# General Poster Abstracts

## *Abstracts of the General Posters attended by authors on MONDAY (23 Oct 2006)*

### *ANIMAL MODELS OSTEOPOROSIS*

**P-MON-01** **Comparison of effects of gamma-linolenic, eicosapentaenoic and docosahexaenoic acids on bone post-ovariectomy in rats**
Poulsen, R., Firth, E., Rogers, C. and Kruger, M. (New Zealand)

**P-MON-03** **Modified goat milk raises peak bone mass and reduces OVX induced bone loss in the female rat**
Chua, W-H., Booth, C.L., Prosser, C., Lowry, D. and Kruger, M.C. (New Zealand)

**P-MON-04A** **Epimedium-derived flavonoids ameliorated trabecular bone of proximal tibia through a dual mode of action in established ovariectomy-induced osteoporotic rats**
Zhang, G., Qin, L., Peng, S.L., Chao, W.Y., Hung, W.Y., Yeung, H.Y., Chan, C.W., Cheung, W.H., Leung, K.S. and Leung, P.C. (Hong Kong, PR China)

### *NEURAL CONTROL*

**P-MON-05** **DXA and PQCT techniques identify mouse strains with bone deficits due to sodium valproate treatment**
Senn, S., Kantor, S., Andrikopoulos, S., O'Brien, T.J., Morris, M.J. and Wark, J.D. (Australia)

### *MINERAL METABOLISM*

**P-MON-07** **In vitro actions of FGF23 on phosphate uptake and vitamin D metabolism.**
Stewart, I.J., Nelson, A., Robinson, B.G., Mason, R.S. and Clifton-Bligh, R.J. (Australia)

**P-MON-09** **Suspected Inherited Rickets in Corriedale Sheep**
Dittmer, K.E., Thompson, K.G. and Blair, H.T. (New Zealand)

**P-MON-11** **Assessment of proliferative activity of glandular cells in hyper functioning parathyroid gland using flow cytometric analysis**
Gupta, S.K., Agarwal, A., Godbole, M.M., Naik, S., Mishra, S.K., Tewari, M. and Srivastava, M. (India)

### *CYTOKINE, GROWTH FACTOR AND HORMONE MECHANISMS OF ACTION*

**P-MON-14** **Patterns of $[Ca2^{+}]_{I}$ oscillations in calcium-sensing receptor-expressing HEK293 cells exposed to agonists and allosteric activators**
Brennan, S.C., Mun, H-C. and Conigrave, A.D. (Australia)

**P-MON-16** **Direct control of osteoclastogenesis by β2 adrenergic signal**
Kato, M., Takaishi, H., Matsumoto, M., Chiba, K. and Toyama, Y. (Japan)

**P-MON-18** **Intermittent and continuous PTH treatments act on differentiated osteoblasts which induce bone formation and resorption through distinct mechanisms**
Shinoda, Y., Kawaguchi, H., Higashikawa, A., Nakamura, K., Chung, U.I. and Ogata, N. (Japan)

**P-MON-20** **Impact of L-amino acids on human calcitonin-secreting thyroid C-cells**
Mun, H-C. and Conigrave, A.D. (Australia)

**P-MON-22** **Effect of adiponectin on bone cells**
Lin, J-M., Williams, G., Watson, M., Callon, K.E., Reid, I.R. and Cornish, J. (New Zealand)

**P-MON-24** **Chondromoduline-1 directly suppresses proliferation of human cancer cells in vitro.**
Mera, H., Kawashima, H., Yoshizawa, T., Ishibashi, O., Hayami, T., Ali, M.M., Endo, N. and Kawashima, H. (Japan)

**P-MON-26** **α1 Adrenergic agents modify RANKL/OPG expression and cell proliferation in osteoblastic cells**
Huang, H.H., Brennan, T.C., Muir, M.M. and Mason, R.S. (Australia)

## *CELL BIOLOGY*

**P-MON-28** **Decreased mesenchymal stem cells and increased adipogenic differentiation in stem cell pool**
Sheng, H., Qin, L., Zhang, G., Cheng, W.H., Wang, H.F., Lee, K.M. and Leung, K.S. (Hong Kong, PR China)

**P-MON-30** **The histone deacetylase inhibitor, Suberoylanilide hydroxamic acid blocks osteoclastogenesis**
Liapis, V., Vincent, T., Hay, S., Atkins, G., Butler, L., Findlay, D. and Evdokiou, A. (Australia)

**P-MON-32** **TWEAK expression in tissues adjacent to pathogenic bone loss**
Holding, C.A., Atkins, G.J., Dharmapatni, K., Ueno, T., Findlay, D.M., Zheng, T. and Haynes, D.R. (Australia and USA)

