed that changes in muscle CSA were positively related to changes in diaphyseal CortAr and L1-L3 bone strength, and inversely to changes in mid-femur MedAr (5.3-14.9% of the variance). In conclusion, at axial and appendicular sites there were uniform losses in muscle CSA with age, but not bone geometry, vBMD or strength. Changes in muscle CSA were related to changes in bone geometry in the lower limbs, and bone density and strength at the spine.

P77

Effect of fatigue-induced microdamage on the fracture resistance of human cortical bone

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Microdamage can be both detrimental and beneficial to the mechanical competence of bone. The accumulation of fatigue-induced microdamage can result clinically as fragility and stress fractures. Conversely, the formation of microdamage at highly stressed locations within the bone microstructure provides a mechanism to redistribute stresses and dissipate fracture energy. Microdamage formation is also the basis of various fracture toughening mechanisms including ligament bridging and crack deflection.

This study aimed to investigate the effects of the extent and distribution of fatigue-induced microdamage on the fracture resistance of human cortical bone. *In vitro* mechanical tests were undertaken using compact tension specimens (twenty per tibia) machined from the diaphysis of fresh-frozen human tibial bone (three tibiae, age at amputation 60-66) with the main crack orientated transverse or parallel to the bone long-axis. Microdamage was introduced into the specimens prior to fracture testing via fatigue loading. Sequential fluorochrome staining was used to label the main crack growth and microdamage prior to and during testing.

Preliminary results show a general decrease in the fracture resistance with an increase in the pre-fatigue cycles and thus amount of microdamage. Initiation fracture toughness for the specimens with the main crack orientated longitudinally ranged from 1.2 to 1.9 MPa$\sqrt{m}$ for no pre-fatigue cycling and from 0.5 to 1.5 MPa$\sqrt{m}$ after fatigue cycling to a 10% stiffness loss. Similar trends were displayed by the transversely orientated specimens. Continued experiments and analysis in this study will elucidate the effect of fatigue-induced microdamage on the fracture resistance of human cortical bone.

P78

Prognosis of fracture risk by quantitative ultrasound measurement and bone mineral density

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We sought to determine whether the combined use of calcaneal QUS and BMD measurements could improve the accuracy in fracture risk prediction, and to develop a nomogram based on the predictive model.

The study was designed as a population-based prospective investigation, which involved 407 women and 421 men aged 62-89 year, who had been followed for a median of 13 years during the period of 1994-2009. BMD was measured at femoral neck by DXA using GE Lunar DPX-L densitometer and BUA was measured at the calcaneus using CUBA sonometer.

During the follow-up period, 18% men (n=77) and 38% women (n = 154) had sustained a fragility fracture. Each standard deviation decrease in BUA was associated with a hazard ratio [HR] of fracture 1.86 (95%CI, 1.57-2.27) in women and 1.50 (95% CI, 1.19-1.94) in men. After adjustment for BMD, BUA remained significantly associated with fracture risk in women and men with reduced magnitude (HR 1.57, 95%CI, 1.26-1.95 in women; HR 1.32, 95% CI, 1.03-1.69 in men). Reclassification analysis also yielded a total net reclassification improvement (NRI) of 9.7% (p = 0.02) and 3% (p = 0.69) for women and men respectively. Overall, 21% of women and 33% of men were reclassified into a different risk category. Based on the estimated parameters of the final model, two nomograms were constructed for individualizing fracture risk prediction.

These results suggest that combination of QUS and BMD in form of a nomogram can enhance the accuracy of categorizing individuals according to their risk of fracture.

P79

Functional involvement of the microtubule-binding dynein-dynactin complex in osteoclast formation and bone resorption

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Osteoclastic bone resorption requires the co-ordinated interplay between acidified carrier vesicles laden with osteolytic enzymes (e.g. Cathepsin K), motor proteins and the underlying cytoskeleton in order to sustain the specialized structural and functional polarization of the ruffled border membrane. Cytoplasmic dynein, a mechanochemical motor comprising heavy, intermediate and light chains coupled to the dynactin co-factor complex, powers retrograde motility of diverse cargos to microtubule minus ends. Although cytoplasmic dynein is known to be essential for many cellular functions, its functional involvement in osteoclasts remains unclear. Here, we investigate the expression, localization and functional contribution of the dynein-dynactin complex in osteoclast formation and function. We demonstrate that dynein-dynactin complex is highly expressed in mature osteoclasts and is intimately coupled to the microtubules, undergoing drastic reorganization following the onset of osteoclastic polarisation. In bone-resorbing osteoclasts, $p150^{Glued}$, a major subunit of dynactin, exhibits distinct polarization of plus-end caps at the osteoclastic resorptive front, thus orientating the ruffled border as the microtubule plus-end domain. Global disruption of the dynein-dynactin complex via p50/dynamitin-overexpression significantly delays osteoclastogenesis, owing to an increase the mitotic stasis of mononuclear precursor cells. Moreover, disruption of dynein-dynactin motor leads to marked relocalization of intracellular organelles in osteoclasts including the Golgi and lysosomes as evidenced by confocal and live cell microscopy. Finally, we demonstrate that cytoplasmic dynein is required for the efficient delivery of Cathepsin K to the ruffled border membrane and thus osteoclastic resorptive function. Collectively, these data unveil the multifaceted roles of the dynein-dynactin complex in osteoclast formation and function.

P80

Ac45 mediates acidification and endocytosis during osteoclast bone resorption

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Solubilization of bone mineral by osteoclasts is dependent on the acidification of the extracellular resorption lacuna by means of a multimeric vacuolar type proton pump (V-ATPases). V-ATPases are also essential for acidification of diverse intracellular compartments that includes the Golgi, endosomes, lysosomes and secretory granules. Ac45 constituents an accessory subunit of the V-ATPase complex in all higher eukaryotes, however, its function remains incompletely understood. Previously, we have established that Ac45 is up-regulated in osteoclasts and that truncation of the 26 residue-cytoplasmic tail of Ac45 impairs bone resorption. Here, we have employed a gene silencing-based approach to further investigate the functional contribution of Ac45 in osteoclast formation and function. We demonstrate that knockdown of Ac45 severely disrupts osteoclastic resorptive function owing to impaired osteoclast maturation, intracellular acidification and receptor-mediated endocytosis, each consistent with a loss of VTPase function in osteoclasts. Moreover, examination of the interaction between Ac45 and other V-ATPase subunits revealed close proximity of Ac45 with subunits c", e and a3 of the V0 domain of the V-ATPase complex. Consistent with this, we found that Ac45 partially co-fractionates with the V0 domain subunits throughout the secretory pathway. We propose that Ac45 modulates V-ATPase function through its specific association with the V0 domain, thereby regulating intracellular acidification and receptor mediated endocytosis, both of which are crucial for osteoclast formation and function.

P81

The effects of arachidonic acid and docosahexaenoic acid on proliferation of and osteoclast formation from RAW 264.7 murine monocytes

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Several animal studies have shown that n-3 PUFAs may have a positive effect on bone. Recent findings indicate that docosahexaenoic acid (DHA) may inhibit osteoclast-mediated bone resorption while the effect of arachidonic acid (AA) is unknown. The aim of the study was to compare the effects of AA and DHA on proliferation of RAW 264.7 monocytes and osteoclastogenesis. For proliferation studies, RAW 264.7 cells were exposed to ethanol (vehicle control), AA and DHA at 5µg/ml to 20µg/ml. After 72 hours exposure, proliferation was quantified using a standardised crystal violet staining procedure. A triple-stain fluorescent procedure was performed to investigate possible mechanisms by which AA or DHA affected proliferation. Osteoclasts were generated by exposing RAW 264.7 cells to RANKL for 5 days in the absence or presence of AA or DHA at 5µg/ml to 20µg/ml. Tartrate-resistant acid phosphatase (TRAP) activity in the conditioned media and presence of osteoclasts were determined by a standardised colorimetric method and TRAP staining respectively. Proliferation was inhibited by AA at concentrations above 10µg/ml with DHA having an inhibitory effect across all concentrations. The triple-stain procedure revealed an increase in cellular acid content at 20µg/ml DHA compared to controls. AA had a slight inhibitory effect in contrast to DHA that dose-dependently reduced osteoclastogenesis. Both fatty acids inhibited proliferation; the triple stain suggested increased numbers of lysosomes or the formation of autophagic vacuoles in the presence of DHA, an indication of a form of cell death. Both fatty acids inhibited osteoclastogenesis, with DHA having a more pronounced effect.

P82

Grape seed proanthocyanidin extract inhibits osteoclast differentiation and resorption while having no effect on osteoblast proliferation *in vivo*

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The antioxidant properties of plant proanthocyanidins have been widely studied and used for their purported therapeutic properties. Grape seed proanthocyanidin extracts (GSPE) have been demonstrated to have an effect on bone metabolism in animal models *in vivo*. GSPE share common properties with other plant polyphenols that have been shown to have an effect on bone cells *in vitro*. In this study we tested the effect of a GSPE on osteoclast formation and bone resorption in two different *in vitro* models of receptor activator for nuclear factor-κB ligand (RANKL) activated osteoclast differentiation, and cell proliferation in osteoblasts. Osteoclast differentiation was stimulated by treating murine macrophage-like RAW 264.7 cells with murine RANKL, whilst CD14+ human peripheral blood mononuclear cells were treated with human RANKL and M-CSF. Non-differentiated cells or mature osteoclasts were treated with GSPE in the presence of differentiation factors. GSPE inhibited tartrate-resistant acid phosphatase (TRAP) activity and osteoclast formation in RAW 264.7 cells at concentrations of 1 µg/ml and 0.1 µg/ml respectively. GSPE also inhibited TRAP activity, mature osteoclast numbers and resorption in the CD14+ osteoclast model. In the osteoblast model, GSPE had no effect on osteoblast cell proliferation in murine MC3T3-E1 osteoblast-like cells at concentrations up to 1 µg/ml, and inhibited cell proliferation at concentrations above 10 µg/ml. The results of this study show that GSPE inhibits osteoclast differentiation whilst having no effect on osteoblast activity. Further studies are being undertaken to determine the mechanistic basis for the inhibition of osteoclast differentiation and function by GSPE.

