ORALS

001

SECULAR TRENDS IN FRACTURE INCIDENCE: WHY ARE FRACTURES INCREASING IN ASIA BUT FALLING IN THE WEST?

C. Cooper1,2
1MRC Life Course Epidemiology Unit, University of Southampton, Southampton, Great Britain
2Institute of Musculoskeletal Sciences, University of Oxford, United Kingdom

Osteoporosis constitutes a major public health problem through its association with age related fractures. These fractures typically occur at the hip, spine and distal forearm. It has been estimated from incidence rates derived in North America that the lifetime risk of a hip fracture in Caucasian women is 17.5%, with a comparable risk in men of 6%. Age and sex-adjusted hip fracture rates are generally higher in Caucasian than in Asian populations. Furthermore, the pronounced female preponderance in fracture incidence observed in white populations is not seen amongst blacks or Asians in whom age-adjusted female to male incidence ratios approximate unity. Life expectancy is increasing around the globe and the number of elderly individuals is rising in every geographic region. Assuming constant age-specific incidence rates for fracture, the number of hip fractures occurring worldwide among people aged 65 years and over will rise from 1.66 million in 1990 to 6.26 million in 2050. Studies performed in the United States, Scandinavia, and the United Kingdom, between 1930 and the late 1980s, consistently reported increases in the age-adjusted incidence of hip fractures among men and women. This increase appears to have levelled off, in the northern regions of the United States, as well as more recently in Europe. Rates in Asian populations continue to show substantial rises between the 1960s and the present time. In the most recent data available from the United States, the incidence of first ever hip fracture declined by 1.37% per year among women and 0.06% per year among men. The cumulative incidence of a second hip fracture after 10 years was 11% among women and 6% among men, when death was treated as a competing risk. The reduction in hip fracture occurrence was even greater than that expected from the declining incidence of hip fractures more generally. Age-period-cohort models have suggested influences of all three contributors to these secular trends. Among current risk factors for low bone density and trauma (low body mass index, cigarette smoking, alcohol consumption, physical inactivity and dietary calcium intake) the trends are best explained by physical inactivity. Developmental contributors to peak bone and muscle mass, for example maternal nutrition and lifestyle, also appear capable of contributing to cohort effects. Finally, debate continues on the role of more aggressive osteoporosis risk assessment and therapeutic strategies in contributing to the secular decline in hip fracture rates generally. Although pharmacologic intervention might be efficacious, only a minority of hip fracture patients remain so treated, and the scope for even greater reductions in incidence remains an enticing prospect.

002

STRATEGIES TO OVERCOME EPIDEMIC OF VITAMIN D DEFICIENCY IN ASIA

A. Mithal
Endocrinology and Diabetes Division, Medanta, Haryana, India

Vitamin D is an integral determinant of musculoskeletal health. Reports from across the world indicate the presence of widespread Vitamin D deficiency. A 2009 IOF review of global vitamin D status highlights the prevalence and severity of vitamin D deficiency in Asia. Despite abundant sunshine, populations from many countries in Asian region, in particular in South Asia and possibly China are severely affected by vitamin D deficiency. In India, for example, all ages (newborns, children, adults, pregnant women and elderly) have been shown to be affected. Among factors that have been blamed for this epidemic in Asia is skin pigmentation, cultural practices like avoidance of sun exposure, and traditional clothing that allows limited skin exposure. Fortification of food products with Vitamin D is not widely practiced in Asia.

The majority of the world's population lives in this region of the world, and, given the diverse nature of the countries in the region, each with their own set of unique issues and problems, it is difficult to envisage a uniform strategy for the region as a whole. However, the approaches can broadly be divided into 3 parts-

1. Increasing awareness about importance of sun exposure and outdoor activities in the population, particularly in children.
2. Designing and implementing vitamin D fortification programs. This requires combined expertise of ‘vitamin D’ experts, nutritionists, chemists, food technologists and social workers, so that a comprehensive plan can be presented to policy makers. Choice of the appropriate food items to be fortified and monitoring strategies are essential to the success of such a program.
3. Selective and judicious use of vitamin D supplements in populations ‘at risk’. Typical examples include schoolchildren and the elderly. The dose required to achieve adequate 25(OH) D levels may be much higher than internationally recommended, due to low baseline levels. Studies from India show that at least 2000 IU of cholecalciferol is required daily to achieve optimum serum 25(OH) D levels.

There is an urgent need for Asian countries to formulate strategies for combating this epidemic, based on country specific needs and situations.
STRATEGIES FOR PREVENTING OSTEOPOROSIS IN ASIA-WHERE DO WE GO FROM HERE?
E. Lau
Asian Pacific Osteoporosis Foundation, Changing District, China

Osteoporotic fracture in Asia is a major public health challenge for several reasons:
- The incidence is rising rapidly.
- The absolute number is very large due to a large aging population.
- In countries in which the problem is most frequent eg in India and China, many patients cannot afford the drugs for osteoporosis.

The approach to this problem can be divided into 3 levels:
- National level: Countries should recognize the problem and have policies to address this in the short, medium and long term.
- Health care provider: Should put this problem as a priority and work as a team to prevent fractures. They should also adopt innovative and cost-effective approaches eg fracture prevention clinics.
- The public: Should contribute to national policies and adopt public health approaches to prevent fractures eg exercise regularly, increase calcium intake, and ensure that their vitamin D level is adequate.

International organizations such as the IOF have a major role to play in co-ordinating and enhancing the above efforts.

MEN, OSTEOPOROSIS AND BODY COMPOSITION
E. Orwoll
Oregon Health and Science University, Portland, United States

Osteoporotic fractures in older men are heavily influenced by body size and composition. Underweight men are at higher risk of bone loss and fracture. On the other hand, most fractures, including most hip fractures, happen in men who are overweight or obese. To some extent this is because there are few underweight men and many who are overweight. In addition, in obese men (unlike in obese women) there is actually an increased risk of fracture compared to normal weight men - especially after adjustment for BMD. Moreover, whereas high BMI results in slightly greater mean BMD, many overweight/obese men have low BMD. The reasons obesity imparts increased risk are unclear. Unlike in obese women, trochanteric tissue thickness in overweight/obese men is not sufficient to protect the hip against the forces of a fall that are greater with higher body mass. Gain of fat mass is associated with an increased rate of bone loss. Although estradiol levels tend to be higher in obese men, SHBG levels are also higher, potentially blunting the positive effects of estrogen on bone mass. To some extent, the increased risk of fractures in overweight/obese men appears to be due to impaired physical performance and increased falls. In fact, muscle function may be impaired in obese men. Insulin resistance (common in overweight/obese men) is associated with greater losses in lean mass. Testosterone levels are reduced in obese men and are associated with more falls in men.

Whereas most osteoporotic fractures in men occur in the overweight or obese, public health recommendations currently focus on the high risk associated with low weight. How should overweight/obese men at risk of fracture be identified? Should weight loss be considered, when it's clear that weight loss for any reason is associated with BMD loss? Are osteoporosis treatments effective in overweight/obese men?

OSTEOPROTECTIVE ACTIONS OF SEX HORMONES MEDIATE THEIR NUCLEAR RECEPTORS IN BONE CELLS
S. Kato, S. Kondoh, Y. Imai
Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan

Sex steroids, estrogens and androgens, display an osteoprotective effect and prevent bone loss associated with post-menopausal osteoporosis. However, the molecular mechanism of how this is accomplished remains to be elucidated. To address this issue, we have selectively ablated nuclear sex hormone receptors in bone cell-type specific manners by means of a Cre-loxP system. Using Cathepsin K-Cre recombinase knock-in mouse line (Ctsk-Cre), osteoclastic estrogen receptor (ER a ) and androgen receptor (AR) were ablated by crossing with the floxed ER a and AR lines in mature osteoclasts in male and female mice. Bone loss in ER a D/ D males and AR D/ D females was evident in X-ray and 3D-CT, while the bone in ER a D/ D and AR D/ D females looked normal. Similarly when AR was selectively ablated in mature osteoclasts ( AR D/ D, D/ D), bone loss was evident in males, but not in females. Bone histomorphometric analysis of AR D/ D females and AR D/ D males revealed significant increases in osteoclast surface, osteoclast number and eroded surface in with increased MAR and BFR.

Similarly, osteoblastic ER a and AR were ablated by a Cre transgenic line driven by a collagen type I gene promoter [Col1a1(2.3 kb)-Cre] . Femoral distal and lumbar BMD of ER a D/ D and AR D/ D mice were decreased in males, but not females. By bone histomorphometry of ER a D/ D males, low turnover bone loss was found. AR D/ D females looked normal, but male mutants exhibited decreased BMD in diaphysis of long bones and calvaria. Furthermore, bone histomorphometric analyses revealed that MAR and BFR were
reduced in both cortical and trabecular bone of ARD Ob/Db. All together, these findings have uncovered that osteoprotective actions of sex hormones mediate sex dimorphic and bone cell type-specific functions of nuclear receptors.

ANDROGENS ARE CRITICAL TO NEUROPEPTIDE Y MEDIATED CONTROL OF BONE FORMATION

A. Zengin1,2, R. F. Enriquez1,2, A. D. Nguyen3, N. J. Lee3, A. Sainsbury2,4, H. Herzog2,3, J. A. Eisman1,3, P. A. Baldock1,2,3

1Osteoporosis & Bone Biology Program, Garvan Institute of Medical Research, Sydney, NSW, Australia
2Neuroscience Program, Garvan Institute of Medical Research, Sydney, NSW, Australia
3Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia
4School of Medical Sciences, University of New South Wales, Sydney, NSW, Australia

Background: Neural signals regulate bone mass through a Neuropeptide Y (NPY) dependent pathway involving hypothalamic signalling via Y2 receptors and direct osteoblastic signalling via Y1 receptors. Moreover, this pathway has important interactions with endocrine factors. Indeed, the bone anabolic phenotype of Y1 receptor null mice (Y1−/−) is completely attenuated by orchidectomy (ORX). In contrast, this effect was not attenuated following ORX in wild type (wt) or Y2−/− mice, or in hypothalamus-specific Y2−/− mice. This suggests a critical interaction between the androgen and NPY pathways in the osteoblast.

Aim: To investigate the interaction between androgens and osteoblastic Y1 receptors in the regulation of bone homeostasis.

Method: Mice with selective deletion of Y1 receptor in osteoblasts (ObY1−/−) was achieved by crossing Y1 receptor floxed mice with 2.3kb α1(I)-collagen Cre expressing mice. Mice underwent ORX or sham-operation at 8 weeks and skeletal responses were examined at 16 weeks.

Results: In sham-operated groups, ObY1−/− mice had increased cancellous bone volume compared to wt (14.1±0.8% vs 11.0±0.5, p=0.02), associated with greater mineral apposition rate (MAR) (1.9±0.04μm/d vs 1.7±0.05, p=0.005). There was no difference in mineralising surface (30.2±1.7% vs 27.6±1.1), osteoclast surface (7.4±1.2% vs 5.8±1.3) or osteoclast number (3.9±0.6/mm vs 3.1±0.6). Importantly 8 weeks following ORX, this ObY1−/− effect was markedly attenuated. Cancellous bone volume (4.2±0.4 vs 3.5±0.3) and MAR (2.2±0.1 vs 2.3±0.1) were similar in both ORX groups. Moreover, osteoclast surface (16.9±1.0 vs 15.6±2.3), osteoclast number (8.3±0.7 vs 8.1±1.3) and bone formation rate (0.63±0.06μm2/μm/d vs 0.58±0.03) were similar between ObY1−/− and wt following ORX.

Conclusion: Both germline Y1−/− and ObY1−/− mice require intact androgen signalling for the anabolic phenotype. Together these data suggest it is the androgen dependent regulation of osteoblast activity that is modulated by the NPY Y1 receptor pathway. Both neural and endocrine pathways interact within the osteoblast to regulate bone formation.

OSTEOMMUNOLOGY

H. Takayanagi
Tokyo Medical and Dental University, Bunkyo-ku, Japan

The interaction between the immune and skeletal systems has long been acknowledged, but investigation into autoimmune arthritis as well as the various bone phenotypes found in mice deficient in immunomodulatory molecules has highlighted the importance of the dynamic interplay between the two systems. This has led to the recent emergence and subsequent rapid evolution of the field of osteoimmunology. In the bone destruction associated with autoimmune arthritis, IL-17-producing helper T (Th17) cells play a major role by inducing receptor activator of nuclear factor-κ B ligand (RANKL). RANKL stimulates osteoclastogenesis through nuclear factor of activated T cells cytoplasmic 1 (NFATc1), which is well known as a crucial regulator of immunity. In addition to cellular interactions via cytokines, the immune and skeletal systems share various molecules, including transcription factors, signaling molecules and membrane receptors. Recent reports suggest that maintenance and mobilization of hematopoietic cells are regulated by bone cells. Thus, the scope of osteoimmunology has grown to encompass a wide range of molecular and cellular interactions, the elucidation of which will provide a scientific basis for future therapeutic approaches to diseases of both the immune and skeletal systems. Here I will discuss emerging topics in osteoimmunology.

(1) Nat Rev Rheumatol. 5, 667-76 (2009)
008

INFLAMMATION AND BONE RESORPTION IN RHEUMATOID ARTHRITIS
S. Goldring  
Hospital for Special Surgery, New York, United States

Rheumatoid arthritis (RA) represents a paradigm of an inflammatory condition that markedly alters the equilibrium of physiological bone remodeling. In this condition, the inflamed synovial lining of diarthrodial joints undergoes immune-mediated deregulated proliferation and growth and produces a variety of immunomodulatory and proinflammatory cytokines that are potent regulators of bone cell function. These include factors that modulate the activity of both osteoclasts and osteoblasts. The joint margins at the integration sites of the RA synovium are the initial skeletal regions adversely affected by the inflammation, which leads to progressive focal erosion of the bone surfaces. The adjacent marrow space also is involved in the inflammatory process leading to an advancing front of bone resorption that destroys the subchondral bone. RA is a systemic disease and the inflammatory process is accompanied by a generalized defect in bone remodeling associated with systemic as well as periarticular bone loss. Multiple lines of investigation have demonstrated that osteoclasts are essential for the focal articular and systemic bone resorption. The process of osteoclastic bone resorption can be attributed to potent cytokines and immunomodulatory factors that are produced in the inflamed synovium, including receptor activator of NF-κB ligand (RANKL) that is required for osteoclast differentiation and activity. Targeting RANKL with its physiological inhibitor osteoprotegerin (OPG) in animal models of inflammatory arthritis markedly inhibits focal bone resorption at sites of joint inflammation. In a recent study, treatment of RA patients with the fully human monoclonal antibody denosumab resulted in inhibition of focal joint erosions and attenuation of systemic bone loss. In addition to the localized increase in bone resorption in inflamed joints, there is also evidence that bone formation is impaired. Recent studies suggest that the defect in bone formation results at least in part from the production in the inflamed synovium of molecules, including secreted Frizzled-related protein and members of the Dickkopf family, which are inhibitors of the Wnt signaling pathway that regulates bone formation. A better understanding of the molecules and signal pathways that regulate bone resorption and repair should lead to the development of new and more effective strategies for preventing bone destruction in RA and related forms of inflammatory joint diseases.

009

INFLAMMATION AND BONE FORMATION IN ANKYLOSING SPONDYLITIS
G. Thomas  
Diamantina Institute, University of Queensland, Australia

The spondylarthropathies are a group of arthritic diseases in which the inflammatory process is targeted to the spine and pelvis. The prototypic example of these conditions is ankylosing spondylitis (AS). In this common condition, inflammation ultimately leads to bone formation around and ultimately across affected joints, resulting in joint fusion (ankylosis). No therapies are currently available that prevent or even slow this inevitable progression that results in significant disability. It is well established that AS has two phases, an initial inflammatory phase followed by an osteoproliferative/ankylosing phase where the joint fusion is initiated and progresses. Very little is known about the progression from the initial inflammatory stages of the disease to this pathologic bone formation. The inflammatory phase has similarities with other inflammatory arthritides such as RA with high levels of pro-inflammatory cytokine production and joint damage through osteoclast activity. However, the extent of joint destruction though the cytokine/osteoclast axis is significantly less than in RA with the dominant joint impact stemming from ankylosis occurring as a result of osteoproliferation.

Recent work has suggested that changes in Wnt signalling, the key bone regulatory pathway, may contribute to joint ankylosis in AS. Using the proteoglycan-induced mouse model of AS (PGISp) we have demonstrated ectopic matrix formation in the axial joints and characterised the underlying molecular changes. Both SOST and DKK1, inhibitors of the Wnt signaling pathway, are downregulated in affect joints thereby providing a permissive environment where excessive bone formation might occur. These changes provide potential new targets for novel therapeutic approaches for this debilitating disease.

010

DOUBLE STRANDED RNA INCREASES BONE MASS IN OSTEOPOROSIS MODEL MICE BY INHIBITING OSTEOCLASTOGENESIS VIA INTERFERON-B/STAT1 PATHWAY
A. Miyamoto1, M. Takami1, Y. Miyamoto2, A. Yamada1, M. Yim2, T. Tamura2, R. Kamijo1
1Department of Biochemistry, School of Dentistry, Showa University, Tokyo, Japan  
2College of Pharmacy, SooMyung Women’s University, Seoul, Sth Korea

Double-stranded RNA (poly(I:C)), a double stranded RNA that functions as a ligand for Toll-like receptor is known to induce antiviral responses in the innate immune system. However, the effects of poly(I:C) on bone metabolism are unknown. We found that poly(I:C) markedly increased bone mass by inhibiting osteoclastogenesis. Six-week-old female mice were subjected to an ovariectomy (OVX) for an osteoporosis model or a sham operation for the control groups. After surgery, we intravenously administrated poly(I:C) (250 μg) or PBS to the
mice every 3 days for 4 weeks. Analysis of tibia using microcomputed tomography revealed that trabecular bone volume per tissue volume (BV/TV%) in the sham/poly(I:C) group was significantly greater than that in the sham/PBS group (13.5±11.9% vs. 10.8±12.2%, P<0.05). Similarly, BV/TV in the OVX/poly(I:C) group was significantly greater than that in the OVX/PBS group (13.5±11.4% vs. 8.3±10.6%, P<0.05). Histomorphometric analysis of femur sections revealed a significantly decreased number of osteoclasts in the OVX/poly(I:C) and sham/poly(I:C) groups as compared to the OVX/PBS and sham/PBS groups, respectively. In vitro osteoclastogenesis induced by RANKL was inhibited by poly(I:C) in a dose-dependent manner. Since osteoclast precursors produced interferon (IFN)-β, an inhibitor of osteoclastogenesis, in response to poly(I:C), we added antibodies for IFN-β or IFN-α/β receptor to block IFN-β action. In the presence of these antibodies, osteoclastogenesis was successfully accomplished, even in the presence of poly(I:C). Subsequently, we prepared osteoclast precursors from mice deficient of STAT1, an intracellular signal transducer of IFN-α/β receptor. Osteoclastogenesis of STAT1-deficient osteoclast precursors was not inhibited by poly(I:C). Furthermore, administration of poly(I:C) as well as PBS to STAT1-deficient mice failed to increase bone mass in either the OVX or sham groups. These results suggest that poly(I:C) induces IFN-β production in osteoclast precursors and inhibits osteoclastogenesis via the STAT1 signaling pathway, resulting in an increase of bone mass in sham and OVX mice.

011

NORMATIVE ULTRASOUND DATA FOR POPULATION-BASED AUSTRALIAN MEN AND WOMEN: THE GEELONG OSTEOPOROSIS STUDY

H. Gould1,2, J. A. Pasco1,2, S. L. Brennan1,2, G. C. Nicholson1,2,4, M. A. Kotowicz1,2, M. J. Henry2

1NorthWest Academic Centre, Department of Medicine (Western Section), The University of Melbourne, Footscray, Melbourne, VIC, Australia
2Epidemiology and Biostatistics Unit (Barwon Health), School of Medicine, Deakin University, Geelong, VIC, Australia
3Department of Diabetes and Endocrinology, Barwon Health, Geelong, VIC, Australia
4Rural Clinical School, School of Medicine, The University of Queensland, Toowoomba, QLD, Australia

Aim: The aim of this study was to develop normative data for quantitative calcaneal ultrasound for the Australian population using a population-based random sample of men and women.

Methods: Using a GE Lunar Achilles Insight Bone Ultrasonometer, heel ultrasound parameters of broadband ultrasound attenuation (BUA) and speed of sound (SOS) were measured for men (n=1104) and women (n=914) aged 20-93 yr. Participants were randomly selected from the Barwon Statistical Division (BSD) in Victoria using the Australian Commonwealth electoral roll. Linear regression was used to examine the association between BUA and SOS and age, weight and height for both genders.

Results: The relationship between age and BUA differed between genders. For men the relationship was linear and negative across the age range. BUA decreased by 0.17 dB/MHz per year (95% CI: -0.22,-0.13 dB/MHz, P<0.001), however the strength of this association was weak (r=-0.22 [95% CI: -0.27,-0.16]). For women the association between age and BUA was also negative but non-linear as BUA decreased at a greater rate after menopause. These patterns of association were also observed for SOS and age: SOS decreased linearly by 0.51 m/s (95% CI: -0.63,-0.38 m/s, P<0.001) per year in men and the decline in SOS per year in women was non-linear. The parsimonious multivariable models for BUA and SOS were different for both genders. For men, the model for BUA included the variables, age, weight and height (R², 6.3%) but only age and height for SOS (7.3%), whereas for women, models for both BUA and SOS included age and weight (37.0% and 25.9%, respectively).

Conclusions: In men, there is a weak linear age-related decline in BUA and SOS, whereas in women the decline is greater and non-linear. As with normative data for BMD, these data for quantitative calcaneal ultrasound may be used to optimise algorithms for fracture risk prediction.

012

THE TRANSCRIPTION FACTOR ARID5B MODULATES ENDOCHONDRAL BONE FORMATION IN COOPERATION WITH SOX9

R. Takahashi1, K. Hata1, K. Ono1, M. Wakabayashi1, K. Amano1, M. Nakanishi1, Y. Maeda1, R. H. Whitson2, R. Nishimura1, T. Yoneda2

1Biochemistry, Osaka University graduate school of dentistry, Suita, Japan
2Division of Molecular Biology, Beckman Research Institute of the City of Hope, Duarte, United States

The transcription factor Sox9 plays an essential role in chondrocyte differentiation. Mutations of Sox9 gene in human cause Campomelic dysplasia characterized by severe chondrodysplasia and mice in which Sox9 is conditionally knocked out exhibited profound skeletal malformations. Sox9 overexpression in the mesenchymal stem cells C3H10T1/2 induced chondrocyte differentiation by up-regulating the expression of chondrogenic genes including Col2a1 and aggregan. However, introduction of Sox9 alone does not necessarily induce chondrocyte differentiation, suggesting that Sox9, in some situations, requires a cooperative molecule to induce chondrocyte differentiation. Based on this hypothesis, genome wide expression profiles of C3H10T1/2 and non chondrogenic cell lines including NIH3T3 and BALB/3T3 were compared with Solexa sequencing technology. As a result we identified the transcription factor Arid5b (AT-rich interactive domain 5b). Whole mount in situ hybridization demonstrated a discernible expression of Arid5b mRNA in the developing limbs where Col2a1 and Sox9 were specifically expressed. Arid5b was also expressed in the resting and proliferating chondrocytes as determined by immunohistochemistry. These data suggest that Arid5b is a transcription factor critical for chondrogenesis. To verify the functional role of Arid5b in chondrocyte differentiation, we overexpressed Arid5b in C3H10T1/2 cells using adenovirus system. Arid5b enhanced Sox9-induced Col2a1 mRNA expression,
although Arid5b alone failed to alter Col2a1 expression. Of interest, Arid5b showed no effects on Sox9 expression, while transcriptional activity of Sox9 was markedly up-regulated by Arid5b. Interestingly, Sox9 dependent chondrogenesis was reduced in primary chondrocytes isolated from Arid5b KO mice ribs compared to WT mice. Pull-down and two-hybrid analyses showed that Arid5b physically associated with Sox9. Direct binding of Arid5b to the Col2a1 gene promoter was shown by DNAP and ChIP experiments. Moreover, overexpression of dominant-negative mutant (DN) Arid5b inhibited BMP2-induced chondrocyte differentiation of C3H10T1/2 cells and mouse embryo limb bud cells. More importantly, Arid5b deficient mice exhibited dwarfism and shortened limbs with decreased width of proliferating chondrocytes. Consistently, DN-Arid5b transgenic mice driven by the Prx-1 gene promoter showed delayed endochondral ossification and reduced Col2a1 mRNA expression. In conclusion, our results suggest that Arid5b promotes chondrocyte differentiation as a transcriptional partner of Sox9 and provide further insights into the molecular mechanism by which Sox9 regulates chondrogenesis.

013

THE ROLE OF EPHB/EPHRINB MOLECULES IN FRACTURE REPAIR

A. Arthur1, R. Panagopoulos1, L. Cooper1, A. Zannettino2, S. A. Koblar3, H. A. Sims4, K. Matsuo5, S. Gronthos1

1Haematology, Institute of Medical and Veterinary Science/SA Pathology, Robinson Institute, Adelaide, SA, Australia
2Haematology, Centre for Cancer Biology, Institute of Medical and Veterinary Science/SA Pathol, Adelaide, SA, Australia
3School of Molecular and Biomedical Science, School of Medicine, University of Adelaide, Adelaide, SA, Australia
4Bone, Joint & Cancer Unit, St Vincent's Institute of Medical Research, Fitzroy, VIC, Australia
5Laboratory of Cell and Tissue Biology, School of Medicine, Keio University, Shinjuku-ku, Tokyo, Japan

Aims: The Eph family of receptor tyrosine kinases have been shown to contribute to bone homeostasis and remodelling, osteoarthritis, bone cancer prognosis, in addition to mesenchymal stem cell attachment, migration and osteochondral differentiation. However, the involvement of these molecules in the skeletal repair process following trauma or injury to long bones has not been investigated. The aim of this study was to identify the function of the EphB/ephrinB molecules during fracture repair.

Methods: A fracture was created in the right femur of C57Bl/6 mice and stabilised via internal fixation. Mice were assessed at various stages post-fracture: one week (haematoma), two weeks (callus) and 4 to 8 weeks (remodelling). Animals were assessed by histology (n=6 mice), RT-PCR (n=6) and micro-computed tomography (μCT) imaging (n=6-12).

Results: The receptor EphB4 was the most highly expressed Eph family member in the fracture callus and was significantly increased at the gene level at one, two and eight weeks post-fracture when compared with the contralateral uninjured femur, as demonstrated by RT-PCR. EphrinB1 (4-fold), EphB2 (8-fold) and ephrinB2 (4-fold) gene expression was also significantly up regulated one and two weeks post-fracture compared to the contralateral uninjured femur. To investigate the contribution of EphB4 to the fracture repair process, fracture surgery was carried out on Collagen-type 1-EphB4 transgenic mice which expressed high levels of EphB4 in their osteoblasts. Preliminary analysis of live µCT reconstructed images revealed a 20% larger callus size in EphB4 pre-fracture mice compared with their wild type littermate controls two weeks post-fracture. Notably, the Collagen-type 1-EphB4 pre-fracture mice also exhibited significantly higher numbers of cortical bone-derived fibroblast colony forming units (CFU-F) compared with wild type control pre-fracture mice.

Conclusions: Our observations suggest that EphB/ephrinB molecules are important during the bone repair process and that overexpression of EphB4 enhances endochondral bone formation following bone injury.

014

BROWN ADIPOSE TISSUE DYSFUNCTION CAUSES BONE LOSS

M. Kawaji1, C. J. Rosen2

1Dpt of Bone and Mineral Research, Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Osaka, Japan
2Center for Clinical and Translational Research, Maine Medical Center Research Institute, Scarborough, Maine, United States

Aim: Brown adipose tissue (BAT) is critical for energy homeostasis and BAT dysfunction causes low body temperature, which drives sympathetic nervous system (SNS) to maintain the core body temperature. Accumulating evidence demonstrates that the skeletal metabolism is under the control of the SNS, although it remains to be determined whether increased sympathetic tone caused by impaired BAT function influences bone mass.

Methods: To evaluate the role of BAT in skeletal mass, Misty mice, a spontaneous mutant that exhibits an age-associated impairment in BAT function, and Ucp1-deficient mice that has defects in BAT function from birth were studied. Energy expenditure, a marker for sympathetic tone and bone mass were analyzed in these mice. Histomorphometric analysis was also performed.

Result: Misty mice showed impaired BAT function, low body temperature, increased energy expenditure and an age-dependent increase in sympathetic tone. Concurrent with the increase in sympathetic tone, expression of target genes of sympathetic activation, such as Rankl, was enhanced in the femur. Skeletal phenotyping revealed that 8 wk female Misty mice had low total areal bone mineral density and distal femoral trabecular bone volume fraction vs controls. This difference was even greater at 16 weeks and was associated with impaired bone formation and accelerated bone resorption. Importantly, reduced trabecular bone mass of Misty mice was in part reversed by the blockade of sympathetic tone by a β-blocker. To confirm the indirect effects of BAT dysfunction on bone mass, we found that Ucp1-deficient mice had very low femoral bone volume fraction that also worsened with age.
Conclusion: Reduced BAT function causes bone loss in part through activating SNS activity. Given the emerging evidence of an age-dependent decrease in BAT function and an increase in the incidence of osteoporosis, agents that enhance BAT function could be a promising therapeutical approach for the treatment of osteoporosis.

A RANDOMIZED CONTROLLED TRIAL ON SAFETY AND EFFICACY OF SINGLE INTRAMUSCULAR V/S STAGGERED ORAL DOSE OF 6,00,000 IU VITAMIN D IN TREATMENT OF NUTRITIONAL RICKETS

K. Mondal1, A. Seth1, D. Dhanwal1, R. K.R. Marwah1, S. Aneja4, R. Singh1, P. Sonkar1

1Pediatrics, Lady Hardinge Medical College and Kalawati Saran Children’s Hospital, New Delhi, India
2Thyroid Research Centre, Institute of Nuclear Medicine and Allied Sciences (INMAS), New Delhi, India
3Department of Medicine, Maulana Azad Medical College, New Delhi, New Delhi, India
4Department of Biochemistry, Lady hardinge Medical College, New Delhi, India

Background: Nutritional rickets is commonly treated by administering 6,00,000 IU of vitamin D parenterally as a single dose. However, doubts have been raised about safety of this regime. There is scant research on comparative efficacy and safety of oral and intramuscular routes for administering vitamin D.

Methods: Children (6 months to 5 years) with nutritional rickets were included in the study and randomized to receive one of the two treatment protocols using block randomization (1) 60,000 IU vitamin D orally every week for 10 weeks (2) 6,00,000 IU single i/m injection. Serum calcium (total and ionic), phosphate, ALP, urinary calcium/creatinine ratio was measured at baseline, after 7 days, after 4 weeks and at 12 weeks. Serum 25(OH)D was measured at baseline and at 12 weeks. A 10 point radiological score was done at baseline, after 4 weeks and at 12 weeks.

Results: 61 cases (30 oral, 31 i/m) were followed up till 12 weeks. Combined end point of ALP>420 IU/L, and radiological score <1.5 was achieved in 93.3% cases in oral group and 90.3% cases in i/m group. No difference was found in efficacy of the 2 regimes on comparing serum calcium, phosphate, ALP levels and radiological score measured at 12 weeks. At baseline, 96.67% in oral group and 93.55% in i/m group had serum 25(OH)D > 20 ng/ml, at the end of treatment 70% from oral group and 71% from i/m group achieved serum 25(OH)D level >20 ng/ml. After completion of treatment 2 children from oral group and 1 child from i/m group had excess serum 25(OH)D level (>100 ng/ml) without any symptom or hypercalcemia, 2 children from each group developed asymptomatic hypercalcemia. 9 children with baseline elevated urinary calcium/creatinine ratio continued to have elevated urinary calcium/creatinine ratio throughout the study period. No child other than these 9 children developed hypercalciuria during the study period.

Conclusion: Both staggered oral dose and single i/m dose of 6,00,000 IU in vitamin D are equally effective and safe in treatment of nutritional rickets.

FUNCTIONAL REDUNDANCY OF GSK-3A AND GSK-3B TO CONTROL CHONDROCYTE DIFFERENTIATION THROUGH PHOSPHORYLATION OF RELA/NF-KB

S. Itoh1, T. Saito1,2, M. Hirata1,3, K. Nakamura1, U. Chung1, H. Kawaguchi1

1Sensory & Motor System Medicine, University of Tokyo, Tokyo, Japan
2Bone and Cartilage Regenerative Medicine, University of Tokyo, Tokyo, Japan
3Center for Disease Biology and Integrative Medicine, University of Tokyo, Tokyo, Japan

Several principal signals in chondrocytes are mediated by glycogen synthase kinase-3 (GSK-3). Here we examined individual roles of the two mammalian isoforms, GSK-3α and GSK-3β, in chondrocyte differentiation and skeletal development. Both isoforms were largely unphosphorylated and active in the early differentiation stages during SOX9, type II collagen (COL2A1), and aggrecan expression in cultured chondrogenic ATDC5 cells and in the mouse limb cartilage. Although Gsk3a−/− mice and Gsk3b−/− mice showed normal skeletal development, their compound Gsk3a−/−;Gsk3b−/− mice exhibited dwarfism with impairment of early chondrocyte differentiation without affecting the later differentiation or proliferation. Gain- and loss-of-function analyses using cultures of ATDC5 cells and primary chondrocytes from the knockout mice with overexpression of GSK-3 isoforms and their siRNAs revealed that GSK-3α and GSK-3β induced early chondrocyte differentiation with functional redundancy in a cell-autonomous fashion. This was independent of the canonical Wnt signaling, since none of the transcriptional activity (TOPflash), protein level, nor subcellular localization of β-catenin was altered by the Gsk3 genotypes. Instead, computational predictions followed by SOX9 and COL2A1 promoter assays identified RelA (NF-kB p65) as a key phosphorylation target of GSK-3, and further analyses by mutagenesis in the 12 putative phosphorylation sites of RelA revealed that the Thr254-phosphorylation was essential for GSK-3 to induce chondrocyte differentiation. The gain- and loss-of-function analyses above showed that GSK-3α and GSK-3β caused the Thr254-phosphorylation in early differentiation stages without affecting the RelA protein level, subcellular localization, IκB phosphorylation, or IKK level. The Cre-mediated deletion of RelA in the Rela−/− chondrocyte culture caused suppressions of the early differentiation markers, which was restored by the RelA overexpression, but not by the Thr254 mutant or the GSK-3 overexpression. Lastly, we created conditional knockout mice of Rela in undifferentiated limb mesenchyme (Prx1-CreRelafl/fl) and in cartilage (Col2a1-CreRelafl/fl), and confirmed that both mouse models exhibited dwarfism with impairment of early chondrocyte differentiation, similarly to the Gsk3a−/−;Gsk3b−/− mice. In conclusion, the redundant functions of GSK-3α and GSK-3β through phosphorylation of RelA at Thr254 play a crucial role in early stages of chondrocyte differentiation.
SERUM SCLEROSTIN LEVEL WAS POSITIVELY CORRELATED WITH BONE MINERAL DENSITY IN CENTRAL SOUTH CHINESE POSTMENOPAUSAL WOMEN

Z. Sheng1,2, D. Tong3, Y. Ou4, H. Zhang1, Z. Zhang4, S. Li1, H. Zhou4, X. Wu3, H. Xie1, L. Yuan4, E. Liao1

1 Institute of Metabolism and Endocrinology, changsha, China
2 Key Laboratory of Protein Chemistry and Developmental Biology of Education Min, changsha, China
3 3 Hospital Infection Control Center, the Second Xiang-Ya Hospital, Central South, changsha, China
4 College of Public health, Central South University, changsha, China

Aim: To elucidate the relationship between serum sclerostin level, body composition and bone mineral density in central south Chinese postmenopausal women.

Methods: A cross-sectional study was conducted on 260 healthy central southern Chinese postmenopausal women, aged 50–76 years old. Total body, lumbar spine, and left femur BMD and total body soft tissue composition were measured by dual X-ray absorptiometry. Serum sclerostin levels were measured by ELISA kits.

Results: In contrast to the women without osteoporosis, the osteoporotic women were older, shorter, and thinner, and they had an earlier age at menopause, a lower BMD and bone mineral content (BMC) of the total body and at different sites, and also had lower body mass and body mass components. Serum sclerostin level in the women without osteoporosis was higher than that in the osteoporotic women (P<0.0001). Moreover, serum sclerostin level was positively correlated with body weight, BMI, and fat mass, and the BMD of the total body and at different sites (P<0.05). After controlling for age, age at menopause, height and body weight, serum sclerostin level were still positively correlated with the BMD of the total body and at different sites(P<0.05). Multiple linear stepwise regression analysis showed that serum sclerostin level was the most significant determinant of the BMD at total body and the lumbar spine as compared with age, age at menopause, fat mass and lean mass, while age had similar impact as serum sclerostin on the BMD at total hip.

Conclusions: The present study showed that in central south Chinese postmenopausal women, serum sclerostin level was positively correlated with the BMD of the total body and at different sites. These seem to be conflicted with our predictions since sclerostin is a potent inhibitor of bone formation.

2 Regulation of circulating sclerostin levels by sex steroids in women and in men.J Bone Miner Res. 2011:26:27-34.
3 Serum sclerostin levels positively correlate with lumbar spinal bone mineral density in postmenopausal women-the six-month effect of risedronate and teriparatide. Osteoporos Int. 2011 Jan 11.PMID:2130

A RANDOMIZED CONTROLLED TRIAL OF ANNUAL LOW DOSE ZOLEDRONATE IN POSTMENOPAUSAL WOMEN

I. R. Reid, M. Bolland, S. Wong, A. Horne, G. Gamble, A. Grey

Medicine, University of Auckland, Auckland, New Zealand

Aim: To investigate the effects on BMD and bone turnover of annual administration of doses of zoledronate (Zol) lower than 5mg.

Methods: Randomized placebo-controlled trial of intravenous Zol 1mg, 2.5mg and 5mg in 180 osteopenic postmenopausal women. The primary endpoint was change in lumbar spine BMD at 12 months. Secondary endpoints were change in total hip BMD, and changes in markers of bone turnover.

Results: After 12 months, change in spine BMD was greater in each of the Zol groups than the placebo group [mean (95% CI) Zol 1 3.5% (2.2, 4.8); Zol 2.5mg 4.0% (2.7, 5.3); Zol 5mg 3.6% (2.3, 4.9), P<0.001 vs placebo for each Zol dose]. Change in BMD at the total hip was also significantly greater in each of the Zol groups than the placebo group [Zol 1mg 2.7% (1.9, 3.5); Zol 2.5mg 3.6% (2.8, 4.4); Zol 5mg 3.6% (2.8, 4.4), P<0.001 vs placebo for each Zol dose]. Each of the turnover markers, β-CTX and P1NP, decreased significantly in each of the Zol groups [β-CTX, Zol 1mg -44% (-55, -33); Zol 2.5mg -68% (-78, -57); Zol 5mg -73% (-83, -63); P1NP, Zol 1mg -41% (-55, -28); Zol 2.5mg -58% (-72, -44); Zol 5mg -64% (-78, -51), P<0.001 vs placebo for each marker for each Zol dose]. Markers of bone turnover in each Zol group were stably decreased between 6 and 12 months.

Conclusions: Annual administration of doses of Zol of either 1 or 2.5mg produces substantial anti-resorptive effects that approximate those of the 5mg dose. Annual zoledronate doses of 1 or 2.5mg may confer significant anti-fracture efficacy.

EFFECT OF ODANACATIB ON BONE DENSITY AND BONE TURNOVER MARKERS IN POSTMENOPAUSAL WOMEN WITH LOW BONE MINERAL DENSITY: YEAR 4 RESULTS

J. A. Eisman1,2,3, N. Binkley4,5,6, H. Bone1, D. Hosking7, B. Langdahl3, I. Reid3, H. Resch1,8, J. Rodriguez Portales9, C. Le Bailly10, E. Liao11, Z. Sheng12, D. Tong3, Y. Ou4, H. Zhang1, Z. Zhang4, S. Li1, H. Zhou4, X. Wu3, H. Xie1, L. Yuan4, E. Liao1

1 Osteoporosis & Bone Biology, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia
2 Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia
3 Endocrinology, St Vincent’s Hospital, Darlinghurst, NSW, Australia
4 Osteoporosis Clinical Center and Research Program, University of Wisconsin, Madison, Wisconsin, United States
Cortical bone constitutes 80% of skeletal mass, and 70% of bone loss during ageing is the result of intracortical remodeling which results in porosity that compromises bone strength. Denosumab increased volumetric bone mineral density (vBMD) of the cortical bones of postmenopausal women with low bone mass and was generally well tolerated. Bone formation was relatively unaffected. Discontinuation led to resolution of BMD & bone biomarker effects to near baseline.

PARATHYROID HORMONE 1-84 ACCELERATES FRACTURE HEALING IN PUBIC BONES OF ELDERLY OSTEOPOROTIC WOMEN

G. Holzer1, P. Peichl2, L. A. Holzer3, R. Maier3
1Orthopaedics, Medical University of Vienna, Vienna, Austria
2Rheumatology, Evangelisches Krankenhaus Wien, Vienna, Australia
3Traumatology, Thermenklinikum Baden, Baden, Australia

Background: Parathyroid hormone (PTH) was shown to increase bone mineral density (BMD) and reduce fractures in patients with osteoporosis, but also to improve fracture healing. Osteoporotic pelvic fractures are less frequent, but require a longer healing period with immobilisation. In postmenopausal women with PTH 1-84 for the treatment of osteoporosis and conservatively treated pelvic fractures without requiring surgery, the effect of PTH 1-84 on the course of fracture healing and functional outcome was tested in a randomized controlled study prospectively. Methods: 65 patients (mean age: 82.8 years) had plain x-rays and a computer tomography (CT) scan to verify fractures and were scanned for osteoporosis. 21 patients received a once daily injection of 100μg PTH 1-84 starting within two days after admission to the hospital, 44 patients without PTH treatment served as a control group. All patients received 1000 mg Calcium and 800 IU Vitamin D. CT scans were repeated every fourth week until radiographic evidence of cortical bridging was confirmed. Functional outcome was assessed using a pain Visual Analogue Scale (VAS) and a Timed Up and Go (TUG) test. Results: In all 21 patients treated with PTH 1-84 pelvic fractures were healed at a mean of 7.8 weeks, whereas in patients with no PTH treatment fractures had healed after 12.6 weeks (p<0.001). At week 8 all fractures in the treatment group were healed and four fractures in the control group (healing rate 100% versus 9.1%; (p<0.001). Both the VAS and TUG improved statistically significant (p<0.001) compared to control. Conclusions: In elderly patients with osteoporosis, PTH 1-84 accelerates fracture healing in pelvic fractures and improves functional outcome.

EDENOSUMAB DECREASES CORTICAL POROSITY IN POSTMENOPAUSAL WOMEN WITH LOW BONE MINERAL DENSITY

1Instituto de Investigaciones Metabolicas, Buenos Aires, Argentina
2Michigan Bone and Mineral Center, Detroit, Michigan, United States
3University of Calgary, Calgary, Canada
4INSERM U890 and University Hospital of St-Etienne, St-Etienne, France
5INSERM U831 and Universite de Lyon, Lyon, France
6Thousand Oaks, Amgen Inc., United States

Cortical bone constitutes 80% of skeletal mass, and 70% of bone loss during ageing is the result of intracortical remodeling which results in porosity that compromises bone strength. Denosumab increased volumetric bone mineral density (vBMD) of the cortical bones of postmenopausal women with low bone mass and was generally well tolerated. Bone formation was relatively unaffected. Discontinuation led to resolution of BMD & bone biomarker effects to near baseline.
Long-term denosumab treatment in postmenopausal women with osteoporosis: Results from the first two years of the Freedom trial extension


1Michigan Bone and Mineral Clinic, Detroit, United States
2Hôpital Édouard Herriot, Lyon, France
3University of Florence, Florence, Italy
4CHUQ Research Centre, Quebec City, Canada
5Krakow Medical Centre, Krakow, Poland
6Amgen Inc., Thousand Oaks, CA, United States
7University Hospital of Lausanne, Lausanne, Switzerland
8Centro TIEMPO, Buenos Aires, Argentina
9Sahlgrenska University Hospital, Göteborg, Sweden
10Universidade Federal do Paraná, Curitiba, Brazil
11University of Liège, Liège, Belgium
12St. Vincent’s Hospital, Vienna, Austria
13Hospital Universitario La Fe, Valencia, Spain
14Paris Descartes University, Paris, France
15CPMC Research Institute, San Francisco Coordinating Center, San Francisco, United States
16Leiden University Medical Center, Leiden, Netherlands
17Amgen Australia Pty Ltd, Sydney, Australia

AIM
We report the 2 yr interim results of an open-label extension study designed to evaluate up to 10 years of long-term efficacy and safety of denosumab in the treatment of postmenopausal osteoporosis.

METHODS
Postmenopausal women who completed the Freedom study were invited to participate in the extension study. All women receive denosumab (60 mg) every 6 months and daily calcium and vitamin D. For women who received placebo during FREEDOM, the data presented here reflects 2 years of denosumab treatment (cross-over group). For women who received denosumab during FREEDOM, the data presented here reflects 5 years of continuous denosumab treatment (long-term group).

RESULTS
Of the women who completed FREEDOM, 70% (4550) enrolled in the extension (2207 cross-over; 2343 long-term). Similar to those in the denosumab group in FREEDOM, the cross-over group in the extension study had significant gains (P<0.0001) in the lumbar spine (7.9%) and total hip (4.1%) BMD in the first two years. In the long-term group, there were further significant increases (P<0.0001) in BMD to a total of 13.7% (lumbar spine) and 7% (total hip) from FREEDOM baseline. Serum C-telopeptide (CTX) was rapidly reduced following denosumab dosing in both groups, with the characteristic attenuation of CTX reduction at the end of the dosing period. New vertebral and nonvertebral fracture incidence remained low in both groups. Incidences of adverse events (AEs) and serious AEs (SAEs) were similar to or lower than in the FREEDOM study. In particular, incidence rates of SAEs of infection in the long-term group were similar to or lower than in the FREEDOM study.
CONCLUSIONS
The interim safety and efficacy results from this extension study are consistent with the original FREEDOM study results and provide long-term exposure data for up to 5 years.
(1) Cummings NEJM 2009; 361:756

### INCREASED INTRACORTICAL POROSITY IS ASSOCIATED WITH HIGHER SERUM UNDERCARBOXYLATED OSTEOCALCIN IN MIDDLE-AGED MEN

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tibia</th>
<th>Radius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porosity within compact-appearing cortex</td>
<td>r=0.57***</td>
<td>r=0.60***</td>
</tr>
<tr>
<td>Porosity of the transitional zone</td>
<td>r=0.59***</td>
<td>r=0.63***</td>
</tr>
<tr>
<td>Fraction of the trabecular (medullary) compartment that is void</td>
<td>r=0.59***</td>
<td>r=0.46*</td>
</tr>
</tbody>
</table>

* Indicates p≤ 0.05, ** p≤0.01, *** p≤0.001. OC ucOC (undercarboxylated osteocalcin). (Data were adjusted for age and BMI).

Conclusion: A larger intracortical surface facilitates remodelling and as such may, indirectly, be involved in glycaemic control.

### LOWERING THE EFFICACIOUS DOSE OF BMP-2 USING HEPARAN GLYCOSAMINOGLYCANS

Aim:
Autologous bone graft remains the most reliable method for treatment of orthopaedic trauma, however donor-site morbidity and insufficient graft material limit its use. Graft substitutes containing BMP-2 are efficacious, however supraphysiological doses are required due to its inherent instability. As such, BMP-2 treatment is expensive and associated with unwanted dose-related osteopathologies. The ability to lower the efficacious dose of BMP-2 represents an important therapeutic aim. Heparin has been shown to bind and enhance growth factor activity. Due to >90% sulfation, heparin readily interacts with various proteins to both agonize and antagonize them. Heparan sulfate (HS), a structural analog of heparin, may provide greater selectivity in protein interactions due to its lower, yet greater specificity of sulfation that can be exploited to improve the therapeutic utility of BMP-2.

Method:
In this study we compared heparin and HS that we tuned to avidly bind BMP-2 (HS3), on their ability to enhance BMP-2 activity both in vitro and in vivo. Osteoprogenitor cells were treated with varying doses of BMP-2 in the presence or absence of heparin/HS3 and the mechanism of action determined. Effects were assessed by FACS, immunochemistry, Western blot, ELISA and qPCR-based assays. In vivo testing of was performed using a rabbit ulna segmental defect model and healing determined over an 8-week period as determined by Xray, μCT, histology and mechanical testing.

Results:
HS3 dose-dependently enhanced BMP-2-induced osteogenic differentiation in a manner comparable to heparin. Importantly, BMP-2 activity was greatly enhanced in the presence of heparin/HS3 through a mechanism that involved the inhibition of noggin's antagonism of BMP-2, resulting in sustained ALP activity in the presence of increasing concentrations of noggin. Co-immunoprecipitation revealed that heparin/HS3 reduces noggin/BMP-2 interactions. Heparin/HS3 increased the bioavailability of BMP-2 and sustained pSMAD 1/5/8 signalling over a 72 h period resulting in accelerated osteogenic differentiation. In vivo assessment showed that HS3 alone was capable of stimulating robust bone formation that was similar to exogenous dosing with BMP-2.

Conclusions: These results suggest that heparin/HS3 interacts with BMP-2 and enhances its activity and provides a means to explore the clinical use of HS3 as a bone healing agent.

ADIPose STEM CELLS AND CRANIOFACIAL REGENERATION

Y. Lin
State Key Laboratory of Oral Diseases, West China School of Stomatology, Sichuan University, Chengdu, Sichuan, China

Aim: Craniofacial tissue defects are major and difficult problems in clinic work, currently regenerative medicine approaches are being developed to reconstruct and restore the function of damaged or diseased tissues. Seeding cell is the key factor of the tissue regeneration.

Methods: The multilineage differentiation ability of adipose stem cells (ASCs) was systematically studied, and different cell labeling methods were compared in order to find an ideal marker for transplantation. Furthermore, ASCs were applied in craniofacial tissue regeneration including bone, cartilage, tooth, fat regeneration. Then we tried to elucidate the origination of ASCs and application in vascularization.

Results: ASCs come from pericytes and can contribute to vascularization. ASCs can be induced into osteoblast, chondrocyte, odontoblasts and adipocytes. Loading on different scaffolds, the ASCs can regenerate bone, cartilage, tooth and fat tissue which can be used to repair the different craniofacial tissue defects.

Conclusions: ASCs are ideal seeding cells in the craniofacial tissue regeneration. The multilineage differentiation ability of ASCs supports the feasibility of regenerating multiphasic tissue using a single cell source. ASCs can enhance the vascularization of engineered tissue due to the pericytes origination.

Acknowledgement: NSFC 30801304, 81071273, FANEDD 200977, NCET-08-0373, 2010JQ0066.

A BASIS FOR BUILDING BONE: NOVEL BIOMATERIALS FOR NEW BONE GROWTH

H. Zreiqat, S. Roohani-Esfahani, C. Dunstan, Z. Lu
Biomedical Engineering, University of Sydney, Sydney, NSW, Australia

Clinically available modalities for treating large bone defects are limited in their success. Significant challenges remain in the regeneration of biomechanically functional bone tissue. There is increasing demand for synthetic materials that can regenerate lost or diseased bone. Ideal scaffolds for skeletal tissue regeneration need to have the combined properties of being biocompatible, porous, interconnective degradable, osteo-inductive, osteoconductive, mechanically compatible with bone and bioactive rendering them suitable for treating large bone defects in load-bearing applications. Using the basis of “functional tissue engineering” we have developed novel nanocomposite 3D scaffolds with clinically relevant attributes for skeletal tissue and vascular ingrowth. These scaffolds exhibited mechanical properties and elasticity that are superior to the clinically available ones. This presentation will highlight some of our newly developed nanocomposite scaffolds for effective skeletal tissue integration and vascularization. Innovative biodegradable and bioactive biomaterials for bone augmentation will provide a promising route towards individualized bone tissue regeneration.
THE CONTRIBUTION OF SOFT TISSUE OSTEOPROGENITORS TO SPINAL FUSION


Orthopaedic Research & Biotechnology Unit, The Children’s Hospital at Westmead, Sydney, NSW, Australia
Faculty of Medicine, University of Sydney, Sydney, NSW, Australia
Faculty of Engineering, University of Sydney, Sydney, NSW, Australia

Background: Spinal fusion surgery has been performed for almost a century to prevent the deterioration of spinal anomalies. This highly invasive surgical procedure uses bone graft and/or bone morphogenetic proteins (BMPs) to stimulate fusion (union) of multiple vertebrae. The identity of osteoprogenitors that these osteogenic stimuli act upon is poorly understood. We have hypothesized that myogenic and/or vascular progenitors from the adjacent musculature may contribute to fusion.

Methods: We created a newly refined surgical methodology for induction spinal fusion in mice. This procedure features a midline approach and delivery of BMP-2 via collagen sponges inserted between the spinal processes and the adjacent muscles. BMP-induced bone was visualized in 3D by micro-computed tomography. Experiments were performed in MyoD-cre+;Z/AP+ and Tie2-cre+;Z/AP+ transgenic conditional reporter mice. In these mice, staining for the heat-stable human alkaline phosphatase reporter permitted lineage tracking of myogenic and vascular progenitors in vivo.

Results: Dose response experiments with rhBMP-2 have illustrated that robust bone formation can be achieved with 10µg rhBMP-2, however fusion can still be obtained with doses as low as 1µg rhBMP-2. Histological staining with Picrosirius red/Alcian blue show the early presence of cartilage, which is later replace with bone. Lineage stains have shown that MyoD-lineage cells contribute significantly to bone formation in this model. Data from tracking of Tie2-lineage cells is expected to also be extremely informative.

Conclusion: These data suggest a contribution from cells from the soft tissue adjacent to the spine to the fusion process. These cells may represent a novel target cell population for therapeutic intervention to maximise bone formation.

GETTING TO GRIPS WITH FRAX®: HOW IT REALLY WORKS

J. Kanis

University of Sheffield Medical School, Sheffield, Great Britain
WHO Collaborating Centre for Metabolic Bone Diseases, Australia

FRAX® is a computer based algorithm (http://www.shef.ac.uk/FRAX) that calculates the 10-year probability of a major fracture (hip, clinical spine, humerus or wrist fracture) and the 10-year probability of hip. Fracture risk is calculated from age, body mass index and dichotomized risk factors comprising prior fragility fracture, parental history of hip fracture, current tobacco smoking, long-term oral glucocorticoid use, rheumatoid arthritis, other causes of secondary osteoporosis and alcohol consumption. Femoral neck bone mineral density (BMD) can be optionally input to enhance fracture risk prediction. The risk of death is calculated from the same array of risk factors. Fracture probability is computed taking both the risk of fracture and the risk of death into account. This is important because some of the risk factors affect the risk of death as well as the fracture risk. Examples include increasing age, sex, low BMI, low BMD, use of glucocorticoids and smoking. Other risk algorithms calculate the probability of a clinical event without taking into account the possibility of death from other causes.

The relationships between risk factors and fracture probability have been constructed using information derived from the primary data of nine population based cohorts from around the world, including centres from North America, Europe, Asia and Australia. Clinical risk factors for fracture were identified that provided independent information on fracture risk based on a series of meta-analyses. The FRAX algorithm has been validated in 11 independent cohorts with a similar geographic distribution with in excess of 1 million patient years. The use of primary data for the model construct permits the determination of the predictive importance in a multivariable context of each of the risk factors, as well as interactions between risk factors, and thereby optimises the accuracy by which fracture probability can be computed.

In addition, the FRAX models are calibrated for different countries using country-specific fracture and mortality rates. Models are currently available for 31 countries across the world. FRAX is now, or is in the process of being, incorporated in many clinical guidelines. Despite the wide acceptance of the tool, FRAX should not be considered as a gold standard in patient assessment, but rather as a reference platform. The same argument applies to BMD testing. Thus, the fracture risk estimates derived from FRAX (or BMD alone) should not be uncritically used in the management of patients without an appreciation of its limitations as well as its strengths. In some instances, limitations (e.g. to experts in bone disease) are perceived as strengths to others (e.g. primary care physicians). Several of these limitations (perceived and real) are discussed.
EFFECT OF CO-MORBIDITIES ON FRACTURE RISK: FINDINGS FROM THE GLOW STUDY


1 MRC Lifecourse Epidemiology Unit, Southampton General Hospital, Southampton, Great Britain
2 School of Clinical Medicine, Addenbrooke’s Hospital, Cambridge University, Cambridge, United Kingdom
3 University of Massachusetts Medical School, Worcester, United States
4 Department of Medicine, Columbia University Medical Center, United States
5 Centre for Outcomes Research, UMASS Medical School, Worcester, United States
6 St. Joseph’s Hospital, McMaster University, Hamilton, Canada
7 Division of Geriatric Medicine, Leuven University Center for Metabolic Bone Diseases, Leuven, Belgium
8 INSERM U831, Université de Lyon, Lyon, France
9 Hospital del Mar-IMIM-Autonomous, University of Barcelona, Barcelona, Spain
10 Fred Hutchinson Cancer Research Center, Seattle, United States
11 Department of Endocrinology, VU University Medical Center, Amsterdam, Netherlands
12 Department of Internal Medicine III, Alfréd Krupp Krankenhaus, Essen, Germany
13 Department of Rheumatology, University of Verona, Verona, Italy
14 Cochin Hospital, Paris Descartes University, Paris, France
15 University of Alabama-Birmingham, Birmingham, United States
16 Royal North Shore Hospital, University of Sydney, Sydney, Australia
17 Department of Rheumatology, Cedars-Sinai/UCLA, Los Angeles, United States
18 Bone Health and Osteoporosis Center, University of Cincinnati, Cincinnati, United States
19 University of Pittsburgh, Pittsburgh, United States
20 Institute of Musculoskeletal Sciences, University of Oxford, Oxford, United Kingdom

Introduction. Greater awareness of the relationship between co-morbidities and fracture risk can improve fracture prediction in algorithms such as FRAX®. We utilised a large, multinational cohort study to investigate the effect of co-morbidities on fracture risk.

Methods. 52,960 women were recruited from The Global Longitudinal Study of Osteoporosis in Women (GLOW). GLOW is an observational prospective study of women aged 55 years and older recruited through 723 primary physician practices in 17 sites in 10 countries. At baseline, women completed a questionnaire that recorded co-morbidity history and prevalent fragility fracture. Incident clinical fracture history was recorded annually. A co-morbidity index, defined as number of baseline co-morbidities from the following was derived: high blood pressure, high cholesterol, heart disease, stroke, asthma, chronic obstructive pulmonary disease (COPD), arthritis (reported osteoarthritis & rheumatoid arthritis), inflammatory bowel disease, coeliac disease, cancer, diabetes, multiple sclerosis and Parkinson's disease.

Results. 3224 (6.1%) women sustained an incident fracture over 2 years of follow-up. Co-morbidities were common: 26,215 women (49.5%) reported hypertension, and 26,084 women (49.3%) high cholesterol levels. All recorded co-morbidities were significantly associated with fracture except high cholesterol, and coeliac disease; the strongest association was seen with Parkinson's disease [HR 2.13 (95% CI 1.51, 3.01), p<0.0001]. The HR of fracture increased with increasing co-morbidity index [HR 1 versus 0 co-morbidities: 1.08 (0.93, 1.25); HR 4+ versus 0 comorbidities: 1.49 (1.27, 1.74), after adjustment for age, BMI, prior fracture, current steroid use, alcohol more than 3 drinks/day and current smoking]. The co-morbidities that contributed most to fracture prediction, in order of importance, were: arthritis, Parkinson’s disease, COPD, diabetes and multiple sclerosis.

Conclusion. Co-morbidities as captured in a comorbidity index, contribute significantly to fracture risk. Arthritis and Parkinson’s disease carry a particularly high risk of fracture. Increasing co-morbidity index was associated with increasing fracture risk.

ABSTRACT OF THE GLOW STUDY


Introduction. Greater awareness of the relationship between co-morbidities and fracture risk can improve fracture prediction in algorithms such as FRAX®. We utilised a large, multinational cohort study to investigate the effect of co-morbidities on fracture risk.

Methods. 52,960 women were recruited from The Global Longitudinal Study of Osteoporosis in Women (GLOW). GLOW is an observational prospective study of women aged 55 years and older recruited through 723 primary physician practices in 17 sites in 10 countries. At baseline, women completed a questionnaire that recorded co-morbidity history and prevalent fragility fracture. Incident clinical fracture history was recorded annually. A co-morbidity index, defined as number of baseline co-morbidities from the following was derived: high blood pressure, high cholesterol, heart disease, stroke, asthma, chronic obstructive pulmonary disease (COPD), arthritis (reported osteoarthritis & rheumatoid arthritis), inflammatory bowel disease, coeliac disease, cancer, diabetes, multiple sclerosis and Parkinson’s disease.

Results. 3224 (6.1%) women sustained an incident fracture over 2 years of follow-up. Co-morbidities were common: 26,215 women (49.5%) reported hypertension, and 26,084 women (49.3%) high cholesterol levels. All recorded co-morbidities were significantly associated with fracture except high cholesterol, and coeliac disease; the strongest association was seen with Parkinson's disease [HR 2.13 (95% CI 1.51, 3.01), p<0.0001]. The HR of fracture increased with increasing co-morbidity index [HR 1 versus 0 co-morbidities: 1.08 (0.93, 1.25); HR 4+ versus 0 comorbidities: 1.49 (1.27, 1.74), after adjustment for age, BMI, prior fracture, current steroid use, alcohol more than 3 drinks/day and current smoking]. The co-morbidities that contributed most to fracture prediction, in order of importance, were: arthritis, Parkinson’s disease, COPD, diabetes and multiple sclerosis.

Conclusion. Co-morbidities as captured in a comorbidity index, contribute significantly to fracture risk. Arthritis and Parkinson’s disease carry a particularly high risk of fracture. Increasing co-morbidity index was associated with increasing fracture risk.
6%-10% in men. Clinical decision based on prior fracture alone yielded suboptimal NB for hip fracture. For all models and for a given threshold, the NB was greater in women than in men.

Conclusion. These results suggest that a 10-year risk of any fracture between 10-20% or hip fracture risk between 6-10% provides optimal net benefit.

031

TRANSCRIPTIONAL INDUCTION OF ADAMTS5 BY AN NF-KB FAMILY MEMBER RELA/P65 IN CHONDROCYTES DURING OSTEOARTHRITIS DEVELOPMENT

H. Kobayashi

Departments of Sensory & Motor System Medicine, The University of Tokyo hospital, Hongo, Bunkyo-ku, Tokyo, Japan

ADAMTS5 (aggrecanase-2) is known to be a crucial proteinase that degrades joint cartilage during osteoarthritis (OA) development. To elucidate the molecular network as a therapeutic target of OA, the present study attempted to identify transcription factors to induce ADAMTS5 expression and examined the underlying mechanism. Exhaustive comparison of the genomic sequences of about 2 kb of 5'-end flanking regions of human, macaca, and mouse ADAMTS5 genes revealed that the 1.4 kb region upstream of the transcriptional start site was highly conserved among species. The sequence search in this region predicted the consensus binding motifs of NF-xB, C/EBP, GATA, RUNX, AP-1, OCT, SOX, STAT, and HIF. We therefore created expression vectors of 12 representative transcription factors for these sites, and transfected them in chondrogenic ATDC5 and non-chondrogenic HeLa cells with a luciferase reporter construct containing the 1.4 kb ADAMTS5 gene fragment. Among the transcription factors, an NF-xB family member, RELA/p65, most strongly stimulated the luciferase activity in both cells. In the ADAMTS5 genes, there were three NF-xB binding motifs: −1,196/−1,187, −896/−887, and −424/−415 bp, in which deletion, mutagenesis, and tandem-repeat analyses of the luciferase assay identified the core responsive regions of RELA/p65 to be the two upstream motifs. Electrophoretic mobility shift assay revealed the binding of nuclear extracts of RELA/p65-overexpressed COS-7 cells with the two NF-xB motif oligonucleotide probes. The specificity of the binding was verified by the cold competition with excess amount of the unlabelled wild-type probe and by the supershift with an antibody to RELA/p65. Retroviral overexpression of RELA/p65 markedly increased the Adamts5 expression in ATDC5 cells. Furthermore, IL-1β, a putative inducer of the NF-xB signal as well as OA development, enhanced Adamts5 and Rela/p65 expressions in ATDC5 cells. The Adamts5 induction by IL-1β was suppressed by the knockdown of Rela/p65 with its specific siRNA transfection. Finally, in the experimental OA model by surgical induction of instability in the knee joints of 8-week-old mice, Adamts5 and Rela/p65 were co-expressed in chondrocytes of the degraded joint cartilage. In conclusion, we identified RELA/p65 as a potent transcriptional activator of ADAMTS5 in chondrocytes during OA development. The molecular network related to the RELA/p65-ADAMTS5 axis may thus represent a therapeutic target for OA.

032

EFFECTS OF MONOSODIUM URATE (MSU) CRYSTALS ON CHONDROCYTE VIABILITY AND FUNCTION; IMPLICATIONS FOR DEVELOPMENT OF JOINT DAMAGE IN CHRONIC GOUT

A. Chhana1, K. E. Callon1, B. Pool1, D. Naot2, G. Gamble3, F. McQueen3, M. Dray3, J. Cornish3, N. Dalbeth1

1Medicine, University of Auckland, Auckland, New Zealand
2Molecular Medicine & Pathology, University of Auckland, Auckland, New Zealand
3Anatomical Pathology, Middlemore Hospital, Auckland, New Zealand

Aim: Chondrocytes, the stromal cells of cartilage, are important mediators of cartilage degradation in arthropathies such as osteoarthritis and rheumatoid arthritis. In gout, focal cartilage damage occurs within the joint at sites of urate crystal deposition. We hypothesised that interactions between chondrocytes and monosodium urate monohydrate (MSU) crystals contribute to cartilage damage in chronic gout.

Methods: MSU crystals were prepared by recrystallisation of uric acid. Cultures of primary human chondrocytes were prepared from cartilage obtained from patients undergoing knee or hip arthroplasty. These cells were cultured under non-adherent conditions using tissue culture plates coated with poly-(2-hydroxyethyl methacrylate), PicoGreen and alamarBlue assays were used to assess chondrocyte viability following culture with MSU crystals. Quantitative real-time PCR was used to determine changes in gene expression in chondrocytes cultured with MSU crystals. Joint samples from patients with gout were stained with toluidine blue and analysed for cartilage morphology.

Results: MSU crystals rapidly reduced chondrocyte viability in a dose-dependent manner. The reduction in chondrocyte viability was specific to MSU crystals, as soluble uric acid did not alter cell viability. Culture with MSU crystals reduced mRNA expression of matrix proteins and increased mRNA expression of degradative enzymes such as MMPs (matrix metalloproteases) and ADAMTS (A disintegrin and metalloproteinase with thrombospondin motifs) peptidases. In cartilage samples from patients with gout, cartilage adjacent to tophus was highly disordered with loss of normal architecture and reduced proteoglycan staining.

Conclusions: These data indicate that MSU crystals may contribute to cartilage damage in gout through reduction of chondrocyte viability and promotion of a catabolic state.
THE CROSS TALKS BETWEEN WNT/B-CATENIN AND RAC-1 SIGNALING IN REGULATION OF MAINTENANCE AND FUNCTION OF SUPERFICIAL CELL LAYER IN ARTICULAR CARTILAGE

R. Yasuhara1, M. Enamoto-Iwamoto2, D. Suzuki3, A. Yamada4, A. Aiba5, S. Takeda4, R. Kamijo3

1Department of Oral Pathology and Diagnosis, School of Dentistry, Showa University, Tokyo, Japan
2Division of Orthopedic Surgery, Department of Surgery, Children’s Hospital of Philadelphia, Philadelphia, PA, United States
3Department of Biochemistry, School of Dentistry, Showa University, Tokyo, Japan
4Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
5Division of Endocrinology, Metabolism and Nephrology Department of Internal Medicine, Keio University, School of Medicine, Tokyo, Japan

• yAim: Articular cartilage has poor ability to regenerate and repair, and thereby proceed toward osteoarthritis. The superficial layer (SFL) in articular cartilage has received much attention as an initial site of cartilage degeneration, however, the exact regulation of SFL function remain largely unclear. We have found that Wnt/β-catenin signaling is critical for maintenance of SFL in articular cartilage using the loss- and gain-of-function approaches of β-catenin (Col2CreER/β-catenin and Col111-/-/β-cateninER, respectively). Rac-1, one of the small GTPase has been shown to regulate β-catenin signaling pathway. In this study, we tested whether β-catenin and Rac-1 signaling pathways have a crosstalk in regulation of SFL function in articular cartilage.

• yMethods: zWe isolated the superficial cells (SFCs) that show strong and rapid attachment on fibronectin substrate from mouse articular cartilage and determine the effect of β-catenin and Rac-1 signaling on proliferation and differentiation in SFCs. We also examined the regulation of SFL function by analyzing the articular cartilage in cartilage-specific Rac-1 deficient mice (Col2Cre-Rac-1fl/fl). yResults: zSFCs expressed higher levels of Wnts and receptors for Wnt ligands as well as articular surface markers such as Lubricin and Asporin as compared with chondrocytes. Treatment of Wnt3a, an inducer of Wnt/β-catenin signaling, stimulated proliferation and maintained high expression of Lubricin in SFCs over passages. In contrast, Rac-1-deficient-SFCs expressed lower levels of Lubricin and Asporin than the control cells, and failed to activate β-catenin signaling even after treatment of Wnt3a. At 3- and 6-month-old of ages, Rac-1 deficient articular cartilage was deformed and contained significantly thinner SFL with less number of cells as compared with the control articular cartilage. Interestingly, the similar phenotypes have been also observed in the articular cartilage in β-catenin deficient-mice. yConclusions: zThese results suggest that Rac-1 and Wnt/β-catenin signaling could cooperatively regulate SFL proliferation and function.

INHIBITION OF PROTEIN KINASE-D PROMOTES CARTILAGE REPAIR AT INJURED GROWTH PLATE IN RATS

R. Chung1,2, B. K. Foster3, C. J. Xian1,3

1Sansom Institute, University of South Australia, Adelaide, SA, Australia
2Orthopaedic Surgery, Women’s and Children’s Hospital, North Adelaide, SA, Australia
3Physiology, Adelaide University, Adelaide, SA, Australia

Injured growth plate cartilage is often repaired by bony tissue, impairing bone growth and causing growth defects in children. Currently, molecular events leading up to the undesirable bony repair remain unclear. Aim: This study utilised a rat growth plate injury model to investigate the potential role during growth plate bony repair of protein kinase-D (PKD) which is known to regulate osteoblast differentiation transcription factor osteonectin. Methods: Following surgical injury at the proximal tibial growth plate, young rats received four once-daily injections during days 5-9 of vehicle or 2.35mg/kg g60976 (a PKD inhibitor known for its inhibitory effects on osteonectin), and injured growth plate samples were collected at day 10. Results: Micro-CT analysis revealed that bone volume at the injury site was significantly lower following g60976 treatment compared to the vehicle control (P<0.05). Histological analysis showed that PKD inhibition resulted in an increase in % of mesenchymal repair tissue (P<0.001), a decrease in bone trabeculae and bone marrow tissues, and more cartilaginous tissue within the injury site. Consistently, g60976 treatment decreased mRNA expression at the injury site of bone related genes (osterix and osteocalcin) and increased levels of cartilage related genes (collagen-2a and Sox9). In support, in vitro experiments with rat primary bone marrow stromal progenitor cells showed that addition of g60976 promoted chondrogenic differentiation resulting in a significant increase in collagen-2a expression (P<0.05). Conclusions: These results suggest that PKD is an important factor for growth plate bony repair and blocking PKD activity after growth plate injury may result in less bone formation and potentially more desirable cartilage repair.
NOTCH/RBPJ/HES1 SIGNAL IN CHONDROCYTES MODULATES THE TERMINAL STAGE OF ENDOCHONDRAL OSSIFICATION DURING SKELETAL GROWTH AND OSTEOARTHRITIS DEVELOPMENT

Y. Hosaka1, T. Saito1, S. Sugita1, A. Fukai1, T. Hikata2, H. Akiyama3, T. Nakamura1, K. Nakamura1, U. Chung4, H. Kawaguchi5

1Graduate school of medicine, University of Tokyo, Tokyo, Japan
2Orthopaedic Surgery, Keio University, Tokyo, Japan
3Orthopaedic Surgery, Kyoto University, Kyoto, Japan
4Center for Disease Biology and Integrative Medicine, University of Tokyo, Tokyo, Japan

Here we have examined the role of the Notch signaling pathway in chondrocytes during the endochondral ossification process that is essential for skeletal growth and osteoarthritis (OA) development. In cultures of mouse primary costal chondrocytes and chondrogenic ATDC5 cells, Notch1, 2, the transcriptional effector Rbpj, and the target transcription factor Hes1 were strongly expressed in their terminal differentiation stages during Mmp13 and Vegfa expression, while Notch3, 4 and other target Hes/Hey members were little expressed throughout the differentiation stages. In the limb cartilage of mouse embryos and in the knee joint cartilage of a mouse experimental OA model with surgical induction of instability, intracellular domains (ICD) of Notch1, 2 were localized in the nucleus of highly differentiated chondrocytes in the hypertrophic zone and in the degraded cartilage, respectively, while they remained in the cytoplasm of less differentiated chondrocytes of the proliferative zone and undegraded cartilage. Rbpj and Hes1 were also co-expressed in the nucleus of highly differentiated chondrocytes, while other Notch ICDs and Hes/Hey members were not detected in either cartilage. We then created conditional knockout mice of Rbpj in chondroprogenitor cells (Sox9-Cre;Rbpjfl/fl) and chondrocytes (Col2a1-Cre;Rbpjfl/fl). Although the Sox9-Cre;Rbpjfl/fl mice died shortly after birth, the embryos exhibited dwarfism with impaired matrix degradation and vascular invasion into the cartilage primordia due to decreases of Mmp13 and Vegfa expression. When we created the experimental OA model in a Col2a1-Cre;Rbpjfl/fl mouse line with partial Rbpj inactivation causing normal skeletal growth, the knee OA development was suppressed as compared to the Rbpjfl/fl littermates, with prevention of the terminal stage of endochondral ossification, similar to the Sox9-Cre;Rbpjfl/fl limb cartilage. Retroviral overexpression of Notch1-ICD or Notch2-ICD in ATDC5 cells caused enhancement of Alizarin red and ALP stainings, as well as Mmp13, Vegfa, and Hes1 expression. On the contrary, a Notch inhibitor DAPT suppressed these markers in immature murine articular chondrocytes. Luciferase analyses revealed that the Hes1 transfection enhanced the MMP13 and VEGFA promoter activity most potently among the Hes/Hey members. In conclusion, the Notch/Rbpj/Hes1 signal in chondrocytes modulates the terminal stage of endochondral ossification during skeletal growth and OA development, indicating it to be a possible therapeutic target of OA.

SARCOPENIA, AGING AND SARCO-OSTEOPOROSIS

N. Binkley

Osteoporosis Clinical Center and Research Program, University of Wisconsin, Madison, United States

It is axiomatic that chronologic age is a poor predictor of an individual's functional status. Nonetheless, age is included in a variable in FRAX as fracture risk increases with age. This fracture risk increase with age is much greater than the corresponding BMD decline. Thus, “age” encompasses non-BMD factors that increase fracture risk, likely including lower muscle strength and higher falls risk; factors that differ between individuals. Sarcopenia, the age-related decline in muscle mass and function, is associated with increased falls and fracture risk and could well explain much of the increased fracture risk currently attributed to “age.” It is likely that older adults with both osteoporosis and sarcopenia (sarco-osteoporosis) are at an even higher fracture risk. Consistent with this, a recent report found 45% of women with hip fracture to have sarco-osteoporosis. Is it possible to improve sarco-osteoporosis detection, and thus, potentially, fracture risk prediction?

Sarcopenia definitions, including recent European guidelines, often include low lean mass as measured by DXA. With this approach, appendicular lean mass (ALM) is adjusted for current height (ALM/Height2) and compared to a young adult population with a “low” value, e.g., 2 SD below young adult mean, being part of a sarcopenia definition. However, height loss is common with advancing age; on average 80+ year-old women have lost 8 cm. It is apparent that an older adult who has lost height would have a better ALM/Height2 value than if no loss had occurred and would therefore be less likely to be classified as sarcopenic. Additionally, DXA is often confounded in older adults by spinal degenerative changes that elevate BMD. Proximal femur BMD measurements may also be affected by degenerative changes, whereas the one-third radius site is unaffected. Prior work documents the ability of radius BMD measurement to predict fracture risk. Thus, it is possible that use of historical tallest height and radius BMD measurement might improve sarco-osteoporosis diagnosis and therefore fracture risk prediction in older adults.

In summary, while intuitively logical, it is necessary to determine if diagnosing sarco-osteoporosis enhances fracture prediction. If so, whether additions to the sarco-osteoporosis diagnostic approach (e.g. use of tallest height and inclusion of radius BMD) facilitates risk prediction. Clinical consideration of sarco-osteoporosis seems likely to enhance fracture risk reduction strategies.
SARCOPAENIA AND VITAMIN D

D. Scott
Institute for Health and Social Science Research, CQU, University Australia, Rockhampton, QLD, Australia

Sarcopenia is the term used to described age-related declines in skeletal muscle mass and function, and has been demonstrated to be associated with an increased risk of mobility limitations, disability and loss of independence, falls and fractures, and mortality (1). Whilst sarcopenia appears to represent a ubiquitous and continuous process, the associated functional declines may be largely preventable and even reversible through behavioural modifications such as resistance training and adequate nutrition (2).

There has recently been a great deal of interest in the physiological actions of vitamin D, a secosteroid hormone produced in the epidermis following ultraviolet B light exposure and also obtained in small amounts from some foods (3). Older adults, particularly those with mobility limitations and those who are institutionalised, may be at increased risk of vitamin D deficiency due to inadequate sun exposure and there is evidence to suggest that this may contribute to sarcopenia progression (4).

Higher circulating levels of vitamin D have been associated with improved physical performance (5) and reduced falls (6) in epidemiological studies of older adults. It is likely that these functional benefits are explained at least in part by maintenance of muscle function, with prospective studies demonstrating a reduced loss of strength in older adults with higher baseline vitamin D levels (7-8).

These findings suggest that vitamin D supplementation may represent a potential therapeutic strategy for sarcopenia prevention. However, evidence from randomised controlled trials of vitamin D supplementation suggests that improvements in muscle function may only be obtained in those with vitamin D deficiency or through high-dose supplementation (9-10). The evidence for the role of vitamin D in sarcopenia prevention and implications for future research will be further discussed in this presentation.

(6) Snijder MB, van Schoor NM, Pluijm SMF, van Dam RM, Visser M, Lips P. Vitamin D status in relation to one-year risk of recurrent falling in older men and women. JCEM 2006;91:2980-5.
(7) Visser M et al. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and mass (sarcopenia): The Longitudinal Aging Study Amsterdam. JCEM 2003;88:5766-72.
Conclusions: The prevalence of sarcopenia was greatest for age 70-79. Prevalence data age-standardised to national levels (2001) suggest that sarcopenia affects 15.2% of Australian women aged 60+. Cross-sectional analyses reveal that women with sarcopenia are habitually less active and more likely to be at risk of falling.

ROLE OF TMEM119 IN THE MUSCLE OSSIFICATION SIGNALING
K. Tanaka1,2, Y. Inoue1, G. N. Hendy3, L. Canaff4, T. Katagiri5, R. Kitazawa5, T. Komori5, T. Sugimoto5, S. Seino17, H. Kaji1,7
1Diabetes and Endocrinology, Kobe University Graduate School of Medicine, Kobe, Japan
2Internal Medicine I, Shimane University Faculty of Medicine, Izumo, Japan
3McGill University, Montreal, Quebec, Canada
4Pathophysiology, Saitama Medical University, Saitama, Japan
5Diagnostic Molecular Pathology, Kobe University Graduate School of Medicine, Kobe, Japan
6Basic Medical Sciences, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
7Cellular and Molecular Medicine, Kobe University Graduate School of Medicine, Kobe, Japan

Fibrodysplasia ossificans progressive (FOP) is a genetic disease in which heterotopic ossification occurs in muscle. However, the details of the heterotropic ossification in FOP remain unclear. Our previous studies suggested that Smad3 independently of TGF-β is related to the bone anabolic action of PTH. We identified the PTH-responsive Smad3-related molecule, Tmem119, as an osteoblast differentiation factor. The constitutively activating mutation (R206H) of the bone morphogenetic protein (BMP) receptor, ALK2, underlies the molecular pathogenesis of FOP. In the present study, we performed a DNA microarray analysis between empty vector and ALK2 (R206H)-transfected mouse myoblastic C2C12 cells, and Tmem119 was one of the genes identified, whose expression was increased >3.5 times in the experimental versus control group. Stable Tmem119 overexpression induced the commitment of C2C12 cells into osteoblasts and their mineralization. On the other hand, differentiation of myoblastic cells into myotubes was suppressed, and differentiation into chondrocytes was little affected. Moreover, transcriptional activity of the BMP-2 signaling molecules, Smad1/5, was increased in the absence of exogenous BMP-2 in C2C12 cells. We then analyzed the mechanism whereby Tmem119 induced the commitment of myoblasts into osteoblasts by using C2C12 cells. Stable Tmem119 overexpression enhanced endogenous BMP-2 levels, and a reduction in endogenous Tmem119 by specific siRNA suppressed BMP-2 levels. BMP-2/4 neutralizing antibody and dorsomorphin, an ALK2 inhibitor, antagonized the enhancement by Tmem119 of alkaline phosphatase (ALP) and osteocalcin (OCN). Although Tmem119 interacts with Runx2, Smad1 and Smad5, Tmem119 siRNA antagonized the BMP-2-induction of ALP and OCN, but not Runx2 and Osterix, mRNAs, in C2C12 cells. In conclusion, we showed that Tmem119 promotes the differentiation of myoblasts into osteoblasts and the interaction with the BMP signaling pathway occurs downstream of Runx2 and Osterix in myoblasts. Tmem119 may play a critical role in the commitment of myoprogenitor cells to the osteoblast lineage.

1) Hisa I, J Biol Chem. 2011;286:9787-9796
2) Sowa H, J Biol Chem. 2002;277:36024-3603
4) Sowa H, J Bone Miner Res. 2002;17:1190-1199
5) Tobimatsu T, Endocrinology 2006;147:2583-2590

NON CANONICAL BONE: BONE FAT BRAIN
P. A. Baldock
Garvan Institute, NSW, Australia

Skeletal research is currently undergoing a period of marked expansion. The boundaries of “bone” research are being re-evaluated and are revealing a more complex and interconnected bone biology than previously imagined. One aspect that has become the focus of particular attention is the relationship between bone and fat homeostasis. It is clear that weight exerts a powerful effect upon bone mass; the skeletal response to loading is well known. However, it is increasingly apparent that the relationship is more complex than the response to strain. Emerging evidence indicates that bone and adipose tissue regulation is both related and independent. Signals from fat cells are known to regulate bone mass and signals from bone cells are now being identified that are capable of regulating fat cells. Moreover, this relationship further involves central hypothalamic signalling.

Our laboratory investigates a fundamental controller of skeletal and energy homeostasis, the neuropeptide Y system (NPY). Importantly, the NPY system regulates bone and fat mass through a combination of local and central signalling, and thus presents an opportunity to view the regulatory interactions between Bone and Fat and Brain.

Altering hypothalamic NPY expression exerts powerful effects within bone (reducing osteoblast activity by up to 7-fold) and fat tissue (4-fold increase in fat mass), involving local and central NPY signalling. Moreover, recent work shows osteoblast-specific NPY signals induce changes in energy homeostasis, thereby revealing the interdependence of bone and fat mass, and the role of neural signalling in the regulatory connections.

Clinically, body mass is a strong predictor of fracture, with a marked increase in fracture risk at lower body weights. Importantly, low weight/weight loss as well as low bone density/bone loss are independent predictors of mortality. At this exciting time, the potential to identify novel therapeutic agents has never been greater.
BONES AND SALT: THE RENIN-ANGIOTENSIN-ALDOSTERONE AXIS IN BONE

C. Nowson

Centre for Physical Activity and Nutrition Research, Deakin University, Burwood, Australia

Osteoporosis and hypertension are characterised by abnormalities in calcium metabolism, with emerging evidence of cellular and molecular mechanisms that underlie the co-morbidity of both conditions. Increased urinary calcium has been associated with hypertension, which in some studies has also been associated with increases in parathyroid hormone. Additionally high salt intake is associated with increased urinary calcium losses and reducing salt intake can reduce urinary calcium. Evidence from epidemiological studies suggest a link between hypertension and osteoporosis e.g. higher blood pressure in elderly women was associated with increased bone loss at the femoral neck. Clinical studies indicate a benefit of angiotensin converting enzyme inhibitors (ACE) and angiotensin receptor blockers (ARBs) in reducing fracture risk and improving bone metabolism. It appears that the renin angiotensin system (RAS) may be one of the several factors involved in bone metabolism. RAS plays an important role in regulating blood volume, total body sodium and systemic vascular resistance. As vasculature has an important role in bone remodelling, and alterations in RAS could alter the regulation of blood flow to bone, impacting on bone turnover. Additionally, angiotensin I and II (Al, All) have been found to be potent stimulators of osteoclastic bone resorption. Experiments using transgenic hypertensive mice expressing both the human renin and human angiotensinogen genes indicate that activation of RAS induces high turnover osteoporosis with accelerated bone resorption. Furthermore their results suggest that A II indirectly promotes the differentiation and activation of osteoclasts responsible for bone resorption through the up-regulation of the activator of Receptor Activator of Nuclear factor Kappa-B Ligand (RANKL). Therefore, strategies such as reducing dietary salt intake and the use of specific antihypertensive agents, in addition to thiazide diuretics, such as ACE inhibitors and ARBs which reduce blood pressure and cardiovascular risk may provide additional benefits in reducing osteoporosis and fracture.

LRP5, SEROTONIN AND BONE

M. Warman

Childrens Hospital Boston, MA, United States

Abstract text not available at time of print.

THE ROLE OF OSTEOCALCIN IN GLUCOCORTICOID-INDUCED METABOLIC DYSFUNCTION

T. C. Brennan-Speranza1, K. I. Blankenstein1, C. Gundberg2, C. R. Dunstan1,2, H. Zhou1, M. J. Seibel1

1Bone Research Program, ANZAC Research Institute, University of Sydney, Concord, NSW, Australia
2Department of Orthopaedics, Yale University School of Medicine, New Haven, CT, United States

Biomedical Engineering, Faculty of Engineering, University of Sydney, Sydney, NSW, Australia

We recently demonstrated that osteoblast-targeted disruption of glucocorticoid (GC) signaling attenuates GC-induced bone loss, metabolic dysfunction and obesity, while preventing the marked decrease in circulating osteocalcin usually seen with GC treatment. In the present study, we investigated the role of osteocalcin in GC-induced insulin resistance and glucose intolerance.

Seven-week-old CD1 outbred mice were treated with placebo or corticosterone (1.5mg/week) for 28 days. On day 7, osteocalcin was replaced via hepatic transfection of a non-viral DNA plasmid containing the osteocalcin gene driven by the albumin promoter (pLIVE, Mirus). We transfected either a wild-type osteocalcin construct (wt-OCN) able to undergo gamma carboxylation, or a mutant osteocalcin construct (mOCN) which cannot be carboxylated. Empty vectors were used as controls. Insulin tolerance tests (ITT) and oral glucose tolerance tests (oGTT) were performed 0, 7, 14, 21 and 28 days into GC treatment. Weight, body composition and food intake was monitored throughout. Successful transfection was confirmed via detection of the GFP-containing pLIVE-vector in mouse hepatocytes 7 to 28 days post transfection.

GC-treatment resulted in complete suppression of serum osteocalcin levels at d7 and increasing insulin resistance over the 4-week observation period. GC-treated mice receiving the mOCN construct on day 8 regained their insulin tolerance in a time-dependent manner, with glucose levels falling to 60% of baseline in response to insulin on day 28. Glucose tolerance followed the same pattern. In contrast, GC-treated mice transfected with either the empty vector or the wt-OCN construct remained insulin resistant and glucose intolerant.

In conclusion, the adverse effects of exogenous GC on insulin sensitivity and glucose tolerance in mice can be overcome by replacing non-carboxylated (but not carboxylated) osteocalcin in the circulation. These data provide evidence that the osteoblast-specific peptide, osteocalcin, plays a central role mediating the effects of exogenous GC on systemic energy metabolism.
PATHOPHYSIOLOGY OF BONE DISEASE IN MULTIPLE MYELOMA

A. Zannetino1,2
1SA Pathology-RAH, Centre for Cancer Biology, Adelaide, SA, Australia
2Centre for Translational Cancer Research, University of Adelaide, SA, Australia

Multiple Myeloma (MM) is an incurable haematological malignancy characterised by the clonal proliferation of malignant plasma cells (PC) within the bone marrow (BM). MM accounts for approximately 1% of all cancers and is the second most common haematological malignancy after non-Hodgkin’s Lymphoma. Each year in Australia, approximately 1,100 people are diagnosed with MM, almost 80% of whom are over the age of 60. Alarminglly, in the period between 1993 and 2003, there was a 44% increase in the number of Australians diagnosed with MM. The main clinical manifestations of MM are the development of devastating osteolytic bone lesions, bone pain, hypercalcaemia of malignancy, renal insufficiency and increased BM angiogenesis. MM is the most common cancer to metastasize to bone, with up to 90% of patients developing bone lesions. The bone lesions in MM are purely osteolytic in nature and up to 60% of patients develop a pathologic fracture over the course of their disease. The MM-induced bone lesions are a result of increased osteoclast (OC) activity in areas adjacent to MM PC. This increase in OC activity is also accompanied by a MM PC-mediated suppression of osteoblast differentiation and activity, resulting in severely impaired bone formation. This presentation will cover our current understanding of the pathophysiology underlying bone disease in MM.

MECHANISMS AND PREVENTION FOR CANCER CHEMOTHERAPY-INDUCED BONE DEFECTS

C. Xian1,2
1Sansom Institute for Health Research, University of South Australia, ADELAIDE, Australia
2Disciplines of Physiology and Paediatrics, University of Adelaide, Adelaide, Australia

Cancer chemotherapy often induces bone loss in cancer patients and survivors; yet the underlying mechanisms remain unclear and there are no specific preventative treatments. Aim: This talk summarises our recent studies addressing how the commonly used anti-metabolites cause bone loss and exploring potential therapeutic effects of some adjunct treatments. Methods: Using chemotherapy models in rats treated with methotrexate (MTX) for short-term (5 daily injections at 0.75mg/kg) or long-term (1.5mg/kg twice weekly 6 weeks), or acutely with 5-fluorouracil (5-FU) (single dose at 150mg/kg) , we investigated cellular and molecular mechanisms for chemotherapy-induced growth plate dysfunction, bone formation/resorption, and bone – fat switch, and explored effects of supplementary treatments with folic acid and some anti-inflammatory and/or anti-oxidant nutraceuticals. Results: Chemotherapy induces growth plate chondrocyte apoptosis and suppresses proliferation and collagen-2 expression, leading to thinner growth plate and depressed endochondral bone formation. Chemotherapy attenuates Wnt/ b-catenin signaling and increases expression of osteoclastogenic cytokines (TNF-a , IL-1 b , IL-6, RANKL) in the bone and bone marrow stromal cells. We have found that chemotherapy causes bone loss by decreasing pool of marrow osteoprogenitor cells, reducing osteoblast but enhancing adipocyte differentiation, increasing formation of osteoclasts, leading to reduced bone formation and increased bone resorption and marrow adiposity. Supplementation with folic acid (antidote for MTX) attenuated MTX damaging effects on growth plate and bone formation. Oral doses of some nutraceuticals preserved osteoprogenitor content and bone formation, suppressed expression of osteoclastogenic factors and osteoclast density in bone, and minimized marrow adiposity. In addition, preserving Wnt signaling by blocking its antagonist(s) also abrogated the bone defects. Conclusions: Our studies indicate that cancer chemotherapy causes bone defects by damaging multiple compartments in the bone, and that some supplementary treatments may be beneficial in preserving bone integrity during chemotherapy. Acknowledgements: These studies were funded in part by NHMRC, Bone Growth Foundation, and Channel-7 Children’s Research Foundation. (Disclosure: No conflicts of interest.)