P83

Culture of non-bone cells on bisphosphonate-coated bovine bone – an *in vitro* model of osteonecrosis of the jaw (ONJ)

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Osteonecrosis of the jaw (ONJ) is defined as an area of non-healing, exposed bone in the mouth present for more than several weeks. Cancer patients with tumours that metastasise to bone are commonly treated with high-dose bisphosphonate and ~5% of these patients develop ONJ often following invasive dental procedures. We hypothesise that ONJ is caused by toxicity to the soft tissues of the mouth, in particular epithelial cells, due to the very high concentrations of bisphosphonate deposited in the adjacent bone. During oral trauma and resulting bone damage, exposed bisphosphonate may have an effect on nearby cells in the area of injury.
To test our hypothesis *in vitro* we cultured epithelial cells (Caco-2 and CHO-S cell lines) on cortical bovine bone slices pre-coated with bisphosphonates. Cell growth was assessed by cell counts and thymidine incorporation at 24-72 hours. Pre-coated bone slices showed a significant reduction in cell number and thymidine incorporation at 48 hours and 72 hours (>60% reduction with zoledronate in both cell types; $P<0.05$) compared to control bones. These reductions were related to the clinical potency of the bisphosphonate used.
We have developed an *in vitro* model of ONJ that exposes epithelial cells to bisphosphonate bound to the bone surface. Our model suggests that the presence of bisphosphonate on bone inhibits epithelial cell growth which may contribute to the failure of the oral epithelium to heal in cancer patients treated high-dose bisphosphonates.

P84

Osteoblast growth and differentiation in two- and three-dimensional cultures

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Culturing osteoblastic cells on plastic provides useful information about their biology. However 2D plastic surfaces poorly mimic the complex 3D microenvironment where osteoblasts operate *in vivo*. Type I collagen, which sets as a gel at appropriate temperature and pH, can be used as a scaffold for 3D culture of cells. This study aimed to compare growth and differentiation of osteoblasts cultured in collagen gels with cells cultured on plastic.
Primary rat osteoblasts were cultured in parallel in plastic dishes and in 3mg/ml collagen minigels. Cell proliferation was measured by thymidine incorporation and differentiation was assessed using von Kossa and alizarin red staining. Gene expression during differentiation was examined by qRT-PCR.
Cells cultured in 3D responded to anabolic factors like TGFβ, PDGF and lactoferrin over a similar concentration range as in 2D, but the magnitude of the response increased in the gels. For example, 10pM TGFβ increased thymidine incorporation 1.6x in 2D and 3.3x in 3D. The targeted tyrosine kinase inhibitor imatinib, whose targets include the PDGF receptor, inhibited cell proliferation in both systems, with inhibition at concentrations 10x lower in 3D. Effects of 3D culture on differentiation included up-regulated expression of osteoblast markers alkaline phosphatase (20x), bone sialoprotein (40x) and osteocalcin (250x) in the first week of the assay and mineralisation occurred earlier in 3D than 2D.
Collagen minigels are simple to set-up and produce reproducible results. 3D culture of rat osteoblasts appears to amplify the effect of selected anabolic agents and promote osteoblast differentiation.

P85

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

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In vivo, blood calcium (Ca2+) levels are strictly regulated and total calcium levels usually lie within the range 2.2 – 2.6 mM in healthy individuals. The skeleton is the major reservoir for calcium and osteoblastic incorporation of calcium into bone matrix is an important homeostatic mechanism.(1) Mineralisation, the deposition of Ca2+ as hydroxyapatite-like calcium phosphate, occurs in lamellar bone concurrent with osteoblast to osteocyte transition. We hypothesised that Ca2+ is a potential anabolic stimulus of osteoblast differentiation. The aim of this study was to investigate the effect of Ca2+ on adult human primary osteoblasts (NHBC) in vitro and their differentiation into osteocyte-like cells.

NHBC were cultured under conditions permissive for mineralisation in the presence of a range of total Ca^{2+} levels (1.8 - 26.8 mM). Cultures were assayed for *in vitro* mineralisation by examining levels of cell associated calcium and phosphate using histochemical analyses and by using electron dispersive spectroscopy. The expression of osteoblast differentiation genes, including dentin matrix protein 1 (DMP1), E11 and SOST/sclerostin were measured by real-time RT-PCR. Supernatants were assayed for OPG secretion.

Treatment with Ca^{2+} demonstrated a striking dose- and time-dependent increase in mineralisation. The mineralizing cultures also expressed elevated mRNA levels of sclerostin, E11 and DMP1, all markers of osteocytes. Increasing calcium levels decreased expression of the mineralization inhibitor MEPE and increased levels of mineralisation promoter PHEX, suggestive of a mechanism by which cells could incorporate more mineral. Consistent with a net anabolic effect, Ca^{2+} promoted increased OPG secretion in the first 14 days of culture. This study provides new insight into the effect of Ca^{2+} on cells of the osteoblast lineage, and suggests the possibility that Ca^{2+} promotes the differentiation and/or survival of osteocytes.

1. Parfitt 2003 Bone 33:259.

P86

EphB/ephrin-B interactions mediate MSC recruitment and differentiation: potential implications in bone remodelling

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Mesenchymal stem cells (MSC) that reside within their perivascular niche of the bone marrow are essential for regulating skeletal tissue homeostasis, including bone formation and repair. The processes of bone homeostasis is well defined and is essentially regulated by two cell types, osteoblasts that give rise to MSC that produce bone, and osteoclasts that originate from the hematopoietic lineage and resorb bone. However, the molecular signals that maintain multi-potential MSC populations within the stem cell niche and the mechanisms that drive the mobilization of MSC towards the bone surfaces are not well defined. The Eph/ephrin family of receptor tyrosine kinases have been implicated in regulating bone homeostasis and the maintenance of stem cell niches in neural, intestinal and dental tissue. We characterized the gene and protein expression of EphB/ephrin-B molecules for human culture expanded MSC populations and in situ on tissue sections of human trephines. EphB2 displayed the most abundant expression, localising to the perivascular marker, CD146. EphB2 or ephrin-B1 fusion proteins, which bind and signal through their cognate ephrin-B ligand or EphB receptor, respectively, demonstrated that reverse ephrin-B signalling inhibits human MSC attachment and spreading while EphB forward signalling promotes MSC migration. Furthermore, activated ephrin-B molecules expressed by MSC promoted osteogenic differentiation. To elucidate the contribution of EphB/ephrin-B molecules to MSC skeletal tissue homeostasis, a murine femoral fracture model was used. Both EphB and ephrin-B genes significantly increased during the early stage of bone repair and remodelling and returned to steady-state levels at late stages of bone remodelling. EphB2 and ephrin-B1 were most abundantly expressed within the callus site, including blood vessels, hypertrophy and calcified chondrocytes, osteoblasts, osteocytes, and newly forming bone. In this study we have demonstrated the role of EphB/ephrin-B family members in human MSC migration and osteogenic differentiation, which may be extrapolated to the processes of bone fracture healing using a murine femoral fracture model. These observations suggest that EphB/ephrin-B interaction potentially maintain human MSC within their niche under steady state conditions, while promoting migration and skeletal tissue homeostasis following injury.

P87

Both class I and class II histone deacetylases (HDACs) are important during osteoclast development

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Histone deacetylases (HDACs) are enzymes that play an important role in regulating gene transcription and are increasingly becoming a therapeutic target for a wide variety of diseases. This study investigates the expression of various HDACs throughout osteoclast development and the effect of a novel dual class I and II HDAC inhibitor (1179.4b) on osteoclast formation. By identifying the key HDACs we may be able to target specific HDACs and minimize chances of side effects associated with broad spectrum inhibitors.
Osteoclasts were generated from human peripheral blood mononuclear cells (PBMCs) *in vitro* using receptor activator Nuclear Factor κB ligand and other factors over 17 days. Tartrate resistant acid phosphatase (TRAP) expression and resorption of dentine were used to assess osteoclast activity. Throughout the assay RNA was collected at various time points and the expression of each HDAC assessed using real time PCR.
1179.4b at 20nM was shown to result in a significant reduction in osteoclast numbers and activity as well as mRNA for NFATc1 ($p<0.01$) at day 17. The class I, HDAC 8 and class II HDAC 5 were significantly elevated at day 17 ($p<0.05$).
The results of this study indicate that inhibition of both class I and class II HDAC suppressed osteoclast formation by inhibiting NFATc1 formation during the terminal stages of osteoclast formation. In addition, the high mRNA expression of HDAC5 and 8 at day 17 is consistent with HDACs of both class I and II playing a key role during the terminal stages of osteoclast development.