BONE PAIN ASSOCIATED WITH CANCER METASTASIS TO BONE

T. Yoneda, M. Nakanishi, T. Nishisho, H. Wakabayashi, K. Hata
Department of Biochemistry, Osaka University Graduate School of Dentistry, Suita, Osaka, Japan

Bone pain is one of the major complications that deleteriously affect QOL, prognosis and management of cancer patients with bone metastases. Although the precise mechanism of bone pain is still unclear, the longstanding clinical observations that specific inhibitors of osteoclastic bone resorption such as bisphosphonate (BP) effectively reduce bone pain suggest a potential role of osteoclasts, which play a central role in the pathophysiology of bone metastases. Osteoclasts dissolve bone minerals by releasing protons through the a3 isoform vacuolar type proton pump (a3V- H--ATPase). Moreover, metastatic cancer cells and inflammatory cells also produce protons. Thus, the microenvironment of bone metastases is most likely acidic. Since local acidosis is a well-known algesic factor, we reasoned that acidic microenvironment was involved in causing bone pain in bone metastases. Recent studies have shown that protons activate the acid-sensing receptors including the transient receptor potential channel-vanilloid subfamily member 1 (TRPV1), which converts the noxious acid signal into electrochemical signals and transmits it to brain as pain via dorsal root ganglion (DRG) and spinal cord. Our immunohistochemical (IHC) examination showed that TRPV1 was expressed on the calcitonin gene-related protein (CGRP)-positive sensory neurons innervating bone. To study the role of TRPV1, we developed an animal model of cancer-induced bone pain by inoculating cancer cells into the bone marrow cavity of tibiae in wild-type (WT)
and TRPV1-deficient (TRPV-/−) mice. In WT mice, cancer-bearing tibiae showed hyperalgesia and increased hind-limb lifting (flinching) compared with non-cancer-bearing tibiae, indicating cancer colonization induced bone pain. The BP zoledronic acid and an inhibitor of V-H+-ATPase bafilomycin A1 significantly reduced the hyperalgesia and flinching, suggesting a critical role of protons released by osteoclasts. Of note, however, there were no differences in hyperalgesia and flinching between cancer-bearing and non-cancer-bearing tibiae in TRPV1-/− mice, demonstrating an essential role of TRPV1 in cancer-induced bone pain. Acid (pH 5.5) increased Erk phosphorylation and transcription activity of CREB, leading to an up-regulation of CGRP production in DRG in organ culture. IRTX, a specific inhibitor of TRPV1 activation, inhibited these events. In contrast, acid failed to activate these events in TRPV-/− DRG. In conclusion, our results suggest that the activation of TRPV1 on the sensory neurons innervating bone by acidic microenvironment of bone metastasis plays a critical role in cancer-induced bone pain.

047

TREATMENT WITH INTERLEUKIN 6 RECEPTOR ANTIBODIES INHIBITS PROSTATE CANCER GROWTH IN A MURINE MODEL OF BONE METASTASIS

D. Basel1,2, Y. Zheng1,2, J. Kelly1, F. Buttgereit1, R. L. Sutherland2, C. R. Dunstan1,4, H. Zhou1, M. J. Seibel1,2

1Bone Research Program ANZAC Research Institute, The University of Sydney, Concord, Sydney, NSW, Australia
2Cancer Research Program, Garvan Institute of Medical Research, Darlinghurst, Sydney, NSW, Australia
3Dept of Rheumatology and Clinical Immunology, Charite University Medicine, Berlin, Germany
4Department of Biomedical Engineering, The University of Sydney, Sydney, NSW, Australia

Aim: In patients with metastatic prostate cancer, high circulating interleukin-6 (IL-6) levels have been associated with poor clinical outcomes. IL-6 has also been linked to a more aggressive phenotype and progression of hormone refractory prostate cancer. In this study, we investigated the effect of the IL-6 on human prostate cancer growth in vitro and in vivo.

Methods & Results: Treatment of the human prostate cancer cell line, PC3, with RANKL up-regulated IL-6 mRNA expression 2-fold within 4 hrs. Interestingly, treatment of PC3 cells with IL-6, in turn, increased RANK mRNA expression, and PTHrP expression 2-fold. The effects of IL-6 on RANK expression were blocked by treatment of PC3 cells with the anti-human IL-6 receptor antibody, tocilizumab. These data suggest that RANKL, IL-6 and RANK (or PTHrP) may form a ‘feed-forward’ loop that promotes cancer growth in bone.

In-vivo studies: PC3 cells were implanted intra-tibially into 5-week-old nude male mice. Mice were then randomized into 2 groups (n=8 each), receiving either tocilizumab (50mg/kg/3d) or vehicle. Zoledronic acid (ZA) (100 µg/kg/3d) was co-administered in a subset of mice (n=8) to determine the contribution of the bone microenvironment to tumour growth. Mice were monitored by X-ray imaging on d17, d24 and d30 (sacrifice). Compared to controls, treatment with tocilizumab significantly inhibited radiographic osteolysis from d17 onwards. Co-treatment with ZA completely prevented osteolysis.

Conclusions: As tumour-derived IL-6 increased tumour cell RANK expression, and bone-derived RANKL increased tumour cell IL-6 expression, the inhibitory effects of tocilizumab on tumour growth may be due to the interruption of a ‘feed-forward’ loop between tumour and bone cells which involves RANKL, IL-6 and RANK (or PTHrP). Our data indicate that IL-6 plays an important role in the metastatic growth of prostate cancer cells in bone and may be a potential therapeutic target in prostate cancer bone metastasis.

048

TO BMD OR NOT TO BMD?

J. Kanis1,2

1University of Sheffield Medical School, Sheffield, Great Britain
2WHO Collaborating Centre for Metabolic Bone Diseases, Australia

The major clinical applications for BMD testing is to diagnose the disease, assess fracture risk and monitor treatment. The use of BMD in monitoring treatment is controversial and there is a case to be made for abandoning its use for this purpose, in much the same way as the use of aspirin to prevent cardiovascular disease is not monitored. At present, its major use is in patient assessment, and treatment is commonly recommended on the basis of a BMD threshold which in different countries varies from a T-score of -1.5 to -3.0 SD. The development of FRAX, increasingly incorporated into practice guidelines, has meant that patients are treated on the basis of fracture probability of which only a component is derived (optionally) from the measurement of BMD. Thus, intervention thresholds will, in the future, be less reliant on the T-score than on the 10 year probability of fracture.

The ability to assess fracture probability in the absence of BMD is providential for the very many countries that have very limited or no access to BMD testing. For the purpose of risk assessment, a characteristic of major importance is the ability of a technique to predict fractures, traditionally expressed as the increase in relative risk per standard deviation (SD) unit decrease in risk score - termed the gradient of risk. The gradient of risk with the use of FRAX without BMD is comparable to the use of BMD without FRAX. Under such circumstances, FRAX identifies high risk patients that respond to pharmaceutical interventions. In addition, the selection of high risk individuals with FRAX, without knowledge of BMD, preferentially selects for low BMD.

Notwithstanding, the addition of BMD into the FRAX calculation enhances fracture risk prediction, but this does not mean that BMD testing is required in all that might be considered for treatment. Many guidelines recommend treatment in the absence of information on BMD in women with a previous fragility fracture (a prior vertebral or hip fracture in North America). There will be other instances where the probability will be so low that a decision not to treat can be made without BMD. The most efficient use of BMD is to limit testing to individuals who lie close to an intervention threshold, in whom there is a likelihood of reclassifying individuals...
from high to low risk (and vice versa). This parsimonious approach forms the basis of the guidelines adopted by the National Osteoporosis Guideline Group (NOGG) in the UK and avoids unnecessary testing in the majority.

In short, where intervention thresholds are based on fracture probability, treatments can be directed without BMD, but assessment is enhanced with the judicious use of BMD where this would affect a management decision. The wider acceptance of probability-based assessment will erode the clinical need for the T-score and relegate this to epidemiology (for which it was originally developed).

THE ROLE OF FRACTURE LIAISON SERVICES IN THE MANAGEMENT OF PATIENTS WITH OSTEOPOROSIS
M. Seibel
ANZAC Research Institute and Concord Hospital, Australia

Minimal trauma fractures predispose to further fractures, significant morbidity and pre-mature death. Recently published data from NSW reveal that 35% of patients who presented with a first minimal trauma fracture between 2002 and 2008 were re-admitted to hospital with a re-fracture. This accounted for 16,225 admissions, with an average length of stay of 22 days. Of those with re-fractures, 17% died during the time under study (1). Despite the availability of advanced medical care and medications that reduce the risk of refracture, most patients with incident osteoporotic fractures are neither investigated nor treated for their underlying condition. The fundamental need to improve early diagnosis and access to appropriate osteoporosis services has been recognised world-wide. Attempts to address these issues through simple educational campaigns have failed to translate into significant improvements in osteoporosis diagnosis and treatment. In contrast, there is now high quality evidence that the implementation of appropriate models of care for patients with osteoporotic fractures reduces re-fractures, bed days in hospital and other health system usage. The most effective interventions found to improve the health of people with osteoporosis are dedicated fracture liaison services. These models include targeted case-finding, systematic diagnostic assessment, appropriate treatment as well as access to self-management educational programs and support systems, exercise and nutritional advice. Clinical care programs aimed at ensuring appropriate management of patients following an osteoporotic fracture have been developed by individual clinicians throughout Australia but most health services continue to fail in meeting the financial and logistic requirements for the targeted and systematic management of patients with osteoporotic fractures.


INCIDENCE AND PREVENTION OF SECOND HIP FRACTURE IN JAPAN
H. Hagino
School of Health Science and Rehabilitation Division, Tottori University, Yonago, Japan

Hip fractures are a common cause of morbidity and mortality in the elderly and are associated with considerable health expenditures in most industrialized countries. Previous epidemiological studies concluded that the incidence rates of hip fractures for Asian people including Japanese are lower than those for Caucasians living in North Europe and North America. The residual lifetime risk of hip fracture for individuals at 50 years of age for Japanese was estimated as 5.6% for men and 20.0% for women in 2006 and the age- and gender-specific incidence has not plateaued as it has for populations in Northern Europe and North America. Since only a few epidemiological studies have been conducted to determine the incidence of sustaining a second hip fracture within the Asian population, a survey was conducted for all female patients who had experienced hip fracture due to minor trauma for the first time at 25 hospitals in five geographic areas in Japan. The analysis was performed on 2,328 patients and it was found that the rate ratio of a second hip fracture was as high as 4.0 times for women aged 65 years or older after their first hip fracture in comparison to the incidence of hip fractures in the general population. The rate ratio was higher in patients under 75 years of age than that in 75 years or older. Anti-osteoporosis pharmacotherapy was prescribed in 18.7% during the one-year period. Since hip fracture patients are the most plausible candidates in the prevention of subsequent fractures, appropriate osteoporosis treatments are essential, and more aggressive interventions are needed.
TIMING OF ADVERSE OUTCOMES FOLLOWING OSTEOPOROTIC FRACTURES IN ELDERLY WOMEN AND MEN: IMPLICATION FOR LONG TERM MANAGEMENT

D. Blythe1, N. D. Nguyen1, T. V. Nguyen1,2, J. A. Eiseman1,2,3, J. R. Center1,2,3
1Osteoporosis & Bone Biology, Garvan Institute of Medical Research and St Vincent's Hospital, Darlinghurst, NSW, Australia
2Endocrinology, St. Vincent's Hospital, Darlinghurst, NSW, Australia
3University of New South Wales, Sydney, NSW, Australia

Background: The long term risks of re-fracture and mortality following osteoporotic fracture in the elderly are not clear, partly due to their interdependency (ie risk of re-fracture depends upon survival). In this situation, Kaplan-Meier analysis can be unreliable. Thus cumulative incidences of re-fracture, mortality and sustaining both outcomes in elderly men and women using competing risk analyses were examined.

Methods: Subjects from the Dubbo Osteoporosis Epidemiology Study were followed (1989-2007). Initial and subsequent fractures and mortality status obtained. Competing risk models with 4 possible outcomes: death without re-fracture, death following re-fracture, re-fracture but alive, and event–free were considered.

Results: Of the 2245 women and 1760 men (29,660 and 20,171 p-ys, respectively), 952 women and 342 men had an initial osteoporotic fracture. Of these 23% women and 21% men re-fractured and 28% women and 39% men died within 5 yrs. After 5yrs both mortality and re-fracture rates decreased significantly. Long term (>5-10yr) cumulative re-fracture incidence was reduced in both sexes by the competing risk of death, particularly in older age groups. Following re-fracture 50% of women and 75% of men died within 5 yrs so total 5-yr mortality was 41% in women and 58% in men. However, total mortality (post initial and re-fracture) was elevated above population mortality for ≥10 yrs following initial fracture with most of the 5-10yr excess mortality due to that following re-fracture. Re-fracture within 5 years was associated with a higher mortality than re-fracture after 5yrs [adjusted HR 2.44 (95%CI, 1.73-3.45)].

Conclusion: Re-fracture and mortality were highest immediately post fracture, however, excess mortality exists up to 10yrs post fracture, primarily due to that following re-fracture. However, those who survived remaining fracture-free 5-10 yrs post initial fracture had a very low risk of further adverse outcomes, suggesting a less aggressive approach may be appropriate for this population.

ALTERED OSTEOCYTE FUNCTION IN OSTEOARTHRITIS: A POSSIBLE PATHOLOGICAL ROLE IN SUBCHONDRAL BONE SCLEROSIS

Y. Xiao1, A. Jaiprakash1, I. Prasadam1, R. Crawford1, J. Feng2
1Institute of Health and Biomedical Innovation, Queensland University of Technology, Kelvin Grove, QLD, Australia
2Baylor College of Dentistry, Texas A&M Health Science Center, Hoston, Texas, United States

Background and aim: Subchondral bone sclerosis is a recognised manifestation of osteoarthritis (OA) joint. The osteocyte cell network is now considered to be central to the regulation of bone homeostasis but it is still unknown whether the integrity of the osteocyte cell network is altered in OA patients.

Methodology: The pathophysiology of the osteocyte network in OA tissues were studied in tibial knee specimens obtained from patients undergoing knee replacement surgery. Type 1 control (n=5) vs type 4 OA (n=5) subchondral bone volumes was assessed using microCT. Osteocyte cell number and lacunae per unit area were counted. Scanning electron microscopy (SEM) was performed to detect any morphological variations. Immunostaining techniques were applied to observe the relative expression strength of osteocyte specific markers and matrix metalloproteinase’s (MMP’s) in samples graded according to disease severity.

Results: Compared with type 1 controls, type 4 OA subjects showed significant increase in the average number of osteocyte lacunae. There was an increase in the number of average osteocyte nucleus in the type 4 OA patient group. Morphological scanning electronic microscopy images showed defective organization of osteocyte-canalicular system in type 4 OA patients compared to controls. Type 4 OA patients had a lower proportion of osteocytes expressing sclerostin compared to type 1 controls. Conversely, the expression of dentin matrix protein -1, matrix metalloproteinases -1 (MMP-1), MMP-9, and ADAMTS4 were all significantly higher in type 4 OA osteocytes. MicroCT results showed a 20% increase in bone volume in the type 4 OA patient group compared to type 1 controls (p = 0.049).

Conclusion: Dysregulation of osteocytic proteins occur in the course of OA development and appears to be central to altered bone and mineral metabolism in this patient population and is likely to be a critical determinant contributing to pathological changes in OA subchondral bone.
A GAIN-OF-FUNCTION TYPE MUTATION OF THE NATRIURETIC PEPTIDE RECEPTOR B CAUSES ACCELERATION OF SKELETAL GROWTH AND OSTEOPOROTIC CHANGE IN HUMANS AND MICE

K. Miuра1, N. Namba1, M. Fujiwara1, T. Kitakura1, Y. Ohata1, H. Hirai1, C. Higuchi2, N. Tsumaki2, H. Yoshikawa2, T. Michigami3, K. Ozono1

1Pediatrics, Osaka University Graduate School of Medicine, Suita city, Osaka, Japan
2Orthopaedics, Osaka University Graduate School of Medicine, Suita city, Osaka, Japan
3Bone and Mineral Research, Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi city, Osaka, Japan

[Background] C-type natriuretic peptide (CNP)/Natriuretic peptide receptor type B (NPR-B) signaling pathway is known to play an essential role in endochondral ossification.

[Clinical report] The proband is a 12-year-old boy. He had fractures at ages 11 and 12 years and was referred to the pediatric department for evaluation of suspected skeletal dysplasia with advanced growth, fragile bones, and arachnodactyly of hands and feet. Height was 177.0 cm (+2.7 SD). Weight and arm span were in the normal range. Blood pressure was normal. Physical examination showed Marfanoid habitus and markedly longer and wider halluces. Laboratory findings were normal except for increased bone formation and resorption markers. BMD Z-score corrected for his height was -3.9. His mother and maternal grandmother showed the same phenotype as well as severe scoliosis. Although the phenotype resembled overproduction of CNP due to a chromosomal translocation, his karyotype was normal.

[Objective] To determine whether the patient's phenotype results from excessive CNP/NPR-B signaling.

[Design and Method] (1) Direct sequencing of the coding regions of the Natriuretic peptide precursor C (NPPC) and NPR-B genes was performed. (2) To compare cGMP production between wild-type and mutant NPR-B (WT and Mut, respectively), HEK293A cells were transfected with vectors containing WT and Mut and cGMP concentrations were measured after CNP incubation. (3) Furthermore, transgenic mice in which Mut was expressed in chondrocytes under the control of Col11a2 promoter/enhancer were generated.

[Results] (1) We identified a heterozygous missense mutation resulting in a p.Val883Met substitution within the catalytic domain of NPR-B. (2) Treatment with CNP increased HEK293A cGMP levels in a dose-dependent manner. Mut always showed concentrations significantly higher than WT even without CNP (p<0.05). Moreover, circulating levels of cGMP were also increased in the patients. (3) Mut transgenic mice exhibited a phenotype similar to that of the patients. Soft X-rays showed that the mineralized cancellous bone mass was significantly decreased. Stronger osteoporotic change/bone deformity was observed in mice with the highest Mut mRNA expression and advanced with aging.

[Conclusions] We present the first report of a 3-generation family with tall stature due to a gain-of-function NPR-B mutation. Although NPR-B mRNA expression is not restricted to bone, the patients' phenotype seems to be confined only to the bone. Similarly, Mut transgenic mice demonstrated acceleration of skeletal growth and osteoporotic change.

DUAL EFFECTS OF PIM INHIBITION ON MYELOMA: INDUCTION OF BONE FORMATION AND TUMOR SUPPRESSION

M. Hiasa1,2, M. Abe3, A. Nakamo1, K. Watanabe1, C. Qu1, T. Harada1, S. Fujii1, H. Miki1, S. Nakamura1, K. Kagawa1, K. Takeuchi1, E. Tanaka1, K. Asaoka2, S. Ozaki1, T. Matsumoto1

1Department of Medicine and Bioregulatory Sciences, University of Tokushima Graduate School, Tokushima, Japan
2Department of Biomaterials and Bioengineering, University of Tokushima Graduate School, Tokushima, Japan
3Department of Orthodontics and Dentofacial Orthopedics, University of Tokushima Graduate School, Tokushima, Japan

Multiple Myeloma (MM) closely interacts with bone marrow microenvironment to enhance tumor growth along with progression of devastating bone destruction. We recently reported that MM-bone marrow stromal cell (BMSC) interaction potently up-regulate in MM cells the serine/threonine kinase Pim-2 which acts as a critical anti-apoptotic regulator in MM cells (Leukemia, 2011). We also found that the MM cells suppressed osteoblast differentiation from BMSCs along with up-regulation of Pim-2 in BMSCs. In the present study, we therefore explored the role of Pim-2 in osteoblastogenesis and the effects of Pim inhibition on tumor growth and bone destruction in MM. Treatment with Pim-2 siRNA or the Pim inhibitor SMI-16a facilitated mineralized nodule formation by BMP-2 in MC3T3-E1 cells. However, enforced expression of Pim-2 in MC3T3-E1 cells abrogated the mineralized nodule formation, suggesting antagonism of bone formation by Pim-2. The Pim inhibition further up-regulated smad1/5 and p38MAPK phosphorylation as well as osterix expression induced by BMP-2 in MC3T3-E1 cells. Importantly, the Pim inhibition restored mineralized nodule formation in MC3T3-E1 cells suppressed by MM cell conditioned media. Furthermore, treatment with SMI-16a 20 mg/kg i.p. every other day markedly decreased MM tumor size without apparent loss of bone both in MM mouse models with intratibial injection of murine 5TGM1 MM cells and in human INA6 MM cell-bearing SCID-rab MM models, while control mice exhibited extensive bone destruction along with tumor expansion in the bone marrow and outside the bone in microCT images and in bone sections. These results suggest that Pim-2 induced in BMSCs by MM cells plays as a negative regulator for bone formation in MM, and that Pim inhibition is able to resume bone formation while reducing tumor burden in MM. Therefore, Pim inhibitors may become a candidate of novel therapeutic agents targeting the MM-BMSC interaction.
INHIBITION OF RETINOIC ACID RECEPTOR (RAR) SIGNALLING POTENTIATES OSTEOBLAST DIFFERENTIATION AND BONE FORMATION

A. C. Green, T. Jovic, L. E. Purton, E. K. Baker

Stem Cell Regulation Unit, St. Vincent’s Institute, Fitzroy, VIC, Australia

Aim: To investigate the effects of a pan-retinoic acid receptor (RAR) agonist (all-trans retinoic acid; ATRA) and a pan-RAR antagonist (NRX194310) on osteoblast differentiation. Methods: We investigated the effects of ATRA and NRX194310 on osteoblast differentiation of Kusa4b10 stromal cells. We also determined the effects of ATRA and NRX194310 on the differentiation of primary osteoblast lineage populations obtained from collagenase-digested bone. These cells are as follows: mesenchymal stem cells (Sca-1+, CD51-), osteoprogenitors (Sca-1+, CD51+) and osteoblast (Sca-1-, CD51+) cell populations. The effects on osteoblastic differentiation were assessed by Alizarin Red staining for mineralisation and qRT-PCR. To further assess the effects of the RAR pan-agonist on bone, we gavage fed C57BL/6 mice daily for 10 days with NRX194310 (0.5mg/kg/day).

Results: RARα and RARγ subtypes were highly expressed in all osteoblast lineage populations. ATRA (1µM) significantly inhibited mineralisation and expression of genes associated with mature osteoblasts, with a more pronounced effect observed in immature progenitor cells. Supportive of this, ATRA inhibited and NRX194310 significantly potentiated parathyroid hormone (PTH)-stimulated cAMP production in Kusa4b10 cells (P<0.01, n=4). Histomorphometry indicated a significant increase in mineralised bone surface in mice treated with NRX194310 compared to DMSO controls (P<0.05, n=4). However, micro computed tomography (µCT) data showed no significant change in bone volume with 10 days of NRX194310 treatment. Conclusions: These results demonstrate that ATRA inhibits differentiation of osteoblast lineage cells whereas NRX194310 promotes osteoblast differentiation in vitro and bone formation in vivo. Our data also help to explain why high doses of vitamin A are associated with risk of osteoporosis.

Acknowledgments: Dr R.A.S. Chandraratna kindly provided the NRX194310 for these studies.

References:

B CELLS DO NOT INFLUENCE INTRAMEMBRANOUS BONE MODELLING IN VIVO

A. R. Pettit1,2, S. Kaur1, K. A. Alexander1,2,3, K. P.A. MacDonald1, L. J. Raggatt1,2

1UQ Centre for Clinical Research, The University of Queensland, Herston, QLD, Australia
2Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD, Australia
3Queensland Institute for Medical Research, Herston, QLD, Australia

Aim: Fracture healing in mice is accelerated in the absence of B and T cells, but dissection of the independent role of each of these lymphoid populations has not been undertaken. Osteoblasts support B cell development therefore we were interested to determine if B cells reciprocate any contribution to osteoblast maintenance and function.

Methods: Immunohistochemistry was used to examine distribution of cell populations of interest in either wild-type or μMT deficient mice (lack mature B cells). Adapted histomorphometry was used to quantify the extent of physiological endocortical osteoblast bone surface and osteal macrophage (osteomac) canopy. Bone repair was assessed in a stabilized tibial injury model that heals predominantly via intramembranous ossification resulting in complete intra-medullar and inter-cortical bridging of the defect site by 7 days post injury.

Results: Immunohistochemistry for the B220 antigen indicated that mature B cells were randomly distributed throughout bone marrow of wild-type mice with no propensity or aversion for endosteal regions or sites of bone modelling and/or remodelling. In the endocortical diaphyseal region, adapted histomorphometry demonstrated that wild-type and μMT deficient mice had a similar extent of osteocalcin+ osteoblast bone surface (63±3.4% versus 74±7%, respectively, p=0.13). The extent of the osteoblast-associated osteomac canopy was also comparable in these mice (77±1.6% versus 71±4.3%, respectively, p = 0.13). In a tibial injury model, B220+ B cells were occasionally scattered within areas of high anabolic activity in wild-type mice. Boney bridging (collagen type 1+ matrix area within the injury site), area of F4/80 (macrophage marker) staining and area of TRAP (osteoclast marker) staining within the injury site were similar in μMT deficient and wild-type mice.

Conclusions: Osteoblast bone forming surface and intramembranous ossification during bone healing are unimpeded in the absence of mature B cells suggesting that these lymphoid cells do not influence anabolic bone modelling in vivo.

SELECTIVE SEROTONIN RE-UPTAKE INHIBITORS (SSRIS) INHIBIT HUMAN OSTEOCLAST AND OSTEOBLAST FORMATION AND FUNCTION

J. M. Hodgkinson1, Y. Wang1, L. J. Williams2, F. M. Collier1, T. J. Fernandes1, M. J. Constable1, J. A. Pasco2, S. Dodd2, G. C. Nicholson3, M. Berk2

1Barwon Biomedical Research, Geelong Hospital, Geelong, Australia
2School of Medicine, Deakin University, Geelong, VIC, Australia
3Rural Clinical School, School of Medicine, University of Queensland, Darling Heights, QLD, Australia

SSRIs are widely used antidepressants and one of the most commonly used medications. Our group was amongst the first to document a link between SSRI use and reduced bone mineral density. Limited studies in animal and human models indicate that
SSRIs may directly regulate serotonin signalling in bone cells. However the mechanism of action of SSRIs on human osteoclast (OC) and osteoblast (OB) formation and function remains unclear. Gene expression levels of serotonin (5-HT) receptors, serotonin transporter (5HTT) and tryptophan hydroxylase-1 (Tph1) were assessed in OC precursors, mature OC, non-mineralising and mineralising primary human OB by real time PCR. OC formation and resorption in the presence of a number of SSRIs was assessed in CFU-GM-derived cells treated with RANKL and M-CSF for 14d. OB were cultured with SSRIs for 28d and assessed for alkaline phosphatase (ALP) activity and bone mineralisation. Cell viability and apoptosis was determined by flow-cytometric annexin V assessment. OC (precursor and mature) and OB (non-mineralising and mineralising) expressed Tph1, 5HTT and 5-HT1B. 5HT2B was expressed only in OB, whereas 5HT2B expression increased dramatically from precursor to mature OC. Except for citalopram (C), the SSRIs all dose-dependently inhibited OC formation and resorption over the range of 1-10μM in the order of potency: sertraline (S) > fluoxetine (Fx) > paroxetine (P) > fluvoxamine (Fvm). SSRIs (except C) also inhibited ALP and bone mineralisation by OB in a similar order, but only at 30μM. SSRIs induced apoptosis in both OC precursors, and OB in an identical pattern to inhibitory effects observed in OC and OB. Treatment with serotonin alone had no effect on either OC or OB parameters. These data demonstrate that SSRIs inhibit bone cell function via apoptosis, but with differing potencies. Given the capacity of SSRIs to sequester in the bone marrow at high concentrations over several months, these data may explain the loss of BMD with chronic use.

C-FOS PLAYS AN ESSENTIAL ROLE IN UP-REGULATION OF RANK EXPRESSION IN OSTEOCLAST PRECURSORS

A. Arai1,2, T. Mizoguchi1, Y. Kohbayashi1, T. Yamashita1, K. Yamada2, J. M. Penninger4, N. Udagawa3, N. Takahashi1

1Institute for Oral Science, Matsumoto Dental University, Shiojiri, Nagano, Japan
2Department of Orthodontics, Matsumoto Dental University, Shiojiri, Nagano, Japan
3Department of Biochemistry, Matsumoto Dental University, Shiojiri, Nagano, Japan
4Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Vienna, Austria

Aim: We previously reported that osteoclast precursors were detected as RANK-positive cells in bone tissues (J Cell Biol 184:541, 2009). RANK-positive cells were observed in bone tissues in RANKL-1 mice, but not in c-Fos-1 mice. On the other hand, F4/80-positive macrophages were similarly observed in bone tissues in both osteopetrotic mice. These results suggest that c-Fos, but not RANKL, is required for the up-regulation of RANK in osteoclast precursors. Then, we analyzed the mechanism of c-Fos-mediated up-regulation of RANK in osteoclast precursors. Methods: Frozen tibial sections were prepared from wild-type mice. RANKL-1 mice, and c-Fos-1 mice and subjected to immunostaining for c-Fms (a receptor of M-CSF). Spleen macrophages (SPMs) were prepared by the treatment with M-CSF of spleen cells obtained from wild-type mice and c-Fos-1 mice. Those SPMs were used for experiments on RANK expression, osteoclast differentiation, and c-Fos and RANK over-expression. Results: (1) c-Fms-positive cells were detected in bone tissues of c-Fos-1 mice and RANKL-1 mice as well as wild-type mice. (2) The expression levels of RANK and c-Fos in wild-type SPMs were increased by the treatment with M-CSF. In contrast, the up-regulation of RANK was not observed in c-Fos-SPMs. (3) The RANK expression in c-Fos-SPMs was increased by the over-expression of c-Fos. (4) Osteoclastic differentiation of c-Fos-SPMs could not be rescued by the over-expression of RANK. Conclusions: We showed for the first time that c-Fos induced by M-CSF plays an essential role in the up-regulation of RANK in osteoclast precursors. Researchers have believed that c-Fos plays an essential role under the RANK-mediated signals in osteoclast precursors to differentiate into osteoclasts. Our results suggest that c-Fos plays essential roles not only in RANKL-induced formation of osteoclasts but also in M-CSF-induced formation of osteoclast precursors.

ONCOSTATIN M POTENTLY INDUCES IL-6 AND RANKL EXPRESSION IN MOUSE SYNOVIAL FIBROBLASTS AND SYNERGIES WITH IL-1

B. Le Goff1, B. A. Tomkin1, S. Singhrant1, T. J. Martin1,2, E. Romas3, N. A. Sims1,2, N. C. Walsh1,2

1St Vincent's Institute for Medical Research, Melbourne, VIC, Australia
2Dept. of Medicine, St Vincent's Hospital, University of Melbourne, Melbourne, VIC, Australia

AIM: Oncostatin M (OSM) is a multi potent cytokine expressed in rheumatoid arthritic and osteoarthritic synovial tissues. OSM alone, or together with pro-inflammatory cytokines like IL-1, can stimulate synovial fibroblasts (SFs) to promote inflammation and joint destruction. We examined acute effects of OSM, IL-1 and their combination on SF expression of IL-6 and RANKL.

METHODS: SFs were isolated from non-arthritic mice and stimulated with mouse OSM (2 ng/mL), mouse IL-1 (10 ng/mL) and their combination for 1, 6 and 24 hrs. Gene expression was assessed by quantitative RT-PCR; protein by flow cytometry and ELISA.

RESULTS: In SFs, OSM and IL-1 increased IL-6 mRNA expression 80-fold at 6 hrs; OSM further increased expression 135-fold at 24 hrs. Profound synergistic upregulation of IL-6 mRNA and protein occurred when submaximal doses of OSM and IL-1 were combined (>1000-fold, mRNA; ~150-fold, protein). OSM and IL-1 both increased RANKL mRNA at 6 hrs (OSM, 9-Fold; IL-1, 4-Fold), with OSM increasing RANKL expression 20-fold at 24 hrs. Combining OSM and IL-1 enhanced RANKL mRNA expression at 24 hrs (100-fold), but without synergism. OSM also stimulated mRNA expression of its co-receptors (OSMR, 6-fold; gp130, 3-fold). Furthermore, OSM increased IL-1 receptor mRNA and protein expression. While IL-1 did not regulate its own receptor, it induced OSMR expression 3-fold. Importantly, the effects of OSM were dependent on OSMR expression.
CONCLUSIONS: OSM, acting alone or in synergy with IL-1, potently stimulates IL-6 and RANKL expression in SFs, with its actions dependent on OSMR expression. The synergism between OSM and IL-1 may be due to the cross-regulation of their respective receptors. This study suggests a significant role for OSM, acting through OSMR, in contributing to inflammation and bone destruction in arthritic joints.

060

EPIGENETIC REGULATION OF OSTEOCLAST DIFFERENTIATION: POSSIBLE INVOLVEMENT OF JMJD3 IN THE HISTONE DEMETHYLATION OF NFATC1
J. Hirose, T. Yasui, T. Matsumoto, H. Masuda, K. Nakamura, S. Tanaka
Sensory & Motor System Medicine, University of Tokyo, Bunkyo-ku, Tokyo, Japan

Recent studies have revealed that gene expression is controlled by epigenetic mechanisms such as chromatin histone modifications and DNA methylation, and that the expression of key developmental genes tend to be regulated by the trimethylation and demethylation of histone H3 lysine 4 (H3K4me3) and lysine 27 (H3K27me3). Osteoclast differentiation is tightly controlled by two essential cytokines, macrophage colony-stimulating factor and receptor activator of nuclear factor kappa B ligand (RANKL). However, the role of epigenetic regulation in osteoclast differentiation is poorly understood. We applied massively parallel sequencing of the chromatin immunoprecipitation products to investigate the H3K4me3 and H3K27me3 modification patterns around the transcription start site (TSS) of several transcription factors known to be important for osteoelasticogenesis, i.e. Mef2, Nfkb1, Nfkb2, Mif, Fox, and Nfatc1. H3K4me3 was present in both osteoclast precursors and osteoclasts in TSS of all of these transcription factors, except for Mitf. The H3K27me3 marks were present in a relatively broad peak centered on the TSS of Nfatc1, but not of the other transcription factors. Following the treatment with RANKL and subsequent osteoclast differentiation, a marked reduction in the level of H3K27me3 at the Nfatc1 locus was observed. Since the most likely explanation of H3K27me3 demethylation at the Nfatc1 locus is the involvement of H3K27me3 demethylases, we examined the expression of H3K27me3 demethylases during osteoclast differentiation. The expression of Jumonji domain containing 3 (Jmjd3), but not Utx, was time-dependently increased in osteoclast precursors and recruited in the vicinity of the TSS of Nfatc1 after stimulation with RANKL. In addition, gene silencing of the Jmjd3 gene by short hairpin RNA reduced demethylation of H3K27me3 around the TSS of Nfatc1 and markedly suppressed RANKL-induced osteoelasticogenesis. These results suggest that demethylation of H3K27me3 in the vicinity of the TSS of the Nfatc1, regulated by Jmjd3, plays a key role in RANKL-induced osteoclast differentiation.

061

TARGETED DISRUPTION OF THE GLUCOCORTICOID RECEPTOR IN ADIPOCYTES RESULTS IN, AN OSTEOSCLEROTIC PHENOTYPE, INCREASED FAT MASS AND GROWTH RETARDATION IN MICE
Y. Zhang\(^1\), J. Tu\(^1\), J. Kelly\(^1\), C. R. Dunstan\(^2\), A. Rauch\(^1\), J. Tuckermann\(^1\), M. J. Seibel\(^1\), H. Zhou\(^1\)
\(^1\)Bone research program, ANZAC Research Institute, University of Sydney, Sydney, NSW, Australia
\(^2\)Department of Biomedical Engineering, University of Sydney, Sydney, NSW, Australia

Aim: The mechanisms by which glucocorticoids exert their receptor-mediated effects on bone and fat cells are poorly understood. In the present study we aimed to elucidate the role of the glucocorticoid receptor (GR) in adipocytes, and its interaction with bone, through characterisation of an adipocyte GR-deficient mouse line.

Methods: GR\(^{floxed}\)/floxed mice were crossed with Fabp4-Cre mice to generate GR\(^{Fabp4Cre}\) mice, in which the cre recombinase is under the control of the mouse fatty acid binding protein 4 (Fabp4) promoter. Cre-negative-GR\(^{floxed}\)/floxed mice served as denoted wild-type (WT) controls. Mice were analysed for post-natal skeletal changes (by whole body bone and cartilage staining), body composition (by DAX) and bone volume (by micro-CT).

Results: GR\(^{Fabp4Cre}\) and their WT littermates had a similar phenotype at birth, with normal skeletal size and regular bone and cartilage staining. Both groups developed normally until day 6, when GR\(^{Fabp4Cre}\) mice started to display a pleiotropic phenotype with significant growth retardation, pronounced alopecia followed by premature death within 2 weeks after birth. On day 10, skeletal size and body weight were significantly reduced in GR\(^{Fabp4Cre}\) mice when compared to WT littermates (p<0.05). Analysis of body composition revealed a significant increase in total body fat mass and a significant decrease in total body lean mass in GR\(^{Fabp4Cre}\) mice compared to WT littermates (p<0.05 for both). In contrast, trabecular bone volume was significantly increased in Fabp4-GR\(^{ko}\) mice (p<0.05). Despite delayed secondary calcification (Figure, arrows), GR knock-out in adipocytes significantly increased tibial BV/TV, compared to WT mice (p<0.05 compared to WT littermates). In addition, calvaria bone density was increased in GR\(^{Fabp4Cre}\) mice (Figure), indicating that both endochondral and intramembranous bone formation are altered by adipocyte specific GR knock-out mice.

Conclusion: Adipocytic glucocorticoid signalling through the GR may play an important role in the post-natal development and growth of mice with profound skeletal effect.
ANALYSIS OF THE ROLES OF FGF23 IN FETUS-SPECIFIC MINERAL METABOLISM USING HYP MOUSE

Y. Ohata,1,2 M. Miyagawa,1 M. Yamazaki,1 T. Okada,1 M. Kawai,1 K. Ozono,1 T. Michigami1
1Bone and Mineral Research, Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Japan
2Pediatrics, Osaka University Graduate School of Medicine, Suita, Japan

[Aim] Serum levels of phosphate (Pi) in fetuses are maintained higher than maternal levels during late gestation, although the underlying mechanism remains unclear. We have previously reported that placenta expressed Klotho and might be a target of FGF23 signaling. In the current study, we have investigated whether FGF23 plays a role in fetus-specific mineral metabolism, using Hyp mice with high levels of serum FGF23.

[Methods] Blood samples from E18.5 pregnant Hyp (Phex<sup>Hyp</sup>) and wild-type (WT) mice, and their male fetuses were subjected to the measurement of calcium (Ca), Pi, and FGF23. Genotyping was performed by genomic PCR to distinguish male Hyp (Phex<sup>Hyp</sup>) fetuses from WT fetuses delivered from Phex<sup>Hyp</sup> mothers. Gene expression in placenta was analyzed by real-time PCR.

[Results] Although the Pi level in Phex<sup>Hyp</sup> mothers was lower than that in WT mothers, Pi levels in fetuses were comparable among the 3 groups; those from WT mothers and Phex<sup>Hyp</sup> and WT fetuses from Phex<sup>Hyp</sup> mothers. The Ca level in Phex<sup>Hyp</sup> fetuses was significantly lower than that in WT littersmates. The FGF23 level in Phex<sup>Hyp</sup> mothers was higher than that in WT mothers. WT fetuses from both Phex<sup>Hyp</sup> and WT mothers had equivalently low levels of FGF23. On the other hand, FGF23 level in Phex<sup>Hyp</sup> fetuses was about 20-fold higher than that in Phex<sup>Hyp</sup> mothers. The expression of vitamin D receptor (Vdr) was decreased in placenta from Phex<sup>Hyp</sup> mothers. In isolated trophoblasts, FGF23 induced the phosphorylation of ERK1/2 and the expression of Egr-1, and decreased the expression of Vdr.

[Conclusions] Materno-fetal Pi transport is accelerated in Phex<sup>Hyp</sup> mothers with high FGF23 levels, independently of the genotype of fetuses, suggesting that maternal FGF23 might play a role in Pi transport. On the other hand, FGF23 in fetuses may be involved in vitamin D metabolism rather than Pi transport.

A COMPUTATIONAL APPROACH TO UNDERSTANDING FUNCTIONAL BEHAVIOUR OF BONE MULTICELLULAR UNITS

P. R. Buenzli1, P. Pivonka1, J. Jeon2, D. W. Smith1, P. T. Cummings2,3
1Faculty of Engineering, Computing and Mathematics, The University of Western Australia, Crawley, WA, Australia
2Department of Chemical and Biomolecular Engineering, Vanderbilt University, Nashville, TN, United States
3Center for Nanophase Materials Sciences, Oak Ridge National Laboratory, Oak Ridge, TN, United States

Bone remodelling maintains the functionality of skeletal tissue by locally coordinating bone-resorbing cells (osteoclasts) and bone-forming cells (osteoblasts). This coordination is operated within so-called Bone Multicellular Units (BMUs). While several properties of bone cell-cell communication have been assessed experimentally, a comprehensive understanding of the functional behaviour of a BMU from its cells and regulatory factors in a spatio-temporal framework remains to be elucidated. In this contribution, we will present two computational models of cortical BMUs that address this question. In the first model, we show how some of the most important cell communication pathways currently known to exist between osteoblasts and osteoclasts (such as RANK-RANKL-OPG, TGFβ) are able to organise the cells into a travelling structure corresponding to the progression of a cortical BMU. This model allows to understand the spatio-temporal mechanisms of action of the regulatory factors, leading to segregated but functionally-coordinated cells (1). In the second model, we study microscopic bone resorption mechanisms in cortical BMUs and show how the life history of the osteoclasts (generation, apoptosis, nuclei renewal) influences their movement pattern and collective behaviour. These properties strongly influence the shape and extent of the developing resorption cavity, and so functional resorption by BMUs.

(1) P.R. Buenzli, P. Pivonka and D.W. Smith, Spatio-temporal structure of cell distribution in cortical Bone Multicellular Units: A mathematical model, Bone 48 (2011) 918-926

VISCOELASTIC RESPONSE OF PTH, IBANDRONATE AND COMBINATION TREATMENT IN OVARIECTOMIZED RAT FEMUR CORRELATING WITH BMD

X. XANG, T. LEE

BIOENGINEERING, NATIONAL UNIVERSITY OF SINGAPORE, SINGAPORE, SINGAPORE, Singapore

Viscoelastic response upon loading is one of the properties exhibited by bone. Little has been reported on viscosity changes existing in osteoporotic bone or with treatments. Since bone viscoelasticity correlates to load-bearing capacity, the aim of this study is to investigate the viscoelastic properties of ovariectomized and drug-treated rat femurs.

15 SD rats were divided into 5 groups: (1) SHM: sham surgery; (2) O VX: ovariectomy surgery and treated with vehicle saline; (3) PTH: 10μg/kg PTH treated ovariectomized rats; (4) IBN: 7μg/kg ibandronate treated ovariectomized rats; (5) COM: ibandronate and PTH concurrent treated ovariectomized rats. Rats were euthanized at week 12. After metaphyseal region scanning by μCT and
pQCT, femurs were embedded in epoxy and polished. After rehydration, nanoindentation was conducted using CSM mode to determine elastic modulus ($E$) and hardness ($H$). Basic creep test was conducted using the Voigt model. $V$ isocity ($\nu$) is computed based on the curve fitting of displacement by non-linear regression.

μCT analysis suggested that IBN and COM group had a better effect than PTH in preserving trabecular bone in terms of BV/TV, Tb.Th, SMI, BS/BV, Tb.Sp and Tb.N. In BMD, viscosity and SSIy, COM group was significant ly higher than the other two monotherapy groups. In 12 wks, BMD- $\nu$, SSIy- $\eta$ are both positively correlated (R=0.844 and 0.863 respectively, p<0.01 for both), whilst the $E$ or $H$ showed weak correlation with BMD or SSIy.

Our results suggest that:
1. The osteoporotic deterioration does exist in bone viscoelasticity while treatments can dramatically restored decreased $\eta$ and $E$.
2. $\eta$, which strongly correlated with BMD and SSIy, presented its potential to be another bone quality surrogate.
3. PTH has the highest $E$ among treatments. However, $\eta$ of PTH group was significantly lower than the other two. We hypotheses that different drugs have various specialties in improving nano-level bone quality.


065

WHOLE VERTEBRAL BODY STRENGTH PREDICTED BY BONE MINERAL DENSITY FROM DXA AND BY BONE MICROARCHITECTURE FROM MICRO-CT

E. Perilli$^{1,2}$, A. M. Briggs$^{3,5}$, J. D. Codrington$^{1,2}$, S. Kantor$^{1,2}$, I. H. Parkinson$^{1,2}$, N. L. Fazzalari$^{1,2}$, J. D. Wark$^{5}$

$^1$Bone and Joint Research Laboratory, Surgical Pathology, SA Pathology and Hanson Institute, Adelaide, SA, Australia
$^2$Discipline of Anatomy and Pathology, The University of Adelaide, Adelaide, SA, Australia
$^3$Curtin Health Innovation Research Institute, Curtin University, Perth, WA, Australia
$^4$School of Mechanical Engineering, The University of Adelaide, Adelaide, SA, Australia
$^5$Department of Medicine, University of Melbourne, Bone & Mineral Service, Royal Melbourne Hospital, Melbourne, VIC, Australia

The positive relationship between areal bone mineral density (aBMD) derived from dual energy X-ray absorptiometry (DXA) and bone strength underpins aBMD as a good predictor of fracture risk. However, the predictive validity of aBMD for osteoporotic vertebral fractures remains suboptimal. The diagnostic sensitivity of DXA may be improved by assessing vertebral aBMD from lateral projections, compared to the commonly-used posterior-anterior (PA) projections. X-ray micro-computed tomography (micro-CT) allows non-destructive three-dimensional structural characterisation of entire bone segments at high resolution. The aim of this study was to assess vertebral aBMD by both PA- and lateral-projection DXA and bone volume (BV) by micro-CT, and to compare their ability to predict whole vertebral body strength determined experimentally.

Eight human cadaver spines (mean age at death 78±10 years) were immersed in a water bath and scanned by DXA in PA and lateral projections; aBMD for L2 and L3 vertebrae was calculated. The L2 and L3 vertebrae were then dissected from each spine and entirely scanned by micro-CT (18µm pixel size). BV was calculated over the micro-CT trabecular bone volume of the entire vertebrae. The vertebral bodies were then tested to failure in uniaxial compression to determine ultimate load.

aBMD by lateral-projection DXA and BV by micro-CT were both highly predictive of ultimate load ($r^2=0.70$, and $r^2=0.81$, both p<0.01). aBMD by lateral-projection DXA was highly predictive of BV assessed by micro-CT ($r^2=0.68$, p<0.01). Conversely, aBMD by PA-projection DXA had a lower coefficient of determination with ultimate load ($r^2=0.37$, p<0.05) and with BV ($r^2=0.29$, p<0.05). The standard-error-of-the-estimate in predicting ultimate load decreased by 31% when using aBMD from lateral-projection DXA, compared to PA-projection DXA.

These findings highlight the capability of aBMD assessed using lateral-projection DXA to predict vertebral strength, and provide a basis for further exploring the clinical application of lateral-projection DXA analysis.

066

BIOMATERIAL SCAFFOLDS FOR MUSCULOSKELETAL REGENERATIVE MEDICINE: AN IN VITRO ANALYSIS

D. S. Musson$^1$, B. G. Matthews$^1$, V. Terremi$^2$, K. E. Callon$^1$, D. Naot$^1$, J. Cornish$^1$

$^1$Department of Medicine, University of Auckland, Auckland, New Zealand
$^2$Department of Molecular Biology, University of Siena, Siena, Italy

Background:
Injuries to bone and tendons can cause major morbidity in healthy, active people. The ability to provide a scaffold that encourages appropriate cell attachment, growth, and ultimately tissue regeneration, could improve the clinical outcomes from injuries such as rotator cuff tears and non-union fractures.

Aim:
Several scaffold materials of both natural and synthetic origin have been tested in this study to evaluate their potential utility in musculoskeletal regenerative medicine.
Methods:
Four different scaffolds were evaluated as biomaterials: Spidrex 543 (Oxford Biomaterials Ltd, UK), a spider-like silk fabric; Endoform® (Mesynthes, NZ), a decellularised ovine forestomach matrix; three-dimensional (3D) collagen gels and FiberWire® (Athrex, Inc, US), a polyethylene and polyester composite, commercially available suture currently utilised in orthopaedic surgery. Attachment and growth of primary osteoblasts and tenocytes were assessed using live-dead staining and alamar blue fluorescence. Morphological phenotype was assessed using confocal microscopy and cell differentiation was evaluated by differential gene expression.

Results:
Osteoblasts and tenocytes both successfully adhered to and grew on the Endoform®, the silk and within the 3D collagen gels, whereas the orthopaedic suture material proved unsuitable for cell attachment/growth. Gene analysis and morphology in the three permissible scaffolds suggest cells retain their phenotype when cultured in them. The 3D culture systems support increases in proliferation and differentiation, notably, gene expression of key osteoblastic markers alkaline phosphatase, osteocalcin and bone sialoprotein were increased 33-, 240- and 34-fold, respectively, in osteoblasts cultured within 3D collagen gels for 72hrs (P<0.05) compared to osteoblasts in 2D cultures.

Conclusions:
We have identified a number of biomaterial scaffolds that have potential for use in bone and tendon regeneration. Further testing is required to determine if they support tissue formation.
Results. GC and hTNF transgene additively decreased mechanical strength, rigidity/stiffness, and energy to yield in both tibiae and vertebral bodies. In tibial torsion test, GC reduced energy to failure without changing the ratio of post-yield energy to total energy in WT mice, while GC increased energy to failure and the ratio of post-yield energy to total energy in hTNFtg mice. In compressive test of vertebral body, the ratio of post-yield energy to total energy was decreased by GC in hTNFtg mice but not in WT mice. Microstructures of bone were deteriorated mainly by hTNF transgene, while degree of mineralization was decreased by both hTNF and GC.

Conclusions. The results of this study suggest that GC additively decreases bone strength in RA and that hypomineralization of bone in GC-treated RA, which increases ductility of bone, are associated with increased risk of insufficiency fracture.

PREVENTION OF WEAR PARTICLE-INDUCED OSTEOLYSIS BY A NOVEL V-ATPASE INHIBITOR SALIPHENYLHALAMIDE (SALIPHE) THROUGH INHIBITION OF OSTEOCLAST MATURATION AND BONE RESORPTION

A. Qin1,2, T. S. Cheng1, Z. Lin1, L. Cao2, S. M. Chim3, N. J. Pavlos1, J. Xu1, M. H. Zheng1, K. R. Dai2

1Orthopaedic Research, University of Western Australia, Perth, WA, Australia
2Department of Orthopaedics, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China
3School of Pathology and Laboratory Medicine, University of Western Australia, Perth, WA, Australia

We report that particle-induced aseptic loosening of prosthetic devices is associated with osteolysis which is partially attributed to a disruption in osteoclast acidification and polarization, both a prerequisite for osteoclast bone resorption. Here, we demonstrate that two selective V-ATPase inhibitors, saliphenylhalamide and bafilomycin, attenuate wear particle-induced osteolysis in a mouse calvarial model. In vitro biochemical and morphological assays revealed that the inhibition of osteolysis is partially attributed to a disruption in osteoclast acidification and polarization, both a prerequisite for osteoclast bone resorption. Interestingly, V-ATPase inhibitors also impaired osteoclast differentiation via the inhibition of RANKL-induced NF-κB signaling pathway. In conclusion, we showed that V-ATPase inhibitors affected multiple physiological processes including osteoclast differentiation, acidification and polarization, leading to inhibition of osteoclast bone resorption in vitro and wear particle-induced osteolysis in vivo. The results of the study provide proof that V-ATPase inhibitors, such as saliphenylhalamide, are potential anti-resorptive agents for treatment of wear particle-induced osteolysis to prevent aseptic prosthetic loosening.

THE EPIDEMIOLOGY OF PAGET'S DISEASE

T. Cundy

Medicine, FMHS, University of Auckland, Auckland, New Zealand

Archaeological evidence suggests that Paget's disease first appeared in Western European populations in the Roman era. Numerous radiographic surveys carried out since the 1970s have demonstrated considerable between-country variation in prevalence – for example, it was greater in the UK than that in other Western European nations; and uncommon in Scandinavia, Eastern Europe and Asia. Within countries certain regions have high prevalence; for example Lancashire (England) and Campania (Italy). Outside Europe, the epidemiology of Paget's disease closely parallels patterns of emigration from Western Europe. Australia and New Zealand have a relatively high prevalence in descendants of migrants coming from the United Kingdom in the late 19th century, whereas it is rare amongst the indigenous peoples. Similarly, the prevalence is higher in Queensland than other Canadian provinces, and in New England than in the rest of the USA. Other clusters apparently associated with migration include a kindred from Belize, the Argentinian population of Italian descent, and the Jewish population of Recife (Brazil).

In the UK, various Western European centres and New Zealand repeat radiological prevalence surveys have been undertaken in recent years. Compared to the estimates of 25-30 years earlier, the later studies have reported ~50% lower prevalence. It has been argued that the later studies may have underestimated prevalence by the inclusion of non-European subjects, but in the New Zealand studies we only included subjects self-identifying as of European descent, and we have also observed the recent emergence of Paget's disease in migrants of Asian descent, suggesting that this explanation cannot account for the changing epidemiology. Consistent with a falling prevalence is the observation that severe polyostotic disease is becoming something of a rarity. Over a 30 year period in our clinic the severity of disease in newly presenting subjects (judged by plasma ALP and disease extent on scintigraphy) fell steadily, with a reciprocal increase in the proportion of subjects having monostotic disease (now nearly 40% of patients). This is not reflect earlier recognition - the mean age at diagnosis increased from 62 to 74 years over the same period. Because of their greater life expectancy women now form a greater proportion of the affected population. We have also found that in subjects inheriting SQSTM1 mutations Paget's disease emerges significantly later and in a more attenuated form than in their affected parents, suggesting an important gene-environment interaction.

Exactly when the secular change in disease severity and prevalence began is uncertain, but it was noted in 1978 that the proportion of death certificates mentioning Paget's disease had declined progressively in cohorts born between 1870 and 1915, as had the number of adult deaths attributed to osteosarcoma (a Paget's-related condition). The secular changes suggest that there is a significant environmental factor in its etiology. The identity of that factor is unknown, but viruses remain popular candidates. Epidemiological
evidence suggests associations with rural life, and contact with farm animals so a zoonosis remains a possibility. Epidemiological evidence relating Paget's disease to contact with domestic animals has not been corroborated in all studies.

071

GENETICS OF PAGET'S DISEASE OF BONE
S. H. Ralston

Molecular Medicine Centre, Western General Hospital, The University of Edinburgh, South Bridge Edinburgh, Great Britain

Over the past 10 years, tremendous advances have been made in understanding the role that genetic factors play in the regulation of Paget's disease of Bone (PDB).

Current evidence suggests that classical PDB is caused by a combination of rare alleles of large effect size that cause autosomal dominant inheritance of the disease and commoner alleles of smaller effect size. In addition, a number of rare inherited bone disorders have been described with clinical similarity to PDB which are caused by mutations affecting the protein coding regions of the TNFRSF11A, TNFRSF11B and VCP genes. Mutations of the SQSTM1 gene are the most common cause of classical PDB, occurring in about 40-50% of patients with a family history of the disease and 5-20% of those with “sporadic” disease. Most SQSTM1 mutations cluster in the ubiquitin associated (UBA) domain of the protein and impair or abolish the ability of the gene product, p62 to bind ubiquitin. This in turn results in activation of NF k B signaling by mechanisms that are still not completely understood, causing increased osteoclast formation. Knock-in of the common P392L mutation of SQSTM1 into the germ line of mice causes a bone disorder with remarkable similarity to PDB indicating that these mutations are sufficient to cause the disease in the absence of an environmental trigger. Having said that, environmental factors also clearly play a role in PDB, since carriers of SQSTM1 mutations develop the disease about 5-10 years later than their parents did. Other genes and loci that predispose to PDB have recently been identified by genome wide association studies. The susceptibility alleles lie within or close to the TNFRSF11A, CSF1, OPTN, TM7SF4, NUP205, PML and RIN3 genes. Individually these increase the risk of PDB by 35-75% but collectively the effect is large accounting for about 86% of the population attributable risk of the disease. Although many advances have been made in understanding the pathophysiology of PDB much work needs to be done to identify the causal variants and explore the mechanisms by which they interact with environmental factors to cause the disease.

072

PAGET'S DISEASE OF BONE-ASSOCIATED SEQUESTOSOME 1/P62 MUTANT PROTEINS INCREASE RANKL-INDUCED AP-1 SIGNALLING AND AFFECT CELLULAR CO-LOCALISATION WITH KEY SIGNALLING INTERMEDIATES AJUBA AND TRAF6
S. L. Rea1,2, J. Xu1, J. P. Walsh1, T. Ratajczak1,2

1Centre for Medical Research, University of Western Australia, Crawley, WA, Australia
2Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia

School of Pathology and Laboratory Medicine, University of Western Australia, Crawley, WA, Australia

Aim: Paget's disease of bone patients commonly harbour a mutation in the Sequestosome 1/p62 (p62) gene. Most known mutations manifest within or cause deletion of the Ubiquitin-associated (UBA) domain of p62, a scaffold protein that forms complexes with both TRAF6 and the LIM-protein Ajuba in RANKL-induced signalling to NF- k B. We have previously shown that p62 mutant proteins increase NF- k B signalling compared with wild-type p62. The aim of this study was to investigate the effect of p62 mutations on AP-1 activity, and the interaction of p62 with key signalling intermediates in the AP-1 and NF- k B pathways, important for osteoclastogenesis.

Methods: HEK293 cells stably expressing RANK were transfected with an AP-1 luciferase reporter with empty vector or p62 (wild type or mutant). Cells were treated with RANKL or left untreated. Lysates were prepared and tested for AP-1 activity. For co-immunoprecipitations, p62 (wild type or mutant) was co-expressed with either TRAF6 or Ajuba. p62 protein was immunoprecipitated, bound to beads and, following extensive washes, p62 and binding interacting proteins were eluted and processed for Western blot analysis.

Results: We observed that p62 mutant proteins are associated with increased AP-1 activity compared with wild type p62 and the empty vector control. Additionally, we observed that Ajuba induces AP-1 activity under basal and RANKL-induced conditions, an effect that is abrogated by p62 co-expression. We also found that wild type p62 appears to aggregate with key signalling intermediates Ajuba and TRAF6 in the nucleus, whereas UBA-deficient p62 interacts with these proteins primarily in the cytosol.

Conclusions: We conclude that aggregation of signalling intermediates by p62 is the potential mechanism underlying signalling repression that is observed with p62 over-expression. Furthermore, the increased signalling observed with p62 mutant proteins may be due to decreased capacity for client-protein aggregation and/or altered cellular localisation.
WHY BONES FAIL: STRUCTURE AND MECHANICS

M. Forwood
School of Medical Science, Griffith University, Gold Coast, QLD, Australia

Whether bones fail depends on how much energy they can absorb, and how much loading we apply to them. Our bones, therefore, must achieve an adequate safety factor while minimising the energy cost of movement: a trade off between strength and lightness. As we age, or develop skeletal disorders, our bone strength may diminish, reducing the safety factor to the point where activities of daily living can cause fracture. The robusticity of bones per se depends on the properties of their material, and the build of the whole bone structure. This offers three possible mechanisms to reduce their risk of fracture. First, increase bone mass – larger bones can resist greater loads. Second, distribute that mass most effectively – small additions to bone mass can substantially increase bending and torsional strength if placed strategically. Third, improve bone material properties – make the bone matrix, itself, stronger or capable of absorbing more energy. Adding, or redistributing, bone mass influences structural properties, affecting the behaviour of bones as an organ. Alterations in the bone material are manifested in the mechanical properties of bone per unit volume, reflected in measures of stress, strain, modulus of elasticity and modulus of toughness. The influence of such factors that affect bone fragility, but are not accounted for by bone mass or quantity, has been termed bone quality. These variables explain the disparity between the change in BMD and the reduction in fracture risk in response to treatment. Although they are necessary to understand the risk of fracture, they are more inscrutable than mass to measure in vivo. Such factors include true mineral density, maturation and chemical composition; collagen structure and biochemistry; osteocyte viability; porosity, microdamage; and, micro-architecture. The technologies that measure bone quality to assess fracture risk are emerging, but embryonic. Greater understanding of the material properties of bone, and its interaction with structure, will ultimately improve the assessment of fracture risk and monitoring of patients being treated for metabolic bone disease.

QUANTIFYING THE MATERIAL COMPOSITION AND STRUCTURE OF BONE NON-INVASIVELY

E. Seeman1,2
1University of Melbourne, VIC, Australia
2Austin Health, Australia

Bone is constituted by differing proportions of water, mineral and collagen phases assembled in three dimensional space. Each phase differentially attenuates photons. The ability to accurately quantify the degree of attenuation produced by the differing proportions of each of the three phases within each of the ~5 million voxels embracing a region of interest, allows the accurate reconstruction of the external dimensions and shape of the region and its internal microarchitecture voxel by voxel, using noninvasive methods in vivo provided the information contained within each voxel is not disregarded or misclassified.

Current image processing techniques use fixed arbitrary thresholds to segment (separate) bone from soft tissue and segment cortical from trabecular bone within a region of interest. Fixed thresholds incorrectly binarize bone, assuming it exists as two discrete compartments; cortical (compact) bone and trabecular bone. In advancing age, cortical bone is trabecularized by intracortical remodeling producing a transitional zone that is the source of most bone loss with advancing age. Segmenting using fixed arbitrary thresholds results in errors in the quantification of cortical and trabecular compartments by either including fragmented cortical bone in the trabecular compartment overestimating its trabecular density in old age and so understimating the age-related decline in trabecular density four-fold, or omitting intracortical porosity understimating the rise in intracortical porosity that occurs during aging and disease. Porosity below 100 microns, which constitutes 80% of intracortical porosity, is not quantified. The increase in porosity across age due pores >82 microns is only ~15%. When pores <82 microns are included, porosity increases across age by ~70%. Threshold based methods also obscure the effect of disease and treatment on bone microarchitecture and so fail to accurately quantify fracture risk in during age, disease and therapy.

Segmentation without using fixed thresholds allows accurate quantification of bone morphology without loss of information. Nonthreshold based methods include all voxels and quantify the differing attenuation of voxels containing only fully mineralized bone, voxels contain only fluid or soft tissue (porosity in cross-section, canals in cortex), voxels containing incompletely mineralized bone (that has undergone partial primary and secondary mineralization) and so allow accurate separation of bone within the periosteal envelope from surrounding muscle, and segmentation of bone into compact cortex, the transitional zone containing varying proportions of cortical fragments that look like trabecular (trabecularized cortex), and the medullary cavity containing cancellous bone. This method also allows quantification of tissue mineral density in vivo. Bisphosphonates produce a right shift in the frequency distribution curve reflecting an increase in the proportion of voxels containing predominantly bone and a decrease in proportion of voxels occupied by void; 55% of the bone is mineralized at 70-95% while in age-matched untreated peers, mineralization is normally distributed and only 23% of the bone mineralized at 70-95% range. The higher cortical area is due to filling of intracortical pores as voxels within cortex adjacent to marrow fill with bone. The change at the voxel level precedes changes in other morphological parameters and is detected with this new method. Nonthreshold based image analysis is applicable to many tissue as well as bone that is applicable to research and day to day decision making in assessing fracture risk in vivo.
OSTEOCYTES AND CALCIUM: A LITTLE BIT OF GIVE AND TAKE
D. Findlay, G. J. Atkins
Orthopaedics and Trauma, University of Adelaide, Adelaide, Australia

Osteocytes are the most abundant cells in bone and represent osteoblasts that are incorporated into bone during bone formation, forming a dense network of cell bodies and interconnecting cell processes. Their location within the mineral matrix of bone gives them the unique ability to sense and/or respond to environmental influences that are important to the maintenance and wellbeing of bone. For example, osteocytes can detect and initiate bone repair in response to microcracks in the bone matrix, which prevents the accumulation of microdamage. In response to increased loading of bone, osteocytes are able to orchestrate the formation of the additional amount of bone required to meet the increased load demands. Osteocytes are also part of the complex metering system of the body that ensures homeostatic control of circulating calcium and phosphate. As such, they appear to be able to regulate the removal of calcium from the bone matrix and its replacement. Thus, it appears that systemic demand for calcium can be met in real time by osteocytic mobilisation of calcium from bone immediately adjacent to them. Although the mechanisms for this remain to be worked out, there is evidence for the involvement of PTH. These ‘osteolytic’ osteocytes, such as have been described in lactation or in PTH treated animals, express genes associated with resorbing osteoclasts. Whether the replacement of this ‘lost’ calcium is a cell-mediated event, or is a purely physico-chemical event, is not clear. We and others have also shown that osteocytes can drive osteoclast formation and activity, and in fact may be largely responsible for osteoclastic resorption. There is also evidence for osteocytic regulation of phosphate metabolism, via their production of FGF-23, which in turn controls the renal production of 1,25(OH)2 vitamin D. Whether osteocytes contribute positively to bone formation is not clear but they do appear to exert a tonic negative control on bone formation via production of sclerostin. Antagonism of sclerostin leads to a dramatic increase in bone formation. Both the mineralisation of bone and, as we have found, the control of mineralisation, seem to be functions of osteocytes, the latter at least partly due to the action of sclerostin to inhibit several key steps in the mineralisation process.

ANALYSIS OF OXIDATIVE STRESS AND ANTIOXIDANT ENZYMES IN MECHANICAL STRESS RESPONSE
D. Morikawa1,2, Y. Saita1,2, H. Nogiri1,2, K. Kobayashi1,2, K. Watanabe1, Y. Asou1, K. Kaneko2, T. Shimizu1
1Molecular Gerontology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan
2Department of Orthopaedics, Juntendo University, Tokyo, Japan

[Aim] Mechanical loading plays an important role in maintaining homeostasis both in skeletal muscle and bone. Reduced mechanical stimulation leads to the enhancement of oxidative stress in skeletal muscle, and the alteration of the expression pattern of antioxidant enzymes, resulting in muscle atrophy. However, the relevance of mechanical stimulation and oxidative stress in bone remains to be fully elucidated.

[Method] This study investigated the oxidative stress level and the gene expression pattern of antioxidant enzymes in bone using tail suspension to clarify whether skeletal unloading regulates oxidative stress and antioxidant capacity in bone.

[Result] Hindlimb unloading significantly increased the level of reactive oxygen species in bone marrow cells as well as the serum level of an oxidative stress marker. Hindlimb unloading also up-regulated the expression of CuZn-SOD (Sod1), one of the major antioxidant enzymes in the cytoplasm, but not any other antioxidant enzymes (Sod2, Cat and Gpx1). The tails of Sod1- deficient (Sod11) and wild-type mice (WT) were suspended for 2 weeks to investigate the physiological role of Sod1 on mechanical unloading. Dual X-ray absorptiometry revealed that Sod11 mice showed a significant decrease by 1.7-fold in the femur BMD in comparison to the WT mice. Similarly, a micro CT analysis showed that Sod1 deficiency significantly reduced BV/TV by unloading (Sod11: -48%, WT: -24%, p<0.01). The dynamic bone formation parameters revealed that the Sod1 deficiency exacerbated the decline of the bone formation rate and mineralizing surface by unloading, while no difference was observed in the bone resorption parameters between Sod11 and WT, thus indicating that Sod1 insufficiency exacerbated bone loss under mechanical unloading conditions due to the suppression of osteoblastic bone formation activity.

[Conclusion] These results indicate that reduced mechanical stimulation modified the antioxidant capacity and Sod1 plays a protective role in oxidative stress due to unloading in bone.

SKELETAL DYSPLASIAS: A NEW APPROACH
A. Zankl
Queensland Genetics, Royal Brisbane and Women’s Hospital, QLD, Australia

Osteoarthritis and osteoporosis are major public health problems, but their molecular genetic basis remains poorly understood. Genome-wide association studies have identified common variants that contribute to these common disorders, but the effect size is usually small.
Skeletal Dysplasias are rare genetic disorders that disrupt normal skeletal development in a major way. Patients with skeletal dysplasias experience symptoms such as severe short stature, early joint degeneration or increased bone fragility. The identification of mutations of large effect in monogenic disorders such as the skeletal dysplasias has the potential to substantially improve our understanding of the pathogenic mechanisms that lead to more common bone and cartilage disorders such as osteoarthritis and osteoporosis and thereby identify potential targets for future therapeutic intervention.

The complexity of skeletal dysplasias and the relatively small number of affected individuals have hindered the identification of the genetic basis of many skeletal dysplasias. As there are over 400 bone dysplasias, many of which look superficially similar, correct diagnosis is essential to achieve a homogenous study population.

While many skeletal dysplasia genes have been identified through linkage analysis in the past, there is a lack of suitable multiplex families to perform linkage analysis in the remaining conditions. We have therefore explored the possibility of using Next Generation Sequencing technology to identify the disease causing genes in small families or sporadic cases with rare bone dysplasias. We have successfully used this approach to identify the disease causing gene in a novel bone dysplasia affecting a single family with two affected individuals. We have also used this approach to identify the gene responsible for another bone dysplasia in 5 unrelated individuals.

We are also exploring the use of Next Generation Sequencing to identify mutations in patients with bone dysplasias for which the responsible gene(s) are already known. We present our data on establishing Next Generation Sequencing as a faster, more comprehensive and more cost-efficient way of genetic testing for clinical and research purposes.

HYPOPHOSPHATAEMIA AND FGF23

S. Fukumoto
Department of Internal Medicine, University of Tokyo Hospital, Bunkyo-ku, Tokyo, Japan

FGF23 is a hormone produced by bone and regulates serum phosphate and 1,25-dihydroxyvitamin D [1,25(OH)2D] levels by binding to Klotho-FGF receptor complex. This implies that circulatory levels and actions of FGF23 are physiologically tightly controlled. Several genetic and acquired hypophosphatemic diseases have been shown to be caused by excess actions of FGF23. These FGF23-related hypophosphatemic diseases include X-linked hypophosphatemic rickets/osteomalacia (XLH), tumor-induced rickets/osteomalacia and so on. However, it is not completely understood what causes dysregulation of FGF23 actions in these diseases. We have previously established sandwich enzyme-linked immunosorbent assay for FGF23 and shown that FGF23-related hypophosphatemic diseases can be diagnosed by chronic hypophosphataemia with high FGF23 levels. On the other hand, FGF23 levels in hypophosphatemic patients from other causes such as Fanconi syndrome and vitamin D deficiency are rather suppressed suggesting that hypophosphatemia suppress FGF23 production. Usual medical treatment of FGF23-related hypophosphatemic diseases includes neutral phosphate and active vitamin D3. However, these medications sometimes cause tertiary hyperparathyroidism and seem to further increase FGF23 levels. We have examined effects of anti-FGF23 antibodies in Hyp mouse, a murine homologue of XLH. Administration of anti-FGF23 antibodies increased serum phosphate and 1,25(OH)2D levels, corrected impaired mineralization and improved longitudinal growth of long bones. In addition, these antibodies also increased grip power and spontaneous movement of Hyp mice. These results suggested that the inhibition of FGF23 activity is promising as a new therapeutic tool for FGF23-related hypophosphatemic diseases. In this session, I would like to discuss the pathogenesis, diagnosis and treatment of FGF23-related hypophosphatemic diseases.

MOUSE MODELS FOR SKELETAL DYSPLASIAS

M. Warman
Childrens Hospital Boston, MA, United States

Abstract text not available at time of print.
080

VARIABLE OSTEOGENESIS IMPERFECTA PHENOTYPE RESULTING FROM A FOUNDER FKBP10 MUTATION IN SAMOA

T. Cundy, U. Schwarze, S. Pyott, E. C. Davis, M. Hegde, P. H. Byers

1 Medicine, FMHS, University of Auckland, Auckland, New Zealand
2 Pathology and Medicine, University of Washington, Seattle, United States
3 Anatomy and Cell Biology, McGill University, Montreal, Canada
4 Genetics, Emory University, Atlanta, United States

Mutations in FKBP10, which encodes the collagen prolyl cis-trans isomerase chaperone protein FKBP65, have recently been discovered to cause a recessively-inherited variant of osteogenesis imperfecta. We have identified 17 individuals in 10 independent families originating from the Samoan islands who share one FKBP10 mutation. One group presents at birth with Bruck syndrome-like features of talipes and flexion contractures; and/or neonatal fractures – these patients do not attain independent mobility. Patients in the second group present later in childhood, typically with pain on walking or long bone fractures aged 4-18 years. The difficulty with ambulation is the result of progressive acetabular protrusion which leads in some to severe impairment of mobility. The diversity of phenotype was present even within families. Short stature, macrocephaly, platybasia, Wormian bones, white sclerae with normal hearing and teeth were common to all patients. The short stature is exacerbated by progressive scoliosis, a major cause of reduced life expectancy. The Samoan mutation [c.948_949insT] in FKBP10 has not been previously described. It creates a frameshift with a premature stop codon in exon 7 and results in mRNA instability, so that no protein is produced. In all but one of these families, affected individuals were homozygous for this mutation. Two affected siblings from a family with one Samoan parent were compound heterozygous for the Samoan mutation and the previously described c.831_832insC mutation. We estimate this mutation has a frequency of ~1 in 50-100 in the Samoan population; it is probably a founder mutation carried by early settlers to Samoa ~1000 BCE.

081

BISPHOSPHONATES: THE GOOD, THE BAD AND THE UGLY

P. R. Ebeling

NorthWest Academic Centre, University of Melbourne, Western Hospital, Footscray, VIC, Australia

Current osteoporosis therapy has focused on the osteoclast and is “anti-catabolic”. Amino-bisphosphonates such as alendronate, risedronate and zoledronic acid (ZOL) diffuse through the actively resorbing osteoclast cell membrane and inhibit the HMG CoA reductase, reducing osteoclast activity. They have differing potencies and skeletal half-lives. Clinical trials and systematic reviews with amino-bisphosphonates show risk reductions for all major types of fractures, including vertebral, non-vertebral and hip fractures. Mortality risk is increased for up to 5 years, even after minor fractures. Evidence from trials and observational studies show that bisphosphonates may reduce mortality. Early pre-clinical and trial evidence also suggest ZOL may also have direct anti-cancer effects on breast cancer, and reduce breast cancer recurrence.

Important beneficial effects of amino-bisphosphonates need to be balanced against less common adverse effects. The most common are upper gastrointestinal, including oesophagitis and gastritis. These have been reduced by less frequent oral dosing and enteric-coated tablets. A rare association with oral bisphosphonates is oesophageal cancer, but the absolute risk is low, being <1/1000 after 5 years. Atrial fibrillation was associated with intravenous ZOL in the HORIZON trial, but was not increased in the hip fracture study, including older patients. Systematic reviews have not shown an increase in risk of atrial fibrillation with bisphosphonate therapy. Jaw osteonecrosis (ONJ) has been associated with bisphosphonate and denosumab use, but is more common in patients with cancer. In osteoporosis, estimates of ONJ frequency range from 1:952 to 1:10,000. This risk is increased following dental extraction. Atypical femoral fractures have been associated with long-term bisphosphonate therapy (median duration 7 years), however, other co-treatments and co-morbidities may also be present (glucocorticoids, proton pump inhibitors, diabetes mellitus, rheumatoid arthritis). 70% of patients with atypical fractures have a prodrome of thigh or groin pain, while bilateral symptoms and radiological signs occur in about 30%.

082

FRACTURE HEALING -MECHANICAL AND HISTOLOGICAL CHANGES WITH BISPHOSPHONATE TREATMENT

S. Mori

Bone and Joint Surgery, Seirei Hamamatsu General Hospital, Hamamatsu, Shizuoka, Japan

Anti-resorptive agents are widely used for the treatment of osteoporosis. However, osteoporotic patients are prone to fracture. While bisphosphonates (Bis.) can reduce 50% fracture risk, the rest of the patients sustain fractures under drug treatment. Bone resorption is a important function of fracture repair. The questions arise whether they disturb fracture repair process, whether or not they should be stopped when the patients on these drug treatment sustained fracture. To answer these questions rat's osteotomy model was used to test whether bisphosphonates disturb 1) restoration of mechanical integrity, 2)callus formation and remodeling. Incadronate (3rd generation Bis.) 10 or 100 ug/kg was injected 3times a week for 2weeks as pretreatment. Then osteotomy at the middle of the femur was performed. Incadronate treatment was either continued (C-) or stopped (P-) until sacrifice at 2, 4, 6, 16, 25,
and 49 weeks after surgery. Animals were double labeled with calcein before sacrifice. Femora was extracted and 3 point bending test was performed as mechanical evaluation, and callus area and remodeling was histologically evaluated. Ultimate strength was highest in C-100 among the groups at 16, 25, 49 weeks. Cross sectional area was largest in C-100 through the periods. Eroded surface in callus was significantly less in C groups compared to vehicle (V). % lamellar bone in cortical shell decreased in C groups compared to V Suppression of bone resorption induced by high dose Bis. treatment increased woven bone formation and delayed woven to lamellar bone remodeling. Increased ultimate load by Bis treatment may be caused by the enlarged cross sectional area despite poor woven to lamellar callus remodeling, suggesting that mechanical adaptation is the most powerful gear for controlling fracture repair process under any metabolic conditions.

083

ATYPICAL SUBTROCHANTERIC AND DIAPHYSSEAL FEMORAL FRACTURES - WHAT WE KNOW AND WHAT WE DON'T

A. Ng
Singapore General Hospital, Singapore, Singapore

Recent reports linking long-term use of bisphosphonates (BP) with atypical fractures has been a cause for concern. These fractures were considered atypical because they often occurred spontaneously or under circumstances of minimal trauma and because of their unusual morphology and location (in the subtrochanteric and shaft of femur). Despite preclinical data lending biologic plausibility to a potential association between long-term BP use, a causal association between BPs and atypical fractures has not been established. Recent observations inform regarding the possible risk factors for the development of these fractures but we are still far from a clear understanding of this condition. The continued surveillance and gathering of more epidemiologic and clinical data will hopefully shed more light. Based on currently available data, the incidence of atypical femoral fractures appears to be very low, although under-reporting may be a factor. There is a need to cultivate a greater awareness and understanding amongst physicians and patients to improve surveillance of these fractures while avoiding an irrational fear of bisphosphonates therapy.

084

ADVERSE CARDIOVASCULAR EFFECTS OF CALCIUM SUPPLEMENTS MAY NOT PERSIST AFTER DISCONTINUATION OF SUPPLEMENTS: 5-YEAR FOLLOW UP OF THE AUCKLAND CALCIUM STUDY

University of Auckland, Auckland, New Zealand

Aims: In a 5y randomized placebo-controlled trial of 1g/day calcium citrate in 1471 postmenopausal women, relative risks for myocardial infarction (MI) and stroke with calcium were 1.49 and 1.37, respectively.1,2 These findings are further supported by the results from meta-analyses of placebo-controlled trials of calcium supplements.3,4 We wished to determine whether the adverse cardiovascular effects of calcium supplements persist after discontinuation of supplements.

Methods: Approximately 5y after completion of the trial, we collected information on MI, stroke, and post-trial calcium supplement use in the 1408 surviving participants. 1174 participants were contacted by telephone. Information on all 1408 participants was obtained from national databases of hospital admissions and deaths.

Results: During an average of 9.1y of follow-up, 138 women (52 during, 86 post-trial) had an MI, 158 had a stroke (59 during, 99 post-trial) and 257 women died (63 during and 194 post-trial). Post-trial calcium supplement use was similar in both treatment groups (35-37%). When analysed on an intention to treat basis (n=1471), there was no difference in the risk of myocardial infarction (HR 1.04, CI 0.74-1.45), stroke (HR 1.04, CI 0.76-1.42), or death (HR 1.16, CI 0.91-1.48) between women originally allocated to calcium and those allocated to placebo.

There were no differences in the risk of MI (HR 0.82, CI 0.55-1.22) or stroke (HR 0.84, CI 0.58-1.22) in the post-trial period between women originally allocated to calcium and those allocated to placebo. There were also no differences in the risk of MI or stroke between women who took calcium post-trial and those who did not, in either treatment group.

Conclusion: The adverse cardiovascular outcomes seen in the original clinical trial did not persist in the post-trial period. Confounding by post trial calcium supplementation did not influence this conclusion.

DOES CALCIUM SUPPLEMENTATION WITH AND WITHOUT VITAMIN D INCREASE CARDIOVASCULAR RISK? A CLINICO-BAYESIAN INTERPRETATION

T. S. Thach, N. D. Nguyen, J. R. Center, J. A. Eisman, T. V. Nguyen

Osteoporosis and Bone Biology, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia

Background:
Recent analyses suggest that calcium supplementation+/−vitamin D (Ca+/−D) is associated with increased risk of myocardial infarction (MI). However, the effect size was modest, and could be due to bias. In this study, we re-examined the likelihood that such a clinically relevant association exists using a Bayesian meta-analysis approach.

Methods:
Data from 13 randomized controlled trials published between 1993-2011 were synthesized using Bayesian random-effects meta-analysis. The studies included 29,276 individuals followed up for between 1 and 9.5 years. The endpoints were MI, stroke, composite cardiovascular events, and death. Review of the cardiology literature suggests that a >10% increase in risk would be considered clinically significant. Thus, posterior probability that the risk exceeds the specified difference (d>10%) was estimated via the Bayes’ theorem. Evidence of risk (or benefit) was inferred at a posterior probability of risk >0.95, i.e. that there is a >0.95 probability that the increased risk is >10%. Sensitivity analysis of possible bias was also carried out.

Results:
Under the assumption of no bias, the pooled rate of MI was higher in the Ca+/−D compared with placebo (relative risk: 1.18, 95% Critical interval: 1.01-1.39 ) ; however, under the assumption that the true relative risk was over-estimated by at least 5%, the difference was no longer statistically significant (1.14, 0.77-1.7). The probability that Ca±D increases the risk [at least 10%] of MI, stroke, composite outcomes, and death was 0.79, 0.55, 0.59, and 0.37, respectively. When the analysis was limited to individuals receiving both calcium and vitamin D, the respective probabilities were 0.61, 0.64, 0.61, and 0.38. Under the assumption that bias overestimates the true odd ratios by 20%, there was <0.3 probability that Ca+/-D increases the risk of any cardiovascular outcome by >10%.

Conclusions:
These results suggest a low likelihood of cardiovascular risk with Ca+/−D compared to placebo.

QUIESCENT OSTEOCLAST PRECURSORS
N. Takahashi
Institute for Oral Science, Matsumoto Dental University, Shiojiri, Nagano, Japan

Osteoblasts are believed to play a role in the determination of correct sites for osteoclast formation through the expression of RANKL and M-CSF. However, using RANKL-deficient mice, we showed that even RANKL-deficient osteoblasts also determined the correct sites for osteoclastogenesis ( Endocrinology 147:3366-3374, 2006) . On the other hand, cell cycle-arrested quiescent osteoclast precursors (QOPs) were detected along bone surfaces as the lineage committed osteoclast precursors (J Cell Biol 184:541-554, 2009). M-CSF administration into op/op mice induced osteoclast formation in bone tissues through the systemic transfer of QOPs from spleen to bone. Osteoclasts appearing in BMP-induce ectopic bone were derived from circulating QOPs. RANK-positive cells obtained from bone marrow and peripheral blood possessed characteristics of QOPs. These results suggest that QOPs circulate in blood and settle down to bone. The expression level of RANK in QOPs detected on bone surfaces is much higher than that in QOPs in blood, suggesting that some cytokines expressed by osteoblasts must be involved in the up-regulation of RANK expression in QOPs. We recently found that some Wnt signals enhanced RANK expression in osteoclast precursors, thereby enhancing RANKL-induced osteoclastogenesis. These results suggest that osteoblasts are involved in homing of QOPs to bone, and in up-regulation of RANK expression in bone-homed QOPs through the Wnt5a-Ror2 axis.
Osteoclast differentiation is tightly controlled by two essential cytokines, macrophage colony-stimulating factor and receptor activator of nuclear factor kappa B ligand (RANKL). However, the role of epigenetic regulation in osteoclast differentiation is poorly understood. The expression of key developmental genes tend to be regulated by the trimethylation and demethylation of histone H3 lysine 4 (H3K4me3) and lysine 27 (H3K27me3). We applied massively parallel sequencing of the chromatin immunoprecipitation products using the Illumina cluster station and 1G Analyzer to investigate the H3K4me3 and H3K27me3 modification patterns around the transcription start site (TSS) of several transcription factors known to be important for osteoclastogenesis. The H3K27me3 marks were present in a relatively broad peak centered on the TSS of Nfatc1, but not of the other transcription factors. Following the treatment with RANKL and subsequent osteoclast differentiation, a marked reduction in the level of H3K27me3 at the Nfatc1 locus was observed. The expression of Junonji domain-containing-3 (Jmjd3), a H3K27me3 demethylase, was time-dependently increased in osteoclast precursors and recruited in the vicinity of the TSS of Nfatc1 after stimulation with RANKL. In addition, gene silencing of the Jmjd3 gene by short hairpin RNA reduced demethylation of H3K27me3 around the TSS of Nfatc1 and markedly suppressed RANKL-induced osteoclastogenesis. These results suggest that demethylation of H3K27me3 in the vicinity of the TSS of the Nfatc1, regulated by Jmjd3, plays a key role in RANKL-induced osteoclast differentiation.

Interleukin-6 (IL-6), IL-11, leukemia inhibitory factor (LIF), cardiotrophin-1 (CT-1), oncostatin M (OSM) and ciliary neurotrophic factor (CNTF) all influence cell function by complexing with the gp130 coreceptor. IL-11, IL-6 and, most potently, OSM, are well-established as strong stimuli of osteoclastogenesis in vitro. In addition gp130 neutralising antibody studies revealed that signalling through gp130 plays a role in osteoclast formation in response to PTH, I.25D and IL-1. It was a surprise then when genetic deletion of gp130 resulted in increased osteoclast formation in vivo. However, these findings were complicated by the neonatal lethality of the mutation, dwarfism due to chondrocyte defects, and haemopoietic defects. Generation of adult mice with specific intracellular mutations of gp130 showed gp130 influences osteoclast formation and chondrocyte differentiation by independent pathways. Studies of expression patterns of gp130 family members in bone, and bone phenotypes of mice with global genetic deletions of many of these have revealed distinct functions of each family member, and distinct communication pathways between osteoblasts and osteoclasts. For example, IL-11, OSM and LIF are expressed by osteoblasts and act through receptors on osteoblasts and osteocytes, but absent in mature osteoclasts. These cytokines are required for normal osteoclast formation, but their roles differ. In OSMR and IL-11R deficiency, osteoclast formation is impaired throughout life. Furthermore, with PTH treatment, osteoblastic OSMR expression is upregulated, and anabolic PTH treatment of OSMR null mice caused bone loss by stimulating osteoclastogenesis. In contrast, LIF null mice demonstrate region-specific changes in osteoclast formation; only formation of osteoclasts that destroy calcified cartilage (chondroclasts) is changed by LIF deletion, while osteoclasts in remodelling bone are unaffected. These region-specific changes relate to LIF's effect on chondrocytes to modify vascular invasion of the growth plate. This confirms independent, and important roles of gp130-signalling cytokines in osteoclast formation during bone development, growth and maintenance.

Bone is constantly remodeled through resorption of old bone by osteoclasts and deposition of new bone by osteoblasts. The net bone mass is regulated through a balance between preceding resorption and subsequent formation; however, the factor(s) and mechanism that couple the two processes are not fully understood. We hypothesized that such coupling factor is secreted or presented by osteoclasts and stimulates osteoblastic differentiation. Through global gene expression analysis during osteoclastogenesis, we have identified collagen triple helix repeat containing-1 (Cthrc1) encoding a 245-amino acid protein secreted by mature bone-resorbing osteoclasts which targets stromal/osteoblastic cells so as to stimulate osteoblastic differentiation. Cthrc1 expression in osteoclasts is robustly induced in resorbing condition on bone or dentin, compared to modest expression on plastic plate in vitro. Its expression in vivo increases in a high bone turnover state induced by RANKL injection, whereas it decreases with aging or following alendronate treatment. Osteoclast-specific as well as systemic deletion of the Cthrc1 gene results in osteopenia due to reduced bone formation; it also impairs bone mass recovery following RANKL-induced bone resorption, pointing to a defect in coupling. These data demonstrate that Cthrc1 acts as an osteoclast-derived coupling factor which regulates bone remodeling and hence, skeletal integrity.
PREVENTION OF SKELETAL DAMAGES WITH FOLINIC ACID SUPPLEMENTATION IN YOUNG RATS RECEIVING LONG-TERM METHOTREXATE CHEMOTHERAPY

C. Fan¹, M. A. Scherer³, B. K. Foster³, C. J. Xian²

¹Sansom Institute for Health Research, University of South Australia, Adelaide, SA, Australia
²Physiology, Adelaide University, Adelaide, SA, Australia
³Orthopaedics, Women’s and Children’s Hospital, North Adelaide, SA, Australia

With the development of chemotherapy and increasing childhood cancer survivor rates, skeletal complications such as bone growth arrest and fractures during and after chemotherapy have become significant problems for paediatric cancer survivors. It is therefore important to develop preventative strategies for these skeletal defects. Methotrexate (MTX), a commonly used chemotherapeutic agent in paediatric cancers, has been shown to cause bone growth defects both clinically and in experimental animals.

**Aim:** The current study examined the effects of chronic high-dose methotrexate (MTX) chemotherapy on bone growth of young rats, and potential protective effects of antidote folinic acid (FA) in protecting bone growth during long-term MTX chemotherapy.

**Methods:** During the induction phase, rats received injections of saline, MTX or MTX+FA (MTX at 0.65mg/kg, FA at 0.87mg/kg, 5 days on/9 days off), followed by the maintenance phase of twice weekly injections for 4 weeks (MTX at 1.3mg/kg and FA at 1.3mg/kg).

**Results:** Histological analysis revealed that at the growth plate, chronic MTX treatment caused reduction in columnar chondrocyte numbers, induction of chondrocyte apoptosis and chondroclast recruitment. In the metaphysis, ex-vivo x-ray microtomography (μCT) revealed MTX caused overall reduction of trabecular bone volume, which was due to increased osteoclast density, induction of osteoblast apoptosis and increased adipocytes. Furthermore, plasma from MTX-treated rats was able to induce ex-vivo osteoclast formation from normal bone marrow cells, suggesting systemic contribution to bone resorption. FA supplementary treatment was able to alleviate MTX-induced histological and cellular damages in both the growth plate and metaphysis.

**Conclusion:** These findings indicate that FA supplementation can prevent growth plate and metaphyseal damages from chronic MTX administration, and may potentially be useful in paediatric patients who are at risk of skeletal growth suppression as a result of chronic MTX chemotherapy.

METHOTREXATE CHEMOTHERAPY-INDUCED BONE LOSS AND MARROW ADIPOSY IS ASSOCIATED WITH DEREGLULATION OF THE WNT/B-CATENIN SIGNALLING PATHWAY

K. R. Georgiou¹, T. J. King¹,², M. A. Scherer¹, B. K. Foster³, C. J. Xian¹²³

¹Sansom Institute, School of Pharmacy and Medical Science, The University of South Australia, Adelaide, SA, Australia
²Discipline of Physiology, The University of Adelaide, Adelaide, SA, Australia
³Department of Orthopaedic Surgery, Women’s and Children’s Hospital, Adelaide, SA, Australia

The chemotherapeutic agent methotrexate (MTX), commonly used to treat a variety of cancers, has been shown to cause bone defects including osteoporosis. The current study sought to characterise the effects of MTX treatment on bone/fat volume and differentiation potential of bone marrow stromal progenitor cells in a rat model and to identify potential regulatory mechanisms. The Wnt/β-catenin signalling pathway is an integral regulator of bone formation and adipogenic differentiation, therefore this study aimed to investigate the impact of its modulation for the prevention of MTX-induced bone loss and marrow adiposity. MTX treatment (5 consecutive daily doses at 0.75mg/kg) caused a significant reduction in trabecular bone volume p<0.001 in vivo compared to controls. FA supplementary treatment was able to alleviate MTX-induced histological and cellular damages in both the growth plate and metaphysis.