P88

Role of neuropeptide Y1 receptor signalling blockade on osteoblast activity

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Neuropeptide Y (NPY) and Y1 receptor (Y1-R) have been shown to play an important role in the local control of bone remodelling. Mice lacking Y1-R gene display an increase of bone mass and Y1-R gene is expressed locally in bone marrow stromal cells and osteoblasts[1,2]. These evidences suggest that an anti-receptor strategy may be a useful therapeutical approach to bone regeneration. Nevertheless, the mechanisms underlying the action of local Y1 receptor signalling blockade on osteoblast activity are still unknown. The aim of our study was to evaluate the local effects of Y1-R signalling blockade in the regulation of osteoblast activity. Therefore, MC3T3-E1 pre-osteoblast cells were treated with a range of concentrations of NPY_{1-36} or co-treated with NPY_{1-36} + Y1-R antagonist BIBP3226, for 24 hours. The proliferation and survival rates, the gene expression profile of Y1-R and the triggered-downstream mechanisms, namely cAMP inhibition and MAPK/ERK pathway, were evaluated. Our results showed that NPY_{1-36} induced a slight increase on osteoblast proliferation, which was enhanced by the pharmacological blockade of Y1-R. This anabolic effect occurs with a concomitant down-regulation of Y1-R gene expression and is not mediated by cAMP inhibition but partially mediated through ERK1/2 activation. Moreover, Y1-R blockade in the presence of NPY_{1-36} also enhanced the survival rates of osteoblast-induced apoptosis. Taken together, the present data bring new insights into the therapeutic potential of Y1-R targeting on bone repair processes.

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P89

17-allylamino geldanamycin (17AAG) increases osteoclast formation in vitro through the induction of a cell stress response

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We previously found 17AAG (an HSP90 inhibitor and potent anti-tumour agent) increases both bone loss in mice and MDA-MB-231 breast cancer cell invasion of bone in a mouse cardiac injection model. 17AAG also elevates osteoclast formation *in vivo* and *in vitro*, including RANKL-stimulated bone marrow macrophages (BMM) and RAW264.7 cultures. However, the reason for this is unclear as many RANKL-dependent signals are at least partly HSP90-dependent. Since 17AAG blockade of HSP90 provokes cell stress responses, we investigated whether 17AAG actions on osteoclasts require stress responses dependent on transcription factor HSF1.

17AAG treatment elevated HSP70 and HSF1 levels in RAW264.7 cells and BMM, consistent with stress response; HSP70 induction was seen in murine embryonic fibroblasts (MEF) but not $HSF1^{-/-}$ MEF. HSF1-response element (HSE) luciferase reporter assays in UMR106 cells showed strong responses to 17AAG. Novabiocin, an HSP90 inhibitor that does not induce stress responses did not increase osteoclast formation. In contrast, HSF1 stimulator celastrol did, as did ethanol (1%) and H_2O_2 (400μM) which both induce stress responses. HSF1 inhibitor KNK437 blocked 17AAG induced stress responses and blocked 17AAG- and ethanol-enhanced osteoclast formation in RAW264.7 cells. In contrast, TGFβ-enhanced osteoclast formation was unaffected by KNK437. However, 17AAG did not enhance (indeed slightly reduced) RANKL-induced NF-κB and NFATc1 signals in RAW264.7 cells.

These data indicate that, although the intracellular pathways mediating 17AAG influences on osteoclast formation are unclear, they depend on stress responses mediated by HSF1, rather than reduced HSP90 function. This may suggest that stress responses could elicit general pro-osteolytic influences.

P90

Critical molecular regulators, histomorphometric indices and their correlations in the trabecular bone in primary hip osteoarthritis

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There is increasing evidence that systemic bone changes are an underlying cause of primary osteoarthritis (OA). In this study, differential gene expression, histomorphometric indices and linear correlations between these were investigated in primary hip OA patients and control (CTL) subjects; with the aim of identifying potential molecular drivers consistent with the changes in structural and remodelling indices in the OA pathology. Bone samples from the proximal femur intertrochanteric region were obtained from age and sex-matched cohorts of 23 OA patients and 21 CTL subjects. Real-time PCR and histomorphometric analysis were performed on each sample, and correlations between gene expression and histomorphometric variables determined. In OA bone, expression of critical regulators of osteoblast differentiation and function (TWIST1, PTEN, TIMP4) were decreased. Bone structural and formation indices (BV/TV, Tb.N, OS/BS) were increased, resorption indices (ES/BS, ES/BV) were decreased. Importantly, correlations in CTL bone between CTNNB1 expression and formation indices (OS/BS, OS/BV, OV/BV) were absent in OA bone, indicating altered Wnt/β-catenin signalling. TWIST1 expression and BV/TV were correlated in CTL bone, but not in OA bone, consistent with altered osteoblastogenesis in OA (fig.1). MMP25 expression and remodelling indices (ES/BS, ES/BV, ES/TV) were correlated only in OA pointing to aberrant bone remodelling in this pathology. These findings indicate an altered state of osteoblast differentiation and function in OA driven by several key molecular regulators. In association with this differential gene expression, an altered state of both trabecular bone remodelling and resulting microarchitecture were also observed, further characterising the pathogenesis of primary hip OA.

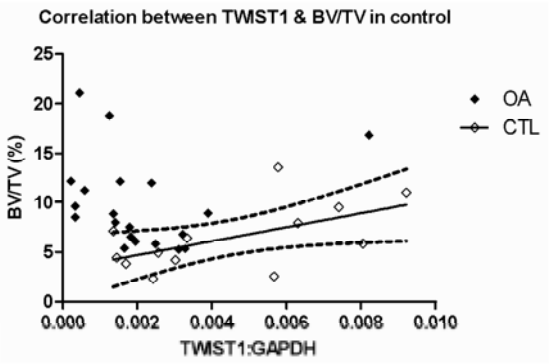

Figure1.

P91

The TWIST family of basic-helix-loop-helix transcription factors, Twist-1 and-2, mediate mesenchymal stromal/stem cell proliferation and differentiation

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The TWIST family of genes, Twist -1 and Dermo-1, are basic helix-loop-helix transcription factors that mediate mesodermal tissue development and contribute to correct patterning of the skeleton. The present study found that freshly purified human bone marrow-derived MSC express high levels of Twist-1 and Dermo-1. However, following ex vivo expansion these genes are down-regulated. Enforced expression of Twist-1 or Dermo-1 in human MSC cultures increased expression levels of the MSC marker, STRO-1, and early osteogenic transcription factors, Runx2 and Msx2. Conversely, we observed a decrease in the gene expression of osteoblast associated markers, bone morphogenic protein-2, bone sialoprotein, osteopontin, alkaline phosphatase and osteocalcin. Enforced expression of Twist-1 or Dermo-1 by cultured MSC resulted in an increase in proliferation potential of approximately 2.5 fold higher than vector control MSC lines. The growth differential was also shown to be associated with elevated levels of Id-1 and Id-2 gene expression. Functional studies demonstrated a reduction in the osteo/chondrogenic differentiation capacity of high expressing Twist-1 and Dermo-1 MSC, and an enhanced capacity of these cells to undergo adipogenesis. These findings implicate the TWIST gene family members as potential mediators of MSC self renewal and lineage commitment in postnatal skeletal tissues by exerting their effects on genes involved in the early stages of osteo/chondrogenic and adipogenic development.

P92

Differential gene expression in osteochondrosis lesions

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Osteochondrosis occurs in a number of species including horses; it is a developmental orthopaedic condition involving defective endochondral ossification and retention of cartilage in subchondral bone. The pathophysiology of this condition is poorly characterised, although our recent studies suggest that it does not result from failure of chondrocytes to undergo normal hypertrophy. The aim of the current study was to characterise changes in chondrocyte gene expression associated with the initiation of osteochondrosis. Early lesions were induced in an equine model of osteochondrosis by feeding foals a high energy diet. RNA was extracted from lesions in articular-epiphyseal growth cartilage and used for subtractive hybridization experiments. Eighty genes were identified as candidates for differential expression in osteochondrosis lesions compared to normal articular cartilage. The candidates include genes involved in development, cell death, chondrocyte hypertrophy, and osteoclast function, as well as extracellular matrix proteins. Quantitative PCR to confirm differential expression has shown that the expression of ATPase, H+ transporting, lysosomal 38 kDa V0 subunit D2 (ATP6V0D2), cathepsin K, integrin alpha V, low density lipoprotein receptor protein 4, thymosin beta 4, bone sialoprotein, osteopontin, lumican and ribophorin II was significantly increased in lesions compared to normal articular cartilage. These observations are likely to reflect genes undergoing primary changes in expression related to the disease, rather than secondary degenerative changes. Of additional interest, some genes such as ATP6V0D2, which is involved in osteoclast fusion and function, have not been previously described in chondrocytes, while others have been poorly characterised.

P93

JNK inhibitors promote osteogenic differentiation in NF1-deficient cells

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Neurofibromatosis (NF1) is a complex disorder with characteristic symptoms including skeletal manifestations such as scoliosis and congenital tibial dysplasia. The lack of standardized adjunctive pharmacotherapies combine with poor bone healing in these patients to make orthopaedic operations challenging. NF1 encodes neurofibromin, a negative regulator of Ras activation. This protein can signal through several downstream pathways including c-Jun N-terminal kinase (JNK). This study aimed to determine whether inhibition of the JNK pathway could be beneficial to bone cell differentiation in NF1-deficient osteoprogenitors.

Primary bone marrow stromal cells (BMSCs) isolated from *Nf1+/-* mice were differentiated under osteogenic conditions. The cells were treated with a range of concentrations of the JNK inhibitors SP600125 and CC401. The effects of co-treatment with BMP-2 were also examined. JNK inhibition was found to have a pro-osteogenic effect as measured by alkaline phosphatase assays, matrix mineralization and gene expression of both early (*Alp*) and late (*Ocn*) osteogenic markers. Synergistic effects were seen with BMP-2 treatment. To conclude, JNK inhibitors may be new and potentially useful adjunctive agents for treating the orthopaedic complications of NF1. This will need to be further evaluated in cell culture and pre-clinical models.