The Wnt/β-catenin signalling pathway is an important regulator of bone formation and adipogenic differentiation and therefore this study aimed to investigate the impact of its modulation for the prevention of MTX-induced bone loss and marrow adiposity. MTX treatment (5 consecutive daily doses at 0.75mg/kg) caused a significant reduction in trabecular bone volume compared to controls. FA supplementary treatment was able to alleviate MTX-induced histological and cellular damages in both the growth plate and metaphysis.

**Conclusion:** These findings indicate that FA supplementation can prevent growth plate and metaphyseal damages from chronic MTX administration, and may potentially be useful in paediatric patients who are at risk of skeletal growth suppression as a result of chronic MTX chemotherapy.
THE VITAMIN D RECEPTOR PROMOTES HUMAN PROSTATE CANCER CELL GROWTH VIA A LIGAND INDEPENDENT PATHWAY

Y. Zheng1,2, D. Deng1, D. Basel1, T. Trivedi1, J. Kelly1, R. L. Sutherland2, C. R. Dunstan1,4, H. Zhou1, M. J. Seibel1,5

1Bone Research Program ANZAC Research Institute, The University of Sydney, Concord, Sydney, Australia
2Cancer Research Program, Garvan Institute of Medical Research, Darlinghurst, Sydney, NSW, Australia
3Dept of Rheumatology and Clinical Immunology, Charite University Medicine, Berlin, Germany
4Department of Biomedical Engineering, The University of Sydney, Sydney, NSW, Australia
5Dept of Endocrinology & Metabolism, Concord Hospital, Concord, Sydney, NSW, Australia

Aim: Bone is a frequent site for prostate cancer metastasis. We have previously reported that vitamin D deficiency promotes human prostate cancer cell growth in bone. However, little is known about the role of the vitamin D receptor (VDR) in this context. The present study aimed to define the role of the VDR in human prostate cancer growth in-vitro and in-vivo.

Methods & Results: VDR expression was knocked down by stable expression of shRNA in PC3 cells (PC3-VDR-KD), with non-target cells (PC3-NT) generated as controls. VDR mRNA knock down was 85% and induction of CYP24 mRNA expression by 1,25(OH)2D3, normally seen in VDR expressing cells, was abrogated in PC3-VDR-KD cells, indicating effective disruption of VDR signaling.

Treatment of PC3-NT cells with 1,25(OH)2D3 significantly reduced cell growth by up to 51% as compared to untreated PC3-NT cells. Surprisingly, growth of PC3-VDR-KD cells in ligand-free cultures was also reduced by 49% (compared to NT cells). Moreover, cell migration was increased by 10% in PC3-VDR-KD cells. Of note, PC3-VDR-KD cells did not respond to treatment with 1,25(OH)2D3.

To further investigate the effects of VDR knockdown in vivo, PC3-NT and PC3-VDR-KD cells were implanted subcutaneously in nude mice. and tumor growth was monitored for 69 days. Compared to NT cells, VDR knockdown resulted in significantly smaller tumors from day 12 onwards. Similarly, when PC3-NT or PC3-VDR-KD cells were implanted into the tail of vitamin D sufficient nude mice, disruption of VDR signaling resulted in significantly smaller osteolytic lesions from day 17 onwards (X-ray analysis).

Conclusion: These results suggest a novel ligand-independent role of the VDR in promoting prostate cancer cell growth and suppressing invasive cell potential (migration). This novel function of the unliganded VDR contrasts with the known anti-proliferative actions of the liganded VDR and may offer new therapeutic approaches in cancer treatment.

HOW WELL DO THE FRAX (AUS) AND GARVAN CALCULATORS PREDICT FRACTURES FROM THE GEELOG OPSEOPOROSIS STUDY (GOS)

Y. Zhang1, J. A. Pasco1, M. A. Kotowicz3, K. M. Sanders3, G. C. Nicholson4, M. J. Henry1

1Department of Medicine, NorthWest Academic Centre, The University of Melbourne, Footscray, VIC, Australia
2Epidemiology and Biostatistics Unit (Barwon Health), School of Medicine, Deakin University, Geelong, VIC, Australia
3Dept Endocrinology and Diabetes, Barwon Health, Geelong, VIC, Australia
4Rural Clinical School, School of Medicine, The University of Queensland, Toowoomba, VIC, Australia

The FRAX calculator (AUS)1 estimates the 10yr absolute risk of fractures at the hip, spine, humerus and wrist (“major osteoporotic fractures”) whereas the Garvan calculator2 predicts the 10-year absolute risk of fracture at the hip, spine, wrist, meta-carpal, humerus, scapula, clavicle, lower limb, pelvis and sternum (“osteoporotic fractures”). The calculators use 11 and 5 risk factors respectively. This study aims to assess the ability of both calculators to predict fracture in a cohort of women followed prospectively for 10yr.

An age-stratified random population-based sample of women was recruited by GOS during 1993-7 (n=587; age 60-90yr). Risk factors measured at baseline visit included: femoral neck BMD, falls, prior fracture, weight, height, parental fracture, smoking, glucocorticoid usage, secondary osteoporosis, and alcohol consumption. Subjects were followed biennially for 10yr (median 9.1yr, IQR: 4.79-10). Fractures documented and verified radiologically were only those sustained after a low trauma event. Absolute risk of fracture was calculated using both calculators. Number of predicted fractures was calculated by the sum of the absolute risks adjusted by time in the study. A one-sample chi-squared test assessed the difference between the observed and predicted number of fractures. The areas under the receiver operating characteristic curves (AUC) were calculated.

There were 38 hip, 15 major osteoporotic, and 127 osteoporotic fractures observed. The FRAX calculator (with BMD) predicted 20.1 hip fractures (p<0.01) and 49.5 major osteoporotic fractures (p<0.0001) whereas the Garvan calculator predicted 54.8 hip (p=0.02) and 127.5 osteoporotic fractures (p=0.96). There was no significant difference in AUC using FRAX (hip: AUC=0.75, 95%CI=0.68-0.82, major osteoporotic: AUC=0.63, 95%CI=0.60-0.75) whereas the Garvan calculator predicted 54.8 hip (p=0.02) and 127.5 osteoporotic fractures (p=0.96). There was no significant difference in AUC using FRAX (hip: AUC=0.75, 95%CI=0.68-0.82, major osteoporotic: AUC=0.63, 95%CI=0.60-0.75) whereas the Garvan calculator predicted 54.8 hip (p=0.02) and 127.5 osteoporotic fractures (p=0.96). There was no significant difference in AUC using FRAX (hip: AUC=0.75, 95%CI=0.68-0.82, major osteoporotic: AUC=0.63, 95%CI=0.60-0.75) whereas the Garvan calculator predicted 54.8 hip (p=0.02) and 127.5 osteoporotic fractures (p=0.96). There was no significant difference in AUC using FRAX (hip: AUC=0.75, 95%CI=0.68-0.82, major osteoporotic: AUC=0.63, 95%CI=0.60-0.75) whereas the Garvan calculator predicted 54.8 hip (p=0.02) and 127.5 osteoporotic fractures (p=0.96). There was no significant difference in AUC using FRAX (hip: AUC=0.75, 95%CI=0.68-0.82, major osteoporotic: AUC=0.63, 95%CI=0.60-0.75) whereas the Garvan calculator predicted 54.8 hip (p=0.02) and 127.5 osteoporotic fractures (p=0.96). There was no significant difference in AUC using FRAX (hip: AUC=0.75, 95%CI=0.68-0.82, major osteoporotic: AUC=0.63, 95%CI=0.60-0.75) whereas the Garvan calculator predicted 54.8 hip (p=0.02) and 127.5 osteoporotic fractures (p=0.96). There was no significant difference in AUC using FRAX (hip: AUC=0.75, 95%CI=0.68-0.82, major osteoporotic: AUC=0.63, 95%CI=0.60-0.75) whereas the Garvan calculator predicted 54.8 hip (p=0.02) and 127.5 osteoporotic fractures (p=0.96). There was no significant difference in AUC using FRAX (hip: AUC=0.75, 95%CI=0.68-0.82, major osteoporotic: AUC=0.63, 95%CI=0.60-0.75) whereas the Garvan calculator predicted 54.8 hip (p=0.02) and 127.5 osteoporotic fractures (p=0.96). There was no significant difference in AUC using FRAX (hip: AUC=0.75, 95%CI=0.68-0.82, major osteoporotic: AUC=0.63, 95%CI=0.60-0.75) whereas the Garvan calculator predicted 54.8 hip (p=0.02) and 127.5 osteoporotic fractures (p=0.96). There was no significant difference in AUC using FRAX (hip: AUC=0.75, 95%CI=0.68-0.82, major osteoporotic: AUC=0.63, 95%CI=0.60-0.75) whereas the Garvan calculator predicted 54.8 hip (p=0.02) and 127.5 osteoporotic fractures (p=0.96). There was no significant difference in AUC using FRAX (hip: AUC=0.75, 95%CI=0.68-0.82, major osteoporotic: AUC=0.63, 95%CI=0.60-0.75) whereas the Garvan calculator predicted 54.8 hip (p=0.02) and 127.5 osteoporotic fractures (p=0.96). There was no significant difference in AUC using FRAX (hip: AUC=0.75, 95%CI=0.68-0.82, major osteoporotic: AUC=0.63, 95%CI=0.60-0.75) whereas the Garvan calculator predicted 54.8 hip (p=0.02) and 127.5 osteoporotic fractures (p=0.96).
FRAGILITY FRACTURE AND OSTEOSTEOARTHRITIS: INTERACTIONS BETWEEN BONE MINERAL AND BONE MASS INDEX

M. Y. Chan1, N. D. Nguyen1, J. R. Center1,2, J. A. Eisman1,2, T. V. Nguyen1,2,3
1Osteoporosis and Bone Biology, Garvan Institute, Darlinghurst, NSW, Australia
2St Vincent’s Clinical School, St Vincent’s Hospital, Darlinghurst, NSW, Australia
3School of Public Health and Community Medicine, University of New South Wales, Kensington, NSW, Australia

Increased bone mineral density (BMD) and bone mass index (BMI) are associated with increased risk of osteoarthritis (OA) and reduced risk of fragility fracture. However, little is known about the relationship between fragility fracture and osteoarthritis. This study sought to examine the interactions between BMD and BMI in the determination of the OA-fracture relationship. The study was part of the ongoing Dubbo Osteoporosis Epidemiology Study, which involved 2,412 women and 1,452 men aged between 46 to 99 years. Baseline BMD was measured at femoral neck (FNBMD) and lumbar spine (LSBMD) by dual energy X-ray absorptiometry. Osteoarthritis was ascertained by self-report. The incidence of fragility fracture was ascertained by X-ray report. A total 1,077 participants (691 women and 386 men) had a diagnosis of OA. Overall, the risk of OA was associated significantly with increased LSBMD in men (odds ratio [OR] 1.34, 95%CI 1.19-1.52) and women (1.20; 1.09-1.32). Elevation in FNBMD was significantly associated with increased risk of OA in men (OR 1.16; 1.02-1.32), but not in women. When stratified by BMI, significant association remained between OA risk and high LSBMD amongst women with BMI < 25 kg/m² (OR 1.26; 1.07-1.47) and BMI > 30 kg/m² (1.26; 1.05-1.51); and in men with BMI < 25 kg/m² (OR 1.75; 1.37-2.27) or BMI 25-30 kg/m² (1.21; 1.02-1.43). OA was associated with an increased risk of fracture. After adjusting for LSBMD, women with OA had significant increased fracture risk (OR 1.41; 1.16-1.72), particularly in those with BMI 25-30 kg/m² (OR 1.45; 1.05-1.99). However, the association was not significant in men. These data suggest that high BMI is associated with a greater risk of OA in both men and women, especially those with low BMI; and hence, BMD could be a useful measure for identifying individuals at high risk of OA, particularly among those at lower BMI spectrum. Despite having higher bone density, women with self-reported OA, especially those overweight, have an increased risk of fragility fracture, suggesting that the OA-fracture association is mediated via non-BMD factors.

ENDOGENOUS PARATHYROID HORMONE IS ASSOCIATED WITH REDUCED CARTILAGE VOLUME IN VIVO IN A POPULATION-BASED SAMPLE OF ADULT WOMEN

S. L. Brennan1,2, F. M. Cicuttini1, G. C. Nicholson1, J. A. Pasco3,4, M. A. Kotowicz5, M. J. Henry1, A. E. Wulka2
1Northwest Academic Centre, Department of Medicine, The University of Melbourne, Footscray, VIC, Australia
2Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, VIC, Australia
3Rural Clinical School, The University of Queensland, Toowoomba, QLD, Australia
4Epidemiology and Biostatistics Unit, School of Medicine, Deakin University (Barwon Health), Geelong, VIC, Australia
5Department of Endocrinology and Diabetes, Barwon Health; The Geelong Hospital, Geelong, VIC, Australia

Aim: Parathyroid hormone (PTH) has complex actions on bone, and recent animal and in vitro studies also suggest that PTH may affect articular cartilage. However, little is known of the relationship between PTH and joint structure in vivo, thus, the aim of this study was to examine the association between endogenous PTH and cartilage volume in vivo in a healthy adult population with no symptoms of knee OA.

Methods: Magnetic resonance imaging of the dominant knee was performed on 101 asymptomatic females aged 35-49 years (2007-9), from which knee cartilage volume was determined. Blood samples were obtained 10 years prior (1994-7), and stored at -80°C for random batch analyses. Serum intact PTH was quantified by chemiluminescent enzyme assay. Serum 25-hydroxyvitamin D (25(OH)D) was assayed using an equilibrium radioimmunoassay after extraction with acetonitrile.

Results: A 1-unit (pmol/L) increase in PTH was positively associated with reduced medial cartilage volume [regression coefficient ± standard deviation, p value] (-0.7±0.3, p=0.03), after adjustment for age, BMI and bone area. The association was sustained after further adjustment for season (−0.8±0.3, p=0.02), and for 25(OH)D with a sinusoidal adjustment (−0.06±0.4, p=0.04), and calcium supplementation (−0.9±0.3, p=0.01). No associations were observed with lateral cartilage volume (0.2±0.3). After excluding subjects with osteophytes, to account for the possibility of subjects having signs of pre-clinical OA, results remained similar (−0.9±0.4, p=0.01), including after further adjustment for season (−1.2±0.4, p=0.007), and 25(OH)D (−1.0±0.4, p=0.01).

Conclusions: This study suggests increased levels of PTH might be detrimental to cartilage in humans in vivo. Animal studies suggest that increased PTH may reduce the ability of cartilage to heal following minor injury. This may explain our results, particularly given the effect we observed in the medial compartment which is exposed to higher loads during weight bearing compared to the lateral compartment.
FAT MASS AND FRACTURE RISK IN ELDERLY MEN AND WOMEN: A PROSPECTIVE STUDY
S. Yang1,2, N. D. Nguyen1, J. R. Center1,3, J. A. Eisman1,3, T. V. Nguyen1,2
1Garvan Institute of Medical Research, Sydney, NSW, Australia
2School of Public Health and Community Science, UNSW, Sydney, NSW, Australia
3St Vincent’s Hospital, Sydney, NSW, Australia

Background and aim: The relationship between body fat mass and fracture risk is controversial, due primarily to lack of prospective data. The present study sought to examine the association between whole body and abdominal fat mass and fracture risk in postmenopausal women and elderly men.

Materials and methods: The present study was part of the ongoing Dubbo Osteoporosis Epidemiology Study (DOES), in which a random sample of more than 2000 men and women aged 60+ years has been continuously followed up for 21 years. The present study was based on a cohort of 1129 participants (361 men and 768 women), whose total body bone mineral density (BMD) scans were available. BMD at the femoral neck and lumbar spine, total body fat mass and abdominal fat mass were measured by dual energy X-ray absorptiometry (GE-LUNAR Corp, Madison, WI). Baseline characteristics of participants including age, height, physical activity, history of falls, smoking and prior fracture were ascertained at the initial visit. The incidence of low-trauma and non-pathological fractures was ascertained from X-ray reports. The Cox’s proportional hazards regression was used to evaluate the association between fat mass and fracture risk, with adjustment for baseline covariates.

Results: During the median 5 years of follow-up, 19 (5%) men and 107 (14%) women had sustained a fragility fracture. Women with fracture had lower BMD, lower body fat mass and body weight than those without a fracture. In women, increased risk of fracture was associated with lower abdominal fat mass (hazard ratio/standard deviation[HR/SD]: 1.33; 95% CI: 1.07-1.65), after adjusting for age (HR/SD: 1.32; 1.08-1.63), femoral neck BMD (HR/SD:1.26; 1.03-1.56), and prior fracture (HR/SD:1.41; 1.15-1.73). Compared with women in the highest tertile of abdominal fat mass, those in the lowest tertile had a 2.1-fold (95% CI: 1.25-3.55) increase in fracture risk. Further analyses revealed that lower body fat mass was also associated with increased fracture risk, but the association was not independent of BMD. The magnitude of association between fat mass and fracture risk (HR/SD: 1.32; 1.08-1.63) was greater than that of body weight and fracture risk (HR/SD: 1.15; 0.94-1.41). Approximately 27% of fracture liability was attributable to abdominal fat mass.

Conclusion: Lower total body fat mass, particular lower abdominal fat, was significantly associated with increased fracture risk in women, not in men. These results suggest that the incorporation of fat mass into existing fracture prognostic models may enhance their predictive accuracy.

SCLEROSTIN INDUCES OSTEOCYTE SUPPORT OF OSTEOCLAST FORMATION AND OSTEOCLAST ACTIVITY
A. R. Wijenayaka1, M. Kogawa1, H. P. Lim1, L. F. Bonewald2, D. M. Findlay1, G. J. Atkins1
1Bone Cell Biology Group, Discipline of Orthopaedics and Trauma, University of Adelaide, Adelaide, SA, Australia
2School of Dentistry, Department of Oral Biology, University of Missouri - Kansas City, Kansas City, Missouri, United States

Sclerostin is a product of mature osteocytes and a potent negative regulator of bone formation (1). Our recent study showed that sclerostin affects osteoblasts in an anti-anabolic manner (2). However, studies employing a neutralising antibody against sclerostin have reported decreased bone resorption markers (3), indicating that sclerostin may also have a catabolic action. The aim of this study was to investigate potential catabolic actions of sclerostin via the RANK-RANKL pathway.

The effect of recombinant human sclerostin (rhSCL) on pro-osteoclastic gene expression was tested in cultures of human primary immature osteoblasts and differentiated late-osteoblast/pre-osteocyte cultures, as well as the mouse osteocyte-like cell line, MLO-Y4. To examine the functional effects of rhSCL on resulting pro-osteoclastic activity, MLO-Y4 cells plated onto a bone-like substrate were primed with rhSCL for three days and then either mouse spleenocytes or human peripheral blood-derived mononuclear cells (PBMC) were added. Resorptive activity was analysed after 14 days of culture. As apoptosing osteocytes have been shown to support osteoclast formation, the effect of rhSCL on MLO-Y4 apoptosis was assessed by caspase assays and nuclear morphology.

Sclerostin dose-dependently up-regulated the expression of RANKL mRNA and down-regulated that of OPG, increasing the RANKL:OPG mRNA ratio in late osteoblast/pre-osteocyte-like cultures and in MLO-Y4 cells. In co-cultures of rhSCL treated MLO-Y4 cells and osteoclast precursors, osteoclast resorptive activity increased approximately 7-fold. The increased resorption was abolished by co-addition of recombinant OPG. rhSCL treatment also increased TRAP-positive multinucleated cell formation, and significantly increased the size of the formed cells. No detectable increase of caspase activity was observed in rhSCL-treated MLO-Y4 cells and the nuclear morphology did not change, indicating that the pro-osteoclastic effect was not as a result of MLO-Y4 cell death.

Our findings show for the first time that sclerostin, in addition to its anti-anabolic activity, acts on viable osteocytes to promote osteoclast formation and activity, and does so in a RANKL-dependent manner.

(1) Li X et al Sclerostin antibody treatment increases bone formation, bone mass, and bone strength in a rat model of postmenopausal osteoporosis. JBMR (2009)

(2) Atkins GJ et al. Sclerostin is a locally acting regulator of late-osteoblast/pre-osteocyte differentiation. JBMR EPub ahead of print (2011)

(3) Eddleston A et al. A short treatment with an antibody to sclerostin can inhibit bone loss in an ongoing model of colitis. JBMR (2009)
LOWER SERUM 25-HYDROXYVITAMIN D LEVELS ARE ASSOCIATED WITH GREATER ALL-CAUSE AND CANCER-RELATED MORTALITY AMONG AUSTRALIAN ADULTS: FINDINGS FROM THE AUSTRALIAN DIABETES, OBESITY AND LIFESTYLE STUDY (AUSDIAB)

R. M. Daly1,2, D. J. Magliano3, C. Gagnon2,4, Z. X. Lu5,6, D. W. Dunstan3, K. A. Sikaris5, P. Z. Zimmet1, P. R. Ebeling2, J. E. Shaw6

1School of Exercise and Nutrition Sciences, Deakin University, Burwood, VIC, Australia
2Department of Medicine, NorthWest Academic Centre, The University of Melbourne, Western Health, Melbourne, VIC, Australia
3Baker IDI Heart and Diabetes Institute, Melbourne, VIC, Australia
4Centre de recherche du CHUQ, Laval University, Quebec City, Quebec, Canada
5Melbourne Pathology, Melbourne, VIC, Australia
6Department of Medicine, Monash University, Melbourne, VIC, Australia

Aim: Low serum 25OHD levels have been associated with morbidity and mortality, but the relationship with mortality in Australia has not been investigated. Thus, we examined the association between 25OHD and overall and cause-specific mortality in Australian adults. Methods: Multivariate-adjusted Cox proportional hazards regression models were used to estimate the relative mortality risk of adults (n=10,542) aged ≥25 years, using data from the 1999-2000 AusDiab study linked to mortality records [National Death Index] for deaths until 16/7/2007 for all-cause, CVD and cancer-related mortality. The fully adjusted model included: age, sex, season, latitude, ethnicity, education, smoking, waist circumference, exercise, diabetes status, hypertension, use of lipid-lowering medication, serum cholesterol, triglycerides, HDL-C, history of CVD and eGFR. Results: During follow-up (median 7 years), 530 (5.0%) participants died (173 CVD- and 213 cancer-related deaths) and these participants had lower 25OHD levels compared to those who survived (57 vs 64 nmol/L, P<0.001). All-cause mortality risk was increased by 60% in the lowest compared to highest 25OHD quartile, and cancer-related mortality was increased by 79-88% across the lowest three quartiles (Table). There was a trend for CVD and non-CVD/cancer related deaths to be greater in those in lowest quartile, but the HRs were not significant. Similar results were observed after excluding the 101 participants who died within 2-years of follow-up. Conclusion: Lower serum 25OHD levels were independently associated with an increased risk for all-cause and cancer-related mortality in Australian adults.

Table: Hazard ratios (95% CI) for mortality by quartiles of 25OHD

<table>
<thead>
<tr>
<th>Mortality</th>
<th>Serum 25OHD Quartiles, nmol/L</th>
<th>&lt;47</th>
<th>48-61</th>
<th>62-77</th>
<th>&gt;77</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-cause</td>
<td></td>
<td>1.60 (1.20, 2.14)</td>
<td>1.29 (0.96, 1.73)</td>
<td>1.21 (0.90, 1.61)</td>
<td>1.00</td>
</tr>
<tr>
<td>CVD</td>
<td></td>
<td>1.51 (0.92, 2.48)</td>
<td>1.08 (0.65, 1.80)</td>
<td>0.98 (0.59, 1.62)</td>
<td>1.00</td>
</tr>
<tr>
<td>Cancer causes</td>
<td></td>
<td>1.86 (1.13, 3.04)</td>
<td>1.88 (1.16, 3.04)</td>
<td>1.79 (1.11, 2.88)</td>
<td>1.00</td>
</tr>
<tr>
<td>Non-CVD/cancer</td>
<td></td>
<td>1.49 (0.86, 2.60)</td>
<td>1.10 (0.62, 1.95)</td>
<td>1.08 (0.61, 1.91)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

VITAMIN D INSUFFICIENCY IN OLDER WOMEN: PREVALENCE AND IMPACT ON BONE DENSITY, FRACTURE RISK AND MORTALITY

K. Zhu1,2, J. R. Lewis1,2, R. L. Prince1,2

1Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Perth, WA, Australia
2School of Medicine and Pharmacology, University of Western Australia, Perth, WA, Australia

Aim: Low vitamin D status may have negative effects on health outcomes in older people. However, there are few longitudinal data in older Australian women. This study aimed to examine the prevalence of vitamin D insufficiency and its association with bone density, 10-year fracture risk and mortality in older community-dwelling Western Australian women.

Methods: The study subjects were 1383 women aged 70-85 years when recruited in 1998 from the population. After finishing a five year RCT of calcium supplementation (CAIFOS) (1), they were then recruited into a five year epidemiology study. Baseline serum 25(OH)D concentration was determined using the LC-MS/MS method. The total hip DXA BMD was measured at year one. Clinical incident osteoporotic fractures were ascertained by adverse events diary returned to the study centre every four months and confirmed by radiographic report. Mortality data were obtained from the WA mortality registry.

Results: 400 (28.9%), 504 (36.4%) and 479 (34.6%) subjects had insufficient (serum 25(OH)D ≤ 50 nmol/L), sufficient (serum 25(OH)D 50-75 nmol/L) and ideal vitamin D status (serum 25(OH)D ≥ 75 nmol/L), respectively. Adjusting for baseline age, weight, calcium intake, physical activity, season and calcium treatment, subjects with vitamin D insufficiency had 3.6% lower total hip BMD compared to those with ideal vitamin D status (794 ± 6 vs 823 ± 6 mg/cm², P=0.001). Vitamin D insufficiency was associated with 131% higher risk for vertebral fracture (Hazard ratio 2.31, 95% CI 1.20-4.47) and 43% higher risk for all-cause mortality (Hazard ratio 1.43, 95% CI 1.03-1.98) compared to those with ideal vitamin D status. There was no association between vitamin D status and non-vertebral fracture.

Conclusions: Approximately one third older community-dwelling Western Australian women had vitamin D insufficiency, which is related to lower BMD and increased risk of vertebral fracture and all-cause mortality. Vitamin D nutrition is important for maintaining health in the elderly.

SERUM 25 HYDROXY VITAMIN D (25OHD) AND INCIDENT OR WORSENING KNEE PAIN IN OLDER ADULTS: A FIVE YEAR LONGITUDINAL STUDY

L. L. Laslett¹, C. Ding², S. J. Quinn³, J. Burgess⁴, V. Parameswaran⁴, T. M. Winzenberg⁴, G. Jones¹

¹Menzies Research Institute Tasmania, University of Tasmania, HOBART, TAS, Australia
²Department of Epidemiology and Preventive Medicine, Monash University, MELBOURNE, VIC, Australia
³School of Medicine, Flinders Clinical Effectiveness, Flinders University, ADELAIDE, SA, Australia
⁴Diabetes and Endocrine Services, Royal Hobart Hospital, HOBART, TAS, Australia

Vitamin D is important for bone, cartilage and muscle function. However, there is little data on its association with pain. The aim of this study was to describe the association between serum 25OHD and change in knee pain over five years.

Methods: Longitudinal population-based study of randomly selected older adults (n=766). Serum 25OHD was assessed by radioimmunoassay and knee pain using the WOMAC questionnaire at baseline and again after five years. We used linear regression with adjustment for season, age, sex and, BMI. We also examined potential structural mechanisms for any effect by additionally adjusting for radiographic osteoarthritis, bone marrow lesions, chondral defects and muscle strength.

Results: Participants were aged 50-80 years (mean 62 years), 50% were male with a mean WOMAC score of 3.2 (range 0-39). Mean serum vitamin D was 53.8 nmol/l (range 13-166 nmol/l), with 4.2% of participants having moderate deficiency (<25 nmol/l). Knee pain (total WOMAC score) was stable in participants with vitamin D 25-50 and ≥50 nmol/l but worsened over five years in persons with vitamin D <25 nmol/l (b=-1.02, p=0.002), with consistent results within each of the pain subscales. This association persisted after adjustment for covariates. When vitamin D was analysed as a continuous measure, there were no associations between vitamin D and change in WOMAC score (b=-0.12, p=0.2). This effect was largely independent of structural factors.

Conclusions: Serum vitamin D level in the osteomalacic range (<25 nmol/l) is an independent predictor of worsening or incident knee pain over five years suggesting a lag time between the development of low levels and pain. This suggests supplementing levels below this will prevent worsening knee pain.

ANNUAL HIGH-DOSE ORAL VITAMIN D₃: IS THE INCREASED RISK OF FALLS ATTRIBUTABLE TO CHANGES IN MUSCLE STRENGTH?

K. M. Sanders¹,²,³, G. Duque², T. McCorquodale³, A. L. Stuart¹,², E. J. Williamson², M. A. Kotowicz⁵,⁶, G. C. Nicholson⁷

¹Medicine, NorthWest Academic Centre, Melbourne, VIC, Australia
²The University of Melbourne, VIC, Australia
³Medicine, University of Sydney, Sydney, NSW, Australia
⁴School of Population Health, Centre for Molecular, Environmental, Genetic and Analytic Engineering, Melbourne, VIC, Australia
⁵Barwon Health, Geelong, VIC, Australia
⁶School of Medicine, Deakin University, Geelong, VIC, Australia
⁷School of Medicine, Rural Clinical School, The University of Queensland, Toowoomba, QLD, Australia

We have previously reported increased falls and fractures in a RCT using a single annual dose of 500,000IU cholecalciferol (D₃) administered orally for 3 to 5 years to 2,256 older women. The increased rate of falling in the D₃ group was higher in the first 3 months post-dosing (p=0.017).

Aim: To investigate if the increased falls are associated with muscle function.

Methods: Serial biochemistry and physical assessments were done on a sub-study of 97 randomly-selected participants. Serum 25-hydroxyvitamin D (25D) and muscle marker alpha antichymotrypsin (ACT) were measured using immunnoassay and ELISA(G Biosciences), respectively. Hip flexion muscle strength was measured using Nicholas™ Manual Muscle Tester and is reported as change at 3-month post dose, ≥2 years after baseline. The peak force (kg) required to break an isometric muscle contraction was measured as the examiner applied force against the participant (average of 3 trials/participant). Our post-hoc analysis used a regression model at 3-months post-dose and included age, baseline strength and change in 25D as covariates. The analysis was stratified by whether or not the change in 25D was more than 150% of the baseline 25D.

Results: Baseline 25D was 49nmol/L and increased to 124, 93 and 62nmol/L in the D₃ group at 1-, 3- and 12-month post-dose. Increases in 25D up to 150% were associated with progressively increased strength whereas larger increases in 25D (>150%) were associated with decreasing strength (mean strength change associated with 10 nmol/L unit increase in 25D = 0.38 kg [95% CI: 0.1, 0.7] vs –0.26 kg [95% CI: -0.6, 0.06]; >150% vs >150% increase in 25D, respectively; heterogeneity p=0.003). Change in ACT also suggests a threshold (p=0.029).

Conclusion: These findings suggest a threshold effect of vitamin D status following annual high-dose D₃ and are consistent with a U-shaped association reported between frailty status and 25D levels.

(1) Sanders KM, Stuart AL, Williamson EJ, Kotowicz MA, Young D, Nicholson GC. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. JAMA. 2010
(2) Ensrud KE et al, JCEM 2010
MATERNAL VITAMIN D SUPPLEMENTATION DURING PREGNANCY PREVENTS VITAMIN D DEFICIENCY IN THE NEWBORN. A RANDOMISED CONTROLLED TRIAL

C. P. Rodda, J. E. Benson, A. J. Vincent, L. C. Whitehead, A. Polyakov, B. Vollenhoven

Women’s and Children’s Program, Southern Health, Clayton, VIC, Australia
Clinical Nutrition and Metabolism, Southern Health, Clayton, VIC, Australia
Jean Hailes Clinical Research Unit, Monash University, Clayton, VIC, Australia
The Ritchie Centre, Monash Institute for Medical Research, Clayton, VIC, Australia
Department of Paediatrics, Monash University, Clayton, VIC, Australia
Department of Obstetrics and Gynaecology, Monash University, Clayton, VIC, Australia

Vitamin D (Vit D) deficiency is increasing due to lifestyle factors affecting sun exposure. Aim: To determine if maternal Vitamin D (Vit D) supplementation during pregnancy prevents neonatal Vitamin D deficiency, in Vit D deficient mothers. Methods: A randomised controlled trial was conducted over 12 months from 2008 to 2009 in a metropolitan Melbourne (latitude 38°S) tertiary maternity hospital antenatal clinic. 48 of 70 mothers with singleton pregnancies diagnosed with Vit D deficiency (serum 25-OH Vit D <75 nmol/L) at 12–16 weeks gestation, consented to be randomised to Vit D supplementation with 2000 IU cholecalciferol orally daily until delivery (n=23), or no supplementation (n=25). At 28 weeks, those remaining Vit D deficient on Vit D retesting in the treatment group received doubled cholecalciferol (4,000iu) until delivery. Results: Mean maternal 25-OH Vit D concentration at 28 weeks gestation was significantly higher (p=0.0004) in the treatment group (65nmol/L, 95% CI 54 - 76nmol/L) compared with the control group (41nmol/L, 95% CI 33 - 48nmol/L). Mean umbilical cord 25-OH Vit D concentration at delivery was significantly higher (p<0.0001) in neonates of supplemented mothers (81 nmol/L, 95% confidence interval 70 - 91nmol/L) compared with neonates of control mothers (42 nmol/L, 95%CI 34 - 50nmol/L). There was a significant positive correlation between maternal 25-OH Vit D and umbilical cord 25-OH Vit D concentrations at delivery (Spearman Rank correlation coefficient 0.88; p=0.0001). Mean supplemented maternal 25-OH Vit D concentration at delivery was significantly higher (p<0.0001) (71nmol/L, 95%CI 62 - 81nmol/L) compared with control mothers (36nmol/L, 95%CI 29 - 42nmol/L). Conclusion: Vit D supplementation of Vit D deficient pregnant women prevents neonatal Vit D deficiency.

TREATMENT WITH ORAL CHOLECALCIFEROL 2000 IU AND 5000 IU ON SERUM VITAMIN D, PTH, BONE TURNOVER AND MUSCLE STRENGTH IN PATIENTS WITH VITAMIN D DEFICIENCY

T. Diamond, Y. Wong, T. Golombick
Endocrinology, St George Hospital, Kogarah, Australia

Aim : To determine the optimal dose of cholecalciferol required to achieve a target serum 25OHD level > 75nmol/L and its relationship to both bone turnover and muscle strength.

Methods : 30 vitamin D deficient patients (serum 25OHD < 50nmol/L) randomly assigned to two groups – ie 2000 IU/day and 5000 IU/day. Collected at baseline, at 2 months and 3 months post therapy: (a) clinical demographics (b) dietary calcium recall (c) physical tests of muscle function and (d) biochemistry. Statistical analysis using paired student T-test and analysis of variance (ANOVA). Regression analysis was used to determine relationship between serum 25OHD, PTH and bone turnover.

Results : 26 (87%) patients completed 3 months of therapy. Percentage increase in serum 25OHD (compared to baseline) was 82.7% in 2000 IU group and 219.5% in 5000 IU group. While all participants (100%) achieved a serum 25OHD concentration > 50 nmol/L, only 5 subjects (45.4%) in 2000 IU group compared to 14 subjects (93.3%) in 5000 IU group achieved final 25OHD concentration > 75 nmol/L (p<0.01). Mean serum calcium increased from 2.35±0.09 mmol/L to 2.39±0.08mmol/L after cholecalciferol 2000 IU daily (p=0.55) and from 2.35±0.10 mmol/L to 2.38±0.08 mmol/L after cholecalciferol 5000 IU daily (p=0.55). Serum PTH levels normalised in most patients (n=19; 73.0%). In the regression analysis, the serum PTH levels began to rise as the serum 25OHD concentrations decreased below 70-75 nmolar range. All parameters of muscle strength showed trends in improvements following the administration of both the 2000 IU and 5000 IU doses. No patient reported untoward side effects and no patient developed hypercalcaemia.

Conclusion : Treatment for 3 months with oral cholecalciferol 5000 IU daily may be more effective than 2000 IU daily in achieving optimal serum 25OHD concentrations in vitamin D deficient patients.

MEASURING A GROWING SKELETON, AND THE EFFECT OF ETHNICITY ON BONE AND BODY COMPOSITION

C. Cowell
Institute of Endocrinology and Diabetes, The Children’s Hospital at Westmead, NSW, Australia

Abstract text not available at time of print.
**NUTRITION AND CHILDREN'S BONE**

T. Winzenberg, G. Jones  
Menzies Research Institute, Australia

Bone mineral density (BMD) in later life, an important risk factor for osteoporotic fracture, depends both on peak bone mass (PBM) and the rate of subsequent bone loss. Low BMD in childhood is also a risk factor for childhood fracture. An estimated 90% of PBM is gained by age 18 years and modeling suggests that increasing could substantially delay the onset of osteoporosis. Therefore, childhood is a critical time to intervene to improve PBM and potentially reduce the risk of both childhood and osteoporotic fractures. Important modifiable influences on childhood bone development include nutritional factors such as maternal diet in utero, breast feeding, calcium and dairy intake, vitamin D, fruit and vegetable intake, and possible adverse effects from high dietary sodium intake and intake of carbonated beverages. However, the roles of these factors and the benefits from improving them remain incompletely understood. Interventions with childhood fractures as an outcome are lacking so clinical trial evidence relies predominantly on BMD outcomes. Calcium and vitamin D supplementation have the most extensive evidence base. A meta-analysis of calcium supplement RCTs in healthy children showed no benefits for BMD at the hip or lumbar spine and only a small benefit unlikely to be clinically significant at the upper limb. Another meta-analysis of vitamin D trials showed no benefit at any site, although a subgroup analysis of studies with low mean baseline serum vitamin D levels suggested that targeting deficient children could result in clinically important BMD improvements. The few trials of maternal calcium supplementation in pregnancy give mixed results. Other nutritional influences lack RCT evidence though observational data support their further examination. In conclusion, the best nutritional approach to improving PBM is yet to be determined and should be a focus of future research to reduce the burden of osteoporotic fracture in the long-term.

**OSTEOPOROSIS AND OSTEOPEROSIS IN THE YOUNG**

K. Ozono  
Department of Pediatrics, Osaka University Graduate School of Medicine, Osaka, Japan

Bone mineral content increases associated with growth during childhood, although there is dissociation between the peak height velocity and bone mineral accrual. Peak bone mass is affected by various factors including genetic background, nutrition, endocrine status and life-style. Since bone mass is controlled by osteoblastic bone formation and osteoclastic bone resorption, differentiation failure and impaired function of these bone-specific cells result in abnormal bone mineral density (BMD). Osteogenesis imperfecta (OI) is a representative congenital disorder associated with bone fragility. Up to 90% of patients with OI harbor mutations affecting structure or expression of collagen type I, and the remaining are caused by mutations of CRTAP, LEPRE1, PPIB, SERPINH1 and FKBP10. Some OI patients show short stature due to bone deformity and undefined mechanism. Bisphosphonate (BP) treatment improves BMD and reduces bone fracture in OI patients. Contrary to initial concerns, we observe rather favorable effects on growth following BP treatment. Infantile cortical hyperostosis may be associated with other type mutation of COL1A1. Osteoporosis-pseudoglioma syndrome (OPPG) is caused by the defect of the low density-lipoprotein receptor-related protein 5 (LRP5) gene. LRP5 acts as a co-receptor of the canonical Wnt signaling, while inhibition of serotonin synthesis by Lrp5 in intestine has been reported. Hypomorphic mutation of LRP6, encoding another co-receptor of Wnt signaling, leads to osteoporosis. On the other hand, the gain-of-function mutation of LRP5 is responsible for high-bone-mass phenotype and autosomal dominant osteopetrosis type 1. Recently, we have found that the gain-of-function mutation of NPR-B, the C-type natriuretic peptide receptor, causes overgrowth and low BMD. Bone resorption is performed by acid environment and proteases secreted from osteoclasts. Osteopetrosis is a heterogeneous bone-sclerosing disorder characterized by malformation or developmental defect of osteoclasts. To date, several genes including TCIRG1, CLCN7, OSTM1, TNFSF11 and TNFRSF11A have been identified to be responsible for osteopetrosis.
GROWTH FROM BIRTH TO ADULTHOOD AND BONE MINERAL DENSITY DATA FROM THE NEW DELHI BIRTH COHORT


1Endocrinology and Metabolism, All India Institute of Medical Sciences, New Delhi, Delhi, India
2MRC LifeCourse Epidemiology Unit, University of Southampton, Southampton, United Kingdom
3Pediatrics, Sitaram Bhartiya Institute of Science and Research, New Delhi, India
4Preventive Cardiology and Epidemiology, Centre for Chronic Disease Control, New Delhi, New Delhi, India
5Statistics, Indian Council of Medical Research, New Delhi, Delhi, India
6Pediatrics, Maulana Azad Medical College, New Delhi, Delhi, India
7Pediatric Cardiology, The Heart Centre, New Delhi, Delhi, India
8Pediatrics, Fortis Hospital, New Delhi, Delhi, India
9Preventive Cardiology and Epidemiology, Public Health Foundation of India, New Delhi, Delhi, India
10Pediatrics, Sunder Lal Jain Hospital, New Delhi, Delhi, India

We studied the relationship of height and body mass index (BMI) during childhood with adult bone mineral content (BMC) and areal and volumetric bone density (aBMD, vBMD) in the New Delhi Birth Cohort, India. Participants were 565 men and women aged 33-39 years, whose weight and height was recorded at birth and annually during infancy (0-2 years), childhood (2-11 years) and adolescence (11 years-adult). Lumbar spine, femoral neck and forearm BMC and aBMD were measured using dual X-ray absorptiometry; lumbar spine and femoral neck vBMD were calculated. Birth length, and height and height gain during infancy, childhood and adolescence were positively correlated with adult BMC (p<0.01 all sites except birth length with femoral neck). Correlations increased with height from birth-6 years, then remained constant for later height measurements. There were no associations with vBMD. BMI at birth, and during childhood and adolescence was also positively correlated with BMC (p<0.01 for all sites). BMI at 11 years, and BMI gain in childhood and adolescence, were correlated with aBMD and vBMD (p<0.001 for all); these correlations strengthened with increasing age of BMI measurement. All associations with height and BMI in early life were attenuated after adjustment for adult height and BMI respectively. We conclude that greater skeletal growth in utero and during infancy are associated with higher peak BMC, sharing a causal pathway with attainment of adult height. Greater BMI gain in childhood and adolescence is associated with higher peak aBMD and vBMD, sharing a causal pathway with attainment of adult BMI.

LONGITUDINAL CHANGE OF BONE GEOMETRY IN THE MID FEMUR OF GROWING CHILDREN

P. Bridge1, N. Pocock2, K. Atkins3, T. Nguyen2, C. Munns2, C. Cowell2, M. Thompson1

1Discipline of Health Science, The University of Sydney, Australia
2Garvan Institute, Darlinghurst, Australia
3The Children’s Hospital @ Westmead, Westmead, Australia
4Department of Health and Ageing, Canberra, Australia
5St Vincents Hospital, Darlinghurst, Australia

Introduction and aim: Long bones of the appendicular skeleton are often assumed to be symmetrical particularly in the mid-shaft section which is predominantly cortical bone. This region of bone may however respond asymmetrically to pubertal maturation and gender influences. In this study we tested the hypothesis that the femoral mid-shaft is growing in a symmetrical pattern along its length.

Method: Pre-pubertal healthy children (26 boys and 20 girls) aged 8-11 years were studied at baseline and after 26-48 months (pubertal stage Tanner 2 to 4). MRI was used to acquire serial contiguous slices (6 mm) of the entire femur. Bone geometry of the proximal two thirds, mid section (50%) and distal third were compared longitudinally across genders.

Results: Changes in total area (TA), cortical area (CA) and midshaft area (MA) were all significant (p<0.001) from baseline at the three sites (Table 1) without gender differences. When the three sites were compared and analysed separately for gender, there was no difference between enlargement of CA at proximal and distal slices for boys or girls. At the distal slice MA expansion was highly significant (p<0.0001) in both genders compared to the mid and proximal slices. In girls, there was no significant difference between proximal and mid slices for MA. The changes in TA were significantly different (p<0.001) between all sites for both genders.

| Table 1 | Bone Area Change from Baseline: Mean (CI 95% lower bound, upper bound) |
|-----------------|-------------|-------------|-------------|
| Site            | Total Bone area (mm²) | Cortical Area (mm²) | Medullary Area (mm²) |
|                 | Male         | Females     | Male         | Females     | Male         | Females     |
| Proximal Slice  | 127.8 (90.5,155.6) | 110.8 (90.5,131.0) | 92.2 (74.0,124.4) | 82.7 (64.9,100.4) | 61.3 (53.5,69.0) | 50.7 (41.0,60.4) |
| Mid Slice       | 144.9 (116.8,173.0) | 127.0 (109.3,144.7) | 113.6 (88.1,139.1) | 98.6 (83.5,113.7) | 55.0 (48.8,61.2) | 56.4 (39.7,53.0) |
| Distal Slice    | 166.6 (135.6,197.5) | 165.1 (141.2,189.1) | 90.9 (69.0,112.7) | 89.1 (74.9,103.2) | 120 (97.142) | 103 (84,120) |
Conclusion: Growth induces long bone geometrical adaptation in response to stimuli in a site specific pattern. These results confirm that the mid femoral shaft is not a symmetrical hollow cylinder. Caution should be used in interpreting results of single slice examinations (eg pQCT) within this section of bone.

THE ROLE OF OSTEOCYTES IN THE SKELETAL PATHOLOGY OF NEUROFIBROMATOSIS TYPE 1 (NF1)
J. V. Kühnisch1, C. Lange2, J. Seto3, J. Grohmann3, S. Stumpp3, D. Stevenson4, F. Elefteriou6, U. Kornak5, P. Fratzl5, M. Kolanczyk1,2, S. Mundlos1,2,4
1Max Planck for Molecular Genetics, Berlin, Berlin, Germany
2Institute for Medical Genetics, Charité, Universitätsmedizin, Berlin, Germany
3Max-Planck-Institute for Colloids and Interfaces, Department of Biomaterials, Potsdam, Germany
4Berlin-Brandenburg Institute for Regenerative Therapies (BCRT), Berlin, Germany
5Shriners Hospitals for Children Salt Lake City, Salt Lake City, United States
6Vanderbilt University Medical Center, Center for Bone Biology, Nashville, United States

Osteocytes are critically important for bone metabolism as they not only regulate local bone mineralization but also mediate mechanosensory signalling. Still, relatively little is known about these specialised cells, due to technical difficulties posed by analysis of the mineralized bone in which they are embedded.

The NF1 gene is required for normal bone development and skeletal manifestations, such as low bone mass, long bone bowing, focal bone changes, and dystrophic scoliosis, are common in neurofibromatosis type 1 (NF1) [1] [2] [3]. Spontaneous and non-healing bone fractures (pseudarthrosis) constitute additional clinical challenge. While NF1 in chondrocytes, osteoblasts, and osteoclasts critically regulates proliferation and differentiation, a potential role in the fourth bone cell, the osteocyte, has not been studied so far. In humerus of NF1-Prx1 mice, an animal model for NF1, we found increased osteocyte lacuna size, altered canalicul network shape, and impaired mechanical bone material properties. Comprehensive analysis of the NF1-Prx1 and NF1-Col1I bone phenotypes, revealed dramatic disarrangement of bone organization and osteocyte morphology especially pronounce in the proximity of the muscle attachment sites and vessels. In the humerus midshaft region large areas of demineralised bone were observed in the proximity of vessels, suggesting local osteolysis, likely involving "miscommunication" between vessels and osteocytes. Collectively our data indicate that NF1 is required for normal functioning of the lacuno-canalicul system revealing yet another aspect of the complex NF1 pathology.

(3) Brunetti-Pierri et.al - Mol Genet Metab 2008

MUSCLE-SPECIFIC KNOCKOUT OF NF1 CAUSES NEONATAL LETHALITY
K. Sullivan1, J. El-Hoss1, J. Seto1, M. Gdalevitch1, K. N. North2,3, D. G. Little1,2, A. Schindeler1,2
1Orthopaedic Research and Biotechnology, The Children’s Hospital at Westmead, Sydney, NSW, Australia
2Discipline of Paediatrics and Child Health, University of Sydney, Sydney, NSW, Australia
3Institute for Neuromuscular Research, The Children’s Hospital at Westmead, Sydney, NSW, Australia

Aim: Neurofibromatosis (NF1) is an autosomal dominant disorder that features a diverse series of characteristics, each with limited penetrance. The skeletal manifestations such as scoliosis and tibial dysplasia/pseudarthrosis are recalcitrant to surgical intervention and can have profound negative effects on quality of life. There is some evidence that muscle function is also affected in these individuals based on several small clinical studies and reduced muscle differentiation in a limb-specific NF1 knockout mouse. The importance of muscle contribution to the skeletal phenotypes of scoliosis progression and deficient bone healing are also poorly understood. In this study we aimed to examine muscle function in NF1 heterozygous and NF1/MyoD−/− homozygous mice.

Methods: Grip strength testing studies and botaX-induced atrophy/regeneration experiments were performed in the NF1/MyoD−/− mouse strain. NF1/MyoD−/− mice were generated by crossing the MyoD−/− mouse strain with the NF1+/− mice to induce a muscle-specific knockout. Phenotyping experiments were performed in cultured NF1/MyoD−/− myoblasts and examining NF1/MyoD−/− embryos and neonates. Results: No significant decrease in muscle strength was seen in the NF1/MyoD−/− mouse. However, compared to wild type control mice, NF1/MyoD−/− mice showed a lower recovery following bottoX treatment and injected muscles exhibited evidence of extensive fibrosis. Primary NF1/MyoD−/− myoblasts were examined for their ability for myogenic as well as osteogenic differentiation. Notably, NF1/MyoD−/− myoblasts showed decreased alkaline phosphatase and matrix mineralization under pro-osteogenic conditions.

Conclusions: These data show further evidence for a key role for NF1 in muscle development and/or maintenance. Further rescue experiments and studies examining the importance of NF1 muscle deficiency in the NF1 skeletal phenotype are underway.
RAP-011 AUGMENTS CALLUS FORMATION IN CLOSED RAT FRACTURES

A. Morse1,2, A. Schindeler1,2, L. Peacock1, K. Mikulec1, M. M. McDonald1, D. G. Little1,2

1Orthopaedic Research and Biotechnology, The Children’s Hospital at Westmead, Westmead, NSW, Australia
2University of Sydney, Sydney, NSW, Australia

Aim

RAP-011, a fusion of the extracellular domain of the activin type IIA receptor to a murine IgG-Fc fragment, antagonizes Activin signaling. We hypothesized RAP-011 could be used to augment fracture healing.

Methods

52 male Wistar rats underwent closed femoral fractures by three-point bending. Rats received twice-weekly subcutaneous injections of RAP-011 (10 mg/kg) or Vehicle. End-points were 2, 4, and 6 weeks for radiography and histology outcomes.

Results

Earlier bony union with RAP-011 was indicated by X-ray at 2 and 4 weeks. Histomorphometry indicated hastened cartilage removal, with a 49% reduction in callus percent cartilage at 2 weeks with RAP-011 (p<0.05). At 6 weeks, RAP-011 resulted in a superior bony callus. QCT of the fractured femora revealed increases in total bone mineral content (BMC) (31%, p<0.01), total bone volume (BV) (36%, p<0.05), and periosteal bone circumference (16%, p<0.05). These resulted in a 93% increase in calculated polar moment of inertia (p<0.01). Comparable results were seen with microCT. Callus length, by X-ray, increased by 32% (p<0.01), 18% (p<0.05), and 16% (p<0.01) at 2, 4, and 6 weeks respectively. RAP-011 treatment produced mild systemic effects by 6 weeks, measured in the contralateral femora by QCT. Small but statistically significant increases in total BV (8%, p<0.05), periosteal perimeter (4%, p<0.05), and predicted moment of inertia (15%, p<0.05) were observed.

Conclusions

These data suggest significant early and late stage affects of RAP-011 in the promotion of fracture repair. Early union, more rapid cartilage removal, increased callus length and size, and a calculated stronger callus were all promoted by RAP-011. These data suggest that Activin signaling may be a valuable pathway for targeting for orthopaedic intervention. Future studies will confirm mechanical strength, optimize dosing regimens, and test alternative surgical models.

Acknowledgements

Funding and reagents from Acceleron Pharma. ACE-011 (the human analogue to RAP-011) currently under clinical development by Celgene Corporation.

A GENOME-WIDE ASSOCIATION STUDY OF BONE MINERAL DENSITY: RESULTS FROM THE ODENSE ANDROGEN STUDY

C. L. Brasen1, T. L. Nielsen2, K. Wraae2, B. Abrahamsen3,4, L. Christiansen5, M. Andersen2, K. Brixen2, L. Bathum6

1Department of Clinical Biochemistry and Pharmacology, Odense University Hospital, Odense, Denmark, Denmark
2Department of Endocrinology, Odense University Hospital, Odense, Denmark, Denmark
3Department of Medicine F, Gentofte Hospital, Hellerup, Denmark, Denmark
4OPEN, Institute of Clinical Research, University of Southern Denmark, Odense, Denmark, Denmark
5Department of Epidemiology, Institute of Public Health, University of Southern Denmark, Odense, Denmark, Denmark
6Department of Clinical Biochemistry, Slagelse Hospital, Slagelse, Denmark, Denmark

Aim: To identify genetic variants affecting peak bone mass in a cohort of young men by using genome-wide genotyping and to test association between the associated SNPs and bone mineral density (BMD) in elderly men.

Methods: We used a two-step study design comprised of a discovery cohort of 783 young men aged 20-30 and a replication cohort of 600 elderly men aged 60-76. In the discovery cohort, participants were selected on the basis of BMD of the hip; genome-wide genotyping using Affymetrix 5.0 Array was performed in the 100 participants with the highest and lowest BMD, respectively. We tested the ten SNPs with the lowest p-values in the elderly cohort for association with BMD of the hip and lumbar spine as well as occurrence of potentially osteoporotic fractures.

Results: Of the ten SNPs selected from the microarray analysis, three SNPs reached significance in the replication cohort. We found rs1335858 (p=0.001) and rs8021947 (p=0.026) significantly associated to BMD of the hip and rs8112088 (p=0.028) associated to BMD of the lumbar spine. The effect size of rs1335858 was in order of a change in BMD of 5.5% across genotypes explaining 1.2% of the total variance of BMD. None of the SNPs were found significantly associated with fracture occurrence although fracture frequency changed by a factor of two across genotypes for rs1335858. All three SNPs are in areas not previously connected to BMD by genome-wide association studies.

Conclusions: We found three SNPs associated with BMD of the hip/lumbar spine. Rs1335858 was significantly associated with BMD of the hip after correction for multiple testing in the replication cohort and had a substantial effect size. The impact on fractures, however, did not reach significance.
FRACTURES AND FALLS WITH CHRONIC ANTI-EPILEPTIC DRUG USE AND PATIENT AWARENESS OF THE ISSUE

B. Shiek Ahmad1, J. D. Wark1,4, K. D. Hill1,2, T. J. O’Brien1,5
1Department of Medicine, The Royal Melbourne Hospital, University of Melbourne, Parkville, VIC, Australia
2La Trobe University and Northern Health, Musculoskeletal Research Centre and School of Physiotherapy, Bundoora, VIC, Australia
3National Ageing Research Institute, Parkville, VIC, Australia
4Bone and Mineral Service, The Royal Melbourne Hospital, Parkville, VIC, Australia
5Department of Neurology, The Royal Melbourne Hospital, Parkville, VIC, Australia

Aim:
To evaluate the prevalence of fractures and falls in epilepsy patients taking antiepileptic drugs (AED) and assess their level of awareness about AED-related bone health and fracture risk.

Methods:
A cross-sectional survey was conducted in epilepsy clinic out-patients and a non-epileptic comparison sample. Detailed information on their fall and fracture history was collected. Data of non-epileptic non-AED-user subjects from other studies approved by the Melbourne Health Human Research Ethics Committee who met the selection criteria were included for comparison.

Results:
150 AED-users (72 males, 78 females, median age = 39.3 years, IQR: 28.1) and 506 healthy comparison subjects (314 female, 192 male non-AED-users, median age = 41.8 years, IQR: 27.7) were studied. The prevalence of previous fractures was increased in users at vertebrae (p=0.009), clavicle (p=0.013) and ankle (p=0.039). Users had significantly greater history of multiple fractures (p=0.001) than non-users. Within users, fracture risk increased with age (p=0.032), longer therapy duration (p=0.001) and polytherapy (p=0.007). Non-seizure-related fractures (69% of cumulative fractures during therapy) occurred more than seizure-related fractures. In female users the prevalence of falls (p=0.027) and multiple falls (p=0.028) in the preceding year was significantly higher than in female non-users. In all users, non-seizure-related falls were more frequent than seizure-related falls. Less than 30% of epilepsy patients were aware of the association of AED use with increased risk for fractures, decreased bone mineral density or falls.

Conclusion:
A clinical sample of patients with epilepsy taking AEDs has increased fracture risk. Those who are older, receiving longer term AED treatment and on polytherapy are at particular risk of fractures. Female AED-users have an increased prevalence of falling and of multiple falls. Patients on chronic AED therapy need information about their increased risk of falling and fractures, and strategies to minimize these major adverse effects.

SERUM URIC ACID IS ASSOCIATED WITH BONE LOSS AND BODY COMPOSITION IN WOMEN: A LONGITUDINAL STUDY

J. Makovey1, M. Macara1, J. Chen1, C. S. Hayward3, L. March1, M. J. Seibel3, P. N. Sambrook1
1Department of Rheumatology, Royal North Shore Hospital, Institute of Bone and Joint Research, Kolling Institute, University of Sydney, Sydney, Australia
2Department of Cardiology, St Vincent’s Hospital, Victor Chang Cardiac Research Institute, Sydney, NSW, Australia
3Bone Research Program, ANZAC Research Institute, University of Sydney, Concord C, Sydney, NSW, Australia

Oxidative stress has been linked to osteoporosis. Serum uric acid (UA), a strong endogenous antioxidant, has been associated with higher bone mineral density (BMD), lower bone turnover and lower prevalence of fractures in a large cross-sectional study of men. Whether this relationship is present in women and how UA relates to changes in BMD longitudinally has not been examined.

A sample of 356 peri- and postmenopausal women, mean age 60.5 years was studied. Each individual had baseline BMD and body composition measurements by dual energy x-ray absorptiometry and at least one repeat measure, on average 9.7 years later. Rate of change in BMD was expressed as percent gain or loss per year. UA, calcitriol hormones and bone turnover markers were measured at the final visit.

Cross-sectional data analyses revealed that women with higher UA levels had significantly higher absolute BMD measures at all skeletal sites. Multiple regression analyses showed a strong association between UA and BMD at all skeletal sites at baseline and follow-up visits. Body weight and its components such as lean mass (LM) and fat mass (FM) were also significantly related to serum UA. The association between serum UA and BMD remained significant in multiple regression analyses after accounting for possible confounders including LM and FM. Regression analyses of the longitudinal BMD data demonstrated significant associations between serum UA levels and rates of change in BMD at all skeletal sites. Rates of change in body weight and LM, but not FM, were also significantly associated with serum UA levels. However after adjustment for changes in LM, associations remained significant for lumbar spine, forearm and whole body BMD but not for hip BMD. Higher serum UA levels appear to be protective for bone loss in peri- and postmenopausal women and this relationship does not appear to be explained by changes in body composition measures. This commonly measured biochemical parameter may be a useful marker of risk of osteoporosis in peri and postmenopausal women.
SAFETY AND EFFICACY OF DENOSUMAB IN GIANT CELL TUMOUR OF BONE (GCTB)

D. Thomas1, J. Y. Blay2, S. Chawla1, J. M. Broto1, E. Choy2, M. Dominkus6, J. Engellau7, R. Grimer8, R. Henshaw9, E. Palmerini10, P. Reichardt11, P. Rutkowski12, K. Skubitz13, Y. Zhao14, Y. Qian16, I. Jacobs14

1Peter MacCallum Cancer Centre, East Melbourne, VIC, Australia
2Department of Medicine, Claude Bernard Lyon I, Centre Léon Bérard, Lyon, France
3Sarcoma Oncology Center, Santa Monica, California, United States
4Hospital Son Dureta, Palma de Mallorca, Spain
5Dana Farber/Harvard Cancer Center, Massachusetts General Hospital, Boston, MA, United States
6Medizinische Universität Wien, Wien, Vienna, Austria
7Skåne Universitetssjukhus, Lund, Sweden
8Royal Orthopaedic Hospital, Birmingham, United Kingdom
9Georgetown University College of Medicine, Washington, DC, United States
10Istituti Ortopedici Rizzoli, Bologna, Italy
11HELOYS Klinik Bad Saarow, Bad Saarow, Germany
12Department of Soft Tissue/Bone Sarcoma and Melanoma, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland
13University of Minnesota, Masonic Cancer Center, Minneapolis, MN, United States
14Amgen Inc, Thousand Oaks, California, United States

AIM
GCTB is an osteolytic tumour that contains osteoclast-like giant cells and mononuclear cells expressing RANKL. Denosumab is a fully human monoclonal antibody that binds RANKL, inhibiting osteoclast activity. We report the safety and efficacy results of a 12-month interim analysis in an open-label, phase 2 study of denosumab in GCTB.

METHODS
Eligible subjects with surgically unsalvageable GCTB (cohort 1) or salvageable GCTB with planned surgery (cohort 2) received subcutaneous denosumab 120mg 4-weekly (120mg loading dose on days 8 and 15). The primary objective was to evaluate the safety of denosumab. Analyses also included physicians' subjective assessments of disease progression and the proportion of cohort-2 subjects for whom surgery was delayed, reduced in scope, or not required. Safety analyses included all subjects who received denosumab; efficacy analyses included subjects who received denosumab for ≥6 months.

RESULTS
Most enrolled subjects (87%) had a Karnofsky status ≥80% at baseline and 52% had recurrent unresectable disease. AEs were reported in 126/158 subjects (80%) who received denosumab; most frequent were fatigue (15%) and headache, back pain, and extremity pain (13% each). Osteonecrosis of the jaw was reported in 3/158 subjects (1.9%). No other serious AEs were attributed to denosumab. Two subjects died on study; neither death was attributed to denosumab. Hypocalcaemia was reported in 7 subjects (4%). Based on physicians' subjective assessment of disease status at 12 months, there was no disease progression in 72/73 evaluable cohort-1 subjects (99%). Among 23 cohort-2 subjects who had planned surgery at baseline, 15 (65%) did not undergo surgery within the first 12 months of the study; 5/8 subjects (62%) underwent less morbid surgical procedures than planned.

CONCLUSION
Denosumab was well tolerated in subjects with GCTB and was associated with inhibited disease progression and reduced requirements for surgery.

PARATHYROID HORMONE EXCESS AND DEFICIENCY: IS PETER REALLILY ROBBED OR PAUL PAID?

Department of Medicine and Endocrinology, Austin Health, University of Melbourne, Melbourne, VIC, Australia

Parathyroid hormone (PTH) excess is held to be anabolic at trabecular sites and catabolic at cortical sites. We hypothesize that (i) the purported anabolic effect is the result of an erroneous increase in trabecular density produced by intra-cortical remodelling which fragments cortex producing remnants that look like trabeculae (‘trabecularization’). (ii) Correction of PTH excess will only partly reverse the cortical and trabecular deficits because the negative bone balance produces irreversible bone loss. (iii) PTH deficiency produces lower intra-cortical porosity due to reduced remodelling.

We assessed 30 patients with untreated primary hyperparathyroidism (HyperP, 64.4±13.9yrs), 15 with treated HyperP (59.2±14.5yrs), 17 with hypoparathyroidism (HypoP, 57.1±15.3yrs) and 45 controls (61.0±14.9yrs). Images of the distal radius and tibia acquired using high-resolution peripheral computed tomography were analysed using a new software (Strax1.0) which quantifies intra-cortical porosity.

At the tibia, relative to controls, cortical area in untreated HyperP was reduced by 22% (p=0.003), medullary area was increased by 10% (p=0.069), trabecular volumetric vBMD was reduced by 15% (p=0.023) and intra-cortical porosity was increased by 14% (p=0.012) which resulted in a cortical vBMD deficit of 6% (p=0.005). As shown, there was a negative correlation between intra-cortical porosity and cortical vBMD across all subjects (r = -0.591, p<0.001).

Relative to controls, treated HyperP had 3% smaller cortical area (p=0.099), 29% smaller medullary area (NS) and 22% lower trabecular vBMD (p=0.008). Intra-cortical porosity was 6% (NS) higher and cortical vBMD was 2% (NS) higher.
Relative to controls, HypoP had 13% higher cortical area (p=0.107), 4% smaller medullary area (NS) and 9% higher vBMD (p=0.215). Intra-cortical porosity was 13% lower (p=0.067) and cortical vBMD was 3% higher (p=0.110). Similar trends were observed at the radius.

We infer PTH excess is deleterious to cortical and trabecular bone, correction of PTH excess partially reverses the deficits while PTH deficiency partly preserves bone volume.

117

NEW STRATEGIES FOR TREATMENT OF OSTEOPOROSIS
T. Matsumoto
Department of Medicine and Bioregulatory Sciences, University of Tokushima Graduate School of Medical Sciences, Tokushima, Japan

Bisphosphonates have been the mainstay of osteoporosis treatment, with strong evidence for the prevention of vertebral and non-vertebral fractures. However, because bisphosphonates are deposited in bone for a long time, there are concerns about the development of brittle bone by long-term use of potent bisphosphonates. Teriparatide is the only anabolic agent available for osteoporosis treatment, and has a robust effect in increasing bone mass and strength. However, its use is restricted to patients with high fracture risk. In addition, in order for these agents to be effective in increasing bone strength, substrates for bone mineralization need to be supplied. As a result, vitamin D and calcium are supplemented along with all these treatments.

Active vitamin D binds to vitamin D receptor (VDR) and regulates the transcription of its target genes. After the development of SERMs, it has become clear that actions of ligands for nuclear receptors can be modulated by a tissue-specific manner. Thus, there is a possibility that tissue-specific VDR ligands can be developed with stronger effects on bone remodeling compared with calcitropic effects exerted mainly on intestinal calcium transport. Eldecalcitol was developed under such a concept, and showed superior effects to alfacalcidol in modulating bone turnover and increasing bone strength in rodents. When administered to osteoporotic patients, 0.75 m g/day eldecalcitol was superior to 1 m g/day alfacalcidol in increasing lumbar and femoral bone mineral density, reducing bone turnover markers, and lowering the incidence of new vertebral fractures. The incidence and degree of hypercalcemia developed during the treatment were similar between the two treatment groups.

These results demonstrate that eldecalcitol is more potent than alfacalcidol in increasing bone mass and strength at a dose with similar calcemic effect to alfacalcidol. Because eldecalcitol can modulate bone turnover and increase bone strength, along with its effect in increasing intestinal calcium absorption, eldecalcitol can become a new modality of therapy for osteoporosis.

118

OSTEOPOROSIS MANAGEMENT AND MORTALITY
J. Center
Garvan Institute of Medical Research and St Vincent's Hospital, Darlinghurst, Australia

Premature mortality occurs not only following hip and vertebral fractures but also after other major and even minor fractures in the elderly. This increased mortality risk persists for about 5 years before decreasing to the background population mortality risk but is increased again if a subsequent fracture occurs. It is widely accepted that antiresorptive treatment reduces re-fracture risk. However, there is now emerging data from randomized trial and several cohort studies to suggest that antiresorptive therapy also decrease mortality risk, particular after a fracture. Intriguingly, this reduction in mortality does not appear to be due primarily to a reduction in subsequent fracture risk.

This talk will discuss the evidence surrounding the reduction in mortality with antiresorptive therapy with a focus on data from the Dubbo Osteoporosis Epidemiology Study. The pattern of the increased mortality as well as potential mechanisms of mortality reduction will be discussed with implications for management.

119

OSTEOPOROSIS: THE FUTURE
J. Eisman
Osteoporosis & Bone Biology, Garvan Institute of Medical Research, Darlinghurst, Australia

Osteoporosis and its associated fragility fractures have a major health impact due to morbidity, increased risk of future fractures and increased mortality risk. Although treatments reduce fracture risk, they do not prevent all fractures. Thus some people sustain further fractures on effective treatment while others, at apparently similar high risk, do not. Similarly some at apparently high risk of fragility fracture do not sustain fractures or suffer one but not recurrent fractures. Yet, some do badly with recurrent fractures, major morbidity and premature mortality. Some of these differences in outcome and responses are likely to relate to individual (genetic) predispositions. Reduction of bone turnover and bone loss with anti-resorptive therapy clearly results in fewer fractures and possibly
better survival. However, despite the recognized clinical impact of osteoporotic fractures and the potential for benefit from well-tolerated therapies, a minority of women and an even fewer men receive long-term effective therapy, even after a fragility fracture. At the patient-practitioner interaction, care of osteoporosis seems to suffer not only from being seen to be of limited clinical importance but also from largely unjustified concerns about safety and efficacy.

While “healthy life style” recommendations seem reasonable, these are not supported by RCT evidence of efficacy or safety. Thus the extent to which, even if fully accepted, they would influence the long-term outcomes of osteoporosis in terms of fracture risk is unclear.

Prediction of absolute fracture risk should guide doctor-patient discussions about interventions. These should go beyond the crude criteria of prior fracture, older age and categorical BMD cut-points. More sophisticated measures of bone structure are yet to be shown to provide greater clinical sight. Absolute fracture risk assessment should take into account temporal attributes to improve sensitivity and specificity; while we are yet to identify predictors of good versus adverse outcomes. Novel anabolic therapies becoming more available could allow substantial restoration of bone structure with marked reduction of future fracture risk, even in those presenting with severe osteoporosis. Their use could overcome limitations to response and should become first line, except for mild osteoporosis, and be followed by anti-resorptive ‘maintenance’ therapy.

On-going studies of genetic markers may allow identification of those at greater risk of developing osteoporosis and help predict clinical outcomes and response to specific therapies.

These advances along with the wider community recognition of the associated morbidity and mortality implications of osteoporosis will help overcome the current not-so-benign neglect of osteoporosis, such that osteoporosis prevention and treatment will receive the appropriate clinical and public attention.
CONTROL OF INTRACELLULAR CAMP LEVELS BY CALCIUM-SENSING RECEPTOR AGONISTS AND MODULATORS: ROLES OF GI/O AND PHOSPHODIESTERASES

V. A. Avlani, A. D. Conigrave
School of Molecular Bioscience, The University of Sydney, Sydney, NSW, Australia

The extracellular calcium-sensing receptor (CaR) is a class C G Protein-Coupled Receptor (GPCR) with multimodal sensing properties. The CaR responds to multivalent cation agonists including Ca\(^{2+}\), Mg\(^{2+}\) and Cd\(^{2+}\) and is subject to allosteric modulation by L-amino acids, glutathione and its analog S-methylglutathione (SMG) that bind in the Venus Fly Trap domain as well as phenylalkylamines including NPS R-467 and Cinacalcet that bind in the heptahelical domains. The CaR couples to the heterotrimeric G-proteins G\(_{q/11}\), G\(_{i/o}\) & G\(_{12/13}\) resulting in the activation of various signaling pathways leading to intracellular calcium mobilization, ERK\(_{1/2}\) activation and suppression of cAMP levels. In the present study we show that CaR-mediated suppression of cAMP arises from both a reduction in synthesis by adenylyl cyclases and enhanced degradation by phosphodiesterases. Real-time measurement of changes in intracellular CAMP levels was achieved using the cAMP biosensor CFPnd-Epac1-cpVenus in a Fluorescence Resonance Energy Transfer (FRET)-based microfluorescence assay. EPac is a guanine nucleotide exchange protein directly activated by cAMP. All allosteric modulators tested including L-Phenylalanine, SMG and cinacalcet enhanced extracellular calcium (Ca\(^{2+}\)) induced decreases in cAMP levels. Overnight treatment of cells with pertussis toxin, an inhibitor of G\(_{i/o}\), abolished not only Ca\(^{2+}\) mediated cAMP inhibition but also the effects of the allosteric modulators suggesting that G\(_{i/o}\) activation is critical to the receptor's control of cAMP levels. Isobutyl Methyl Xanthine (IBMX), a phosphodiesterase (PDE) inhibitor significantly reduced the potency of Ca\(^{2+}\). The results suggest that CaR mediated reduction in cAMP is dependent on inactivation of adenylyl cyclase via G\(_{i/o}\) and supported by PDE activation.

FOCAL SUBCHONDRAL BONE ATTRITION OF THE DISTAL METACARPAL CONDYLEs IN THOROUGHBRED RACEHORSES IS ASSOCIATED WITH MICROFRACTURE ACCUMULATION AND INCREASED BONE VOLUME FRACTION OF SURROUNDING BONE

E. Bani Hassan\(^1\), M. Mirams\(^1\), E. J. Mackie\(^1\), A. Ghasem-Zadeh\(^2\), R. C. Whilton\(^1\)
\(^1\)Department of Veterinary Science, The University of Melbourne, Werribee, VIC, Australia
\(^2\)Department of Endocrinology and Medicine, The University of Melbourne, Austin Health, Melbourne, VIC, Australia

Background and aims: Focal subchondral bone attrition is a common feature of osteoarthritis of the human knee. A naturally occurring model of subchondral bone (SCB) injury and attrition occurs in the distal metacarpal condyles of up to 70% of Thoroughbred (TB) racehorses. We hypothesised that subchondral bone attrition is due to fatigue injury of subchondral bone in racehorses.

Methods: Metacarpal bones from 39 racing Thoroughbred cadavers were examined for gross articular surface injury and imaged by high resolution peripheral quantitative computed tomography (HR-pQCT) and backscattered scanning electron microscopy (BSEM). Bone volume fraction (BV/TV) was calculated using HR-pQCT. The proportion of articular surface collapse (attrition) was measured using HR-pQCT images and microfracture density was measured on BSEM images. Statistical analysis was by Pearson and Spearman correlations.

Results: Intersecting oblique microfractures in the superficial SCB were observed in all cases with gross articular surface injury, and greater microfracture density was associated with higher grades of articular surface injury and focal SCB attrition in the lateral and medial condyles (r\(_{xy}\) = 0.55, P < 0.001). BV/TV of trabecular bone surrounding lesions was also higher with greater gross pathology grade (r = 0.40, P = 0.005), microfracture density (r = 0.50, P = 0.001) and proportion of articular surface collapse (r = 0.46, P < 0.001).

Conclusions: Microfractures appear to contribute to SCB attrition in racehorses, and SCB modelling is likely an important response. These findings are typical of fatigue injury of SCB, demonstrating that this is one possible mechanism by which SCB attrition may occur.

ADIPOSE STEM CELLS ORIGINATE FROM PERICYTES WHICH CONTRIBUTE TO VASCULARIZATION

X. Cai, Y. Lin
State key Laboratory of Oral Disease, Sichuan University, Chengdu, Sichuan, China

Aim:
Recent research has shown that adipose tissues contain abundant mesenchymal stem cells. The origin and location of the adipose stem cells, however, remain unknown presenting an obstacle to the further purification and study of these cells. In this study, we aimed to investigate the origins of adipose stem cells.
Methods:
Smooth muscle actin alpha (α-SMA) is one of the markers of pericytes. We harvested adipose stromal cells from α-SMA-GFP transgenic mice and sorted them into GFP positive and negative cells by FACS. Multilineage differentiation tests were applied to examine the pluripotent ability of the α-SMA-GFP positive and negative cells. Immunofluorescent staining for α-SMA and PDGF-Rβ were applied to identify the α-SMA-GFP positive cells. Then α-SMA-GFP positive cells were loaded on a collagen-fibronectin gel with endothelial cells to test their vascularization ability both in vitro and in vivo.

Results:
Results show that in the adipose tissue, all of the α-SMA-GFP positive cells congregate around the blood vessels. Only the α-SMA-GFP positive cells have multilineage differentiation ability while the α-SMA-GFP negative cells can only differentiate in an adipogenic direction. The α-SMA-GFP positive cells maintained expression of α-SMA during multilineage differentiation. The α-SMA-GFP positive cells can promote the vascularization of endothelial cells in 3-D culture both in vitro and in vivo.

Conclusions: We conclude that the adipose stem cells originate from pericytes and congregate around blood vessels.

Acknowledgement: National Natural Science Foundation of China (30801304, 81071273).

Reference

204
HIGHLY EXPRESSED CKIP-1 AND LOWLY EXPRESSED SMA1/5 IN AGED CALLUS SPECIMEN
B. GUO1, B. Zhang1, H. Wu1, T. Tang1, Y. He1, G. Li2, L. Hung1, X. Xie1, F. He3, L. Zhang2, L. Qin1, G. Zhang1
1Department of Orthopedics & Traumatology, Musculoskeletal Research Laboratory, HK, Hong Kong
2, Beijing Institute of Radiation Medicine, State Key Laboratory of Proteomics, Beijing Proteome Research Center, Beijing, China
3Longguang District People’s Hospital of Shenzhen, Shenzhen, China

Introductions: Either osteopenia or aged fracture healing characterized as impaired bone formation is a great threat to aged people with the population aging. The only FDA-approved anabolic agent capable of stimulating bone formation is parathyroid hormone (PTH). But dominant bone resorption after 18-month-duration with PTH is a great concern (1). Recently, we have identified CKIP-1 is an intracellular negative regulator of osteoblast differentiation for bone formation (2). The established CKIP-1 deficient mice showed increased bone mineral density (BMD) and enhanced osteoblast activity but without activating bone resorption. Mechanistically, CKIP-1 interacts with the ubiquitin-protein ligase (E3) (Smurf1), which has been well-defined as a specific suppressor of postnatal bone formation and enhances the ligase activity of Smurfl through targeting the linker region between the substrate-recogonizing WW domains. However, the expression pattern of CKIP-1 and its biological mechanism in aged women hasn't been well established for therapeutic target validation in human.

Objective: To validate the therapeutic target of CKIP-1 for impaired bone formation disease, we will explore the gene expression pattern of CKIP-1, Smad1/5 and smurf1 in aged women.

Methodology: The callus from female patients (age range: 55–80) was collected with the exclusion criteria included subject with malignancy, diabetes and other severe diseases. Fracture callus biopsies were obtained at surgery for internal fixation or for malposition as part of routine treatment. The callus was randomly divided into two groups: Aged group (age range: 65–80; n=8) and Young group (age range: 55–64; n=8). Then the callus was subjected to western blotting for protein expression quantification of CKIP-1, Smad1/5 and Smurf1.

Results: We found the protein expression of CKIP-1 in Aged group was impaired well compared to the young group. It implied a negative role of CKIP-1 in aged fracture healing or oseopenia.

Acknowledgement: National Natural Science Foundation of China (30801304, 81071273).

Reference

205
NEUROFIBROMIN (NF1) IS REQUIRED FOR NORMAL SKELETAL MUSCLE DEVELOPMENT
M. Kolanczyk1, N. Kossler1, S. Stricker1, C. Rödelsperger2, P. N. Robinson1, J. Kim1, C. Dietrich1, M. Osswald1, J. Kühnisch2, D. A. Stevenson1, T. Braun1, S. Mundlos1,2
1Max Planck for Molecular Genetics, Berlin, Germany
2Institute for Medical Genetics, Charité, Universitätsmedizin, Berlin, Germany
3Cardiac Development and Remodeling, Max-Planck-Institute for Heart and Lung Research, Bad Nauheim, Germany

Introductions: Either osteopenia or aged fracture healing characterized as impaired bone formation is a great threat to aged people with the population aging. We harvested adipose stromal cells from α-SMA-GFP transgenic mice and sorted them into GFP positive and negative cells by FACS. Multilineage differentiation tests were applied to examine the pluripotent ability of the α-SMA-GFP positive and negative cells. Immunofluorescent staining for α-SMA and PDGF-Rβ were applied to identify the α-SMA-GFP positive cells. Then α-SMA-GFP positive cells were loaded on a collagen-fibronectin gel with endothelial cells to test their vascularization ability both in vitro and in vivo.

Results: We found the protein expression of CKIP-1, Smad1/5 and Smurf1 in aged women.

Acknowledgement: National Natural Science Foundation of China (30801304, 81071273).

Reference
Neurofibromatosis type 1 (NF1) is a multi-system disease caused by mutations in the NF1 gene encoding a Ras-GAP protein, neurofibromin, which negatively regulates Ras signalling. Besides neuroectodermal malformations and tumours, the skeletal system is often affected (e.g., scoliosis and long bone dysplasia) demonstrating the importance of neurofibromin for development and maintenance of the musculoskeletal system. We have previously shown that NF1 is required for normal bone development where it regulates differentiation and proliferation of cartilage cells and osteoblasts. Since muscle strength is one of the factors determining bone quality, we hypothesised that at least part of the skeletal manifestations in NF1 might also relate to the yet unrecognized skeletal muscles phenotype. Here we focus on the role of neurofibromin in skeletal muscle development. NF1 gene inactivation in the early limb bud mesenchyme using Prx1-cre (NF1Prx1) resulted in muscle dystrophy characterised by fibrosis, reduced number of muscle fibres, and reduced muscle force. This was caused by an early defect in myogenesis affecting the terminal differentiation of myoblasts between E12.5 and E14.5. In parallel, the muscle connective tissue cells exhibited increased proliferation at E14.5 and an increase in the amount of connective tissue as early as E16.5. These changes were accompanied by excessive MAPK pathway activation. Our results demonstrate a requirement of neurofibromin for muscle formation and maintenance. This previously unrecognized function of neurofibromin may contribute to the skeletal problems in NF1 patients.

---

**CYTOPLASMIC SUPEROXIDE CAUSES BONE FRAGILITY DUE TO LOW TURNOVER OSTEOPOROSIS AND IMPAIRED COLLAGEN CROSS-LINKING**

H. Nojiri1,2, Y. Saita1,2, D. Morikawa1,2, K. Kobayashi1,2, T. Miyazaki2, M. Saito3, K. Marumo3, K. Kaneko1, T. Shimizu2

1Department of Orthopaedics, Juntendo University, Bunkyo-ku, Tokyo, Japan
2Molecular Gerontology, Tokyo Metropolitan Institute of Gerontology, Itabashi-ku, Tokyo, Japan
3Department of Orthopaedics, Jikei University School of Medicine, Minato-ku, Tokyo, Japan

Copper/zinc superoxide dismutase (CuZn-SOD, encoded by the Sod1 gene) is the enzyme that exists in cytoplasm and scavenges superoxide. Although previous studies have reported that SOD1-deficient mice (Sod1−/−) showed various age-related degeneration, little evidence of pathological effects from SOD1 deficiency in bone has been found. In the present study, we analyzed the bone tissue of Sod1−/− mice and investigated the pathological role of cytoplasmic superoxide in bone.

Sod1−/− mice showed in vivo decreased bone mineral density in DEXA and decreased both cortical and cancellous bone mass in pQCT analysis. It also showed decreased physiological cross-links and increased pathological cross-links of collagen in bone. These alterations caused a distinct weakness in bone strength. The histomorphological analyses revealed decreased parameters of both bone formation and resorption, indicating the occurrence of low turnover osteoporosis. In vitro experiments demonstrated that intracellular superoxide induced cell death and suppressed the proliferation in primary osteoblasts. Interestingly, no difference was observed in RANKL/M-CSF-induced osteoclastogenesis, osteoclast survival and function. The expression levels of Rankl and Csf1 mRNA were significantly down-regulated in the whole bones of the Sod1−/− mice, thus indicating that impaired osteoblast viability caused the decrease in the osteoblast number and suppressed RANKL/M-CSF osteoclastogenic signaling in bone. Furthermore, treatment with an antioxidant, vitamin C, effectively improved the bone fragility and osteoblastic survival.

These results imply that an intracellular redox imbalance caused by SOD1 deficiency plays a pivotal role in the development and progression of bone fragility both in vivo and in vitro. This is the first report to demonstrate that intracellular oxidative stress causes impaired bone quality in rodents. We herein present a valuable model for investigating the effects of oxidative stress on bone fragility to develop suitable therapeutic interventions.

---

**EFFECT OF GENISTEIN ON OSTEOBLAST AND OSTEOCLAST NUMBER IN HYPOESTROGENIC RAT**

N. NURDIANA1, S. BASOEKI2, M. YHONI3

1Pharmacology, Faculty of Medicine, University of Brawijaya, Malang, East Java, Indonesia
2Pathologic Anatomy, Faculty of Medicine, University of Brawijaya, Malang, East Java, Indonesia
3Faculty of Medicine, University of Brawijaya, Malang, Indonesia

Aim: To evaluate the effect of genistein on osteoblast and osteoclast number in hypoestrogenic rat.

Methods: Five groups involved in this study, consist of control group (P1), ovariectomized group (P2), ovariectomized group + genistein 0.1 mg/kgBW/day (P3), ovariectomized group + genistein 0.5 mg/kgBW/day (P4), and ovariectomized group + genistein 1 mg/kgBW/day (P5). Administration of genistein was done for 4 weeks after 4 weeks ovariectomized rats. The histologic preparation of tibial bone stained with Hematoxylin-Eosin then count for osteoblast and osteoclast number.

Results: One way ANOVA test conclude there is significant different of osteoblast and osteoclast (p=0.002) number in each groups. There is positive correlation between genistein administrations with osteoblast number (p=0.036; r=0.608). There is negative correlation between genistein administrations with osteoclast number (p=0.000; r=-0.888).

Conclusion: Genistein administration repair bone turn over in hypoestrogenic rat.

Key words: genistein, osteoblast, osteoclast, ovariectomized rat
DAIDZEIN PREVENTS THE INCREASE IN CD4+CD28NULL T CELLS IN OVARIECTOMIZED MICE: A KEY MECHANISM FOR ANTI-OSEOCLASTOGENIC EFFECT

D. Singh, A. M.A. Tyagi, K. Srivastava
Endocrinology, Central Drug Research Institute, Lucknow, Uttar Pradesh, India

Aim: Estrogen deficiency increases the population of premature senescent CD4+CD28null T cells which secrete higher amount of TNF-α, a major osteoclastogenic cytokine. Isoflavonoids like daidzein share structural similarity with 17 b -estradiol (E2) and have osteoprotective role. Aim of this study was to explore the effect of daidzein on the proliferation of TNF-α producing T cells and premature T cell senescence under estrogen deficient conditions.

Methods: For this study adult Balb/c mice were treated with Daidzein at 10 mg/kg body weight dose by oral gavage daily post ovariectomy (Ovx). After six weeks animals were autopsied and bone marrow (BM) and spleen cells were collected for FACS analysis. Pure CD4+CD4+CD28nullCD28null T cells were retrieved from BM by positive and negative selection for FACS analysis and qPCR. Blood serum was collected for ELISA.

Results: It was observed that Ovx mice treated with Daidzein for six weeks show reduction in Ovx induced expansion of CD4+ T cells in bone marrow and spleen when analysed by flow cytometry. Estrogen deficiency led to increased prevalence of TNF-α secreting CD4+CD28null T cells, however, treatment with Daidzein increased the percentage of CD4+CD28+ T cells. Daidzein inhibited the Ovx induced decrease in transcript levels of nucleolin and hnRNP-D0A transcription factors important for CD28 expression. Co-culture of CD4+CD28null T cells and bone marrow cells resulted in enhanced osteoclastogenesis as evident by increased tartarate resistant acid phosphatase (TRAP) expression. Treatment with Daidzein abrogated this effect thereby reducing osteoclastogenesis.

Conclusion: Based on our findings, we propose that one of the mechanism by which Daidzein prevents bone loss is by (i) inhibiting the proliferation of TNF-α producing CD4+CD28null T cells and (ii) preventing premature T cell senescence via increasing mRNA levels of nucleolin, hnRNP-D0A, and CD28 in BM T cells.


(1) AM Tyagi et al. Premature T Cell Senescence in Ovx Mice is Inhibited by Repletion of Estrogen and Medicarpin: A Possible Mechanism for Alleviating Bone Loss. Osteopor International (In press)

SUPPLEMENTATION OF ACETONE SOLUBLE FRACTION OF BUTEA MONOSPERMA CRUDE EXTRACT TO GROWING RATS PROVIDES GREATER PROTECTION AGAINST BONE LOSS AFTER OVARIECTOMY

D. Singh, K. Srivastava, A. M. Tyagi
Endocrinology, Central Drug Research Institute, Lucknow, Uttar Pradesh, India

Aim: Optimising peak bone mass can reduce the risk of developing osteoporosis later in life. Studies in our lab identified osteogenic activity in acetone soluble fraction (ASF) of Butea monosperma crude extract. We hypothesized that supplementation with ASF may have beneficial effect in later life in reducing bone mineral loss due to menopause.

Methods: For this study, immature female Sprague–Dawley rats were used. The groups were vehicle group and vehicle + ASF treated groups at 50 and 100 mg/kg body weight dose. A soy extract treated group was taken as positive control. After three months in vivo micro-CT scanning of rat femora and tibiae was done. Following this the animals fed with ASF and one of the control groups were ovariectomized. These animals were then left for another three months without ASF supplementation. After three months the animals were autopsied, femur and tibia dissected and various parameters like trabecular microarchitecture and bone formation were determined. Blood serum and urine was collected for assessing various biochemical markers.

Result: Three month treatment of ASF to growing rats significantly increased cortical bone volume and cortical thickness. It was observed that ovariectomized rats previously fed with ASF showed improved trabecular microarchitecture compared to control ovariectomized animals in which no prior ASF supplementation was given. This was evident with increased trabecular bone volume, trabecular thickness and trabecular number in ASF fed Ovx rats. In ASF fed Ovx rats there was less tendency towards bone loss. There was increased bone formation rate in the ASF fed ovariectomized rats. ASF fed Ovx rats also inhibited increased bone turnover rate under estrogen deficiency.

Conclusion: We conclude that ASF treatment to growing animals lowered the chances of bone loss under estrogen deficiency. Bone loss caused by osteoporosis in the post menopausal women may be thus prevented by raising peak bone mass.
CHOLINE KINASE BETA IS AN IMPORTANT REGULATOR OF BONE HOMEOSTASIS

J. Kular1, J. Tickner1, N. Pavlos2, T. Abel1, B. Lim2, M. Zheng2, J. Xu1
1School of Pathology and Lab. Medicine, University of Western Australia, Crawley, WA, Australia
2Centre for Orthopaedic Research, University of Western Australia, Crawley, WA, Australia
3Centre for Microscopy Characterisation and Analysis, University of Western Australia, Crawley, WA, Australia

The maintenance of bone homeostasis requires a tight balance between bone formation and bone resorption by osteoblasts and osteoclasts. The molecular mechanism(s) 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 an osteoporotic phenotype as evidenced by microCT and histological assessment. In vivo and in vitro analysis reveals elevated osteoclast numbers in the mutant mice. Osteoclast precursors, derived from the bone marrow of choline kinase beta mutant mice, have an increased sensitivity to RANKL during osteoclastogenesis. Furthermore, osteoclasts from choline kinase beta mutant mice exhibit increased resorptive activity as compared to those of littermate controls. Treatment with CDP-choline in vivo and in vitro reduces osteoclast numbers, thereby rescuing the osteoclast phenotype. In vitro assays show a reduction in bone formation in osteoblast cultures derived from the bone marrow and calvaria of mutant mice. Taken together, our data document, for the first time, that choline kinase beta plays an important role in bone homeostasis by regulating both osteoclasts and osteoblasts.

X-RAY RADIOGRAPHY, MRI AND MULTIDEDECTOR CT OF SPINE

H. Uzkeser
Ataturk University, Erzurum, Turkey

A 45-year-old woman admitted to our hospital with low back pain. She has been suffering for five years and the complaint was intensified over the past month. Low back pain was increasing by walking and decreasing by resting. Reduced lumbar lordosis and paravertebral muscle spasm were observed in her examination. Neurological examination and laboratory tests were all normal. Lumbar X-ray radiography was also normal. Fusion of three consecutive lumbar spines was identified in the longitudinal sections in lumbar MRI. Finally, the 3-dimensional view of vertebrae was obtained by multidetector CT and lumbar fusions were more precisely viewed. As a result, the X-ray radiography may be useful to view the bone structure, but it could easily be affected by factors such as position of the spine and obesity. Thus, multidetector CT can view the spine pathologies more definite.