P94

Localisation of RBP4 in human suture derived cells from craniosynostosis patients suggests a site specific function

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Craniosynostosis occurs in 1 in 2500 live births and is the premature fusion of the cranial sutures, which are the growth regions separating calvarial bones. Although the cause of most craniosynostosis is unknown, both genetic and environmental factors have been implicated. In a recent microarray study of fused and fusing tissue from craniosynostosis patients we discovered that RBP4 is expressed in cranial sutures and that it is downregulated 37 fold in fused sutures. Hepatic RBP4 transports Vitamin A via serum to target tissues, however, the function of extra-hepatic RBP4 is unknown. Vitamin A metabolite retinoic acid is a potent inducer of osteogenesis and exposure to high levels during pregnancy has been linked to suture fusion and craniosynostosis.

Using primary cells grown from human suture tissue we have used immunocytochemistry to elucidate the localisation of RBP4 in these cells. Human suture cells display a distinct staining pattern, with RBP4 being polarised to one side of the cell and always surrounding the nucleus. The localisation of RBP4 in these cells differs from human hepatic cells in which RBP4 elicits a known function from which RBP4 is known to be secreted. Co-localisation studies suggest that RBP4 is located in the Endoplasmic Reticulum. This staining suggests that RBP4 is playing a localised role in the suture cells.

P95

Microtubule motor protein Tctex-1 interacts with Rab3D and is required for osteoclastic bone resorption

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Vesicular transport along microtubules must be strictly regulated to sustain the unique structural and functional polarisation of bone-resorbing osteoclasts. However, the molecular mechanisms bridging these vesicle-microtubule interactions remain largely obscure. Rab3D, a member of the Rab3 subfamily (Rab3A/B/C/D) of small exocytotic GTPases, represents a core component of the osteoclastic vesicle transport machinery. Here, we identify a new Rab3D-interacting partner, Tctex-1, a light chain of the cytoplasmic dynein microtubule motor complex by a yeast two-hybrid screen. We demonstrate that Tctex-1 binds specifically to Rab3D in a GTP-dependent manner and co-occupies Rab3D-bearing vesicles in bone-resorbing osteoclasts. Furthermore, we provide evidence that Tctex-1 and Rab3D intimately associate with the dynein motor complex and microtubules in osteoclasts. Finally, targeted disruption of Tctex-1 by RNA interference significantly impairs bone resorption capacity, attesting to the notion that components of the Rab3D-trafficking pathway contribute to the maintenance of osteoclastic resorptive function. Hence, we propose that Tctex-1 serves as a molecular adaptor, linking Rab3D-bearing vesicles to cytoplasmic dynein, thus facilitating microtubule-mediated transport during bone resorption.

P96

Mechanisms of erosive gout: monosodium urate monohydrate crystals inhibit osteoblast viability and function

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Gout is an inflammatory arthritis that is triggered by monosodium urate monohydrate (MSU) crystals within the joint. Bone erosion is a frequent manifestation of chronic gout, and leads to joint damage and deformity, with subsequent disability. We have previously shown that patients with erosive gout have disordered osteoclastogenesis and that MSU crystals indirectly promote osteoclast formation through interactions with stromal cells.

In this study we investigated the effects of MSU crystals on osteoblast cell viability and function. MSU crystals were prepared by recrystallisation of uric acid. Flow cytometry and the MTT assay were used to assess cell viability following MSU treatment in MC3T3-E1 and ST2 osteoblast-like cells, and in primary rat and human osteoblasts.

MSU crystals rapidly reduced viability in all cell types in a dose-dependent manner. The inhibitory effect was maximal at the higher concentrations of 0.3 and 0.5mg/mL MSU crystals and was independent of crystal endocytosis. The reduction of osteoblast cell viability was a specific effect of MSU crystals, as soluble uric acid did not affect cell viability. Furthermore, the effects on cell viability did not alter with different crystal lengths, or addition of serum. Long term culture of MC3T3-E1 cells with MSU crystals showed decreased mineralisation (bone nodule formation) and reduced expression of genes related to osteoblast differentiation.

These results indicate that MSU crystals contribute to bone erosion in gout not only through promotion of osteoclast formation, but also through reduction of osteoblast viability and differentiation.

P97

Mangiferin attenuates osteoclastogenesis, bone resorption and RANKL-induced activation of NF-κB and ERK

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Osteolytic bone diseases such as osteoporosis have a common pathological feature in which osteoclastic bone resorption outstrips bone synthesis. Bone resorption is dependent on osteoclast formation and its activity is regulated by a key TNF family cytokine known as receptor activator of nuclear factor κB ligand (RANKL). The induction of RANKL signaling pathway occurs following the tight interaction of RANKL to its cognate receptor, RANK. This specific binding drives the activation of downstream signaling molecules, which ultimately induce the formation and activation of osteoclasts. The aim of this study was to investigate the effect of a natural immunomodulator, mangiferin, on RANKL-induced osteoclastogenesis, bone resorption and signaling pathways. Mangiferin dose-dependently attenuated RANKL-induced osteoclastogenesis in primary bone marrow macrophage (BMM) cultures. In addition, mangiferin was shown to decrease the ability of BMM-derived mature osteoclasts to resorb bone. Results obtained from reverse-transcriptase polymerase chain reaction (RT-PCR) revealed that mangiferin diminish expression of osteoclast gene markers, including cathepsin K and calcitonin receptor, as well as decrease the expression of osteoclast cell-fusion gene markers, DC-STAMP and V-ATPase d2. Our mechanistic studies revealed that mangiferin inhibited RANKL-induced activation of NF-κB, concomitant with the inhibition of IκB-α degradation and p65 nuclear translocation. In addition, mangiferin also exhibited an inhibitory effect on RANKL-induced ERK phosphorylation. Collectively, our data demonstrate that mangiferin exhibit excellent anti-resorptive properties, supporting its use as a natural compound for the treatment and prevention of bone diseases involving excessive osteoclastic bone resorption.

P98

Homozygous deletion of Dickkopf-1 results in a high bone mass phenotype

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Dickkopf-1 (DKK1) is an antagonist of osteoblast differentiation through interaction with the LRP5 co-receptor, with complete deletion of *Dkk1* embryonically lethal. Recently, adult mice with complete absence of *Dkk1* have been generated by reducing the activity of Wnt3. Over 50% of mice of *Dkk1*-/-; *Wnt3*+/- genotype are viable. We examined the bone phenotype in *Dkk1*-/-; *Wnt3*+/- (HOM/HET) compared to *Dkk1*+/+;*Wnt3*+/+ (WT/WT).
Analysis of calvarial RNA showed no postnatal expression of Wnt3 in either genotype. Body mass did not differ. Whole body BMC was increased in both male (12%) and female (16%) HOM/HET mice compared to WT/WT ($p<0.05$). QCT scans of metaphyseal bone revealed increases in trabecular BMC in female HOM/HET (67%, $p<0.01$ vs WT/WT). Diaphyseal cortical bone volume was increased in female (31%) and male (27%) HOM/HET mice ($p<0.01$ vs WT/WT). Cortical thickness was increased in both female (22%) and male (20%) HOM/HET mice ($p<0.05$ vs WT/WT). MicroCT analysis of metaphyseal bone revealed a 3-fold increase in female and a 2-fold increase in male HOM/HET mice for BV/TV ($p<0.01$ vs WT/WT), which were associated with increases in trabecular number ($p<0.05$). Furthermore, trabecular BMD was increased 83% in female and 104% in male HOM/HET mice ($p<0.05$ vs WT/WT).
Preliminary histological analysis showed a 48% increase in trabecular MAR in female HOM/HET mice compared to WT/WT ($p=0.06$, N=4) but no alterations in osteoclast parameters.
In conclusion, our findings to date have revealed that the absence of DKK1 results in a robust high bone mass phenotype due to enhanced bone formation.

P99

The impact of pregnancy and lactation on bone in rat mothers exposed to uteroplacental insufficiency

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Pregnancy and lactation effect maternal bone mass providing offspring with calcium requirements. Uteroplacental insufficiency (UPI) complicates 10% of human pregnancies causing intrauterine growth restriction, lower pup body calcium and programming of bone deficits. We determined whether mothers exposed to UPI have altered skeletal phenotype.
Bilateral uterine vessel ligation (Restricted) or sham surgery (Control) was performed on gestational day 18 (term=22 days) in rats. Post mortem of Restricted and Control mothers was performed on prenatal day 20, postnatal day 1 and 7 and weeks 5, 7 and 9, and in non-pregnant rats. Right femur dimensions, mineral content and density were measured (peripheral quantitative computed tomography).
Trabecular content and density were lower on postnatal day 1 ($p<0.05$) in Control compared to Restricted. In Control rats only, cortical bone content and density increased by prenatal day 20, following the normal profile allowing fetal skeletal mineralisation, with content falling below non-pregnant by postnatal day 1 ($p<0.05$). These deficits in trabecular and cortical bone content and density did not occur in Restricted mothers. The stress strain index of bone strength decreased in Control rats only from prenatal day 20 to postnatal day 1 ($p<0.05$). By postnatal day 7, bone parameters in both groups were not different to non-pregnant, with complete restoration of bone occurring after weaning.
Mothers suffering UPI did not undergo the normal skeletal changes seen in Control rats. Presumably calcium supply to offspring was reduced during pregnancy and lactation, further limiting postnatal skeletal growth. The adverse maternal bone consequences were not long-lasting.