THE EFFECTS OF ANTI-PYCHOTIC MEDICATIONS ON BMD AND BONE MICROARCHITECTURE IN RATS FED HIGH-FAT DIET

M. Watson1, E. Rizal2, J. L. Costa1, G. C. Smith2, P. Shepherd2, K. E. Callon1, A. Grey1, J. Cornish1
1Dept of Medicine, University of Auckland, Auckland, New Zealand
2Dept of Molecular Medicine and Pathology, University of Auckland, Auckland, New Zealand

Aim
Fracture risk is increased in patients with schizophrenia, but the mechanism by which skeletal fragility is increased has not been determined and includes the possibility of direct skeletal toxicity by anti-psychotic drugs. In an earlier study, we reported that the antipsychotic drug clozapine but not haloperidol, reduces BMD and bone volume in rats. We have now examined the skeletal effects of anti-psychotic drugs in rats receiving a high fat diet, an experimental design which may more accurately reflect the human situation.

Methods
Six week old male Sprague-Dawley rats were administered intravenous clozapine, quetiapine, haloperidol or vehicle once daily for a period of 42 days. BMD was assessed by DXA and tibiae were examined using μCT.

Results
Whole body BMD was significantly lower in rats treated with clozapine than in those treated with vehicle (p<0.05). In rats treated with haloperidol and quetiapine, there was a significant reduction in BV/TV in the proximal tibia by 21% and 25% respectively (p<0.05 vs vehicle) and a reduction in Tb.N by 20% and 23% respectively (p<0.05 vs vehicle). There was also an increase in structure model index (SMI) the haloperidol and quetiapine-treated animals by 14% and 16% respectively (p<0.05 vs vehicle), indicating a more rod-like structure and less plate-like structure compared to controls.

Conclusions
This data demonstrates that in rats receiving a high fat diet, both haloperidol and quetiapine have an adverse effect on bone microarchitecture. Long term administration of both typical and atypical anti-psychotics may have a negative effect on bone health and further studies to investigate this possibility are warranted.
MEMANTINE POTENTLY INHIBITS HUMAN OSTEOCLASTOGENESIS

T. Yag, Y. Nanke, M. Kawamoto, H. Yamanaka, S. Kotake

Institute of Rheumatology, Tokyo Women's Medical University, Shinjuku-ku, Japan

[Background] Memantine, an antagonist of N-methyl-D-aspartate (NMDA) glutamate receptors, is used for treating moderate to severe Alzheimer's disease (AD). Recently, it has been approved in Japan and its clinical efficacy is expected. It was reported that murine macrophages (RAW264.7 cells) express NMDA receptors and that a specific antagonist of NMDAR, MK801, inhibits murine osteoclastogenesis through activation of the NF-κB pathway.

[Objective] To investigate the effect of memantine on human osteoclastogenesis.

[Methods] Human CD14+ cells (monocytes) were cultured with macrophage colony stimulating factor (M-CSF; 100 ng/ml) for 3 days. Monocytes were cultured with a soluble receptor-activator of NF-κB ligand (sRANKL) and/or memantine (300 or 1000 μM) for 10 days to induce osteoclast differentiation. Cells were then stained with osteoclast-specific CD51/61 (vitronectin receptor) antibody.

[Results] Memantine potently inhibited human osteoclastogenesis. Furthermore, the inhibition effect of memantine was dose-dependent.

[Conclusions] We have demonstrated that memantine, an antagonist of the NMDA receptor, inhibits human osteoclastogenesis. Our results suggest that memantine has the therapeutic potential to prevent bone destruction in rheumatoid arthritis.


ASSESSMENT OF PANORAMIC RADIOMORPHOMETRIC INDICES IN A LARGE MIXED BRAZILIAN POPULATION

M. B.C. Alonso1, G. M.B. Ambrosano1, F. Hailer-Neto1, S. A.C. Monteiro2, P. C.A. Watanabe2

1Oral Diagnosis-Radiology, State University of Campinas/Piracicaba Dental School, Piracicaba, São Paulo, Brazil
2Morphology, Stomatology and Physiology, University of São Paulo/ Ribeirão Preto School of Dentistry, Ribeirão Preto, São Paulo, Brazil

The aim of this study was to evaluate the mandibular cortical bone thickness (MCBT) in the mental foramen region (Mentalis Index-MI) and in the gonial region (Gonial Index-GI) on panoramic radiographs in Brazilians and check how they relate to gender and age. A total of 1,287 digital panoramic radiographs of patients, age 17 to 90 years, both genders, were selected and assigned to five groups of age: 17–20 years; 21–35; 36–55; 56–69 and 70 or older. The MI and GI were evaluated using the Radioimp ® software and the measurements of the cortical bone thickness were made bilaterally by one experience researcher. Mean values and standard deviations for both MI and GI were obtained. ANOVA, Tukey’s t and the intraclass correlation coefficient were used for the statistical analyses. All the quantitative indices were significantly correlated with gender and age (p <0.05); however, no significant differences were found among some age groups, considering both genders. Intraclass and inter-rater agreement was excellent. The MCBT is influenced by age and gender. The mean values obtained in young Brazilian men (MI=4.19mm; GI=1.37mm) and young Brazilian women (MI=3.60mm; GI=1.25mm) can help establish a standard tool to assess bone quality in patients belonging to all groups of age.


VALIDATION OF LONGITUDINAL DXA CHANGES IN BODY COMPOSITION FROM PRE-TO MID-ADOLESCENCE USING MRI AS REFERENCE

P. Bridge1,4, T. N. Nguyen2, N. A. Pocock3, C. T. Cowell1, C. Munns4, N. Forwood3, M. W. Thompson1

1Dept of Health Science, The University of Sydney, Lidcombe, NSW, Australia
2The Garvan Institute, Darlinghurst, NSW, Australia
3Nuclear Medicine, St Vincents Hospital, Darlinghurst, NSW, Australia
4Endocrinology, The Children’s Hospital at Westmead, Westmead, NSW, Australia

Introduction:
Dual energy X-ray absorptiometry (DXA) has been used extensively for bone mineral density and body composition assessments. Surprisingly, the role of DXA in monitoring changes in children’s body composition, using direct imaging methods such as magnetic resonance imaging (MRI) as reference, is still yet to be validated.

Objective:
We aimed at validating the use of DXA in monitoring change in the thigh lean soft tissue mass (LSTM) and fat mass (FM), in comparison to thigh skeletal muscle mass (SM) and FM, measured using MRI as the reference standard, from childhood to mid-adolescence.

Method:
At Baseline, 22 healthy children (16 boys- 6 girls) aged 8-11 years were included, and then recalled at pubertal stage Tanner2-Tanner4. LSTM-DXA and FM-DXA of the mid-third femur and SM-MRI and FM-MRI of the same region were measured on the same day. The same protocol was repeated 26-48 months later.

Results:
At baseline, DXA over-estimated LSTM-DXA on average by 222g (95% CI: 33-410g) with a concordance C-LSTM = 0.576. FM-MRI and FM-DXA were not significantly different (95% CI: 213 to 199g, the C-FM = 0.907 ).

At follow-up, change in LSTM-DXA and FM-DXA were not significantly different to change in SM-MRI and FM-MRI respectively (95% CI of the difference was -278 to 208g for LSTM, and -148 to 256g for FM). The coefficient of concordance between the two techniques was 0.88 for both LSTM and FM.

Conclusion:
This study validates the use of DXA in monitoring changes in LTM and FM in children, confirming its significant potential in clinical and research roles in Paediatric body composition.

QUANTITATIVE ULTRASOUND AND FRACTURE PREDICTION IN NON-OSTEOPOROTIC MEN AND WOMEN

M. Y. Chan1, N. D. Nguyen1, J. R. Center2, T. V. Nguyen3, J. A. Eisman2

1Osteoporosis and Bone Biology, Garvan Institute, Darlinghurst, NSW, Australia
2St Vincent’s Clinical School, St Vincent’s Hospital, Darlinghurst, NSW, Australia
3School of Public Health and Community Medicine, University of New South Wales, Kensington, NSW, Australia

This study aimed to examine whether lower calcaneal QUS is associated with increased fracture risk in non-osteoporotic individuals. It is part of the on-going Dubbo Osteoporosis Epidemiology Study, which included 702 participants (312 women and 390 men) aged 62-90 with BMD T-score > -2.5, who had QUS assessment. QUS was measured in Broadband ultrasound attenuation (BUA) at calcaneus using CUBA sonometer, BMD was measured at the femoral neck (FNBMD) by dual energy X-ray absorptiometry (DXA) using GE Lunar DPX-L densitometer. The incidence of fragility fracture during the follow-up period (median 12 years) was ascertained by X-ray report. Overall, 26% of women (n = 80) and 14% of men (n = 53) had experienced at least one fragility fracture during the follow up.

Results:
Subjects with fracture had lower baseline FNBMD and BUA than those without. In women, increased fracture risk was associated with decrease in BUA (HR= 1.74, 95% CI: 1.33-2.29), and reduced FNBMD (HR =1.40; 95% CI, 1.02-1.94) before adjustment. After adjusting for age, falls, prior fracture and osteoarthritis, fracture risk remained significantly associated with BUA (HR =1.50; 95% CI, 1.13-1.99), but not with FNBMD (HR= 1.24; 95% CI, 0.89-1.73). The association between BUA and fracture was also observed for hip fracture (HR= 4.17; 1.67-10.43), but not for vertebral fracture (HR =1.51; 0.96-2.38. In men, the association between BUA and fracture risk was insignificant using BMD T-score of -2.5 as cut off point; but the association had become significant when the cut off was changed to -1.0 (adjusted HR= 1.69; 95% CI, 1.12-2.54). The ROC curve analysis revealed similar AUC values of BUA in men (0.71, 95% CI, 0.64-0.78) and women (0.71, 95% CI, 0.64-0.78), with the highest AUC value at hip fracture in women (0.85, 95% CI, 0.75-0.95).These results suggest that calcaneal BUA is an independent predictor of any and hip fracture in women without osteoporosis; but result was inconclusive in men.
IS ABMD A PREDICTOR OF MICROARCHITECTURE?
A. Ghaseem-Zadeh, T. Vu, Q. Wang, R. Zebaze, E. Seeman
Austin Health, University of Melbourne, West Heidelberg, VIC, Australia

Background Bone mineral density (BMD) is commonly used in clinical and research to identify individuals at risk for fragility fracture. However, it lacks sensitivity and specificity, in part, because the aBMD is a 2D projection of a 3D structure. aBMD is dependent on bone size; individuals with larger bones have higher aBMD. We therefore hypothesized that the variance in aBMD is to a large degree is explained by differences in bone size in individuals.

Material & Methods To test this hypothesis, we studied 85 women (mean age 66 yrs (range 45 to 89). Images were acquired at the non-dominant ultra-distal radius using high resolution peripheral computed tomography (XtremeCT, Scanco) and processed following the manufacturer's protocol to quantify total cross-sectional area (T-CSA), cortical volume, trabecular volume, cortical vBMD, trabecular BV/TV. aBMD was measured at the same location using DXA (Lunar Prodigy). Multivariate stepwise regression models were used to assess the relative contribution of bone size and other structural parameters to the variance in aBMD.

Results Together structural parameters and bone size explained 72% of the variance in aBMD. T-CSA independently explained only 3% of the variance in aBMD. 45% of the variance was accounted for by total cortical volume (which includes matrix volume plus void volume (e.g., haversian canals)) and 24% was explained by trabecular volumetric density. Neither cortical vBMD (a composite of void volume and tissue mineralization density), nor medullary volume independently predicted aBMD.

Conclusion Contrary to our hypothesis, aBMD at the ultra-distal radius largely reflects cortical and trabecular bone structure, not bone size. The structural basis of the remaining 28% of the variance requires further studies.

DEFINING CUT-OFF VALUES FOR THE DIAGNOSIS OF OSTEOPOROSIS IN POSTMENOPAUSAL WOMEN USING THE DXL METHOD
Z. Hamidi, R. Hafezi, A. Keshkhtar, B. Lartjani
Endocrinology and Metabolism Research Institute of Tehran University of Medical, Tehran, Iran

DXL of the calcaneus is a portable and easily accessible method that can be used extensively for bone mineral densitometry. This study was designed to determine a cut-off point for DXL of the calcaneus in the diagnosis of osteoporosis in postmenopausal women. In 510 healthy, postmenopausal women, the bone mineral density (BMD) of the spinal and femoral region was determined using DXA; the BMD of the heel was determined using DXL. The agreement between the two methods in the diagnosis of osteoporosis and the optimum cut-off point for DXL in defining osteoporosis was obtained.

Osteoporosis existed in 34.3% of the subjects using the DXA method (32.2% based on L2-L4 and 11% based on the total region of the femur) and in 26.1% of the subjects using the DXL method. The agreement between the two methods in the diagnosis of osteoporosis (kappa score) was 0.407 for the lumbar region and 0.347 for the total region of the femur. Using ROC curves, we found that a T-score of -1.8 was the optimum cut-off point for DXL in the diagnosis of osteoporosis in the lumbar spine; the sensitivity and specificity were 84 and 60%, respectively. We also found that a T-score of -2.2 was the optimum cut-off point for DXL in the diagnosis of osteoporosis in the total region of the femur; the sensitivity and specificity were 84 and 70%, respectively. The area under the curve for the spine and total regions was 0.807 (P value<0.000) and 0.859 (P value<0.000), respectively.

The results of this study demonstrate a moderate agreement between the two methods for the diagnosis of osteoporosis, suggesting that DXL cannot be used as a replacement for the DXA method, but it may be able to be used as a screening method for identifying osteoporosis.

ULTRASTRUCTURAL ASSESSMENT FOR VASCULAR CALCIFICATION IN THE AORTA OF KLOTHO−/− MICE
T. Hasegawa1, M. Li1, M. Sasaki1, T. Chihiro1, I. Ookido2, Z. Liu1, T. Yamamoto1, K. Yokoyama2, N. Amizuka1

1Department of Developmental Biology of Hard Tissue, Graduate School of Denial Me, Hokkaido University, Sapporo, Japan
2Department of internal medicine, The Jikei University School of Medicine, Tokyo, Japan

Vascular calcification is a major risk factor for cardiovascular morbidity and mortality. Several investigators have demonstrated that in vitro cultured vascular smooth muscle cells (VSMCs) appear to mineralize in the presence of β-glycerophosphate and undergo a phenotypic differentiation into osteoblast-like cells. In this study, in order to clarify underlying ultrastructural mechanisms, we have examined morphologically Mönckeberg's calcification in the aorta of klotho−/−mice.

Six 5-weeks old klotho−/− male mice and the age-matched wild-type mice were fixed with paraformaldehyde solution, and their aortas were extracted for histochemical examination, e.g., von Kossa staining (calcification), van Gieson staining (elastic fiber), and immunohistochemistry for type I collagen, alkaline phosphatase (ALP), Runx2/Cbfa-1, matrix-Gla-protein (MGP) and a smooth muscle actin (α SMA). Immunogold electron microscopic technique was employed for localizing MGP on the GMA-embedded ultrathin sections of the aortas.

Von Kossa staining demonstrated a markedly calcified layer of vascular smooth muscle in the tunica media, i.e., medial calcification of klotho−/− aorta. Elastic lamellae lost wavy patterns and some of them were fragmented in the klotho−/− aorta, which tends to show an intense immunoreactivity of type I collagen but a decrease reactivity of α SMA. Although several reports suggested trans-differentiation of VSMCs into osteoblastic phenotype, VSMCs of klotho−/− aorta did not show immunoreactivity of ALP and
Runx2/Cbfa-1, hallmarks of osteoblasts. However, observing under transmission electron microscopy, VSMCs appears to show many rough endoplasmic reticulum and Golgi apparatus, indication of active synthesis of extracellular matrices. There were many particles like matrix vesicle and calcifying organic materials surrounding the VSMCs. Immuno-electron microscopy revealed MGP, which is assumed to inhibit calcification, onto calcifying organic materials and in the periphery of the well-calciﬁed elastic lamella. Thus, despite the expression of MGP, the aorta was actively calcified in the klotho deﬁcient circumstance. In conclusion, vascular calcification shows a biological process, in part, resembling osteogenesis.

220

EFFECTIVE DOSE AND QCT OF THE PROXIMAL FEMORA: WHAT ARE THE FACTS?
J. J. Hislop-Jambrich¹, D. Jackson²
¹Medical, Toshiba Australia, North Ryde, NSW, Australia
²Diagnostic Imaging, Southern Health, Clayton, VIC, Australia

Aim: The objective of this paper was to determine the ranges of effective dose (E) that could reasonably be expected for a quantitative computed tomography (QCT) examination of the hips for osteoporosis screening.

Method: The tool for this evaluation was a commercial dose modelling software designed to estimate doses delivered during a computed tomography (CT) scan (1). Doses were calculated using exposure factors sourced from Australian clinical centres. Doses are therefore presented with regard to the unique geometry of modern multi-slice clinical CT scanners (2).

Results: This paper provides data on the effective doses that can reasonably be expected using current CT equipment for osteoporosis screening and applying QCT methods. Results demonstrate signiﬁcant differences in effective doses between males and females. Results also demonstrate that effective dose may be signiﬁcantly mitigated by adjustments in acquisition techniques, which do not affect image quality for the extraction of QCT data.

Conclusion: Contemporary evaluations of the amount of ionising radiation delivered per capita by medical procedures in the developed world have alarmed referrers and patients, with a reported doubling of medical dose per capita over the last 10-15 years (3). However, a recent study evaluating the cancer-risk and clinical beneﬁt of using CT for screening of bowel cancer, provided strong evidence to use the ionising radiation CT method for screening, especially after the age of 50 years (4). These proximal femur screening dose data are presented to support and stimulate a complete discussion in evaluating the most appropriate method of screening for osteoporosis, and thus best determining fracture risk.


221

A NEW STOCHASTIC MODEL FOR HIP FRACTURE RISK
J. J. Kaufman¹, Z. Scheckner³, G. M. Luo³, R. S. Siffert²
¹CyberLogic, Inc., New York, United States
²Orthopedics, The Mount Sinai School of Medicine, New York, NY, United States

Fracture risk assessment in osteoporosis relies primarily on measurement of bone mass, as for example with X-ray bone densitometry (DXA). Estimation of fracture risk is most often evaluated using non-mechanistic statistical approaches, such as proportional hazards models. Notwithstanding the success of these models at estimating fracture risk, there is still much uncertainty as to who will or will not suffer a fracture. This has led to a search for other components besides mass that affect bone strength, such as trabecular architecture and degree of mineralization. The purpose of this paper is to introduce a new mechanistic stochastic model that characterizes the risk of hip fracture in an individual. A Poisson process is used to model the occurrence of falls, which are assumed to occur at a rate, λ. The load induced by a fall is assumed to be a random variable that has specific probability distribution. The combination of falls together with loads leads to what is known as a compound Poisson process. By retaining only those occurrences of the compound Poisson process that result in a hip fracture, a thinned Poisson process is defined that can be shown to be a Poisson process as well. The fall rate is modeled as an increasing function of age, and hip strength is modeled as a power law function of bone mineral density (BMD) as determined, for example, by DXA. The risk of hip fracture can then be computed as a function of age and BMD. By extending the analysis to a Bayesian framework, the conditional densities of BMD given a prior fracture and no prior fracture can also be computed, and also demonstrate results similar to clinical data. The model elucidates the fact that the hip fracture process is inherently random and improvements in hip strength estimation over and above that provided by BMD operate in a highly “noisy” environment and may therefore have little ability to impact clinical practice. Note that the results do not contradict that there indeed may be other bone-specific factors besides bone mass that have an impact on bone
strength. Rather, the results show that the occurrence of a hip fracture is *intrinsically stochastic*, and that the ability to identify accurately who will and who will not fracture may be inherently limited.

## RESPONSE TO LOADING IN EX VIVO BOVINE BONE

K. A. Khalid1,2, R. T. Ormsby1, K. J. Welldon2, H. P. Lim1, S. Syazwani1, S. FatinNabilah3, G. J. Atkins1, D. M. Findlay1

1Orthopaedics & Trauma, University of Adelaide, Adelaide, SA, Australia
2Orthopaedics & Trauma, Royal Adelaide Hospital, Adelaide, SA, Australia
3Orthopaedics, Traumatology & Rehabilitation, International Islamic University Malaysia, Kuantan, Pahang, Malaysia

Aims: The response of bone in vivo towards mechanical loading is widely reported(1). However, mechanotransduction studies performed in vitro cannot measure biomechanical parameters in real-time or emulate the 3D structure of bone matrix. The Zetos™ system is able to overcome the above limitations(2). Prior studies have investigated the effects of loading ex vivo bone with intact marrow(3-5). The aim of this study was to investigate the effect of mechanical loading on ex vivo trabecular bone without marrow.

Methods: Trabecular bone cores (10 x 5 mm) were prepared from a fresh 9-month old steer sternum. Care was taken to maintain sterility and viability at all times. Half of 24 bone cores had their marrow removed. Using custom-made bone culture chambers, they were perfused with culture media (7ml/hr) at 37°C. Three treatment groups of eight samples (four with marrow and four without) were either unloaded or mechanically loaded (2,000 μstrain, 1 Hz, 300 cycles daily or 2,000 μstrain, 1 Hz, 100 cycles thrice a day) for 10 consecutive days. Young's Modulus was measured daily. μCT images before and after the 10 days were taken and analysed. Media was changed daily and its pH measured. Results: Although the initial stiffness and μCT measurements varied widely, despite using a single bone from the same animal, the samples without marrow were more responsive to loading than those with intact marrow. An initial drop in the stiffness of the bone cores was followed by an increase in all treatment groups. Correlation between stiffness and all μCT measurements were superior in samples without marrow. Daily pH measurement indicated increased metabolic activity in the samples. Conclusions: The results suggested that a component of the increase in stiffness was independent of loading. Loading in the physiological range increased stiffness and more so in bone without marrow. Removal of bone marrow is beneficial in terms of uniformity of effect and better correlation between mechanical and structural parameters, perhaps due to better diffusion of growth media.

(2) Jones DB, Broeckmann E, Pohl T, Smith EL. Eur Cell Mater.2003 Jun 30;5:48-59; discussion -60

## PREVALENCE OF MORPHOMETRIC VERTEBRAL FRACTURES USING AN ADVANCED SEMI-AUTOMATED METHOD IN HEALTHY INDIANS

R. K. Marwaha1, N. Tandon2, Y. Gupta2, A. Mithal2, S. C. Kukreja3, R. S. Kanwar2, K. Bhadra1

1Endocrinology and Thyroid Research, Institute of Nuclear Medicine & Allied Sciences, Delhi, Timarpur, delhi, India
2department of Endocrinology & Metabolism, all India Institute of Medical sciences, delhi, delhi, India
3Department of Endocrinology, Medcity Medanta Hospital, Gurgaon, Haryana, India

4Department of Endocrinology, university of Illinois, Chicago, Chicago, chicago, United States

Aim: To study the prevalence of morphometric vertebral fractures using an advanced semi-automated method in healthy Indians.

Methods: We recruited 808 healthy subjects aged 50 years or more, residing in 3 residential colonies in Delhi. India who voluntarily agreed to participate in this study. All subjects underwent lateral X-rays of the lumbar and thoracic spine according to standardized protocol. All X-rays were evaluated by a single trained person by an, advanced semi-automated software (Optasia Medica). Recruited subjects underwent anthropometric, biochemical, and hormonal evaluation. BMD was measured by DXA at lumbar spine, hip, and distal radius. 45 subjects (5.6%) were excluded from final analysis in view of technical/quality issues with the obtained radiographs.[1,2]

Results: 348 males and 415 females, with mean age of 64.9 (6.7) years were evaluated. Vertebral fractures were present in 17.95% subjects (Males=18.96%, Females=17.10%). Prevalence of osteoporosis increased with age in females from 14.68% in 50-60 years age group to 21.27% in >70 years age group, but not in males (21.6% in 50-60 years age group to 20.76% in >70 years age group). Among females, there was no correlation between vertebral fractures and BMD at any site, or any anthropometric, biochemical or hormonal parameter. In males, BMD at femur neck was found to be significantly higher (p<0.002) in subjects with fracture.

Conclusions: A high prevalence of vertebral fractures was observed in elderly Indian subjects. Prevalence of vertebral fractures increased with age in females, but not in males. The constancy of fracture prevalence across age groups among men suggests a prominent contribution by traumatic fractures, as has also been reported in a large multi-centric European study.[3]

IS DIFFERENCE BETWEEN THE EFFECTS OF BODY MASS INDEX AND BODY FAT DISTRIBUTION ON BONE MINERAL DENSITY?

A. Hossein-nezhad, K. Mirzaei, Z. Maghbooli, H. Ansar, M. Khosrofar

Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, Tehran, Iran

Aim: Despite the protective role of BMI (body mass index) on bone turnover, the relationship between bone mineral density (BMD) and body fat distribution remains under debate. The aim of this study was to investigate the relationships between bone mineral density with fat mass and visceral fat in obese.

Methods: The participants were 220 adult obese (30 men and 190 women). The mean of age and BMI were 36.24±11.75 and 31.31±3.24 respectively. Lumbar spine (L2-L4) and Total hip BMD were measured by dual energy X-ray absorptiometry (DEXA). Absolute (kg) and relative fat and lean body mass (LBM) were assessed by means of electric bioimpedance method.

Results: We showed both total hip and spine BMD were positively correlated with fat mass but BMI and visceral fat only correlated with hip BMD (r=0.44, p<0.001 and r=0.40, p=0.001). Regression analysis confirmed these correlations after adjustment for age. Based on BMD T score the prevalence of osteopenia in lumbar spine and hip were 30.6% and 8.5% respectively. We found significant lower LBM in osteopenic patients in both region of BMD. But regarding BMI, fat mass and visceral fat this significant reduction only found in hip osteopenia.

Conclusion: These finding indicated that body composition and fat distribution may influence on BMD but the effects of each component in fat evaluation may influence on specific region of BMD. Among them, LBM may have important effect on BMD in all regions.

Key words: Body mass index, body fat distribution, bone mineral density, lean body mass

ASSOCIATION BETWEEN BONE MINERAL DENSITY, LEAN MASS AND FAT MASS IN BRITISH AFRICAN MEN, WOMEN AND CHILDREN LIVING IN LONDON, U.K.

S. R. MITRA, A. HANLEY

School of Human Sciences, London Metropolitan University, London, Great Britain

Studies have suggested age specific association between different body compartments and that lean mass is the main predictor of bone mineral density (BMD)\(^1\). Aim

The purpose of this study is (ongoing) to explore the association between BMD with fat mass (FM), lean mass (LM) and skeletal muscle mass (SMM) in British African men, women and children living in London, UK.

Materials and Methods:

Whole body scans were conducted using dual-energy X-ray absorptiometry (Norland XR800). Scanned images were analysed for total body and segmental FM, LM and BMD. Total SMM (kg) was determined by adding appendicular LM of arm and leg regions and multiplying by 1.33 (assuming this represents 75% of total SMM)\(^2\).

Pearson correlation coefficients were used to evaluate univariate relationships between BMD, SMM, LM and FM. In order to evaluate the relative contribution of FM, LM and SMM to BMD, multivariate linear regression models were developed with BMD as the dependent variable and LM, SMM and FM as independent variables using a stepwise process. Men and women were modelled separately.

Results:

8 men, 15 women and 8 children were measured. Mean ages were 28.6y (S.D. 10.3); 27.2y (S.D. 7.6); and 8.3y (S.D. 2.3y) respectively. BMD (g/cm\(^2\)) ranged from 0.979 – 1.214; 0.866 – 1.166; and 0.629 – 1.001 respectively.

Univariate correlation between BMD and LM was significant in women (r=0.54, P=0.04) and in children (r=0.95, P<0.001). SMM was significantly correlated with BMD in children (r=0.98, P<0.001) but less so for adults. When both LM and FM were entered into the regression model in women, LM was a better predictor of BMD, accounting for 29% of variance (R\(^2\)=0.289; P=0.02). In children, LM accounted for 91% of variance in BMD (R\(^2\)=0.909; P<0.001). Our analysis failed to show similar association in men.

Conclusion

The data suggests that in our British African population, LM is a better determinant of BMD in children and adult women.


RADIATION STERILIZATION REDUCES THE ENERGY ABSORPTION CAPACITY OF MORSELLIZED CANCELLOUS BONE ALLOGRAFTS DURING COMPACTION

H. Nguyen1, 2, D. A. F. Morgan1, M. R. Forwood1
1School of Medical Science, Griffith University, Gold Coast, QLD, Australia
2Queensland Bone Bank, Queensland Organ and Tissue Donation Services, Brisbane, QLD, Australia

Morsellized cancellous bone allografts are commonly used in joint revision to fill bone gaps and to stabilize the implanted stem. To minimize the risk of infection, bone allografts are sterilized using gamma radiation. However, radiation also affects bone mechanical properties. Many studies are reported for radiation effects on cortical and cancellous bone allografts, but data for morsellized bone is limited. We aimed to investigate the mechanical response of morsellised cancellous bone irradiated at 15, 25 and 50 kGy using a compaction test. Ten femoral heads were processed to obtain morsellized bone samples. Bone material from each femoral head was allocated to four irradiation groups: 0, 15, 25, and 50 kGy. Morsellised bone (5 grams per sample) was placed in a 14 millimetre aluminium tube and compacted at load of 100N for 150 cycles by a piston attached to an Instron dual column material testing machine (3365A series) and Bluehill software was used to calculate strain, elastic modulus, and energy. Test results revealed that the difference in strain between control (0 kGy) and irradiated groups was not significant. However, the modulus of elasticity increased significantly after the 30th cycle at the low gamma dose (15 kGy, P < 0.05), and after the 10th cycle for the standard dose (25 kGy) and high dose (50 kGy) groups (P < 0.01). More importantly, energy absorption (toughness) was significantly lower in all irradiated bone groups, even at early cycles compared to the control group (P < 0.01). These data illustrate that morsellized bone becomes more brittle following gamma irradiation, reducing its ability to absorb energy. As a result, it is less effective as stabilizing agent in joint revision, increasing the risk of loosening following impaction grafting.

BONE STRENGTH AND ATOMIC MINERAL CHARACTERS IN OSTEOPOROSIS

ZH. NOOR1, S. B. SUMITRO1, M. HIDAYAT2, A. H. RAHIM1, A. SABARUDIN3, 4, T. UMEMURA5
1Orthopedics, Ulin General Hospital, Faculty of Medicine, University of Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia
2Biology, Faculty of Mathemathic and Natural Science, University of Brawijaya, Malang, East Java, Indonesia
3Orthopedics, Saiful Anwar General Hospital, Faculty of Medicine, University of Brawijaya, Malang, East Java, Indonesia
4Orthopedics, Hasan Sadikin Hospital, Faculty of Medicine, University of Padjadjaran, Bandung, West Java, Indonesia
5Chemistry, Faculty of Mathematics and Natural Science, University of Brawijaya, Malang, East Java, Indonesia
6Nanomaterial, EcoTopia Science Institute, Nagoya University, Nagoya, Japan

Aim: To find relationship between bone strength (trivial and non trivial injury) and atomic mineral characters in trabecular osteoporosis bone.

Methods: Two groups involved in this study such as trivial injury group and non trivial injury group. Inclusion criteria consist of post menopausal and non menopausal woman, trabecular bone fracture, osteoporosis Bone Mass Density (BMD) score, and no history of previous disease. The study was conducted in Department of Orthopedics Ulin General Hospital of Banjarmasin and Department of Orthopedics Saiful Anwar Hospital of Malang from September 2010-April 2011. Bone was obtained in surgery room then analyzed for atomic mineral characters by High Resolution Inductively Coupled Plasma Mass Spectrometry in Division of Nanomaterial Sciences, EcoTopia Science Institute, Nagoya University.

Results: Concentration of Li, Na, Mg, Al, P, K, Ca, Cr, Cu, Zn, As, Rb, Sr, Pd, Ag, Te, Ba, Pb, and Se in non trivial injury group is higher than trivial injury group. Many references indicated that these atomic minerals have positive (synergistic) role on bone strength. Concentration of B, S, V, Mn, Fe, Co, and Ni in non trivial injury group is lower than trivial injury group. These atomic minerals have negative (antagonistic) role on bone strength.

Conclusion: Bone trabecular strength of osteoporosis patients is in accordance with the characters of atomic composition found, indicating that these atomic minerals should be considered in any discussion related to the bone strength.

Key words: atomic mineral, trabecular bone, osteoporosis, HR ICP-MS

A PHANTOM FOR DETERMINING ACCURACY OF DXA-DERIVED FEMORAL NECK STRUCTURAL GEOMETRY

B. C. C. Khoo1, R. L. Price1, 2, R. L. Prince3, T. J. Beck2
1Medical Technology & Physics, Sir Charles Gairdner Hospital, Perth, WA, Australia
2Quantum Medical Metrics (QMM), Baltimore, MD, United States
3School of Medicine and Pharmacology, University of Western Australia, Perth, WA, Australia
4School of Surgery, University of Western Australia, Perth, WA, Australia

Aim: There has been an increase in the use of structural geometry, measured by DXA or QCT methods to facilitate understanding of bone fragility in the hip. However, methods for assessing the accuracy of structural geometric methods are lacking. This study reports the development of a 3D anthropometric proximal femoral phantom for calibration of measurements of femoral neck structural geometry variables derived from DXA scans.
Methods: An anthropometric phantom was constructed from materials that radiologically simulate trabecular & cortical bone. It consisted of a femoral shaft and head, plus seven interchangeable neck modules varying in size and shape, to span the range of geometries commonly observed in the necks of adult femurs; from a cortical thickness of 0.7 mm defining a circular annulus (Module 1) to thicknesses of 3 and 8 mm in an asymmetric elliptical cross-section (Module 7). The phantom was scanned (each module 10 times) in a water bath using an Hologic Discovery DXA scanner in hip-array mode; the data then analysed using hip structural analysis (HSA) software (QMM).

Results: All DXA-derived structural geometrical variables were very highly correlated (r~1.00) with the equivalent 'gold-standard' values derived from the phantom's known physical properties (shape and composition). Though systematic errors were up to 98%, these high correlations validate the use of the phantom as an accuracy calibration tool. Linear predictive equations were generated for each variable (exponential for buckling ratio [BR]), then used to predict the 'correct' values derived from DXA. After this calibration was applied, maximum accuracy errors for DXA measurements on the module with the thinnest cortex (1) ranged from -22% for average cortical thickness (aCt) to +6% for areal (a) BMD. All variables in other modules showed a maximum accuracy error of 5%, with most values <3%. The Table summarises correlations and percentage (%) differences between calculations of structural geometrical variables based on phantom physical properties and corresponding DXA-based HSA measurements for aBMD, cross-sectional area (CSA), cross-sectional moment of inertia (CSMI), outer diameter (OD), section modulus (Z), estimated aCt and BR, following calibration. The pre-calibration value is in parentheses.

Conclusions: Accuracy errors were largest for measurements of aCt, Z & aBMD in the femoral neck module with the thinnest cortex (0.7 mm), but acceptable for other geometrical variables, plus generally all variables in the other modules with progressively thicker cortices.

Table: DXA-derived structural geometrical accuracy, expressed as % difference from direct phantom calculations, following adjustment for the pre-calibration value in parentheses*

<table>
<thead>
<tr>
<th>Module</th>
<th>aBMD</th>
<th>CSA</th>
<th>CSMI</th>
<th>OD</th>
<th>Z</th>
<th>aCt</th>
<th>BR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6(16)</td>
<td>4(18)</td>
<td>-3(23)</td>
<td>-1(1)</td>
<td>5(19)</td>
<td>-22(30)</td>
<td>-1(-20)</td>
</tr>
<tr>
<td>2</td>
<td>2(8)</td>
<td>3(10)</td>
<td>4(7)</td>
<td>&lt;1(1)</td>
<td>-1(3)</td>
<td>1(-6)</td>
<td>4(11)</td>
</tr>
<tr>
<td>3</td>
<td>-3(1)</td>
<td>-2(1)</td>
<td>&lt;1(-4)</td>
<td>&lt;1(-1)</td>
<td>2(2)</td>
<td>&lt;1(-6)</td>
<td>-1(-26)</td>
</tr>
<tr>
<td>4</td>
<td>3(-2)</td>
<td>-2(-2)</td>
<td>1(-10)</td>
<td>&lt;1(-1)</td>
<td>&lt;1(-4)</td>
<td>4(-37)</td>
<td>-1(-49)</td>
</tr>
<tr>
<td>5</td>
<td>-1(-2)</td>
<td>-1(-3)</td>
<td>-2(-15)</td>
<td>&lt;1(-1)</td>
<td>-3(8)</td>
<td>5(-43)</td>
<td>-3(63)</td>
</tr>
<tr>
<td>6</td>
<td>-1(-3)</td>
<td>&lt;1(-4)</td>
<td>&lt;1(-15)</td>
<td>&lt;1(-2)</td>
<td>-2(-10)</td>
<td>1(-50)</td>
<td>1(83)</td>
</tr>
<tr>
<td>7</td>
<td>2(-1)</td>
<td>1(-5)</td>
<td>&lt;1(-19)</td>
<td>-1(-3)</td>
<td>2(-8)</td>
<td>-3(-56)</td>
<td>1(98)</td>
</tr>
</tbody>
</table>

Correl (r )
1.00  1.00  1.00  1.00  1.00  1.00  1.00

* BR required an exponential calibration function; all other variables used a linear function.

Differences in Structural Geometrical Outcomes at the Neck of the Proximal Femur Using Two-Dimensional DXA-Derived Projection (APEX) and Three-Dimensional QCT-Derived (BIT QCT) Techniques

B. C.C. Kho1, K. Brown2, K. Zhu3, M. Pollock4, K. E. Wilson5, R. I. Price6, R. L. Prince5,4
1Medical Technology and Physics, Sir Charles Gairdner Hospital, Perth, WA, Australia
2Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Preth, WA, Australia
3Mindways Software, Austin, TX, United States
4School of Medicine and Pharmacology, University of Western Australia, Perth, WA, Australia
5Hologic Inc., Bedford, MA, United States
6School of Surgery, University of Western Australia, Perth, WA, Australia

Aim: There has been increasing interest in using bone structural geometry to assess bone fragility to complement bone mineral mass. The aim of this study is to compare structural geometrical differences between “2D” DXA-derived and “3D” QCT-derived techniques in unselected clinical cases.

Methods: 237 females mean age 77.6 (SD 5.1) years had both DXA and QCT assessments of femoral neck structural geometry using manufacturer supplied instructions for determination of area of interest and orientation. QCT structural geometrical variables calculated by BIT QCT (Mindways Software Inc., Austin, TX, USA) were compared to those obtained from DXA Hip Structure Analysis [APEX 3 (HSA option of APEX version 3.0)] (Hologic, Bedford, MA, USA). The variables compared were areal bone mineral density (aBMD), cross-sectional area (CSA), cross-sectional moment of inertia (CSMI), section modulus (Z), averaged cortical thickness, endosteal width (ESW), sub-periosteal width (W) and buckling ratio (BR).

Results: Correlation coefficient of BIT QCT compared to APEX 3 femoral neck variables ranged from 0.45 for ESW to 0.90 for CSA. APEX 3 and BIT QCT derived femoral neck sub-periosteal width values were numerically similar. However, CSA, CSMI, Z and averaged cortical thickness values measured by APEX version 3.0 were higher and ESW and buckling ratio values were lower than corresponding BIT QCT.

Conclusions: Two dimensional DXA structural analysis of the neck of femur is related to but different from the same parameters calculated from true three dimensional images obtained by CT. Femoral neck size values are similar for DXA and QCT but structural geometrical variables dependent on mass calibration standards, location of neck ROI and mathematical derivation techniques are different.
QCT AND DXA HIP SIZE AND STRENGTH MEASUREMENTS ARE HIGHLY CORRELATED
K. Ramamurthi, O. Ahmad, K. Engelke, R. H. Taylor, K. Zhu, S. Gustafsson, R. L. Prince, K. E. Wilson

1Hologic, Inc., Bedford, MA, United States
2Engineering Research Center for Computer-Integrated Surgical Systems and Technol, The Johns Hopkins University, Baltimore, MD, United States
3Institute of Medical Physics, University of Erlangen, Erlangen, Germany
4School of Medicine and Pharmacology, University of Western Australia, Perth, WA, Australia
5Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Perth, WA, Australia

Aim: Hip Structure Analysis (HSA), a method of assessing proximal femur strength using two dimensional dual-energy x-ray absorptiometry (DXA) technology, has been criticized as not correctly assessing in vivo engineering strength. This study was designed to examine the correlations between DXA HSA measurements with those calculated from true three dimensional QCT.

Methods: Forty-one elderly women mean age 82.8 ± 2.5 years had standard PA hip DXA and 64 slice CT measured. DXA HSA values at the Narrow Neck (NN) and Intertrochanter (IT) were assessed by APEX 3.0 software (Hologic Inc., Boston, MA). The DXA and QCT datasets were then co-registered using four DXA images acquired at different angles and the equivalent PA QCT HSA values calculated using in house software.

Results: Femoral Neck Axis Length (FNAL) and width of the bone at the NN and IT regions, physical measurements were identical and highly correlated (r = 0.90 – 0.95). HSA and QCT strength variables were highly correlated at both the NN and IT regions for Cross Sectional Area (CSA) (0.95 and 0.93), Cross Sectional Moment of Inertia (0.94 and 0.93) and for Section Modulus (Z) (93 and 0.89). The small differences in slope and offset between the two techniques were due to differences in calibration materials for bone mineral content.

Conclusions: DXA hip size and strength measured using the Hologic emulation of the Beck algorithm correlates highly with variables measured at the same site by QCT.

ASSESSMENT OF ABSORBED DOSE IN POSTMENOPAUSAL WOMEN, USING DXA, AND ITS RELATIONSHIP WITH HEIGHT, WEIGHT AND BODY MASS INDEX
M. R. Salamat, M. B. TAVAKKOLI, L. Abedi

Medical Physics and Medical Engineering, Isfahan University of Medical Sciences, Isfahan, Iran

Objective: The purpose of this study was to assess the entrance surface dose (ESD) and effective dose (ED) in postmenopausal women, using dual energy x-ray absorptiometry (DXA) and the correlation of ESD and ED with height, weight and body mass index.

Materials and Methods: 43 female volunteers were recruited. ESD and ED were assessed, using thermo luminescent dosimeters (TLDs) and the abdominal CT scans of the patients. The correlations between the ESD and ED with height, weight and BMI were determined.

Results: Pearson’s correlation analysis showed that there was a significant correlation between ESD and BMI (r = 0.885, P< 0.001) and weight (r = 0.745, P< 0.001), but, there was an indirect correlation between ESD and height (r = -0.258, P= 0.047). Pearson’s correlation analysis also showed that there was an indirect correlation between the ED and BMI (r = -0.651, P< 0.001), height (r = -0.346, P= 0.001) and weight (r = -0.811, P= 0.001), which may be due to the scanner filtration. A mean ED and ESD of 0.50 µSv (0.26 – 0.95) and 9.54 µSv (7.25 – 12.65) were found for the anterior posterior spine scans, respectively.

Conclusion: Our results showed that the patients ionizing radiation exposure using a DXA technique, a Norland XR_46 system is very low (0.50 µSv), and much lower than the average daily background in the UK of 7 µSv.

EPIPHYSYAL TRABECULAR BONE MINERAL DENSITY COULD PROVIDE A MURINE OVARIECTOMY MODEL OF PRE-OSTEOARTHRITIS PATHOLOGY
P. Salmon, L. Oste

1SkyScan, Kontich, Belgium
2Galapagos, Mechelen, Belgium

Osteoarthritis as well as osteoporosis are skeletal consequences of postmenopausal estrogen deficiency [1], and the early etiology of osteoarthritis has been associated with decreased mineral density of the subchondral bone and adjacent epiphysial trabecular bone [2] leading to impaired mechanical performance of the same. In this study, the mineral density of epiphysial trabecular bone was assessed by micro-CT in a murine model of ovariectomy (OVX) and drug treatment. C3H mice 3 month old were either sham operated (n=6) or OVX, and three OVX groups (each n=6) were administered vehicle, alendronate and estradiol respectively, intravenously for 5 weeks. Then the mice were sacrificed and the right femurs harvested. Both metaphysial and epiphysial trabecular bone were analysed in anatomically referenced volumes of interest.

OVX decreased metaphysial trabecular BV/TV significantly, and this loss was partially and fully prevented by alendronate and estradiol treatment respectively. At the epiphysis, there were no morphometric changes in the trabecular bone associated with OVX or treatment. However tissue mineral density (TMD) of the trabecular bone decreased by 2 percent (p<0.0005) in the OVX vehicle
group compared to sham. Alendronate increased trabecular TMD by about 1% with marginal significance (p<0.06) relative to the OVX vehicle group, while estradiol treatment fully prevented the TMD decrease from OVX (p<0.02). Epiphyseal trabecular TMD changes mirrored those of metaphyseal trabecular architecture. This study indicates that the murine OVX model can be applied to assessment of mineral diminution in epiphyseal trabecular bone that may be a precursor to osteoarthritis.

(2) Day JS et al. A decreased subchondral trabecular bone tissue elastic modulus is associated with pre-arthritic cartilage

EXPERIMENTAL VALIDATION OF A MULTISCALE MODEL OF MINERALIZED COLLAGEN FIBERS AT TWO LEVELS OF HIERARCHY
E. M. Spiesz, P. K. Zysset
Institute of Lightweight Design and Structural Biomechanics, Vienna University of Technology, Vienna, Austria

Recently a model of mineralized collagen fibril-array present in bone was proposed (1). This mean field homogenization model predicts transverse-isotropic elastic properties of the fibril using mechanical properties of its constituents at two hierarchical levels. The aim of the study was to validate this model experimentally in micro- and macroscopic scale.

Methods
Mineralized turkey leg tendons (MTLT) were used in the study, as they consist of unidirectional arrangements of mineralized collagen fibrils-arrays. 49 samples were extracted from 10 tendons. Validation of the fibril array model at the micro-level involved microindentation. Further, elastic properties obtained with the model were used in micro finite element simulations to predict overall stiffness of MTLT samples. Results of these simulations were compared with macroscopic tension tests preformed on the samples modelled, which allowed for validation of the model at the macro-scale.

Results
Good agreement between the experimental and numerical results was reached at both micro- and macrolevel. Correlation coefficient of indentation moduli predicted with the fibril-array model and those obtained with microindentation was 0.619. Macroscale validation - comparison between the experimental and numerical tensile tests resulted in correlation coefficient of 0.957.

Conclusions
Fibril-array model may, next to microindentation, serve as a competitive method of obtaining elastic properties of mineralized tissues for the use in finite elements models. Further improvements of the model that account for the complex structure of the mineralized collagen fibril-arrays present in lamellar bone, have the potential to predict elastic properties and improve our understanding of the structure-function relationships in bone at multiple hierarchical levels in health and disease.

Acknowledgments
We acknowledge grant no P19009-N20 of the Austrian Science Foundation (FWF).

CORRELATION BETWEEN BMD MEASURED BY PERIPHERAL AND CENTRAL DXA IN HEALTHY INDIAN CHILDREN AND ADOLESCENTS AGED 10-18 YEARS
1Endocrinology and Metabolism, All India Institute of Medical Sciences, New Delhi, Delhi, India
2Department of Endocrinology and Thyroid Research Centre, Institute of Nuclear Medicine and Allied Sciences, New Delhi, Delhi, India
3Department of Biostatistics, All India Institute of Medical Sciences, New Delhi, Delhi, India
4Department of Medicine, Sur Homeopathic College, New Delhi, Delhi, India

Several predisposing factors of osteoporosis, a disease which mostly manifests in the elderly, arise in childhood, necessitating studies evaluating pattern of bone mineralisation in children. The objectives of the present study were to assess the correlation between measures of bone mineral density (BMD) assessed by central DXA (cDXA) and peripheral DXA (pDXA) in children and adolescents and to determine the optimal Z-score thresholds of pDXA for predicting two predefined Z-score cutoffs (≤1, and ≤2) of cDXA. A total of 844 (441 boys, 403 girls) children and adolescents between 10-18 yrs of age were recruited from Delhi schools. BMD of the antero-posterior lumbar spine (L1-L4), left proximal femur and forearm was measured by cDXA (Prodigy Oracle, GE Lunar). Peripheral BMD of left radius and left calcaneus was estimated in using pDXA (Osteosys EXA 3000, Osteosys Corporation). Pearson’s correlation was used to estimate the correlation between pDXA and cDXA BMD measures. Receiver operating characteristic curve analysis was used to determine optimal Z score thresholds of peripheral DXA for predicting Z-score cutoffs (≤1, and ≤2) of central DXA. Correlation was statistically significant at all sites (p value <0.01 at all sites). Correlation coefficients in boys ranged from 0.56 to 0.79 whereas in girls they varied from 0.17 to 0.32, demonstrating a gender difference. Area under curves of LCBMD and LFBMD Z scores were significantly smaller in girls as compared to boys at both Z-score cut offs. Area under curves in boys ranged from 0.67 to 0.79 and in girls from 0.57 to 0.7 between different sites. Significant positive correlation was found between BMD measurements of peripheral and central DXA in healthy Indian children in this study. Peripheral BMD in girls had significantly lower correlation and lesser predictability of central BMD as compared to males.
RACIAL DIFFERENCE IN BONE MICROSTRUCTURE AND DENSITY AT DISTAL RADIUS AND TIBIA BETWEEN YOUNG CHINESE AND CAUCASIAN MEN

X. Wang, Q. Wang, A. Ghasem-Zadeh, E. Seeman
Endocrine Centre, Austin Health, University of Melbourne, West Heidelberg, VIC, Australia

Hip and forearm fracture rates are lower in Asians than Caucasians. We reported Chinese women have thicker cortices and trabeculae within a smaller bone than their Caucasian counterparts (Wang et al 2009). To define racial differences in bone microstructure in men, we studied 50 young healthy Chinese and 62 Caucasian men aged 18 to 50 years (mean age 36.3 vs 38.3 years) using high-resolution peripheral quantitative computed tomography (pQCT, XTreme CT, Scanco Medical AG, Bassersdorf, Switzerland). Chinese men were shorter and weighed less than Caucasians. Radius total bone cross-sectional area (CSA) was less by 12.1% and (p<0.001) in Chinese than Caucasian men (5.7% after height and weight adjustment, p=0.075). Cortical and trabecular thickness and volumetric bone mineral density (vBMD) was similar by race. Trabecular number at the radius was less by 6.0% (p<0.05) in Chinese men but similar after height and weight adjustment. Similarly, trabecular number and bone volume/tissue volume (BV/TV) were also less by 7.3-7.8% in Chinese men at the distal tibia before but not after height and weight adjustment. The other bone microarchitecture at the tibia was similarly presented by race. Tibial CSA was 6.4% (p<0.05) smaller in Chinese men but not differed after height and weight adjustment.

In conclusion, unlike women, Chinese and Caucasian men have very similar bone microarchitecture and density. We infer that the lower fracture risk in Chinese men may be explained by factors other than their bone structure in the young adulthood.


QUANTITATIVE HEEL ULTRASOUND (QUS) AS A MEASURE OF BONE QUALITY AMONG MEN AND WOMEN WITH DEPRESSION: GEELONG OSTEOPOROSIS STUDY (GOS)

L. J. Williams1,2, J. A. Pasco1, F. N. Jacka1,2, G. C. Nicholson3, M. A. Kotowicz4, M. Berk1,2
1School of Medicine, Deakin University, Geelong, VIC, Australia
2Department of Psychiatry, The University of Melbourne, Parkville, VIC, Australia
3School of Medicine, The University of Queensland, Darling Heights, QLD, Australia
4Department of Endocrinology and Diabetes, Barwon Health, Geelong, VIC, Australia

Aim: Previous research has demonstrated depression to be a risk factor for low bone mass as a result of disease and/or medication-related processes. QUS is a portable and relatively cheap screening tool for determining bone quality and fracture risk. The value of QUS in screening psychiatric cohorts remains to be established. We aimed to investigate the association between QUS parameters and depression in a population-based sample of men (n=745) and women (n=897) participating in the GOS.

Methods: Lifetime depression was diagnosed using a semi-structured clinical interview (SCID-I/NP). Bone quality was determined by QUS (Achilles Express, GE Medical Systems) and included the following parameters: Broadband Ultrasound Attenuation (BUA), Speed of Sound (SOS) and Stiffness Index (SI). Anthropometric measurements and socio-economic status (SES) were determined. Information on medication use and lifestyle was obtained via questionnaire. Linear regression models were used to test the association, after adjusting for age, weight and SES.

Results: Mood disorders were identified in 126 (17%) men and 255 (28%) women. In men, depression were associated lower adjusted mean SOS [1562.0 (95%CI 1552.0-1572.0) vs 1570.3 (1562.5-1578.0), p=0.04], BUA [118.7 (114.8-122.3) vs 121.3 (118.3-124.3), p=0.09] and SI [96.3 (91.4-101.2) vs 100.5 (96.7-104.2), p=0.03] parameters. In women, age was an effect modifier. Among younger women (≤40yr), depression were associated with lower adjusted mean SOS [1581.9 (1570.9-1593.0) vs 1594.2 (1583.8-1604.5), p=0.007] and SI [99.4 (94.0-104.9) vs 104.0 (98.9-109.0), p=0.04] but not BUA [115.1 (110.7-119.5 vs 116.9 (112.8-121.0), p=0.32]. No differences were detected in older women (>40yr). These patterns persisted after further adjustment for physical activity, alcohol and calcium intake and medication use.

Conclusions: Our data suggest that bone quality, as measured by QUS, is reduced among men and younger women with a lifetime history of depression. Thus, QUS may be a useful screening tool for determining fracture risk within these populations.
THE HISTOMORPHOMETRIC AND COMPOSITIONAL CHARACTERISTICS OF BONE IN AN OSTEOPOROTIC SHEEP MODEL

M. R. Zarrinkalam1,2, A. Mulaibrahimovic1,2, R. J. Moore1,2,3
1The Adelaide Centre for Spinal Research, SA Pathology, Adelaide, SA, Australia
2Hanson Institute, SA Pathology, Adelaide, SA, Australia
3Discipline of Pathology, University of Adelaide, Adelaide, SA, Australia

Aim: The mechanical properties of bone are dependent on its architecture, the degree of mineralisation and its chemical composition. The aim of this study was to investigate the effect of induced osteoporosis on the morphology and composition of vertebral and iliac crest bone.

Methods: Osteoporosis was induced in 10 mature ewes (1). Five age matched sheep were used as controls. The lumbar spines (LS) and iliac crest (IC) were collected and processed undecalcified for standard bone histomorphometric analysis. The surface of the specimens was polished with fine grade abrasive paper and then carbon coated for electron probe Microanalysis (EPMA) at up to 30 randomly selected sites using a Cameca SX51 Microprobe (beam current 20 nA, accelerating voltage 15 kV). Calcium, phosphorus and oxygen content were estimated as a percentage of total bone mass.

Results: Trabecular bone volume and trabecular thickness in both anatomical regions reduced over 35% in osteoporotic sheep (p<0.01). Trabecular spacing was increased by 25% (p<0.05) in the LS but not in the IC. Trabecular bone of osteoporotic sheep in LS and not IC had less phosphorus (P) than control sheep (p<0.01) but the calcium level was identical in each group. Consequently trabecular bone of the osteoporotic sheep in LS had higher Ca/P ratio than the control sheep (p<0.01).

Conclusion: Osteoporosis in this experimental model influenced not only the bone architecture but its elemental composition. Further investigation is required to determine significance of these effects on the mechanical properties of the bone.

References


ENHANCEMENT OF INFLAMMATORY OSTEOCLASTOGENESIS BY LYSINE-SPECIFIC GINGIPAIN

T. Akivama1,2, Y. Miyamoto1, A. Yamada2, M. Takami1, K. Yoshimura2, K. Baba1, R. Kamijo2
1Department of Prosthodontics, Showa University School of Dentistry, Tokyo, Japan
2Department of Biochemistry, Showa University School of Dentistry, Tokyo, Japan

Periodontitis is a chronic inflammatory disease accompanied by alveolar bone resorption by osteoclasts. Porphyromonas gingivalis, a potent etiological agent for periodontitis, secretes cysteine proteases called gingipains, which are classified by their cleavage site specificities, i.e., arginine-specific gingipains and lysine-specific gingipain (Kgp). We found that Kgp degrades osteoprotegerin (OPG) and suppresses osteoclast differentiation induced by active vitamin D and various Toll-like receptor (TLR) ligands in vitro (1). In this study, we investigated the effects of Kgp on osteoclast differentiation induced by proinflammatory cytokines, such as tumor necrosis factor-α (TNFα), interleukin (IL)-1β, and IL-17, in vitro. In a co-culture system of mouse calvarial osteoblasts and bone marrow cells, differentiation of osteoclasts was induced by TNFα, IL-1β, or IL-17 in the presence or absence of Kgp. Kgp augmented the osteoclast differentiation induced by TNFα and IL-1β. On the other hand, IL-17-induced osteoclast differentiation was suppressed by Kgp. Proteolytic degradation of these cytokines by Kgp was compared with that of OPG. TNFα and IL-1β were less susceptible, but IL-17 was more susceptible to the degradation by Kgp than OPG. These results indicate that the enhancing and suppressing effects of Kgp on inflammatory cytokine-induced osteoclast differentiation are dependent on the difference in degradation efficiencies of each cytokine and OPG. The present study reinforced the idea that degradation of OPG by Kgp is a crucial event in the development of bone loss in periodontitis. Then we investigated the cleavage sites of OPG by Kgp. Analysis of N-terminal amino acid sequence of OPG fragments revealed that Kgp degraded OPG in a region containing Lys-258 and Lys-262, resulting in prevention of dimer formation of OPG required for RANKL inhibition. These results suggest a potential utility of recombinant OPGs resistant to hydrolysis by Kgp for prevention of bone loss in periodontitis.


RESURFACING ARTHROPLASTY IN OSTEOPOROSIS HIP OSTEOARTHRITIS

M. Cirstoiu, C. Cirstoiu, D. Popescu, A. Popescu, R. Ene
University Hospital Bucharest, Bucharest, Romania

Aim: Patients with incipient hip arthrosis may benefit from a relatively new therapeutic approach using resurfacing total hip replacement but in those with associated osteoporosis this type of surgical intervention is contraindicated given the poor quality of osteoporotic bones. We assessed the efficacy of the antosteoporotic pharmacological therapy to improve bone quality and bone strength in postmenopausal women diagnosed with hip arthrosis and osteoporosis thus facilitating the hip surgical intervention. Methods: We evaluated 20 postmenopausal women aged between 53-60 years diagnosed with osteoporosis according to the WHO...
criteria using dual-energy X-ray absorptiometry (DXA) for bone mineral density measurements. All these patients had low hip T score (osteopenia/osteoporosis) and also incipient hip arthrosis. The surgical approach was delayed for 12 months and all the patients received bisphosphonate therapy with calcium and vitamin D supplements. In all the patients DXA scans were performed after 12 months of therapy. Results: A surgical intervention with resurfacing total hip replacement was performed in 12 of the 16 patients presenting with increasing BMD, 4 of them showing elements of rapidly advancing hip arthrosis to a stage that made this type of intervention impossible. We chose not to use this technique in the group with stable BMD (4 patients). All 12 women surgically treated had a favorable post-operative outcome without experiencing a femoral neck fracture during the surgical intervention or during the twelve-month follow-up. All 20 patients continued to receive bisphosphonate therapy. Conclusion: In postmenopausal women with osteoporosis and associated hip arthrosis, improving bone mass and bone quality with bisphosphonate therapy is necessary and important in order to allow hip arthroplasty using the technique of resurfacing avoiding the risk of intra-operative fractures


(2) Cătălin Cristoiu, Radu Rădulescu, Dan Popescu, Razvan Ene, Monica Cristoiu, Valeriu Horhoianu, FRACTURILE PE OS OSTEOPOROTIC LA FEMEIA ÎN POST-MENОPAUZĂ - PROVOCARE TERAPEUTICĂ? - REVISTA PHARMARON

ENDOCHONDRAL OSSIFICATION IN MICE OVER-EXPRESSING TISSUE NON-SPECIFIC ALKALINE PHOSPHATASE DRIVEN BY TYPE II COLLAGEN PROMOTER

T. Hasegawa1, K. Oda1, M. Sasaki1, C. Tabata1, Z. Liu1, Y. Guo1, K. Inoue3, M. Li1, T. Komori4, T. Yamamoto1, N. Amizuka1

1Department of Developmental Biology of Hard Tissue, Graduate School of Dental Medicine, Hokkaido University, Sapporo, Japan
2Department of Oral Functional Anatomy Division of Oral Functional Science, Graduate School of Dental Medicine, Hokkaido University, Sapporo, Japan
3Department of Oral Biochemistry, Niigata University Graduate School of Medical and Dental Science, Niigata, Japan
4Department of Cell Biology, Unit of Basic Medical Sciences, Graduate School of Biomedical Sciences, @Nagasaki University, Nagasaki, Japan

During endochondral ossification, one of the most important enzymes must be ALPase. We generated transgenic mice over-expressing tissue non-specific alkaline phosphatase (TNApase) driven by type II collagen promoter, in order to examine if over-expression of TNApase could give rise to premature calcification in cartilage.

Eighteen days-old transgenic fetuses and their wild-type littermates were fixed with paraformaldehyde solution. Paraffin sections of transgenic and wild-type fetuses were examined for the localization of matrix Gla protein (MGP), osteocalcin and type II collagen, as well as calcification by von Kossa staining. Ultrathin sections of epoxy resin-embedded specimens were examined under transmission electron microscopy.

Transgenic mice revealed an intense TNApase immunopositivity in resting and proliferative chondrocytes, while the wild-type counterparts did not express this. TNApase over-expressing mice showed the similar morphology and the distribution of type II collagen in the epiphysis compared to those of wild-type fetuses. Likely, immunohistochemistry demonstrated the similar distribution of osteocalcin and MGP between the wild-type and transgenic fetuses. Taken together, the over-expression of TNApase did not appear to influence the morphogenesis of epiphyseal cartilage and the distribution of matrix proteins such as osteocalcin and MGP that affect calcification in bone and cartilage. However, the transgenic epiphyseal cartilage revealed less number of calcified nodules, and they were composed of very fine needle-like crystals compared with those of the wild-type. In general, TNApase is believed to hydrolyze various phosphate esters including pyrophosphates, an inhibitor of calcification, which however would be immediately converted into monophosphate by TNApase. Therefore, it is possible that the over-expression of TNApase might cause unbalanced production of pyrophosphates and monophosphates, consequently, influencing calcification in cartilage.

In conclusion, an excess amount of TNApase appears to inhibit calcification in cartilage.

ALENDRONATE TREATMENT REDUCES TIBIAL SUBCHONDRAL BONE DAMAGE IN EARLY-STEM OSTEARTHRITIS: AN IN VIVO MICRO-CT STUDY IN A RODENT MODEL

G. Mohan1,2, E. Perilli1,2, J. Kuliwaba1,2, I. Parkinson1,2, N. Fazzalari1,2

1Bone and Joint Research Laboratory, SA Pathology, Adelaide, SA, Australia
2Discipline of Anatomy and Pathology, University of Adelaide, Adelaide, SA, Australia

In this study, the efficacy of Alendronate (ALN) treatment on tibial subchondral bone was evaluated using in vivo micro-CT in a rat model of low-dose monosodium iodoacetate-induced (MIA) osteoarthritis (OA), at an early stage of the disease. Rats (n=24) received a single intra-articular injection of 0.2mg MIA in the right knee to induce OA, and sterile saline in the left knee. Twelve out of the 24 rats (OA+ALN group) received twice-weekly subcutaneous injections of 15μg/kg ALN for 2 weeks. Control rats (n=4) received saline injection in the right knee and received no treatment. All the rats were scanned in vivo by micro-CT at 2 weeks post MIA-injection (early-stage OA), to assess architectural changes in the tibial subchondral bone. The serum bone turnover marker, C-
terminal telopeptide of type I collagen (CTX-I) was assessed at 2 weeks. Changes in hind paw weight distribution was determined up to day 14 as an index of OA joint discomfort. Micro-CT analysis showed that subchondral bone volume fraction, trabecular number, and trabecular separation in the OA+ALN group did not differ significantly compared to the control group. Conversely, in the untreated OA group there was significantly decreased subchondral bone volume fraction (p<0.05), trabecular number (p<0.001) and increased trabecular separation (p<0.0001) compared to the OA+ALN and control groups. In addition, the serum CTX-I level of the untreated OA group was significantly elevated (p<0.05) compared to the OA+ALN and control groups. ALN also reversed a shift in weight-bearing of MIA-injected knee observed in the OA group (p<0.0001 for day 3). These findings demonstrate that ALN treatment prevents tibial subchondral bone changes observed in early-stage OA. Therefore, ALN could be used as an OA disease-modifying drug to enhance subchondral bone quality. Further investigations are planned, which will determine if ALN halts disease progression in this rat model of OA.

A NOVEL MOUSE MODEL OF TIBIAL PSEUDARTHROSIS FEATURING LOCALIZED DOUBLE INACTIVATION OF THE NFI GENE

A. Schindeler1,2, J. El-Hossi1,2, K. Sullivan1,2, T. Chen1,2, K. Mikulec1, L. Peacock1, P. A. Baldock3, I. A. Alexander4, D. G. Little1,2

1 Orthopaedic Research & Biotechnology, The Children’s Hospital at Westmead, Westmead, NSW, Australia
2 Faculty of Medicine, University of Sydney, Sydney, NSW, Australia
3 Bone Unit, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia
4 Children’s Medical Research Institute, Gene Therapy Unit, Westmead, NSW, Australia

Aim: Congenital tibial dysplasia (CTD) is a severe orthopaedic condition. The majority of clinical cases are observed in children with Neurofibromatosis type 1 (NF1) and it has been previously reported that osseous lesions in CTD exhibit local double inactivation of NFI. We have developed in vitro and surgical models that recapitulate the double inactivation of NFI.

Methods: Nf1flour/flox, Nf1flour/flour, and wild type mice had closed and open fractures performed; an AdenoCre (Cre-expressing adenovirus) was injected into the fracture site at d0 to induce local recombination (Nf1mut). Fracture healing was analysed by X-ray, microCT, and histology. Neonatal calvarial cell culture experiments were also performed with and without AdenoCre.

Results: In closed fractures, bridging was 100% in control fractures and <40% in Nf1mut fractures (P<0.05). In open fractures, bridging was 75% in control fractures and <30% in Nf1mut fractures (P<0.05). In both open and closed fracture repair models the Nf1mut state was associated with a significant increase of up to 15-fold more fibrotic tissue invading the callus by week 3. TRAP+ cells were observed histologically in the Nf1mut fibrotic tissue. Closed Nf1mut fractures also showed a significant increase in proliferating BRDU labelled cells in the callus. No statistically significant differences were seen between Nf1flour/flour and Nf1flour/flour mice suggesting that a heterozygous background is not critical for impaired healing in this model. In vitro experiments demonstrated that cultured Nf1mut calvarial pre-osteoblasts were impaired in their capacity to differentiate into osteoblasts.

Conclusions: Local Nf1 double inactivation in a fracture callus was sufficient to induce poor rates of bridging and can lead to fibrous non-union. The fibrous tissue has characteristics of a NF1 pseudarthrosis such as proliferative fibrous tissue surrounding TRAP+ cells. This model provides valuable insight into the pathobiology of the disease and will be helpful for trials in new therapeutics.

EVALUATION OF A NEW AUTOMATED CHEMILUMINESCENCE IMMUNOASSAY FOR FGF23

Y. Shimizu, S. Fukumoto, T. Fujita

Division of Nephrology & Endocrinology, Department of Medicine, University of Tokyo Hospital, Tokyo, Japan

FGF23 is a hormone regulating phosphate and vitamin D metabolism. Excess and deficient actions of FGF23 result in several kinds of hypophosphatemic rickets/osteomalacia and familial hyperphosphatemic tumoral calcinosis, respectively. We have previously established an enzyme-linked immunosorbent assay (ELISA) for full-length active FGF23. Using this assay, we have shown that hypophosphatemia with FGF23 levels above 30 pg/ml indicates the disease caused by excess actions of FGF23. However, this ELISA has a rather narrow assay range, a lower detection limit and shorter time to get the first result. The assay was linear up to about 15,000 pg/ml and had the detection limit of 1 pg/ml. In addition, this assay showed coefficients of variation of less than 5% using samples with average FGF23 levels of 10 - 2500 pg/ml. When FGF23 levels in more than 200 samples from patients with chronic hypophosphatemia by various cases were measured by this assay and the previous ELISA, there was a good correlation with R² of more than 0.9. Moreover, when these samples were divided into two groups with FGF23 levels above or below 30 pg/ml, there was no discrepancy in the discrimination of samples using these two assays. These results indicate that this new assay has a wider assay range, a lower detection limit and shorter time to get the first result. Therefore, this assay seems to be ideal for both clinical use and clinical studies especially measuring many samples with high FGF23 levels.
TRANSGENIC DISRUPTION OF GLUCOCORTICOID SIGNALING IN OSTEOBLASTS ATTENUATES INFLAMMATION AND BONE LOSS IN COLLAGEN ANTIBODY-INDUCED ARTHRITIS

J. Tu1, Y. Zhang1, J. Kelly1, C. R. Dunstan2, M. J. Seibel3, H. Zhou1
1Bone Research Program, ANZAC Research Institute, Concord, NSW, Australia
2Department of Biomedical Engineering, University of Sydney, Sydney, NSW, Australia
3Dept of Endocrinology & Metabolism, Concord Hospital, Concord, NSW, Australia

Background: Transgenic (tg) overexpression of the glucocorticoid (GC) inactivating enzyme, 11beta-hydroxysteroid dehydrogenase type 2 (HSD2), under the control of a 2.3Kb collagen type I promoter (Col2.3-HSD2), abrogates intracellular GC signalling exclusively in mature osteoblasts. Using the T cell-independent K/BxN serum transfer model of autoimmune arthritis, we previously reported that osteoblast-targeted disruption of endogenous GC signalling attenuated arthritis in Col2.3-HSD2-tg K/BxN mice (1). In the present study, we aimed to further elucidate the role of the endogenous GCs in a T cell-dependent model of inflammatory joint disease, namely collagen antibody-induced arthritis (CAIA).

Methods: Arthritis was induced in 6-week-old male Col2.3-HSD2-tg mice (tg-CAIA, n=8) and their wild-type (WT) littermates (WT-CAIA, n=10). Four tg and 8 WT mice receiving carrier (PBS) only served as controls (CTR). Body weight and the degree of arthritis (clinical score, paw swelling) were assessed daily from induction to endpoint (day 14). Micro-CT of the tibia was employed to assess for morphological skeletal changes.

Results: Both tg-CAIA and WT-CAIA developed acute arthritis. However, the inflammatory response was significantly blunted in tg-CAIA mice from day 9 onward (p<0.05). At endpoint, tibial trabecular separation was significantly increased in WT-CAIA compared to WT-CTR (p<0.05), and BV/TV and trabecular bone number were reduced (p=0.32 and p=0.051, respectively), consistent with increased bone resorption. In contrast, trabecular thickness was increased in WT-CAIA (p<0.05) but not tg-CAIA, consistent with the presence of continuing bone formation.

In summary, our results demonstrate that disruption of GC signalling in mature osteoblasts attenuates arthritis not only in T cell-independent but also in T cell-dependent models of arthritis. We therefore conclude that osteoblasts modulate local inflammatory responses via a glucocorticoid-dependent pathway in arthritis models with distinct pathogenetic mechanisms.


MODEST REVERSAL OF METABOLIC SYNDROME MANIFESTATIONS WITH VITAMIN D CORRECTION: A 12-MONTH PROSPECTIVE STUDY

N. Al-Dagher1,2, K. Alkhalty1,2, Y. AlSaleh2,4, O. Al-Attas1,2, A. Alothman2,5, O. Moharram1,2, S. Sabico1,2, S. Kumar4, G. Chrousos5, G. Chrousos2
1Biomarkers Research Program, Biochemistry, King Saud University, Riyadh, Saudi Arabia, Namibia
2Prince Mataib Bin Abdulaziz Chair for Osteoporosis, King Saud University, Riyadh, Saudi Arabia, Namibia
3Pharmacy, King Saud University, Riyadh, Saudi Arabia, Namibia
4Endocrinology, King Abdulaziz Medical City, Riyadh, Saudi Arabia, Namibia
5Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia, Namibia
6Diabetes and Metabolism Unit, Warwick University, Warwick, NA, Namibia
7King Abdulaziz University Hospital, Riyadh, Saudi Arabia, United Kingdom
8Pediatrics, Athens University, Athens, Greece

Aims: Numerous cross-sectional studies have noted significant negative associations between circulating levels of vitamin D and cardio-metabolic risk factors, highlighting potential extra-skeletal functions of this sterol hormone. Prospective studies, however, have been limited and hence no cause and effect relations can be inferred. This study aims to determine whether vitamin D correction can reverse already established manifestations of the metabolic syndrome (MetS). Methods: A total of 59 adult non-diabetic, overweight and obese Saudis (31 males, 28 females) were randomly recruited in this prospective 1-year intervention study. Anthropometry and biochemical evaluation were performed, including determination of serum vitamin D, calcium and phosphorous concentrations, as well as fasting blood glucose and lipid profile. Results: At the initial baseline visit, the prevalence of both low HDL-C and hypertension was significantly increased among patients with vitamin D deficiency (p<0.05), even after adjusting for gender and BMI. Subjects were advised to regularly expose themselves to sunlight and increase intake of vitamin D-rich foods. All measurements were repeated 6 and 12 months later. Overall prevalence of MetS patients by the modified NHANES ATP III definition decreased from 25.2% to 13.0% and this was largely due to a parallel decrease in the prevalence of low HDL-cholesterol, triglycerides and hypertension. Conclusion: Optimization of vitamin D levels through sun exposure and increased intake of a vitamin D-rich diet can lead to an improved cardio-metabolic profile, offering a promising non-pharmacologic approach in the prevention of MetS manifestations.
INTRODUCTION
Polycystic Ovary Syndrome (PCOS) is one of the most common endocrine-metabolic diseases, affecting reproductive-age women. The pathogenesis of the syndrome is multifactorial and polygenic, having PCOS a number of different clinical, reproductive, metabolic and cardiovascular implications. Recent studies have shown that women with PCOS do have hypovitaminosis D and that 25(OH)D levels are negatively correlated with BMI. The mechanisms underlying the association of low 25(OH)D levels and PCOS are not fully understood.

AIM OF THE STUDY
Purpose of the study was to investigate serum vitamin D in women with PCOS compared with healthy control women and the association of 25(OH)D levels with metabolic parameters of PCOS.

PATIENTS AND METHODS
Fifty-two patients with PCOS and 20 age- and BMI-matched controls, matched were studied. Rotterdam Criteria were used for the diagnosis of PCOS; two of the 3 prerequisites were required excluding other etiologies: a. clinical and/or biochemical features of hyperandrogenism; b. evidence of oligo/anovulation; c. polycystic ovaries. None of the patients had diabetes or were taking hormones or other medications interfering with the interpretation of our results. Vitamin D was measured in all patients from November to March and these values were correlated with BMI, HOMA and fasting and stimulated insulin and glucose.

RESULTS
25(OH)D levels were significantly lower in PCOS (60.6±28 nmol/L) than in controls (80.5±41.8 nmol/L). 25(OH)D levels negatively correlated with BMI (p<0.03). HOMA was positively correlated with insulin after oral glucose load in PCOS subjects. Vitamin D was negatively correlated with HOMA (p<0.05) in PCOS with BMI < 25.

CONCLUSIONS
These results suggest that PCOS patients have low levels of Vitamin D independently of obesity. The negative correlation with HOMA suggests that 25(OH)D might be involved in the insulin-resistance in PCOS.
IDENTIFICATION OF TARGET GENES IN MINERALISING OSTEOBLASTS THAT ARE DIRECTLY REGULATED VIA THE ANDROGEN RECEPTOR

Medicine (All/NH), University of Melbourne, Heidelberg, VIC, Australia

Aim: To identify target genes that are regulated by the androgen receptor (AR) in osteoblasts using a mouse model in which the AR is deleted in mineralising osteoblasts (mOBL-ARKOs) (1).

Methods: Microarray was performed on RNA from bones of mOBL-ARKOs and WT littermates at 6 and 12 weeks of age (n=3/group). Genes were considered to be regulated if P<0.05 and expression differed from WT by >1.5 fold.

Results: 20 and 353 genes were regulated in mOBL-ARKOs at 6 and 12 weeks of age respectively, with 13 genes being regulated at both 6 and 12 weeks of age. The regulation of candidate genes in the bones of mOBL-ARKOs compared to WTs were confirmed by quantitative real time PCR (n=20/group). Type 1a1 collagen and osteocalcin gene expression were upregulated in mOBL-ARKOs (type 1a1 collagen - 2 fold increase at 6wks; osteocalcin – 2.2 fold increase at 12wks), consistent with the increased matrix development and mineralisation we observed in these mice (1). Further evidence for the direct regulation of these genes by androgens was the increase in Col1a1 and osteocalcin mRNA levels observed in male WT mice following orchidectomy, which were normalised to control levels upon treatment with the non-aromatisable androgen, DHT (P<0.05, n=10/group). Other genes identified to be regulated in the bones of mOBL-ARKOs include those involved in; growth and skeletal development (growth hormone - ↑3.5 fold at 12wks, Wnt4 - ↑2.2 fold at 12wks, TGFB2 – ↑1.9 fold at 12wks, LTBP2 – ↑3.5 fold at 6wks and 2.6 fold at 12wks); and the regulatory genes (Dpp4 – ↑2.7 fold at 6wks, adiponectin - ↑2.5 fold at 12wks).

Conclusion: Androgens act directly via the AR in mineralising osteoblasts to regulate a number of target genes, in particular those associated with the development and regulation of osteoblasts and skeletal growth.


STATE OF THE ART OF VITAMIN D ASSAYS: LATEST GENERATION ASSAYS

C. Farrell1,2, S. Martin3, I. Straub4, P. Williams5,6, B. McWhinney7, M. Herrmann1,7
1Chemical Pathology, Lavery Pathology, North Ryde, NSW, Australia
2Biochemistry, PaLMS Pathology, St Leonards, NSW, Australia
3Immunology, Lavery Pathology, North Ryde, NSW, Australia
4Faculty of Medicine, University of Sydney, Sydney, NSW, Australia
5Endocrinology, Royal Prince Alfred Hospital, Camperdown, NSW, Australia
6Chemical Pathology, Royal Brisbane Hospital, Herston, QLD, Australia
7Chemical Pathology, Royal Prince Alfred Hospital, Camperdown, NSW, Australia

Background: A number of pre-existing automated vitamin D assays have recently been modified and several new assays have been launched. This study aimed to compare latest generation automated vitamin D immunoassays with liquid chromatography tandem mass spectrometry (LCMS) measurement.

Methods: 170 randomly selected serum samples were divided into 6 aliquots, stored at -20°C and analysed in batches, with freshly thawed aliquots used for all analyses. Vitamin D was measured by LCMS (2 different methods at independent laboratories), a radioimmunoassay (RIA) from Diasorin as well as 5 automated chemiluminescent immunoassays from Abbott, Diasorin, IDS, Roche and Siemens. The Roche assay used was the recent monoclonal vitamin D assay which specifically detected 25-hydroxy vitamin D3. All other immunoassays detected both 25-hydroxy vitamin D3 and D2 and reported a total vitamin D result. The Diasorin immunoassay was a pre-market assay. To assess intra- and inter-assay variability we measured 5 replicates of a high and of a low serum pool over 5 consecutive days.

Results: The two LCMS methods correlated well with r = 0.984 and an average bias of 2.0%. In the cohort tested, all immunoassays correlated well with LCMS with the exception of Roche (table 1). At vitamin D levels between 25-120nmol/L Diasorin, Siemens and RIA showed excellent accuracy, while Abbott, IDS and Roche exhibited variable bias (table 1). Vitamin D levels < 20 nmol/L were only measured accurately by Diasorin, IDS and RIA, while Siemens, Roche and Abbott showed substantial bias at these concentrations. Intra- and inter-assay precision differed between assays but was within the acceptable range for all tests.

Conclusion: Latest generation automated vitamin D immunoassays demonstrated significantly improved performance and compared well with LCMS. While most automated assays produced acceptable results across the clinically relevant range Siemens and Diasorin demonstrated superior accuracy. The assays tested showed variable performance at low vitamin D concentrations and laboratories should adapt their reportable range accordingly.

<table>
<thead>
<tr>
<th>Versus Avg. of 2 LC Tandem MS Methods</th>
<th>Correlation coefficient</th>
<th>Avg. bias if vit D &gt;20nmol/L</th>
<th>Avg. bias if vit D &lt;20 nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott</td>
<td>0.932</td>
<td>25.21%</td>
<td>102.62%</td>
</tr>
<tr>
<td>Liaison</td>
<td>0.954</td>
<td>-0.48%</td>
<td>32.21%</td>
</tr>
<tr>
<td>Siemens</td>
<td>0.941</td>
<td>3.97%</td>
<td>119.30%</td>
</tr>
<tr>
<td>Roche</td>
<td>0.678</td>
<td>-17.62%</td>
<td>32.57%</td>
</tr>
<tr>
<td>IDS</td>
<td>0.954</td>
<td>13.44%</td>
<td>14.82%</td>
</tr>
<tr>
<td>RIA</td>
<td>0.984</td>
<td>-5.38%</td>
<td>10.26%</td>
</tr>
</tbody>
</table>
VITAMIN D INSUFFICIENCY IN HEALTHY AUSTRALIAN OFFICE WORKERS

F. Fayel1, J. Wright1, L. Ridges3, P. Small3, M. J. Seibel2, A. D. Conigrave5, R. S. Mason4
1Nutrition and Metabolism, The University of Sydney, Camperdown, NSW, Australia
2ANZAC Institute, The University of Sydney, Camperdown, NSW, Australia
3Nestle Ltd, Rhodes, NSW, Australia
4Bosch Institute, The University of Sydney, Camperdown, NSW, Australia
5School of Medical Science, The University of Sydney, Camperdown, NSW, Australia

Low vitamin D status has been linked to increased risk of osteoporosis and various other disorders. Sun exposure is a key determinant of vitamin D production. Due to the indoor nature of office work, there may be an increased risk of vitamin D insufficiency in this group. There are limited data on the vitamin D status of office workers in Australia and New Zealand. We assessed the vitamin D status of healthy office workers at the end of summer and winter in 2010. Anthropometric (n=104; age 36.2 ± 8.6y; BMI 24.6 ± 4.2 kgm-2; 61% female), physical activity, dietary intake, sun exposure and skin phototype data were collected by examination and questionnaires. Serum 25-hydroxy vitamin D was measured by radioimmunoassay (Diasorin) in late summer (March, 2010) and late winter (August/September 2010). Mean 25-hydroxy vitamin D concentration in late summer was 68 ± 3nmol/L (range: 24-160 nmol/L), and in late winter was 60 ± 4 nmol/L (range: 15-174 nmol/L). Insufficiency (25-OH D < 50 nmol/L) was noted in 83% and 88% patients in summer and winter, respectively.

HIGH PREVALENCE OF VITAMIN D DEFICIENCY IN ASIAN-INDIAN PATIENTS WITH FRAGILITY HIP FRACTURE

M. Gupta, R. Jindal, M. A. Siddiqui, S. K. Wangnoo
Endocrinology, Indraprastha Apollo Hospital, New Delhi, India

AIM

METHODS
The study subjects included 48 (M: 17; F: 31) patients with non-traumatic hip fracture admitted in our hospital. The exclusion criteria include traumatic fracture, history of previous non-traumatic fracture or intake of systemic steroids, anti-osteoporotic medication, anti-tubercular or antiepileptic drugs. Routine biochemistry, [25(OH)D] and BMD (DXA) were measured in all patients. Diagnosis of vitamin D deficiency (VDD) was considered when serum 25(OH)D levels were < 20 ng/ml. Age and sex matched apparently healthy subjects (without history of fracture at any site) were selected from general population. All controls underwent BMD measurement at hip and measurement of 25(OH)D.

RESULT
The mean age of patients was 58+/−9.6 years. History of adequate sun exposure was obtained in 31% cases only. The mean serum 25(OH)D level was 11.6+/−3.9 in cases and 23+/−5.4 ng/ml in controls. All patients except one had VDD. No significant difference in serum 25(OH)D levels was observed between patients with and without adequate sun exposure. BMD of patients with fragility fractures were significantly low in comparison to BMD of healthy controls. (cases −0.7600+/−0.1 gm/sq cm vs controls 0.894+/−0.1 gm/sq cm). The mean t-score of hip BMD of cases was −0.99+/−1.2. No significant difference was observed in the BMD of patients with or without adequate sun exposure and with or without calcium and vitamin D supplementation at the time of fracture. Similarly, no significant difference was noted in BMD of patients with severe VDD and patients with mild to moderate VDD.

CONCLUSION
A high prevalence (98.7%) of vitamin D deficiency in Asian-Indian patients with fragility hip fracture was seen in our study. The BMD of these patients is significantly low in comparison to age and sex matched healthy controls.

ASSOCIATION STUDY OF PLASMA SEROTONIN LEVELS WITH BONE TURNOVER STATUS BEFORE AND AFTER HORMONE THERAPY IN POSTMENOPAUSAL WOMEN

K. Han1, H. Kim2, M. Park1, J. Kim1, S. Park1, C. Yim1, S. Kim1, H. Yoon1
1Endocrinology and Metabolism, Cheil General Hospital, School of Medicine, Kwandong University, Seoul, Sth Korea
2Endocrinology and Metabolism, Wonkwang University, School of Medicine, Seoul, Sth Korea

Selective serotonin reuptake inhibitors (SSRIs), widely used antidepressants, have been shown to be associated with reduced bone mass and increased risk of fractures. Circulating serotonin has been suggested as an important regulatory factor to inhibit bone formation. Estrogen may regulate the bone metabolism through the serotonin pathway. In this study, we analyzed the association between plasma serotonin levels and bone metabolism before and after hormone therapy (HT) in healthy postmenopausal women.

Methods: We measured plasma serotonin levels in seventy-three postmenopausal women (14 placebo, 59 on HT), aged 46-64 years, at baseline and after 3 months and 1 year of HT. Serum concentrations of osteocalcin (OCN) and carboxyterminal telopeptides (CTx) were determined by electrochemiluminescence immunoassays. Bone mineral density (BMD) at the lumbar spine and femoral neck was measured by dual-energy X-ray absorptiometry (DXA).

Results: Baseline plasma serotonin tended to inversely correlated to serum concentrations of OCN (r=-0.225, p=0.055), TALP (r=-0.228, p=0.054) and CTx (r=-0.0221, p=0.060). Baseline TALP levels were significantly associated with age (r=0.277, p=0.019) and
showed a significant correlation with baseline plasma serotonin levels after adjusting for age (r=−0.235, p=0.048). Plasma concentrations of serotonin did not correlate with BMD measured at either the lumbar spine or femoral neck. After 3 months of HT, the median decrements of plasma serotonin level from baseline were -25.0% (−48.7-73.3%, IQR) and -20.2 % (-52.8-15.2%, IQR) at 3 months and 1 year of HT, respectively. In placebo group, the median decrements of plasma serotonin were -9.3% (-34.0-53.6%, IQR) and -7.1% (-25.5-64.5%, IQR) after 3 months and 1 year of follow-up, respectively. Plasma serotonin levels after HT were not significantly different from those at baseline.

Conclusions: In postmenopausal women, baseline plasma serotonin levels were weak and inversely associated with TALP. These finding may support the possible role of plasma serotonin in bone metabolism. However, estrogen replacement therapy did not change plasma serotonin levels. Further large scaled studies are needed do to confirm our results.

PHOSPHATE 'SENSING' BY HUMAN BONE

Discipline of Orthopaedics and Trauma, The University of Adelaide, Adelaide, SA, Australia

Aim: Osteocytes and osteoblasts produce the humoral factor, FGF23, which maintains serum levels of phosphate and 1, 25 dihydroxy vitamin D within the physiological range. This implies the ability to sense the levels of phosphate in blood and interstitial fluid, however there is no phosphate sensing mechanism has been identified in humans. In this study we investigated the ability of bone tissue to sense phosphate.

Methods: We obtained human trabecular bone samples from patients undergoing total hip replacement. Samples were incubated with different concentrations of phosphate with or without dihydroxy vitamin D in the media (phosphate: 1.0μM to 10μM, 1, 25 dihydroxy vitamin D: 0 or 10μM). After 24 to 72 hours, RNA was extracted for measurement by qPCR of mRNA corresponding to phosphate homeostasis or bone formation related genes, such as FGF23, PHEX, DMP1, GALNT3 and SOST.

Results: Gene expression in human bone samples was differentially regulated by phosphate in a time and dose dependent. The clearest effects were seen after 72 h treatment and the co-presence of 1, 25 dihydroxy vitamin D (10nM) increased the responses of FGF23, PHEX, DMP1 and SOST mRNA levels to phosphate. After 72 hours treatment with phosphate at 2.5 or 5.0μM, expression of each of FGF23, PHEX, DMP1 and SOST mRNA was increased. GALNT3 was up-regulated by phosphate at 24 hour.

Conclusions: These results showed the possibility that extracellular phosphate can be sensed by cells in the bone and that a high phosphate level can stimulate the production of FGF23 or perhaps prevent the cleavage of FGF23 via up-regulation of GALNT3. High levels of 1, 25 dihydroxy vitamin D enhanced these responses. The results also suggested that high phosphate may prevent bone formation by increasing sclerostin expression.

SARCOPENIA AND VITAMIN D DEFICIENCY ARE RISK FACTORS FOR FALLS BUT REMAIN UNDETECTED IN AGED CARE RESIDENTS

S. Juliano-Burns1, E. Seeman2
1University of Melbourne / Austin Health, West Heidelberg, Australia
2Monash University, Clayton, Australia

Aim: Institutionalised elderly, in whom sarcopenia and vitamin D deficiency are common, are at high risk for falls. We aimed to determine the contribution of sarcopenia and vitamin D deficiency to falls risk and to identify ways of assessing the risk of falling in elderly aged-care residents.

Methods: 80 ambulatory female residents age 86 years (range 67-99 years) from 18 low-level aged care facilities participated. Body composition was determined using DXA, ankle, knee and hip strength measured using the Nicholas manual muscle tester, balance assessed using the Lord's Balance test, and physical function reported using TUG and walking speed over 6 metres. Serum 25(OH)D was measured from morning blood samples. Basic anthropometry was performed. Falls were recorded prospectively during 12-months. Medical records were reviewed for medical conditions and medication use. Relative sarcopenia was determined using the Janssen method. Chi square distributions and logistic regression analysis were performed.

Results: Sarcopenia was associated with an increased risk for falls (OR 10.7; 95% CI 1.1–99.9, p<0.05). Higher serum 25(OH)D levels had a small but protective effect against risk for falls (OR 0.97; 95%CI 0.94–0.99, p<0.05). Age, strength, function, balance, and number of medications or medical conditions were not predictors for falls. Sarcopenic women were heavier (but not taller), had higher BMI (27.8 ± 4.3 v 22.1 ± 2.4, p<0.001) and had greater % body fat (40.8 ± 5.4 v 26.2 ± 5.1, p<0.001) than non-sarcopenic women, but groups did not differ in lean mass. Sarcopenic women were not distinguishable using limb circumferences or functional measures, and despite being sarcopenic most were in the overweight to obese weight range.

Conclusion: Sarcopenia is a risk factor for falls but is not readily identified from basic anthropometry therefore goes undetected. Routine screening of serum 25(OH)D levels and correction of deficiencies may also contribute to falls risk reduction in elderly aged care residents.
255

25(OH)-VITAMIN D3 ACTS DIRECTLY ON OSTEOCLASTS TO REDUCE RESORPTION: WHAT IS THE MECHANISM?

M. Kogawa1, D. M. Findlay1, P. H. Anderson2, H. A. Morris1,3, G. J. Atkins1
1Orthopaedics & Trauma, University of Adelaide, Adelaide, SA, Australia
2Endocrine Bone Research Laboratory, SA Pathology, Adelaide, SA, Australia
3School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA, Australia

We have reported the metabolism of 25D into active 1,25D by osteoclast lineage cells, which optimizes osteoclast differentiation, and inhibits osteoclast activity. 525D 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[11]. However the mechanism, by which 25D reduces osteoclast resorption is not clear and the aim of the experiments reported here was to elucidate this action of 25D. Firstly, the effect of 25D on osteoclast morphology was examined. No obvious differences were observed between control and 25D treated cells as assessed by Crystal Violet staining. Actin ring formation was also apparently unperturbed. Secondly, treatment with 25D had no apparent effect on osteoclast survival or apoptosis, as assessed by cell counting and caspase-3 activity, respectively. Thirdly, to investigate the effect of 25D on osteoclast gene expression, we performed RT-PCR for osteoclast function-related genes. Unexpectedly, the expression of all genes thought to be related to resorption, such as TRAP, carbonic anhydrase 2, cathepsin K, V-ATPase and CIC-7, was elevated in the presence of 25D. Subsequently, we have focussed on the 25D-induced increase of TRAP mRNA levels because it has been reported that osteoclast migration is regulated by TRAP through osteopontin dephosphorylation[12-13]. Support for this in our model system was our finding that GCT osteoclast area was smaller in the presence of 25D. We speculate that TRAP dephosphorylation of substrate binding proteins modifies osteoclast attachment, thereby reducing resorptive activity. Additional studies are now aimed at investigating the putative role of 25D to modulate osteoclast attachment and/or migration.