P100

The angiotensin converting enzyme inhibitor, captopril, reduces bone quality in ovary-intact rats but not in ovariectomized rats

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Angiotensin converting enzyme inhibitors (ACEIs) reportedly reduce the risk of fracture in humans and prevent osteoporosis in hypertensive rats. In this study we investigated the effect of short-term (4 week) and longer term (12 week) treatment of the ACEI, captopril (CAP), on bone in both ovary-intact (Sham) rats and ovariectomized (OVX) rats.
Eighty, 13 week-old female rats were divided into 8 groups of 10 consisting of 2 groupings each for Sham+saline, Sham+CAP, OVX+saline and OVX+CAP. One set of groupings was treated for 4 weeks and the other for 12 weeks. Both saline and CAP (2.5 mg/kg/day) were delivered via subcutaneously implanted mini-osmotic pumps. Histomorphometric analysis was performed on metaphyseal trabecular bone from femora. Biomechanical analysis was undertaken on humeri from the 12 week experimental groups.
CAP treatment suppressed bone remodelling in both Sham and OVX rats. There was almost a 10% decrease in trabecular bone volume in Sham+CAP compared to Sham+saline and bone formation rates were suppressed in Sham+CAP compared to Sham+saline over the 12 week period ($p < 0.05$).
Four weeks of CAP treatment to OVX animals reduced bone loss compared to OVX+saline; OVX+CAP contained about 90% more trabecular bone ($p < 0.01$) with almost a 55% reduction in bone formation rate ($p < 0.001$) compared to OVX+saline. But by 12 weeks, both OVX+CAP and OVX+saline exhibited similar, low trabecular bone volumes (i.e. around 10% each).
Most notably, breaking strains of humeri in Sham+CAP were about 25% less than Sham+saline ($p < 0.05$) and these low breaking strains in Sham+CAP were similar in magnitude to both OVX+saline and OVX+CAP.
Although there were neither beneficial nor detrimental effects on bone quality due to CAP treatment in OVX rats, the current data implicate that long term use of ACEIs might reduce bone quality in animals with normal ovarian function.

P101

Endothelin-1 receptor and myofibroblast-like cells in early soft callus: a potential role in fracture healing

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Smooth muscle-like viscoelastic and contractile properties have been demonstrated in early, soft fracture callus *ex vivo.* Cells responsible for this contractility are likely to be myofibroblast-like osteoprogenitor cells within fibrous matrix. In healing of soft tissues, myofibroblastic contraction and migration is vital and the peptide, endothelin-1 appears to be one of the most important factors that influences these processes. Accordingly, we aimed to investigate the presence of myofibroblast-like cells in early callus and explore whether endothelin-1 may also play a role in fracture healing.
Unfractured rat ribs along with calluses from rat rib fractures were removed 7, 14 and 21 days post-fracture and analysed using RT-PCR, Western blot and immunohistochemistry. Specific myofibroblastic markers investigated were alpha smooth muscle actin (αSMA), non-muscle myosin, fibronectin extra domain A variant (EDA fibronectin) as well as the receptor for endothelin-1 induced contraction i.e. endothelin receptor type A (ETA receptor).
mRNA expression for the above markers and ETA receptor was up-regulated in callus compared to unfractured bone; peaking at 7 days ($p < 0.05$) and returning towards unfractured levels by 21 days post-fracture. Western blot analysis exhibited an obvious increase in protein expression of αSMA, EDA fibronectin and ETA receptor in 7 day callus tissue. Immunohistochemistry localised expression of these proteins in osteoprogenitor cells of fibrous regions of callus. Notably, there was extensive co-immunolabelling of OB cadherin and αSMA in these cells, which is a distinguishable indicator of myofibroblasts.
This study provides further evidence that myofibroblast-like cells are present in early callus and may play a role in healing. Specifically we were able to localise these cells to the fibrous regions of callus, signifying that callus osteoprogenitor cells may be myofibroblast-like in nature. These results, together with callus expression of ETA receptor, suggests that endothelin-1, similar to that in soft tissue wound repair, may play a role in healing of bone fractures by inducing myofibroblast-like cell contraction.

P102

Increased bone VDR impairs osteoclast and osteoblast activities with low dietary calcium in a mouse model

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Transgenic mice with over expression of the vitamin D receptor in osteoblasts (OSVDR) have lower bone resorption and increased bone formation resulting in increased bone volume and strength compared to wild-type (WT) mice when fed normal dietary calcium. The OSVDR bone phenotype, however, is diminished to WT levels when fed a low dietary calcium. Thus we aimed to investigate the cellular and molecular mechanisms for this reduced bone volume. 6-week old female WT and OSVDR mice fed either low (0.1%) (WT-LCa, OSVDR-LCa) or high (1%) calcium diet for 3 months, after which animals were killed for analyses. The reduced bone volume in OSVDR-LCa mice occurred without increased serum PTH or RANKL-mediated osteoclastogenesis but with lower NFATc1 expression compared to WT-LCa levels ($P<0.001$), suggesting these OSVDR-LCa mice are less capable of osteoclastogenesis than WT mice. The reduced bone volume in OSVDR-LCa mice was associated with reduced mineralising surface and reduced Runx2, ALP, Col1, and osteocalcin mRNA levels compared to all other groups ($P<0.05$). Furthermore, OSVDR-LCa mice had markedly reduced serum 1,25D levels compared to WT-LCa mice ($P<0.001$), which was likely to be due to higher serum FGF23 levels ($P<0.05$). The inappropriately low 1,25D levels in OSVDR-LCa mice is associated with reduced intestinal calcium absorption, as indicated by CaBP9k expression ($P<0.01$). Thus, under conditions of low dietary calcium, increased sensitivity to vitamin D in osteoblasts results in increased FGF23 levels, inhibition of renal vitamin D synthesis and intestinal calcium absorption, impaired osteoclast and osteoblast activities, resulting in reduced bone mineral volume.

P103

Glutathione analogs are potent suppressors of PTH secretion from human parathyroid cells

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The human calcium-sensing receptor (CaR) plays a central role in calcium homeostasis and is allosterically modulated physiologically by L-amino acids and pharmacologically by phenylalkylamines, including cinacalcet, a clinically effective agent in hyperparathyroidism. Recent work has identified γ-glutamyl peptides as novel positive allosteric modulators of the CaR. We have tested the physiological significance of γ-glutamyl peptides, including the dipeptides γ-Glu-Cys and γ-Glu-Ala, and tripeptide, γ-Glu-Cys-Gly (glutathione) and analogs, S-methylglutathione (SMG) and S-propylglutathione, on intracellular Ca^{2+} (Ca^{2+}_i) mobilization and parathyroid hormone (PTH) secretion in normal human parathyroid cells. In addition, to explore their mechanism of action, we examined the effects of the potent γ-glutamyl peptide, SMG, on HEK293 cells that stably expressed either the wild-type CaR or the double mutant T145A/S170T CaR, which exhibits selectively impaired responses to L-amino acids. The γ-glutamyl peptides (0.1-10 μM) potently enhanced Ca^{2+}_i mobilization and inhibited PTH secretion in human normal parathyroid cells. Furthermore, the T145A/S170T CaR exhibited markedly impaired responses to SMG. The results indicate that extracellular glutathione and its natural analog, SMG, are potent suppressors of human PTH secretion and that glutathione analogs share a common mechanism of action with L-amino acid modulators.

P104

Volume resuscitation confounds the interpretation of serum vitamin D concentrations in critically ill patients

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Background: Recent reports have highlighted the prevalence of vitamin D deficiency and suggested an association with excess mortality in critically ill patients. Serum vitamin D concentrations in these studies were measured following resuscitation. It is unclear whether aggressive fluid resuscitation independently influences serum vitamin D.

Methods: A model of cardiopulmonary bypass (CPB) was chosen because of its finite volume load and a defined insult. It also allowed assessment of the effect of inflammation on vitamin D. Nineteen patients (14M,5F) were studied. Serum $25(OH)D_3$, $1,25(OH)_2D_3$, parathyroid hormone, CRP, and ionised calcium were measured at five defined timepoints: T1 - baseline, T2 - 5 minutes after onset of CPB (time of maximal fluid effect), T3 - on return to ICU, T4 - 24 hrs after surgery and T5 - 5 days after surgery. Linear mixed models were used to compare measures at T2-T5 with baseline measures.

Results: Hemodilution resulted in significant reductions in $25(OH)D_3$ (35%), $1,25(OH)_2D_3$ (45%) and i[Ca], with elevation in PTH. Serum $25(OH)D_3$ returned to baseline only at T5 whilst $1,25(OH)_2D_3$ demonstrated an overshoot above baseline at T5. There was a delayed rise in CRP at T4 and T5; this was not associated with a reduction in vitamin D levels at these time points.

Conclusion: Large volume fluid administration can significantly lower vitamin D levels independent of other factors; and therefore the timing of blood sampling to determine serum vitamin D is critical. Contrary to earlier reports, serum $25(OH)D_3$ was not reduced by inflammation.