256

THE ASSOCIATION BETWEEN VITAMIN D SERUM LEVELS AND BLOOD PRESSURE STATUS: AN IRANIAN STUDY

Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, Tehran, Iran

Aim- Vitamin D deficiency has been proposed as an associating factor with increased blood pressure. We studied the relationship between serum vitamin D and blood pressure levels among a large representative sample of Iranian population. Methods- In this cross sectional study, based on data of 2508 individuals (aged between 20 and 70) from the Iran Multicenter Osteoporosis Study (IMOS), the association between vitamin D and four categories of blood pressure (based on JNC-7(1)) was investigated. We compared the mean transformed scales (Neperian Logarithm) of vitamin D serum levels in different stages of blood pressure using One-Way ANOVA test. Results- The mean age of the studied population was 42.4 ± 13.9 years. 67.8% had vitamin D deficiency and 27.6% were hypertensive (17.9% and 9.7% for stage-I and -II respectively). We found a significant difference between mean (± SD) vitamin D levels of individuals in stage-I hypertension and that of the three other groups (Normal: 32.9 ± 27.5; Prehypertension: 34.4 (± 27.2); Stage-I: 38.7 (± 29.2); Stage-II: 34.7 (± 24.0) ng/mL; p-value < 0.05 for all of the comparisons). Conclusion- It could be concluded that despite the raise noted in vitamin D levels as the BP stage increases, there is a considerable decline moving from stage-I to stage-II. The values reported in individuals with stage-I hypertension is significantly higher than that of other stages. Considering the difference noted between our results and previous studies, further research is needed in this field to assess the effect of ethnicity and possible genetic on this findings. It should, however, be noted that the difference in the methodology of these researches and the fact that none of the previous studies, to our knowledge, had omitted the skewness effect of vitamin D are the main reasons contributing to this difference.

IMPROVING MOBILITY AND REDUCING DISABILITY IN OLDER PEOPLE THROUGH EARLY HIGH-DOSE VITAMIN D REPLACEMENT FOLLOWING HIP FRACTURE (THE REVITAHIP TRIAL): PRELIMINARY RESULTS

J. C. S. Mak 1, 2, I. D. Cameron 3, R. S. Mason 1, L. Klein 4, M. Soong 5, K. Ohn 6

1Department of Geriatric Medicine, Central Coast Health, Gosford Hospital, Gosford, NSW, Australia
2Rehabilitation Studies Unit, University of Sydney, Ryde, Sydney, NSW, Australia
3Bosch Institute, University of Sydney, Camperdown, Sydney, NSW, Australia
4Office of Medical Education Sydney Medical School, University of Sydney, Sydney, NSW, Australia

Background: Hypovitaminosis D is particularly common among older people with a proximal femoral (hip) fracture and has been linked with poorer lower extremity functioning, falls, and fractures. There is evidence that disability severity, fall rates and fractures may be reduced by adequate vitamin D replacement (1,5) but at higher doses may contribute to falls and fractures (4). The ideal regimen for vitamin D administration to have these benefits in older people who have been in hospital has not been established (2,3,5). This randomized controlled trial will investigate the effects of an oral vitamin D loading dose with maintenance oral vitamin D and calcium on lower extremity function (gait velocity), correction of hypovitaminosis D, falls, and fractures among older people after hip fracture surgery. The cost-effectiveness of the REVITAHIP program from the health and community service provider's perspective will also be established, as will predictors of adherence with the treatment. Methods and Design: A total of 600 older people who have recently had a hip fracture requiring surgical intervention will be screened to achieve 250 participants for the study. Participants will have no medical contraindications to vitamin D replacement. The primary outcome measure will be mobility-related disability as measured with the 2.4-m gait velocity test. Secondary measures will be 25-hydroxyvitamin D (25-OHD) levels at 2, 4, and 26 weeks, number of falls and fractures, and additional measures of mobility, disability, quality of life, health system and community–service contact, adherence to the intervention, and adverse events. After surgical fixation and deemed medically stable, participants will be randomly allocated to an intervention or placebo-control group. Participants of the intervention group will receive initial oral vitamin D3 (250000 IU (5 x 50,000 IU) vitamin D3 tablets. Both groups will receive oral maintenance vitamin D3 and calcium and will follow the usual hip fracture rehabilitation pathway. Discussion: The study will determine the impact of a vitamin D loading dose on mobility-related disability in older people following hip fracture, and will discuss the efficacy and cost-effectiveness of a loading dose vitamin D replacement more generally. The preliminary results will be discussed.

1 Trivedi DP, Doll R, Khaw KT. Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community. BMJ. March 2003;326(7387):469.
4 Sanders KM, Stuart AL, Williamson EJ, et al. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. JAMA. May 2010;303(18):1815-1822.

WIDESPREAD VITAMIN D DEFICIENCY AMONG INDIAN HEALTH PROFESSIONALS

M. Beloyartseva 1, A. Mithal 1, S. Kalra 1, M. P. Baruah 1, S. Mukhopadhyay 1, T. R. Bandgar 6

1Endocrinology, Medanta The Medicity, Gurgaon, Haryana, India
2Endocrinology, Bharti Hospital, Karnal, India
3Endocrinology, Excel Care Hospitals, Guwahati, Assam, India
4Endocrinology, Institute of Postgraduate Medical Education, Kolkata, West Bengal, India
5Endocrinology, St. John’s Medical College & Hospital, Bangalore, Karnataka, India
6Endocrinology, USV Ltd, Mumbai, Maharashtra, India

Several single centre studies from India have shown high prevalence of vitamin D deficiency (VDD) among health professionals in different regions of India. Aim: To determine prevalence of VDD among health professionals in different regions of India.

Method: In this multicentric study we enrolled 933 apparently healthy medical and paramedical personnel from 15 Indian cities. Blood samples were collected from December 2010 to March 2011 and analyzed in a central laboratory by RIA. VDD was defined as 25(OH)D<20 ng/ml, insufficiency - as 25(OH)D=20-30 ng/ml and sufficiency - as 25(OH)D>30 ng/ml.

Results: Mean±SD age of subjects was 42±11.9 years. Mean 25(OH)D level was 11.6±8.36ng/ml (median 9.8). We found that 88% of subjects were vitamin D, 9% were insufficient, and just 3% were sufficient.

<table>
<thead>
<tr>
<th>City</th>
<th>N</th>
<th>Age</th>
<th>Mean VitD</th>
<th>VitD deficientN%</th>
<th>VitD insufficientN%</th>
<th>VitD sufficientN%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahmedabad</td>
<td>16</td>
<td>43.3±7.5</td>
<td>6.6±5.5</td>
<td>3.8</td>
<td>15(94%)</td>
<td>1(6%)</td>
</tr>
<tr>
<td>Aurangabad</td>
<td>24</td>
<td>28.8±4.8</td>
<td>15.1±4.0</td>
<td>14.8</td>
<td>22(92%)</td>
<td>2(8%)</td>
</tr>
<tr>
<td>Bangalore</td>
<td>71</td>
<td>44.2±14.9</td>
<td>14.6±8.8</td>
<td>10.6</td>
<td>65(92%)</td>
<td>5(7%)</td>
</tr>
<tr>
<td>Bhopal</td>
<td>10</td>
<td>32.8±4.5</td>
<td>12.0±4.1</td>
<td>11.9</td>
<td>10(100%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Chandigarh</td>
<td>55</td>
<td>49.3±11.9</td>
<td>7.4±12.3</td>
<td>7.6</td>
<td>44(80%)</td>
<td>3(5%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>City</th>
<th>N</th>
<th>Age</th>
<th>Mean VitD</th>
<th>VitD deficientN%</th>
<th>VitD insufficientN%</th>
<th>VitD sufficientN%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahmedabad</td>
<td>16</td>
<td>43.3±7.5</td>
<td>6.6±5.5</td>
<td>3.8</td>
<td>15(94%)</td>
<td>1(6%)</td>
</tr>
<tr>
<td>Aurangabad</td>
<td>24</td>
<td>28.8±4.8</td>
<td>15.1±4.0</td>
<td>14.8</td>
<td>22(92%)</td>
<td>2(8%)</td>
</tr>
<tr>
<td>Bangalore</td>
<td>71</td>
<td>44.2±14.9</td>
<td>14.6±8.8</td>
<td>10.6</td>
<td>65(92%)</td>
<td>5(7%)</td>
</tr>
<tr>
<td>Bhopal</td>
<td>10</td>
<td>32.8±4.5</td>
<td>12.0±4.1</td>
<td>11.9</td>
<td>10(100%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Chandigarh</td>
<td>55</td>
<td>49.3±11.9</td>
<td>7.4±12.3</td>
<td>7.6</td>
<td>44(80%)</td>
<td>3(5%)</td>
</tr>
</tbody>
</table>
Aim. To determine the frequency of vitamin D deficiency and insufficiency in people of different age, not previously treated with vitamin D, who living in different regions of Ukraine (west, centre, east) and to determine factors associated with low vitamin D intakes. No correlations between 25 (OH) vitamin D level and ultrasound densitometry data.

Conclusion. Our study reconfirms high prevalence of VDD all across India in apparently healthy, middle aged health professionals.
ANNUAL HIGH-DOSE ORAL VITAMIN D₃: IS INCREASED RISK OF FRACTURES ATTRIBUTABLE TO CHANGES IN BONE TURNOVER MARKERS?

K. M. Sanders¹,²,4, P. R. Ebeling¹,², A. L. Stuart¹,²,4, E. J. Williamson¹,²,3, M. A. Kotowicz¹,4, G. C. Nicholson⁶

¹Medicine, North West Academic Centre, Melbourne, VIC, Australia
²The University of Melbourne, VIC, Australia
³School of Population Health, Centre for Molecular, Environmental, Genetic and Analytic Engineering, Melbourne, VIC, Australia
⁴Barwon Health, Geelong, VIC, Australia
⁵School of Medicine, Deakin University, Geelong, VIC, Australia
⁶School of Medicine, Rural Clinical School, The University of Queensland, Toowoomba, QLD, Australia

We have previously reported increased falls and fractures in a RCT using a single annual oral dose of 500,000IU cholecalciferol (D₃) administered orally in autumn or winter for 3-5 years to 2,256 older women. The increased rate of falling in the D₃ group was higher in the first 3 months post-dosing (p=0.017). This temporal pattern was observed for fractures although not significant.

Aim: To investigate if the increased rate of fractures is associated with change in bone turnover markers (BTM).

Method: Serial biochemistry was performed on a sub-study of 99 randomly selected participants. Serum 25-hydroxyvitamin D (25D: Diasorin) and the BTM, beta C-telopeptide (CTx -resorption) and N-terminal propeptide of type 1 collagen (P1NP -formation) were measured by electrochemiluminescence immunoassay (Roche, Germany). Biochemistry is reported at baseline and ≥2 years later at pre-dose, 1- and 3-month post-dose. Our post-hoc analysis used a linear regression model for change in BTM at 1- and 3-months post-dose and included baseline BTM and %change in 25D as covariates. The analysis also tested for heterogeneity (interaction) between changes in BTM and 25D.

Results: The median baseline 25D was 49nmol/L. In the D₃ group, 1-, 3- and 12-month median post dose levels were 124, 93 and 62nmol/L, respectively. At 3-months post-dose both CTx and P1NP increased with increases in 25D (estimated mean % change from pre-dose per 50% increase in 25D was 28.7% CTx and 8.4% P1NP, p=0.024 and 0.015, respectively). There was no heterogeneity of either marker (p>0.1).

Conclusions: Our findings suggest that annual high-dose D₃ treatment may be associated with increased bone turnover in the 3-month post-dose period. In these older women, higher bone turnover may have contributed to increased fractures.

(1) Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D, Nicholson GC. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. JAMA. 2010

SERUM TESTOSTERONE AND FRACTURE RISK IN MEN: A COMPARISON OF RADIOIMMUNOASSAY VERSUS LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

T. S. Tran¹, N. D. Nguyen¹, J. R. Center¹, M. J. Seibel¹, J. A. Eisman¹, T. V. Nguyen¹

¹Osteoporosis and Bone Biology, Garvan Institute of Medical Research, St Vincent’s Hospital, Darlinghurst, NSW, Australia
²ANZAC Research Institute, Sydney, NSW, Australia

Aim. This study was designed to determine whether the testosterone-fracture relationship is affected by methods of measurement at the population and individual level.

Methods. The study was part of the on-going Dubbo Osteoporosis Epidemiology Study. Total testosterone (TT) was measured by LC-MS and RIA methods from serum samples collected from 609 men, whose incidence of fractures was ascertained (by X-ray reports) from 1989 to 2010. Baseline clinical risk factors were obtained. The concordance in serum levels of TT between the LC-MS, denoted LC-MS and RIA-TT, respectively. The Deming’s equation linking the two measurements was: MS-TT=0.91+0.86*RIA-TT (R²=0.50). During the duration of follow-up, 103 had sustained at least a fracture. After adjusting for age, weight, BMD, fracture history, smoking, calcium intake, and sex hormone-binding globulin, TT was significantly associated with fracture risk. The hazard ratio of fracture per SD decrease in TT was 1.29 (1.09-1.52) for MS-TT, and 1.26 (1.06-1.48) for RIA-TT. The correlation between predicted probabilities of fracture by MS-TT and RIA-TT was high (R² = 0.9, Figure), with average difference in the predicted probability of fracture being 0.01% (-6.1% to 6.2%) for any fracture.

Conclusions. These results indicate that lower serum levels of TT were associated with increased risk of fracture in elderly men. Despite the modest concordance between methods of measurement, TT measured by RIA could predict fracture risk as good as LC-MS method.
AGE AND SKELETAL SITE AFFECT RESPONSIVENESS OF BONE MARROW STROMAL CELLS FROM DIFFERENT TRABECULAR COMPARTMENTS IN DIFFERENT PHYSIOLOGICAL CONDITIONS: EFFECT OF ESTROGEN AND VITAMIN D

R. Trivedi, A. Kumar
Endocrinology, CSIR-CDRI, Lucknow, India

Aim: To study the formation of mineralized nodules from different trabecular compartments under various physiological and hormonal changes.

Methods: BMSC were isolated from femur epiphysis (E), tibia proximal (P), tibio-fibula separating point (TFSP) and lumbar vertebrae (V) from growing (modeling), ovary intact adult (remodeling), ovariectomized (OVx, osteopenic), OVx+aging (aging osteopenic), OVx + estrogen (E2) and growing/aging osteopenic + 1.25 (OH)2 vit D3 (D3) rats. Ex vivo nodule formation and expression of type I collagen (Col1) were quantified.

Results: Compared to adult rats, growing rats had higher mineralized nodule formation at each region studied. TFSP exhibited the highest nodule formation compared to other trabecular compartments of the growing rats. TFSP and P of adult rats had greater nodule formation over V and E, which corroborated with the expression of Col1 protein at the corresponding trabecular bones. In osteopenic rats, nodule formation was reduced in all regions compared to adult group, however, between the various trabecular compartments of osteopenic rats, TFSP had the maximum nodule formation followed by V, E and P. Nodule formation of BMSC of all trabecular regions in the aging osteopenic rats was the least compared to every other group. A striking increase in nodule formation at all trabecular regions was observed in OVx + E2 rats compared to adult, and the response was highest in P followed by TFSP. D3 treatment to growing or aging osteopenic rats augmented nodule formation at all sites compared to adult rats. D3 response was highest in TFSP in aging osteopenic and TFSP and P in growing rats.

Conclusion: Between the various trabecular compartments, BMSC from TFSP generally has the greatest mineralizing ability under different physiological and hormonal alterations. The study importantly reveals the varied response of different trabecular compartments to mineralize, and may help explain the non-uniform skeletal response to anti-osteoporosis therapies.

INCREASED BONE VOLUME IN THE BONE-SPECIFIC CYP27B1 TRANSGENIC MOUSE

A. G. Turner1, P. D. O’boughlin1, M. Kogawa2, G. J. Atkins2, P. H. Anderson1,2, H. A. Morris1,3
1Chemical Pathology, SA Pathology, Adelaide, SA, Australia
2Orthopaedics and Trauma, University of Adelaide, Adelaide, SA, Australia
3School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA, Australia

The endocrine hormone, 1α,25-dihydroxyvitamin D3 (1,25D) is a regulator of calcium homeostasis and bone health. We have shown that osteoblasts, osteocytes and osteoclasts are a major source of 1,25D within the skeleton by virtue of their expression of the 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1).

AIM: To determine the effect of increased synthesis of 1,25D in osteoblasts on bone remodeling and bone volume.

METHODS: We have constructed a plasmid in which transcription of the human CYP27B1 sequence is driven by the human osteocalcin promoter. This construct has been 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 calcium and 1,25D levels in the circulation.

RESULTS: Seven week old male OSC1 mice demonstrate an 11.5% increase in BV/TV of the distal femoral metaphysis when compared with WT mice (n=11/gp, p<0.05). This was associated with an 8.5% increase in trabecular number (p<0.05) with trabecular thickness unchanged. Although at 7 weeks of age, female OSC1 mice showed no difference in BV/TV, at 20 weeks of age, BV/TV was 20% greater than in WT mice (p<0.05) due to a 23% increase in trabecular number (p<0.05).

CONCLUSIONS: These data suggest that bone loss with age which is a normal feature of growing mice is reduced in OSC1 mice. Dynamic histomorphometry will further clarify the mechanism of increased trabecular bone volume in OSC mice. Our data further support the concept of the skeleton as an intracrine organ for vitamin D, with locally synthesized 1,25D exerting important actions for bone remodeling and challenging the long-held notion that 1,25D is solely an endocrine hormone.

VITAMIN D LEVELS IN SUBJECTS WITH TYPE 2 DIABETES MELLITUS

H. Uzkeser1, K. Yildirim1, H. Bilen1, S. Karatay2, M. Akbas3
1Departments of Physical Medicine and Rehabilitation, Numune State hospital, Erzurum, Turkey
2Ataturk University, Erzurum, Turkey
3Internal Medicine, Medical Faculty, Ataturk University, Erzurum, Australia

Aim: More recently, there is accumulating evidence to suggest that altered vitamin D and calcium homeostasis may also play a role in the development of type 2 diabetes mellitus (DM). Observational studies show a relatively consistent association between low
vitamin D status, calcium or dairy intake and prevalent type 2 DM. This study was carried out to investigate the serum 25-hydroxyvitamin D levels of patients with type 2 DM.

Material and Methods: Thirty patients with type 2 DM and twenty matched healthy controls were enrolled in this study. In laboratory analysis, serum 25-hydroxyvitamin D, Ca, P and parathyroid hormone (PTH) levels were measured in both groups.

Results: There were no statistically significant differences between the two groups with respect to demographic data (p>0.05). In patients with type 2 DM, 25-hydroxyvitamin D values were not different than those of the healthy controls (p > 0.05). Also, there were not significantly difference between patients with type 2 DM and healthy controls in terms of serum Ca, P and PTH levels (p > 0.05).

Conclusion: Our results suggest that serum 25-hydroxyvitamin D levels not exchange in patients with type 2 DM according to control cases. Further studies needs to define the association between vitamin D and calcium status and risk of type 2 DM.

Key words: type 2 diabetes mellitus, 25-hydroxyvitamin D

---

266

OSTEOPOROSIS, OSTEOPENIA AND 25-HYDROXY-VITAMIN D LEVELS IN PATIENTS AFTER RENAL TRANSPLANTATION

M. A. Siddiqui, M. Gupta, S. K. Wangnoo
Endocrinology, Indraprastha Apollo Hospital, New Delhi, India

AIM:
Loss of bone mineral density (BMD) is one of the long-term complications as a direct result of disturbances of bone metabolism in pre-transplant period and immunosuppressive therapy after the transplantation. This study investigated the prevalence and contributing factors of loss of bone mineral density after renal transplantation.

PATIENTS AND METHODS:
We investigated the prevalence of osteoporosis, osteopenia and low serum 25-hydroxy-vitamin D levels [25(OH)D] in 53 patients (33 M : 20 F) with mean age of 46.5+/- 8.7 years 1-year post renal transplantation following up in our clinic.

RESULTS:
1-year post renal transplantation, osteopenia or osteoporosis was observed among 42.9% or 22.4% of the patients, respectively. The mean body mass index (BMI) value was significantly higher among the normal (27.7+/-4.6 kg/m^2) than the osteoporotic group (21.9+/-3.5 kg/m^2). The amount of proteinuria was significantly lower in the normal (14.9+/-2.9 mg/d) than the osteopenic (138+/-32.4 mg/d) or osteoporotic group (215.5+/-42.9 mg/d). There was no difference in age, gender, cause of end-stage renal disease, pretransplant dialysis duration or modality, immunosuppressive regimen, levels of iPTH, 25(OH)D, calcium, phosphorus, serum albumin, creatinine clearance, or serum bicarbonate concentrations (P > .05). The T-scores of the femoral neck correlated positively with BMI (r: 0.43; P = 0.001) and 25(OH)D levels (r: 0.31, P = 0.023), and inversely with the amount of proteinuria (r: -0.21, P = 0.028), serum alkaline phosphatase (r: -0.37, P = 0.005), and serum magnesium (r: -0.24, P = 0.031). Upon multivariate analysis, BMI and 25(OH)D level were observed to be independent risk factors for loss of femoral mineral density.

CONCLUSION:
Loss of BMD is a common complication that correlates with low BMI values and decreased 25(OH)D levels in post renal-transplant recipients.

---

267

VITAMIN D 1A-HYDROXYLASE GENE MUTATIONS IN CHINESE PATIENTS WITH PSEUODO-VITAMIN D-DEFICIENCY RICKETS

Endocrinology, Key Lab Endocrinology, Ministry of Health., Peking Union Medical College Hospital, Beijing, China

Pseudo-Vitamin D-Deficiency Rickets (PDDR), is a rare autosomal recessive disorder characterized by the early onset of rickets, hypocalcemia, and hyperparathyroidism. PDDR is caused by mutations of the 25-hydroxyvitamin D 1α-hydroxylase (1α-hydroxylase, CYP27B1) gene. To date, more than 30 mutations have been reported, but efforts around genetic characterization have included only one Chinese PDDR patient. Here, we analyze the CYP27B1 gene in four unrelated Chinese families with PDDR. After genomic DNA extraction, all nine exons and exon-intron boundaries of CYP27B1 gene were amplified by PCR and sequenced. Any purification of PCR products with deletion or insertion mutations was done using colonizing sequencing. All four patients had typical manifestations and radiological evidence of rachitic disease. Four mutations in CYP27B1 gene were identified by sequencing in four families, one missense mutation and three deletion or insertion mutations with frame shift. Three of them are novel. They are one missense mutation c170 G → T in Exon 1, c1310delG in Exon 8, and c1442delA in Exon 9. We confirm again that a 7bp insertion in Exon 8 has no obvious ethnic group homology distribution. A missense mutation in Exon 1 c170 G → T in 8 Chinese alleles, maybe a hot mutation site in Chinese patients.
THE ROLE OF VITAMIN D IN THE PROLIFERATION AND DIFFERENTIATION OF OSTEOBLASTS IN VITRO

D. Yang1,4, G. J. Atkins2, P. H. Anderson3, K. J. Welldon3, H. A. Morris1,4
1School of Medicine, Faculty of Health Sciences, The University of Adelaide, Adelaide, SA, Australia
2Bone Cell Biology Group, Discipline of Orthopaedics and Trauma, University of Adelaide, Adelaide, SA, Australia
3School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA, Australia
4Endocrine Bone Research, Chemical Pathology, SA Pathology, Adelaide, SA, Australia

Aim: Although exogenous calcitriol (1,25D), can suppress osteoblast proliferation and stimulate differentiation, whether 1,25D synthesis in osteoblasts (1) can affect these processes remain unclear. We examined the effects of calcidiol (25D) on the proliferation and differentiation of primary osteoblasts derived from wild-type (WT) and VDRKO mice. Also, we examined these effects in relation to changes in calcium concentration.

Methods: Long bones from 6–8 week-old mice were flushed of marrow and cancellous bone, dissected into ~1mm3 pieces and cultured. At confluence, cells were removed with collagenase for experimentation. Cell proliferation rate was determined by CFSE staining and flow cytometry (2). Mineral deposition was assessed by Alizarin Red staining and gene expression analysed by real-time RT-PCR.

Results: In WT cells, 25D (200nM) suppressed osteoblast proliferation by 54% (p<0.05), comparable to 1nM 1,25D (51% suppression). However, post-proliferative differentiation/mineralisation was stimulated by both 25D and 1,25D, an effect more pronounced when the calcium concentration was increased from 1.8mM to 2.8mM. In VDRKO cultures, while increased Ca2+ levels resulted in enhanced mineralisation, both 25D and 1,25D had no effect. In WT cultures both 25D and 1,25D enhanced osteoblastogenesis, evidenced by increased alkaline phosphatase and osteocalcin mRNA levels. However, in the presence of elevated Ca2+ (2.8mM), alkaline phosphatase and osteocalcin mRNA were markedly suppressed, while the expression of the osteocyte marker Dmp1 increased 3-fold, independently of vitamin D treatments. In VDRKO cultures, neither vitamin D treatments nor calcium facilitated normal osteoblastogenesis or osteocyte transition.

Conclusion: These data indicate that 25D is capable of regulating osteoblastogenesis and mineralisation in a fashion comparable to 1,25D and the effects of 25D are VDR-dependent. Our results imply that there is interplay between 25D metabolism and calcium availability at the level of the osteoblast, with the net cellular response depending on the prevailing concentrations both factors.


VITAMIN D INSUFFICIENCY AND DEFICIENCY AS A RISK FACTOR FOR FAST BONE LOSS AMONG ELDERLY MEN AND WOMEN: THE ROAD STUDY

N. Yoshimura1, S. Muraki2, H. Oka1, H. Kawaguchi3, K. Nakamura3, T. Akune3
1Dept of Joint Disease Research, University of Tokyo, Tokyo, Japan
2Dept of Clinical Motor System Medicine, University of Tokyo, Tokyo, Japan
3Dept of Sensory and Motor System Medicine, University of Tokyo, Tokyo, Japan

Aim: To clarify the influence of vitamin D (VD) insufficiency and deficiency on future bone loss in the Japanese elderly. Methods: We initiated Research on Osteoarthritis/osteoporosis Against Disability (ROAD), a large-scale population-based cohort study in urban, mountainous, and coastal areas of Japan in 2005–7, and a total of 3,040 participants were registred at baseline. We enrolled 967 subjects (368 men; 599 women) aged ≥ 65 years from mountainous and coastal areas. VD deficiency and insufficiency was characterized by a serum level of 25(OH)D < 10 ng/mL, and 10 ng/mL ≤ 25(OH)D < 30 ng/mL, respectively. Of the 967 subjects, 765 (79.1%) participated in the second surveillance visit in 2008 and 725 (75.0%) (263 men; 462 women) underwent BMD examination of the lumbar spine (L2–4) and femoral neck (FN) at both visits. Individuals categorized into the group with the greatest quartile for the loss rate of BMD over 3 years were defined as fast bone losers. Results: The mean (SD) levels of serum 25(OH)D of the baseline participants were 26.1 (6.4) ng/mL in men and 22.3 (6.3) ng/mL in women. The prevalence of VD insufficiency and deficiency was 80.0% (men, 70.1%; women, 85.7%) and 1.4% (men, 0.5%; women, 1.9%), respectively. The rate of BMD change at L2–4 and FN was 0.44%/yr (men, 0.49%/yr; women, 0.41%/yr) and -0.71%/yr (men, -0.42%/yr; women, -0.88%/yr), respectively. Logistic regression analysis, after adjustment for age and weight (kg), showed that the VD status was significantly associated with L2–4 fast bone loss in women. Conclusions: This longitudinal study showed high prevalence of VD insufficiency in the Japanese elderly and its significant influence on lumbar bone loss in women.
We quantified changes in bone microarchitecture at the distal tibia in 6 postmenopausal women with osteoporosis treated for 18 months with PTH. Images acquired using HR-pQCT (Scanco Medicals, Switzerland) were processed using StrAxl.0, a new software capable of separating the layer thick trabecular adjacent to the cortex (transition zone; TZ), from the compact-cortex and, the true trabecular compartment (TB). BMDs and porosities were quantified in the entire (total) bone volume, and in each of these compartments.

Total BMD increased by 3.63% (p=0.07) as a result of an increase in TBBMD (7.01%) (P=0.11), as CCBMD was unchanged (0.77%;NS). There was no change in the porosity of the CC (-0.06%;NS) whereas porosity in the TZ tended to decrease (3.2%;P=0.34). Within the constraints of the small sample size, we inferred that condensation of trabeculae adjacent to the compact cortex during anabolic therapy can mimic an increase in porosity due to intracortical resorption. This may explain the purported ‘increase’ in porosity during PTH therapy.

Acknowledgements: This work was supported by a research grant from Merck Sharp & Dohme
PACLITAXEL INHIBITS OSTEOCLAST FORMATION AND BONE RESORPTION VIA INFLUENCING WIDESPREAD CYTOSKELETAL ABERRATIONS, MITOTIC CELL CYCLE ARREST AND RANKL-INDUCED ACTIVATION OF NF-κB AND ERK


1School of Dentistry, University of Western Australia, Nedlands, WA, Australia
2School of Surgery, University of Western Australia, Nedlands, WA, Australia
3Laboratory for Cancer Medicine, Western Australian Institute for Medical Research, Nedlands, WA, Australia
4School of Medicine and Pharmacology, University of Western Australia, Nedlands, WA, Australia
5School of Pathology and Laboratory Medicine, University of Western Australia, Nedlands, WA, Australia

Pathological bone destruction occurs in many medical conditions such as tumor metastasis to bone, locally osteolytic giant cell tumor of bone, and Paget's disease of bone. Paclitaxel is frequently prescribed in the treatment of several malignant tumors where it has beneficial effects on bone lesions. However, the mechanism by which paclitaxel regulates osteoclasts remains ill-defined. In the present study, we demonstrate that paclitaxel dose-dependently inhibits RANKL-induced osteoclastogenesis in both RAW264.7 cells and mouse bone marrow macrophage (BMM) cultures. In addition, paclitaxel reduced the bone resorptive activity of human osteoclasts derived from Giant cell tumor (GCT) of bone, and attenuated lipopolysaccharide (LPS)-induced osteolysis in a mouse calvarium model. Cellular and biochemical assessment revealed that paclitaxel induced widespread cytoskeletal aberrations in osteoclast like (OCL) cells, and mitotic arrest of the OCL cells, possibly accounting for the observed effects. Furthermore, luciferase reporter gene assays and western blot analysis showed that paclitaxel modulates RANKL-induced activation of NF-κB and ERK. Collectively, our findings indicate a role for paclitaxel in the regulation of osteoclast formation and function and point to potential mechanisms by which paclitaxel alleviates pathological osteolysis.

EPGENETIC SIGNATURES OF OSTEOSARCOMA

E. K. Baker1, D. Strbenac2, T. J. Martin2, L. E. Purton2, M. D. Robinson3,4, C. R. Walkley1

1Stem Cell Regulation Unit, St. Vincent’s Institute, Fitzroy, VIC, Australia
2Bone Cell Biology and Disease Unit, St. Vincent’s Institute, Fitzroy, VIC, Australia
3Epigenetics Laboratory, Garvan Institute, Sydney, NSW, Australia
4Bioinformatics Division, Walter and Eliza Hall Institute, Melbourne, VIC, Australia

Aims: Osteosarcoma (OS) is the most common malignancy of bone, and is the second leading cause of cancer related death in children. OS survival rates have plateaued since the 1980s. Patients presenting with metastatic or recurrent disease have less than 20% survival at 5 years. New therapeutic treatments are desperately needed to improve patient outcomes. It is clear that epigenetic changes play a major role in cancer initiation and progression, however the contribution of epigenetic instability to OS, especially metastatic disease, remains largely unknown. Understanding the specific epigenetic changes associated with OS disease onset, progression and metastasis will be important for developing new treatment options. Methods: We have utilized low passage cell lines established from paired primary and metastatic tumours from a mouse model of OS1, and normal osteoblasts derived by in vitro differentiation of Kusa4b10 cells2 to identify epigenetic signatures that define OS. Genome-wide promoter maps of epigenetic modifications in OS and normal osteoblast cells were generated using NimbleGen ChIP-Chip arrays. Results: Gene expression profiling of OS cells and normal osteoblast cells showed a number of enzymes responsible for mediating epigenetic modifications are differentially expressed. Differences in expression were also apparent in cells derived from primary and metastatic tumours from the same mice. Genome-wide promoter mapping of modifications mediated by these enzymes identified an OS specific epigenetic signature that distinguishes the disease from normal osteoblasts. The signatures encompass both RefSeq annotated genes and miRNAs. Conclusions: Aberrant expression of epigenetic modifying enzymes in OS correlates with a specific epigenetic signature that distinguishes the disease from normal osteoblasts. Further profiling of the metastatic disease is warranted, as disease location appears to be associated with specific epigenetic enzyme gene expression. Identifying OS specific epigenetic signatures will provide invaluable knowledge of OS biology, potential markers of metastatic disease development and new therapeutic targets.


REGULATION OF OSTEOCLAST DIFFERENTIATION BY METASTATIC OSTEOSARCOMA

L. B. Endo-Munoz1, A. Cumming1, D. Loo1, P. Mukhopadhyay1, M. Hill1, S. Sommerville2, A. Evdokiou1, N. A. Saunders1

1The University of Queensland Diamantina Institute, Woolloongabba, QLD, Australia
2Department of Orthopaedics, The Wesley Hospital, Auchenflower, QLD, Australia
3Bone Cancer Research Group, The University of Adelaide, Adelaide, SA, Australia

Osteosarcoma (OS) is the most common primary malignant bone tumour in children and adolescents. Current treatment involving surgery and chemotherapy is ineffective for patients with metastatic disease, the leading cause of death in OS. We have recently
published data gathered from OS patient biopsies, a murine model of OS and in vitro experiments, showing that the loss of osteoclasts at the site of the primary tumour contributes to the development of pulmonary metastasis in OS. We have shown that metastatic OS cell lines can inhibit osteoclastogenesis in vitro and in vivo. Moreover, ablation of osteoclasts with zoledronic acid increases the number of metastatic lung lesions in an orthotopic mouse model of OS, whereas fulvestrant treatment increases osteoclast numbers and reduces metastatic lesions. Thus, metastatic potential can be regulated by the interaction between osteoclasts and OS cells, where osteoclasts suppress pulmonary metastasis and OS cells suppress osteoclastogenesis. In the murine model of OS and in patients, the loss of osteoclasts was only observed at the site of the primary tumour without any evidence of oesteolysis or other skeletal involvement. In addition, the loss of osteoclasts in patients was not associated with changes in RANKL, OPG, CSF-1 or other classic modulators of osteoclastogenesis, which suggests the involvement of novel pathways in osteosarcoma. To identify secreted factors that may be responsible for osteoclast inhibition and which may serve as potential therapeutic targets in OS, we used a combined a secretomic (biotin label-based antibody array) and proteomic (SILAC) approach to interrogate the conditioned medium of metastatic and non-metastatic OS cells. This has led to the identification of a number of molecules which may play a role in osteoclast inhibition and which may be potential novel modulators of osteoclastogenesis within the context of a primary bone tumour such as osteosarcoma.

(1) Endo-Munoz et al. (2010). Osteosarcoma is characterised by reduced expression of markers of osteoclastogenesis and antigen presentation compared with normal bone. British J Cancer 103, 73-81.

**BLOCKADE OF PTH/PTHRP SIGNALING INHIBITS INVASIVE CAPACITY OF GENETICALLY ENGINEERED MOUSE OSTEOSARCOMA IN VITRO**

F. W. M. Ho, P. Kocovski, M. Russell, A. Goradia, T. J. Mutsaers, T. J. Martin, C. R. Walkley

**Bone, Joint & Cancer, St Vincent's Institute, Fitzroy, VIC, Australia**

Although most OS is sporadic, important insights into the genetic basis of OS have been acquired from familial cancer syndromes, especially the predisposition to OS conferred by mutation of either the Rb or the p53 genes. We have used an Osterix promoter transgene to direct Cre expression to committed osteoblast progenitors, and after breeding the transgene to conditional alleles for both p53 (p53fl/fl) and Rb (Rbfl/fl), mice developed OS with a mean latency of ~ 4.5 months, with complete penetrance in Osx-Cre p53fl/fl Rbfl/fl animals (Walkley et al., Genes Devel 22:1662, 2008) and more faithful mimicking of the human disease than any other experimental model. Since parathyroid hormone (PTH) responsiveness in OS is long recognized, we have used this model to begin to understand the requirements for PTH/PTHRP receptor (PTHR1) signaling in the pathogenesis of OS. In several tumours and cell lines from primary and metastatic OS from Oss-Cre p53fl/fl Rbfl/fl mice, we showed production of PTHrP and PTHR1 mRNA and protein, and functional PTHR1 by assaying cAMP responsiveness. We prepared several shRNA's against PTHR1 and tested their efficacy in knocking down PTHR1 mRNA and protein, identifying two that reduced PTHR1 mRNA and protein (western blotting, cAMP response) by more than 80%. Specificity was tested by showing ablation of the cAMP response to PTH but retention of b-adrenergic and PGE2 responsiveness. Blockade of PTHR1 in this way did not influence cell proliferation in vitro but profoundly reduced invasive capacity of the OS cells through collagen. This work will be extended to in vivo growth of OS in which PTHR1 is ablated by shRNA. Since this completely penetrant murine OS model can recapitulate all aspects of the disease process, from initiation and establishment to invasion and dissemination to distant sites, specific pathways influencing pathogenetic mechanisms can readily be investigated.

**INCREASED PRO-INFLAMMATORY CYTOKINE EXPRESSION AND NF-KB ACTIVATION POTENTIATE OSTEOCLAST FORMATION FOLLOWING METHOTREXATE TREATMENT**

T. J. King1,4, K. R. Georgiou1,2,4, M. A. Scherer1, J. C. Cool1, E. Ang1, J. Xu1, B. K. Foster1, C. J. Xian1,4

1Bone Growth and Repair Research Unit, UniSA, Adelaide, SA, Australia
2Physiology, Adelaide University, Adelaide, SA, Australia
3Centre of Orthopaedic Research, University of Western Australia, Nedland, WA, Australia
4Orthopaedic Surgery, Women's and Children's Hospital, Adelaide, SA, Australia

Anti-metabolite therapy with Methotrexate (MTX) is known to cause bone defects including diminished bone volume. **Aim:** The current study sought to investigate possible mechanisms which promote osteoclast development and activity in regions of high bone turnover, focusing on changes in pro-inflammatory cytokine - NF-kB signalling which is known to be crucial for osteoclastogenesis. **Methods:** MTX (0.75mg/kg/day) was administered to rats for 5 consecutive days, and bone and bone marrow specimens were collected on days 6, 9 and 14 to observe time course effects on bone damage and recovery. Histology analysis, expression of osteoclastogenic cytokines, ex vivo osteoclast formation, and induction of osteoclast formation and NF-kB activation with serum from treated rats were performed. **Results:** In comparison to saline control, MTX increased the density of mature osteoclasts within the metaphysis and simultaneously the osteoclastogenic potential of marrow cells 9 days after beginning treatment. Consistently, RT-PCR analysis of mRNA expression for pro-osteoclastogenic cytokines in metaphyseal bone indicated that whilst the RANKL/OPG axis was unaffected by MTX, expression of TNF-α, IL-1 and IL-6 increased also on day 9. ELISA analysis of serum of treated rats
demonstrated that MTX increased TNF-α on day 6 and IL-6 on day 14. Furthermore, serum from treated rats (high in TNF-α) was able to induce osteoclast formation from normal bone marrow mononuclear cells. Indicative of the role of NF-κB signalling, day 6 serum increased luciferase activity in RAW osteoclast progenitor cells transfected with a luc-NF-κB reporter, and the serum-induced formation of osteoclasts was abolished in the presence of NF-κB inhibitor parthenolide. Conclusions: Our results confirm a role for osteoclasts in MTX induced bone loss. We demonstrated that MTX induces osteoclast differentiation by creating a pro-osteoclastogenic environment in the bone and in circulation, particularly with an increased TNF-α level, which can induce NF-κB activity promoting osteoclast formation.

ZOLEDRONIC ACID REDUCES BONE MARROW LESIONS AND KNEE PAIN OVER ONE YEAR

L. L. Laslett1, D. A. Doré1, S. J. Quinn3, T. M. Winzenberg1, P. Boon1, E. Bavage1, G. Jones1
1Menzies Research Institute Tasmania, University of Tasmania, HOBART, TAS, Australia
2Flinders Clinical Effectiveness, Flinders University, ADELAIDE, SA, Australia

Background: Knee osteoarthritis (OA) is a leading cause of chronic disability. Bone marrow lesions (BMLs) are regions of increased signal intensity seen on MR images. Knee BMLs are associated with structural changes in the knee, including joint space loss, cartilage defect progression, and cartilage loss. Knee BMLs are strongly associated with knee pain and are therefore a novel target for treatment of knee OA. Bisphosphonates modify the progression of BMLs as they are less common in persons taking bisphosphonates, and an open label study of IV ibandronate showed resolution of hip BMLs.

Methods: Adults aged 50-80 years (n=59) with clinical knee OA and knee BMLs were randomised to receive either ZA or placebo. BMLs were assessed on T2-weighted MR images at baseline, 6 and 12 months. Pain and function were measured using a visual analogue scale (VAS) and the Knee Injury and Osteoarthritis Outcome Score (KOOS) scale.

Results: After adjustment for potential confounders, VAS pain scores were significantly reduced in the ZA group, compared to placebo after six months (-14.5 mm, p=0.04) but not after twelve months of follow up (-9.0mm, p=0.25). Similarly, the change in the total area of BML was significantly reduced in the ZA group compared to placebo after six (-180.1 mm², p=0.02) and twelve months (-163.3 mm², p=0.04). Improvements in the KOOS pain and symptom scales did not reach statistical significance after either six (6.2, p=0.16 for pain; 3.3, p=0.50 for symptoms) or twelve months of follow up (3.5, p=0.55; 10.2, p=0.09).

Conclusions. One infusion of IV ZA reduced knee pain and BMLs after six months. The reduction in BML size was maintained after twelve months of follow up. This suggests that bisphosphonates, particularly ZA, may be successful novel treatments for BML-associated knee pain in participants with clinical knee OA.


MUSCULOSKELETAL BREAKDOWN DUE TO CANCER CACHEXIA: MOLECULAR AND MORPHOLOGICAL EFFECTS

A. Shum1, P. Camilleri1, S. J. Clarke1, G. Robertson1, P. Polly2
1Inflammation and Infection Research Centre, University of New South Wales, Sydney, NSW, Australia
2Department of Pathology, University of New South Wales, Sydney, NSW, Australia

Background: Cancer cachexia (CC) is a hypercatabolic disorder, affecting multiple tissues with 50-80% prevalence in advanced cancer patients and directly contributes to 20-30% of cancer patient mortality. The upregulation of breakdown processes, coupled with defects in tissue formation, contribute to weakened muscle and bone. However, the molecular progression of cachexia that drives these changes in musculoskeletal integrity due to cancer cachexia are largely unknown. Sarcomeric alterations in skeletal muscle and defective osteogenesis coupled with lysis effects due to cancer are unreported. As pathogenic mechanisms of cancer cachexia are complex and multi-factorial, an effective means of predicting, preventing or treating muscle and bone loss has not yet been established. Studies focusing on molecular regulators, which control sarcomeric integrity and osteogenesis or bone resorption due to cachexia are presented. Furthermore it was anticipated that such changes were associated with morphological changes in bone and skeletal muscle during overt cachexia. Methods and Results: The colon 26 (C26) carcinoma mouse model of cachexia was established to investigate such tumour-mediated changes in muscle and bone. Electron microscopy on skeletal muscle and TRAP staining on trabecular bone revealed morphological changes suggesting breakdown in both skeletal muscle and bone. Skeletal muscle and bone biomarker profiles revealed altered mRNA levels. qRT-PCR analysis of m RNA from gastrocnemius skeletal muscle and tibial heads from C26 mice at day 14 post-tumour inoculation (i.e. 15-20% body weight loss=overt cachexia) and controls...
demonstrated differences in myogenic transcription factors e.g. MEF2C and its target genes i.e. sarcomeric contractile apparatus genes, myozienin and myomesin. Alterations in mRNA for osteogenic bone regulators and targets e.g. VDR, bone morphogenetic protein-1, alkaline phosphatase and bone sialoprotein were also seen. Conclusion: Our results have identified potential skeletal muscle and bone biomarkers for CC which may be utilised as diagnostic tools or treatment targets to prevent breakdown.

Key words: Cancer cachexia, Bone, Muscle, Colon 26 Carcinoma

SINGLE CYTOTOXIC AGENTS DIRECTLY CAUSE BONE LOSS DUE TO INCREASED OSTEOCLASTOGENESIS: IMPLICATIONS FOR PREVENTING BONE LOSS IN CANCER PATIENTS

J. Quach1, M. Askymr1, T. Jovic1, K. E. White1, E. K. Baker1,2, N. Walsh1,2, L. E. Purton1,2

1Stem Cell Regulation Unit, St. Vincent's Institute, Fitzroy, VIC, Australia
2Medicine, The University of Melbourne, Parkville, VIC, Australia

Bone parameters were compared to those of non-treated age-matched littermate controls.

Results: All three cytotoxic treatments caused rapid, irreversible loss of trabecular bone compared to controls as assessed by μCT and histomorphometry. The bone loss was associated with significant increases in the number and size of osteoclasts, and elevated osteoclast activity was confirmed with higher serum CTX levels. Directly CAUSE B

BMT. Collectively these studies suggest that blocking osteoclast activity prior to commencing cytotoxic therapies may reduce cancer complications in cancer survivors following cytotoxic therapy.

Methods: Eight-week old male mice were treated with three different cytotoxic therapies: 1) lethal total body irradiation (TBI; 10Gy); 2) lethal TBI with bone marrow transplantation (BMT) or 3) a single dose (150mg/kg) of the chemotherapeutic agent, 5-fluorouracil. In a separate study, we also investigated the effects of a single dose of ZOL (10μg/kg) injected 3 days prior to BMT.

Conclusions: Significant bone loss occurs in response to a range of cytotoxic therapies. This is likely due to inflammation in response to therapy causing increased osteoclastogenesis. Furthermore, a single dose of ZOL was able to prevent the bone loss observed after BMT. Collectively these studies suggest that blocking osteoclast activity prior to commencing cytotoxic therapies may reduce late fracture risks in cancer patients.

METHOTREXATE TREATMENT RESULTS IN LOSS OF DENDRITIC MORPHOLOGY AND DIFFERENTIAL GENE EXPRESSION IN OSTEOCYTE-LIKE MLO-Y4 CELLS

C. Vincent, B. Hopwood, C. Xian

Bone Growth and Repair Research Unit, University of South Australia, Adelaide, SA, Australia

Methotrexate (MTX), a chemotherapy drug commonly used to treat haematological malignancies, has been shown to cause long-term bone defects including growth defects and bone loss; however the specific effects on individual cell types are not well characterized. Osteocytes are the most abundant cell type in bone tissue and their dendritic processes are believed to play a major role in mechanosensing and regulating bone remodelling (bone formation and resorption) and thus bone mass and bone strength. Aim: The objective of this study was to examine the effects of MTX treatment on dendritic morphology and gene expression in the osteocyte-like MLO-Y4 cells. Methods: MLO-Y4 cells were seeded in either 3-Dimensional collagen gels or directly onto dentine. Cells were allowed to develop their characteristic dendritic morphology for five days, after which they were treated for 48 hours with a control medium or a medium with either 10^{-5} M MTX or 10^{-5} M Dexamethasone (DEX), a known inducer of osteocyte apoptosis. Results: Confocal microscopy revealed that both DEX and MTX treatments resulted in loss of dendrites in cultured MLO-Y4 cells after 48 hours of treatments. Real-time RT-PCR analysis showed early IL-6 induction after 1 hour which remained elevated after 48 hours of treatment. IL-11 cytokine mRNA expression showed an increase only after 48 hours of both DEX and MTX treatments. Small increases in RANKL gene expression were also observed. DEX or MTX treatment induced an increase in transcript of the osteoblast differentiation inhibitor, SOST. Conclusions: These results suggest that the bone loss observed in vivo in response to MTX treatment could potentially be associated with osteocyte signalling to osteoclasts via pro-inflammatory cytokines and to osteoblasts via inhibitors of bone formation, as well as direct effects on osteocyte dendritic networks.
ATTENUATED MEGALIN EXPRESSION IN HYPERFUNCTIONING PARATHYROID TUMORS

M. Yamagata¹, Y. Imanishi², Y. Nagata³, H. Tsutsui¹, M. Inaba², T. Yukimura¹
¹Faculty of Pharmacy, Osaka Ohtani University, Tondabayashi, Japan
²Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan

Aim: Megalin is a multiligand endocytic receptor involving in the reabsorption of calcitriol and vitamin D binding protein in renal proximal tubules. Decrement of vitamin D receptor (VDR) expression enhances the secretion of parathyroid hormone (PTH) from parathyroid tumors in primary hyperparathyroidism (PHPT) and secondary hyperparathyroidism of uremia (SHPT), however, little is known about the involvement of its ligand, calcitriol into the tumors by megalin in these hyperfunctioning parathyroid diseases. Methods: Parathyroid tumors were obtained from 7 PHPT and 9 SHPT patients, and normal parathyroid glands from 11 thyroid carcinoma patients with written informed consents. A polyclonal antibody for megalin was used for the assessment of its expression by immunohistochemistry. The study was approved by the institutional ethics committees and was conducted in accordance with the principles of the Declaration of Helsinki. Results: The megalin expressions were observed in plasma membranes and cytoplasm of parathyroid cells in normal parathyroid glands. The expressions decreased in tumors with PHPT and SHPT compared with strong expression in normal parathyroid tissues. In SHPT, its expression was often particularly depressed in nodular areas, compared with adjacent diffuse hyperplasias. The patterns of the megalin expressions in hyperfunctioning parathyroid tumors were concordant with those of the VDR expressions, which were previously reported. Conclusion: The attenuated megalin expressions were observed in hyperfunctioning parathyroid tumors as well as the attenuated VDR expressions, suggesting the decrement of both VDR and megalin expressions enhances vitamin D resistance and hyper-secretion of PTH in the hyperfunctioning parathyroid tumors.

THE VITAMIN D RECEPTOR HAS A LIGAND-INDEPENDENT ROLE IN HUMAN BREAST CANCER CELL GROWTH

Y. Zheng¹,², T. Trivedi¹, L. Ou¹, C. Fong-Yee¹, C. R. Dunstan¹, M. Seibel¹, H. Zhou¹
¹Bone Research Program, ANZAC Research Institute, The University of Sydney, Concord, Sydney, NSW, Australia
²Cancer Research Program, Garvan Institute of Medical Research, Darlinghurst, Sydney, NSW, Australia
³Department of Biomedical Engineering, The University of Sydney, Sydney, NSW, Australia
⁴Dept of Endocrinology & Metabolism, Concord Hospital, Concord, Sydney, NSW, Australia

Aim: To define the role of the vitamin D receptor (VDR) in human breast cancer growth in-vitro and in-vivo. Methods and Results: Using stable shRNA expression, VDR expression was knocked down by 85% and 80% in MDA-MB-231 (MDA-VDRshRNA) and MCF-7 (MCF7-VDRshRNA) cells, respectively. Non-target (NT) cells served as controls. The induction of CYP24 mRNA by 1,25(OH)₂D₃, normally seen in VDR expressing cells, was completely abrogated in VDR-knock-down cells, indicating effective disruption of VDR signaling. Compared to untreated NT cells, treatment of MDA-NT and MCF7-NT cells with 10⁻⁸ M 1,25(OH)₂D₃ significantly reduced cell growth by 37% and 48%, resp., and induced a 2 to 3-fold increase in apoptosis. Surprisingly, MDA-VDRshRNA and MCF7-VDRshRNA cells also exhibited reduced growth rates (~40% and ~53%, respectively) and increased apoptosis (6 to 7-fold) compared to the corresponding NT cells cultured in ligand free conditions. Treatment of either knockdown cell line with 1,25(OH)₂D₃ treatment had no effect on growth or apoptosis. In MDA-VDRshRNA cells, the reduced cell growth was associated with decreased β-catenin and its target gene, cyclin D1 compared to MDA-NT cells.

In-vivo, MDA-VDRshRNA and NT cells were xenografted orthotopically into the mammary fat pad of nude mice. Again, VDR knockdown significantly reduced tumour growth from day 12 onwards. At day 33 post implantation, tumour weight was reduced by 40% and apoptosis was significantly increased in VDRshRNA compared to NT tumours (p<0.05). Similarly when MDA-NT and VDRshRNA cells were implanted into the tibiae of nude mice, lytic lesion size at endpoint (day 21) was significantly smaller in mice implanted with VDRshRNA than with NT cells (p<0.05).

Conclusion: The VDR has ligand-independent functions in regulating breast cancer cell growth which contrast with its ligand-dependent, anti-proliferative and pro-apoptotic effects. It appears that the ligand-independent actions of the VDR are mediated through the β-catenin signalling pathway.
INTERLEUKIN 6 MODULATES TUMOUR BEHAVIOR IN BONE VIA A RANKL DEPENDENT PATHWAY

Y. Zheng1,2, K. Boernert1,3, A. Mikuscheva1,3, D. Basel1,3, R. L. Sutherland2, F. Buttgereit3, C. R. Dunstan3,4, H. Zhou1, M. J. Seibelt1,2

1Bone Research Program ANZAC Research Institute, The University of Sydney, Concord, Sydney, NSW, Australia
2Dept of Rheumatology and Clinical Immunology, Charite University Medicine, Berlin, Germany
3Department of Biomedical Engineering, The University of Sydney, Sydney, NSW, Australia
4Dept of Endocrinology & Metabolism, Concord Hospital, Concord, Sydney, NSW, Australia

Aim: High levels of circulating interleukin-6 (IL-6) has been associated with poor outcomes in patients with metastatic breast cancer. We investigated the role of tumour-derived IL-6 using a xenograft model of skeletal metastasis.

Methods & Results: In-vitro studies using MDA-MB-231 cells demonstrated that RANKL up-regulates IL-6 expression by the cancer cells, while treatment with IL-6 significantly up-regulates their RANK expression. These results indicate that tumour-derived IL-6 may drive a positive forward-loop sensitizing the tumour cells to RANKL.

To define the role of tumour-derived IL-6 on tumour growth in bone, we knocked down (by 80%) IL-6 expression in MDA-MB-231 cells via lentiviral-transduced stable shRNA expression (MDA-KD). Non-target (NT) cells served as controls. Silencing IL-6 significantly reduced tumour cell invasiveness but not proliferation in-vitro. In-vivo, NT and MDA-KD cells were implanted intratibially into 4-week-old nude female mice kept on a low (0.1%) calcium diet to induce high bone turnover. Compared to NT cells, MDA-KD cells developed significantly smaller osteolytic lesions on day 10, 17 and 21, and tumour area at endpoint on day 21. When NT or MDA-KD cells were implanted into the mammary fat pad of nude mice, tumour growth was similar, indicating that the effects of tumour-derived IL-6 are mediated through the bone microenvironment.

In further experiments, IL-6 receptor signalling was disrupted by treating mice implanted intra-tibially with MDA-MB-231 cells with the anti-human IL-6 receptor antibody, Tocilizumab. Compared to controls, Tocilizumab at 50 mg/kg/3days i.p reduced the size of osteolytic lesions and tumour area. Mitotic activity decreased by 60% while apoptosis increased by 70%.

Conclusion: Our results identify a new ‘vicious cycle’ in bone in which RANKL induces cancer cell IL-6 and subsequent RANK expression leading to enhanced tumour growth. Targeting IL-6 activity is a potential treatment strategy in breast cancer bone metastasis.

EFFECT OF METHYL GLYOXAL EXPOSURE ON HYDROGEN PEROXIDE LEVEL IN PRE-OSTEOBLAST MC3T3E1 CELL LINE

I. Akbar1, H. Kalim2, D. W.A. Soeatmadji2, M. Hidayat3

1Orthopaedic surgery, Ulin General Hospital/ Faculty of Medicine, Lambung Mangkurat university, Banjarmasin, Indonesia
2Department of Internal Medicine, 2. Saiful Anwar General Hospital, Faculty of Medicine, University of Brawiyaya, Malang, Indonesia
3Orthopedic surgery,, 3. Saiful Anwar General Hospital, Faculty of Medicine, University of Brawiya, Malang, Indonesia

Aim: To study an effect of methyl glyoxal exposure on hydrogen peroxide level in pre-osteoblast MC3T3E1 cell line using confocal laser scanning electron microscope.

Methods: Pre-osteoblast MC3T3E1 cell line was obtained from American Type Culture Cell. Pre-osteoblast MC3T3E1 cell line was expose by methyl glyoxal on dose 0; 2.5; 5, 10, 20 mM for one hours then hydrogen peroxide level was analyzed by confocal laser scanning electron microscope. This study was done in Central Laboratory of Hayati, University of Brawiyaya, Malang, East Java, Indonesia.

Results: Non parametric test conclude there is no significant different of hydrogen peroxide level in all groups (p=0.083). Pearson correlation conclude there is positive correlation (r=0.582) significantly (p=0.023). It's mean that increasing of methyl glyoxal dose would be following increasing hydrogen peroxide level.

Conclusion: Methyl glyoxal exposure induces oxidant signaling in pre-osteoblast MC3T3E1 cell line.

Key words: pre-osteoblast, AGEs, oxidant, methyl glyoxal
DIFFERENCES IN IN VITRO PROLIFERATION RATES OF OSTEOPHLASTS FROM POLYNESIAN AND EUROPEAN PATIENTS

U. Baya1, D. Naot1, K. E. Callon1, R. Pitto1, J. Bentley1, J. Cornish1

1Medicine, University of Auckland, Auckland, New Zealand
2Surgery, University of Auckland, Auckland, New Zealand
3Middlemore Hospital, Auckland, New Zealand

Studies of bone tissue in Polynesians and Europeans in New Zealand showed that Polynesians have higher bone mineral density and substantially lower rates of hip fractures. Following joint replacement surgeries, the rate at which new bone over grows metal implants is much higher in Polynesians compared to patients from other ethnic populations.

Aim: Our hypothesis is that osteoblasts from Polynesian patients proliferate faster than those of European patients. To investigate this we compared the fraction of cells in S-Phase in osteoblasts cultured from bone samples taken from Polynesian and European patients.

Method: Trabecular bone samples collected from patients undergoing joint replacement surgery were chopped up, collagenase digested to remove any marrow present then placed in culture flasks to allow osteoblast outgrowth cultures to form. Resulting osteoblasts were grown to 50% confluency then fixed in 100% methanol. Further osteoblast cultures from the same bone pieces were used to make RNA and collect conditioned media. Osteoblasts fixed in methanol were labelled with propidium iodide and sorted using fluorescence-activated cell sorting (FACS). The modelling program ModFit LT was used to determine the proportion of cells in S-Phase.

Results: Osteoblast samples from three European and 13 Polynesian patients were used for FACS analysis up to now. We are currently collecting more osteoblasts to increase our sample size. Preliminary results show a trend towards a higher fraction of cells in S-Phase within Polynesian osteoblasts.

Conclusions: Our results suggest that a higher fraction of osteoblasts from Polynesian bone are in S-Phase than those from European bone indicating a higher rate of proliferation. RNA from these samples will be used to further study our hypothesis by investigating the regulatory mechanisms that determine the different proliferation rates of osteoblasts in the two ethnic populations.

PROTECTIVE EFFECT OF OLEANOLIC ACID ON BONE MESENCHYAL STEM CELLS IN OVX-INDUCED RATS BY STEM CELL MICROARRAY

Q. Bian1, S. F. Liu2, J. H. Huang1, D. Z. Tang2, Z. Yang2, Y. Ning1, Y. J. Zhao3, Y. J. Wang2, Z. Y. Shen1

1Institute of integrated T.C.M. & W.M., Huashan Hospital, Fudan University, Shanghai, China
2Department of Orthopaedics & Traumatology, Longhua Hospital, Shanghai University of T.C.M., Shanghai, China

Objective — It has been reported that oleanolic acid (OA) and its glycosides prevent bone loss by inhibiting osteoclasts formation. However, since bone formation and resorption is a balance in bone metabolism, there is still no evidence of the activities of OA on osteogenesis. The aim of the present study was to evaluate the osteoprotective effect of OA in ovariectomy (OVX) induced osteoporosis rats, and search for its molecular targets in bone mesenchymal stem cells (bMSCs).

Methods — Two-month-old female mice underwent OVX were treated with OA (20mg/kg/day). After 2 weeks (2w) and 3 months (3mon), bone mass was evaluated by microcomputed tomography , morphology and immunohistochemical methods. Meanwhile, 256 genes expression were measured via microarray, and confirmed by real time RT-PCR. The effects of OA on bMSCs activities were also observed in vitro.

Results — micro-CT displayed only bone loss tendency at 2w, but a decrease in bone mass at 3mon after OVX. OA promoted osteogenesis by increasing osteoblasts, elevating osteocalcin (OC) and runt-related protein 2(Runx2) proteins in vivo, and facilitating bMSCs osteoblastic differentiation in vitro at dose of 10-6, 10-5M. Gene expression profile analysis revealed that OVX resulted in a marked dysregulation of gene expression, especially at 2w, some of which were retrieved by OA. Few genes overlapped, but their functions were both involved in Notch signaling pathway between two phases of osteoporosis process.

Conclusion — OA promotes bone formation by promoting bMSCs osteoblastic differentiation. The molecular mechanism might be related to Notch signaling pathway and should be further confirmed.

(6) Stenderup K, Justesen J, Eriksen EF, Rattan SI, Kassem M. Number and proliferative capacity of osteogenic stem cells are
The mechanisms underlying the adverse effects of glucocorticoids on bone are not well understood. We recently demonstrated that osteoblast-targeted disruption of glucocorticoid signaling attenuates glucocorticoid-induced bone loss in mice, indicating that the adverse skeletal effects of glucocorticoids are predominantly mediated by osteoblasts. Here, we aimed to elucidate the effects of glucocorticoids on the gene expression profile in bone cells.

7-week old CD1 outbred mice were treated with corticosterone or placebo over 28 days. Blood was obtained weekly for biomarkers. RNA was isolated from tibia at the endpoint and Affymetrix Gene array analysis was performed (n=3 per group). Genes considered to be regulated were at least 1.5-fold differentially expressed. qRT-PCR was employed to validate the array results. By comparing gene expression profiles in RNA from mice treated with corticosterone or placebo, we found that corticosterone specifically regulated 391 genes. To further investigate the genes targeted by corticosterone treatment we performed gene ontology analysis with the aid of heat maps. We found the expression of genes implicated in osteoblast differentiation and the regulation of bone remodeling were downregulated in mice treated with corticosterone compared to placebo. We observed a downregulation of the osteoblast markers: osteocalcin, ALP, and SOST in corticosterone-treated mice compared to placebo. In addition Runx2, BMP4 and FOXO1 followed the same pattern.

Genes that were most profoundly downregulated in the array analysis were validated by qRT-PCR. Osteocalcin mRNA levels were suppressed to almost undetectable levels, consistent with serum levels of osteocalcin measured by IRMA. In addition, Runx2 expression was confirmed to be 2-fold lower in corticosterone treated mice. These results confirm that glucocorticoids primarily target genes involved in osteoblast differentiation and the regulation of bone remodeling. Gene expression profile analyses may point to the pathways involved in the negative effects of glucocorticoids on bone.

FUNCTION AND REGULATION OF EPITHELIAL SODIUM CHANNELS (ENAC) IN OSTEOBLASTS

J. Chen, L. Lu, G. Z. Yang, X. Y. Lu, C. Wan, J. Huang, Q. N. Li

School of Life Science and Biopharmacy, Guangdong Pharmaceutical University, GuangZhou, GuangDong, China

School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, HongKong, China

Department of Pathology, Medical College of Wisconsin, Milwaukee, WI, United States

The epithelial sodium channel (ENaC) is a new type of non-voltage dependent sodium ion channel and is essential for maintaining sodium homeostasis, transducing and mechanical stimuli. Osteoblasts (Ob) are mechano-sensitive cells which synthesize new bone matrix proteins. The expression of ENaC in bone and the relationship between ENaC and bone disease have been reported. Aim: Our study is to determine the ENaC function and regulation in Ob to discover the inner relationship between ENaC and osteoblasts. Method: The patch clamp, immunostaining, RT-PCR and western blot were used in this study. The siRNA PKGII was transfected into cells following the Lipofectamine protocol. Results: The expression of ENaC subunits in Ob was confirmed. The osteogenesis gene expression (ALP and Runx) were decreased while the silence ENaCα mRNA gene were introduced in the Ob. I

and the osteogenesis gene expression (ALP and Runx) were decreased while the silence ENaCα mRNA gene were introduced in the Ob. In addition Runx2, BMP4 and FOXO1 followed the same pattern.

These results confirm that glucocorticoids primarily target genes involved in osteoblast differentiation and the regulation of bone remodeling. Gene expression profile analyses may point to the pathways involved in the negative effects of glucocorticoids on bone.
MICRORNA-1 MEDIATES OXIDATIVE STRESS-INDUCED APOPTOTIC INSULTS TO OSTEOBlastS

R. Chen1,2, I. Lee1, Y. Lin2

1Graduate Institute of Medical Sciences, Taipei Medical University, Taipei, Taiwan
2Cell Biology and Molecular Image Research Center, Taipei Medical University-Wan Fang Hospital, Taipei, Taiwan

Osteoblasts contribute to bone formation. Injury of osteoblasts causes imbalance of bone remodeling, even leading to bone diseases. Oxidative stress is one of key factors to hurt osteoblasts during bone infection and inflammation. MicroRNA-1 (miR-1) has been reported to regulate cell survival and death. This study was aimed to evaluate the roles of miR-1 in oxidative stress-induced insults to osteoblasts. Exposure of rat osteoblasts prepared from neonatal calvaria to sodium nitroprusside (SNP), a nitric oxide (NO) donor, increased cellular NO levels in concentration- and time-dependent manners. Analysis of flow cytometry revealed that SNP time-dependently augmented amounts of intracellular reactive oxygen species. In parallel, exposure of rat osteoblasts to SNP altered cell morphologies and decreased cell viability. After exposure to SNP, activity of caspase-3 was significantly increased. Sequentially, SNP induced DNA fragmentation and apoptosis of rat osteoblasts. By mechanism, treatment of rat osteoblasts with SNP time-dependently induced miRNA-1 expression. Application of miR-1 antisense inhibitors into rat osteoblasts significantly knocked-down miR-1 expression and simultaneously lessened SNP-induced DNA fragmentation and cell apoptosis. In comparison, overexpression of miR-1 in rat osteoblasts synergistically increased SNP-induced apoptotic insults. Therefore, this study has shown that miR-1 plays critical roles in mediating oxidative stress-induced apoptotic insults to osteoblasts.

VERSATILE ROLES OF V-ATPASE ACCESSORY SUBUNIT AC45 IN OSTEOCLAST FORMATION AND FUNCTION

T. S. Cheng1, A. Qin1,2, N. J. Pavlos1, Q. Jiang2, J. Xu2, K. R. Dai2,3, M. H. Zheng1,3

1Orthopaedic Research, University of Western Australia, Perth, WA, Australia
2Department of Orthopaedics, Ninth People’s Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China
3Australian-China Joint centre for Bone and Joint Research, Model Animal Research Center of Nanjing University, Nanjing, China

Vacuolar-type H⁺-ATPases (V-ATPases) are multisubunit proton pumps that acidify intracellular cargos and deliver protons across the plasma membrane of a variety of specialized cells, including bone-resorbing osteoclasts. In osteoclasts, V-ATPases functions in extracellular acidification a process that initiates the dissolution of mineralized bone matrix and crucial for osteoclastic bone resorption. While the importance of V-ATPases in osteoclastic resorptive function is well-defined, whether V-ATPases facilitate additional aspects of osteoclast function and/or formation remains largely obscure. Here we report that the V-ATPase accessory subunit Ac45 participates in both osteoclast formation and function. Using a siRNA-based approach, we demonstrate that targeted suppression of Ac45 impairs intracellular acidification and endocytosis, both are prerequisite for osteoclastic bone resorptive function in vivo. Interestingly, knockdown of Ac45 also attenuates osteoclast formation owing to a reduced fusion capacity of osteoclastic precursor cells. In an effort to gain more detailed insights into the functional role of Ac45 in osteoclasts, we attempted to generate osteoclast-specific Ac45 conditional knockout mice using a Cathepsin K-Cre-LoxP system. Surprisingly, however, insertion of the neomycin cassette in the Ac45-Flox-lox mice resulted in marked disturbances in CNS development leading to embryonic lethality thus precluding functional assessment of Ac45 in osteoclasts and peripheral bone tissues. Based on these unexpected findings we propose that, in addition to its canonical function in V-ATPase-mediated acidification, Ac45 plays versatile roles during osteoclast formation and function.

LONG-CHAIN SATURATED FATTY ACIDS PROMOTE THE DIFFERENTIATION OF CELLS OF THE MARROW STROMAL LINE KUSA4B10 INTO ADIPOCYTES


Medicine, University of Auckland, Auckland, New Zealand

Body weight, and in particular fat mass, is positively correlated to bone mass. The relationships between fat and bone tissue are mediated directly through skeletal loading, and through hormonal and neuronal pathways. Feeding experiments showing that fat ingestion acutely influences bone turnover have raised the possibility that ingested nutrients have direct bone effects, and could function as an additional link between fat and bone. We have recently reported that long-chain, saturated fatty acids palmitic (C18) and stearic (C16) acids inhibit osteoclastogenesis in vitro, and that G-protein coupled receptors (GPR) for fatty acids are expressed in bone cells. Aim

Here we aim to show the effects of palmitic and stearic acids on adipogenic differentiation using the murine marrow stromal cell line, Kusa4b10.

Method
Kusa4b10 cells were cultured for 11 days in adipogenic medium with BSA-conjugated palmitic or stearic acid at concentrations of 10-75mg/mL. Adipogenesis was measured by Oil Red O staining and quantified by dye release and spectrophotometry. RNA extracted from the cultures at different time points was used for gene expression analysis by quantitative PCR.

Results
We found that palmitic and stearic acids potently induce adipogenesis in KUSA4b10 cells. Analysis of gene expression demonstrated significant induction of mRNA levels of the adipocytic genes adiponectin, AP2, adipin and PPARγ. We found high levels of expression of the long chain fatty acid receptor GPR120 in KUSA4b10 cells, and low expression levels of GPR40/41. Treatment of the cells with the GPR40/41 and GPR120 agonist GW9508 produced similar effects to fatty acid treatment: increases in adipogenesis and in levels of mRNA of adipocyte genes.

Conclusions
Our results identify the marrow stromal cell as a novel target of fatty acids. Palmitic and stearic acids not only impair osteoclastogenesis but also promote adipogenic differentiation of marrow stromal cells, possibly by signalling through GPR120 and GPR40/41.

---

INHIBITION OF ITAM ADAPTOR MOLECULES AND THEIR RECEPTORS BY INHIBITION OF CALCINEURIN-NFAT SIGNALLING DURING LATE STAGE HUMAN OSTEOCLAST DIFFERENTIATION

T. N. Crotti, M. S. Zawawi, A. A. Dharmapatni, M. D. Cantley, D. R. Haynes
Anatomy and Pathology, University of Adelaide, Adelaide, SA, Australia

The transcription factor nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 (NFATc1) is a crucial to osteoclastogenesis and plays a role in the immune system. In the osteoclast, the immunoreceptor tyrosine-based activation motif (ITAM)-dependent pathway co-induces NFATc1 via calcium signalling. ITAM receptors osteoclast-associated receptor (OSCAR) and triggering receptor expressed in myeloid cells (TREM2) pair with adaptor molecules Fc receptor common gamma chain (FcRγ) and DNAX-activating protein 12kDa (DAP12) respectively. Increased expression of ITAM factors in chronic inflammatory diseases like RA may exacerbate bone loss (ANZORS 2010). Inhibition of calcineurin-NFAT signalling reduces osteoclastogenic genes, including Beta 3 integrin, CTR and TRACP. We investigated the effect of inhibiting calcineurin-NFAT signalling on the expression of ITAM factors in human osteoclasts. Human peripheral blood mononuclear cells (PBMCs) were differentiated with RANKL and macrophage-colony stimulating factor (M-CSF) over a 10-day time course in the presence or absence of calcineurin inhibitors, FK506 (0.01, 0.1, 0.5μM) or the 11R VIVIT (1.0, 2.0, 5.0 μM). As expected, treatment significantly reduced osteoclast formation (TRACP staining) and activity (dentine pit resorption). Temporal expression of NFATc1, OSCAR, FcRγ, and TREM2 and the late stage osteoclast marker, Cath K were assessed by quantitative real-time polymerase chain reaction (RT-PCR). FK506 significantly reduced gene expression of NFATc1, OSCAR, FcRγ, TREM and Cath K at Day 10, ie the terminal stage of osteoclast formation. 11R-VIVIT also decreased NFATc1, OSCAR and FcRγ expression Day 10. Thus these inhibitors are most likely acting via the positive feedback loop following NFATc1 initial induction. Interestingly, TREM2 actually increased at Day 10 in the treated groups, reflecting the more specific action of 11R-VIVIT on NFAT-Calcineurin and corroborating reports that NFATc1 TREM2 does not directly induce TREM2. These data suggest calcineurin-NFAT inhibitors suppress key ITAM signalling factors differentially depending on the point at which they act in the calcineurin-NFAT signalling cascade.

---

REGULATION OF HUMAN OSTEOCLAST ACTIVITY AND APOPTOSIS BY IAP INHIBITORS IN VITRO

A. A.S. Dharmapatni1, J. W. Gillard2, K. D. Rainsford3, D. R. Haynes3
1Anatomy and Pathology, The University of Adelaide, Adelaide, SA, Australia
2Aegera Therapeutics Inc, Montreal Qc, Canada
3Biomedical Research Centre, Sheffield Hallam University, Sheffield, United Kingdom

Inhibition of apoptosis has been reported at sites of bone resorption in inflammatory diseases. Findings from our group have indicated that this is due to inhibition of executor caspases by endogenous Inhibitory Apoptotic Proteins (IAPs). This in vitro study investigates whether a novel IAP inhibiting compound induces osteoclast apoptosis and thus reduces bone resorption.

We used a potent small molecule IAP inhibitor to examine the effect of IAP inhibition on osteoclast differentiation and activity. Osteoclasts were derived from human peripheral blood mononuclear cells by treatment with recombinant receptor activator nuclear factor kappa B (RANKL) for 17 days. Osteoclast differentiation and activity was assessed by tartrate resistant acid phosphatase expression and resorption of dentine. The effect of IAP inhibition on mRNA expression of several key osteoclastic factors was assessed by real -time RT PCR. In addition, induction of apoptosis was assessed by Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) stain. The compound was particularly effective at inhibiting resorption, reducing resorptive area by more than 50% at concentrations equal to or above 10nM (p<0.05). The mRNA studies revealed that IAP inhibition did not affect important signaling factors during osteoclast formation or TRAP expression. Interestingly, the level of TUNEL associated with large multinucleated cells was markedly elevated by the IAP inhibitor.

Our results suggest that inhibiting IAPs to promote apoptosis significantly inhibits osteoclast resorption. This apoptosis appears to occur in mature osteoclasts as mRNA levels of factors expressed during osteoclast maturation were not affected whilst apoptosis was
markedly elevated in large multinucleated cells that formed during the assay. These results indicate compounds that inhibit IAPs might be used to inhibit osteoclast resorption in human disease.

294
INCREASED SOFAT MRNA EXPRESSION DURING OSTEOCLAST DEVELOPMENT, EVIDENCE THAT SOFAT IS NOT ONLY ASSOCIATED WITH T CELLS
A. Dharmapatni, D. Fitzpatrick, M. D. Cantley, D. R. Haynes
Anatomy and Pathology, The University of Adelaide, Adelaide, Australia

A novel secretory product of T cells, Secretory Osteoclastogenic Factor of Activated T cells (SOFAT), is reported to induce osteoclastogenesis in the absence of soluble receptor activator nuclear factor kappa B ligand (RANKL). SOFAT is a 27 kd molecules that shows homology with the amino acid sequence of a threonine synthase-like protein (THNSL2). Slight differences in sequence suggest SOFAT is an alternative splice variant of the THNSL2 gene. To date, no studies have been conducted to elucidate the possible role of SOFAT in mediating bone erosion associated with chronic inflammatory conditions, such as chronic periodontitis (CP) and peri-implant osteolysis (PO).

This study aims to investigate SOFAT mRNA expression by CP gingival tissue compare with normal healthy gingiva and PO compare with Osteoarthritic (OA) synovial. In addition, we investigate SOFAT mRNA expression during differentiation and maturation of peripheral blood monocytes (PBMC) derived osteoclast vitro

RNA was isolated from frozen sections of 9 chronic periodontitis and 6 normal gingival tissues and 8 tissue samples associated with PO were compared with 6 osteoarthritic (OA) controls. Culture of PBMC derived OC and Real time RT PCR was performed at day 0, 7, 10, 14, and 17 of culture. Correlation between SOFAT mRNA and CTR, NFATC1 mRNA and dentine resorption was also investigated.

Higher SOFAT mRNA was observed in CP than control, however, this difference was not significant statistically. In contrast, SOFAT mRNA was lower in PP than OA tissue. Interestingly increased SOFAT mRNA was observed in the late stages of osteoclast formation with statistically significantly higher levels found at the time osteoclasts mature. High SOFAT mRNA strongly correlated with CTR mRNA and dentine resorption but not NFATC1 mRNA.

The results indicate that SOFAT expression is not associated strongly with inflammatory induce osteolysis. However, SOFAT is expressed by pre-osteoclastic cells as osteoclasts mature.

295
HUMAN ADIPOSE TISSUE IS A BETTER SOURCE OF MESENCHYMAL STEM CELLS FOR OSTEOGENIC DIFFERENTIATION THAN UMBILICAL CORD TISSUE
T. J. Fernandes1, F. M. Collier2, J. M. Hodge3, M. A. Kirkland4, G. C. Nicholson4
1NorthWest Academic Centre, The University of Melbourne, Footscray, VIC, Australia
2Barwon Biomedical Research, The Geelong Hospital, Geelong, VIC, Australia
3Institute for Technology Research & Innovation, Deakin University, Geelong, VIC, Australia
4Rural Clinical School, School of Medicine, The University of Queensland, Darling Heights, QLD, Australia

Mesenchymal stem cells (MSC) are multipotent precursor cells that can differentiate into various cell types including osteoblasts (OB), adipocytes and chondrocytes. With their potential to form OB, MSC are ideal candidates for use in bone tissue engineering applications, however the optimum source remains controversial. This study aimed to (1) characterise and compare the osteogenic capacity of two sources of MSC: adipose tissue-derived (ATMSC) and umbilical cord-derived MSC (UCMSC); and (2) measure the effect of passage on osteogenic differentiation.

MSC were isolated from human umbilical cord (UC) and adipose tissue (AT) using a two-step digestion process and cultured for 3 consecutive passages. The cell surface markers CD34, CD45, CD73, CD90 and CD105 were analysed by flow cytometry at isolation and up to passage 3 (P0-P3). At P3, cells were cultured for 21d in the absence and presence of osteogenic factors for the assessment of alkaline phosphatase (ALP) production and bone mineralisation.

MSC at isolation expressed surface markers CD73 and CD90 (28% of MSC co-expressed CD73+/CD90+ in UCMSC & ATMSC). The expression of CD105, however, previously identified as a critical MSC marker, was absent at isolation and increased with passage to 84% and 95% of UCMSC and ATMSC, respectively, at P3. Although not considered a MSC marker, CD34 was expressed by ATMSC but not UCMSC. There was a trend in ALP increasing with passage for ATMSC o

passage to 84% and 95% of UCMSC and ATMSC, respectively, at P3. Although not considered a MSC marker, CD34 was expressed

The expression of CD105, however, previously identified as a critical MSC marker, was absent at isolation and increased with passage to 84% and 95% of UCMSC and ATMSC, respectively, at P3. Although not considered a MSC marker, CD34 was expressed by ATMSC but not UCMSC. There was a trend in ALP increasing with passage for ATMSC o

passage to 84% and 95% of UCMSC and ATMSC, respectively, at P3. Although not considered a MSC marker, CD34 was expressed by ATMSC but not UCMSC. There was a trend in ALP increasing with passage for ATMSC o

passage to 84% and 95% of UCMSC and ATMSC, respectively, at P3. Although not considered a MSC marker, CD34 was expressed by ATMSC but not UCMSC. There was a trend in ALP increasing with passage for ATMSC o

passage to 84% and 95% of UCMSC and ATMSC, respectively, at P3. Although not considered a MSC marker, CD34 was expressed by ATMSC but not UCMSC. There was a trend in ALP increasing with passage for ATMSC o

passage to 84% and 95% of UCMSC and ATMSC, respectively, at P3. Although not considered a MSC marker, CD34 was expressed by ATMSC but not UCMSC. There was a trend in ALP increasing with passage for ATMSC o

21d in the absence and presence of osteogenic factors for the assessment of alkaline phosphatase (ALP) production and bone mineralisation.

These data demonstrate that osteogenic potential of MSC varies according to source and passage, with AT possessing greater osteogenic potential. As AT is readily available and can be harvested in large quantities it is an ideal source of MSC for use in autologous bone tissue engineering applications.
INHIBITING EPFRINB2/EPHB4 BINDING PREVENTS PROGRESSION OF OSTEOBLAST DIFFERENTIATION AND ENHANCES OSTEOCLASTOGENIC FACTORS

P. W. M. Ho, S. Tonna, F. M. Takayar, N. A. Sims, T. J. Martin

Bone, St. Vincent’s Institute, Fitzroy, VIC, Australia

AIM: Ephrin/Eph family members are recognised as local mediators of cell function through contact-dependent processes in development and in maturity. A particular feature is their capacity for bi-directional signaling, in that when an ephrin acts upon its corresponding Eph receptor tyrosine kinase, the latter can signal in the reverse direction. EphB4 is the main receptor for ephrinB2; both are produced by osteoblasts, and ephrinB2 by osteoclasts. Published evidence shows that reverse signaling from EphB4 through ephrinB2 inhibits osteoclast formation, and that ephrinB2 formation is up-regulated by PTH and PTHR in osteoblasts in vitro and bone in vivo.

METHODS: Mouse calvarial osteoblasts and a stromal cell line (Kusa 4b10) differentiated in culture for 2, 7 14 and 21 days were treated with a recombinant soluble extracellular domain of EphB4 (sEphB4, an antagonist of both forward and reverse signaling) for 24 hrs.

RESULTS: Results showed inhibition by sEphB4 of mRNA for early and late osteogenic genes, such as osterix, DMP1, osteocalcin, and sclerostin, but increases in mRNA for osteoclastic factors including RANKL (6-8-fold), interleukin 6 (10-20-fold) and the oncostatin M receptor (OSMR) (4-fold). Similar results were obtained in both calvarial osteoblasts and Kusa 4b10 cells. This in vitro data indicates that blocking the interaction of ephrinB2 with EphB4 impedes progression of osteoblast differentiation. This is consistent with the phenotype of osteoblast-specific ephrinB2 knockout mice observed. The findings of increased RANKL, IL-6 and OSMR, together with the recognised inhibitory effect of ephrinB2 on osteoclast formation within the haemopoietic cells, were further tested in co-cultures of calvarial osteoblasts with bone marrow-derived osteoclast precursors, where a two-fold increase in osteoclast formation was observed with sEphB4 blockade of receptor binding.

CONCLUSION: It is concluded that ephrinB2/EphB4 signaling within the osteoblast lineage is important in osteoblast differentiation, as well as contributing to osteostageogenesis through several regulatory mechanisms.

MUTATION OF ALDH2 GENE SUPPRESSES OSTEOBLASTS DIFFERENTIATION BY ACCUMULATION OF ACETALDEHYDE AND PEROXIDATED LIPID-PROTEIN

H. Hoshi, H. Wu, Y. Toyama, T. Miyamoto

Orthopedic Surgery, Keio University School of Medicine, Tokyo, Japan

【Purpose】The increasing number of osteoporosis patients is becoming a health concern in countries with aging populations. It is well known that various factors such as estrogen loss, genetics and aging cause osteoporosis. In this study, we demonstrated about relation of gene mutation of aldehyde degradation enzyme (ALDH2) coding gene and bone homeostasis by utilizing ALDH2 dominant negative (ALDH-DAL) mice and elucidate molecular mechanisms of ALDH-DAL-induced osteoporosis.

【Methods】Two-month-old ALDH-DAL and wild-type mice were sacrificed and observed complete skeleton by soft x-ray, and were measured bone mineral density of individual left femurs by DEXA. Tibial bones dissected from these mice were analyzed morphometry using various parameters. Mouse primary osteoblastic cells were isolated from calvaria of newborn mice or from bone marrow cells of 3-month-old mice, and cultured to differentiation into osteoblast cells. Osteoblastogenesis was evaluated by cell staining such as alkaline phosphatase, Von Kossa and alizarin red, and by expression of osteostageogenesis genes and proteins by RT-PCR and western blotting, respectively.

【Results】ALDH-DAL mice exhibited osteoporosis and showed significantly reduced bone density by DEXA analysis. In morphometrical analysis, ALDH2-DAL trabecular and cortical bone thickness were thinner, and bone formation and mineralized rates were also decreased compared to that of wild type mice. ALDH-DAL mice accumulated acetaldehyde and peroxidated lipid protein, 4HNE, in blood plasma and osteoblasts, respectively. The osteoblastic cells of ALDH-DAL or acetaldehyde-treatment showed differentiation failure and higher expression of PPARγ that induces adipogenesis.

【Conclusion】This study indicates that the dominant negative form of ALDH2, ALDH-DAL, causes osteopenia owing to inhibited osteoblast differentiation and promotes increased aldehyde levels, and in turn induces hyper-accumulation of peroxidated lipid, 4HNE. Additionally, osteoblast cells of ALDH-DAL showed inhibit osteoblastogenesis and increasing expression of PPARγ and shifting to adipogenesis.
IDENTIFICATION OF A NOVEL PARTNER OF DAP12 IN THE REGULATION OF ACTIN-RING FORMATION IN OSTEOCLASTS

N. Ishida-Kitagawa, T. Ogawa
Graduated School of biological Sciences, Nara Institute of Science and Technology, Ikoma, Nara, Japan

Aim: Our group previously reported that transcription factor NFAT2/NFATc1 is essential for inducing the entire processes of multinucleated cell formation in osteoclastogenesis (1). To understand the mechanism of cell fusion and/or bone resorption, we tried to screen and analyze membrane proteins whose expressions are controlled by NFAT2.

Methods: Signal sequence trap by retrovirus-mediated expression screening (SST-REX) was conducted to screen genes encoding secreted/membrane proteins expressed in RAW264-derived osteoclasts. Expression of each gene during osteoclastogenesis was tested by RT-PCR with or without cyclosporine A. The function of gene of interest in mouse osteoclasts was studied by RNA interference.

Results: We identified 56 genes by SST-REX methods. Of these, #16 encoded a Type I membrane protein which was reported to associate with ITAM-harboring adaptor proteins DAP12. #16 mRNA appeared to be induced 48 hours after RANKL treatment in osteoclast precursor bone marrow macrophages and sustained until 96 hours when multinucleated osteoclasts were formed. Inhibition of NFATc1 by cyclosporine A or shRNA significantly decreased RANKL-induced #16 expression at both mRNA and protein level. Although multinucleated osteoclasts were formed from #16 knockdown bone marrow macrophages, cells showed a contracted shape with disordered actin-ring structure, accompanying significant reduction of bone resorption activity. Introducing wildtype #16 could recover the defects observed in #16 knockdown osteoclasts; however, transduction of the mutant form of #16, which loses the ability to interact with DAP12, could not rescue these defects.

Conclusions: Our results suggested that the membrane protein encoded with #16 is essential for the formation of functional osteoclasts in vitro through the regulation of actin-ring formation in cooperation with DAP12.

Acknowledgements: We would like to thank JSBMR for a travel award to this presentation.


IRON OVERLOAD COULD ENHANCE THE OSTEOCLAST DIFFERENTIATION AND ACTIVITY BOTH IN VITRO AND IN VIVO THROUGH THE PRODUCTION OF REACTIVE OXYGEN SPECIES

P. Jia
orthopaedics, No.2 affiliated hospital of Soochow University, Soochow, China

Aims: It has been gradually widely accepted that iron overload could lead to osteoporosis, the exact mechanism of which might comprise the inhibitory effect on osteogenesis. However, the effect of iron overload on osteoclast differentiation and its activity was controversial. This study was performed in order to clarify the association between iron overload and osteoclasts or bone resorption as well as the potential mechanism.

Methods: RAW264.7 were treated with ferric ammonium citrate (FAC, 0uM, 12.5uM, 25uM, 50uM) in the presence of 50ng/ml RANKL to observe the formation of osteoclast by stain for tartrate-resistant acid phosphatase (TRAP). TRAP-positive multinucleated cells having three or more nuclei were considered as osteoclasts. After the treatment with the indicated concentration of FAC for 24 hours, the reactive oxygen species in RAW264.7 was measured by 2,7-DCF-DA fluorescence using flow cytometry. RT-PCR was used to explore the expression level of TRAP, Cathepsin-K, MMP-9, RANK, NFATc1 after RAW264.7 was treated with 0uM FAC, 25uM FAC, 25uM FAC+10mM NAC, 10mM NAC respectively. The iron overload mice model and control group were established by the intraperitoneal injection of FAC (0.04g/kg) and normal for eight weeks, three times a week. Bone density of midportion of femur and the fourth lumbar vertebra were determined by in vivo imaging system. Bone marrow cells were obtained from the femur and tibia of iron overload group and control group, which were further cultured to observe the formation of osteoclasts. The serum ferritin, MDA, 8-OHGD, TRAP-5b, RANKL, OPG concentration were measured by ELISA.

Results: FAC could stimulate the formation of osteoclast and reactive oxygen species in a dose-dependent manner (p<0.05). 25uM FAC upregulated the expression level of TRAP, Cathepsin-K, MMP-9 and NFATc1, while such effect were partly blocked by NAC (p<0.05). The expression level of RANK exhibited no significant differences among groups (p>0.05). The intraperitoneal injection of FAC decreased the BMD of midportion of femur and the fourth lumbar vertebra (p<0.05). The number of TRAP positive cells differentiated from the BMM of iron overload mice was significant more than that of control group (p<0.05). The serum level of ferritin, MDA, 8-OHGD, TRAP-5b, RANKL in the iron overload group were markedly higher than those of control group (p<0.05), whereas iron overload mice had decreased serum OPG concentration (p<0.05).

Conclusion: Iron overload could stimulate the differentiation and activity of osteoclast which might be mediated by the production of ROS.
UMR-106 OSTEOBLAST CELLS EXPRESS COMPONENTS OF THE RENIN-ANGIOTENSIN SYSTEM AND ARE REGULATED BY GLUCOCORTICOIDs

C. Lei, K. MacRae, C. Sernia, W. G. Thomas

School of Biomedical Science, University of Queensland, Brisbane, QLD, Australia

Angiotensin II (AngII), a peptide hormone commonly associated with cardiovascular and renal function. However it is now emerging as a significant regulator of osteoblast function with osteolytic endpoints. There is evidence that osteoblasts express an “intrinsic” renin-angiotensin system (RAS) that results in the local production of AngII. It is known from studies in other tissues that glucocorticoids are powerful stimulators of RAS. It is therefore possible that the osteoporotic effects of excess glucocorticoid concentrations may encompass the stimulation of the osteoblast RAS. To test this hypothesis, the effect of dexamethasone (DEX) on the expression of components of RAS, namely AT receptors (AT1a & b; AT2) angiotensigen (AGT), renin and angiotensin converting enzyme (ACE) was investigated in the osteoblast cell line UMR-106. Methods: Serum starved UMR106 cells were treated with 10-7M DEX for different time intervals (0, 4, 8, 12, 24 hour), or with different doses (10^-4, 10^-6, 10^-7, 10^-8, 10^-10M). Reverse transcription-polymerase chain reaction (RT-PCR) using specific short probes primers for renin, ACE, AGT, AT1a, b, AT2 were used to quantify the response to DEX.