	T1 (Base-line)	T2 (max fluid effect)	T3 (on ICU return)	T4 (24 hr after surgery)	T5 (Day 5)
Fluid balance(L)	0	3.5±1.2	3.0±1.5	2.5±1.2	1.1±0.8
$25(OH)D_3$ (nmol/L)	59±16	38±14*	49±15*	55±19*	63±23
$1,25(OH)_2D_3$ (pmol/L)	99±40	54±22*	74±31*	90±37	214±91*
PTH (pmol/L)	21±19	41±25*	13±10	12±5	5±3*
i[Ca] (mmol/L)	1.1±0.1	0.9±0.1*	1.1±0.1	1.1±0.1	1.2±0.04
CRP(mg/L)	6±9	4±5*	6±9	82±40*	138±58*

* P<0.05, compared with baseline

P105

Vitamin D and bone mineral metabolism in obese patients prior to laparoscopic adjustable gastric banding

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Vitamin D deficiency in obese individuals has been reported but poorly characterized. This study aimed to assess vitamin D concentration and bone metabolism in obese individuals awaiting laparoscopic adjustable gastric banding (LAGB). A cross-sectional study of serum calcium, 25-hydroxyvitamin D (25OHD), parathyroid hormone (PTH), calcium intake, and bone mineral density (BMD) in 23 obese (body mass index, BMI >35 kg/m^2) Caucasian individuals (women 74%, mean age ± SD: 44.3±10.5 years) waiting for LAGB. The mean weight and BMI of this cohort were 124.7±20.7 kg and 46.6±6.7 kg/m^2 respectively. Mean serum 25OHD was 65.3±25.0 nmol/L with 43% of subjects having levels less than 60 nmol/L. Only 4% had severe 25OHD deficiency (<25 nmol/L). The 25OHD concentration was inversely associated with BMI (r = -0.33, P <0.01) and PTH (r = -0.35, P <0.01). Only one individual had mild secondary hyperparathyroidism. Mean alkaline phosphatase (ALP) and serum beta-crosslaps were 80.0±18.5 IU/L and 221±164 ng/L respectively. No significant correlation was observed between 25OHD and serum calcium, ALP or beta-crosslaps levels. All individuals except one had BMD within the normal range (T-score <-1.5). Morbidly obese individuals are at risk of vitamin D deficiency. This is inversely correlated with PTH but no biochemical evidence of increase in bone turnover. Despite the reduced vitamin D level, BMD was still maintained within the normal range in most of these individuals. Further investigations are needed to evaluate the effects of weight loss and LAGB on these parameters and long-term bone health.

P106

Osteoclastic metabolism of 25(OH)-vitamin D3: a potential mechanism for optimization of bone resorption

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The extrarenal synthesis of 1α,25-(OH)2vitamin-D3 (1,25D) has been demonstrated in a number of cell types including osteoblasts and cells of the monocyte/macrophage lineage. The skeleton appears responsive to serum levels of the 1,25D precursor, 25(OH)-vitamin-D3 (25D), in terms of bone mineralisation parameters. The effect of metabolism of 25D into active 1,25D by osteoclast lineage cells is unknown. We found that CYP27B1 mRNA expression increased with exposure of human peripheral blood mononuclear cells (PBMC) to M-CSF in the presence or absence of RANKL. Consistent with this, human osteoclast cultures incubated with 25D produced measurable quantities of 1,25D. Osteoclast formation from either mouse RAW264.7 cells or human PBMC in the presence of physiological concentrations of 25D resulted in significant up-regulation of the key osteoclast transcription factor, NFATc1 in PBMC and a number of key osteoclast marker genes in both models. The expression of the osteoblast coupling factor, ephrin-b2, was also increased in the presence of 25D. Levels of CYP27B1 and NFATc1 mRNA correlated during osteoclastogenesis and also in a cohort of human bone samples. CYP27B1 shRNA knockdown in RAW264.7 cells decreased their osteoclastogenic potential. 25D dose-dependently reduced the resorptive capacity of PBMC-derived osteoclasts without compromising cell viability. 25D also reduced resorption by RAW264.7- and giant cell tumor (GCT)-derived osteoclasts. Conversely, osteoclasts formed from VDR-*null* mouse splenocytes had increased resorptive activity compared with wild-type cells. We conclude that 25D metabolism is an important intrinsic mechanism for optimizing osteoclast differentiation, ameliorating osteoclast activity, and potentially, promoting the coupling of bone resorption to formation.

P107

The role of vitamin D in the proliferation and differentiation of osteoblasts

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Calcitriol (1α,25-dihydroxyvitamin D_3 1,25D), is an important factor that suppresses osteoblast proliferation and stimulates differentiation. However, the role of vitamin D and interactions with calcium in osteoblast activity remains unclear. Wildtype C57BL/6 osteoblasts were isolated from cortices of long bones. Isolated cells were seeded for differentiation assays with various vitamin D and calcium treatments according to experimental requirements. The mineral deposition volume is assessed by Alizarin Red (for calcium) and Von Kossa (for phosphate) staining. Preliminary data indicate that under mitogenic conditions (i.e. 10% FCS), osteoblast mineralisation is reduced when treated with 1,25D, due to the suppression of cell proliferation. However, under conditions when cell proliferation rate is reduced (i.e. 2% FCS), both the substrate for 1,25D (25D) and 1,25D stimulate the maturation of osteoblasts as indicated by pronounced increase in mineralisation and the adoption of a mature cell morphology. Importantly, the effects of vitamin D on stimulating mineralisation are calcium concentration-dependent. The stimulating effects of both 25D and 1,25D on mineralisation are most pronounced in media containing 2.8mM $Ca2^+$. The standard mineralisation culture medium (~1.8mM) contains insufficient calcium for optimal mineralisation, which is consistent with findings with human primary osteoblasts (see Welldon *et al abstract*). These findings indicate that both proliferative and post-proliferative effects need to be taken into account in order to interpret the overall effect of vitamin D and calcium on bone mass. Current studies are employing the VDR*null* mouse to elucidate the direct roles of vitamin D in the above contexts.

P108

Vitamin D and bone health in Australian Aboriginals

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Aims: (a) to establish a normal range for 25D levels in this population;
(b) To test for a relationship between 25D and fasting blood glucose levels;
(c) To establish the 25D level at which bone turnover increases and parathyroid hormone level rises;
(d) To test for relationships between 25D and a range of factors which may influence vitamin D synthesis, storage and metabolism.
Methods: 58 Aboriginal participants were recruited via Nunkuwarrin Yunti (Adelaide) and Tullawon Health Service (Yalata). Participant demographics, smoking status, alcohol consumption, average time spent outdoors each day, body mass index (BMI), skin colour and medication use were recorded. Blood was collected after an overnight fast for 25-hydroxyvitamin D (25D), glucose, parathyroid hormone (PTH) and c-terminal telopeptide (CTX).
Results: Serum 25D values showed a normal distribution with a mean of 56.8 nmol/L. A seasonal variation was found, peaking in late summer/autumn and reaching a trough in late winter/spring. A statistically significant association was found between serum 25D and CTX, but not 25D and PTH. 25D levels were significantly associated with time spent outdoors, but not with level of pigmentation, body mass index, smoking status or level of alcohol consumption. No significant association was found between fasting glucose level and serum 25D.
Conclusions: Aboriginal participants in this study had lower 25D levels than those found in comparable published studies, especially in winter. Small sample size limited study power. The relationships outlined above and their implications for clinical practice and further research will be discussed and elaborated upon.

P109

The effect of receptor density on the function of the extracellular calcium-sensing receptor

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The calcium-sensing receptor (CaSR) is a class C G-protein coupled receptor that is activated not only by calcium, but also by L-amino acids and type II calcimimetics, including cinacalcet (Sensipar). Primary hyperparathyroidism occurs most commonly in patients with adenomatous disease of a single parathyroid gland and arises as a result of impaired Ca^{2+}_o-dependent feedback on PTH secretion in a CaSR mediated process. In addition, adenomatous human parathyroid cells exhibit impaired sensitivity to calcium and L-amino acids[1]. The origins of the impaired sensing by adenomatous parathyroids is still uncertain and while no inactivating mutations of the CaSR have been reported[2], reduced receptor expression is a recognised feature[3].

To determine whether the level of expression plays a role in CaSR function, a tetracycline-induced expression system was developed in HEK-293 cells (T-Rex293-CaSR). Incubation with 50 ng/mL of tetracycline for 10h led to a 7-fold increase in total expression, as determined by western blotting, and a 2.5-fold increase in surface expression in an ELISA-based assay, in comparison to uninduced cells.

Increases in tetracycline concentrations (0 – 500 ng/mL) led to enhanced sensitivity to Ca^{2+}_o and L-Phe in concentration response analysis. In addition, these increases in receptor expression increased the number of activated cells (Table 1) and the oscillation frequency in response to varying concentrations of Ca^{2+}_o and L-Phe.

Taken together these results indicate that CaSR expression levels can affect the sensitivity of the receptor to Ca^{2+}_o and L-Phe and suggest that the impaired sensitivity of adenomatous parathyroid may be due to decreased receptor expression.

Table 1: Effects of various concentrations of tetracycline on the percentage of oscillating T-Rex293-CaSRs. Total cells = 20 – 30 for all experiments

	Ca^{2+}_o (mM)			*L-Phe (mM) at 2.0 mM Ca^{2+}_o*		
Tetracycline (ng/mL)	*2.0*	*6.0*	*10.0*	*0.3*	*3.0*	*30.0*
0	0.00%	16.70%	20.00%	0.00%	0.00%	0.00%
5	0.00%	20.00%	30.00%	0.00%	40.00%	100.00%
50	23.30%	93.30%	73.30%	25.00%	65.00%	25.00%
500	15.00%	80.00%	35.00%	30.00%	100.00%	30.00%

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P110

Intermittent high-dose vitamin D3 corrects vitamin D deficiency in adolescents: results from a randomised controlled trial

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Aims: Vitamin D deficiency is common in adolescents, but the optimum vitamin D dosage regimen to correct deficiency in children is unknown. Adherence to daily supplements is often poor. This study aimed to assess the safety and efficacy of intermittent high-dose vitamin D supplementation to correct deficiency in adolescents.
Methods: In this double-blind randomised controlled trial, healthy adolescents with serum 25 hydroxy-vitamin D (25OHD) 12.5-50 nmol/l were allocated to receiving 300 000IU or 150 000IU of vitamin D3 or placebo orally 6-monthly. We measured outcome factors of serum 25OHD (DiaSorin radioimmunoassay) at baseline, 3, 6 and 12 months and serum calcium at 2 weeks. We performed an intention-to-treat analysis using random effects mixed models.
Results: Baseline characteristics of the 22 participants were similar across groups. The mean increase in serum 25OHD was 35 nmol/l higher in the 300 000 IU group than placebo ($p<0.05$) but was not significantly different from placebo in the 150 000 IU group. At twelve months, 1/5 participants (20%) receiving 300,000 IU were deficient, with a borderline serum 25OHD of 49 nmol/L while 5/6 (83%) and 4/7 (57%) participants remained deficient in the placebo and 150,000 IU group respectively. No participants developed hypercalcaemia and there were no other adverse events. We achieved 100% compliance.
Conclusions: We identified a safe, efficacious and effective dosage regimen for the correction of vitamin D deficiency in adolescents. This is an important step in the development of an appropriate and feasible screening and treatment program for vitamin D deficiency in adolescents.