Results: Dexamethasone induced time-dependent increases in all components of RAS over a 24h period. Using a 24h end-point, DEX induced a significant 220%, 30%, 80%, 100%, 60% and 80% up-regulation in AT1a, b, AT2, AGT, renin and ACE expression respectively when compared to the unstimulated cells.

These data show that Dexamethasone increases the osteoblast expression of all the components of local RAS, which would result in increased local AngII production. Since AngII is a significant osteolytic agent it is possible that part of the osteoporotic effect of excessive glucocorticoid is mediated by AngII. Further studies are planned to directly test the involvement of AngII in DEX action on osteoblasts.


INFLUENCE OF HEPcidin ON INTRACELLULAR CALCIUM OF HUMAN OSTEoblasts

G. Li, Y. Xu

Orthopaedics, Second Affiliated Hospital of Soochow University, suzhou, Jiangsu, China

Hepcidin is known to increase intracellular iron through binding and degrading ferroportin, which is a transmembrane protein that transports iron from the inside of a cell to the outside. However, it is not clear whether hepcidin has similar effects on intracellular calcium. Here, we investigated the influence of hepcidin on intracellular calcium of human osteoblasts, with or without high iron environment. We found that hepcidin(<100nmol/L) could increase intracellular calcium, and it was more significant when cells were exposed to high iron environment. To further explore the underlying mechanisms, we pretreated cells with Dantrolene (ryanodine receptor inhibitor) and Nimodipine (L-type calcium channel blocker). These treatments resulted in no alteration of intracellular calcium. Thus, these findings indicate that the increase of intracellular calcium is probably due to calcium release from endoplasmic reticulum, which is triggered by calcium influx.

DOSE-DEPENDENT EFFECTS OF SELENIUM ON THE DIFFERENTIATION OF OSTEOCLASTS IN VITRO


Department of Toxicology, School of Radiation Medicine and Public Health, Soochow University, Suzhou Industrial Park, Suzhou, 215123, Jiangsu, China

[Selenium (Se) deficiency is an underlying cause of Kashin-Beck disease and bone growth retardation. The dose-dependent effects of Se on differentiation of osteoclasts (OCs) were studied in this study. [Methods] Non-adherent bone marrow cells were collected from a C57BL/6 male mouse. Cells were cultured in a medium containing 10%FBS, with different doses of sodium selenite. Cell Counting Kit-8 assays were performed to analyze the cytotoxicity. Intracellular ROS and mitochondrial membrane potential were detected by flow cytometry. To differentiate OCs, cells were seeded in a 96-well dish in the presence of RANKL under different concentrations of sodium selenite. Tartrate resistant acid phosphate (TRAP) staining was used for identifying OCs precursors; TRAP+ mononuclear cells were determined by enzyme-linked immunosorbent assay. [Results] Supplementary Se+ levels at or more than 200μg/L had cytotoxicity; lower mitochondrial membrane potential was seen in sodium selenite-treated cells when the supplementary Se+ level was less than 100μg/L; intracellular ROS levels in sodium selenite-treated cells were higher than in non-treated cells except when supplementary Se+ was 25μg/L; the quantities of TRAP+ mononuclear cells were (13 ± 3.43), (15.75± 3.83), (8.13 ± 1.90), (21.13 ± 2.80) and (24.87±9.13), respectively under supplementary Se+ levels of 0, 10, 25, 50, 100μg/L. The quantities of TRAP+ cells were (35.88±11.74), (165.75±51.58), (210.25±62.28), (31.38 ±12.42), (209.38 ±69.42), (365.63±68.32) and (357.87±63.98), respectively under supplementary Se+ levels at 0, 5, 10, 25, 50, 100, 150μg/L. The change
tendency of TRAP-5b coincided with the quantity change of OCs. [Conclusions] This study provided the dose-dependent effect of Se levels on the differentiation of OCs. Low and high supplementary Se levels significantly stimulated the OCs and OCs precursor formed. Interestingly, the supplementary Se⁺ level at 25μg/L showed no significant effect on the differentiation of OCs. [Key words] Kashin-Beck disease; osteoclasts; sodium selenite.

303

ANTI-APOPTOTIC BCL-2 FAMILY MEMBER MCL-1 POSITIVELY REGULATES CELL VIABILITY AND NEGATIVELY REGULATES BONE-RESORBING ACTIVITY OF OSTEOCLASTS

H. Masuda, J. Hirose, K. Nakamura, S. Tanaka

Department of Orthopaedic Surgery, Faculty of Medicine, The University of Tokyo, Bunkyo-ku, Tokyo, Japan

Mcl-1 is a member of Bcl-2 families that are known as regulators of apoptosis through mitochondrial pathway. Previous studies have suggested that an anti-apoptotic member Mcl-1 prolongs the cell viability of various cell types. However, only limited studies referred to the function of Mcl-1 in osteoclasts and it remains to be delineated. Our aim of this study was to elucidate the dynamics and the function of Mcl-1 in osteoclasts using techniques as overexpression, knockdown and knockout of Mcl-1 protein.

Mcl-1 protein levels in osteoclasts were increased in response to the addition of inflammatory cytokines such as TNF-α, IL-1α and LPS. Mcl-1 exhibited a short half-life in osteoclasts, which was significantly prolonged by a proteasomal inhibitor MG132. We also found that Mcl-1 was markedly ubiquitinated, indicating the involvement of ubiquitin-proteasome pathways in the degradation process of Mcl-1 in osteoclasts.

When Mcl-1 expression was down-regulated by siRNA retrovirus introduction, osteoclasts had significantly shorter cell life than that of control osteoclasts, in spite of no significantly change in the cell differentiation or cytoskeletal organization. Conversely, Mcl-1 overexpression significantly prolonged cell life without showing significantly change in the cell differentiation.

We then examined the effect of Mcl-1 on the bone-resorbing activity of osteoclasts using pit-formation assay. Mcl-1 overexpressed osteoclasts by the adenovirus vector exhibited reduced bone resoring area than control osteoclasts despite of their prolonged cell life. Mcl-1 knockout osteoclasts were generated by transducing Cre-recombinant adenovirus to osteoclasts from Mcl-1flox/flox mice. Bone resoring area of the Mcl-1 knockout osteoclasts was significantly increased despite of their short life time.

These results demonstrated that anti-apoptotic Bcl-2 family member Mcl-1 positively regulates cell viability and negatively regulates bone-resorbing activity of osteoclasts. Further studies are required to elucidate the role of Mcl-1 in physiological and pathological bone resorption by generating osteoclast-specific Mcl-1 knockout mice.

304

FUNCTIONAL ANALYSIS OF THE MICROTUBULE-BINDING DYNEIN-DYNACTIN COMPLEX IN OSTEOCLASTS

P. Y. Ng¹, T. S. Cheng¹, S. Ye², H. T. Feng¹, E. Ang¹, M. H. Zheng¹, J. Xu¹, H. Zhao³, N. J. Pavlos¹

¹Centre for Orthopaedic Research, University of Western Australia, Perth, WA, Australia
²Centre for Osteoporosis and Bone Metabolic Diseases, Department of Internal Medi, University of Arkansas for Medical Sciences, Little Rock, United States

Bone resorption by osteoclasts relies on 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 segregation of the ruffled border membrane. Cytoplasmic dynein, a processive 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. Despite its crucial involvement in a wide range of fundamental cellular processes, the contribution of the dynein-dynactin motor complex in osteoclasts remains unknown. Here, using a combination of complementary biochemical and morphological assays we have dissected the intracellular localization and function of the dynein-dynactin complex in osteoclasts. By subcellular fractionation and immunofluorescence confocal microscopy we demonstrate that the dynein-dynactin complex is highly expressed in mature osteoclasts and is intimately coupled to microtubules, undergoing dramatic reorganization upon the onset of osteoclastic polarization. In bone-resorbing osteoclasts, p150Glu20 and CLIP170, both major constituents of the dynactin and CAP-Gly domain-containing microtubule plus end-associated proteins, exhibit distinct polarization at the osteoclastic resorative front, thus orientating the ruffled border as the microtubule plus-end domain. Interestingly, disruption of the dynein-dynactin complex via p50dynamin-over-expression retards the formation and maturation of osteoclasts, owing to a delay the mitotic stasis of mononuclear progenitor cells. Moreover, uncoupling of the dynein-dynactin motor from microtubules coincides with a drastic redistribution of key osteoclastic intracellular organelles, including the Golgi and lysosomes. Finally, we provide evidence that the dynein-dynactin complex is required for the targeted positioning and delivery of cathepsin K to the ruffled border membrane, and thus constitutes an integral component of the osteoclastic bone resorption machinery. Collectively, these data unveil an unexpected yet versatile role for the dynein-dynactin motor in osteoclast formation and function.
THE MAMMALIAN TARGET OF RAPAMYCIN COMPLEXES 1 AND 2 MEDIATE RESPONSES TO STRONTIUM RANELATE IN PRIMARY HUMAN OSTEOLASTS

M. S. Rybchyn1, A. D. Conigrave2, R. S. Mason1
1Physiology & Bosch Institute, University of Sydney, Sydney, NSW, Australia
2School of Molecular Bioscience, University of Sydney, Sydney, NSW, Australia

We have recently reported that strontium promotes canonical Wnt signaling in primary human osteoblasts (HOBs) via both the activation of an Akt dependent pathway and a decrease in the protein expression of sclerostin (Rybchyn et al, 2011). We previously showed in HOBs that after strontium treatment, Akt is phosphorylated via two pathways; (1) at Thr208 downstream of PI3-kinase mediated activation of PDK1; and (2) at Ser273, known to be via the mammalian target of rapamycin (mTOR) complex-2 (mTORC2).

In the current study we show strontium-dependent activation of mTOR complex-1 (mTORC1) in HOBs via phosphorylation at Ser248 and increased protein expression of the mTORC1 specific regulatory associated protein of mTOR (Raptor). The expression of the mTORC2-associated rapamycin-insensitive companion of mTOR (Rictor) was unaffected by strontium treatment in HOBs. In the current study we also report that the bone cell-positive effects of strontium in HOBs, including increased replication, increased differentiation and decreased stress-induced apoptosis are significantly inhibited by rapamycin – a drug with a range of effects in humans that inhibits cell growth by binding to FKBP12 and blocking mTORC1 activity. These findings implicate mTOR as an essential regulatory factor in exerting the osteogenic effects of strontium ranelate in humans.

(1) Rybchyn MS et al J Biol Chem Online May 12, 2011 (http://www.jbc.org/cgi/doi/10.1074/jbc.M111.251116)

MEASUREMENT OF INDUCED MEMBRANE-BOUND RANKL ACTIVITY IN STIMULATED OSTEOSTEBLASTS

P. P. Singh1, J. M.W. Quinn1,2, A. G.J. Van Der Kraan1,2, J. Xu3, M. T. Gillespie1,2
1Bone, Joint and Cancer, Prince Henry’s Institute, Clayton, VIC, Australia
2Dept of Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia
3School of Pathology and Laboratory Medicine, The University of Western Australia, Nedlands, WA, Australia

Introduction: Osteoclast formation depends on stimulation by osteoblast-derived membrane bound RANKL that is elicited by numerous osteotropic factors. While RANKL regulation can be studied indirectly by examining mRNA levels, it is highly desirable to determine the net activity of the protein on the osteoblast surface.

Aim: To develop a method of measuring cell surface RANKL activity using a co-culture cellular response assay.

Methods: Mouse osteoblasts and osteoblastic Kusa O cells were cultured 24h with 1,25(OH)2 vitamin D3 (1,25(OH)2D3; a strong osteotropic stimulus); RANKL protein was analysed by western blot/immunoprecipitation. RAW264.7 cells were stably transfected with luciferase reporter constructs under control of either κB (NFκB-RAW) or NFAT (NFAT-RAW) response elements. Co-culture assays consisted of osteoblastic cells stimulated for 24h prior to NFκB-RAW or NFAT-RAW cell addition, with luciferase measurement at 6h or 24h subsequent.

Results : Western blot of 1,25(OH)2D3 stimulated osteoblasts yielded little signal; clear signal for RANKL was seen in immunoprecipitated cell membrane preparations of stimulated osteoblasts, but was difficult to quantify due to proximity to immunoglobulin heavy chain bands. NFκB-RAW and NFAT-RAW cells dose-dependently responded to recombinant RANKL particularly at 24h stimulation (3 to 10 fold induction) and was blocked by RANK.Fc. 1,25(OH)2D3-stimulated Kusa O cells gave a large (4-8 fold) induction in luciferase signal in co-cultured NFκB-RAWs (ablated by RANK.Fc); osteoblasts gave a much weaker (2 to 3.5 fold) response perhaps due to higher OPG production. NFAT-RAWs gave a less responsive signal. Kusa O-NFκB-RAW co-cultures gave highly reproducible responses to strong osteolytic stimuli such as oncostatin M, and showed RANKL suppression by TGβ3.

Conclusions: NFκB-RAW cells gave an easily measurable response to RANKL elicited in stromal cells. Limitations included use of stimuli that directly elicit NFκB in RAW264.7 cells (e.g. TNF). Nevertheless, such co-cultures offer simple and rapid methods of assessing RANKL activity.

EPHRINB2 SIGNALLING IN OSTEOSTEBLASTS IS REQUIRED FOR NORMAL OSTEOSTEBLAST DIFFERENTIATION AND OSTEOSTEBLAST MATURATION

S. Tonna, F. Takyar, I. J. Poulton, P. W.M. Ho, N. E. McGregor, T. J. Martin, N. A. Sims
Bone Cell Biology, St Vincent’s Institute, Fitzroy, VIC, Australia

AIM & METHODS: The ephrin/Eph receptor tyrosine kinases are critical for tissue-remodeling processes including cellular migration, angiogenesis and axon guidance. Through direct cell contact, ephrin ligands bind to multiple Eph receptors, resulting in either forward or reverse signaling. EphrinB2/EphB4 signaling has been reported to regulate osteoblast and osteoclast differentiation in vitro, and osteoblastic ephrinB2 is upregulated by PTH and PTHrP. This study focused on the role of signaling by osteoblastic ephrinB2 in osteoblast and osteoclast differentiation by histology and cell culture studies of mice with an osteoblast-specific deletion of ephrinB2 (OexCre.EphrinB2/ff).
RESULTS: OsxCre.EphrinB2f/f primary calvarial osteoblasts expressed 70% less ephrinB2 mRNA than littermate Cre negative EphrinB2f/f cells at all time points studied; importantly, mRNA levels for EphrinB2-binding receptors, EphB2, EphB4 and EphA4 were unchanged. OsxCre.EphrinB2f/f neonates were osteopetrotic. Although chondrocyte maturation proceeded normally, trabecular bone volume and cartilage remnants were significantly increased and osteoclast number was reduced compared to Cre-negative littermates. In addition, although plentiful osteoblasts were observed in OsxCre.EphrinB2f/f mice, osteoid deposition was delayed, confirming impaired osteoblast function despite enhanced osteoblast proliferation. Consistent with the low level of osteoclast formation in vivo, OsxCre.EphrinB2f/f primary calvarial osteoblasts demonstrated a 70% lower level of RANKL mRNA at day 15 and 21 of differentiation compared to Cre negative controls. In adult OsxCre.EphrinB2f/f mice, osteoclast numbers remained low, and although osteoblast formation was enhanced, osteoblast function, indicated by mineralising surface / bone surface was reduced. CONCLUSIONS: Impaired bone formation in these mice was consistent with effects of the specific EphrinB2:EphB4 antagonists (sEphB4 and TNYL), which impair expression of late osteoblast markers. In contrast, these inhibitors increased expression of RANKL. This suggests that while osteoblast activity depends on EphrinB2:EphB4 signalling, impaired osteoclast formation in Osx.EphrinB2f/f mice may relate to the loss of signaling of EphrinB2 through its alternate receptors, EphB4 or EphA4.

308
HSP90 INHIBITORS ENHANCE OSTEOCLAST FORMATION IN VITRO IN A NFATC1 INDEPENDENT MANNER THROUGH THE INDUCTION OF STRESS RESPONSES
A. G. J. Van Der Kraan1,2, R. Cha1, M. M. Kouspou1, J. Xu1, M. T. Gillespie1,2, J. T. Price2, J. M. W. Quinn1,2
1 Bone Joint and Cancer Group, Prince Henry's Institute, Clayton, Australia
2 Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia

Introduction: Breast cancer cells metastasise to bone where they proliferate and provoke osteoclast-mediated bone destruction. Previously we found that 17-allylamino-17-demethoxygeldanamycin (17-AAG; a Hsp90 inhibitor and anti-cancer therapeutic) increases tumour growth within bone in an intracardiac MDA-MB-231 inoculation mouse model. 17-AAG also increases osteoclast formation in vitro and in vivo despite Hsp90 client proteins being important to tumour progression and osteoclast formation. Aim: To define the effects of Hsp90 inhibition on osteoclast formation and examine the potential roles of heat shock factor-1 (HSF1)-induced cellular stress.

Methods: Osteoclast formation levels were examined using RANKL-stimulated RAW264.7 cells and bone marrow cells. HSF1 activity was inhibited by either knock down of HSF1 by specific GIPZ shRNA mir lentiviral constructs or pharmacological inhibition by KNK437. Western blot and luciferase reporter assays were used to examine NFATc1 and NFXB signals in RAW264.7 cells. Stress responses were observed using Western blot detection of HSF1 and HSP70.

Results: 17-AAG and second-generation but structurally unrelated Hsp90 blockers, CCT018159 and NVP-AUY922, dose-dependently enhanced osteoclast formation from bone marrow and RAW264.7 cells; these compounds induce stress responses [1-2]. The Hsp90 inhibitor novobiocin did not induce stress or osteoclast formation responses. Two shRNA mirs that knocked down HSF1 in RAW264.7 cells greatly decreased 17-AAG actions. Pharmacological inhibition of HSF1 by KNK437 blocked 17-AAG effects, but not TGFβ1-enhancement (which is not HSF1 dependent) of osteoclast formation. 17-AAG treatment did not increase NFXB or NFAT1 activity- critical for osteoclast formation- suggesting that the Hsp90 inhibitor action occurs downstream of these factors in a stress response-dependent manner. Consistent with this, RANKL treatment itself rapidly induced Hsp70, suggesting stress induction.

Conclusions: These results indicate Hsp90 inhibition induces a stress response, which causes osteoclast formation to be enhanced in a HSF1-dependent manner. The mechanism of action does not involve NFATc1 but may enhance some stress-dependent action of RANKL on osteoclast progenitors.


309
CALCIUM AS AN ANABOLIC STIMULUS OF OSTEOBLAST DIFFERENTIATION TO AN OSTEOCYTE-LIKE PHENOTYPE
K. J. Welldon, D. M. Findlay, G. J. Atkins
Orthopaedics and Trauma, University of Adelaide, Adelaide, SA, Australia

Calcium, in combination with vitamin D, has been shown to be effective in the treatment of osteoporosis, a condition typically associated with a low BMD, increased fracture risk and a reduced osteocyte number. Bone mineralization, the deposition of Ca2+ as hydroxyapatite-like calcium phosphate, occurs in lamellar bone concurrent with osteoblast to osteocyte transition. We hypothesised that Ca2+ provides an anabolic stimulus for osteoblast differentiation. The aim of this study was to investigate the effect of Ca2+ on differentiation of adult human primary osteoblasts (NHBC) to osteocytes in vitro.

NHBC were cultured under conditions permissive for mineralization in the presence of a wide range of Ca2+ concentrations (1.8 - 26.8 mM). Experiments were performed in the presence or absence of an inhibitor of the calcium sensing receptor (CaSR), NPS2390.
Cultures were assayed for mineralization by examining levels of cell-associated calcium and phosphate, using histochemical analyses. The expression of genes associated with osteoblast differentiation and mineralization, including dentin matrix protein 1 (DMP1), E11, SOST/sclerostin, MEPE and PHEX, were measured by real-time RT-PCR.

NHBC could tolerate even the highest concentration of Ca2+ used. Treatment with Ca2+ resulted in a striking dose- and time-dependent increase in mineralization. RNA encoding the osteocyte markers sclerostin, E11 and DMP1 were elevated in the mineralized cultures. Gene expression was differentially regulated by Ca2+. For example, at 5mM Ca2+ there was a time-dependent increase in E11 mRNA levels. This response was not changed by the addition of the CaSR inhibitor NPS2390. Increasing calcium levels caused decreased expression of the mineralization inhibitor MEPE and increased levels of mineralization promoter PHEX, consistent with the roles of these molecules to respectively inhibit or promote mineralization.[1] This study suggests that Ca2+ promotes the differentiation of human osteoblasts to the osteocyte phenotype and further work will focus on elucidation of the mechanisms involved.

(1) Atkins, G.J., et al., Sclerostin is a locally acting regulator of late-osteoblast/pre-osteocyte differentiation and regulates mineralization through a MEPE-ASARM dependent mechanism. J Bone Miner Res,
CHANGES OF BLOOD GASES AND HEMODYNAMIC PARAMETERS IN PATIENTS UNDERGOING VERTEBRAL BALLOON KYPHOPLASTY IN MULTIPLE LEVELS

K. Alpantaki1, A. Hadjipavlou1, M. Tzermiadanos1, X. Souvatzis2, P. Katonis1
1Orthopaedics, University of Crete, Heraklion, Greece
2Anesthesiology, University of Crete, Heraklion, Greece

Aim: The purpose of this study was to evaluate the safety of balloon kyphoplasty (BK) by the course of hemodynamic, oxygenation and ventilation parameters in dependence of number of vertebral levels treated, and type of cement.

Methods: This is a prospective study of 58 patients. Twenty two patients treated for compressive fractures (OCF), 8 for traumatic and 24 for osteolytic tumors (OT). Mean patient age was 64.8 years. Twenty six patients were treated at 1 level, 15 at 2, 2 at 3, 5 at 4, 1 at 5, 3 at 6, and 2 at 7. Methylmethacrylate (PMMA) was used in 38 patients whereas calcium phosphate in 20. All patients were treated under general anesthesia with constant monitoring of invasive blood pressure, heart rate, peripheral oxygen saturation and regional cerebral oxygen saturation. Arterial blood gases were measured before balloon inflation, 2 minutes after cement insertion for each level and in the recovery room.

Results: Two patients had a transient drop in blood pressure by ≥ 20% during simultaneous inflation of the balloons at 2 levels and three patients had a drop in blood pressure during cement injection (2 PMMA, and 1 calcium phosphate). Five patients had a cement leak and one of them had a moderate and transient drop in blood pressure. Five patients had changes in blood gases (two of them during balloon inflation and 3 during cement injection).

Conclusion: BK appears to be safe when applied for multiple levels in the same sitting, provided general anesthesia and close monitoring. Its rare circulatory effects are not related with the number of levels treated or the cement type.

TWO-YEAR EXPERIENCE OF INTRAVENOUS ZOLENDRONIC ACID IN A WOMEN'S HOSPITAL SANG, T H YEO

S. Ang
Family Medicine Service, KK Women's and Children's Hospital, Singapore, Singapore

Introduction: Yearly intravenous zolendronate (Aclasta) ensures compliance and avoids the gastrointestinal side effects of oral bisphosphonates. This study aims to look at the adverse reactions and effect on renal function with administration of intravenous Aclasta in the Singapore population. Methods: This is a prospective observational study on the patients who received intravenous Aclasta 5mg for treatment of osteoporosis using a standard protocol established in the KK Women's and Children's Hospital. Adverse reactions and creatinine clearance were monitored from March to December 2010, 16 patients, aged between 56 and 87 were treated with intravenous Aclasta. Patients were loaded with oral Vitamin D3 of 3000 IU and elemental calcium carbonate of 600mg daily for 1 month prior to the intravenous infusion. Pre-infusion tests include serum calcium, phosphate, liver function test and renal function test. Infusion was done over 30 min and patients were monitored for an hour before being discharged. Adverse reactions were monitored via phone on 3rd day after infusion. Results: 62.5% of the patients suffered flu-like symptoms and 12.5% experienced fever, giddiness, joint pain or headache. Most of the symptoms lasted 3 days and there was no disturbance to function after 3 days. 25% of the patients did not report any side effects. 69% of patients who had post creatinine clearance test done, had normal creatinine levels. Conclusion: Minor short term adverse reactions are common in patients undergoing intravenous Aclasta but are short-lived and usually resolve after 3 days. Monitoring of post-infusion renal function did not show any negative effect.

INHIBITING HISTONE DEACETYLASES EXPRESSED IN HUMAN PERIODONTAL TISSUES PREVENTS BONE LOSS IN AN ANIMAL MODEL OF PERIODONTAL DISEASE

M. D. Cantley1, D. P. Fairlie2, P. Bartold3, V. Marino3, D. R. Haynes1
1Discipline of Anatomy and Pathology, School of Medical Sciences, University of Adelaide, Adelaide, SA, Australia
2Institute for Molecular Bioscience, University of Queensland, Brisbane, QLD, Australia
3Colgate Australian Clinical Dental Research Centre, School of Dentistry, University of Adelaide, Adelaide, SA, Australia

Histone deacetylase inhibitors (HDACi) have been shown to suppress bone resorption. Class I HDACs are found in the nucleus and include 1,2,3 and 8 while class II are also found in the cytoplasm and include HDAC 4,5,6,7,9 and 10. We recently reported a novel HDACi, 1179.4b which targets both HDAC classes suppressed human osteoclast resorption in vitro via down regulation of TRAF-6. This current study investigated the expression of specific HDAC enzymes near sites of bone loss in periodontitis human tissues. The effect of 1179.4b compared HDACi MS-275 targeting class I HDACs on bone loss was also assessed in a mouse model of periodontitis.

Expression of HDACs was assessed by real time PCR. Human gingival tissue samples from patients with chronic periodontitis (n=9) were compared with normal gingival tissues (n=8). Periodontitis was induced in mice via oral inoculations with P.gingivalis bacteria over 13 weeks. Once disease was established mice were treated with 1179.4b (1mg/kg/day) and MS-275 (10mg/kg/day) daily for 6 weeks (n=4 per group). Live animal micro CT analysis, stereo imaging and histological analysis were used to assess changes in bone.
HDACs from both classes I (1 and 8) and II (5 and 9) were highly expressed in periodontitis tissues relative to normal tissue. In the animal model 1179.4b treatment significantly reduced bone loss (p<0.01) as assessed by micro CT analysis. MS-275 did not have a significant effect. Histological analysis revealed that 1179.4b had no effect on inflammation. This study demonstrates HDACs in both classes are upregulated in periodontitis tissue compared with normal. The novel dual class HDACi, 1179.4b, was found to reduce alveolar bone loss in an in vivo model of periodontitis. While further studies are needed the findings indicate that HDACi treatment could be used for the treatment of periodontitis in humans in the future.

315

STRONTIUM RANELAT IN MALE OSTEOPOROSIS - PERSONAL EXPERIENCE

C. Cirstoiu1, M. Cirstoiu2, D. Popescu2, A. Popescu2, R. Ene4

1Orthopaedics, University Hospital Bucharest, Bucharest, Romania
2University Hospital Bucharest, Bucharest, Romania
3University Hospital Bucharest, Bucharest, Romania
4University Hospital Bucharest, Bucharest, Romania

Male osteoporosis is an increasingly important public health problem. Osteoporotic fractures occur later in life in men and fracture-related morbidity and mortality are even higher than in women. Diagnosis and treatment of osteoporosis are often delayed especially in men even after fragile fracture caused by osteoporosis. It is frequent that an appropriate treatment for osteoporosis has not been provided in men. Is important that safe and effective therapies for this disabling condition become available. Strontium ranelate (SR) is the first medication to uncouple bone formation from bone resorption. It has shown antifracture efficacy at all sites in a large number of postmenopausal women, but data on its use in male osteoporosis are insufficient. We have studied the efficacy of SR on BMD after 12 months in 20 males aged 70 years with primary osteoporosis. Patients were randomized to SR 2 g/day and supplemented daily with 1000 mg calcium and 800 IU vitamin D. The main outcome measure was percent change in lumbar spine and total hip BMD from baseline. Mean BMD (+/- SD) increased by 5.5 +/- 3.6% at the lumbar spine and 3.4 +/- 2.8% at the total hip with SR. In our study, SR produced significantly increases in BMD over 12 months. Mean increases in BMD with SR in men were similar to those previously documented for this agent in postmenopausal women, suggesting that similar benefits on anti-fracture efficacy may be expected.

(1) C. Cirstoiu, M. Cirstoiu et al - Osteoporoza in Menopauza, Ed Carol Davila Bucuresti

316

PERCUTANEOUS VERTEBROPLASTY: IS IT A CAKE WALK? LESSONS LEARNT

B. Dave

Staya Spine Hospital, Ahmedabad, India

STUDY DESIGN: Retrospective analysis of operated percutaneous vertebroplasty cases - single centre study
AIMS: Vertebral compression is frequently seen in elderly patients due to various causes like osteoporosis and bone marrow disorders (malignancy). We analysed our cases who had undergone percutaneous vertebroplasty for complications and to highlight the pitfalls.
METHODS: 39 patients operated at SSHRI in last 5 years. All these patients had undergone percutaneous vertebroplasty with biopsy for fragility fracture with normal neurology, following conservative treatment for four to six weeks with analgesics and anti-osteoporotic drugs.
RESULTS: Three patients were found to have abnormal biopsy report. Two had infection and one had myeloma. In five patients the cement leaked per-operatively. But, none had adverse systemic effect or neurological consequences. Adjacent near or distal fracture occurred in one patient who was non compliant for medical management of osteoporosis. One patient had symptomatic pulmonary embolism with uneventful further outcome. All patients at one year follow up also never achieved the pre-fracture functional status.
CONCLUSION: Rapid and substantial relief of pain and improvement in the quality of life are observed following percutaneous vertebroplasty, with few technical pitfalls. But, usually the patients never return back to their previous functional level and the fragility fracture is the beginning of gradual deteriorating ordeal of age. Biopsy is always mandatory. Adjacent level fracture not observed. Medical treatment is no substitute.
LOWER FAT MASS AND LOWER BONE FORMATION PREDICT GREATER BONE LOSS WITH TENOFOVIR IN HIV-INFECTED ADULTS

P. R. Ebeling1, H. Haskelberg1, J. Hoy1,4, J. Amin1, S. Emery2, A. Carr5
1NorthWest Academic Centre, University of Melbourne, Western Hospital, Footscray, VIC, Australia
2The Kirby Institute, University of New South Wales, Sydney, NSW, Australia
3Monash University, Melbourne, VIC, Australia
4Alfred Hospital, Melbourne, VIC, Australia
5St. Vincent’s Hospital, Sydney, NSW, Australia

STEAL was a randomised, simplification trial that found bone mineral density (BMD) decreased with tenofovir/emtricitabine (TDF/FTC) compared with abacavir/lamivudine (ABC/3TC). We aimed to examine predictors of TDF/FTC associated bone loss. We determined predictors of change in right-proximal femur and lumbar-spine BMD, by DXA, in STEAL participants (per-protocol [PP]) through Wk96. Pre-defined sub-analysis included participants not on TDF or ABC at baseline (“naïve”). Bone turnover markers (BTMs) tested were: formation (BALP, P1NP), resorption (CTx) and cytokine-signalling (osteoprotegerin). Independent predictors of BMD change were determined using forward stepwise linear regression. Randomised groups were compared at Wk96 for BTM changes and 10-year fracture risk (FRAX®) by t-test, and for proportions warranting antiresorptive therapy (US NOF guidelines) using contingency-table and chi-square test.

Baseline characteristics (98% male, mean age 45 years, current TDF 29%, current ABC 20%, current protease-inhibitor 23%) of PP (n=301, 84%) and ‘naïve’ (n=157, 43%) populations were similar to the main study population. In PP population, baseline predictors of greater femur bone loss were TDF randomisation (p=0.001), lower total fat mass (p trend=0.009), lower P1NP (p=0.015), and higher hip t-score (p trend=0.006). Baseline predictors of greater spine bone loss were TDF randomisation (p=0.013), lower total fat mass (p trend=0.005), PI use (p=0.004), and higher spine BMD (p=0.001). TDF increased BTMs through Wk96 (P1NP and CTx; both p<0.01) but no BTM change at Wk12 predicted bone loss. No significant between-group difference was found in 10-year fracture risk, or in proportions (5% per group) reaching NOF thresholds. BMD and BTM changes in naïve-population were of similar magnitude.

In this study, TDF was associated with bone loss, whereas higher fat mass was protective. Lower baseline bone formation predicted greater femoral BMD decrease. TDF was associated with greater increases in bone formation and resorption, but not fracture risk thresholds, in both per-protocol and TDF-naïve populations.

THE NATURAL HISTORY OF PERIACETABULAR OSTEOLYTIC LESIONS

D. M. Findlay1, S. D. Neale1, R. Stamenkov2, D. W. Howie1,2
1Orthopaedics and Trauma, University of Adelaide, Adelaide, SA, Australia
2Orthopaedics and Trauma, Royal Adelaide Hospital, Adelaide, SA, Australia

Aim
Osteolysis (OL) adjacent to an acetabular component remains the most challenging complication of total hip replacement in the medium to long-term. Knowledge of how osteolysis develops and progresses is critical to develop optimal management and therapeutic approaches to this condition. We have previously shown that OL is strongly related to the extent and rate of wear of the polyethylene (PE) liner (1), and that PE particles have potent bio-activity in bone cell cultures (2). The aim of this study was to perform long-term monitoring of the progression in size of osteolytic lesions and to determine risk factors for progression.

Methods
Using sequential high-resolution multi-slice computed tomography (CT) scans with metal artefact suppression, we measured periacetabular osteolysis volumes over a period of up to nine years in a cohort of 26 patients with 30 cementless acetabular components of more than ten years duration at the initial CT. Associations were determined between the progression in size of osteolytic lesions and osteolysis rate at the initial CT, patient age, gender, walking limitations and activity level.

Results
Progression in size of osteolytic lesions was very variable between patients, with a mean of 1.6cm³/yr (range 0.7-5.7cm³/yr). Of the variables examined, the amount of osteolysis at the initial CT and patient activity gave prediction of osteolytic lesion progression. The strongest predictor of osteolytic lesion progression was when these two risk factors were combined (p=0.0024).

Conclusions
This study provides the first long-term results of monitoring by CT of osteolytic lesions adjacent to radiographically stable cementless acetabular components. These lesions have a variable natural history, which is related to the lesion size and to patient activity. The data suggest that, used together, these factors could be useful in predicting the progression in size of periacetabular osteolytic lesions.

(1) 1. Howie et al., JBJS(Am), 89, 1818, 2007
(2) 2. Atkins et al., Biomat, 30, 3672, 2009
HEALTH KNOWLEDGE AND DIETARY INTAKE RISK FACTORS LEADING TO OSTEOPENIA AND OSTEOPOROSIS IN UNIVERSITY STUDENTS


Clinical nutrition, umm al qura university, Makkah, Makkah, Saudi Arabia

ABSTRACT

Background: Nutrition one of the most important factors influencing human health, nutrition plays a role in the etiology of osteoporosis disease. It's a serious metabolic bone disorder that often results in hip fracture and is usually asymptomatic in its initial stages. Since the majority of bone formation occurs during childhood and adolescence. Objective: Assess the knowledge and health beliefs, on osteoporosis, among 19–24 year olds in full-time education in the KSA. This is believed to be an age where peak bone mass, a significant factor in osteoporosis and fracture risk, can be influenced. Methods: A cross sectional study was carried out during the period from 1/1/2010 to 30/6/2010 among a random sample of (257) university female students were chosen from Umm Al Qura from Makkah. The age for sample from 19-24 years old. Data were collected through an interview with case by using special questionnaires. Bone mineral density (BMD) has been measured using bone densitometry device on students wrist using BeamMed made by (sunlight), 7000/8000 series, Type/CSB serial No.5718. Body composition by using Bodystat®1500. Results: Osteoporosis was present in 7% of cases while, osteopenia was current in 32.3% of cases. Moreover there was a highly positive significant relationship at level (1%) between osteoporosis induced and each of weight, height, waist, hip, fat %, fat weight, lean weight, BMR, waist/hip ratio and BMI. However it was a negative significant correlation at the same level between osteoporosis induced and each of age and BMR/waist ratio. Conclusion: A trend of osteoporosis correlated significantly positive (p<0.001) with body fat however it was negatively correlated with age. By other meaning, prevalence of osteoporosis among university students was significant association with raising of body fat and decrees of age. The study results suggested that inevitable decrease of carbonated beverages, taking into consideration variety and balance of diets and nutrition education programs for improving bone health and nutritional status.

EVALUATION OF BETA TRICALCIALUM PHOSPHATE IN HUMAN INFRABONY PERIODONTAL OSSEOUS DEFECTS: A CLINICAL STUDY

M. Gupta1, P. Kinra2

1Periodontics, Maulana Azad Institute of Dental Sciences, Delhi, India
2Periodontics, Punjab Government Dental College and Hospital, Amritsar, Punjab, India

The present study was conducted to evaluate the efficacy of β-tricalcium phosphate (Synthograft)® as a regenerative material in periodontal osseous defects in comparison with open flap debridement in patients suffering from osteoporosis. Clinical evaluations (Plaque index, Gingival index, Probing depth, Clinical attachment level, Gingival recession) were carried out at baseline and 6 months both for test and control sites. Intrasurgical measurements were done at the time of surgery and at re-entry for both test and control sites to assess the percentage and linear bone fill with the material. 12 patients showing clinical and radiographic evidence of almost identical bilateral infrabony defects were selected from amongst those reporting at the Department of Periodontics and Oral Implantology, Maulana Azad Institute of Dental Sciences, New Delhi. It was observed that β-tricalcium phosphate was well tolerated by the patients with no incidence of any adverse reaction during the entire course of the study. The intrasurgical measurements were evaluated at baseline and at 6 months (re-entry). These measurements were compared using ANOVA for the test and control sites. It was found that the mean percentage bone fill and bone fill (in mm) in test sites was greater than in control sites and the values were statistically significant.

In conclusion, within the constraints of this study, β-tricalcium phosphate has been shown to have a regenerative potential, and hence can be successfully used in the treatment of periodontal infrabony defects even in patients with osteoporosis.
FOQS STUDY (STUDY ON FACTORS FOR OSTEOPOROSIS QUALITY-OF-LIFE IN JAPANESE SUBJECTS • J -INTERIM REPORT

H. Hagino¹, T. Sugimoto², S. Souen³, K. Tanaka⁴, N. Endo⁵, R. Okazaki⁶, T. Nakamura⁷

¹Rehabilitation Division, Tottori University, Yonago, Japan
²Internal Medicine I, Shimane University, Izumo, Japan
³Department of Orthopedic Surgery and Rheumatology, Kinki University, Nara, Japan
⁴Department of Food and Nutrition, Kyoto Women’s University, Kyoto, Japan
⁵Division of Orthopedic Surgery, Niigata University, Niigata, Japan
⁶Third Department of Medicine, Teikyo University, Chiba, Japan
⁷Department of Orthopedics, University of Occupational and Environmental Health, Kitakyushu, Japan

• y Aim ▪ z The aim of this study is to investigate changes of QOL, pain, and relevant factors for Japanese osteoporosis patients on once weekly bisphosphonates (BP) treatment.
• y Methods ▪ z 5,904 Japanese female osteoporosis patients (55 years old and over) on BP treatment were enrolled at 308 study centers between November 1, 2009 and April 30, 2011 and are being followed for two years. Medical record review and patient survey are being conducted at the time of enrollment (M0) as well as 3 (M3), 6 (M6), 12, and 24 months after enrollment. We are collecting information on patient background (e.g., age, concurrent diseases, pharmacotherapy), QOL scores (Japanese Osteoporosis Quality of Life Questionnaire (JOQOL), EQ-5D), and pain scores (Visual Analogue Scale (VAS), McGill Pain Questionnaire (MPQ, Japanese version)). Interim analysis was conducted on data collected from 3,233 patients for M0, 2,091 patients for M3, and 1,264 patients for M6.
• y Results ▪ z The average age was 73.4 (55-98) years old among the 3,233 patients, with 79% having concurrent diseases (e.g., hypertension, diabetes, osteoarthritis). Regarding QOL, the total JOQOL scores of M0, M3, and M6 were 65.9, 66.6, and 67.2, respectively, and the M3 and M6 scores were significantly increased in comparison with M0 (P<0.001). EQ-5D utility scores and EQ VAS of M3 and M6 were also significantly increased from M0 (P<0.001). VAS scores of M3 and M6 were also significantly improved from M0 (P<0.001).
• y Conclusion ▪ z Improvement in QOL and pain scores of Japanese osteoporosis patients on BP treatment for 6 months was observed consistently in all indicators used.

INFLUENCE OF BMI ON BONE STRUCTURE AT THE WEIGHT-BEARING AND NON-WEIGHT-BEARING SKELETAL REGIONS IN PREMENOPAUSAL WOMEN

K. Huh¹, X. Wang², A. Ghasem-Zadeh³, Q. Wang⁴, E. Seeman²

¹Department of Oral and Maxillofacial Radiology, Seoul National University, Seoul, Sth Korea
²Department of Endocrinology and Medicine, Austin Health, University of Melbourne, Melbourne, VIC, Australia

Many, but not all, studies suggest that body mass index (BMI) is a protective factor for fragility fractures. BMI may influence the severity of impact or bone structure partly due to the mechanical load imposed on the skeleton. We hypothesized that BMI is associated with bone structure in upper and lower limbs but the association will be stronger in the lower limb. 138 Caucasian premenopausal women were included with a mean age 35 yrs (range 19-50 yrs). Distal metaphyses of radius and tibia of non-dominant side were scanned using high-resolution pQCT (XtremeCT, Scanco). They were stratified into 4 groups according to their BMI (< 20, 20-25, 25-30, and >30) controlling for age and height.

There was no difference in bone size, cortical volumetric bone mineral density (vBMD) or trabecular thickness between groups at either site. Women with greater BMI had greater cortical volume, trabecular BV/TV and trabecular number and smaller trabecular separation. A greater group difference in the cortical volume was found in tibia than radius (skeletal sites X BMI group interaction p = 0.007) (fig). Thus, BMI was associated with differences in morphology within a site but the only morphological difference between sites to suggest a loading effect was a higher cortical volume. Mechanisms responsible remain to be identified.

EVALUATION OF THE RELATIVE EFFICACY OF AN ALLOGRAFT USED ALONE AND THAT IN COMBINATION WITH SIMVASTATIN IN THE TREATMENT OF HUMAN PERIODONTAL INFRABONY DEFECTS – A CLINICAL AND RADIOLOGICAL STUDY

P. Krishna¹, M. Gupta²

¹Periodontology/Oral Implantology, (Formerly at) Punjab Government Dental College and Hospital, AMritsar, Delhi, Delhi, India
²Periodontology/Oral Implantology, Maulana Azad Institute of Dental Sciences, Delhi, India

Background: Simvastatin has been found to cause increased bone formation in vitro and in animal studies. However, its effect on periodontal reconstruction in humans is yet to be determined. Aims: With this study an attempt was made to evaluate the effects of a combination of this drug with DFDBA and also to compare the efficacy of this combination with that of DFDBA alone in the
Treatment of human periodontal defects. Materials and Methods: Fifteen patients with almost identical bilateral 2-walled or 3-walled infrabony defects were selected. Defects on the right side (Group A) were treated with the placement of DFDBA alone while those on the left side (Group B) were treated using a combination of DFDBA and a 10-8 M solution of the drug simvastatin. Two clinical parameters, namely probing pocket depth and clinical attachment level and one radiographic parameter, namely infrabony defect depth were measured preoperatively and 12 and 24 weeks postoperatively. Statistical Analysis: The data were subjected to two types of statistical analyses, viz. Student's t-test and ANOVA approach in order to evaluate the individual and relative efficacies of the two treatment modalities. Results: DFDBA alone as well as the combination of DFDBA and simvastatin resulted in a highly significant mean reduction in probing depth, gain in clinical attachment level, and linear defect fill. The values of mean changes in parameters were significantly greater with the drug-graft combination in comparison with the graft alone. Conclusions: Combination of DFDBA with a solution of simvastatin leads to significantly greater reduction in probing depth, gain in clinical attachment level, and linear defect fill than when the graft is used alone in the treatment of human periodontal infrabony defects.

324

VASCULAR ENDOTHELIAL GROWTH FACTOR: ASSOCIATION WITH BONE LOSS IN COPD PATIENTS
E. A. Kochechova, L. G. Ugay, K. A. Burya, U. V. Maistrovskaya
Medical State University, Vladivostok, Russian Federation

Aim: to assess the relationship between lung function parameters and bone loss with circulation vascular endothelial growth factor (VEGF) in patients with chronic obstructive pulmonary disease (COPD).

Methods: 47 patients with stable COPD (29 with bronchitis type COPD and 18 patients with emphysema) and 23 controls subjects without airflow obstruction were included in the study. Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (DEXA) at the lumbar spine (LS), left femur neck (FN) using a Lunar Prodigy Densitometer (USA). We also estimated concentration of VEGF in serum (VEGFser) and pulmonary function.

Results: We identified a decreased BMD (T-score < -1 SD) in 43/47 patients, as measured on the FN or LS. T-score was lower in patients with emphysema compared to bronchitis type of COPD (p<0.05). There was a direct correlation with forced expiratory volume in 1 second (FEV1) both bronchitis type of COPD and emphysema (r=0.53, p=0.03 and r=0.57, p=0.02, respectively). The median concentrations of VEGFser were significantly higher in serum of COPD patients, but significantly lower in patients with emphysema compared to normal control (p<0.05 and p<0.01, respectively). The concentration of VEGFser from patients with COPD correlated inversely with FEV1 (r=0.73; p=0.0004); in contrast, there was a positive correlation between these two measurements in patients with emphysema (r=0.79; p=0.0002). The VEGFser correlated with BMD (r=0.64; p=0.002) only in the patients with emphysema.

Conclusion: Thus, our finding suggest that VEGF may affect the pathogenesis of these two disease of emphysema and osteoporosis.

325

ANAMNESTIC RISK FACTORS MORE INDICATE OSTEOPOROSIS (REDUCED BONE MORPHOGENIC DENSITY) THAN BONE SPECIFIC LABORATORY VALUES – A STUDY OF 78 PATIENTS WITH METAPHYSEAL LONG BONE FRACTURES
L. Kolios1, C. Takur2, A. A. Moghaddam2, M. H. Hitzler2, A. Suda2, H. Schmidt-Gayk3, B. Höner4, P. A. Grützner2, C. Wältle1

1Department for Plastic-, Reconstructive and Handsurgery, Burn Care Centre, BG Unfallklinik Ludwigshafen, 67071 Ludwigshafen, Germany
2Department of Trauma and Orthopaedic Surgery, BG Unfallklinik Ludwigshafen, 67071 Ludwigshafen, Germany
3Clinical Laboratory Limbach, 69126 Heidelberg, Germany
4Dpt. of Social and Legal Siences, SRH Hochschule Heidelberg, 69126 Heidelberg, Germany

Objectives: Osteoporosis is a major health problem worldwide, and is included in the WHO list of the top 10 major diseases. However, it is often undiagnosed until the first fracture occurs, due to inadequate patient education and lack of insurance coverage for screening tests.

Study design: In our study of 78 patients with metaphyseal long bone fractures, we searched for a correlation between anamnestic risk factors, bone specific laboratory values, and the bone morphogenic density (BMD). Each indicator was examined as a possible diagnostic instrument for osteoporosis. The secondary aim of this study was to demonstrate the high prevalence of osteoporosis in patients with metaphyseal fractures.

Results: 76.9% of our fracture patients had decreased bone density and 43.6% showed manifest osteoporosis in DXA (densitometry) measurements. Our questionnaire, identifying anamnestic risk factors, correlated highly significantly (p=0.01) with reduced BMD, whereas seven bone-specific laboratory values (p=0.046) correlated significantly.

Conclusions: Anamnestic risk factors correlate with pathological BMD more than bone-specific laboratory values. The medical questionnaire used in this study would therefore function as a cost-effective primary diagnostic instrument for identification of osteoporosis patients.
BONE MINERAL DENSITY, QUANTITATIVE ULTRASOUND MEASUREMENT AND CALCIUM INTAKE AMONG POSTMENOPAUSAL CHINESE WOMEN

M. C. Kruger1, P. Hu2, J. M. Todd3, B. Kuhn-Sherlock4, E. Lau5, J. Ma6, G. Qin7, L. M. Schollum4

1Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand
2CCBR, Beijing, China
3Fonterra Brands Ltd, Auckland, New Zealand
4Fonterra Research Centre, Palmerston North, New Zealand
5Centre for Health and Medical Research, CCBR, Hong Kong
6Beijing Friendship Hospital, Beijing, China

Aim: The purpose of the present study was to measure bone mineral density, blood biochemistry and to assess calcium intake in a cohort of post-menopausal women in Beijing, China.

Methodology: A cohort of 124 Chinese women at least 5 years post-menopause was selected from a research facility database. The women were selected as possible participants in an intervention trial and those with T-score < -2.5 (by dual energy x-ray absorptiometry, DXA) were not included. Bone mineral density was obtained using DXA (GE Lunar Prodigy Advance), heel quantitative ultrasound measurements (QUS)(Achilles Insight, GE Healthcare) were taken, and anthropomorphic parameters were measured. Blood samples were taken for blood minerals, haematology, liver and kidney function and nutritional status. Calcium intake was estimated using a 24 hour recall.

Results: The women had a mean age of 62±5 years old (range 49-78) and a mean BMI of 25.7kg/m²±2.4 (range 17.2-30.0). Fasting blood glucose levels were 5.55±0.90mmol/L (range 3.85-11.88). Serum calcium measurements were normal at 2.42±0.07mmol/L (range 2.22-2.62). Using DXA, the mean femoral neck T-score was -0.61±0.72 (range -2.13-1.59) and for lumbar spine (L1-L4) -0.53±0.45 (range -1.78-1.74). The mean T-score obtained using QUS, was -0.39±1.35 (range -3.1-3.1). Estimated dietary calcium intake was 309±118mg (range 13 - 498). The correlation between the QUS T-score and femoral T score was 0.38 (P=0.00), and with the lumbar spine T-score 0.43 (P= 0.00). In this population, using the WHO T-score of -1 for osteopenia, QUS tended to underestimate the women at risk compared to DXA.

Conclusion: The women had a very low dietary calcium intake, though normal to low bone density. T-score using QUS was correlated with DXA of the lumbar spine and the femoral neck, though QUS tended to underestimate low bone mass.

The study is funded by Fonterra Brands (Singapore) Pte Ltd.

PATIENT CHARACTERISTICS AND RISK FACTORS OF OSTEOPOROSIS IN URBAN INDIAN PATIENTS ATTENDING AN ORTHOPEDIC CLINIC FOR BACK PAIN

S. Lakhotia

Orthopaedic, CMRI, Kolkata, West Bengal, India

Background: Osteoporosis is a silent disease and becomes clinically significant in the presence of fragility fracture. Identifying risk factors that are associated with osteoporosis in the community is important in reducing the incidence of fragility fracture.

Objectives: The objective of this study was to characterize the severity of osteoporosis in urban Indian patients attending an orthopedic clinic for back pain. The other objectives were to characterize the signs and symptoms (back pain, fragility fractures) in patients suffering from osteoporosis, to characterize the patient population (demographics, medical histories, osteoporosis risk factors, prior and co-medications etc.) receiving various treatments for osteoporosis. The effect of available therapies on the evolution in the bone mineral density was also studied in a small cohort of 87 patients. The prevalence of other co-morbid conditions and its possible effects on the therapy were also studied.

Methods: This is a single center study to determine the prevalence of osteoporosis risk factors in sample of urban Indian population aged more than 45 yrs attending a orthopedic clinic for over 4 years. All patients with low back pain, regardless of past or present therapeutic regimens, were eligible for inclusion in the study. The patients were recruited regardless of past or present therapeutic regimens. A structured, interviewer supervised questionnaire was used to collect information on demographic data and details of risk factors. Bone mineral density measurements were carried out using a total body DEXA machine. The study was conducted under normal clinical practice where the available epidemiological and safety data corresponding to certain specific and clinically relevant parameters was captured at relevant time points.

Results: A total of 1162 patients (83% females and 17% males) with low back pain were screened for BMD scores and patient characteristics. The average age of patients screened was 59.28 yrs (45 – 76) S.D ± 10.98 yrs. As per the WHO classification 231 (19.88%) were classified as normal and 931(80.12%) were classified as having osteopenia or osteoporosis with average lumbar spine T-Score of -2.23 with SD of ±1.06 and average right hip femur neck T-Score of -1.68 with SD of ±0.61.

The severity characteristics amongst the 931 patients was 404 (43.4%) had osteopenia, 361 (38.77%) had osteoporosis and 166 (17.83%) had severe osteoporosis. 111 (11.9%) patients had vertebral fractures. Hypertension (29%) and diabetes mellitus (17.9 %) were the most common co-morbid conditions.

Of these 87 patients (89.65% females and 10.35% males) were followed up for the evolution in their BMD. The average age of patients was 60.66 yrs (45 – 76) S.D ± 9.95 yrs.

All the 87 patients received calcium 1000 mg/day, of these 46 patients additionally received alendronate 70 mg/week for 2 years. The average lumbar spine T-Score at baseline of the patients who received only calcium was -2.38 with SEM ± 0.19. The average lumbar spine T-score of patients with calcium and alendronate was -2.57 with SEM ± 0.16.
After 2 years the T-Score of the patients who received only calcium was -1.82 with SEM ± 0.21 and T-score of patients with calcium and alendronate was -2.00 with SEM ± 0.16. The change in both the groups was significant when compared to baseline at P<0.001. But the change in calcium with alendronate group was not significant when compared to the calcium only group.

In the calcium group the BMD of the lumbar spine at baseline was 0.901 gm/cm² with SEM of ± 0.23 and after 2 years was 0.962 gm/cm² with SEM of ±0.26. Whereas the baseline BMD of patients with calcium and alendronate was 0.881 gm/cm² with SEM of ± 0.15 after 2 years was 0.950 gm/cm² with SEM of ±0.18 Both these changes were also significant at P<0.001, but changes in lumbar spine BMD in calcium with alendronate group was not significant when compared to the calcium only group.

The reasons for lack of significant improvement could be severe cases selected for bisphosphonate treatment, lack of compliance with treatment, deficiency of Vit D in patients.

Conclusion: The data from this study shows that Indian patients especially postmenopausal women suffer from osteoporosis and osteopenia higher than those reported in the western population. Compliance is key factor for success of anti-osteoporosis therapy. Necessary steps are needed so as to avoid progression of osteoporosis and its complications.

328

ANALYSIS OF AXIAL SKELETON DEVELOPMENT IN TYPE 2 SPONDYLOCOSTAL DYSOSTOSIS MODEL MESP2(-/-) MOUSE, SUGGESTING AN ESSENTIAL ROLE OF THE SEGMENTATION CLOCK OUTPUT FOR PROPER ALTERNATE ARRANGEMENT OF VERTEBRAL BODIES AND INTERVERTEBRAL DISCS

Y. Makino1,2, Y. Takahashi3, R. Tanabe4, K. Hata5, M. Goseki-Sone4, J. Kanno3, Y. Saga5, K. Kaneko1, A. Yamaguchi2, T. Iimura2

1Department of Orthopedic Surgery, Juntendo University, Bunkyo-ku, Tokyo, Japan
2Section of Oral Pathology, Global Center of Excellence (GCOE) Program, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan
3Cellular & Molecular Toxicology Division, National Institute of Health Sciences, Setagaya-ku, Tokyo, Japan
4Department of Food and Nutrition, Faculty of Human Sciences and Design, Japan Women’s University, Bunkyo-ku, Tokyo, Japan
5Department of Molecular and Cellular Biochemistry, Graduate School of Dentistry, Osaka University, Suita-city, Osaka, Japan
6Division of Mammalian Development, National Institute of Genetics, Mishima-city, Shizuoka, Japan

Segmental feature of spine is endowed by somitogenesis during embryogenesis, which is regulated by the segmentation clock involving periodic on-off of Notch signaling in spine precursors. Mutations in the Notch signaling pathway genes, delta-like 3 (DLL3), mesoderm posterior 2 (MESP2), lunatic fringe (LFNG) and hairy and enhancer-spirit 7 (HES7) in human led to distinct genotype-phenotype classification of spondylocostal dysostosis (SCDO) types 1, 2, 3 and 4, respectively. The clinical features of SCDO include a loss of normal vertebral morphology. Mesp2 is a target of Notch signaling and converts the clock signaling into periodic array of somite formation. To understand how the loss of the segmentation clock output impacts on spine development and differentiation, we analyzed chondrogenesis and osteogenesis in developing vertebrae of Mesp2 (-/-) mice. Wild-type and Mesp2 (-/-) mice (E16.5 and E18.5) were analyzed by 3 dimensional computed tomography (3D-CT) and histological observations. The expression patterns of the molecules related with bone, cartilage and intervertebral disc were assessed by immuno-fluorescence and in situ hybridization.

3D-CT analysis showed that vertebral morphology was most severely affected at the thoracolumbar level in Mesp2 (-/-) mice. Immunofluorescence stainings by anti-phospho-Smad2 and -Smad1/5/8 and successive 3D fluorescence morphometry exhibited that the graded inputs of TGF-beta and BMP could dissociate presumptive region of vertebral disc from intervertebral disc region. The expression pattern of the molecular markers and the 3D fluorescence of anti-phospho-Smads clarified that, in Mesp2 (-/-) mice, vertebral bodies in thoracolumbar level largely fused at mid-axial region without significant changes in the process of endochondral ossification whereas intervertebral discs tended to fuse peripherally in vertebral column. Our observation suggested that the segmentation clock output is crucial for proper alternate arrangement of vertebral bodies and intervertebral discs, which involves spatial regulation of the graded TGF-beta and BMP signalings.

329

CALCIPHYLAXIS IN NORTHERN AUSTRALIA: AN EMERGING CONCEPT

U. H. Malabu1, V. Manickam2, K. S. Sangla1

1Endocrinology, Townsville Hospital, Townsville, QLD, Australia
2Renal Medicine, Townsville Hospital, Townsville, QLD, Australia

Objective: Calciphylaxis is characterised by vascular calcification and painful skin necrosis occurring predominantly in end-stage renal failure (ESRF). Diabetes and ESRF, the two common risk factors of the syndrome are higher among the indigenous Australian subset of the population. Though calciphylaxis is said to be rare whether the high prevalence of risk factors of the disease in the community will result in high rate of the syndrome is not known. The aim of the study was to review course and clinical cases of calciphylaxis at the regional hospital.

Methods: All patients admitted to the hospital from 1st March 2006 to 28th February 2011 with diagnosis of calciphylaxis were studied.
Results: Seven patients were reviewed comprising 5 females and 2 males. All except one were Caucasians. Only one Australian aborigine was recorded. Aetiology of the ESRF was diabetes in 4 subjects while chronic glomerulonephritis and obstructive uropathy contributed one in each. Only one patient confirmed to have calciphylaxis had normal renal function; the cause of it was due to vitamin D deficiency associated with severe weight loss. Five of 7 patients representing 71% died. The causes of death: sepsis in 3 whose wounds failed to heal and acute myocardial infarction following completely healed skin lesions in the other 2. Of the 2 surviving subjects, a male still has partially healed ulcer on follow up as out patient. The other, a female who presented with normal renal function responded well to pamidronate infusion.

Conclusion: Calciphylaxis is rare in subjects with normal renal function and in indigenous Australians occurring predominantly in Caucasians with ESRF. It carries poor prognosis with deaths from sepsis in those with active disease and from myocardial infarction after successful treatment of the skin lesion. Further prospective studies on a larger population are needed to verify our findings.

330

RISK FACTORS FOR MULTIPLE FALLS IN A LONGITUDINAL POPULATION-BASED COHORT STUDY IN JAPAN: THE ROAD STUDY
S. Muraki¹, T. Akune¹, H. Oka², K. Nakamura³, H. Kawaguchi¹, N. Yoshimura²
¹Clinical Motor System Medicine, 22nd Century Medical and Research Center, The University of Tokyo, Tokyo, Japan
²Joint Disease Research, 22nd Century Medical and Research Center, The University of Tokyo, Tokyo, Japan
³Sensory and Motor System Medicine, Faculty of Medicine, The University of Tokyo, Tokyo, Japan

Aim: The aim of the present study was to determine the incidence of multiple falls and the risk factors.

Methods: Among the 3,040 participants in the baseline study of the ROAD study, 2,485 subjects (81.7%) participated in the follow-up study. Among the 2,485 subjects, 2,215 subjects (745 men and 1,470 women; mean age, 68.0 years) were analyzed. These subjects filled questionnaires regarding their falls between the baseline study and the follow-up study and questionnaires regarding their knee and lower back pain in the baseline study. They underwent radiography of the knee and the lumbar spine. At the baseline, physical ability was also estimated by measuring the grip strength and the walking speed at the usual pace. Knee osteoarthritis (OA) and lumbar spondylosis were defined as Kellgren/Lawrence grade ≥ 3.

Results: The mean duration between the baseline and follow-up studies was 3.3 ± 0.6 years. During this period, 261 subjects (11.8%; 84 men and 177 women) fell multiple times. A logistic regression analysis without adjustment showed that age (odds ratio, 1.02; 95% confidence interval, 1.01–1.03), grip strength (0.98, 0.97–1.00), walking speed [m/min] (0.98, 0.97–0.99), radiographic knee OA (1.86, 1.35–2.52), and knee pain (2.07, 1.56–2.73) were significantly associated with multiple falls, while radiographic lumbar spondylosis (0.93, 0.72–1.21) and lower back pain (1.21, 0.88–1.66) were not. Next, to determine the independent associations of each variable with multiple falls, a multiple logistic regression analysis was performed with age, gender, grip strength, walking speed, radiographic knee OA, and knee pain as independent variables. Walking speed [m/min] (0.98, 0.98–0.99) and knee pain (1.27, 1.08–1.48) were found to be independently associated with multiple falls.

Conclusion: This longitudinal study showed that lower walking speed and knee pain were risk factors for multiple falls.

331

COMPUTER SIMULATION-BASED MODELING OF THE PHARMACEUTICAL INTERVENTION OF POSTMENOPAUSAL OSTEOPOOROSIS BY DENOSUMAB
P. Pivonka¹, S. Scheiner¹, D. W. Smith¹, C. R. Dunstan²
¹Engineering Comp Biology Group, University of Western Australia, Perth, WA, Australia
²Dept. of Biomedical Engineering, University of Sydney, Sydney, NSW, Australia

Postmenopausal osteoporosis (PMO) is a bone disease eventually leading to a higher bone fracture risk, caused by a porosity significantly increasing over time. Recently, denosumab, a fully human, monoclonal antibody, has been approved for the treatment of PMO. Denosumab is able to efficiently antagonize PMO by binding to RANKL, the ligand of the receptor nuclear factor kappa beta (RANK), causing inhibition of bone resorption. In order to optimize the design of drug administration (in terms of delivered doses and administration chronology), we propose a fully coupled methodology of bone remodeling, considering the governing biochemical and biomechanical regulation mechanisms, and the targeted action of denosumab. Based on experimental results on the long-term degradation of denosumab in the blood serum, a pharmaco-kinetic model is developed, quantitatively relating the dose and the actual serum concentration, for both single and multiple injection-regimes. The time-dependent serum concentration of denosumab is then fed into a previously developed bone cell population model, allowing for prediction of the bone volume evolution of PMO patients. In order to account for the mechanobiology of bone remodelling, we employ the tool of continuum micromechanics-based homogenization, which provides the actual strain state of the investigated bone, on the observation scale where mechanosensing takes place. After calibrating this novel methodology according to pertinent experimental results, different drug administration regimes are simulated, and their effect on the bone microarchitecture is discussed.
REVIEW OF OSTEOPOROSIS TREATMENT AT DISCHARGE AND 12 MONTHS POST-FRACTURE AT A REGIONAL HOSPITAL (SHOALHAVEN) 2008-2010

J. Potts, D. Cossetto, I. Davison, P. Jarman, J. Christley, C. Davenport, D. Harmelin

Private Practice, Nowra, NSW, Australia

Aim: The percentages of patients leaving a representative regional hospital on osteoporosis treatment, following minimal trauma fracture, and those still taking treatment at 12 months, have been identified.

Method: Ethics approval was obtained from the HREC, University of Wollongong. Patients presenting with a minimal trauma fracture to Nowra Private Hospital, between 1 May 2008 and 1 May 2010 were identified from our database of referred patients and through a search of medical records. Patients were contacted by letter and asked three questions: 1. At discharge from hospital, were you taking any medication for osteoporosis, other than calcium and vitamin D? 2. Were you taking any osteoporosis medication 12 months after discharge? 3. Have you suffered a further fracture since discharge?

Results: A total of 200 admissions with a minimal trauma fracture were identified. Age range from 51-91.

<table>
<thead>
<tr>
<th>Total no. admissions</th>
<th>200</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respondents</td>
<td>146</td>
<td>73%</td>
</tr>
<tr>
<td>Deceased</td>
<td>22</td>
<td>11%</td>
</tr>
<tr>
<td>Returned to sender/uncontactable - either at 12 months or for refracture data</td>
<td>42</td>
<td>21%</td>
</tr>
<tr>
<td>Inappropriate to Treat</td>
<td>14</td>
<td>7%</td>
</tr>
<tr>
<td>Declined (any Step)</td>
<td>5</td>
<td>2.5%</td>
</tr>
<tr>
<td>Missing Data</td>
<td>5</td>
<td>2.5%</td>
</tr>
<tr>
<td>Osteoporosis treatment at discharge</td>
<td>96</td>
<td>48%</td>
</tr>
<tr>
<td>No treatment at discharge</td>
<td>75</td>
<td>37.5%</td>
</tr>
<tr>
<td>Osteoporosis treatment at 12 months</td>
<td>69</td>
<td>34.5%</td>
</tr>
<tr>
<td>Further fracture</td>
<td>13</td>
<td>6.5%</td>
</tr>
</tbody>
</table>

Conclusions:
1. This confirms a high risk population (mortality).
2. In our regional centre, more than 1/3rd who qualify for treatment were not offered treatment.
3. Better flagging is required.


EFFECT OF ZOLENDRONIC ACID IN TREATMENT OF POSTMENOPAUSAL OSTEOPOROSIS

V. Povoroznnyk, N. Grygorieva, V. Vayda, N. Balatska, N. Dzerovych, F. Klimovichkii

Institute of Gerontology AMS Ukraine, Kyiv, Ukraine

Introduction. Zolendronic acid is a new bisphosphonate used for treatment of postmenopausal osteoporosis. We have based our findings on results of intravenous infusions of zoledronic acid in 252 cases, 61 of which – secondary.

Aim. To determine the efficacy and safety of intravenous infusions of zoledronic acid, and effects on vertebral pain, bone mineral density (BMD) in postmenopausal women with osteoporosis.

Object. 89 postmenopausal women with osteoporosis aged 50-79 years (average age – 65.3±0.8 years) were examined.

Methods. Evaluation of pain syndrome and life quality was made with questionnaires. BMD was determined with Dual-energy X-ray absorptiometer “Prodigy” (GE Medical systems). 5 mg of zoledronic acid (Aclasta, “Novartis”) was administrated by intravenous injection. During the complex treatment patients received 1 tablet of calcium combined medicine (Calcium – 500 mg, Vit. D – 400 IU) 2 times a day during 12 months. Examination was performed before and after three, six, nine and twelve months of treatment course.

Results. A reliable decrease of vertebral pain syndrome by visual analogue scale was observed after three months. The pain syndrome decreased and improvement of life quality during all period of treatment. The BMD of spine significantly after three (0,043±0,055 г/см2; F=-7.5; P=0,000), six (0,048±0,054 г/см2; F=-7.2; P=0,000), nine (0,073±0,051 г/см2; F=-9.4; P=0,000) and twelve (0,070±0,057 г/см2; F=-8.5; P=0,000) months. We did not find significant difference in patients depending on age (50-59, 60-69 и 70-79 years) and state of bone (osteoporosis or osteopenia).

Conclusion. Intravenous infusions of zoledronic acid (5 mg) were shown to be effectively increasing BMD, decreasing pronounced vertebral pain syndrome and improving life quality in postmenopausal women with osteoporosis.
Marrow Fat Density Predicts Tibial Bone Geometry and Strength in Young Athletic Women

T. Rantalainen1,2,3, R. Nikander1,5, A. Heinonen3, H. Sievänen6,7, R. M. Daly1

1Centre for Physical Activity and Nutrition Research, School of Exercise and Nutr, Deakin University, Melbourne, Burwood, VIC, Australia
2Department of Mechanical engineering, Lappeenranta University of Technology, Lappeenranta, Finland
3Department of Health Sciences, University of Jyväskylä, Jyväskylä, Finland
4Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä, Jyväskylä, Finland
5Helsinki Metropolia University of Applied Sciences, Helsinki, Finland
6Pirkannaa Hospital District, Science Center, Tampere, Finland
7Bone Research Group, UKK Institute, Tampere, Finland

Aim: Since adipocytes and osteoblasts arise from the same mesenchymal stems cells (MSC) within bone marrow, the ability of exercise to increase bone strength may arise through loading biasing MSC differentiation towards osteoblastogenesis rather than adipogenesis. This study examined whether bone marrow fat differs between athletes involved in five contrasting loading groups, and whether marrow fat is an independent predictor of bone strength. Methods: 221 women aged 17-40 years representing athletes in high-impact (n=60), odd-impact (n=47), high-magnitude (n=15), repetitive low impact (n=16), repetitive non-impact sports (n=42) and non-athletic referents (n=41) were analysed. pQCT was used to assess tibial mid-diaphysis endocortical (EndoD), mid and pericortical vBMD, cortical area (CoA), bone strength index (SSI), muscle (MuCSA) and subcutaneous fat CSA (SubFatCSA) and marrow fat density. Results: Relative to controls, CoA and SSI were greater in high-impact (28-32%), odd-impact (17-20%), and repetitive low-impact (23%) groups (P<0.001); CoA and SSI were similar between high-magnitude, repetitive non-impact and control groups. Cortical vBMD was lower (1%) in high-impact, odd-impact and repetitive low-impact groups compared to controls (P<0.05). SubFatCSA was 14-26% lower and MuCSA was 7-15% greater in all athletic groups (except high-magnitude) relative to controls (P<0.05). Marrow fat density tended to be higher (reduced adiposity) in the high-impact (13%), odd-impact (8%) and repetitive low impact (6%) groups, but the differences were not significant from controls (ANCOVA, P<0.61). Pooled regression analysis showed that reduced marrow adiposity was associated with greater CoA and SSI (P<0.05) but lower EndoD (P<0.001), independent of age, height, group, MuCSA and SubFatCSA. Conclusion: Female athletes with higher tibial bone strength indices did not have significantly reduced marrow adiposity compared to controls. However, in all women lower marrow adiposity was related to greater bone strength, which supports the emerging role of bone marrow fat on bone metabolism.


Screening for Osteoporosis: An Extended Routine Indication?
R. Santosh, R. N.A. Mehrotra
Endocrinology, Apollo Hospitals, Hyderabad, AP, India

The screening criteria for osteoporosis in women include women aged 65 or older, younger postmenopausal women based on specific concerns and women in the menopausal transition with other specific risk factors for fragility fractures. We present here a cohort of women with osteoporosis who may have been missed if not looked for vigilantly.

A 42 year old lady presented to the clinic with history of aches and pains. She had a history of Colles’ fracture in her left wrist after a trivial trauma 1 year ago. She had no family history of fractures. She did not smoke, nor was using corticosteroids. She had undergone total abdominal hysterectomy with preservation of ovaries at age 26 for a large uterine fibroid. She was not advised HRT as her ovaries were intact.

On investigations her calcium and phosphorus were normal. Serum 25(OH)D was 54 nmol/L. Thyroid profile was normal. However BMD testing revealed T Score of -3.5 at the lumbar spine and -4.1 at the left hip. She was counselled for teriparatide, but due to financial reasons, she opted for bisphosphonates. She was given iv Zoledronic acid 5 mg.

She is now pain free after 3 months

Since that we screened 8 ladies who underwent total underwent hysterectomy with preservation of ovaries at a mean of 14 years prior to screening. Six were found to have osteoporosis by WHO criteria and qualified for treatment

After hysterectomy, the ovaries undergo involution in many cases after a period of time. Ther theories of this phenomenon are many which include loss of vascularity (utero ovarian axis). The women are at risk for developing problems related to menopause at a young age, including osteoporosis. Better awareness of this phenomenon will help us to prevent more fractures.
ESTIMATION OF RISK FACTORS AND PREVALENCE OF THE OSTEOPOROSIS AMONG THE COUNTRYMEN OF THE REPUBLIC OF TATARSTAN (RUSSIA)

N. G. Shamsutdinova, I. G. Salikhov

Hospital therapy, Kazan State Medical University, Kazan, Russian Federation

Introduction: Nowadays the osteoporosis (OP) is an actual problem. In diagnostics of OP not only the data of densitometry is of great importance, but also an estimation of risk factors. In this connection it is interesting to study the prevalence of OP and densitometry revealed osteopenia in conjunction with risk factors in patients living in rural areas of the Republic of Tatarstan.

The purpose: To study the frequency of bone mineral density decrease and risk factors of its decrease among the countrymen.

Materials and methods: 1520 persons living in the countryside of the Republic of Tatarstan were examined with the radiological densitometer DTX 200 on a radial bone. Diagnostics was made by T-criterion among the men older than 50 years and among the postmenopausal women; by Z-criterion among the men younger than 50 years and among the premenopausal woman. The patients were interviewed with specially developed questionnaire included 30 questions.

Results and discussion: From all examined 45.4% (690 persons) people had decreased bone mineral density with different degree of expressiveness: OP was revealed among 26.4% of the patients (401 persons), osteopenia was revealed among 19% of the patients (289 persons). In the group with OP 24.3% of the patients marked nontraumatic fractures at near relations in the anamnesis, in the group with osteopenia 18.8% of the patients. In patients with osteoporosis compared with the group with normal bone mineral density was significantly higher (p <0.05) met RA, thyroid disease, diabetes. OP was revealed among 35.1% of the patients who are on constant steroid therapy, osteoporosis was revealed among 23.6% of the patients who are on constant steroid therapy. Obtained a significant correlation between reduced calcium intake and decreased bone mineral density. Significantly more often decrease bone mineral density occurs in patients aged over 50 and in the group of postmenopausal women.

Conclusions:
1. Risk factors of bone mineral density decrease are widely represented in the examined patients with osteoporosis and osteopenia.
2. Positive significant correlation between risk factors and bone mineral density decrease confirms necessity of their revealing and estimation for early diagnostics of OP.

PREVALENCE OF OSTEOPOROSIS IN REPUBLIC OF TATARSTAN (RUSSIAN FEDERATION)

N. G. Shamsutdinova, S. P. Yakupova

Hospital therapy, Kazan State Medical University, Kazan, Russian Federation

Introduction:
To date, osteoporosis (OP) is a topical issue. Epidemiological studies in Russia are carried out only in selected regions, primarily due to the unavailability of instrumental diagnosis. In this connection it is interesting to study the prevalence of OP and densitometry revealed osteopenia in patients living in rural areas of the Republic of Tatarstan.

Purpose:
Identification of the frequency of bone mineral density decrease in Republic of Tatarstan.

Materials and methods:
A total of 1,520 people living in rural areas of Republic of Tatarstan, with the help of X-ray osteodenisitometry DTX 200 on radial bone, diagnosis was carried out by T-test in men older than 50 years and in postmenopausal women, the Z-criterion in men 50 years and in premenopausal women.

Results and discussion:
Of all the surveyed in 45.4% (690 patients) were reducing the bone mineral density: in 26.4% of patients (401 persons) revealed densitometry OP, 19% of patients (289 persons) - osteopenia. Moreover, in men older than 50 years and in postmenopausal women, the OP was detected in 40% and 41.2% respectively. In women before menopause OP densitometry is defined in 1% of cases, osteopenia - in 15.9%.

Conclusions:
1. Reduced bone mineral density in Republic of Tatarstan has a wide dissemination of data and prevalence correlated with the available data of studies in another regions.
2. Significantly more likely decreased bone mineral density occurs in patients aged over 50 and in the group of postmenopausal women.
OSTEOPOROSIS AMONG PATIENTS WITH CORONARY ARTERY DISEASE: AN INDIAN SCENARIO

K. P. Singh1, A. Dhatt1, A. Toor1, G. S. Kalra2, R. Brar3, S. P.S. Chawla4
1Endocrinology, Fortis Hospital, Mohali, Punjab, India
2Cardiology, Fortis Hospital, Mohali, Punjab, India
3Radiology, Fortis Hospital, Mohali, Punjab, India
4Radiodiagnosis, Superb Osteoporosis Detection Centre, Chandigarh, UT, India

Coronary Artery Disease (CAD) and Osteoporotic fractures are important public health problems which carry increased morbidity, mortality and constitute national socio-economic burden. Both diseases are important from point of view of developing countries like India where they occur a decade earlier. Both diseases are common in the elderly; the studies are limited on their interaction and none are from India. We studied 205 patients in age range of 51-75 years (90 females & 115 males) during 2008-2009. While they were being evaluated for CAD, Osteoporosis workup was done. CAD was confirmed by CT and/or catheter coronary angiography. Diagnosis of CAD was made when ≥ 1 arteries showed > 50% stenosis in angiogram. CT coronary angiography was done on 64 slice CT (Siemens sensation 64) using non –ionic (Ioversol) dye. Diagnosis of osteoporosis was made with a value of BMD > 2.5 SD below the young adult mean after a detailed drug history, examination and laboratory workup. A BMD that lies between 1 and 2.5 SD below the young adult mean was considered as having osteopenia. BMD within 1 SD of young adult mean was considered normal. BMD was measured using Dual energy X-ray absorptiometry (DEXA) scan done for bilateral femoral neck, lumbar spine and both wrists on whole body DEXA (GE) machine. The 205 patients were divided into two groups based on presence or absence of CAD. The prevalence of osteoporosis in the two groups was observed as follows - Group CAD (n=135) No CAD (n=70) Normal BMD 43.7% 74.3% Osteopenia 17.7% 10.0% Osteoporosis 38.5% 15.7% In the 70 patients showing normal coronaries, BMD was normal in nearly 75% of cases, while patients having CAD were more likely to have abnormal BMD. These results highlight that the presence of one disease should prompt an investigation for the other.


COMPARISION OF OSTEOPOROTIC DATAS IN ACTIVE AND NON ACTIVE ELDERLY IN OUR REGION

A. T. Széplaki1, A. A. Széplaki2
1Orthopaedics and Traumatology, Rehabilitation Clinical Hospital, Cluj, Romania
2Traumatology, Jávorszky Hospital, Vác, Hungary

The authors measured the bone mineral density in elderly over 65 years separated in two groups. One of patients living in a parish and one of active patients living among their family.

The aim of this poster is to present the differences of bone mineral density between the two groups caused by the different life style. We effectuated röntgenmorfometric and densytometric measurements. The results where in favor of the active group. We have measured in both groups lower mineral bone density comparing with average European datas.

SERUM CALCIUM AS A PREDICTOR OF CARDIOVASCULAR DISEASE IN THE BUSSELTON HEALTH STUDY

J. Walsh1,2, M. Divitini3, M. W. Knunam4
1Department of Endocrinology & Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia
2School of Medicine and Pharmacology, University of Western Australia, Crawley, WA, Australia
3School of Population Health, University of Western Australia, Crawley, WA, Australia

Aim. There is conflicting evidence as to whether serum calcium is a predictor of cardiovascular disease. To address this, we examined serum calcium as a predictor of cardiovascular mortality and events in Busselton Health Study.

Methods. Data were analysed from 3802 individuals (1682 men and 2120 women) aged 25-84 years who participated in the 1994-5 Busselton Health Survey. Serum total calcium was measured from venous blood collected after an overnight fast. The primary endpoints were cardiovascular mortality and cardiovascular events (fatal and non-fatal combined), as determined from the Western Australian Data Linkage System, which records all deaths and hospital admissions in Western Australia. Follow-up was up to the end of 2007. We examined serum calcium (corrected for albumin) as a predictor of outcomes in a multivariate Cox proportional hazards model which also included age, smoking, alcohol, blood pressure, waist circumference, fasting glucose and lipids. Results are reported as hazard ratios (HR) with 95% confidence intervals (CI) for each additional 0.1 mmol/L of serum calcium at baseline.
Results. The mean follow-up time was 12.7 years. In men, there were 96 deaths from cardiovascular disease and 290 cardiovascular events. Serum calcium was not a significant predictor of cardiovascular mortality (HR 1.03, 95% CI 0.78, 1.35) or cardiovascular events (HR 1.03, 95% CI 0.87, 1.21). In women, there were 92 cardiovascular deaths and 251 events; similarly, serum calcium was not a significant predictor of cardiovascular mortality (HR 1.02, 95% CI 0.80, 1.30) or cardiovascular events (HR 1.12, 95% CI 0.98, 1.28). Results were similar after excluding participants who had cardiovascular disease or diabetes at baseline.

Conclusion. Serum calcium is not a significant predictor of cardiovascular disease in the Busselton Health Study.

### BALLOON KYPHOPLASTY AND VERTEBROPLASTY IN THE MANAGEMENT OF VERTEBRAL COMPRESSION FRACTURE: A SYSTEMIC REVIEW

<table>
<thead>
<tr>
<th>J. Yang¹</th>
<th>S. Hou²</th>
<th>C. Hou³</th>
<th>F. Lin¹</th>
<th>C. Lin²</th>
<th>R. Yang²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical Imaging, National Taiwan University Hospital, Taipei, Taiwan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orthopedics, National Taiwan University Hospital, Taipei, Taiwan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cathay General Hospital, Taipei, Taiwan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Aim:
The goal of this review is to demonstrate the efficacy and safety outcome of vertebroplasty (VP) and kyphoplasty (KP) in the management of vertebral compression fracture.

Methods:
Detailed searches of electronic databases (i.e. Pubmed, Cochrane library) were performed. Outcome measures of efficacy included visual analog scale (VAS) decrease, change in kyphotic angle, restoration of vertebral height and improvement of functional capacity. Outcome measures of safety were cement leakage, new vertebral compression fracture and complications.

Results:
No significant difference in VAS decrease was noted between VP and KP groups (p=0.374, table). We found a higher rate of cement leakage, new compression fractures, pulmonary embolism and radiculopathy in VP than in KP (all p < 0.05, table). We also found that reduction in kyphotic angle was larger in KP than in VP (p=0.007, table).

Conclusions:
We found a higher level of cement leakage rate in VP than in KP. We also found that reduction in kyphotic angle was better in KP than in VP. These results were compatible with other literatures.

### Table. Summary of Case Series Efficacy and Safety Outcomes

<table>
<thead>
<tr>
<th></th>
<th>KP (N=37)</th>
<th>VP (N=80)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Efficacy outcome</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in pain (VAS 0-10 mm scale)</td>
<td>5.33 ± 1.35</td>
<td>5.65 ± 1.26</td>
<td>0.374</td>
</tr>
<tr>
<td>Change in quality of life (SF-36)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bodily pain</td>
<td>33.43 ± 13.10</td>
<td>26.0</td>
<td></td>
</tr>
<tr>
<td>physical function</td>
<td>27.33 ± 9.50</td>
<td>10.4</td>
<td></td>
</tr>
<tr>
<td>general health</td>
<td>11.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vitality</td>
<td>15.77 ± 7.56</td>
<td>16.9</td>
<td></td>
</tr>
<tr>
<td>social functioning</td>
<td>28.20 ± 10.77</td>
<td>23.7</td>
<td></td>
</tr>
<tr>
<td>role emotional</td>
<td>24.0</td>
<td>29.0</td>
<td></td>
</tr>
<tr>
<td>mental health</td>
<td>12.45 ± 0.78</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td>role functioning</td>
<td>26.30 ± 3.25</td>
<td>22.7</td>
<td></td>
</tr>
<tr>
<td>Change in vertebral height (% original height)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td>16.00 ± 8.28</td>
<td>18.37 ± 9.51</td>
<td>0.725</td>
</tr>
<tr>
<td>Middle</td>
<td>16.88 ± 9.70</td>
<td>18.43 ± 10.94</td>
<td>0.843</td>
</tr>
<tr>
<td>Posterior</td>
<td>5.50 ± 3.54</td>
<td>6.66 ± 3.07</td>
<td>0.742</td>
</tr>
<tr>
<td>Change in kyphotic angle (reduction in°)</td>
<td>7.49 ± 3.33</td>
<td>4.15 ± 1.76</td>
<td>0.007^</td>
</tr>
<tr>
<td><strong>Safety outcomes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cement leakages</td>
<td>12.26 ± 7.91</td>
<td>36.44 ± 28.76</td>
<td>0.000^</td>
</tr>
<tr>
<td>New VCFs</td>
<td>10.46 ± 2.49</td>
<td>21.47 ± 14.67</td>
<td>0.004^</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>0.58 ± 1.63</td>
<td>3.41 ± 2.35</td>
<td>0.023^</td>
</tr>
<tr>
<td>Radiculopathy</td>
<td>0.06 ± 0.16</td>
<td>4.22 ± 3.87</td>
<td>0.000^</td>
</tr>
</tbody>
</table>

* 2 sample t test was performed
^ P < 0.05 : significant finding
HISTOPATHOLOGY OF FEMORAL HEAD: A RETROSPECTIVE REVIEW OF 6161 CASES
K. E. Mackie¹, Z. Zhou¹,², P. Robbins³, M. Bulsara¹, J. Winter¹, M. H. Zheng¹,⁵
¹Orthopaedic Research, University of Western Australia, Perth, WA, Australia
²Department of Orthopaedic Surgery, Guangzhou Red Cross Hospital, Guangzhou, WA, China
³Division of Anatomical Pathology, PathWest, Perth, WA, Australia
⁴Institute of Health and Rehabilitation Research, University of Notre Dame, Perth, WA, Australia
⁵Perth Bone & Tissue Bank, Perth, WA, Australia

Total joint arthroplasty is one of the most common orthopaedic surgical procedures. We propose that assessment of bone tissues from resected femoral heads may give a profile of femoral head pathology, providing potential benefit to total hip arthroplasty patients and bone donors. This study retrospectively analysed the reported histological findings of 6161 femoral heads donated for allografts between 1993 and 2006. Specimens taken at the time of total hip arthroplasty and bone donation were reviewed. Follow-up investigations from histopathological findings were also reviewed. The Western Australian Cancer Registry was used to investigate all patients with suspected neoplasms. Review of histopathology was conducted to evaluate and re-classify all previous observations. A total of 105 femoral heads demonstrated abnormal or reactive histopathological features, that were not reported prior to surgery, and thus were rejected for allograft. Reactive lymphocytic infiltrates most likely due to osteoarthritis were most commonly identified (45 cases). Observations in 27 cases were most likely due to the presence of severe osteoarthritis. Ten cases showed plasmacytosis which may have been related to osteoarthritis. Two patients were diagnosed with Paget's disease and two with rheumatoid arthritis. There were 19 cases with suspected neoplasms. Of these, 8 cases of Non-Hodgkin's Lymphoma and Chronic Lymphocytic Leukemia, and one case of myelodysplastic syndrome were confirmed upon further investigation. It is noteworthy that confirmed malignancies accounted for 1 in 770 patients undergoing total hip arthroplasty in this cohort. Our findings indicate that, even with detailed historical and medical review, clinically significant diseases, including neoplasms and Paget's disease, are observed in cases diagnosed with osteoarthritides prior to total hip arthroplasty. Histological examination plays an integral part in quality assurance in femoral head banking, and as a possible early diagnostic device of bone and marrow related diseases for total hip arthroplasty patients.

BILATERAL TRANSIENT OSTEOPOROSIS OF THE HIP DURING THE LAST TRIMESTER OF PREGNANCY
T. Baykal¹, O. Baykal², K. Senel³
¹Physical medicine and rehabilitation, Regional hospital, Batman, Turkey
²Gynecology and obstetric department, Regional hospital, Batman, Turkey
³Physical medicine and rehabilitation, Ataturk University, Erzurum, Turkey

Introduction
Transient osteoporosis of the hip (TOH) was first described in 1959 by Curtis and Kincaid in the third trimester of pregnancy. It is usually seen in middle-aged men and women in the third trimester of pregnancy and is associated with hip pain. We report the case who was diagnosed with bilateral TOH.

Case report
We present the patient of 35-year-old female who developed, a sudden onset of bilateral hip pain in the eighth month of pregnancy. She was evaluated in consultation with the gynecology and obstetric department. After delivery, the patient was admitted to our department with bilateral hip pain radiating down the both legs. Physical examination showed that there was severe tenderness in the hip joints. There was a reduction in the range of motion of the hip. There was pain and limited internal rotation of bilateral hip. Straight leg raising was 60 degrees bilaterally with no neurological findings in the lower limbs. Laboratory tests were normal ranges. Standard hip radiographs revealed osteopeni and demineralization in the femoral head and neck. Magnetic resonance imaging (MRI) showed low signal intensity of bone marrow on T1-weighted images, and high signal intensity on T2-weighted sequences suggestive of bone marrow oedema. These findings were consistent with bilateral TOH. She was diagnosed as bilateral TOH by clinical, radiographical and MRI findings. The patient was treated with a complete bed rest for the first two weeks, followed by mobilization with a walking device. Also, she underwent a three monts treatment of non-steroidal anti-inflammatory drugs, alendronic acid (70 mg/week) and vitamin D for 6 months. Follow-up examination, complete clinical improvement was observed in the end of sixth month.

Conclusion
In conclusion, TOH must be considered in the differential diagnosis of the patients with bilateral hip pain during the last trimester of the pregnancy.
A NOVEL AUSTRALIAN CALCIUM-SPECIFIC DIET QUESTIONNAIRE: VALIDITY AND RELIABILITY

B. R. Beck, B. K. Weeks, T. L. Norling

School of Physiotherapy and Exercise Science, Griffith University, Gold Coast, QLD, Australia

Aim: To examine validity and reliability of a simple, inexpensive instrument to determine average daily dietary calcium of Australian research subjects.

Methods: Sixty subjects (24 male, 36 female) were recruited to complete a standard generic Australian dietary instrument (Anti-Cancer Council of Victoria Food Frequency Questionnaire; ACVFFQ®) and a novel Australian calcium-specific diet questionnaire (AusCal), in random order, on a single occasion. Ten subjects additionally completed a 7-day weighed food record the following week. The AusCal was readministered to 48 subjects 3 months later. Comprehensive daily nutritional values were derived from the ACVFFQ by external computer processing. Daily dietary calcium was derived from the AusCal and 7-day weighed food records using Foodworks (Xyris, Brisbane), with data entry performed by a single investigator. Intraclass correlation coefficients were calculated between the AusCal, ACVFFQ and 7-day record estimates of daily calcium consumption. Reliability was determined by comparison of baseline with 3-month AusCal calcium values. Validity was determined by comparisons of AusCal estimates of daily calcium with those derived from the ACVFFQ and the 7-day weighed food record. The costs of determining dietary calcium from the AusCal and the ACVFFQ were compared.

Results:

Table 1. Daily calcium means ± SD and Intraclass Correlation Coefficients for each dietary instrument

<table>
<thead>
<tr>
<th>Instrument</th>
<th>ACVFFQ (n=60)</th>
<th>Day record (n=10)</th>
<th>AusCal BL (n=60)</th>
<th>AusCal FU (n=48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mg)</td>
<td>1011.4 ± 377.5</td>
<td>862.9 ± 258.7</td>
<td>957.2 ± 481.7</td>
<td>880.9 ± 445.3</td>
</tr>
<tr>
<td>ACVFFQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-Day record</td>
<td>0.85 (p&lt;0.001)</td>
<td>0.70 (p&lt;0.03)</td>
<td>0.94 (p&lt;0.001)</td>
<td></td>
</tr>
</tbody>
</table>

ACVFFQ = Anti-Cancer Council of Victoria Food Frequency Questionnaire, AusCal = novel calcium-specific diet questionnaire, BL = Baseline, FU = 3-month follow-up.

Conclusions: The AusCal exhibited excellent daily calcium test/retest reliability, very high validity against the ACVFFQ and high validity against a 7-day weighed food record. Use of the AusCal produced a saving of over AUD$800 per 100 subjects over ACVFFQ costs.

Acknowledgments: Supported by a grant from the Griffith Health Institute, Centre for Clinical and Community Practice


BACK PAIN IN PATIENTS WITH SEVERE OSTEOPOROSIS TREATED WITH TERIPARATIDE OR ANTRESORPTIVES: A PROSPECTIVE, MULTICENTRIC, OBSERVATIONAL STUDY

S. Jasqui1, T. Songpatanasilp2, R. Malhotra3, B. Dave4, M. Mumtaz5, H. Singh Chhabra6, G. Bori7, M. Yu8, J. M. Blair9, S. Sorsaburu10

1Eli Lilly Mexico, Mexico City, Mexico
2Phramongkutklao College of Medicine, Bangkok, Thailand
3All India Institute of Medical Sciences, New Delhi, India
4Stayya Spine Hospital & Research Institute, Ahmedabad, India
5Island Hospital, Georgetown, Malaysia
6Indian Spinal Injuries Centre, New Delhi, India
7Instituto Mexicano del Seguro Social, Mexico City, Mexico
8Eli Lilly Canada, Toronto, Canada
9Eli Lilly Australia Pty Ltd, Sydney, Australia
10Eli Lilly and Company, Indianapolis, United States

Aim

This 1-year study compared the effectiveness of teriparatide over antiresorptives on the relative risk of new/worsening back pain.

Methods

This prospective, multicentric, observational study in 9 countries enrolled 652 men and postmenopausal women (mean age 68.9 years), of whom 230 patients were prescribed and received teriparatide, and 327 patients were prescribed and received antiresorptives, for established severe osteoporosis under regular medical care for up to 12 months. Study treatment switching was permitted under normal clinical care. Assessment tools included the Back Pain Questionnaire, Visual Analog Scale (VAS), and the European Quality of Life questionnaire (EQ-5D). Selection bias was addressed via propensity score. The primary effectiveness measure was relative risk of new/worsening back-pain at 6 months.

Results

At baseline, more subjects on teriparatide versus antiresorptives had severe back pain (30.9% vs 17.7%), extreme pain/discomfort (25.3% vs 16.5%), extreme anxiety/depression (16.6% vs 8.0%), and confinement to bed (10.0% vs 5.2%). At study entry, patients treated with teriparatide differed from those treated with antiresorptives for VAS (5.82 vs 5.04) and EQ-5D scores (37.7 vs 45.0).
With teriparatide and antiresorptives at 6 months, respectively, incidence of new/worsening back pain was 9.8% vs 10.1% [relative risk (95% confidence interval) adjusted for propensity score, country, and baseline back pain severity in patients with severe baseline back pain was 0.99 (0.80, 1.22)] and incidence of severe back pain was 1.3% vs 1.5%. The EQ-5D scores were 46.1 vs 54.8, while VAS scores were 2.71 vs 3.32. More teriparatide-treated patients felt better (82.7% vs 70.9%) and were at least very satisfied with their treatment (49.4% vs 36.4%) at 12 months.

Conclusions
Teriparatide-treated patients had a similar risk of new/worsening back pain as antiresorptive-treated patients at 6 months, though further research is needed for confirmation of results.

346
BRIDGING THE GAP IN THE ANALYSIS OF BONE HEALTH IN YOUNG INDIVIDUALS WITH CYSTIC FIBROSIS
D. S.K. Brookes1, J. N. Briody2, C. J. Munn3, R. J. Hill1, P. S.W. Davies1
1Children’s Nutrition Research Centre, The University of Queensland, Herston, QLD, Australia
2The Children’s Hospital at Westmead, Sydney, NSW, Australia

Aim : To assess if there was a deficit of total body bone mineral content (BMC) in children and adolescents with cystic fibrosis (CF), and if it was due to an intrinsic bone problem, reduced lean tissue mass (LTM), or a combination of both.

Methods : Following stratification by sex and Tanner stage, (pre-pubertal: CF=169(F), controls=181(F)); pubertal: CF=37(F), controls=35(23F)), DXA (GE Lunar Prodigy, v11.4) was used to obtain total body BMC, LTM, age and height ratio Z-scores using paediatric control database [1]. These Z-scores were used in a four step algorithm to answer: (1) was BMC low for age; (2) were they short; (3) was LTM appropriate for height; and (4) was there enough BMC for the amount of LTM?

Results : Compared with controls, pre-pubertal and pubertal males with CF had less BMC and pre-pubertal males were shorter (p=0.05). Pre-pubertal males had more LTM for height (p=0.05), whereas pubertal males had adequate LTM for height. Pre-pubertal and pubertal males with CF had adequate BMC for the amount of LTM. The pre-pubertal females with CF displayed non significant deficits compared to the pre-pubertal female controls. Pubertal females with CF had significantly less BMC for age (p=0.02), adequate LTM for height, were shorter (p=0.03), but significantly lower BMC for the amount of LTM (p=0.01).

Conclusion : Compared to controls, pre-pubertal and pubertal males with CF showed lower BMC primarily due to short stature with a normal muscle/bone interaction. Pubertal females with CF, however, appeared to have a primary bone deficit with the bone not adapting adequately to LTM. These data support the need to correct BMC measures for height and maintain LTM in children and adolescents with CF. Particular attention needs to be payed to pubertal females where the skeleton appears to adapt poorly to muscle strain.


347
EFFECT OF LEVETIRACETAM AND OLDER ANTIADIPLETIC DRUGS ON BONE HEALTH AND BODY COMPOSITION: A COMPARATIVE RANDOMISED TRIAL
T. Hakami1, T. J. O’Brien1,2, S. Petty3, M. Sakellarides1, T. Bright1, J. J. Christie1,3, S. Kantor1,3, M. Todaro1,2, M. J. Seibel1, J. D. Wark1,3
1Medicine (RMH/WH), University of Melbourne, Melbourne, VIC, Australia
2Neurology, The Royal Melbourne Hospital, Melbourne, VIC, Australia
3Bone & Mineral Service, The Royal Melbourne Hospital, Melbourne, VIC, Australia
4ANZAC Research Institute, The University of Sydney, Sydney, NSW, Australia

Aim
Antiepileptic drug (AED) therapy is associated with increased fracture risk but it is uncertain whether newer AEDs carry a similar risk to older AEDs. This first-in-the-field trial compared bone mineral measures, body composition and blood indices in patients randomised to levetiracetam (LEV) versus either carbamazepine (CBZ) or sodium valproate (VPA).

Methods
Patients with partial epilepsy who had failed treatment with older AEDs were randomized to treatment with either LEV or CBZ/VPA. Areal bone mineral density (aBMD) of the lumbar spine, total hip, and forearm and total body bone mineral content; peripheral quantitative computed tomography (pQCT) of the radius and tibia; serum hormones and bone turnover markers; and anthropometry were obtained at baseline and 12 months. Univariate and multiple regression analyses were performed seeking associations between change in those measures with treatment group, age, sex, baseline height and weight, and follow-up interval.

Results
45 patients were enrolled to LEV group [median age (IQR)] [37.2 (23.8-51.8)] and 39 patients to the older AED group [43.0 (30.1-61.4)]. In most, AED substitution was within 3 months before baseline. Baseline measures did not differ between groups. Over 12 months, there were increases in the older AED group in weight (p=0.039), BMI (p=0.035), and leptin (p=0.032) and decreases in forearm BMD (p=0.001) and cortisol (p=0.043) with no changes in the LEV group. At follow up, % abdominal fat (p=0.013) and waist: hip ratio (p=0.019) were higher in the older AED group.
Conclusions
In previously-treated patients, substitution with older AED, but not LEV, caused significant increases in body weight and abdominal fat. There was little rapid bone change, and no difference in the early bone response between groups when treated with LEV compared with CBZ or VPA. While this study provides reassurance, long-term follow-up may better characterize the effects of older and newer AEDs on bone health.
The study is funded by an investigator-initiated research grant from UCB Australia.

348
POSSIBLE EFFECT OF STRONTIUM RANELATE IN BONE HEALING AFTER HIP FRAGILITY FRACTURE
C. Cirstoiu, M. Cirstoiu, D. Popescu, A. Popescu, R. Ene
Orthopaedics, University Hospital Bucharest, Bucharest, Romania

Osteoporosis drugs are prescribed to prevent fragility fractures, which is the principal aim of the management of osteoporosis. However, if fracture does occur, then it is also important to promote a fast and uneventful healing process. Data about the effect of osteoporosis drugs on bone healing in humans is insufficient. Strontium ranelate is an osteoporosis agent that increases bone formation and reduces bone resorption and may therefore be beneficial in fracture healing. We report 10 cases (women aged over 67 years) with hip fracture in which we used hip osteosynthesis and follow them up to 24 months. Treatment with strontium ranelate (2 g/day) for between 6 weeks and 24 months appeared to contribute to bone consolidation in the four cases according clinical and imagistic criteria (classical radiological films and computer tomography). Animal studies support beneficial effects of strontium ranelate on bone healing via improvement of bone material properties and microarchitecture in the vicinity of the fracture, suggesting that strontium ranelate accelerates fracture healing. The clinical cases described provide new information on these effects, in the absence of randomized controlled studies on the clinical efficacy of pharmacological treatments in osteoporosis in fracture repair. Further studies are necessary. Fracture healing is an important topic in orthopedic research and a positive effect of osteoporosis treatments on bone healing is an interesting possibility and merits further clinical research.

(1) C.Cirstoiu, M.Cirstoiu et all - Osteoporoza in Menopauza, Ed Carol Davila Bucuresti

349
ARE THERE ANY RISK FACTORS IN ELDERLY, LEADING TO HYponATREMIA FOLLOWING SPINE SURGERY & CAN IT BE PREVENTED?
B. Dave
Stavya Spine Hospital, Ahemdabad, India

STUDY DESIGN: Combined retrospective-prospective single centre study

AIMS: Hyponatraemia is the most frequent electrolyte imbalance seen in hospitalized/operated patients. Most commonly geriatric patients require spine surgery and the rate of occurrence of hyponatraemia is more common in these patients. This study was conducted to identify the incidence of post-operative hyponatraemia and the risk factors contributing to hyponatraemia in ELDERLY PATIENTS following spinal surgery and subsequent corrective measures.

METHODS: Study was carried out for a period of 18 months. On identification of the frequent appearance of hyponatraemia for retrospective 9 months (502 patients), a prospective study to check the effectiveness of three corrective measures (firstly, increasing perioperative 0.9 % NS infusion, secondly postoperative additional 2 gram salt intake in all patients for a week and third, limiting oral fluid intake) were done for 9 months (453 patients). Plasma serum sodium level was measured before and after surgery. All the patients were analyzed for associated diseases, duration of surgery, types and amount of intravenous fluid infusions peri-operatively and peri-operative steroid administration.

RESULTS: Post-operative hyponatraemia was frequently noted in patients operated for tuberculous spine, peri-operative steroid infusion, surgery duration more than 2.5 hours, on antihypertensive Ca channel blocker and diabetics. Significant reduction (p>0.05) in incidence was observed following the corrective measures.

CONCLUSION: There are specific risk factors like tuberculosis, iv high dose steroid injection (per Op), longer duration of surgery, diabetes and patients on calcium channel blockers, developed hyponatraemia in post operatively. Additional salt intake, restricted fluid intake and increasing peri-operative 0.9 % NS infusion would reduce the incidence of post-operative hyponatraemia.

350
INCIDENCE OF HIP FRACTURE IN ROHTAK, NORTH INDIA
D. Dhanwal 1, R. Siwach 3, V. Dixit 1, A. Mithal 2, C. Cooper 4

1Medicine, Maulana Azad Medical College, New Delhi, India
2Endocrinology, Medanta Medicity, Gurgaon, Haryana, India
Aim: To study hip fracture incidence in Rohtak district of India.

Material and methods: The study was conducted in Rohtak district, Haryana, India located 80 km north of New Delhi. All patients having hip fracture admitted in Pt BD Sharma PGI or one of the four orthopaedic centres located in Rohtak in year 2009 were included. Projected population of year 2009 was used to calculate age specific hip fracture incidence.

Results: A total of 541 patients with hip fracture were hospitalized in Rohtak district in year 2009. Out of these 304 were from Rohtak district. Table 1 shows age specific hip fracture rates in different age groups. Hip fracture crude rates in Indians above the age of 55 years are 142/100,000 and 118.2 and 167 per 100,000 in men and women respectively. Hip fracture incidence was similar in both sexes till age of 55 years and then onwards the rates are significantly higher in women.

Table 1: Hip fracture incidence in women and men per 100,000 from India

<table>
<thead>
<tr>
<th>Age</th>
<th>No of fracture</th>
<th>Population</th>
<th>Incidence rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35</td>
<td>4</td>
<td>355403</td>
<td>1.1</td>
</tr>
<tr>
<td>35-44</td>
<td>66377</td>
<td>355403</td>
<td>13.6</td>
</tr>
<tr>
<td>45-54</td>
<td>40074</td>
<td>355403</td>
<td>57.4</td>
</tr>
<tr>
<td>55-64</td>
<td>29214</td>
<td>355403</td>
<td>106.1</td>
</tr>
<tr>
<td>65-74</td>
<td>23381</td>
<td>23381</td>
<td>162.5</td>
</tr>
<tr>
<td>75+</td>
<td>34</td>
<td>9078</td>
<td>374.5</td>
</tr>
<tr>
<td>Total</td>
<td>139</td>
<td>523527</td>
<td>26.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Men</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;35</td>
<td>29</td>
<td>432053</td>
<td>6.7</td>
</tr>
<tr>
<td>35-44</td>
<td>27</td>
<td>73516</td>
<td>36.7</td>
</tr>
<tr>
<td>45-54</td>
<td>33</td>
<td>49553</td>
<td>66.6</td>
</tr>
<tr>
<td>55-64</td>
<td>21</td>
<td>27234</td>
<td>77.1</td>
</tr>
<tr>
<td>65-74</td>
<td>23</td>
<td>24292</td>
<td>94.7</td>
</tr>
<tr>
<td>75+</td>
<td>32</td>
<td>11054</td>
<td>289.5</td>
</tr>
<tr>
<td>Total</td>
<td>165</td>
<td>617702</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Conclusions: This is the first hip fracture incidence study from India. The hip fracture rates in India are intermediate between industrialised world and Africa and similar to some Asian countries. The hip fracture rates among men are similar to those reported in as reported in Indian men living in Singapore. This study emphasises urgent need for formulating strategies for prevention of hip fracture in India.

PREVENTION OF OSTEONECROSIS OF THE JAW DURING ZOLENDRONATE USE IN THE PATIENTS WITH MULTIPLE MYELOMA – EXPERIENCE FROM ONE CENTRE

Department of Internal Medicine-Hematooncology, Department of stomatosurgery, Masaryk University Hospital, Brno, Czech Republic

BACKGROUND and AIMS: Bisphosphonates are non-metabolized pyrophosphate analogues which inhibit osteoclastic activity. Bisphosphonates containing nitrogen have been recently associated with osteonecrosis of the jaw (ONJ). It is defined as three month non healing defect in a jaw, usually in mandible. The incidence of ONJ is the highest in multiple myeloma (MM) patients from all cancers. Most cases of ONJ is associated with zolendronate use. ONJ develops usually after some stomatological procedure, mostly teeth extraction. We made precautions which was aimed to decrease incidence of ONJ in our routine daily praxis.

METHODS: First group of 43 MM patients were treated with zolendronate with 480 infusions. Than, an ONJ Preventive Program (ONJ PP) was activated. The ONJ PP consists from 4. requirements: 1. Stomatological examination before zolendronate treatment, including X-ray examination; 2. Interruption of zolendrante use two months before planned teeth extraction or other stomatosurgery, and resumption of this treatment two month after jaw is completely healed; 3. Antibiotic prophylaxis with amoxicillin/clavulanate 1g p.o. 2 times per day for 14 days if teeth extraction is made; 4. Regular chlorhexidine use during period after teeth extraction. Similar number of patients (41) was treated with precautions of ONJ PP with total of 465 applied infusion of zolendronate. Zolendronate was administrated in common dosage schedule 4 mg intravenously every month. Retrospective analyses of ONJ incidence during zolendronate use was done.

RESULTS: Together four cases (4/43; 9,3 %) of ONJ we monitored in our patients without precautions. All of these patients have used zolendronate for more than one year (median 13 months; range12-36 month) . ONJ developed after forgoing teeth extraction in all cases.

None case of ONJ (0/41) was reported after precautions was established. Five teeth extraction were planned in patients treated with zolendronate with median of 14 months. These patients had stopped zolendronate treatment and they used ONJ PP as recommended. Neither any case of ONJ was not developed in this patients. Incidenence of ONJ after precautions establishing statistically significantly decrease compare to period when these precautions was not used (p= 0,003)

CONCLUSION: ONJ can be common and dangerous complication in multiple myeloma patients treated with zolendronate. Its incidence rapidly increasing during time of therapy. Our data confirm that ONJ developing usually after one year of the treatment. Any procedure attacking bone seems to be key risk factor for ONJ formation. If zolendronate use is stopped before teeth extraction and antibiotic prophylaxis is used incidence of ONJ is rapidly decreased and the risk becomes acceptable for patients.

LOW BONE DENSITY IN ORTHOPAEDIC OUTPATIENT’S FRACTURE CLINIC

A. Heard1, L. Anderson2, N. Gilchrist1,2, J. McKie3
1CGM Research Trust, Christchurch, New Zealand
2Department of Orthopaedic Medicine and Surgery, Christchurch Public Hospital, Christchurch, New Zealand

Aim: To establish the incidence of low bone density in patients attending the orthopaedic outpatient service with peripheral fractures.

Methods: From March 2010 to February 2011 letters were sent to patients with fractures who had attended the orthopaedic outpatient clinic inviting them to have a bone density scan. The visit included a DXA scan (hip and spine), a detailed questionnaire and a FRAX assessment score.

Results: Two hundred and thirty letters were sent to patients aged 45 to 74 years following a fragility fracture. One hundred and sixteen replied with 104 bone densities being completed (84% females and 16% males). Seventy six per cent of these were wrist fractures and 21% humeral fractures. Only 14 patients were on treatment or had had a prior bone density scan. Bone density at the hip and spine revealed osteoporosis in 26% of patients, osteopenia in 37%, with a further 37% of patients with a normal bone density.

Patients with osteoporosis were treated with Calcium, Vitamin D and Alendronate. Twenty three per cent of the osteopenic and normal bone density patients with a FRAX score of greater than 3 were also put on Calcium, Vitamin D and Alendronate. A total of 63% of patients were recommended for some form of treatment with an additional 23% requiring extra treatment according to their FRAX assessment.

Conclusion: Measuring the bone density in the setting of peripheral fractures managed by an Orthopaedic Outpatient Clinic has revealed a significant (>60%) incidence of osteoporosis and osteopenia requiring treatment. FRAX scores are helpful in this regard. However, 50% of patients are still not having bone densities scans because of non-compliance. This issue needs to be addressed in the future.
25-HYDROXYVITAMIN D LEVELS, EXERCISE CAPACITY AND VASCULAR STIFFNESS IN PATIENTS ON DIALYSIS

G. J. Elder1, N. Hewitt2

1Department of Renal Medicine, Westmead Hospital, Sydney, NSW, Australia
2Student Clinical School, University of Notre Dame, Sydney, NSW, Australia

Patients on dialysis often have insufficient or deficient levels of 25-hydroxyvitamin D (25OHD). Nevertheless, calciferol supplementation is prescribed inconsistently, because evidence for laboratory or patient-level benefits is lacking. Although renal conversion of 25OHD to calcitriol is absent in these patients, local conversion occurs in target tissues including muscles, blood vessels and the parathyroid gland. We therefore undertook a randomised controlled trial of cholecalciferol versus placebo in patients on haemodialysis to assess patient-level and laboratory outcomes. Criteria for study entry included a screening 25OHD level ≤ 50nmol/L, stable dialysis for ≥ 3 months and no acute medical conditions. Sixty haemodialysis patients meeting these entry criteria underwent randomisation in autumn 2011 and their baseline characteristics are reported in this abstract. Volunteers had a median age of 62 years (range 20 - 86), 52% were female, body mass index was 29 ± 8.5 kg/m², 55% had a history of diabetes and in the previous month 10% suffered a fall. At randomisation, levels of 25OHD (mean ± SD) were 43 ± 13 nmol/L and were lower in patients with diabetes (p=0.014). 25OHD levels correlated positively to distance covered in a 6-minute walk (p=0.018), with a trend to improved standing balance (p=0.07) but not to grip strength, to dynamometer testing of other muscle groups or to chair stands. On laboratory testing, PTH levels (31±31 pmol/L) were positively associated to bone specific alkaline phosphatase (BSALP) (19±15 mcg/L; p<0.001) and to serum phosphate (p=0.008). Levels of 25OHD did not correlate to calcitriol (41±19 pmol/L), PTH, BSALP, serum calcium or phosphate. Pulse wave velocity, a test of vascular stiffness, correlated positively to age, negatively to levels of 25OHD but not to other laboratory or demographic data and, by stepwise regression, to 25OHD levels only (p=0.04). These associations are consistent with important end-organ 25OHD influences, supporting the necessity of a randomised intervention study.

UPDATE IN ORTHOPAEDIC SURGERY: REPORTING OF MINIMAL CLINICAL IMPORTANT DIFFERENCES IN SURGICAL TRIALS

I. Kashani

University of Western Australia, Department of Surgery, Royal Perth hospital, como, WA, Australia

Introduction

The minimum clinically important difference (MCID) is the smallest difference in outcome between the groups that would be of clinical interest. It influences the estimates that are made to determine the required sample size. The aim of this study was to explore the reporting of the MCID in surgical trials.

Methods

Surgical trials that were published between January 1981 and December 2010 in five prestigious surgical journals were evaluated. Selected for study were trials that studied two groups and reported the main outcome event as a proportion.

Results

Only 21% (100/486) of the admissible surgical trials mentioned a value for the MCID when estimating the sample size. There was a trend, however, for compliance with these factors to increase during the study period. The present post-hoc calculations of the required sample size, which were based on the observed differences between the groups at the end of the study, suggested that one-third of the trials should have accrued at least fivefold the number of patients. Although reporting an estimate of the sample size was associated with the study of more patients (median sample size 145 vs 100), it was not associated with the reporting of more positive results, that is, 61% (95/155) versus 65% (214/331).

Conclusion

There has been an improvement in the proportion of surgical trials reporting formal estimates of sample size during the last three decades. But the construct of these estimates is often suspect because of a failure to provide realistic values for the MCID. I would like to present an update on the implementation of minimal clinical important differences (MCID) for 2011 based on this recent publication. I would also like to focus on important aspects of how current standardized methods of MCID are being accepted to assist difficulties with MCID determination and how this is influencing sample size prediction and satisfactory results in surgical trials. I believe this topic needs to be highlighted to surgeons who are becoming more involved in the academia of evidence based medicine. I will be including examples of orthopaedic studies which have successfully have been able to implement MCID in demonstrating clinical importance.
A RANDOMIZED CONTROLLED TRIAL ON SAFETY AND EFFICACY OF SINGLE INTRAMUSCULAR V/S STAGGERED ORAL DOSE OF 6,00,000 IU VITAMIN D IN TREATMENT OF NUTRITIONAL RICKETS

K. Mondal1, A. Seth1, D. Dhanwal1, R. K. Marwah1, S. Aneja1, R. Singh1, P. Sonkar1
1Department of Pediatrics, Lady Hardinge Medical College and Kalawati Saran Children’s Hospital, New Delhi, India
2Thyroid Research Centre, Institute of Nuclear Medicine and Allied Sciences, New Delhi, India
3Department of Medicine, Maulana Azad Medical College, New Delhi, India
4Department of Biochemistry, Lady Hardinge Medical College, New Delhi, India
5Department of radiodiagnosis, Lady Hardinge Medical College, New Delhi, India

Background: Nutritional rickets is commonly treated by administering 6,00,000 iu of vitamin D paranterally as a single dose. However, doubts have been raised about safety of this regime. There is scant research on comparative efficacy and safety of oral and intramuscular routes for administering vitamin D.

Methods: Children with nutritional rickets (age group: 6 months - 5 years) were randomized to receive one of the 2 treatment protocols using block randomization (1) 60,000 iu vitamin D orally once a week for 10 weeks (2) 6,00,000 iu single intra-muscular injection. Serum calcium (total and ionic), phosphate, ALP, urinary calcium/creatinine ratio were measured at baseline, after 7 days, 4 weeks and at 12 weeks. Serum 25(OH)D was measured at baseline and after 12 weeks. A 10 point radiological score for severity of rickets was assessed at baseline, 4 weeks and 12 weeks.

Results: 61 cases (30 oral, 31 i/m) were followed for 12 weeks. Combined end point of ALP <420 IU/L and radiological score <1.5 was achieved in 93.3% cases in oral group and 90.3% cases in i/m group. No difference was found in efficacy of the 2 regimes on comparing serum calcium, phosphate, ALP levels and radiological score at 12 weeks. At baseline, 96.67% cases in oral and 93.55% in i/m group had serum 25(OH)D < 20 ng/ml. At 12 weeks 70% from oral group and 71% from i/m group achieved serum 25(OH)D level >20 ng/ml. Serum 25(OH)D >100 ng/ml was found in 2 children in oral group (105.4 ng/ml, 103.6 ng/ml) and 1 child in intramuscular group (106.10 ng/ml) at 12 weeks. None of them were symptomatic or had hypercalcemia. 2 children from each group developed asymptomatic hypercalcaemia (range-11.2-12.1mg/dl). 9 children with baseline elevated urinary calcium/creatinine ratio continued to have elevated urinary calcium/creatinine ratio through out the study period. No child other than these 9 children developed hypercalciuria during the study period.

Conclusion: Both staggered oral administration and single i/m dose of 6,00,000 iu vitamin D are equally effective and safe in treatment of nutritional rickets.

PATIENTS WITH INFLAMMATORY BOWEL DISEASES (IBD) ARE AT RISK OF OSTEOPOROSIS DEVELOPMENT

L. I. Myasoutova, A. G. Vasilyev
Department of Hospital Therapy, Kazan State Medical University, Kazan, Russian Federation

Aim. Evaluation of mineral bone density in IBD patients.

Methods. Small-dosed digital X-ray device “Diascan” was used for screening of bone mineral density. Distal part of radial bone was evaluated. T-criteria > -1.0 – was interpreted as normal bone mineral density, T-criteria from ≤ -1.0 to >-2.5 - osteopenia, T-criteria ≤ -2.5 - osteoporosis.

Results. 81 patients with IBD were enrolled: ulcerative colitis (UC) – 49 (60%), Crohn’s disease (CD) – 32 (40%) cases. Normal bone mineral density was seen in 9 (11%), UC – 7 (78%), CD – 2 (22%). Male - 3 (33%), female - 6 (66%), mean age – 34.1±14.0 years. Severity (UC was evaluated by Truelove&Witt score, CD - the Best index): mild – 1 (11%), moderate – 5 (56%), severe – 3 (33%). Therapy: steroids – 7 (78%) (including combination with 5-aminosalicylic acid (5-ASA)), 5-ASA – 2 (22%).

Osteopenia occurred in 38 (47%), UC – 23 (61%), CD – 15 (39%). Male – 13 (34%), female – 25 (66%), mean age – 33.4±14.2 years. Severity: mild – 11 (29%), moderate – 18 (47%), severe – 9 (24%). Therapy: steroids – 23 (60%), 5-ASA – 9 (26%), azathiopine (AZA) – 1 (3%), infliximab – 2 (6%), without treatment – 2 (6%).

Osteoporosis was revealed in 34 (42%), UC – 19 (56%), CD – 15 (44%). 18 (53%) - male, 16 (47%) – female, mean age – 33.6±14.4 years. Severity: mild – 4 (12%), moderate – 19 (56%), severe – 11 (32%). Therapy: steroids – 18 (53%), 5-ASA – 8 (24%), AZA – 5 (15%), infliximab – 2 (6%), without treatment – 2 (6%).

Conclusions. Reduce of bone mineral density was observed in the vast majority of patients - in 89% of cases, mean age of patients was 33.6±14.3 years.
FOOD CHOICES FOR CALCIUM ADEQUACY IN OLDER FEMALES

C. A. Nowson, A. O. Booth
Centre for Physical Activity and Nutrition Research, Deakin University, Burwood, VIC, Australia

Aim: Adequate dietary calcium intake in older women is essential to reduce menopausal and age related bone loss and prevent fractures. In a sample of older females, we assessed the proportion who met the Estimated Average Requirement (EAR, 1100mg/d) for calcium and the dietary food choice pattern that achieved calcium adequacy.

Methods: Analyses of food and nutrient intake derived from two, twenty-four hour recalls of usual food intake, completed in females aged 50 years and over who volunteered for dietary studies.

Results: The mean age of the 145 participants was 58.2 (SD 6.4) years, range 50-78 years and the mean calcium intake was 814 (323)mg calcium/d. Approximately one quarter met the EAR for calcium (28%, n = 41). Those who met the EAR consumed 2.5 (0.5) servings of dairy/d and dairy products provided an average of 726 (51)mg calcium which represented 66% of the EAR. Milk contributed 33% (413 (72)mg) and cheese contributed 18% (193 (46)mg) of total dietary calcium. Twenty-one percent consumed full fat milk only, 29% consumed reduced fat milk only, 3% consumed skim milk only and 47% consumed a combination. Both groups (calcium adequate/inadequate) exceeded the Suggested Dietary Target, ≤ 10% energy from saturated fat, consuming 13.4 (3.7)% and 11.7 (3.5)% energy from saturated fat respectively. If reduced-fat milk (2% fat or less), replaced full fat milk the percent energy from saturated fat would fall by a mean of 3% (from 13.4% of energy to 10.4% of energy).

Conclusions: Consumption of at least 2 ½ servings per day of dairy products is likely to ensure an adequate calcium intake (>1100mg) in females over 50 years. However consumption of reduced fat milk (2% fat or less) is required to ensure that saturated fat remains within reach of recommended levels.

WHAT DO GPS THINK AND BELIEVE ABOUT OSTEOPOROSIS? A CULTURAL MODELS APPROACH TO OSTEOPOROSIS TREATMENT AND PREVENTION

R. Otmar1, M. A. Kotowicz2, G. C. Nicholson3, J. A. Pasco4
1Medicine, NorthWest Academic Centre, The University of Melbourne, Highton, VIC, Australia
2Endocrinology and Diabetes, Barwon Health, Geelong, VIC, Australia
3Rural Clinical School, School of Medicine, The University of Queensland, Toowoomba, QLD, Australia
4Epidemilogy & Biostatistics Unit (Barwon Health), Dept Medicine, Deakin University, Geelong, VIC, Australia

Aim: The study aims to explore challenges in the treatment and prevention of fracture. Methods: The data comprised field notes and audio-transcripts from focus groups held with primary care physicians (GPs). Spradley's domain analysis was used to identify cultural models shared among the fourteen GP participants from south-eastern Australia. Cultural models are mental constructs about specific domains in everyday life, such as health and illness, which are shared within a community.

Results: Response domains were categorised to either ‘barriers’ or ‘enablers’ in treatment and prevention of osteoporosis. Barriers included inconsistent investigation or treatment of patients potentially at risk of fracture; gaps in GPs’ knowledge about osteoporosis investigation and treatment; and patient choice/autonomy presenting a challenge to the GPs’ role as medical authority. Perceptions shared among GPs included: osteoporosis had low salience, patients did not comply with prescribed treatments and the community lacked awareness of osteoporosis. Enablers included having access to high-quality investigative technologies, availability of a range of effective medications on the market, GPs’ continuing medical education and key messages that could improve community awareness of osteoporosis.

Conclusions: The cultural models identified in the domain analysis provide explanatory models for the investigation and treatment of osteoporosis by GPs in this region. The analysis enabled deep insights into why many GPs did not consistently, broadly and ultimately, osteoporosis has low salience among GPs, because there are gaps in their knowledge of the pharmacotherapies available on the market and because they perceive their patients as lacking in awareness of the condition as well as in their commitment to treatment adherence.


EFFECT OF STRONTIUM RANELATE ON VERTEBRAL PAIN SYNDROME AND FUNCTIONAL ABILITIES IN POSTMENOPAUSAL WOMEN WITH SYSTEMIC OSTEOPOROSIS

Institute of Gerontology AMS Ukraine, Kyiv, Ukraine

Aim. To evaluate the effect of strontium ranelate in treatment of systemic osteoporosis in postmenopausal women.
Materials and methods. There were examined 894 postmenopausal women with systemic osteoporosis (average age 59.97±10.57 years, average height 161.82±7.09 cm, average weight 71.32±13.44 kg). Evaluation of pain syndrome and level of physical activity was carried out with visual analog scale (VAS). Examination was performed before onset of treatment and after a four, eight and twelve month treatment course. Strontium ranelate (Bivalos, « Servier ») was taken in a dose of one 2 g sachet as a suspension in water once a day and 1 tablet of Calcemin-advance (Calcium – 500 mg, Vit. D – 400 IU) 2 times a day during 12 months.

Results. The patients had the risk factors of osteoporosis: 28% of patients had osteoporotic fractures in their anamnesis; 17% – hip fractures in mother or father of patients, 12% – smoking, 8% – alcohol abuse, 27% of patients have taken corticosteroid tablets for more than 3 month. We observed a reliable decrease of vertebral pain syndrome (after treatment – 2.97±0.77; after four months – 2.24±0.85, after eight months – 1.61±0.94; after twelve months – 1.24±1.04; p<0.00001) and increase of functional abilities of patients (after treatment – 1.50±0.67, after four months – 2.08±0.52, after eight months – 2.67±0.53; after twelve months – 2.88±0.63; p<0.00001).

Conclusion. It has been demonstrated that strontium ranelate treatment significantly decreases pronounced vertebral pain syndrome and improves functional abilities of patients in the postmenopausal women.

361

THIRTEEN CASES OF ATYPICAL SUBTROCHANTERIC AND DIAPHYSEAL FEMORAL FRACTURES AMONG THE 2,238 CASES OF FEMORAL FRACTURES OCCURRED DURING 2006 TO 2010 IN JAPAN

Y. Saita1, M. Ishijima1, A. Mogami2, T. Baba1, M. Nagao1, K. Sakai3, Y. Homma4, R. Kato4, K. Miyagawa5, N. Nagura2, T. Wada5, M. Yamanaka1, Y. Sakamoto1,4, O. Obayashi2, H. Gen3, H. Kajiharah5, M. Nozawah, K. Shiotos, K. Kanekoi

1Department of Orthopaedics, Juntendo University School of Medicine, Tokyo, Japan
2Department of Orthopaedic Surgery, Juntendo Shizuoka Hospital, Shizuoka, Japan
3Department of Orthopaedic Surgery, Juntendo Urayasu Hospital, Chiba, Japan
4Department of Orthopaedic Surgery, Juntendo Nerima Hospital, Tokyo, Japan
5Department of Orthopaedic Surgery, Chiba Central Medical Center, Chiba, Japan

The ASBMR task force defined major and minor features of atypical femoral fractures in response to the reports linking long-term use of bisphosphonates (BPs) with these fractures (JBMR, 11, 2267-94, 2010). It recommends that all major features be present to designate as atypical and also developed a case definition so that subsequent studies report on the same condition.

Following this definition, we retrospectively investigated all 2,238 proximal and diaphyseal femoral fractures treated in 6 hospitals related to our university in Japan during 2006-2010.

During this period, 13 cases (9 patients) (0.53%) of atypical fractures were found. All these cases showed major features. While 7 of 13 cases showed subtrochanteric fracture (0.36% of proximal femoral fractures), residual 6 cases were diaphyseal fracture (1.1% of diaphyseal fractures). All the 9 patients were female. While the mean age of 13 cases was 64.5y, that of subtrochanteric fractures (54.0y) were much younger than that of diaphyseal fractures (76.7y) (p<0.05). Bilateral fractures were occurred in 4 patients (44%). The duration of BP therapy was; within one year: 1 patient, 1-5 years: 4, over 5 years: 3. The history of glucocorticoids therapy was found in 6 patients (67%). Among them, 4 patients (44%) were long-term glucocorticoid users due to collagen diseases, such as systemic lupus erythematosus and dermatomyositis from their early-life. It should be emphasized that all these glucocorticoid users showed subtrochanteric fractures. The proton pump inhibitors were used in 2 of 9 patients (22%). Twelve of thirteen cases were operated with nail fixation and residual one case with locking plate (due to narrow medullary space). Prophylactic nail fixation was performed in 2 patients, as they complained prodromal pain and radiographic changes were detected in contralateral side. Delayed healing was observed in 4 cases (31%).

If this study is applied to the ASBMR task force established hierarchy, this study would be corresponded to have the “acceptable evidence”. The atypical femoral fractures were shown to be occurred not only BPs users but also patients who have not been treated with BPs. However, our data suggest that the long-term combination therapy of glucocorticoids and BPs might be one of risk factors for the atypical subtrochanteric fracture.


362

BONE MINERAL HOMEOSTASIS INCLUDING VITAMIN D LEVELS AND BONE DENSITY IN INDIAN HIV POSITIVE SUBJECTS

D. Dhanwal, S. Samad, R. Devan

Medicine, Maulana Azad Medical College, New Delhi, India

Aim: To study vitamin D status and bone mineral density in treatment naïve HIV positive patients and its correlation with immunological status and clinical stages.

Material and method: Study included 60 HIV positive ART naïve patients. Patients classified in different clinical stages of HIV according to National AIDS control organization (NACO) guidelines. Markers of bone mineral homeostasis such as serum calcium,
serum phosphate, alkaline phosphatase (ALP) along with 25 (OH) vitamin D and PTH were analyzed. Immune status was evaluated by CD4 and CD3 counts. DEXA scan was done in 20 patients at hip and lumbar spine.

Results: Mean age of patients was 34.11±8.42 yr. There was high prevalence of vitamin D deficiency (81.6%) in HIV positive ART naïve patients. Fifty five percent of subjects had severe (<10 ng/ml) vitamin D deficiency. Vitamin D deficiency was associated with secondary hyperthyroidism. Vitamin D deficiency did not correlate with CD4 and CD3 counts. There was a trend for association between vitamin D levels and clinical stage of HIV but this was not statistically significant. Osteoporosis was observed in 15% subjects at LS and osteopenia was seen in 55 and 60% subjects at hip and spine respectively.

Conclusions: There is high prevalence of vitamin D deficiency among treatment naïve Indian HIV positive subjects. Significant number of these subjects has low bone density at hip and spine.

### 363

**SERUM IGF-1 LEVELS IN PATIENTS WITH ACROMEGALY**

K. Yildirim¹, H. Uzkeser², G. Akcay³, S. Karatay¹

¹Departments of Physical Medicine and Rehabilitation, Medical Faculty, Ataturk University, Erzurum, Turkey
²Department of Physical Medicine and Rehabilitation, Namune State Hospital, Turkey
³Internal Medicine, Medical Faculty, Ataturk University, Erzurum, Turkey

Aim: In recent years measurement of serum insulin-like growth factor-1 (IGF-1), a reflection of the biological activity of GH secretion, has become a mainstay of diagnosis of acromegaly and, perhaps even more importantly, of the success of treatment. This study was carried out to investigate the serum IGF-1 levels of patients with acromegaly.

Material and Methods: Thirty patients with acromegaly and twenty matched healthy controls were enrolled in this study. Serum IGF-1 were measured in laboratory analysis in both groups.

Results: There were no statistically significant differences between the two groups with respect to demographic data (p>0.05). In patients with acromegaly, serum IGF-1 values were significantly increased than those of the healthy controls (p< 0.05). Serum IGF-1 levels of patients and controls were 450 and 294.3 ng/ml, respectively.

Conclusion: Our results suggest that serum IGF-1 levels may exchange in patients with acromegaly according to control cases. Further long-term studies on the subject are needed to explore relation to between serum IGF-1 levels and bone metabolism or disease activity in patients with acromegaly.

### 364

**VITAMIN D LEVELS IN SUBJECTS WITH ACROMEGALY**

H. Uzkeser¹, K. Yildirim², G. Akcay³, S. Karatay¹, M. Akbas³

¹Departments of Physical Medicine and Rehabilitation, Namune State hospital, Erzurum, Turkey
²Ataturk University, Erzurum, Turkey
³Internal Medicine, Medical Faculty, Ataturk University, Erzurum, Turkey

Aim: In the acromegaly patients, growth hormone stimulates bone turnover by influencing other regulators of bone metabolism such as vitamin D. There is also evidence of increased bone turnover in patients with acromegaly. The purpose of this article is to assess the serum 25-hydroxyvitamin D levels in the patients with acromegaly and healthy controls.

Material and Methods: Thirty patients with acromegaly and 20 matched healthy controls were consecutively included in the study. Serum 25-hydroxyvitamin D, Ca, and P levels were analyzed in both groups.

Results: The demographic variables like age and sex and body mass index (BMI) were similar between acromegaly patients and control subjects (p>0.05). Serum 25-hydroxyvitamin D, Ca, and P levels were not statistically significant in with acromegaly patients than control subjects (p>0.05).

Conclusion: Our results suggest that serum 25-hydroxyvitamin D levels were similar in patients with acromegaly according to control cases. Further long-term studies on the subject are needed to explore relation to between serum 25-hydroxyvitamin D levels and drug treatment or disease activity in patients with acromegaly.

### 365

**EXTERNAL QUALITY ASSESSMENT OF COMMERCIAL ASSAYS FOR THE REFERENCE STANDARD BONE TURNOVER MARKERS SERUM PINP AND SERUM BETA-CTX**

S. D. Vasikaran¹, H. A. Morris², W. Egner³, D. Patel¹, C. Cooper¹, J. A. Kanis⁵

¹Department of Core Clinical Pathology and Biochemistry, PathWest-Royal Perth Hospital, Perth, WA, Australia
²School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA, Australia
³UK NEQAS for Immunology, Immunochemistry & Allergy, Department of Immunology, Northern General Hospital, Sheffield,
United Kingdom
MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton General Hospital, Southampton, United Kingdom

Centre for Metabolic Bone Diseases (WHO Collaborating Centre), University of Sheffield Medical School, Sheffield, United Kingdom

Y. Miao, L. Chen, R. Jia

Department of Geriatrics, Peking University People’s Hospital, Beijing, China

Introduction: The objective of this study was to investigate the effect of polymorphisms in osteoprotegerin (OPG) gene promoter on bone mineral density (BMD) in elderly men.

Methods: The subjects consist of 274 elderly men (aged: 60-94 yrs). BMD of the lumbar spine, femoral neck, Troch and total hip were measured using a Hologic densitometer.

THE ASSOCIATION BETWEEN POLYMORPHISMS IN THE OSTEOPROTEGERIN GENE AND BMD IN ELDERLY MEN

Y. Miao, L. Chen, R. Jia

The objective of this study was to investigate the effect of polymorphisms in osteoprotegerin (OPG) gene promoter on bone mineral density (BMD) in elderly men.

Methods: The subjects consist of 274 elderly men (aged: 60-94 yrs). BMD of the lumbar spine, femoral neck, Troch and total hip were measured using a Hologic densitometer.

SEQUENCING AND GENOME-WIDE ASSOCIATION STUDY IN SPORADIC HIGH BONE MASS CASES

G. R. Clark1, C. Gregson2, K. Addison1, M. Brugmans1, E. L. Duncan1, M. A. Brown1

1Human Genetics Group, University of Queensland Diamantina Institute, Wooloongabba, QLD, Australia

2University of Bristol, Bristol, United Kingdom

Aim: This study aimed to determine the genetic cause for unexplained high bone mass (HBM) in a large cohort with extremely high BMD, by screening known HBM-associated genes and looking for novel HBM-associated genes.

Methods: The HBM cohort was recruited through UK DXA databases. HBM was defined as a) L1 Z score of ≥ +3.5 plus total hip Z score of ≥ +1.2, or b) total hip Z score ≥ +3.5. Cases with known causes of HBM were excluded.

GWAS was performed using Illumina HumanOmniExpress-12 v1.0 chips in 240 HBM cases (without known mutations) analysed against two control groups: a) low BMD arm of the Australian Osteoporosis Genetics Consortium (n=900); and b) UK 1958 birth cohort controls (n=5667). Association was assessed using PLINK and the Cochrane Armitage test of trend.

Conclusions: Our data shows that sporadic cases with HBM rarely harbour mutations in genes previously associated with HBM. We have also shown that HBM is a highly heterogeneous trait, unlikely to be mapped by association studies; and approaches such as next-generation sequencing may be more successful for gene discovery in this cohort.
Polymorphisms in OPG promoter (A163G, T245G, T950C) were analyzed. Genotyping was performed using the ABI3730XL sequencing system or RFLP. One-way ANOVA were used to compare the BMD of different genotypes in elderly men.

Results: The different genotype of A163G has different BMD in lumbar spine and Troch region. The total lumbar spine BMD of genotype AA, AG and GG was 1.15 ± 0.14 , 1.04 ± 0.18, 0.99±0.18 (g/cm²) respectively (p<0.01). The troph BMD of AA, AG and GG was 0.74 ± 0.12 , 0.68 ± 0.13, 0.64±0.12 (g/cm²) respectively (p<0.05). We did not find the association between polymorphism T245G, T950C and BMD in elderly men.

Conclusion: Our result support that polymorphism A163G in the OPG gene play a role in the pathogenesis of osteoporosis in elderly men, but no interaction between T245G, T950C and BMD could be demonstrated.

### RELATIVE RISK FACTORS FOR OSTEOPOROSIS IN YOUNG JAPANESE WOMEN BETWEEN POLYMORPHIC VARIANTS OF IMMUNE CYTOKINE AND BONE MINERAL DENSITY

Y. Oishi1, Y. Watanabe1, S. Shinoda1, Y. Fuke1, M. Naka2, Y. Ozawa1, T. Matsuyama1, K. Morozumi2

1Tokyo Metropolitan University, Hachioji, Tokyo, Japan
2the Marunouchi Integrated Health Clinic for Women, Chiyoda-ku, Tokyo, Japan
3Fussa Hospital, Fussa, Tokyo, Japan

Many factors influence the risk of osteoporosis including diet, physical activity, medication use, coexisting disease, aging, and diminished sex-steroid production. Further one of the most important clinical risk factors is a family history, emphasizing the involvement of genetics in the pathogenesis of osteoporosis. Peak bone mass is a major factor determining the risk of osteoporotic fracture. The bone mass attained early in life is perhaps the most important factor of lifelong skeletal health. Recent studies have highlighted the interaction between bone and immune cells, many of soluble mediators of immune cells, including cytokines, chemokines, and growth factors, regulate the activate cells that control bone turnover. The aim of this study was to investigate the polymorphic variants of IL-6, IL-17F and TNF-α related to bone mineral density (BMD) in young Japanese women. DNA samples were obtained from the fingernail of fifty-seven healthy young women (age: 18-22) in Tokyo Metropolitan University. The extracted genomic DNA was the polymerase chain reaction (PCR) amplified IL-6 gene (nt-634), IL-17F gene (nt 7488) and the TNF-α gene (nt-308). Cleavage of the PCR products treated by BsrB I on the IL-6 gene, by Nla III on the IL-17F gene , and by Nco I on the TNF-α gene. Participants were measured their BMD of the total body, lumbar spine (L1- L4) and femoral neck by dual-energy x-ray absorptiometry. The frequencies of IL-6 genotype were 57.9% (CC), 38.6% (CG) and 3.5% (GG), IL-17F genotype were 5.3% (GG), 14.0% (GA) and 80.7% (AA), TNF-α genotype were 87.7% (GG) and 12.3% (GA). Mean values and standard deviations (SD) of BMD were 1.13± 0.07 g/cm2 for total body, 1.14 ± 0.11 g/cm2 for lumbar spine and 1.00 ± 0.11 g/cm2 for femoral neck. These genotypes were not associated with BMD at total body, lumbar spine or femoral neck.

### EGFL6 PROMOTES ENDOTHELIAL CELL MIGRATION AND ANGIogenesis THROUGH THE ACTIVATION OF EXTRACELLULAR SIGNAL-REGULATED KINASE

S. Chim1, A. Qin1, J. Tickner1, N. Pavlos2, T. Davey2, H. Wang3, Y. Guo4, M. Zheng5, J. Xu1

1School of Pathology and Laboratory Medicine, The University of Western Australia, Perth, WA, Australia
2Centre for Orthopaedic Research, School of Surgery, The University of Western Australia, Perth, WA, Australia
3International Joint Cancer Institute and 301 General Hospital Cancer Center, Second Military Medical University, Shanghai, China

Angiogenesis is required for bone development, growth, and repair. It is influenced by the local bone environment that involves cross-talks between endothelial cells and adjacent bone cells. However, data regarding factors that directly contribute to angiogenesis by bone cells remain poorly understood. Here, we report that EGFL6, a member of the epidermal growth factor (EGF) repeat superfamily proteins, induces angiogenesis by a paracrine mechanism in which EGFL6 is expressed in osteoblastic-like cells but promotes migration and angiogenesis of endothelial cells. Co-immunoprecipitation assays revealed that EGFL6 is secreted in culture medium as a homodimer protein. Using scratch wound healing and transwell assays, we found that conditioned medium containing EGFL6 potentiates SVEC (A simian virus 40-transformed mouse microvascular endothelial cell line) cell migration. In addition, EGFL6 promotes the endothelial cell tube-like structure formation in Matrigel assays and angiogenesis in a chick embryo chorioallantoic membrane. Furthermore, we show that EGFL6 recombiant protein induces phosphorylation of ERK in SVEC endothelial cells. Inhibition of ERK impaired EGFL6-induced ERK activation and endothelial cell migration. Together, these results demonstrate, for the first time, that osteoblastic-like cells express EGFL6 that is capable of promoting endothelial cell migration and angiogenesis via ERK activation. Thus, the EGFL6 mediates a paracrine mechanism of cross-talk between vascular endothelial cells and osteoblasts and might offer an important new target for the potential treatment of bone diseases, including osteonecrosis, osteoporosis, and fracture healing.
ANABOLIC STIMULI ENHANCE OSTEOBLAST IL-33 MRNA EXPRESSION, AND IL-33 ENHANCES WNT RESPONSES AND OSTEOBLASTIC MINERALISATION IN VITRO

D. G. Felea1,2, P. P. Singh1, H. Saleh1, A. C. Wu1, M. R. Forwood3, B. L. Grills3, J. A. Schuijers2, S. J. McDonald2, M. T. Gillespie1,2, J. M.W. Quinn1,4

1Prince Henry's Institute, Clayton, VIC, Australia
2Musculoskeletal Research Centre, La Trobe University, Bundoora, VIC, Australia
3School of Medical Science, Griffith University, QLD, Australia
4Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia

IL-33 is an alarmin and Th2 stimulating cytokine, with actions mediated by receptor ST2L. We have shown IL-33 is expressed by osteoblasts and regulated by PTH and oncostatin M (OSM); IL-33 also inhibits osteoclastic formation, while enhancing osteoblastic mineralisation.

Aim: To investigated how IL-33 might be induced in bone and how it influences osteoblastic activity.

Methods: We employed rat ulna loading and fracture callus models (Kidd et al; McDonald et al) to examine IL-33 levels under these conditions. We obtained primary osteoblasts from digested mouse neonatal calvariae. Osteoblasts were matured with ascorbate and beta-glycerophosphate to observe mineralisation and enhancement of mature osteoblast and osteocytic characteristics. We studied osteoblast mRNA expression by quantitative real-time RT-PCR.

Results: We found IL-33 mRNA levels were elevated (24h) in stressed rat ulnae and also enhanced in bone when callus was formed. In osteoblasts, IL-33 mRNA was enhanced by IL-1 but not by TNF, although TNF treatment increased ST2L mRNA levels, suggesting some direct responses to inflammation.

Although IL-33 enhanced osteoblast mineralisation in vitro, osteoblastic marker (e.g. BRIL and BSP) expression was not greater, suggesting maturation is not generally increased by IL-33. However, IL-33 strongly down-regulated sst mRNA in both osteoblasts and calvarial organ cultures, comparable to PTH and OSM. IL-33 also rapidly (1h) enhanced mRNA expression of Wnt1, 3A and 10B, which have previously been linked to pro-anabolic bone responses. Consistent with these observations, beta-catenin regulated target Axin2 mRNA increased with IL-33 treatment. Wnt3A protein also enhanced osteoblast IL-33 mRNA, suggesting a possible autocrine regulatory loop.

Conclusions: IL-33 expression in bone and osteoblasts is enhanced by bone anabolic influences including mechanical loading, PTH and OSM, but also by inflammatory factors. Enhanced of osteoblastic action in vitro by IL-33 may result from higher Wnt signals due to both increased Wnt and decreased sclerostin production.

(1) Kidd et al, Bone 2010 46:369
(2) McDonald et al J Orthop Res. 2009 27:1508

GLYCICANS MEDIATED REGULATION OF BONE MORPHOGENETIC PROTEIN SIGNALLING AND OSTEOGENESIS IN HUMAN CRANIAL SUTURE CELLS

N. N. Lam1, P. P. Dwived2, R. H. Gross3, C. S.H. Hii4, J. Filmus1, P. J. 4, B. C. Powell2

1Discipline of Physiology, University of Adelaide, Adelaide, SA, Australia
2Craniofacial research group, Women's and Children's Health Research Institute, Adelaide, SA, Australia
3Department of Immunology, Women's and Children's Hospital, Adelaide, SA, Australia
4Australian Craniofacial Unit, Women's and Children's Hospital, Adelaide, SA, Australia
5Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada

Craniocynostosis, the premature bony fusion of one or more of the cranial sutures, is a faci-disfiguring abnormality in infancy and childhood, however the mechanisms that give rise to craniosynostosis are unclear. We have previously shown that glypticin 1 (GPC1) and glypican 3 (GPC3) are down-regulated during premature suture fusion (1), suggesting that reduced levels of GPC1 and GPC3 may play a role in craniosynostosis. Glypicans are well recognized to regulate various growth factors signalling pathways, including that of the bone morphogenetic protein (BMP), which has been shown to play a crucial role in skull development, particularly BMP2 signalling pathway (2). We have therefore investigated the effect of GPC1 and GPC3 on BMP2 signalling in proliferating and osteogenic human primary cranial suture cells.

Using confocal microscopy we demonstrated that in proliferating human cranial suture cells, GPC1 and 3 are co-localised with BMP2 receptors at the cell surface and intracellularly. Transient co-transfection reporter assays demonstrated that induction of BMP2 reporter activity was significantly repressed by ectopic expression of GPC1 and GPC3. Furthermore, recombinant GPC1 and GPC3 proteins dose-dependently repressed BMP2-mediated transcriptional activity and blockaded by anti-GPC1/3 antibody increased BMP2 activity. Moreover, treatment of recombinant GPC3 proteins markedly reduced BMP2-mediated osteogenesis in human suture cells.

We conclude from these data that GPC1 and GPC3 are antagonists of BMP2 signalling and osteogenesis in human suture cells. We are currently examining mechanisms of how GPC1 and GPC3 regulate BMP2 signalling and the role of GPC1 and GPC3 in skull development and craniosynostosis using knock-out mouse models. We hypothesised that loss of endogenous GPC1 and/or GPC3 will lead to craniosynostosis through BMP-mediated signalling.

NOVEL EXTRANEURAL ROLE OF NOGO-A: MODULATION OF OSTEOCLASTOGENESIS VIA POSITIVE FEEDBACK REGULATION OF NFATC1

Y. Lee1,2, H. Kim1
1Cell and Dev Biology, Seoul Nat Univ, Seoul, Sth Korea
2Dental Res Institute, Seoul Nat Univ, Seoul, Sth Korea

Osteoclasts are bone-resorbing cells differentiated from macrophage/monocyte lineage precursors upon receptor activator of NFκB ligand (RANKL) stimulation. In a proteomic approach to identify proteins involved in osteoclastogenesis, we observed a dramatic increase in the expression of Nogo-A upon RANKL stimulation of mouse bone marrow macrophages (BMMs) in a nuclear factor of activated T cell cytoplasmic 1 (NFATc1)-dependent manner. The knock down of Nogo-A in BMMs significantly reduced RANKL-dependent osteoclast differentiation accompanied by diminished NFATc1 induction. Conversely, Nogo-A overexpression in BMMs as well as in RAW264.7 macrophages greatly augmented osteoclastogenesis with concomitant increase in the NFATc1 induction. Both mitogen activated protein kinase pathway and calcium oscillation, which are central to the RANKL-induced bone mineral density that appears to implicate a higher AngII antagonist, were amplified by Nogo-A. Finally, Nogo-A knock down in mouse calvariae prevented IL-1-induced bone loss. These findings not only reveal unprecedented extraneural role of Nogo-A in osteoclastogenesis but also suggest novel drug target against bone mineralisation.

ANGIOTENSIN II REGULATES RANKL:OPG RATIO POTENTIALLY THROUGH TRANSACTIVATION OF ERBB RECEPTORS BY EGF-LIKE LIGANDS

K. E. MacRae, W. G. Thomas, C. Sernia
School of Biomedical Sciences, The University of Queensland, St Lucia, QLD, Australia

Angiotensin II (Ang II) is a potent pressor hormone and a dominant causative factor in cardiovascular disease. Elderly hypertensives show reduced bone mineral density that appears to implicate a higher AngII-induced RANKL:OPG ratio. However, the cellular pathways underpinning these changes are unknown but one hypothesis -which we investigate in this study- involves the transactivation of epidermal growth factor receptors (ErbB receptors) by the angiotensin type 1 receptor (AT1R).

Methods: Serum-deprived UMR-106 osteoblast cells were stimulated with Ang II and the EGF-like ligands; epidermal growth factor (EGF), heparin binding-EGF (HB-EGF), betacellulin (BTC) and neuregulin 1β (NRG1β). Cells were also treated with AG1478 (selective ErbB1 inhibitor) or TAPI-1 (non-selective ADAM inhibitor) 45 minutes prior to adding Ang II. Total RNA was isolated with Trizol and OPG, RANKL, ErbB1, ErbB2, ErbB3, ErbB4, ADAM 17, EGF, HB-EGF, BTC and NRG1β expression was analysed semi-quantitatively by RT-PCR. ERK activation by Ang II was evaluated by Western Blotting.

Results: We confirmed that UMR-106 cells express the four ErBb receptor subtypes and several of their ligands. Western Blotting revealed ERK activation after 1-3 min of Ang II treatment and this may be partially attenuated with the addition of AG1478. Ang II (1x10^{-8}M) increased ADAM 17 expression 2-fold. There was also up-regulation of EGF (3.1 fold), HB-EGF (3 fold), BTC (2.3 fold) and NRG1β (2.3 fold). All ErbB receptor subtype expression increased by 2-fold. In keeping with our hypothesis, the EGF-like ligands induced a significant 2-3 fold down-regulation of OPG expression, a significant 2-3 fold up-regulation in RANKL expression and these effects were partially prevented by inhibitors AG1478 and TAPI-1.

Conclusion: These data support our hypothesis that Ang II increases RANKL:OPG ratio partly via transactivation of ErbB receptors by the AT1R. This novel finding provides an insight into the mechanism through which AngII-associated hypertension induces lower bone mineralisation.

BMP-INDUCED ECTOPIC BONE FORMATION IN C-FOS-DEFICIENT MICE

M. Nakamura, T. Ninomiya, T. Mizoguchi, A. Arai, N. Takahashi, N. Udagawa
Matsumoto Dental University, Shiojiri, Nagano, Japan

We previously examined how osteoblasts are involved in osteoclastogenesis other than RANKL expression, using RANKL-deficient mice (Endocrinology 147:3366-3374, 2006). RANKL-deficient mice showed severe osteopetrosis due to lack of osteoclasts. Injection of RANKL into RANKL-deficient mice induced many osteoclasts in bone but not soft tissues. Most of TRAP-positive osteoclasts localized in contact with ALP-positive cells in rhBMP-2 containing disks in RANKL-deficient mice injected with RANKL. These results suggest that mesenchymal osteoblasts determine the place of osteoclastogenesis from haemopoietic stem cells in bone. We explored roles of osteoclasts in ectopic bone formation induced by BMP using op/op and c-fos-deficient osteopetrotic mice. The ectopic bones formed in op/op mice showed extremely rough surfaces, whereas those in wild-type mice showed smooth ones. Bone mineral density of BMP-induced ectopic bone in op/op mice was about 2-times higher than that in wild-type mice. TRAP-positive osteoclasts in the ectopic bone in the wild-type mice. In op/op mice, although osteoclasts strongly exhibit in inside of the BMP-induced ectopic bone, TRAP-positive osteoclasts did not exhibit in outer of the BMP-induced ectopic bone. Furthermore, the accentuation of the BMP-induced ectopic bone formation did not exist in osteopetrotic c-Fos-deficient mice. These results indicate that BMP-induced ectopic bone formation is accurately enhanced in the absence of osteoclasts in outer part of ectopic bone.
in op/op mice, and osteoclasts are involved in normal bone morphogenesis. In c-Fos-deficient mice, which are completely osteoclasts deficiency, the accentuation of the BMP-induced ectopic bone formation did not exist. Furthermore, there are no RANK-positive osteoclast progenitors in bone derived from c-Fos-deficient mice. These results suggest that RANK-positive osteoclasts and osteoclast progenitors positively regulate the signal of bone formation and osteoclasts are involved in normal bone morphogenesis.

INTERLEUKIN-11 IS REQUIRED FOR THE MAINTENANCE OF AXIAL BONE MASS IN MICE

T. Kondo, S. Omatsu, Y. Ohnishi, S. Aizawa, I. Endo, T. Matsumoto

1Department of Medicine and Bioregulatory Sciences, The university of Tokushima of Graduate School of Medical Sciences, Tokushima city, Tokushima, Japan
2Laboratory for Animal Resources and Genetic Engineering, Center for Developmental, RIKEN Kobe, Kobe city, Hyogo, Japan
3Student laboratory, The university of Tokushima of Graduate School of Medical Sciences, Tokushima city, Tokushima, Japan

Interleukin (IL)-11 is mainly expressed in bone marrow stromal cells, and inhibits adipocytic differentiation while stimulating osteoblastogenesis. The expression of IL-11 is enhanced by mechanical stress, which rapidly induces FoxB gene transcription and Smad1/5 phosphorylation, and Smad1/5 cooperatively up-regulate IL-11 gene expression (Plos ONE 5e:13090,2010). IL-11 activates Wnt signaling by suppressing the expression of its inhibitors including dikkopf1 and 2 (Bone 45:1125,2009). Although those findings suggest the involvement of IL-11 on mechanical stress-induced osteoblastogenesis and bone formation, there was a report that IL-11 receptor knockout (KO) mice showed an increase in bone mass with reduced osteoclastic bone resorption and osteoblastic bone formation (JBM R 20:1093,2005). Thus, controversy remains as to the physiological role of IL-11 in the maintenance of bone mass. In order to address this issue, we created IL-11 KO mice, and compared their bone with that of wild-type (WT) mice.

The growth and appearance of IL-11 deficient mice were indistinguishable from that of WT mice. Bone mineral density (BMD) measured by micro CT revealed that lumbar BMD of female IL-11 KO mice became lower compare with that of WT mice as early as 8 weeks of age (367+5.0 vs 379+2.6, p<0.04). Femoral BMD of female IL-11 KO mice was also lower compare with that of WT mice at 12 weeks of age (572+4.8 vs 584+2.4, p<0.04). The reduction in BMD of IL-11 KO mice was more pronounced in cancellous bone, and the difference in BMD gradually increased with age until 24 weeks. In sharp contrast, BMD of non-weight bearing calvarial bone was not reduced in IL-11 KO mice. Similar results were obtained in male IL-11 KO mice.

These results demonstrate that IL-11 deficiency in mice causes a reduction in the axial bone mass, but does not affect calvarial bone mass. It is suggested that IL-11 plays an important role in the maintenance of bone mass, and that the effect of IL-11 is mediated via a stimulation of bone formation in response to mechanical stress.

IMMUNE AND OSTEOGENIC: THE TWO FACES OF INTERFERON GAMMA

C. Vidal, B. Nanan, R. Nanan, G. Duque

1Ageing Bone Research Program, Sydney Medical School Nepean - The University of Sydney, Penrith, NSW, Australia
2Pediatrics and Immunology, Sydney Medical School Nepean - The University of Sydney, Penrith, NSW, Australia

Recently, interferon gamma (IFNγ) has been identified as a potent bone anabolic agent both in vitro and in vivo (Duque et al., Stem Cells, 2009 & J Bone Miner Res, 2011). However, the specific pathways involved in the osteogenic vs. immunogenic effect of IFNγ have not been differentiated. To elucidate these pathways, we used a gene expression microarray to compare gene expression in IFNγ-treated cultures of early differentiating osteoblasts, mesenchymal stem cells (MSC) and Jurkat cells. MSC were induced to differentiate into osteoblasts for 7 days in the presence or absence of IFNγ (100 ng/ml). Simultaneously, undifferentiating MSC and Jurkat cells were grown in the presence or absence of IFNγ at the same concentration. On day 7, total RNA was extracted and gene expression analysis was performed using the Human Gene 1.0 ST array (Affymetrix). Analysis of data was performed using ArrayTools software comparing IFNγ-treated and non-treated cells for each individual cell type. Upregulated genes were confirmed by real-time PCR. Following treatment with IFNγ, 116, 79, and 71 genes were significantly upregulated (>2 fold) in differentiating osteoblasts, MSC, and Jurkat respectively. Amongst the 116 IFNγ-upregulated genes in differentiating osteoblasts, 92 genes were exclusive to this cell type allowing us to identify 6 major functional clusters. Pathway analysis showed genes involved in cytokine-cytokine receptor interactions (11 genes), Toll-like signaling (5 genes), LDL pathway (3 genes) and tryptophan metabolism (2 genes). In contrast, pathway analysis in IFNγ-treated Jurkat cells showed genes predominantly involved in the immune response (28 genes), host-virus interaction (12 genes) and Jak-STAT signaling (3 genes). In summary, we have identified a new osteogenic non-immune profile for IFNγ, thus providing a better understanding of the effect of IFNγ on bone and identifying a set of selective anabolic targets to treat osteoporosis in the near future.
MCP-1 GENE EXPRESSION DOMINATES CHEMOKINE ACTIVATION OF SKELETAL REPAIR AND REMODELLING

A. C. Weg, N. A. Morrison, M. R. Forwood
School of Medical Science, Griffith University, Southport, QLD, Australia

Monocyte chemotactic protein-1 (MCP-1) belongs to the CC chemokine superfamily and plays a critical role in recruitment and activation of leukocytes during acute inflammation. We hypothesize that MCP-1 is also the dominant chemokine regulating recruitment and activation of bone cells required for skeletal repair and remodelling. We have characterised an innovative stress fracture (SFx) model of the rat ulna, allowing investigation of focal remodelling with a known time course and precise anatomical location. Following SFx initiation, remodelling units migrate along the SFx line to repair the damage. SFx were created in the right ulna of female wistar rats using cyclic end-loading. Unloaded animals were used as a control. Rats were euthanized 4h, 1, 4, 7 and 14 days after loading (n=5/group) and RNA extracted and converted to cDNA for quantitative PCR analysis using TaqMan gene expression assays. Four hours after loading, MCP-1 gene expression was significantly increased ~30-fold (P<0.001), remained elevated at 24 h (~12 fold, P<0.001), then declined by day 14. It's receptor, CCR2, increased expression over the time course of 14 days, being significant at 14 days (P<0.05). Other chemokines (SDF-1, RANTES, MIP1a) were not increased at these early time-points. Using immunohistochemistry in separate groups, MCP-1 was localized in periosteal osteoblasts associated with woven bone formation at the fracture exit point, but not within osteocytes adjacent to the SFx. These data support a key role for MCP-1 in the early phase of SFx repair and activated remodelling. We hypothesise that MCP-1 provides crucial regulation of both chemotaxis and osteoclast differentiation during initiation events of bone remodelling.

POSSIBLE DIRECT ROLE OF DOPAMINE IN HEALING OF FRACTURED BONE

D. J. Doyle, S. J. McDonald, A. C. McDonald, J. A. Schuijers, B. L. Grills

Tissue and Cell Biology Group, Musculoskeletal Research Centre, La Trobe University, Bundoora, VIC, Australia

Osteoprogenitor cells (OPCs) in early fibrous fracture callus contain an abundance of alpha smooth muscle actin (1). This protein allows for *ex vivo* force production within callus and this process is hypothesized to play an important, positive role in fracture healing *in vivo* (2). Due to the known ability of dopamine to facilitate fracture healing as well as contract smooth muscle, it is therefore possible that dopamine may have a direct contractile action on callus to facilitate fracture healing.

Aim: to determine whether dopamine could contract early fracture callus *ex vivo* and possibly identify and localize receptors of dopamine in callus.

Methods: Twenty C57BL/6 mice received a transverse fracture of both fibulae, followed by removal of calluses 7 days post-fracture for force transducer studies as well as qPCR and Western blot analyses. Callus tissue from 14- and 21-day fractured fibulae were also used from previous experiments for gene expression and protein analysis. Unfractured fibulae acted as controls.

Results: Force transducer experiments showed an absence of active force production in callus in response to dopamine (10^{-4} M). qPCR and Western blot analysis revealed that the only dopamine receptor to be present in unfractured fibula and callus was the dopamine 4 (D_{4}) receptor and the mRNA expression for this receptor showed an approximate 5- and 7-fold upregulation in 7 day callus (p < 0.05) and 14 day callus (p < 0.01) respectively compared to unfractured fibula. Immunohistochemistry showed that the D_{4} receptor was mainly localised to OPCs within callus.

Conclusions: The lack of dopamine-induced murine fibula callus contraction *ex vivo* and the observed upregulation and localisation of the D_{4} receptor in callus may signify that dopamine acts on OPCs of callus in a metabolic, rather than a contractile manner to facilitate fracture healing *in vivo*.


HISTOMORPHOMETRIC ANALYSIS SUGGESTS INTERNAL FIXATION STIFFNESS DOES NOT ALTER THE MODE OF FRACTURE HEALING

L. S. Gregory¹, H. Minehara², M. E. Wullschleger¹, M. Itoman³, M. A. Schuetz¹,³, R. Steck¹

¹Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, QLD, Australia
²Kitasato University Hospital, Kitasato, Japan
³Trauma Services & Orthopaedics, Princess Alexandra Hospital, Woolloongabba, QLD, Australia

Aim: This project aimed to investigate the effect of fixation stiffness on the spatial and temporal pattern of tissue regeneration following a femoral diaphyseal fracture in a mouse model (MouseFix™).

Methods: Femoral osteotomies were performed in 62 C56/Black6 mice and stabilised with either a low-flex or high-flex internal fixation plate (bending stiffness 14 times higher in low-flex plate). Mice were sacrificed at 4, 7, 14, 21, 28, 35 and 42 days post-osteotomy. Transverse paraffin sections were collected at 40 intervals of 200μm and stained with Safranin O and Fast Green. This
transverse sectioning approach, arguably underutilised in current bone histology, allowed multiplanar analysis of the fracture callus. Histomorphometric analyses of callus area and tissue composition were tested for significance using 2-way ANOVA.

Results: Animals stabilised with the low-flex plate had a significantly smaller periosteal callus area with bony union preceding that observed in high-flex internal fixation. Furthermore evidence of endochondral ossification was present in both the development of the periosteal and intra-medullary calluses under both high-flex and low-flex stabilisation. One hundred percent of animals at 14 and 21 days in the high-flex group and six of seven animals at 14 days in the low-flex group (ossification complete in 7th animal), displayed cartilaginous tissue at the fracture gap. However, the length of time for endochondral ossification to complete was significantly greater in the high-flex animals. Periosteal callus area was asymmetrically distributed on anterior and posterior cortical surfaces; and remodelling of the callus commenced one week earlier on the proximal side of the fracture gap.

Conclusions: Contrary to previous literature reports, our results demonstrate that intramembranous ossification is not the sole mechanism of fracture healing under low flexibility fixation conditions. Cartilaginous tissue formation was observed as a significant phase following fracture in both low-flex and high-flex internal fixation.

380
HIGHER CKIP-1 EXPRESSION DURING IMPAIRED AGED FRACTURE HEALING

Y. HE1, B. Guo1, T. Tang1, Z. Liu1, L. Zhang2, L. Qin1, G. Zhang1

1Orthopaedics & Traumatology, The Chinese University of Hong Kong, Hong Kong, Hong Kong
2Beijing Proteomics Research Center, The Academy of Military Medical Sciences, Beijing, China

Introduction: Orthopedic surgeons are challenged by impaired or delayed fracture healing in aged skeleton. To find a molecular target is critical to develop drugs for promoting aged fracture healing. Bone morphogenetic protein (BMP) signaling pathway is an important one responsible for fracture healing. Smad 1/5 have been shown to be the major molecules in BMP signaling. Smurf 1 was identified as a HECT-domain E3 ubiquitin ligase to degrade the Smad 1/5. Recently, we have found that Casein kinase-2 interacting protein-1 (CKIP-1) interacts with Smurf1 and enhances the ligase activity of Smurf1 to promote degradation of Smad1/5. Further, active osteogenesis and subsequent high peak bone mass were found in our established CKIP-1 gene knockout mice when compared to the wild type, indicating the potential role of CKIP-1 in normal fracture healing. However, the expression of CKIP-1, Smurf 1, and Smad1 and Smad5 during aged fracture healing have not been studied yet.

Materials and Methods: 20 female 3-month-old C57BL/6 mice (Young) and 20 female 12-month-old C57BL/6 mice (Aged) were used in the study. Transverse fracture creation will be performed in both groups. Ten mice for each group will be sacrificed at 2 and 4 weeks post fracture. After sacrifice, the fractured specimens will be performed by microCT examination and western-blot for evaluating callus mineralization and protein expressions.

Results: At different time points, reconstructed mineralized calluses showed better morphologic characteristics in Young group than Aged group in the 3D micro-CT images. Quantitatively, TV, BVt, BVl, BVl/TV and BMC were significantly larger in the Young group than in the Aged group at all time points. CKIP-1, Smad1 and Smad5 protein expression were higher in the Aged group than in the Young group at all time points, but Smurf-1 expression did not show difference between the two groups.

Conclusion: CKIP-1, Smad1 and Smad5 were high expressed in the aged mice callus, which may contribute to the impaired healing mechanism in aged mice.

381
INTERNAL PLATE FIXATION INDUCES A TISSUE RESPONSE INDEPENDENT OF FRACTURE

R. S. Holyoak1, R. Steck1, M. Uen2, M. Wullschleger1, M. Itoman2, M. A. Schuetz2, L. S. Gregory1

1Institute of Health & Biomedical Innovation, Queensland University of Technology, Brisbane, QLD, Australia
2Kitasato University Hospital, Kitasato, Japan
3Trauma & Orthopaedic Services, Princess Alexandra Hospital, Woolloongabba, QLD, Australia

Aim: Using histomorphometric analysis, this study examined the periosteal and endosteal tissue responses to internal fixation plates of two stiffnesses in an absence of an osteotomy within a mouse model (MouseFix™). Method: An internal fixation plate (high-flex or low-flex) was applied to the right femur of C57/Black6 mice (n=17). Animals were sacrificed at 4, 7 and 14 days post-operation in order to assess the early tissue response to the introduction of the internal fixation screws into the cortical diaphysis. Femora were paraffin-embedded, sectioned longitudinally and stained with Haematoxylin and Eosin, as well as Safranin-O/Fast Green and Toluidine Blue if cartilage was observed. Photomicrographs of the stained slides were subjected to histomorphometric tissue analysis. Analytical measurements were made regarding the dimension and tissue composition of the generated calluses. Results: All of the analysed bones displayed a considerable tissue response to the internal plate fixation, independent of fixation stiffness. Multiple periosteal calluses were observed in each animal, where each callus was coupled to either the distal or proximal fixation screws. Woven bone was observed in the periosteal calluses irrespective of time point, whilst cartilaginous tissue and fibrous were observed only in calluses at days 4 and 7. Cartilaginous tissue was observed in the periosteal calluses of 9 of the 17 animals examined. In addition, endosteal calluses composed largely of woven bone formed around all fixation screws within the medullary cavity and were observed in every animal. Conclusions: These results indicate that a significant periosteal tissue response does not depend on the incidence of a fracture. Therefore previous tissue analysis of fracture healing in internal fixation models may have over-estimated the tissue response attributed to the fracture itself.
CHARACTERISTICS OF BONE METABOLISM MARKERS DURING HEALING OF OSTEOPOROTIC VERSUS NONOSTEOPOROTIC METAPHYSSEAL FRACTURES OF LONG BONES – A MATCHED PAIR ANALYSIS

L. Kotios¹, M. H. Hitzler², A. A. Moghaddam³, C. Takur², H. Schmidt-Gayk³, B. Höner¹, P. A. Grützner², C. Wölfl²
¹Dpt. for Plastic-, Reconstructive and Hand Surgery, Burn Care Centre, BG Unfallklinik Ludwigshafen, 67071 Ludwigshafen, Germany
²Dpt. for Traumatology and Orthopaedic Surgery, BG Unfallklinik Ludwigshafen, 67071 Ludwigshafen, Germany
³Clinical Laboratory Limbach, 69126 Heidelberg, Germany

Introduction: Activity and metabolism of fracture healing can be monitored quantitatively by measuring bone turnover markers (BTMs) in serum or urine. But for osteoporotic bone the exact metabolism processes during healing of its metaphyseal fractures is until now not known. Diagnostic instruments allowing a dynamic insight to fracture healing process and being used as decision criteria for therapy and for monitoring of healing course are missing.

Patients and Methods: In the presented study, we analyse the time course of osteoanabolic marker BAP (bone specific alkaline phosphatase) andosteocatabolic marker ß-CTX (crosslinked C-(CTX) telopeptide of type-I-collagen) during fracture healing process of osteoporotic versus nonosteoporotic fractures in a matched pair analysis. Between March 2007 and February 2009 30 patients aged over 50 years who suffered a metaphyseal fracture of proximal humerus, distal radius or proximal femur were included in our study. Development of BTMs was examined over 8 week duration.

Results: After decrease of BAP in the first week, it increased constantly up to fourth week in both groups, whereas level of osteoporotic group surpassed the healthy group. ß-CTX increased in healthy bone constantly up to the fourth week, whereas it already decreased in osteoporotic bone from the first week on.

Discussion: With this work, first aspects of osteoporotic fracture healing on molecular biological level are found to explain the exact mechanism of its delayed fracture healing. The rapid decrease of ß-CTX in osteoporotic situation may be the reason for the delayed healing due to delayed remodelling processes. Further studies are necessary to achieve more detailed insight to fracture healing and decision criteria for therapy and monitoring of fracture healing.

EVALUATION OF THE SUCHEY-BROOKS METHOD FOR AGING AUSTRALIAN CAUCASIAN POPULATIONS USING MULTISLICE COMPUTED TOMOGRAPHY RECONSTRUCTIONS OF THE PUBIC SYMPHYSEAL SURFACE

N. Lottering², D. M. MacGregor¹, M. Meredith², L. S. Gregory¹
¹Medical Sciences, Queensland University of Technology, Brisbane, QLD, Australia
²Forensic Pathology Mortuary, Queensland Health Forensic and Scientific Services, Coopers Plains, QLD, Australia

Establishing age-at-death for skeletal remains is a vital component of forensic anthropology. The Suchey-Brooks (S-B) method of age estimation has been widely utilised since 1986 and relies on a visual assessment of the pubic symphyseal surface in comparison to a series of casts. Inter-population studies (Kimmerle et al., 2005; Djuric et al., 2007; Sakaue, 2006) demonstrate limitations of the S-B method, however, no assessment of this technique specific to Australian populations has been published.

Aim: This investigation assessed the accuracy and applicability of the S-B method to an adult Australian Caucasian population by highlighting error rates associated with this technique.

Methods: Computed tomography (CT) and contact scans of the S-B casts were performed; each geometrically modelled surface was extracted and quantified for reference purposes. A Queensland skeletal database for Caucasian remains aged 15 – 70 years was initiated at the Queensland Health Forensic and Scientific Services – Forensic Pathology Mortuary (n=350). Three-dimensional reconstruction of the bone surface using innovative volume visualisation protocols in Amira® and Rapidform® platforms was performed. Samples were allocated into 11 sub-sets of 5-year age intervals and changes associated with the surface geometry were quantified in relation to age, gender and asymmetry.

Results: Preliminary results indicate that computational analysis was successfully applied to model morphological surface changes. Significant differences in observed versus actual ages were noted. Furthermore, initial morphological assessment demonstrates significant bilateral asymmetry of the pubic symphysis, which is unaccounted for in the S-B method. These results propose refinements to the S-B method, when applied to Australian casework.

Conclusion: This investigation promises to transform anthropological analysis to more quantitative and less invasive using CT imaging. The overarching goal contributes to improving skeletal identification and medico-legal death investigation in the coronial process by narrowing the range of age-at-death estimation in a biological profile.

PROBABLE ASSOCIATION BETWEEN ANGIOPOIEIN-RELATED GROWTH FACTOR AND BONE MINERAL DENSITY

K. Mirzaei, A. Hossein-nezhad, Z. Maghbbooli, H. Ansar, A. Najmafshar
Tehran University of Medical Sciences, Tehran, Iran

Angiopoietin-related growth factor (AGF or Angptl6) is a liver-derived, circulating factor and is considered to be a regulator of metabolic homeostasis. Correlation between AGF with obesity and obesity-related insulin resistance were shown in previous study. The potential role of AGF has been shown in promote bone formation in vitro and in vivo in animal studies also. The purpose of this study was to evaluate a potential relationship between fasting serum ANGPTL6 and Lumbar spine (L2-L4) and total hip BMD that measured by dual energy X-ray absorptiometry (DEXA) in obese persons. Participants were 150 obese subjects in this cross-sectional study who were assessed following an overnight fasting for laboratory assessments. The mean of age and weight were 35.25±10.75 years and 82.57± 8.83 kg respectively. Serum ANGPTL6 levels were quantified by ELISA method. We demonstrated although there was positive correlation between lumbar spine (L2-L4) and fasting serum ANGPTL6 (r=0.42, p =0.03) but there are not significant correlation between fasting serum ANGPTL6 and total hip BMD. Regression analysis confirmed these correlations after adjustment for age and body mass index (p=0.04). Based on BMD T score the prevalence of osteopenia in lumbar spine and hip were 31.22% and 8.78% respectively. We found significant higher fasting serum ANGPTL6 in healthy subjects and significantly lower fasting serum ANGPTL6 in osteopenic patients (64.4±33.1 vs. 33.3± 23.4). These results suggest that ANGPTL6 plays a potential role in regulating bone metabolism and in the pathogenesis of osteopenia. Future study recommended for elucidating the precise pathway in this procedure.

Key words: ANGPTL6/AGF, bone mass density, obesity

VDR FOKI POLYMORPHISM MAY MODIFY EFFECT OF BODY MASS INDEX ON BONE MINERAL DENSITY IN ADULT OBESE

A. Hossein-nezhad, K. Mirzaei, Z. Maghbbooli, H. Ansar, M. Khosrofar
Tehran University of Medical Sciences, Tehran, Iran

Genetic factors are considered to play an important role in regulating bone mineral density and other determinants of osteoporosis. The vitamin D receptor (VDR) gene is an important candidate gene in bone metabolism investigation; however, notwithstanding widespread studies, debate remains regarding its association with bone mineral density (BMD) variation in obesity. A total of 265 healthy obese adults were genotyped for the VDR FokI using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP). Lumbar spine (L2-L4) and Total hip BMD were measured by dual energy X-ray absorptiometry (DEXA). The mean of age and body mass index were 36.24±11.75 and 31.24±3.24 kg/m² respectively. We showed Lumbar spine BMD was negatively correlated with obesity and effect on osteogenic process in this study who were assessed following an overnight fasting for laboratory assessments. The mean of age and weight were 35.25±10.75 years and 82.57± 8.83 kg respectively. Serum ANGPTL6 levels were quantified by ELISA method. We demonstrated although there was positive correlation between lumbar spine (L2-L4) and fasting serum ANGPTL6 (r=0.42, p =0.03) but there are not significant correlation between fasting serum ANGPTL6 and total hip BMD. Regression analysis confirmed these correlations after adjustment for age and body mass index (p=0.04). Based on BMD T score the prevalence of osteopenia in lumbar spine and hip were 31.22% and 8.78% respectively. We found significant higher fasting serum ANGPTL6 in healthy subjects and significantly lower fasting serum ANGPTL6 in osteopenic patients (64.4±33.1 vs. 33.3± 23.4). These results suggest that ANGPTL6 plays a potential role in regulating bone metabolism and in the pathogenesis of osteopenia. Future study recommended for elucidating the precise pathway in this procedure.

Key words: ANGPTL6/AGF, bone mass density, obesity

POTENTIAL CORRELATION BETWEEN EXPRESSION OF THE ADIPOCYTE-SPECIFIC GENE MARKER AND BONE MINERAL DENSITY

Z. Maghbbooli, K. Mirzaei, A. Hossein-nezhad, H. Ansar, M. Khosrofar
Tehran University of Medical Sciences, Tehran, Iran

Common differentiation pathways that involved in adipocyte and osteoblast cells from stem cells previously discussed. The correlation and balance between expressions of the adipocyte and osteoblast specific gene markers has been proposed. The lipid chaperone proteins, also known as fatty acid–binding proteins (FABPs), are a group of molecules that coordinate inflammatory and metabolic responses in adipocytes and macrophages. Expression of FABP4 may affect in obesity and effect on osteogenic process in throughout of life. In the present study, we tested the hypothesis that the potential correlation between expression of FABP4 in peripheral blood mononuclear cell and bone mineral density (BMD) in Lumbar spine (L2-L4) and Total hip BMD that measured by dual energy X-ray absorptiometry (DEXA) in obese subjects. Ninety-six obese subjects participant in this cross-sectional study. The PBMCs were separated from whole blood by Ficoll-hypaque technique. Total cellular RNA was extracted and the cDNA was synthesized. Real-time PCR using specific primer pairs for determine the FABP4 and beta actin gene expression. The mean of age and BMI were 36.74±10.96 years and 31.24±3.24kg/m² respectively. We showed Lumbar spine BMD was negatively correlated with relative FABP4 gene expression (r=-0.6, p=0.03). Regression analysis confirmed these correlations after adjustment for age. We
found significant higher FABP4 gene expression in osteopenic patients in Lumbar spine (L2-L4) compare to healthy subjects (2.20±0.56 vs. 1.04±0.44). These results demonstrated the similar pathway towards adipogenesis at the expense of osteogenesis in obesity.

Key words: adipocyte-specific gene marker, FABP4, bone mineral density, obesity, gene expression

---

**EFFECTS OF MINODRONATE ON CORTICAL BONE RESPONSE IN RAT TO MECHANICALLOADING**

K. Nagira¹, Y. Kameyama¹, H. Hagino², R. Teshima³

¹Orthopedic Surgery, Faculty of Medicine, Tottori University, Yonago, tottori, Japan
²School of Health Science, Faculty of Medicine, Tottori University and, Faculty of Medicine, Tottori University, Yonago, Tottori, Japan

[Aim] The cortical bone response under minodronate administration was evaluated using a 4-point bending device to clarify the relationship between the effect of minodronate and mechanical loading.

[Animals and methods] Six-month old female Wistar rats were used and randomized into five groups (N=10/group): Vehicle administration (M-V), low dose minodronate administration (M-L, 0.01 mg/kg BW), middle dose minodronate administration (M-M, 0.1 mg/kg BW), high-dose minodronate administration (M-H 1 mg/kg BW), and very high-dose minodronate administration (M-VH, 10 mg/kg BW). Minodronate or vehicle was administered orally using the feeding needle at a dosage 3 times/week for 3 weeks (the animals were fasted for 2 hours before and after the administration). Loads on the right tibia at 38 N for 36 cycles at 2Hz were applied in vivo by 4-point bending on the same day for 3 weeks. After calcein double labeling the rats were sacrificed and tibial cross sections were prepared from the region with maximal bending at the central diaphysis. Histomorphometry was performed at the entire periosteal and endocortical surface of the tibiae, dividing the periosteum into lateral and medial surfaces.

[Results] The mineralizing surface (MS) was reduced significantly in M-H at the medial surface, and in M-H and M-VH at the endocortical surface (p<0.01 vs. M-V). The mineral appositional rate (MAR) was reduced significantly in M-H and M-HV at the medial surface, and in M-VH at the endocortical surface (p<0.01 vs. M-V). The bone formation rate (BFR) was significantly reduced in M-H and M-VH at the medial surface (p<0.01 vs. M-V). However, there were no significant differences between M-M and M-V at all surfaces in MS, MAR, and BFR.

[Conclusion] Minodronate does not reduce the cortical bone response to mechanical loading at the optimal dose for the treatment of osteoporosis in rat model.


---

**GLUTATHIONE REDUCTASE ACTIVITY, SMOKING AND MENOPAUSE IN RELEVANCE TO BONE LOSS**

M. Oveis¹, N. Sadeghi², B. Jannat³, M. Hajimahmoodi¹, F. Sadeghi³

¹Dep. of Drug and food control, Tehran University of medical sciences, Tehran, Iran
²Food and drug laboratory research centre, Ministry of health, Tehran, Iran
³School of pharmacy, Azad university, Tehran, Iran

Introduction: The stress biomarkers and antioxidants have been surveyed for assessing age related diseases such as osteoporosis. The purpose of this study was to consider the plasma Glutathione Reductase (GR) activities in osteoporotic Iranian women comparing to the control, in side glance to smoking and menopause effects.

Material and Method: GR activity was determined spectrophotometrically at 339 nm. Participants were selected by inclusion and exclusion criteria among those who referred to Jamie Clinic in Tehran for BMD evaluation, and classified as Patient group (n=76) against control group (n=76). Standard questionnaire (including smoking habit and menopause condition, etc.).

Results: In total osteoporotic group (-1.0<T-score) plasma GR activity value is 82.35±50.33 U/L and in control group (Femoral and Lumber T-score≥-1) is 64.71±31.26 U/L.

In smoker (n=20) and non-smoker (n=172) participants, GR activity values are 59.58±25.58 and 72.71±42.13 U/L , respectively. In pre-menopausal (n=109) and post-menopausal (n=83) participants, GR activity values are 64.26±28.35 and 80.64±51.75 U/L , respectively. Femur mineral density in smoker and non-smoker are -1.22±1.07 and -0.62±1.32, and in pre-menopausal and post-menopausal are -0.44±1.12 and -0.98±1.45 , respectively.

Discussion and Conclusion:
Control group GR values are significantly less than patient group (p<0.01). No difference in menopause and smoking habit was found between groups, but it is worth saying that in all participants (no attention to control or patient group) , there is less plasma GR activity in the smokers than non- smokers (P<0.05) . GR values is more in post-menopausal compared to the pre- menopausal
DIFFERENT EFFECTS OF TERIPARATIDE AND PTH 1-84 ON BONE AND MINERAL METABOLISM IN PATIENTS WITH SEVERE OSTEOPOROSIS

V. Camozzi1, C. Fiore1, M. Piccolo1, R. De Bastiani1, F. L. Giorgino2, D. Armanini4, A. G. E. O., Padova, Italy

Introduzione. Recently, two new molecules able to stimulate bone formation through the activation of osteoblasts have been proposed for the treatment of severe osteoporosis: recombinant parathyroid hormone (PTH 1-84), and teriparatide (PTH 1-34).

Aim of the study. Purpose of the study is to compare the effect of these two molecules on bone and mineral metabolism in women with severe osteoporosis.

Patients and methods. Fifty -height patients with severe osteoporosis, aged between 69 and 88 years, were the object of the study. Thirty -nine patients were treated with teriparatide (20 µg/day subcutaneously) and 19 with PTH 1-84 (100 µg/day, subcutaneously). Treatment lasted for 18 months. For each patient the following laboratory parameters were assessed: serum calcium (Ca), phosphorus (P), bone alkaline phosphatase (BALP), total alkaline phosphatase (ALP), urinary deoxypyridinoline (DPD), daily urinary calcium excretion (UCa), vitamin D and parathyroid hormone (PTH). Samples were collected before starting therapy and after 2, 6, 12, 18 months.

Results. Ca significantly increased in both groups (p < 0.05 in all cases), but was significantly higher in patients treated with PTH 1-84 than in those treated with teriparatide at month 2 and 6 (p < 0.01). ALP and DPD increased more in PTH 1-84 group than in teriparatide one at month 2 (p = 0.002 and P=0.014 respectively). UCa was significantly increased in both groups with no significant differences between the two groups. Both groups showed a similar increase in BMD at lumbar spine (p <0.05), with no change at femur.

Conclusions. PTH 1-84 seems to determine stronger metabolic effects than teriparatide in patients with severe osteoporosis. These differences are more pronounced in the first months of treatment and are probably due both to different amount of substance injected and to different metabolism of the 2 molecules.