P111

The skeletal response to vitamin D supplementation during sunlight deprivation: a randomised trial in Antarctic expeditioners

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Antarctic expeditioners experience prolonged sunlight deprivation that results in vitamin D insufficiency (25(OH)D <50 nmo/l). The deficiency occurs within 4 months unless baseline values are greater than 100 nmol/L. To test the hypothesis that the maintenance of vitamin D sufficiency (>50 nmol/L) required repeat dosing we randomly assigned 90 expeditioners (mean age 44 yrs) to vitamin D3; 50 000IU at departure, monthly or 2 monthly, for 12 months. Participants provided serum samples at departure, 6 and 12 months for assay of vitamin D (25(OH)D), parathyroid hormone (PTH) and bone markers (Osteocalcin). Differences were assessed using repeated measures ANOVA. At baseline, mean 25(OH)D was 64 ± 2 nmol/L. After 12 months, serum 25(OH)D had decreased by 20 ± 6% ($p < 0.01$) in those receiving a single dose, increased by 26 ± 6%, ($p < 0.01$) in the monthly regimen, and was maintained in the two monthly regimen (2 ± 7%, NS). Bone turnover markers were elevated in the single dose (21 ± 8%, $p < 0.05$) and 2-monthly (16 ± 5%, $p < 0.01$) groups and unchanged using the monthly regimen. No group differences were detected for PTH. During an expedition, 50 000IU vitamin D every 2 months was sufficient to maintain serum 25(OH)D levels, but a monthly dose is required to improve vitamin D status so should be considered for those with vitamin D insufficiency prior to departure.

P112

Reduced mechanical loading during growth results in deficits in cortical bone structure: a study of children with Legg-Calve Perthes Disease

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Bone size and cortical area is greater in the playing, than non-playing arm of tennis players, especially in those who commenced playing before puberty. There is little data assessing the structural basis underlying altered weight bearing in children.

We compared the side-to-side differences in tibial bone structure in children with unilateral Legg-Calve Perthes Disease (limited unilateral weight bearing) to test the hypothesis that relatively increased loading on the unaffected side increases total bone CSA and cortical area, while the affected side will have a smaller total CSA and cortical area relative to controls.

We compared distal tibiae architecture using HR-pQCT in 27 cases (n = 21 male, 11.1 ± 0.5yrs, 68% pre-pubertal, mean disease duration 1.4 ± 0.3yrs) and 27 controls (n = 21 males, 11.6 ± 0.6yrs). In cases, cortical area (8 ± 4%, $p<0.05$) and CSMI (4 ± 3%, $p<0.05$) were lower on the affected than unaffected side. The side-to-side difference for cortical area in cases was greater than controls (8 ± 4% v –2 ± 2%, $p<0.05$). In pre-pubertal cases, there was a trend for reduced cortical area of the affected limb compared to the same side in controls after adjusting for multiple comparisons (53.9 ± 5.1 v 64.6 ± 1.8 mm^2, $p <0.05$).

Unilateral loading did not enhance bone size or cortical area but unloading was associated with reduced cortical area but not total cross-sectional area. Reduced loading on weight bearing bones compromises bone strength and increases fracture risk.

P113

Structural deficit in children with forearm fractures

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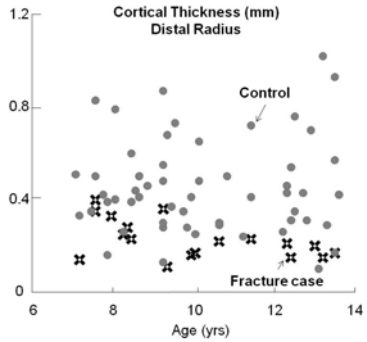
Forearm fractures are common in children and are associated with reduced bone mineral density (BMD). The highest incidence coincides with the pubertal growth spurt, when there is a transient reduction in volumetric BMD (vBMD) and cortical thickness at the distal radius. These observations lead us to hypothesize that bone structure deficits, especially reduced cortical thickness, are present in children with forearm fractures. We recruited 20 children with low trauma forearm fractures and scanned their distal metaphyses of the contralateral radius and tibia using high resolution peripheral quantitative computed tomography within 2 months post-fracture. Each fracture case was matched to 2-4 age and sex-matched controls (n =70). Compared to controls, distal radius vBMD was 14% lower in cases (234 ± 31 vs. 272 ± 49 mg HA/cm^3, $p < 0.001$) due to their 50% thinner cortices (0.22 ± 0.09 vs. 0.45 ± 0.20 mm, $p < 0.001$) (Fig). There was no group difference in trabecular bone volume fraction (BV/TV). However, trabecular architecture was fashioned differently – fracture cases had thicker (81 ± 7 vs. 69 ± 10 µm, $p < 0.001$) but less (1.71 ± 0.20 vs. 2.06 ± 0.26 /mm, $p < 0.001$) trabeculae than controls. In commensurate with the observation at the distal radius, fracture cases also had thinner cortices and differently fashioned trabecular architecture relative to controls at the distal tibia. We conclude that the deficit in cortical thickness partly contributes to the risk of forearm fractures during puberty, while the significance of trabecular architecture needs further investigation.



P114

Wnt7b plays a unique and essential role in osteoblast differentiation

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Activation of the Wnt signaling pathway is vital for osteoblast differentiation. We previously found that the mRNA expression of Wnt7b and Wnt10b increases linearly with osteoblast differentiation (1). To define the individual roles of Wnt7b and Wnt10b in the control of osteoblast differentiation, we knocked down Wnt7b or Wnt10b expression by shRNA stable expression in MC3T3-E1 cells.

Knockdown of Wnt7b in MC3T3-E1 cells resulted in a complete failure of mineralized nodule formation, while non-target (NT) control cells formed significant amounts of nodules by day7. When Wnt10b mRNA was knocked down, the mineralized nodule formation was delayed and reduced by 75% compared to NT control cells. Interestingly, Wnt7b mRNA levels were 4-fold higher in Wnt10b-shRNA cells then seen in NT cells by day8 suggesting Wnt7b may compensate for reduced Wnt10b expression during osteoblast differentiation. In contrast, Wnt10b is unable to compensate for a reduced expression of Wnt7b in Wnt7b-shRNA cells. Consequently, while mRNA for ALP and osteocalcin was suppressed in Wnt7b-shRNA cells, expression was delayed in Wnt10b-shRNA cells. In addition, the osteopontin mRNA levels were 2- and 2.5-fold higher in Wnt7b-shRNA cells than seen in NT cells at day2 and day4 respectively, indicating that differentiation in these cells was arrested at an early stage. Importantly, treatment of Wnt7b-shRNA cells with rBMP2 rescued the phenotype as the cells formed same amount of mineralized nodules as seen in BMP2 treated NT cells, suggesting that BMP may be an important downstream mediator of Wnt7b signaling. We conclude that both members of the Wnt family are relevant to normal osteoblast differentiation and nodule formation but that Wnt7b has a unique function in the control of bone formation, as a "feed-forward" loop that promotes osteoblast differentiation and mineralization.

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P115

Nilotinib inhibits osteoblast proliferation and differentiation, and inhibits osteoclastogenesis

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Nilotinib (AMN107) is a second generation tyrosine kinase inhibitor (TKI) developed to manage imatinib-resistance in patients with chronic myeloid leukaemia (CML), with enhanced activity against the Abl family of tyrosine kinases (TK) (including Bcr-Abl), and activity also against the c-kit and platelet-derived growth factor receptor (PDGFR) tyrosine kinases. Nilotinib exhibits off-target effects in other tissues, and of relevance to bone metabolism, up to 16% of patient receiving nilotinib develop hypophosphataemia (1). Nilotinib has been shown to inhibit osteoclast formation and function, and promote osteoclast apoptosis *in vitro* (2). The aim of our study was to assess the actions of nilotinib on bone cells *in vitro*. We therefore investigated the effects of nilotinib on proliferating and differentiating osteoblastic cells, and on osteoclastogenesis in murine bone marrow cultures and RAW264.7 cells. Nilotinib potently inhibited osteoblast proliferation (0.01-1uM), predominantly through inhibition of the PDGFR. In osteoblastic cells, differentiation was reduced by lower concentrations of nilotinib (0.05-0.5uM), with no effect at higher concentrations (1uM), possibly due to the relative potency of action against Abl and the PDGFR. Nilotinib also potently inhibited osteoclastogenesis, by stromal-cell dependent mechanisms. Thus, nilotinib decreased osteoclast development in murine bone marrow cultures, but did not affect osteoclastogenesis in RAW264.7 cells. Nilotinib treatment of osteoblastic cells increased expression of OPG and decreased expression of RANKL. Thus, *in vitro* nilotinib has significant inhibitory effects on osteoblast growth, and osteoclast development, and has a neutral or inhibitory effect on osteoblast differentiated function. These data suggest that nilotinib may affect skeletal function in vivo, a possibility that warrants further investigation.

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P116

Endogenous opioids regulate bone remodelling via actions on hypothalamic Neuropeptide Y

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Exogenous opioids exert powerful effects on bone mass via endocrine and non-endocrine effects, increasing hip fracture by around 2-fold. We examined the bone phenotype of mice null for the endogenous opioid Dynorphin ($Dyn^{-/-}$) and the potential involvement of NPY.

Cancellous bone volume was elevated in $Dyn^{-/-}$ (wt: 8.8 ±0.6 vs $Dyn^{-/-}$: 11.9% ±1, $p<0.02$). Osteoclast surface (7.9% ±0.7 vs 11.9 ± 0.7, $p<0.01$) and osteoclast number (3.3/mm ±0.3 vs 4.4 ± 0.2, $p<0.05$) were elevated in $Dyn^{-/-}$. However, these changes were overridden by increased mineral apposition rate (MAR) in $Dyn^{-/-}$ (1.6μm/d ±0.1 vs 2.4μm/d ±0.2, $p<0.02$).

Dynorphins signal mainly through the kappa opioid receptor (KOR); however, this receptor does not appear involved in the skeletal changes. There was no skeletal phenotype in $KOR^{-/-}$ mice. Moreover, KOR was expressed in brain not bone, and KOR agonism induced a response in neurons but not osteoblasts, thereby indicating an indirect action.

Loss of dynorphin signalling is known to reduce NPY expression in the hypothalamus, a change known to elevate bone formation. Both $NPY^{-/-}$ and $Dyn^{-/-}$ mice have elevated bone mass. However, $NPY^{-/-}/Dyn^{-/-}$ mice showed no further increase compared to single mutant mice (MAR wt: 1.3μm/d ±0.06, $NPY^{-/-}$: 1.5μm/d ±0.06, $Dyn^{-/-}$: 1.6μm/d ±0.08, $NPY^{-/-}/Dyn^{-/-}$: 1.5μm/d ±0.04). This indicates a critical role for NPY in the transmission of the central dynorphin pathway.

The dynorphin system, acting via NPY, may represent a pathway by which higher processes including stress, addiction and depression influence skeletal metabolism and may enable modulation of the adverse effects of exogenous opioids.

P117

Methionine199 in the TNF-like core domain of RANKL is crucial for RANKL function

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Patients with autosomal recessive osteopetrosis (ARO) present with elevated numbers of non-functional osteoclasts classically termed osteoclast-rich ARO. Recently, mutations within the TNF-like core domain of Receptor Activator of NF-κB Ligand (RANKL) have been shown to underlie a subset of ARO characterized by the absence of osteoclasts (osteoclast-poor ARO). The TNF-like core domain (aa160-318) constitutes the structural core of RANKL activity owing to its role in homotrimerization, RANK receptor binding and activation. In fact, we have previously shown that this domain alone is sufficient to support osteoclast formation *in vitro*. However, the structural and functional consequence of TNF-like core domain mutations has not been well characterized. Here we describe the functional propensity of human M199K (methionine199 to lysine) amino acid substitution mutation in osteoclast differentiation, and its impact on RANKL-mediated signaling. The M199K mutation results from a single-nucleotide-change (596T→A) in exon 8, a region not predicted to be essential for RANKL homotrimerization nor RANKL-RANK interaction but highly conserved throughout evolution. We showed that recombinant M199K RANKL mutant exhibits diminished osteoclastogenic potential and blunted activation of key osteoclastogenic signaling cascades including NF-κB, NFATc1, ERK, and c-Fos. Interestingly, single amino acid substitution of the methionine199 to alanine (M199A) or to glutamic acid (M199E) also showed diminished osteoclast formation and lack of activation of RANKL-induced signaling pathways. Using BIAcore SPR analysis, the binding properties of M199K, M199A and M199E to RANKL and receptor RANK is being explored. Taken together, our data demonstrates that methionine199 is critical for RANKL functions and might serve as therapeutic target for osteoclast inhibition.

P118

Regulation of OPG expression in UMR-106 osteoblast cells by angiotensin II

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Angiotensin II (Ang II), a peptide hormone comonly associated with cardiovascular and renal function, is the major regulator of cardiovascular homeostasis. High blood pressure has been negatively correlated with the development of osteoporosis. Ang II stimulates osteoclastic bone resorption via up-regulation of RANKL and decreases osteoblast differentiation and activation. The aim of this study was to investigate the regulation of osteoprotegerin (OPG) expression in osteoblast-like cells by Ang II.
We found that the UMR-106 osteoblast cell line express angiotensin receptor subtypes AT_1 and AT_2 and OPG. UMR-106 cells were stimulated with Angiotensin II [Ang II] and its bioactive fragments; Angiotensin II (1-7) [Ang (1-7)] and Angiotensin (3-8) [Ang IV]. Total RNA was isolated with Trizol and OPG expression analysed semi-quantitatively by RT-PCR.
Ang II ($1x10^{-8}$M) induced a significant 2-fold up-regulation in AT_1 expression and a 50% reduction in OPG expression when compared to unstimulated cells. No significant change in OPG expression was observed when UMR-106 cells were treated with Ang (1-7) or Ang IV.
These data show that Ang II, but not its bioactive fragments Ang (1-7) or Ang IV inhibits the expression of OPG in UMR-106 cells. This inhibition, together with the Ang II induced stimulation of RANKL could explain the decreased bone mineralisation reported in hypertensive patients.

P119

Role of glypicans in regulation of bone morphogenetic protein signaling during skull bone growth

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Craniosynostosis is the premature bony fusion of cranial sutures, a developmental disorder that affects one in 2500 live births. It can have dramatic consequences for affected children, including raised intracranial pressure, impaired vision and hearing, intellectual disability and the psychological problems due to different head shapes.
We have previously demonstrated using microarray studies that the heparan sulfate proteoglycan, glypicans 3 (GPC3) is down regulated during human suture fusion (Coussens et al. 2007). GPC3 has been shown to regulate BMP activity in non osteoblastic cells (Midorikawa et al. 2003). We propose that reduced levels of GPC3 are one of the possible causes of suture fusion. Using confocal microscopy and flow cytometry analysis, we have demonstrated that GPC3 is localized at the cell surface and intracellularly in human suture cells. Transient transfection analysis of a GPC3 expression vector and BMP responsive promoter-reporter construct, suggests that the BMP pathway is repressed by ectopic expression of GPC3 in human suture cells. The data favor our proposed model that reduced GPC3 may lead to a hyperactive BMP pathway and contribute to craniosynostosis in children.

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P120

Oncostatin M is a potent regulator of IL-6 and RANKL mRNA expression in synovial fibroblasts

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In rheumatoid arthritis (RA), TNF augments osteoclastogenesis and bone erosions in part by stimulating the osteoclast support function of osteoblasts and synovial fibroblasts (SFs). Oncostatin M (OSM) stimulates osteoclast formation via osteoblasts and is produced at high levels by macrophages and T cells in RA, but its effects on SFs are unknown. This study used qRT-PCR to compare the acute effects of murine (m) OSM or TNF on IL-6, RANKL and OPG mRNA levels in mouse SFs.
OSM mRNA was not expressed by normal SFs, but OSM treatment (2 and 50ng/mL) rapidly induced RANKL mRNA 10-fold at 1 h and 100-fold at 6 h, when there was also a 2-fold increase in OPG. In contrast, the maximal dose of TNF (7.5ng/mL) increased RANKL 10-fold and reduced OPG 2-fold. OSM treatment also increased IL-6 mRNA levels 100-fold, compared to a 10-fold induction by TNF. OSM is therefore a more potent stimulus of IL-6 and RANKL mRNA expression in SFs than TNF.
OSM acts through gp130 bound to either OSM receptor (OSMR) or leukemia inhibitory factor receptor (LIFR); SFs express each of these receptor components. mOSM stimulated OSMR mRNA levels 4-fold at 1 and 6 hrs, but gp130 and LIFR mRNA levels were not significantly altered. TNF did not modify SF OSMR or gp130 mRNA expression but increased LIF and reduced LIFR expression at 6 hrs. Synovial fibroblasts are abundant in RA synovium and these data suggest that targeting OSMR signaling may potentially limit SF-mediated inflammation and bone erosion in RA.

P121

Actions of novel adipokines on bone cells

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Fat mass is positively related to bone density and inversely to fracture risk. This relationship is partly due to some adipokines, such as leptin, resistin and adiponectin based on the studies from our and other groups. In this study, we screened effects of novel adipokines, at peri-physiological concentrations, using *in vitro* assays: osteoclastogenesis, in mouse bone marrow cultures, and rat osteoblast cultures. The adipokines included visfatin, retinol-binding protein 4 (RBP4) and fasting-induced adipose factor (FIAF). The novel adipokines were also screened for their presence in bone marrow.
Our results show that visfatin and RBP4 had no effect on osteoblast mitogenesis. In addition, visfatin had no effect in osteoclast formation. FIAF had no action on osteoblasts, but potently inhibited osteoclastogenesis by about 30 and 50% at 250 and 500 ng/mL, respectively. Microarray analysis and real-time PCR showed that visfatin was highly expressed in bone marrow, while RBP4 was expressed at low levels and FIAF was not expressed.
Screening of further novel adipokines in bone cells indicates that FIAF could be another factor involved in the fat mass/bone mass relationship. Interestingly, FIAF is up-regulated in the adipose tissue in a mouse model of obesity as reported previously. Further studies will explore the mechanism of FIAF's action in bone and its interaction with other adipokines.