INTRAPERITONEAL INJECTION OF (-)-EPIGALLOCATECHIN-3-GALLATE (EGCG) INCREASE BONE VOLUME IN OVARIECTOMIZED RATS

C. Chen, Y. Lin, R. Wu, M. Ho, J. Chang, R. Lin, Y. Fu, G. Wang

Kaohsiung Medical University, Kaohsiung, Taiwan

INTRODUCTION:

Green tea is one of the most popular beverages in the world. Among the catechins, (-)-epigalloncatechin -3-gallate (EGCG) has received by far the most attention. Our previous showed EGCG can enhance osteogenesis in a murine bone marrow cell line (1). Beside, we also found EGCG can inhibit osteoclastogenesis via NF-kB (2). In this study, we evaluated the in vivo effects of EGCG.

METHODS:

Twenty-seven female Sprague-Dawley rats received ovariectomy at 6-month-old. Three months later, all rats were randomly divided into 2 groups. Thirteen rats received intra-peritoneal injection of EGCG in DMSO solution (0.24 mg/kg/day) and fourteen rats received EGCG (2.4 mg/kg/day) for 3 months. The other fourteen rats received same concentration of DMSO as control group.

After treatment, BMD, μCT and histology over proximal tibia were examined. Besides, the biochemical profiles including liver function, renal function and electrolyte were also examined.

RESULTS:

The increase was significant in BMD, trabecular thickness in μCT and and bone volume in histology with the treatment of EGCG. Besides, trabecular separation in μCT decreased after the treatment of EGCG. At the end of treatment, there was no obvious liver and renal toxicity. In the autopsy study of liver and kidney section, there was no obvious necrosis.

DISCUSSION:

Our previous results indicated that EGCG significantly enhanced osteogenesis of murine marrow mesenchymal cells 10 µmol/L in murine bone marrow mesenchymal cells (1). We also found EGCG (10:100 µmol/L) significantly suppressed the RANKL-induced differentiation of osteoclasts and the formation of pits in murine RAW 264.7 cells and bone marrow macrophages (2). In this study, we confirm the proximal tibia BMD and bone volume can be increased with intra-peritoneal injection of EGCG at the dose of 2.4 mg/kg/day with the estimated peak serum concentration of 10 umol/L. The effects in human need further studies.
391

EFFECT OF A GREEN TEA EXTRACT ON BONE HEALTH AND BODY COMPOSITION IN OVARIECTOMISED SPRAGUE DAWLEY RATS

W. Chua1, C. Norris2, C. Lloyd-West1, B. Kuhn-Sherlock2, L. Schollum2, M. C. Kruger1

1Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand
2Fonterra Research Centre, Palmerston North, New Zealand

Aim: Test the effect of a green tea extract (GTE), high in total phenolic compounds, on osteoblast proliferation, osteoclastogenesis, and bone in ovariectomised rats. Methods: The effect of GTE on osteoblast proliferation and osteoclastogenesis was tested using the MC3T3-E1 and RAW 264.7 cell lines respectively. In the animal model, three groups of six-month old rats were ovariectomised and fed control diet, or diets containing GTE (1, 2%) for 24 weeks. Bone density and body composition were assessed at 8, 16 and 24 weeks by dual energy x-ray absorptiometry. Femur biomechanics and tibia microarchitecture were assessed after 24 weeks. Results: GTE had no effect on MC3T3-E1 cell proliferation at concentrations up to 10 μg/ml, while inhibition occurred at 100-1,000 μg/ml. Fewer osteoclasts were formed in the RANK-L induced RAW 264.7 model when cells were treated with GTE at 0.1-10 μg/ml. In contrast, there was no significant effect on the measured bone parameters in the ovariectomised rats. However, GTE had a significant effect on lean and fat mass body composition. Rats fed the GTE were lighter, gained less body fat, and had greater lean body mass than ovariectomised controls despite having similar food intakes. Conclusions: The GTE inhibited osteoclastogenesis while having no effect on osteoblast proliferation at the same concentration. Dietary GTE intake had no effect on bone density in ovariectomised rats fed GTE (1, 2%) for 24 weeks, but had a significant effect on minimising the post-ovariectomy increase in body fat, and increased lean mass.

392

PREVENTING BONE LOSS IN POSTMENOPAUSAL WOMEN WITH LOW BONE MASS AND LOW-TRAUMA FOREARM FRACTURES

M. Cirstoiu, C. Cirstoiu, D. Popescu, A. Popescu, R. Ene

University Hospital Bucharest, Bucharest, Romania

Aim: To evaluate the efficacy of early therapeutic intervention in preventing bone loss and reducing the risk of future fragility fractures in postmenopausal women with low bone mass and a history of forearm low-trauma fractures. Methods: 34 postmenopausal women aged from 51-56 were admitted in our hospital for low-trauma forearm fractures and treated with closed reduction and AO external fixation. 12 of this patients had a medical history of pharmacological therapies (chronic glucocorticoid therapy) or medical condition with potentially negative effects on bone mass. After the orthopedic intervention , bone mineral density (BMD) was measured at lumbar spine and proximal femur using dual energy X-ray absorptiometry with results suggestive for low bone mass. 20 of this patients (especially those with osteoporotic risk factors) were included in to a program aiming to prevent further bone loss using different therapeutic strategies ( hormonal replacement therapy or bisphosphonate therapy associated with calcium and vitamin D supplements) and the rest of 14 women had received only calcium and vitamin D supplements. They all made periodical medical checks and radiological evaluation at 6 weeks, 3, 6, 12 and 18 months postoperator.DXA scan was performed in all patients 2 years after initial evaluation. Results: At 3-4 month after orthopedic intervention, all patients had a favorable fractures healing without pseudarthrosis, osteoporotic therapy having no influence on this healing process. In all patients we performed BMD measurements at 2 years after initiating therapy, with DXA scan showing a tendency of losing bone mass in the group of patients without pharmacological therapy (8 patients-57,14%) and with stable BMD (8 patients-40%) or increasing BMD (12 patients -60%) in the group receiving pharmacological therapy. Conclusion: Early pharmacological intervention in women with low-trauma fractures and low bone mass may be beneficial in reducing the risk of further fractures and improving life quality.

(1) C.Cirstoiu - Osteoporosis in menopausa- Ed.Univ. Carol Davila Bucharest

393

IMPROVING MANAGEMENT OF LOW TRAUMA FRACTURES IN A TERTIARY HOSPITAL THE "FRACTURE CAPTURE" PROJECT


University of Melbourne / Austin Health, West Heidelberg, Australia

Aims: Follow up after admission for a low trauma fracture is low with a previous audit of the Austin Hospital indicating that less than 1% of in-patients were discharged with treatment related to a fragility fracture, and only 6% had follow up investigation. We
aimed to determine if a designated fracture identification and treatment program improved treatment rates following a low trauma fracture.

Methods: Patients admitted through the emergency department (ED) with a low trauma fracture (hip, spine upper and lower limbs) were identified weekly. Inpatients had clinical assessment and biochemical investigations for secondary causes of osteoporosis. Treatment was commenced according to standardised guidelines. After discharge, endocrine clinic review was scheduled following outpatient DXA and pathology assessments. Patients discharged directly from the ED were contacted via mail to undergo secondary screening. Follow up reminder letters were sent to patients who failed to respond.

Results: Over a 24 month period, 955 females (mean age 74.8±11.4 years) and 325 males (mean age 71.1±11.8 years) with fragility fractures were identified. 587 were inpatients (309 hip, 35 wrist, 243 other) and 693 discharged directly from the ED (18 hip, 294 wrist, 381 other). 51% of inpatients were discharged with treatment, compared to <1% previously and 40% of inpatients underwent assessment and clinic review compared to 6% observed in the prior audit. 53% of those discharged directly from the ED underwent investigations and clinic review compared to no patients prior to the program. 14% elected to be treated by their own GP or specialist. Of all the low trauma fracture admissions, only 12% potentially went untreated (failed to respond to correspondence).

Conclusions: Implementation of a dedicated bone fragility identification and treatment program significantly improved initiation of therapy. Whether this translates into improved compliance and fracture risk reduction required further investigation.

**EFFECTS OF BISPHOSPHONATE MONOTHERAPY OR COMBINED THERAPY WITH BISPHOSPHONATE AND VITAMIN K2 ON SERUM UNDERCARBOXYLATED OSTEOCALCIN AND OSTEOCALCIN IN OSTEOPOROTIC WOMEN**

Y. Kasukawa1, N. Miyakoshi1, T. Ebina2, T. Aizawa2, Y. Shimada3

1Department of Orthopedic Surgery, Akita University Graduate School of Medicine, Akita, Japan
2Department of Orthopedic Surgery, Kakunodate General Hospital, Kakunodate, Akita, Japan

Aim: Serum undercarboxylated osteocalcin (ucOC), a vitamin K2 deficiency marker, is also considered to be a bone mineral density-independent marker of bone quality. Its cut-off value for osteoporotic fracture is 4.5 mg/ml, but the value changes under bisphosphonate therapy. This study aimed to evaluate the effects of bisphosphonate monotherapy or combined therapy with bisphosphonate and vitamin K2 on serum ucOC, osteocalcin (OC) and other bone metabolic markers prospectively.

Methods: Ninety-five osteoporotic women were randomly stratified into a Bis-group (mean age: 74 years; n=48) treated with risedronate (17.5 mg/week) and a Bis+K2-group (mean age: 76 years; n=47) treated with risedronate and menatetrenone (45 mg/day). Serum ucOC, OC, cross-linked N-telopeptide of type 1 collagen (NTx) and bone alkaline phosphatase (BAP) were measured before and after 6 and 12 months of treatment. The change rates of these markers were evaluated.

Results: The therapy persistence rates at 6 and 12 months were 83% and 75% in the Bis-group and 74% and 68% in the Bis+K2-group, respectively. The ucOC decrease rates at 6 and 12 months did not differ significantly between the groups (Bis-group: 31% and 51% versus Bis+K2-group: 34% and 56%, respectively). However, the OC decrease rates were significantly higher (p<0.01) in the Bis-group at 6 and 12 months (25% and 36%, respectively) than in the Bis+K2-group (0.4% and 17%, respectively). The ucOC/OC change rates were significantly lower (p<0.05) in the Bis-group at 6 and 12 months (9.3% and 23%, respectively) than in the Bis+K2-group (36% and 48%, respectively). The NTX and BAP decrease rates did not differ significantly between the groups at both time points.

Conclusions: Although the serum ucOC decrease rates did not differ between the bisphosphonate monotherapy and combined therapy with bisphosphonate and vitamin K2, the serum OC decrease rates were significantly lower for the combined therapy than for the monotherapy.

**LONG-TERM INTERMITTENT MINODRONIC ACID (ONO-5920/YM529) TREATMENT COMPARABLE TO DAILY TREATMENT IN SUPPRESSING INCREASED BONE TURNOVER WHILE PREVENTING REDUCTION IN BONE MASS AND STRENGTH IN OVARIECTOMIZED RATS WITH ESTABLISHED OSTEOPENIA**

A. Kimoto1, M. Tanaka1, M. Mori1, K. Nozaki1, H. Mori1

1Drug Discovery Research, Astellas Pharma Inc., Tsukuba, Ibaraki, Japan
2Research Promotion, Ono Pharmaceutical Co.,Ltd., Mishima, Osaka, Japan
3Discovery Research Laboratories, Ono Pharmaceutical Co.,Ltd., Mishima, Osaka, Japan

Aim: To compare the effects of intermittent (once/4 weeks) and daily administration of the highly potent nitrogen-containing bisphosphonate minodronic acid (ONO-5920/YM529) on bone mineral density (BMD), strength, turnover, and histomorphometry in ovariectomized (OVX) rats.

Methods: Female rats ovariectomized at 14 weeks showed increased bone turnover and decreased bone mass and strength 12 weeks later. Minodronic acid was orally administered once every 4 weeks at 0.2, 1, and 5 mg/kg or once daily at 0.006, 0.03, and 0.15 mg/kg from 12 weeks after ovariectomy for 52 weeks.
Results: In both treatment regimens, minodronic acid dose-dependently ameliorated the decrease in BMD of lumbar vertebrae and femurs and restored serum levels of osteocalcin, a bone formation marker, while reducing elevated urinary levels of deoxypyridinoline, a bone resorption marker. A mechanical test at 52 weeks of treatment found that minodronic acid dose-dependently ameliorated the reduction in bone strength (maximum load) of lumbar vertebral bodies and mid femur, with significance up to 5 mg/kg with intermittent administration. Bone histomorphometric analysis of lumbar vertebral bodies after 52 weeks of treatment showed that minodronic acid significantly ameliorated the decrease in bone mass and increases in trabecular separation, bone resorption, and bone formation in OVX rats. Minodronic acid suppressed OVX-induced increases in bone turnover at the tissue level and ameliorated all structural indices, thereby improving the deterioration in bone quality under OVX-induced disease conditions.

Conclusions: These results suggest that intermittent administration of minodronic acid may be as clinically useful in treating osteoporosis as its daily administration regimen, provided the total administered dose over a specified period of time is the same in both regimens.

396

EFFECT OF A ONCE-YEARLY ZOLEDRONIC ACID ON BMD AND THE RISK OF FALLING IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS

H. LIN, T. XU, L. Fan, H. Yang, X. Chen
The Center of Research for Metabolic Bone Disease, The Affiliated Drum Tower Hospital of Medical School, Nanjing University, nanjing, jiangsu, China

Objective To determine the effects of once yearly zoledronic acid improves bone density and decreases the risk of falling in postmenopausal women with osteoporosis. Methods Randomized controlled trial with one-year follow-up, carried out in the study. A total of 91 postmenopausal osteoporosis women were enrolled. The 91 postmenopausal osteoporosis subjects were randomized to either the intervention group (Group A, n =45) or the control group (Group B, n =46). Group A: a single, 30 minute intravenous infusion of zoledronic acid (5 mg), supplement 1,25-dihydroxyvitamin D0.25μg and calcium 600mg with VitD 125IU daily. Group B: supplement 1, 25-dihydroxyvitamin D0.25μg and calcium 600mg with VitD 125IU daily only. During one year of intervention, BMD and risk of falling were measured by dual-X-ray absorptiometry (DXA) at lumbar spine and hip and the balance test (Sunlight Tetraz) at pre-intervention and 12 months after intervention. Adverse events occurring of zoledronic acid infusion were measured. Results After one year, 41 subjects in group A and 41 subjects in group B completed the follow-up. In groups A, BMD were significantly increased at the lumbar spine (5.8%), total hip (3.9%), and femoral neck (2.9%), and in groups B, BMD was significantly increased at the lumbar spine (4.4%), compared with themselves at pre-intervention. The risks of falling were significantly reduced in both group, and there was no statistical significance between group A and group B. Some adverse events were tolerated in group A. Conclusion A once-yearly infusion of zoledronic acid during a 1-year period significantly increased BMD at the lumbar spine, total hip, and femoral neck. It also reduced the risk of falling in postmenopausal women with osteoporosis. The treatment of once-yearly infusion of zoledronic acid ensures the effectiveness of therapy and also increase the compliance of patients. It’s a very important assurance of curative effect for long-time treatment of osteoporosis.

(4) Hwang JS, Chin LS, Chen JF, Yang TS, Chen PQ, Tsai KS, Leung PC. The effects of intravenous zoledronic acid in Chinese women with postmenopausal osteoporosis. J Bone Miner Metab. 2010 Oct 5. [Epub ab
(5) Cauley JA, Black D, Boonen S, Cummings SR, Mesenbrink P, Palermo L, Man Z, Hadji P, Reid IR; on behalf of the HORIZON Pivotal Fracture Group. Once-yearly zoledronic acid and days of disability, bed re
(8) Gallagher JC.The effects of calcitriol on falls and fractures and physical performance tests. The Journal of Steroid Biochemistry
(10) Ringe JD, Farahmand P, Schacht E, Rozehnal A. Superiority of a combined treatment of Alendronate and Alfacalcidol compared to the combination of Alendronate and plain vitamin D or Alfacalcidol alone i
THE EFFECTS OF GALNON ON THE OSTEOPOROTIC RAT MODEL

H. W. McGowan, S. J. McDonald, J. A. Schuijers, B. L. Grills, A. C. McDonald

Tissue and Cell Biology Group, Musculoskeletal Research Centre, La Trobe University, Bundoora, VIC, Australia

Aim: Galanin (GAL) is a naturally occurring neuropeptide and, when injected locally, results in decreased expression of cytokines responsible for stimulating bone breakdown, increases size and number of bone forming cells (osteoblasts) plus increases bone formation rate (J). The aim of this experiment was to determine whether systemic treatment with the GAL receptor agonist, galnon (Galn), could offset bone degeneration experienced in osteoporosis.

Materials and Methods: 20 fourteen week-old female rats were ovariectomised (OVX) with half the animals receiving, via mini-osmotic pumps, 0.2 mg/kg/day Galn in vehicle and half receiving a vehicle solution of 10% DMSO in 0.9% saline. Following 6 weeks of treatment plasma was analysed for osteocalcin concentration plus bone tissue was analysed via qPCR, histology and biomechanical loading.

Results: No significant difference in plasma osteocalcin levels was detected between the two groups. qPCR indicated that OVX rats treated with Galn had increased gene expression of IL-1β (p < 0.005) and RANKL (p < 0.05), however no significant change was seen in TNF-α or OPG expression. Galn-treated rats also had significantly decreased trabecular bone volume (p < 0.05), cortical area (p < 0.05), periosteal radius (p < 0.05) and a significantly increased ultimate displacement when tested using a 3-point bending apparatus (p < 0.005) compared to OVX vehicle rats.

Conclusions: Previous research has shown locally injected GAL increases calvarial bone formation relative to vehicle injections. Contrary to expected outcomes, results indicate Galn increases bone resorptive genes IL-1β and RANKL and also enhances resorption of trabecular and periosteal cortical bone in the osteoporotic rat model. Further research using both GAL and its agonist, Galn, must take into consideration their potential effects on the skeletal system.

(1) McDonald, A. C., Schuijers, J. A., Gundlach, A. L., and Grills, B. L. (2007) Galanin treatment offsets the inhibition of bone formation and downregulates the increase in mouse calvarial expression

NEFFECTS OF COMBINED JUMPING EXERCISE AND HONEY SUPPLEMENTATION ON TIBIA BONE HISTOMORPHOMETRY PROPERTIES IN YOUNG FEMALE RATS

F. K. Ooi1, S. S. Tavafzadeh1, L. K. Hung2, K. M. Chan2, Y. X. He2

1Sports Science Unit, Universiti Sains Malaysia, Kota Bharu, Kelantan, Malaysia
2Department of Orthopaedic and Traumatology, The Chinese University of Hong Kong, Hong Kong, Shatin, Hong Kong

Aim: This study investigates the effects of 8 weeks of combined jumping exercise and honey supplementation on bone histomorphometry properties of the tibias in young female rats.

Methods:
Forty eight 12-week old female rats were divided into four groups: Sedentary without supplementation control group (C), sedentary with honey supplementation group (H), jumping exercise group (J), and combined jumping exercise and honey supplementation group (JH). Jumping exercise consisted of 40 J/day for 5 days/week at a height of 40 cm. Oral honey supplementation was given to the rats at a dosage of 1g/kg body weight/rat/day, for 7 days/week. At the end of the study, bone structural analysis was performed on trabecular bone in tibial proximal and cortical bone in tibial midshaft by using Micro-computed Tomography (µCT). Bone volume (BV), tissue volume (TV), bone volume/tissue volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular space (Tb.S) and polar moment of inertia (MOI) were obtained for comparison.

Results:
It was found that in tibial proximal, bone volume fraction (BV/TV), trabecular number (Tb.N) and thickness (Tb.Th.) were found to be the highest in JH group. Contrary, the trabecular space in this bone region was found to be the lowest in JH group. In tibial midshaft cortical bone, MOI in JH was found to be the highest among the experimental groups.

Conclusion:
The present study found that there are more discernable beneficial bone effects in tibial proximal, i.e. bone volume fraction (BV/TV), trabecular number (Tb.N) and thickness (Tb.Th), and MOI at tibial midshaft in combined jumping exercise and honey supplementation group compared to the other groups. These findings imply that combination of jumping exercise and honey may elicit additional beneficial bone effects than jumping exercise and honey supplementation alone in rats.
THE CHAOS CLINIC – A RANDOMIZED CONTROLLED TRIAL OF A FALLS CLINIC FOR PREVENTION OF FALLS AND RELATED FRACTURES

M. Palvanen1, P. Kannus1, J. Parkkari2, S. Niemi1, M. Piirtola1, M. Järvinen2,3

1UKK Institute, Tampere, Finland
2Tampere University Medical School, Tampere, Finland
3Tampere University Hospital, Tampere, Finland
4Tampere Research Center of Sports Medicine, Tampere, Finland

The purpose of a Finnish falls clinic system (Chaos Clinic) is to prevent falls and fall-related injuries (fractures) among high-risk elderly individuals. A pragmatic randomized controlled trial assessed the effectiveness of this system in the cities of Tampere and Lappeenranta in Finland between January 2005 and August 2010.

The Chaos Clinic has a multidisciplinary approach to evaluate and treat the intrinsic and extrinsic risk factors for falls and related injuries. 1314 persons aged 70 years or more with increased risk for falling and fractures were first interviewed and examined at the Chaos Clinic. After a comprehensive and individual assessment of the risk factors, the participants were randomized to the intervention group (n=661) and control group (n=653). Thereafter, the personnel of the Chaos Clinic decided, on individual basis, the falls prevention measures needed and supervised their execution in the intervention group. The intervention program included measures such as strength and balance training, guidance to increase physical activity in general, guidance to proper nutrition, review of medications, treatment of fall-related illnesses, hip protectors, and home hazard assessment and modification. The control group received general injury prevention guidelines only. All the participants were then followed-up for 12 months (in three months intervals) for falls and related injuries.

At the one-year follow-up, the number of fallers was 297 in the intervention group and 349 in the control group. The numbers of falls were 609 and 825, respectively. These falls caused 821 injuries altogether, 352 in the intervention group and 469 in the control group. The numbers of fractures were 33 and 42, respectively.

The multifactorial Chaos Clinic program is effective in preventing falls of older adults. The program reduces the risk of falls and related injuries by almost 30%.

COST EFFECTIVENESS OF A TARGETED INTERVENTION TO REDUCE THE RATE OF REFRACTURE: RESULTS OF A 4-YEAR PROSPECTIVE CONTROLLED STUDY

M. Cooper2, A. Palmer2, K. Ganda1, M. J. Seibel1

1ANZAC Res Institute, University of Sydney, Sydney, NSW, Australia
2Menzies Research Institute, University of Tasmania, Hobart, TAS, Australia
3Dept of Endocrinology, University of Birmingham, Birmingham, United Kingdom, United Kingdom

We recently reported the clinical effectiveness of a Minimal Trauma Fracture (MTF) service. The MTF intervention is offered on an outpatient basis to patients presenting with a minimal trauma fracture to Concord Repatriation General Hospital (CRGH) in New South Wales, Australia. In a 4-year prospective observational study, that was unique in having a control group treated in primary care, it was demonstrated that the MTF service significantly reduced the risk of refracture by 80%. The MTF service involves input from a specialist clinic and greater use of bone density assessment, laboratory tests and medications. It was thus unclear whether the benefits seen would be cost-effective. Our aim was therefore to determine the costs and improvements in quality-adjusted life years (QALYs) associated with the MTF service, allowing a comparison of value for money versus standard practice. We developed a Markov, 2nd-order Monte Carlo simulation model to project the 4-year clinical and cost outcomes to 10 years. The model accounted for hip, forearm and humerus fractures; their associated direct costs; changes in health utility; and in the case of hip fracture, increases in mortality. Fracture probabilities were calculated directly from the recently published clinical trial. Direct medical costs (expressed in 2010 Australian Dollars) for the MTF intervention and the control arm were derived from reported health resource consumption. Outcomes were discounted at 5% annually. Total 10-year costs (C), quality-adjusted life years (QALY) and incremental C/QALY-gained were calculated for MTF versus control. Extensive sensitivity analyses were performed e.g. varying anti-fracture effectiveness, intervention costs, number of patients treated and level of compliance with therapy.

Reducing recurrent fracture rates with the MTF service versus control led to an improvement of 0.07±0.003 QALYs per patient over 10 years. Despite higher treatment costs, total costs in the MTF treatment arm were only increased by $1,333±437 per patient over the 10-year period, due to reduced costs from fractures avoided. The incremental cost-effectiveness ratio for the MTF service versus control was $20,210/QALY gained, indicating that the MTF intervention represents excellent value for money. Sensitivity analysis demonstrated that the results were robust under all plausible assumptions.

The MTF service is thus a cost-effective intervention to reduce recurrent osteoporosis fractures in a high risk population.
INTRANASAL SALMON CALCITONIN FOR THE TREATMENT OF OSTEOPOROTIC BONE PAIN AFTER BILATERAL OOPHORECTOMY

H. B. Zeng¹, Y. Z. Li²
¹gynecology, people’s hospital affiliated to Quanzhou medical college, Quanzhou, China
²orthopedics, The second affiliated hospital of Fujian medical university, Quanzhou, Fujian, China

Aim: To study the effectiveness of intranasal salmon calcitonin for treatment of osteoporotic bone pain after the bilateral oophorectomy. Methods: 25 patients with the osteoporotic bone pain after the bilateral oophorectomy were included in our series. The patients received intranasal salmon calcitonin 200 IU/day, calcium and vitamin D for 3 months. The intensity of pain was evaluated with VAS pain score before treatment, one week, four weeks and twelve weeks after the use of calcitonin. Results: The bone pain was diagnosed between 2 months and 96 months after the bilateral oophorectomy. Three patients had the history of osteoporotic fracture of spine or hip. The patients were often treated with NSAIDS for relieving pain. BMD suggested 15 patients had osteoporosis with T-score ≤ -2.5SD and 10 had osteopenia with T-score between -1 and > -2.5. The VAS pain score was between 4 and 9 ( 6.1 ± 1.2 ) before the calcitonin use. The intensity of pain began to reduce one week after the treatment of calcitonin with VAS pain score 4.6 ± 1.1 and the VAS pain score was between 1 and 5 ( 3.1 ± 0.8 ) at four weeks. All patients had VAS pain score ≤ 3 at 12 weeks after use of intranasal salmon calcitonin. Discussion: Bone pain was often in the patients after bilateral oophorectomy and was not paid enough attention. Intranasal calcitonin was very effective for relieving the bone pain. We emphasize that the prevention of osteoporosis is important for the patients after the bilateral oophorectomy.

EFFECT OF LONG-TERM ANTICONVULSANT THERAPY ON BONE MINERAL DENSITY AND SERUM 25-HYDOXYVITAMIN D LEVEL

T. Baykal¹, K. Senel²
¹Physical medicine and rehabilitation, Regional Hospital, Batman, Turkey
²Physical medicine and rehabilitation, Ataturk University, Erzurum, Turkey

The long-term use of anticonvulsants can affect bone and mineral metabolism. Several studies report that 20 to 60% of anticonvulsant users can developed rickets or osteomalacia. Anticonvulsant therapy can cause the osteomalacia due to alteration of the vitamin D metabolism inducing hepatic microsomal enzymes, inhibiting 25-hydroxylation of vitamin D. Long-term anticonvulsant therapy also can inhibit intestinal calcium transport and bone mineral mobilization. Here, we report a young male patient with seizure who was treated with anticonvulsant (diphenylhydantoin) for 14 years and was admitted with the complaints of the difficulty to walk and generalized bone pains due to anticonvulsant therapy induced osteomalacia and seconder osteoporosis in our department.

In conclusion, we recommend evaluating bone by quantifying bone mineral density as measured by DEXA and 25-hydroxyvitamin D measurement after anticonvulsant therapy periodically.

THE RELATIONSHIP BETWEEN SMOKING CESSION, BODY FAT AND BONE MINERAL DENSITY

J. J. Christie¹, S. Maran¹, C. Segan², S. Kantor¹, R. H. Osborne³, J. D. Wark¹,4
¹QUIT Victoria (RMH/WH), University of Melbourne, Melbourne, VIC, Australia
²QUT Victoria, Cancer Council Victoria, Melbourne, VIC, Australia
³Public Health Innovation, Deakin University, Melbourne, VIC, Australia
⁴Bone and Mineral Service, The Royal Melbourne Hospital, Melbourne, VIC, Australia

Aim

We investigated the effect of smoking cessation on total body and abdominal fat and their associations with total hip (TH) BMD and total body bone mineral content (TBBMC) in Quiltine Victoria callers aged over 40 years.

Methods

249 of 419 participants were re-assessed 1-3 years post baseline. Total fat mass (FM), abdominal fat (AF), TH BMD, and TBBMC were measured (Hologic QDR4500A DXA). Health-related questionnaires were administered. Age-, height-adjusted changes (visit 2–visit 1) were annualized.

Results

Independent t-tests revealed quitters had a 20% increase in FM and 18% increase in AF between visits. Female quitters had a significant increase in AF compared to smokers between visits (+23.4% quitters; -1.6% smokers; p = 0.006). Male smokers reduced FM by 4.5% whilst quitters increased by 20.7% (p = 0.03). Pearson’s correlation revealed quitters had positive moderate to strong correlations between bone and fat measures at follow-up: In females, TH BMD with AF (r=0.376, p=0.027) and FM (r=0.467, p=0.06); In males, TH BMD with AF (r=0.527, p<0.001) and FM (r=0.630, p<0.001), and between FM and TBBMC (r=0.516, p=0.001). Smokers had weak positive correlations between bone and fat measures in both genders.

Conclusions
As expected, smoking cessation was associated with increased weight and fat mass in both genders. Of concern, AF increased significantly in quitters. Moreover, TH BMD and TBBMC were positively associated with abdominal fat and fat mass in quitters. Results showed the effect of quitting on bone to be largely associated with changes in fat mass. Although multiple mechanisms are involved in the effects of smoking on bone, changes in fat mass appears to be a key factor. Early exercise and dietary interventions in quitters may be useful to prevent weight gain, especially abdominal fat. However, BMD should be monitored especially in females.

ASSOCIATION BETWEEN ALCOHOL CONSUMPTION AND BONE MINERAL DENSITY IN A COMMUNITY-SAMPLE OF ELDERLY AUSTRALIAN MEN: GEELONG OSTEOPOROSIS STUDY (GOS)

C. E. Coulson1, M. J. Henry2, L. J. Williams2, M. Kotowicz2, M. Berk2, D. I. Lubman1, J. A. Pasco3
1Psychiatry, The University of Melbourne, Melbourne, VIC, Australia
2Medicine, Deakin University, Geelong, VIC, Australia
3Endocrinology and diabetes, Barwon Health, Geelong, VIC, Australia
4Turning Point Drug and Alcohol Centre, Eastern Health and Monash University, Melbourne, VIC, Australia

Aim: The relationship between alcohol consumption and bone mineral density (BMD) is unclear. The aim of this study was to investigate the association between alcohol consumption and BMD in a population-based sample of 534 men aged 65 years and older (median age 76.7yr, IQR 71.3-82.5) enrolled in the Geelong Osteoporosis Study (GOS).

Methods: Alcohol intake was ascertained from a food frequency questionnaire and categorised into non-drinkers and alcohol users on usual drinking days of 1-2, 3-4 and ≥5 standard drinks daily. BMD at the PA-spine, femoral neck, total body, midforearm and ultradistal forearm was determined using dual energy X-ray absorptiometry (Lunar DXP-L and Prodigy). Age and weight adjusted BMD was estimated using linear regression analysis. Cigarette smoking, physical activity, height and daily energy intake were tested as confounders.

Results: There were 90 non-drinkers (16.9%), 266 (49.8%) consuming 1-2 drinks/day, 104 (19.5%) having 3-4 drinks/day (n=104, 19.5%) and 74 (13.8%) having ≥ 5 drinks/day. There was an inverse relationship between alcohol consumption and BMD at the midforearm. BMD at the midforearm for non-drinkers was 0.801g/cm² ± 0.009 (mean ± SE) and decreased for those consuming 1-2 drinks (0.784 g/cm² ± 0.005), 3-4 drinks (0.776 g/cm² ± 0.008) and for those consuming 5+ drinks (0.768 g/cm² ± 0.010), all p<0.05. Interactions and confounding variables were tested and non-significant. No association was detected between alcohol intake and BMD at the PA-spine, femoral neck, total body or ultradistal forearm.

Conclusions: Apart from an inverse relationship at the midforearm site, our data provide little support for an association between alcohol consumption and BMD in elderly men. The reason for an association at one site that comprises mainly cortical bone remains unclear. This may suggest an effect of alcohol on bone but the possibility of a type 1 error cannot be excluded.

RELATIONSHIP OF POSTMENOPAUSAL CIRCULATING MATRIX METALLOPROTEINASE-3 AND ITS INHIBITOR LEVELS WITH BONE MINERAL DENSITY AND OSTEOPONTIN LEVEL

Y. DAI1, L. SHEN2, H. ZHANG3
1Orthopaedics and Trauma, Wuhan Hospital of Traditional Chinese Medicine, Wuhan, Hubei, China
2Orthopaedics and Trauma, Wuhan Union Hospital,Huazhong Science and Technology University, Wuhan, Hubei, China

Objective: To study the serum matrix metalloproteinase-3 (MMP-3) and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) and the correlations of MMP-3 and TIMP-1 with osteopontin and bone mineral density (BMD) in aged postmenopausal Chinese women.

Methods: The serum MMP-3, TIMP-1 and OPN of 210 postmenopausal Chinese female volunteers were measured using ELISA. And the ratios of MMP-3 to TIMP-1 were calculated. BMD were measured using dual energy X-ray absorptiometry. Results: (1) Serum MMP-3 concentrations were significantly higher in osteoporosis (153±121 ng/ml than in age-matched normal controls (125±101) ng/ml, as well OPN were higher in osteoporosis (56±20) ng/ml than normal (26±11) ng/ml. But Serum TIMP-1 concentrations were lower in osteoporosis (13±106) µg/L than in age-matched normal controls (147±130) µg/L. (2) In osteoporosis, notable negative correlations between ratio of MMP-3/TIMP-1 and BMD were found (P<0.05) as well as positive correlations were found between MMP-3, ratio of MMP-3/TIMP-1 and sOPN (P<0.05), and the positive relations of TIMP-1 and the BMD of Ward’s triangle, the negative relations of TIMP-1 and sOPN were existed (P<0.05). Conclusion: There are significant correlations between ratios of MMP-3 and TIMP-1 and sOPN and BMD, and MMP-3 may increase with increases in bone-metabolism. The increases of ratio of MMP-3/TIMP-1 appear possibly as a concomitant event in high bone turnover state, such as postmenopausal osteoporosis.

(1) Minenna GE, DaMore SE, Maggiodini PE, et al.® RANKL/RANK® OPG and OPT in a group of patients affected by chronic arthritis Preliminary report@® Recenti Prog Med® 2005; 96(9): 431-432®
(2) Huong W, Cadsen BE, Rudkin GE, et al.® Osteopontin is a negative regulator of proliferation and differentiation in MC3T3-E1 preosteoblastic cells® Bone® 2004; 34(5): 799-808®

(6) Manicourt DH, Fujimoto N, Obata K, Thonar EJ. Levels of circulating collagenase, stromelysin-1, and tissue inhibitor of matrix metalloproteinases 1 in patients with rheumatoid arthritis. Relationship


406

ASESSMENT OF DIETARY INTAKE OF CALCIUM AND MAGNESIUM BY SINGAPOREAN CHINESE

E. Dewi1, S. Y. Wijaya2, C. S. Yeoh2, T. Walczyk1,2,3

1 NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore, Singapore
2 Chemistry (Science), National University of Singapore, Singapore, Singapore
3 Biochemistry (Medicine), National University of Singapore, Singapore, Singapore

Aim: To assess dietary calcium (Ca) and magnesium (Mg) intakes by Singaporean Chinese and to compare them against current estimated average requirements (EAR).

Methods: A comprehensive database for Ca and Mg content of more than 2,700 food and beverage entries was developed based on six food composition tables, food labels, in-house chemical analysis and other relevant sources. Ca and Mg intake by females aged 18-30 years (n=47) and ≥55 years (n=51) were assessed using 3-day food records, that had been validated through a duplicate diet study.

Results: Ca and Mg intake of the younger group (495±37 and 192±11 mg/day, respectively) were significantly lower than those of the elderly (721±60 and 244±15 mg/day, respectively) (p<0.01). Ca and Mg intakes of both groups were lower than current EAR of 800 and 1,000 mg/day for Ca and 255 and 265 mg/day for Mg for adults 18-30 and ≥55 years old, respectively. Differences in Ca and Mg intake between young and elderly females could be largely attributed to differences in intake from supplements and fortified foods. Intake from supplements was about six times higher in the elderly for Ca (229 vs 38 mg/day) and eight times higher for Mg (69 vs 9 mg/day). Intake from fortified foods was about two times higher in the elderly for Ca (105 vs 41 mg/day) and Mg (16 vs 9 mg/day).

Conclusions: Average Ca and Mg intakes of Singaporean Chinese females were below current EAR, with that of the younger females being significantly lower. High Ca and Mg intakes in the elderly could be related to supplementation, fortified foods and beverages consumption and/or a greater awareness of Ca and Mg importance for health. However, it remains open if low Ca and Mg intakes are of concern as current EAR have largely been derived from studies in non-Asian populations.

407

ARE WRIST FRACTURES STILL A USEFUL PREDICTOR OF FUTURE HIP FRACTURE IN 2011?

S. Dholakia, M. Thilagarajah, R. Singh

Trauma & Orthopaedics, NHS, Dartford, Kent, Great Britain

Introduction

Hip fractures among elderly people are a major cause of morbidity and mortality. There are an estimated 70,000 hip fractures occurring each year in the United Kingdom. Studies show fractures of the wrist present with a relative risk rate of 1.54 for future hip fracture. The aim of the study was to identify the relative risk rate for hip fracture following wrist fracture in our local population group and to correlate this with the accepted literature values, with a view to evaluating the benefits of bone protection.

Methods

We identified hip and wrist fracture patients retrospectively over a two year period. Using our trauma admission records, medical notes and the hospital picture archiving and communication system (PACS), we identified those patients who suffered either wrist or hip fracture or both. Male patients were excluded to enable direct comparison with previous literature. Statistical analysis for relative risk was calculated for hip fracture following wrist fracture.

Results

The incidence of patients with a history of wrist fracture prior to hip fracture in our female sample group was 13.1%, 57 patients. Of the total 239 wrist fracture patients presenting to our A&E, 177 were female and 62 were male. The relative risk ratio for female patients who went on to fracture their hip was calculated at 1.31 with a 95% confidence interval of 0.05

Conclusions

The incidence of wrist fractures as a marker of future hip fracture is low and the relative risk offered in its predictive value is falling making it less sensitive.

Empirical treatment of bone protective agents prior to admission that has shown to be beneficial and cost effective. Having not offered routine DEXA scanning for wrist fractures we have not seen a rise in relative risk for future hip fracture.
Significantly large proportions of hip fractures are occurring despite being on bone protective prior to admission, requiring further evaluation.

(1) Bogoch Er, Elliot-Gibson V, Escott BG, Beaton DE: The osteoporosis needs of patients with wrist fracture. J Orthop Trauma 2008 (8Suppl): S73-78


(3) Earnshaw SA, Cawte SA, Worley A, Hosking DJ: Colle’s fracture of the wrist as an indicator of underlying osteoporosis in postmenopausal women: a prospective study of bone mineral density and bone t

(4) Klotzbuecher CM RP, Landsman PB, Abbott TA III, Berger M: Patients with prior fractures have increased risk of future fractures: A summary of the literature and statistical synthesis. J Bone Miner


(7) Mallmin H, Ljunghall S, Persson I, Naessen T, Kru semen UB, Bergstrom R. Fracture of the distal forearm as a forecaster of subsequent hip fracture: a population-based cohort study with 24 years of f


STUDY OF BONE MINERAL DENSITY AND FACTORS AFFECTING SAME IN TYPE 1 DIABETES MELLITUS

A. S. JOSHI¹, P. K. VARTHAKAVI¹, M. D. CHADHA¹, N. M. BHAGWAT¹, J. P. DHOYLE¹, B. JHANKARIA¹

¹ENDOCRINOLOGY, TOPIWALA NATIONAL MEDICAL COLLEGE AND BYL NAIR CHARITABLE HOSPITAL, MUMBAI CENTRAL, MAHARASHTRA, India

²RADIOLOGY, JHANKARIA DIAGNOSTIC CENTRE, MUMBAI, MAHARASHTRA, India

AIM: To study bone mineral density(BMD) and factors affecting same in type 1 diabetes mellitus(T1DM).

INTRODUCTION: Most studies have reported a strong association between poor BMD and increased fracture risk in T1DM. T1DM and fractures are major causes of morbidity in our population. We studied the BMD and factors affecting same in T1DM.

METHODS: 86 consecutive T1DM patients who satisfied the inclusion criteria and 140 age and sex matched nondiabetic controls were measured for BMD and body composition by dual energy X-ray absorptiometry. HbA1C, antimicrosomal and IgA tissue transglutaminase(IgA TTG) antibodies, serum cortisol, FSH, LH, T4, TSH, GH, IGF1, IGFBP3, calcium, phosphorus, albumin, alkaline phosphatase, 25(OH) Vitamin D3 and intact PTH levels estimated.

RESULTS: Expressed as BMD Z score mean + standard deviation

<table>
<thead>
<tr>
<th></th>
<th>T1DM(n=75)</th>
<th>NONDIABETIC CONTROL(n=140)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumbar spine*</td>
<td>-1.10+1.50</td>
<td>-0.57+1.39@</td>
</tr>
<tr>
<td>Total body*</td>
<td>-1.03+1.19</td>
<td>-0.57+1.04@</td>
</tr>
<tr>
<td>Hip *</td>
<td>-0.85+0.95</td>
<td>-0.29+1.03@</td>
</tr>
<tr>
<td>Fractures 5(6.7%)</td>
<td>1(0.71%)@</td>
<td></td>
</tr>
</tbody>
</table>

Patients who were positive for celiac autoimmunity had worse BMD as compared to age and sex matched T1DM controls and were analyzed as separate sub group (below)

<table>
<thead>
<tr>
<th></th>
<th>T1DM IgA TTG+(n=11)</th>
<th>T1DM IgA TTG-(n=20 age and sex matched)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumbar spine*</td>
<td>-1.47+0.59</td>
<td>-0.21+0.86@</td>
</tr>
<tr>
<td>Total body*</td>
<td>-1.66+0.65</td>
<td>-0.48+0.62@</td>
</tr>
<tr>
<td>Fractures 4(36%)</td>
<td>1(5%)@</td>
<td></td>
</tr>
</tbody>
</table>

*adjusted for height, weight and pubertal status (warner model).1 @ p<0.05

On multivariate linear regression analysis low BMD was associated with celiac autoimmunity, early onset diabetes, physical inactivity, uncontrolled glycaemia, lower body fat mass and lower IGF-1 levels (p<0.05).
Concomitant hypothyroidism or vitamin D deficiency did not have a statistically significant impact on BMD.

CONCLUSIONS: Patients with T1DM have a lower BMD and greater fracture incidence as compared to controls. Poor glycaemic control, low IGF 1 levels, less physical activity and concomitant presence of celiac disease contributed to worse BMD and increased fracture risk.

VITAMIN D STATUS IN HEALTHY INDIANS AGED 50 YEARS AND ABOVE
R. Kanwar
Endocrinology, DRDO, INMAS, New Delhi, India

Introduction: There is widespread prevalence of vitamin D deficiency from new-born to infancy, childhood and adult male and females (non-pregnant, pregnant and lactating). However, there is limited information of the vitamin D status in elderly Indians.

Material & Methods: The study was carried in 1346 healthy subjects more than 50 years of age residing in Delhi, India. These subjects, who were divided in two groups: Group-1 (50 - <65 years) and Group-2 (≥65 years), underwent anthropometric, biochemical and hormonal evaluation for vitamin D status Bone mineral density was measured by dual x-ray absorptiometry.

Results: There were 643 males and 703 females, with a mean age of 58.0 ± 9.5 years (range 50-84 years). Vitamin D deficiency [VDD, serum 25(OH)D levels < 20 ng/ml] was present in 1228 (91.2%) and Vitamin D insufficiency [VDI, serum 25(OH)D levels 20-30 ng/ml] in 92 (6.8%). There was no significant difference in prevalence of either VDD or VDI between two age groups and sexes. Serum 25(OH)D levels were negatively correlated with PTH levels (r = -0.027, p <0.00001) and BMI (r = -0.128, p 0.05).

Prevalence of secondary hyperparathyroidism increased from 14.1% to 43.1% from VDI to severe VDD, PTH levels started rising at vitamin D level < 30 ng/ml. However, more than 50% of subjects with severe VDD had PTH levels within normal range. High prevalence of osteopenia (50.2%) and osteoporosis (31.2%) was observed in this population.

Conclusion: Hypovitaminosis D is universal above the age of 50 years in north India. Absence of a PTH response was observed in more than 50% of individuals with VDD, the cause of which merits further evaluation. Normal bone mass was observed in only 18.6% of study subjects.

Key Words: Vitamin D insufficiency, Vitamin D Deficiency, serum 25(OH)D Levels, Secondary hyperparathyroidism.

THE EFFECT OF AGE ON PARATHYROID HORMONE LEVELS AND BONE TURNOVER IN BLACK POSTMENOPAUSAL WOMEN
I. M. Kruger1, A. Kruger1, M. C. Kruger2

1AUTHOR, North-West University, Potchefstroom, South Africa
2Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand

Aim: This study sought to compare the effects of age on PTH concentration as well as bone turnover in black postmenopausal women from urbanized and rural communities.

Method: Black postmenopausal South African women from rural (N=306) and urban (N=293) communities were included. Biochemical markers (PTH, albumin-corrected serum calcium, 25 (OH)D3, CTX) were determined as well as renal function expressed as estimated creatinine function (eCcr). Associations between PTH and respective biochemical markers were assessed.

Results: Urban women revealed a significant age-increase in PTH (p<0.01) and CTX levels (p<0.02). CTX increased significantly in rural women (p=0.02) despite no significant increase in PTH levels (p=0.65). No significant change was observed for serum calcium in either group. Furthermore, only urban women revealed a significant age-related decrease in 25(OH)D3 (p<0.01). A significant decrease in eCcr (p<0.01) was observed only in rural women. Single regression analysis revealed a positive correlation between PTH and age (r=0.29; p<0.01) for urban women only. Weak positive correlations between age and CTX was found for urban (r=0.17; p<0.01) and rural (r=0.15; p=0.01) women. In addition rural women revealed a strong inverse relation between age and eCcr (r=-0.30; p<0.01) which was not noticeable in urban women. Additionally, urban women revealed a positive association between PTH and CTX (r=0.29; p<0.01).

Standard multiple regression models revealed 25(OH)D3 as the strongest predictor of PTH level variance in rural women (β=0.15; p=0.01) whereas age per se (β=0.23; p<0.01) was the most significant determinant in urban women. Declining renal function (eCcr) was the strongest determinant of CTX levels in rural women (β=0.25; p<0.01). PTH levels was the most significant predictor of CTX variance in urban women (β=0.26; p<0.01).

Conclusion: Despite a significant age-related rise in PTH levels observed only within urban women, both urban and rural women revealed a significant increase in CTX levels. Determinants of CTX levels seem to differ between these two groups.

FACTORS AFFECTING BONE MINERAL DENSITY IN KOREAN ADULTS
H. Lee, S. Kim, W. Bae, J. Kim
Health promotion center, Seoul National University of Bundang Hospital, Seongnam-si, Gyeonggi-do, Sth Korea

Bones are an important component in maintaining the shape of the human body. The purpose of this study was to determine the importance of a healthy lifestyle in relation to the role it plays on the bone density of Korean adults. The research involved such factors as dietary habits, nutritional intake, anthropometry data, and indeed environmental factors.

We performed this cross-sectional study on 144 adults who had visited the University Hospital Promotion Center. (Male: 66, Female:77)
The Correlation analysis revealed that the femoral neck in the Z-score was positively associated with vegetable protein, vegetable calcium, iron, vegetable iron, potassium and folate. The score of dietary habits appear normal and osteopenia did not show any, but the T-score and the correlation were positive. Smoking showed negative correlation with lumbar BMD, T-score, Z-score and drinking showed a negative correlation with lumbar T-score. (p<0.05) Also exercise has a significant influence on bone density.

In conclusion, this study reveals that people who have a balanced diet including beneficial dietary habits, and who have an active lifestyle have healthy bones.

412

EVALUATION OF MILK BASIC PROTEIN SUPPLEMENTATION ON BONE DENSITY AND BONE METABOLISM IN CHINESE YOUNG WOMEN

X. Lin

Department of Nutrition and Food Hygiene, School of Public Health, Peking University, 38 Xueyuan Road, Haidian District, China

Introduction Milk is a good source of bioavailable calcium compared with other foods. Recent in vitro and in vivo studies have shown that milk whey protein, especially its basic protein fraction (milk basic protein, MBP), contains several components capable of promoting bone formation and inhibiting bone resorption. The objective of this study was to examine the effects of MBP on bone mineral density (BMD) and bone metabolism of healthy young women.

Methods Eighty-four healthy young women were randomly assigned to three groups: control group, whole milk group or MBP group treated with milk containing 40 mg MBP for 8 months. The bone mineral density of total body, the lumbar vertebrae L2–L4 and the left forearm of each subject were measured by dual-energy X-ray absorptiometry (DEXA) at 0 and 8 months of treatment. Serum indexes of bone metabolism were measured at 0, 3, 6 and 8 months. Eighty-one subjects who completed the study in accordance with the protocol were included in the analysis.

Results Total BMD in all groups significantly increased compared with baseline values. However, no significant difference on the mean rate of gain of total BMD was observed among the MBP group (2.19%), the whole milk group (2.63%) and the control group (1.61%). Serum crosslinked N-telopeptides of type-I collagen (NTx) in MBP group at 8 months and in whole milk group at 6 months were significantly decreased from baseline. There were no significant differences between whole milk group and MBP group; however, combining the milk groups, NTx had significantly decreased from baseline. No significant increase was observed in serum bone-specific alkaline phosphatase (BAP) in both whole milk group and MBP group.

Conclusion No significant effect of MBP on bone mineral density and bone metabolism was observed, but milk supplementation was effective in suppressing bone resorption.

413

THYROID FUNCTION AND BONE MINERAL DENSITY AMONG INDIAN SUBJECTS

R. K. Marwaha1, M. K. Garg1, N. Tandon2, R. S. Kanwar1, A. Sastry1, K. Bhadra1

1Endocrinology and Thyroid Research, Institute of Nuclear Medicine & Allied Sciences, Delhi, Timarpur, India

2ENDOCRINOLOGY AND METABOLISM, All India institute of Medical sciences, DELHI, India

Introduction: Thyroid hormones affect bone remodeling in patients with thyroid disease by acting directly or indirectly on bone cells.[1-4] There is limited information on correlation of thyroid function with BMD in euthyroid subjects hence; we undertook a study to evaluate the correlation of thyroid function with BMD in subjects with normal thyroid function and subclinical hypothyroidism. Material & Methods: 1290 subjects included in this cross sectional study, were divided in Group1 with normal thyroid function and Group 2 with subclinical hypothyroidism (normal FT4 and high TSH). Fasting blood samples were drawn for the estimation of serum 25(OH)D, intact parathyroid hormone, total and ionized calcium, inorganic phosphorus, and alkaline phosphatase (ALP). Bone mineral density (BMD) at lumbar spine, femur and forearm was measured. Results: BMD at all sites (radius, femur and spine) were comparable in both groups. There was no difference in BMD within both groups, when subjects were divided in tertiles of TSH levels. In group-1 FT4 and TSH were positively associated with BMD at distal 33% radius; and FT3 was negatively associated with BMD at lumbar spine after adjustment for age, sex, BMI, 25(OH)D and PTH levels. In group-2 FT4 and FT3 were negatively correlated with BMD at lumbar spine and radius respectively. In this group, there was no association between TSH and BMD at any site. Conclusion: Thyroid hormones have positive effect on cancellous bone and adversely affect trabecular bone in euthyroid subjects. TSH does not affect BMD in euthyroid subjects and subjects with subclinical hypothyroidism.

(1) Biondi B, Cooper DS. The clinical significance of subclinical thyroid dysfunction. Endocr Rev 2008, 29: 76-131
RESTING METABOLIC RATE: IS A STRONG MARKER FOR BMD PREDICTION IN OBESE SUBJECTS?
K. Mirzaei, A. Hossein-nezhad, Z. Maghbooli, H. Ansar
Tehran University of Medical Sciences, Tehran, Iran

Significant correlation between basal metabolic rate (BMR) and bone mineral density (BMD) has been reported in postmenopausal women and elderly men. However, the relationship between BMR and BMD in adult obese subjects has not been studied yet. In this study, we aimed to investigate the relationship between RMR and Lumbar spine (L2-L4) and Total hip BMD in adult obese and to determine whether obesity contribute to this relationship. The participants were 235 adult obese (45 men and 190 women). The mean of age and BMI were 35.14±10.35 years and 33.38±3.24 kg/m2 respectively. Lumbar spine (L2-L4) and Total hip BMD were measured by dual energy X-ray absorptiometry (DEXA). Participants were assessed following an overnight fasting for resting metabolic rate (RMR) by means of indirect calorimetry. The mean of Lumbar spine (L2-L4) and Total hip BMD were 1.2±0.17g/cm2 and 1.08±0.18 g/cm2 respectively. Based on BMD T score the prevalence of osteopenia in lumbar spine and hip were 32.1% and 9.3% respectively. We found significant lower computed RMR in compare with predicted value in osteopenic patients in Lumbar spine (L2-L4) BMD. Also mean of RMR/kg was significant lower in osteopenic person compare to healthy controls. These finding indicate that possible responsibility of bone metabolism as a major component of RMR; however, the clear pathway and clinical application of this relationship is not understood yet. Future studies should focus on the relationship between RMR and the markers of bone turnover. Key words: Resting metabolic rate, BMD prediction, obesity

INDEPENDENT VALIDATION OF THE GARVAN NOMOGRAMS FOR PREDICTING ABSOLUTE FRACTURE RISK: TROMSO STUDY
L. A. Ahmed1, N. D. Nguyen1, A. Bjørnerem2, R. M. Joakimsen3, L. Jørgensen2, D. Blinc1, J. R. Center1, J. A. Eisman1, T. V. Nguyen4, N. Emaus2
1Osteoporosis and Bone Biology, Garvan Institute of Medical Research, St Vincent's Hospital, Darlinghurst, NSW, Australia
2Department of Health and Care Sciences, Faculty of Health Sciences, University of Tromso, Tromso, Norway
3Department of Clinical Medicine, Faculty of Health Sciences, University of Tromso, Tromso, Norway

Aim. The present study was designed to validate and evaluate the performance of the Garvan nomograms for predicting 10-year risk of fragility fracture in an independent Norwegian cohort.

Methods. The study was based on the prospective cohort Tromsø study in Norwegians. Clinical data were obtained from 2928 (1614 women) participants aged 60+, who have been followed for 8 years. The incidence of fracture was ascertained by radiographic archives. Predicted 10-year probabilities of hip and non-hip non-vertebral (NHNV) fractures were determined using Garvan's models with and without BMD. The concordance between observed and predicted incidence of fracture was used as a measure of fit. Reclassification analysis was used to compare the performance of models.

Results. The overall incidence (1/1000 person-years) of NHNV and hip fracture during the follow-up was 34.6 and 9.1 in women, respectively; and 12.3 and 5.4 in men, respectively. In both sexes, the predicted 10-year probability of fractures in the fracture group was consistently higher than the non-fracture group for all models. For hip fracture, the predicted probabilities of fracture in the fracture group was 2.8 (women) to 3.1-times (men) higher than those in the non-fracture group. There was a close agreement between predicted and actual risk of fracture in both men and women. For example, in women, the average predicted risk for the model with BMD in quartile groups were 9%, 16%, 24% and 48%, respectively; and corresponding observed risks were 13%, 17%, 25% and 31%, respectively. However, among those in the highest quartile of risk, the model over-estimated the risk of fracture. Models with BMD+clinical risk factors performed better than models with body weight+clinical risk factors in terms of correct classification of fracture and non-fracture cases in their risk.

Conclusions. The Garvan nomograms are valid and reasonably accurate in identifying individuals at high risk of fracture.

BONE TURNOVER WITH COMBINED AEROBIC DANCE EXERCISE AND HONEY SUPPLEMENTATION IN ADULT WOMEN
F. K. Ooi5, R. Marhasiyah1, W. A.H. WanZuraidda6
1Sports Science Unit, Universiti Sains Malaysia, Kota Bharu, Kelantan, Malaysia
2Immunology Department, Universiti Sains Malaysia, Kota Bharu, Kelantan, Malaysia

AIM:
This study investigates the effects of 8 weeks of combined jumping exercise and honey supplementation on bone turnover markers in women.

METHODS:
Forty four healthy sedentary women (25-40 year-old) were age and weight matched, and subsequently being assigned into four groups with n=11 per group: Control (C), honey supplementation (H), aerobic dance exercise (Ex) and combined aerobic dance
exercise with honey supplementation (HEx) groups. Aerobic dance exercise was carried out for one hour/session, three times/week for eight weeks. Honey drink was consumed by H and HEx groups, in a dosage of 20g of honey diluted in 300ml of plain water, for 7 days/week for 8 weeks. In HEx group, the subjects were required to consume honey drink 30 minutes before performing exercise. Before and after 8 weeks of experimental period, subjects' percentages of body fat were measured. Additionally, blood samples were taken to determine the concentrations of bone osteocalcin (bone formation marker), serum C-terminal telopeptide of type 1 collagen (1CTP) (bone resorption marker), and parathyroid hormone (PTH). Repeated measure ANOVA was performed to determine the significance of the differences between and within groups.

RESULTS: At the end of 8 weeks of experimental period, it was found that there was significant increase of percentage of body fat compared to the pre-test scores in H group. However, this observation was not evident in C, Ex and HEx groups. At post test, the percentages of increment in 1CTP, a bone resorption marker, and PTH concentrations in HEx group were the lowest compared to the other experimental groups. Nevertheless, there was no significant change in serum osteocalcin in HEx.

CONCLUSION: The present results suggest that combination of aerobic dance exercise and honey supplementation may elicit effects on reducing increment in bone resorption compared to honey supplementation and aerobic exercise alone in adult women.

RISK FACTORS FOR FRAGILITY HIP FRACTURES IN ELDERLY ASIAN INDIANS

R. Ramot1, M. Gahlot2, V. Sharma3, R. Rajput4, R. Khadgawat1
1Endocrinology & Metabolism, AIIMS, New Delhi, India
2Dietetics, AIIMS, New Delhi, India
3Orthopedics, AIIMS, New Delhi, India
4Endocrinology, PGI, Rohtak, Haryana, India
5Endocrinology, AIIMS, New Delhi, India

Objective: To study the risk factors associated with fragility hip fractures in elderly Asian Indians aged ≥60 years. Materials and methods: This cross sectional observational study was carried out in 150 consecutive subjects (4 excluded) aged ≥60 years, presenting with non-traumatic hip fracture. After informed written consent, detailed history and tailored clinical examination, samples were collected for routine biochemistry and serum 25 hydroxy vitamin D. Results: Of 146 subjects included in analysis, the mean age was 71±9.15 (range 60-105, 88 females, age 71.2±8.7 years; 62 males, age72±9.2 years). More fractures were reported in intertrochantric region (64%) as compared to Neck of femur (31%). Majority of fractures (60%) occurred while subjects were at home, of which 44% were around home premises and 32% in bedroom. Slipping on wet floor (27%) was the commonest single cause of fall followed by obstruction by some object while walking and imbalance due to knee joint pain were other common causes of fall, resulting in fracture. Majority of falls occurred while walking (65%) and more falls occurred in evening/night (43%). As part of first aid at home only analgesics were given in 85% of cases, no cast or tractions were applied. Post-operative DVT prophylaxis was provided only to 23% of cases. Follow-up data (6 months, available in 71) showed second fracture in 2 cases, 73% were walking with stick/walker support while 21% were able to walk without any support. One year follow-up (45 subjects) showed second fracture (hip, humerus& toe) in 3 subjects while 50% of cases were able to walk without support. Total 10 subjects died within one year after surgery, of these, 50% died within one month of surgery while another 50% within 4 months of surgery.

PREVALENCE OF VERTEBRAL FRACTURES AMONG ELDERLY ASIAN INDIANS PRESENTING WITH FRAGILITY HIP FRACTURES

R. Ramot1, V. Sharma2, S. Gamanagatti3, M. Gahlot4, R. Khadgawat1
1Endocrinology & Metabolism, AIIMS, New Delhi, India
2Orthopedics, AIIMS, New Delhi, India
3Radiology, AIIMS, New Delhi, India
4Dietetics, AIIMS, New Delhi, India
5Endocrinology, AIIMS, New Delhi, India

Objective: To assess vertebral fractures in elderly Asian Indians (aged ≥60 years) presenting with fragility hip fractures. Materials and methods: The study population comprised of 100 consecutive subjects (2 subjects excluded) admitted with fragility hip fractures. Of 98 subjects, 64 underwent X-rays of dorso-lumbar spine. All X-rays were assessed by single trained radiologist for presence of vertebral fracture. Same radiographs were also assessed by vertebral morphometry software (Optasia- spine analyser 3.2.2.7) based on Genant semiquantitative grading scale for vertebral fracture by single investigator. The level of vertebrae analysed by software ranged from T4 to L4. Results: Of 64 x-rays analysed, vertebral fractures were reported in 45% (n=29) cases. There were total 586 vertebral fractures among the 64 cases, of which 54% had fractures. In thoracic spine, 78% fractures were seen between T9 to T12 vertebrae, where in 72% were wedge type and mild intensity fractures (56%). Majority of fractures in lumbar spine were seen in L1 vertebra (72%), mostly wedge type (61%) and of moderate intensity (55%). There were total 26 consecutive vertebrae with fractures, mainly between T9 to L1. Radiologist's analysis revealed significant number of fractures in L5 (23%) vertebral also, which was not analysed by software. Of 45% of study subjects with vertebral fractures, 48% were reported in age group of 60-70 years, 65% were females. Subjects presenting with intertrochantric fractures (55%) reported more vertebral fractures.
than subjects with fracture of neck of femur (37%). Conclusion: There is high prevalence of vertebral fracture in elderly subjects with fragility hip fracture. The majority of vertebral fractures occurred in lower thoracic and upper lumbar region.

**RELATION OF OSTEOPOROSIS TO CATALASE ACTIVITY, SMOKING AND MENOPAUSE**

N. Sadeghi1, M. Oveis1, B. Jannat2, M. Hajimahmoodi1

1Dep. of drug and food control, Tehran university of medical sciences, Tehran, Iran
2Food and drug laboratory research centre, Ministry of Health, Tehran, Iran

Introduction: Osteoporosis is a major metabolic bone disease causing enhanced bone fragility. Free radicals have an important role in many age related diseases and Catalase can change them to less harmful compounds. The purpose of this study was to consider the erythrocytes Catalase activity in osteoporotic Iranian women comparing to the control, by glance to smoking and menopause effects.

Material and Method: Catalase activity was measured spectrophotometrically at 240 nm. Participants were selected by inclusion and exclusion criteria among those who referred to Jamie Clinic in Tehran for BMD evaluation, and classified as Patient group (n=76) against control group (n=76). Standard questionnaire (including smoking habit and menopause condition, etc.) were used in this study.

Results: In total osteoporotic group (T-score ≥−1) Catalase activity value is 369.62±63.10 k/gHb and in control group (Femoral and Lumber T-score ≥−1) is 390.62±60.78 k/gHb. In smoker (n=20) and non-smoker (n=172) participants, Catalase activity values are 268.18±40.49 and 265.88±43.18 k/gHb, respectively. In pre-menopausal (n=109) and post-menopausal (n=83) participants, Catalase activities are 268.17±44.17 and 263.42±41.06 k/gHb, respectively. Femur mineral density in smoker and non-smoker are 1.22±1.07 and -0.62±1.32, and in pre-menopausal and post-menopausal are -0.44±1.12 and -0.98±1.45, respectively.

Discussion and Conclusion:
Catalase activity values were markedly lower in the patients than the controls (p<0.05). Catalase activity values have positive relationship with T-score in total participants (r=+0.001; p=0.16).

No difference in menopause and smoking habit was found between groups, but it is worth saying that in all participants (no attention to control or patient group), Catalase values is more in pre-menopausal compared to the post-menopausal, though it is not significant. Femur mineral density is lower in smokers than non-smokers (P<0.05), also in post-menopausal than pre-menopausal women (p<0.01).

It seems that catalase decrease make it possible to depict a relation between osteoporosis occurrence and antioxidants deficiency.

**COMPARISON OF BONE MINERAL DENSITY, MUSCLE STRENGTH AND SOME HORMONAL AND BIOCHEMICAL FACTORS OF BLOOD SERUM IN ATHLETIC (VOLLEYBALL PLAYERS) AND NON ATHLETIC GIRLS**

M. R. Salamat, M. Kargarfard, N. Bijeh, I. Abedi

Medical Physics and Medical Engineering, Isfahan University of Medical Sciences, Isfahan, Iran

Objective: The aim of this study was to assess bone mineral density (BMD), anthropometrical characteristics and muscular strength of female volleyball players and compares them with non-athlete females of the same age. Materials and Methods: 15 healthy female volleyball players and 12 non-athlete females participated in this study. The BMDs of femoral neck (FN) and lumbar spine, L2-L4 (LS) were measured by DXA method. The strength of quadriceps muscle and hamstring were measured by means of Kin-COM. Calcium and phosphor level of blood serum and alkaline phosphates and parathormone hormone activity were measured before having breakfast. Results: The findings showed that there were no significant differences between the BMDs of LS and FN of volleyball players and non-athletes, though the BMDs of volleyball players were greater than non-athletes. The mean muscular strength of hamstring and forceps of the volleyball players were significantly higher than the non-athletes. Discussion: In general, our results showed that FN and LS BMDs and muscular strength of hamstring and quadriceps were greater in volleyball players compared to non-athletes. This reveals the impact of exercise and physical activities in preventing disease and improving society health.

**MEDICATION USAGE FOUR MONTHS POST FRACTURE- RESULTS FROM AUSICURS**

A. L. Stuart1, S. Iuliano-Burns2, E. Seeman2, R. Prince3, G. Duque4, T. Winzenberg1, L. March5, P. R. Ebeling1, G. C. Nicholson1, F. Borgstrom2, K. M. Sanders1

1Medicine, NorthWest Academic Centre, University of Melbourne, Melbourne, VIC, Australia
2Austin Health, University of Melbourne, Melbourne, VIC, Australia
Aim
To document new medications commenced in the first four months following fracture of the wrist, hip or spine. The Australian Study of Costs and Utilities Related to Osteoporotic Fracture (AusICUROS) is being conducted at 7 sites across Australia and recruits adults aged 50+ years within 2 weeks of a low-energy fracture.

Methods
AusICUROS will establish the cost and quality of life impact of fragility fractures in Australian fracture patients over the immediate 18-month period following fracture. The study is based on the IOF-endorsed International Costs and Utilities Related to Osteoporotic Fractures Study. We present results of self-reported medication that were commenced ‘as a consequence of the fracture’ in participants who have completed their 4-month post-fracture interview. Chi square analysis was used to determine differences in proportion of patients taking medication.

Results
To date, 256 participants (84 hip, 141 wrist and 31 vertebral fracture patients) have completed the 4-month interview. The proportion of participants who commenced pain medication post-fracture does not vary by fracture site (hip - 74%, wrist - 70%, vertebral - 65%, p = 0.596). Fewer wrist fracture participants commenced either calcium/vitamin D supplementation (total 23%, n = 60/256: hip - 36%, wrist - 14%, vertebral - 32%, p = 0.001) or bisphosphonate treatment (total 10%, n = 28/256: hip - 17%, wrist - 6%, vertebral - 20%).

Conclusion
These results demonstrate that less than 40% of hip or vertebral fracture patients commence treatment for bone fragility (calcium/vitamin D supplementation or bisphosphonates) following fracture and the proportion of wrist fracture patients commenced on bisphosphonates or supplements is less than 15%.

422

POPULATION PREVALENCE OF FRAGILITY FRACTURES IN INDIA BASED ON A NATIONWIDE QUESTIONNAIRE BASED EPIDEMIOLOGICAL STUDY

N. Tandon1, A. Mithal2, R. M. Anjana3, R. Pradeepa3, M. Deepa3, K. Mani4, V. Mohan1

1Endocrinology and Metabolism, All India Institute of Medical Sciences, New Delhi, Delhi, India
2Endocrinology, Medanta-The Medicity, Gurgaon, Haryana, India
3Epidemiology and Research Operations, Madras Diabetes Research Foundation, Chennai, Tamil Nadu, India
4Biostatistics, All India Institute of Medical Sciences, New Delhi, Delhi, India

There is no community-based information on the population prevalence of fragility fractures in India. In an attempt to acquire such information, we added a set of questions to an ongoing nation-wide study designed to evaluate the national prevalence of diabetes and pre-diabetes - The India Diabetes Study (INDIAB). The first phase of INDIAB involved 3 states and one Union Territory – one each from North (Chandigarh), East (Jharkhand), South (Tamil Nadu) and West (Maharashtra) regions of India. This study utilizes a stratified multi-stage design, with households serving as ultimate stage units in both areas. One individual from each household, randomly selected using the WHO Kish method was asked the following three questions: have you had a fracture in the last 10 years?; where was this fracture?; and in case of hip, spine and wrist fractures was the causative trauma trivial or significant?

A total of 14271 subjects (7116 males; 7155 females) responded to the questions. A total of 491 fractures were reported [3.44%; 95% CI 3.14-3.74]. Fractures at the hip, wrist and spine were reported by 31 (0.2%), 50 (0.35%) and 36 (0.25%) subjects respectively; and 42% of these were due to trivial trauma, giving an overall prevalence of 34.3/100000 population. The prevalence of fractures in males was 4.8% [95% CI 4.31-5.30] and in females 2.08% [95% CI 1.75-2.41]. The population was divided in 7 age categories starting from 20 years, each encompassing a decade, with the oldest category including all subjects 80 years or older. The fracture prevalence ranged from 2.39% (20-30 years) to 8.93% (80 years and older), with no difference in prevalence between the four regions. In conclusion, this large questionnaire based study in the community across four regions of India reports the population prevalence of trivial trauma fractures at hip, spine and wrist to be 34.3/100,000.
RELATIONSHIP BETWEEN SERUM 25-HYDROXYVITAMIN D LEVELS AND PREVALENT FRACTURE RISK IN POSTMENOPAUSAL WOMEN

M. Yamauchi1, K. Nawata1,2, H. Kaji3, S. Takaoka1, T. Yamaguchi1, T. Sugimoto1
1Internal Medicine 1, Shimane University Faculty of Medicine, Izumo, Japan
2Health and Nutrition, The University of Shimane, Matsue, Japan
3Division of Cellular and Molecular Medicine, Kobe University Graduate School of Medicine, Kobe, Japan

Aim: Vitamin D insufficiency is frequently observed in general population, especially in elderly. The importance of vitamin D status in bone health has been noted. Vitamin D insufficiency is associated with an increase in PTH, which might be critical for an increase in bone fragility. However, the role of endogenous PTH in vitamin D insufficiency-induced fracture risk still remains unclear. The present study was performed to examine the relationships among vitamin D insufficiency, bone fragility and PTH in 202 postmenopausal women. Methods: Bone mineral density (BMD) was measured using the DXA method at the lumbar vertebrae and femoral neck (FN). The presence of vertebral fractures was confirmed on X-ray and nonvertebral fractures were assessed by the clinical interview. Serum levels of Cr, CTX, intact PTH and 25-hydroxyvitamin D [25(OH)D] were measured. Motor function tests included tandem walk test and measurements of grip strength. Results: The percentages of subjects with 25(OH)D levels below 20 ng/ml were 80.7%. Serum 25(OH)D levels were negatively related to age, Cr, CTX and PTH, although it was positively related to BMD. In multiple regression analysis, BMD was significantly related to 25(OH)D levels, when adjusted for age, BMI, Cr, CTX and PTH. Multiple logistic regression analysis showed that lower 25(OH)D levels were significantly related to prevalent fracture risk, when adjusted for age, BMI, tandem walk test, grip strength, Cr, CTX, PTH as well as FN BMD. The rate of the subjects with prevalent fractures was significantly higher in the group with lower PTH and lower 25(OH)D, compared to that of the groups with lower PTH and higher 25(OH)D or higher PTH and higher 25(OH)D. Conclusions: Vitamin D insufficiency was related to prevalent fracture risk independently of PTH. Functional hypoparathyroidism rather than functional hyperparathyroidism might be a risk factor for bone fragility in vitamin D insufficiency.

CONTRIBUTIONS OF LEAN MASS AND FAT MASS TO BONE LOSS: THE DUBBO OSTEOPOROSIS EPIDEMIOLOGY STUDY

S. Yang1,2, N. D. Nguyen1, J. R. Center1,3, J. A. Eisman1,3, T. V. Nguyen1,2
1Garvan Institute of Medical Research, Sydney, NSW, Australia
2School of Public Health and Community Science, UNSW, Sydney, NSW, Australia
3St Vincent’s Hospital, Sydney, NSW, Australia

Introduction: Greater bone loss is associated with increased fracture risk. Although it is well known that higher body weight is associated with reduced bone loss, the contribution of body composition components to bone loss has never been studied. The present study was designed to examine the contribution of fat mass and lean mass to bone loss in the elderly.

Materials and methods: The study was part of ongoing Dubbo Osteoporosis Epidemiology Study (DOES), which is a perspective population based cohort study. This study involved 717 (204 men) participants aged 50+ years, whose BMD had been measured at least 3 visits. BMD was measured at the femoral neck (FN), lumbar spine (LS), and whole body (WB) by DXA method (GE-LUNAR Corp, Madison, WI). Lean mass and fat mass were derived from whole body scan. Baseline risk factors, including age, weight, height, history of fracture, smoking and physical activity were recruited at initial visit. The association between lean mass, fat mass and bone loss was analyzed by a mixed-effects model, with adjustment for participants’ baseline characteristics.

Results: The median follow-up of the study participants was 5 years (range: 2-10 years). In men, after adjusting for age, height, prior fracture, smoking and physical activity, each standard deviation (SD) increase in lean mass was associated with a 0.03 mg/cm² increase in FNBMD, and this magnitude of association was greater than that for fat mass (regression coefficient: 0.02 mg/cm²). However, in women, the effect of lean mass on FNBMD change was similar to that of fat mass (each factor has a regression coefficient of 0.04 mg/cm²). The proportion of variance in bone loss attributable to lean mass and fat mass was 5% and 0.5%, respectively.

Conclusion: Greater fat mass and particularly greater lean mass are associated with reduced bone loss; however, their contributions to variance in BMD change is modest.
LONGITUDINAL VBMD CHANGES IN PERI-PUBERTAL CHILDREN

P. Bridge, T. Nguyen, N. Pocock, C. Munns, C. Cowell, M. Thompson, K. Atkins

1The Children’s Hospital at Westmead, Westmead, Australia
2Garvan Institute, Darlinghurst, Australia
3The University of Sydney, Australia
4Department of Health and Ageing, Canberra, ACT, Australia
5St Vincents Hospital, Darlinghurst, Australia

Introduction and aim: True volumetric BMD (vBMD) is an important parameter in bone research. A few longitudinal studies report a modest but significant increase in vBMD with increasing maturation during growth. Attention has however focused on a number of cross sectional studies suggesting that vBMD is relatively constant during childhood and throughout puberty. This study tested two hypotheses:

1) vBMD increases from childhood to adolescence.
2) The degree of changes in vBMD is skeletal site and gender specific.

Method: At baseline pre-pubertal healthy children (26 boys and 20 girls) aged 8-11 years were recruited, and recalled 26-48 months later at pubertal stage Tanner 2 to 4. vBMD was measured at both visits in the mid-third femur by combining DXA, to derive Bone Mineral Content (BMC), and MRI to derive volume. Spine (L2-L4), and femoral neck, vBMD were calculated using BMC and bone volume derived using DXA.

Results: In girls, significant increases in vBMD (g/cm3) from baseline were observed at the spine (0.029±0.030), hip (0.033 ± 0.045) and femoral shaft (1.406 ± 0.262). In boys there was a significant (p≤0.001) increase in vBMD at the mid femoral shaft (0.177 ± 0.111) from baseline. The degree of vBMD change was significantly different between boys and girls (p≤0.001) at all skeletal sites, but were not for bone volume and BMC changes. These differences were explained by the ratio of change between BMC and bone volume which is specific to skeletal sites and genders (Table 1).

Conclusion: vBMD is increasing with pubertal maturation following a site specific pattern different in boys and girls. vBMD increase is particularly significant in cortical bone at the mid femur. Since boys and girls display a very different graphic of bone growth, vBMD should be interpreted within the context of changes in bone volume and BMC accretion.

Table 1: Longitudinal % change (boys and girls). Mean (CI 95% lower bound, upper bound)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Spine L2-L4</th>
<th>Hip (Femoral Neck)</th>
<th>Mid-1/3 Femoral Shaft</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Boys n=26</td>
<td>Girls n=19</td>
<td>Boys n=26</td>
</tr>
<tr>
<td>vBMD %</td>
<td>2.4 (2.0,6.7)</td>
<td>12.5 (6.2,18.8)</td>
<td>-4.8 (-13.8,4.0)</td>
</tr>
<tr>
<td>Bone volume %</td>
<td>74.6 (62.9,86.3)</td>
<td>73.4 (61.0,85.9)</td>
<td>43.9 (36.1,51.7)</td>
</tr>
<tr>
<td>BMC %</td>
<td>82.9 (68.2,97.5)</td>
<td>94.0 (77.5,110.3)</td>
<td>43.0 (35.4,50.6)</td>
</tr>
</tbody>
</table>

Conclusion: vBMD is increasing with pubertal maturation following a site specific pattern different in boys and girls. vBMD increase is particularly significant in cortical bone at the mid femur. Since boys and girls display a very different graphic of bone growth, vBMD should be interpreted within the context of changes in bone volume and BMC accretion.

BONE STRUCTURAL PARAMETERS IN PRE AND PUBERTAL INDIVIDUALS WITH CYSTIC FIBROSIS (CF)

D. S.K. Brookes, J. N. Briody, C. J. Munns, R. J. Hill, P. S.W. Davies

1Children’s Nutrition Research Centre, The University of Queensland, Herston, QLD, Australia
2The Children’s Hospital at Westmead, Sydney, NSW, Australia

Aim: To assess bone structural parameters using peripheral quantitative computed tomography (pQCT) in individuals with CF and controls, 7.00-17.99 years.

Methods: Groups were stratified by sex and Tanner stages (TS), (TS1: CF=16(9F), controls=18(11F); TS 2-5: CF=37(25F), controls=35(23F)) . pQCT (XCT3000, Stratec) was used to measure bone mineral content (BMC), total volumetric bone mineral density (vBMD) and cross sectional area (CSA) of the bone at both distal (4%) and shaft (66%) sites of the tibia and radius. Additionally, trabecular vBMD was measured at the distal sites, and cortical vBMD and muscle CSA were measured at the shaft sites.

Results: Compared with controls, pre-pubertal males with CF had 17% greater trabecular vBMD (p=0.01) and 16% greater total vBMD (p=0.006) at the distal tibia, and 14% greater total vBMD (p=0.02) at the distal radius. Pre-pubertal females with CF had 8% (p=0.02) and 11% (p=0.04) greater total vBMD at the tibia and radius (shaft), respectively, and 5% greater cortical vBMD (p=0.04) at the radius. In the pubertal group, control females and males had 15% (p=0.006) and 20% (p=0.02) greater BMC, respectively, compared to individuals with CF at the distal tibia. Control females had 15% larger bone CSA at distal tibia (p=0.001) and distal radius (p=0.06); this was only a trend in the males. At the tibia shaft, muscle CSA was 16% (p=0.009) and 29% (p=0.02) larger in the control females and males, respectively. A trend to greater BMC and bone CSA was shown in the controls for both limbs.
Conclusion: B one structural parameters were not compromised in pre-pubertal patients with CF, while, in contrast, pubertal patients displayed several deficits compared with controls. These apparent reductions in bone structural parameters in pubertal individuals with CF may indicate optimal peak bone mass/structure will not be achieved and poses a threat for bone health in later life.

EFFECT OF ADIPOSITY ON BONE MASS IN PRE-PUBERTAL OBESE CHILDREN
M. Gahlot*, R. Khadgawat, D. Khandelwal, R. Ramot, A. Bakshi
Dept.of Endocrinology, *Dept. of Dietetics, All India Institute of Medical Sciences, Delhi, India

Background - The effect of obesity on bone mass accrual is not well understood due to confounding effect of growth, maturation and body composition.

Objective - The objective of this study was to determine the effect of obesity on total body bone mineral content (TB BMC) relative to age, height, lean body mass (LBM), weight, and fat mass in pre-pubertal obese children.

Methods - TB BMC (g), LBM (g) and fat mass (g) were measured in 25 pre-pubertal obese and 75 pre-pubertal controls (aged 4-12 years) by using dual energy X-ray absorptiometry (DXA). Predictive values for TB BMC adjusted for age, height, LBM, weight and fat mass were calculated by multiple linear regressions using control population. Muscle and bone relationship was studied by first assessing LBM for height and then determining TB BMC for LBM. All values were converted to Z score and compared with control.

Results - Obese children had significantly higher Z-Score for TB BMC adjusted for age (1.01±1.2, p<0.0001), TB BMC adjusted for height (0.67±1.4, p<0.01) and LBM for height (4.51±2.4, p<0.0001) where as TB BMC for LBM Z-Score was comparable to control. Further adjustment of TB BMC for weight and fat mass showed significantly lower Z-score (-2.89±0.69, p<0.0001 & -1.12±0.89, p<0.001) when compared with control.

Conclusion - Obese children have increased absolute bone mass for age and height and LBM for height. Bone mass is also well adapted to increased muscle mass but it did not adapt well to the increased body weight and fat mass. Finally skeletal adaptations in the obese children are not sufficient to compensate for excess load on the whole body rather it was well adapted for increased muscle mass. Further studies are needed to see the effect of these differences on fracture risk.

EFFECTS OF MATERNAL UNDERNUTRITION AND HYPOXIA DURING PREGNANCY ON BONE DEVELOPMENT OF OFFSPRING
A. M.C. Lee1,2, J. L. Morrison2,3, K. J. Botting2,3, T. Shandala1,2, C. J. Xian1,2
1Sansom Institute for Health Research, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia
2Discipline of Physiology, School of Medical Sciences, University of Adelaide, Adelaide, SA, Australia
3Early Origins of Adult Health Research Group, SHHR, School of PMB, University of South Australia, Adelaide, SA, Australia

Epidemiological studies suggest that weight at birth is associated with bone mass and density in adult life. Low birth weight can be induced by fetal hypoxemia and/or undernutrition. The independent effects of maternal hypoxia and maternal undernutrition on bone structural development are not known. We hypothesise that fetal growth restriction due to maternal hypoxia or undernutrition may reduce bone mass both before and after birth in the guinea pig. Aim: The current study aimed to investigate the effects of fetal growth restriction induced by either maternal undernutrition or maternal hypoxia during pregnancy on bone structure and volume of the offspring before and after birth. Methods: At 35d gestation date-mated guinea pigs were provided either ad libitum feed at 21% O2 (control), housed at 12% O2 with a subsequent reduction in food intake per body weight (hypoxic), or exposed to 21% O2 and feed restricted (matched to the food intake per body weight of hypoxic mothers; undernutrition). To examine treatment effects on offspring growth plate/bone structure and bone volume, femur and tibia were collected at 62d gestation and 129d guinea pigs for bone histomorphometric measurements, and bone marrow cells were isolated for assessing osteoprogenitor cell contents (CFU-f assays). Results: CFU-f alkaline phosphatase-positive colony formation assays showed no changes in osteogenic potential pools in the fetal bone with maternal undernutrition or maternal hypoxia in comparison to normal controls. Histological analysis showed that maternal undernutrition and maternal hypoxia did not result in significant changes in growth plate thickness and primary spongiosa heights in offspring before (62d gestation) or after birth (120d). Conclusion: These results suggest that neither maternal hypoxia nor undernutrition during gestation changes the pool of osteogenic cells in early life and trabecular bone volume in the offspring. More studies are required to examine potential effects for maternal undernutrition and hypoxia during pregnancy on offspring's bone development.
CHANGES IN BONE MASS AND BONE BIOMARKERS IN CHILDREN WITH ATTENTION-DEFICIT HYPERACTIVITY DISORDER RECEIVING STIMULANT MEDICATION

A. S. Poulton1, T. McCorquodale2, M. Herrmann2, G. Duque2

1Paediatrics, Sydney Medical School Nepean-The University of Sydney, Penrith, NSW, Australia
2Ageing Bone Research Program, Sydney Medical School Nepean-The University of Sydney, Penrith, NSW, Australia

Attention-deficit hyperactivity disorder (ADHD) is a very common behavioral disorder among children. Symptoms associated with ADHD have been treated with stimulant medications. Despite their effectiveness, there are some concerns about the effect of stimulant medications on the growth of the children and particularly in their bone development. To identify the potential effect of stimulant medications on bone mass and bone biomarkers, a cohort of 31 children (27 boys) with diagnosis of ADHD (age 7.4 ± 1.2 years) were treated with either methylphenidate (22.0±6.5 mg/day, n=22) or dexamphetamine (10.8±4.0 mg/day, n=9) for up to three years. Total bone mineral content (BMC) and density (BMD) were measured by densitometry and serum concentrations of bone biomarkers for bone formation (P1NP) and bone resorption (CTX) were quantified at time 0, 6m and 36m of treatment. In addition, serum concentrations of vitamin D and parathyroid hormone (PTH) were also assessed. At month 6 of treatment, there was a significant increase in total BMC (∼4%) and CTx (from 40±3 to 50±6 pg/mL, p<0.05) without a significant change in either total BMD or P1NP. However, at month 36 there was a significant increase in both BMC (∼29.2%) and total BMD (∼6.3%) that was also associated with a significant increase in CTx (from 50±6 to 89±8 pg/mL, p<0.01) and P1NP (from 54±4 to 64±3 ng/mL, p<0.01). This increase in bone biomarkers indicated higher levels of bone resorption than formation at two time points after treatment. In addition, no difference in serum concentrations of vitamin D and PTH was observed at the three time points. These results were similar in both treatment groups. In conclusion, our data support a significant effect of long-term (3 years) use of stimulant medication on bone mass and turnover that goes beyond the normal effect of growth on bone in children with ADHD.

EFFECTS OF AGE ON THE DEVELOPMENTAL TIMELINE FOR THE MANIFESTATION OF THE PROGRAMMED BONE DEFICITS ASSOCIATED WITH FETAL GROWTH RESTRICTION

T. Romano1,2,3, J. D. Wark2, M. E. Wlodek1

1Department of Physiology, The University of Melbourne, Parkville, VIC, Australia
2Department of Medicine, The University of Melbourne, Bone and Mineral Service, Royal Melbourne Hospital, Parkville, VIC, Australia
3Tissue and Cell Biology Group, Musculoskeletal Research Centre, La Trobe University, Bundoora, VIC, Australia

Aim: Recent evidence links low birth weight and poor adult bone health. Uteroplacental insufficiency (UPI) complicates 10% of human pregnancies causing intrauterine growth restriction and programming of bone deficits. We characterized bone size, mineral content, density and stress strain index of strength (SSI) in rats from weaning to 12 months.

Methods: 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. Post mortem of Restricted and Control male and female offspring was performed at postnatal day 35 (weaning), 2, 4, 6 and 12 months. Right femur mineral content, density and strength were measured (pQCT).

Results: Male and female Restricted pups were born 10-15% lighter, remaining smaller and having shorter femurs than Controls to 12 months (p<0.05). Male and female Restricted rats had lower trabecular content compared to Controls across all ages (p<0.05), without trabecular density changes. Cortical content at day 35, and 6 and 12 months was reduced in Restricted males, this deficit increasing between 6 (7%) and 12 months (11%). Cortical content was reduced across all ages in Restricted females (p<0.05). Cortical density was lower at day 35 in Restricted males only (p<0.05). Cortical thickness was reduced in Restricted males on day 35 and 12 months (p<0.05). SSI was lower at day 35, and 6 (13%) and 12 months (15%) in Restricted males, and across all ages for Restricted females (p<0.05).

Conclusions: Skeletal deficits were observed in Restricted males as early as 35 days, and at 6 and 12 months. Restricted females demonstrated deficits across all ages, highlighting gender differences regarding programming of adult bone. The findings that deficits observed at 6 months in males are increased at 12 months indicate that aging may be important in the manifestation of programmed adult bone phenotypes.
EFFECTS OF PREGNANCY AND LACTATION ON THE BONE OF NORMAL RAT MOTHERS AND THOSE EXPOSED TO UTEROPLACENTAL INSUFFICIENCY: A MECHANISM PROGRAMMING OFFSPRING BONE HEALTH

T. Romano1,2,3, J. D. Wark2, M. E. Wlodek1

1Department of Physiology, The University of Melbourne, Parkville, VIC, Australia
2Department of Medicine, The University of Melbourne, Bone and Mineral Service, Royal Melbourne Hospital, Parkville, VIC, Australia
3Tissue and Cell Biology Group, Musculoskeletal Research Centre, La Trobe University, Bundoora, VIC, Australia

Aim: During pregnancy and lactation, maternal bone provides offspring with calcium requirements. Uteroplacental insufficiency (UPI) complicates human pregnancies causing growth restriction, low maternal milk calcium content, lower pup body calcium and programming of bone deficits. We determined whether UPI mothers have altered skeletal phenotypes during pregnancy and lactation, and how this relates to programming offspring skeletal deficits.

Methods: 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 pregnancy day 20, postpartum days 1 and 7, weeks 5, 7, 9, and in non-pregnant rats. Right femur pQCT measures were quantified.

Results: Trabecular content and density in Control were not different between non-pregnant and pregnant day 20, but decreased between pregnant day 20 and postpartum day 1 (p<0.05). Cortical content and density increased from non-pregnant to pregnant day 20 by 7% and 2%, respectively (p<0.05). Cortical content and density decreased between pregnant day 20 and postpartum day 1 by 22% and 3%, respectively (p<0.05). Control stress-strain index of bone strength increased by 5% between non-pregnant and pregnant day 20, then decreased between pregnant day 20 and postpartum day 1 (p<0.05). The skeletal gains and losses in Control followed the normal profile required for fetal skeletal mineralization, and were absent in Restricted and significantly different to Control (p<0.05). By postpartum day 7, bone parameters in both groups were not different to non-pregnant, indicating restoration of maternal bone.

Conclusions: Control mothers demonstrated normal skeletal gains and losses associated with pregnancy and lactation, ensuring adequate pup skeletal mineralization. Mothers suffering UPI did not undergo these skeletal changes, indicating a lack of calcium transfer to their pups. This reduction in calcium availability to pups is most likely involved in programming of their poor adult bone health.

BONE BENEFITS OF AN EIGHT-MONTH IN-SCHOOL JUMPING INTERVENTION ARE MAINTAINED AFTER THREE YEARS: POWER PE FOLLOW-UP

B. K. Weeks, B. R. Beck

Musculoskeletal Research Program, Griffith Health Institute, Griffith University, QLD, Australia

Aim: To determine if the musculoskeletal benefits of a twice-weekly, school-based, jumping regime in healthy adolescent boys and girls were maintained three years later. Methods: Subjects of the original POWER PE trial (n = 99) were contacted and asked to participate in 36-month follow-up testing. All original measures were completed including: sitting height, standing height, weight, calcaneal broadband ultrasound attenuation (BUA), whole body, hip and spine bone mineral content (BMC), lean tissue mass, and fat mass. Physical activity was recorded with the bone-specific physical activity questionnaire (BPAQ) and calcium intake was estimated with a calcium-focused food questionnaire. Maturity was determined by Tanner staging and estimation of the age of peak height velocity (PHV). Muscle power was assessed using a vertical jump test. Results: Twenty-nine adolescents aged 17.3 ± 0.4 years agreed to participate. At 36 months, there were no differences in subject characteristics between control and intervention groups (p > 0.05). Three-year change in weight, lean mass, and fat mass were similar between groups (p > 0.05), however, height increased more for the intervention group (+5.1%) compared with controls (+2.6%) (p = 0.05). There were no significant group differences in three-year change in BUA or BMC at any site (p > 0.05). Conclusion: Findings suggest that adolescents will maintain osteogenic benefits from an in-school jumping intervention at least into young adulthood.

Acknowledgements: The study was supported by an Arthritis Australia Grant-in-Aid.