**P-MON-34** **OCIL regulates targets of the BMP and notch signaling pathways in osteoblasts**
Kartsogiannis, V., Ly, C., Cipetic, M., Saleh, N., Quinn, J.M., Nakamura, A., Zhou, H., Martin, T.J., Ng, K.W. and Gillespie, M.T. (Australia)

**P-MON-36** **IMATINIB mesylate inhibits osteoclastogenesis *in-vitro* and may promote bone formation *in-vitro* and *in-vivo***
O'Sullivan, S., Naot, D., Callon, K., Porteous, F., Watson, M., Reid, I.R., Cornish, J., Browett, P. and Grey, A. (New Zealand)

**P-MON-38** **Granulocyte colony stimulating factor enhances human osteoclast formation and function**
Granfar, R.M.S., Day, C.J. and Morrison, N.A. (Australia)

**P-MON-40** **Osteoblast-adipose tissue interaction is involved in regulatory mechanisms of bone marrow homeostasis**
Uchihashi, K., Aoki, S., Shigematsu, M., Sugihara, H. and Toda, S. (Japan)

**P-MON-42** **Gene expression profiling of osteosarcomas provides insight into therapeutic response and the development of metastasis**
Endo-Munoz, L., Sommerville, S., Dickinson, I., Guminski, A., Jones, L., McLachlan, G., McMillan, N.A.J. and Saunders, N.A. (Australia)

**P-MON-44** **Microarray study of circulating monocytes in searching for functional genes of osteoporosis**
Lei, S-F., Deng F-Y., Wu1, S. , Li, L-M., Chen, X-D. and Deng, H-W. (PR China and USA)

**P-MON-46** **Role of caveolin in mineralization by murine osteoblastic MC3T3-E1 cells**
Sawada, N., Taketani, Y., Amizuka, N., Isshiki, M., Ichikawa, M., Okawa, C., Nashiki, K., Shuto, E., Sato, T., Yamamoto, H., Arai, H. and Takeda, E. (Japan)

**P-MON-48** **Retrovirus-mediated conditional immortalization and analysis of osteoclast precursor cell lines**
Kawata, S., Suzuki, J. and Shishido, T. (Japan)

**P-MON-50** **Establishment of a rat culture system for studying the morphological diversity of hypertrophic chondrocytes**
Chen, K-S., Tatarczuch, L., Ahmed, Y., Mirams, M., Pagel, C.N. and Mackie, E.J. (Australia)

**P-MON-52** **Physiological death of hypertrophic chondrocytes**
Ahmed, Y., Tatarczuch, L., Chen, K-S., Pagel, C.N., Davies, H.M., Mirams, M. and Mackie, E.J. (Australia)

**P-MON-54** **Extracellular inorganic phosphate alters gene expression in early chondrocytes through phosphorylation of ERK**
Kimata, M., Tachikawa, K., Ozono, K., Kogo, M. and Michigami, T. (Japan)

**P-MON-56** **Expression of cytokine, chemokine and osteoclast differentiation-related genes in monocytes from Chinese females with high or low bone mineral density**
Chen, X-D., Tan, L-J., Lei, S-F., Deng, F-Y., Xiao, S-M., Jiang, C., Sun, X., Yang, F., Guo, Y-F., Wu, S., Li, L-M., Chen, Y., Jiang, H., Tang, Z-H., Wang, X-L., Chen, F-R. and Deng, H-W. (PR China and USA)

## *BIOMATERIALS*

**P-MON-58** **Calcium-silicate based ceramics for bone regeneration**
Ramaswarmy, Y., Wu, C.T. and Zreiqat, H. (Australia)

**P-MON-59** **Analysis of *in vivo* responses to purified PHBV and hydroxyapatite/PHBV composite implanted in a murine tibial defect**
Wu, C.K.A., Grøndahl, L., Trau, M., Mackie, E.J. and Cassady, A.I. (Australia)

## *ORTHOPAEDICS, RHEUMATOLOGY AND TISSUE BIOLOGY*

**P-MON-61** **Zoledronic acid modulates the expression of S100A8 In adjuvant arthritis**
Zreiqat, H., Binder, W., Little, C.B., Geczy, C.L. and Youssef, P. (Australia)

**P-MON-63** **Characterisation of RING finger protein Praja1 in RANKL-induced osteoclastogenesis**
Rampellini, J., Yip, K., Zheng, M-H. and Xu, J. (Australia)

**P-MON-65** **Zoledronic acid enhances femoral head ossification and retards osteonecrotic collapse in a rat model of Perthes disease**
McDonald, M.M. and Little, D.G. (Australia)

**P-MON-67** **Non-steroidal antinflammatory drugs can delay bone formation during healing of experimental stress fractures**
Kidd, L.J., Haji-Mat-Rasil, N.R., Kelly, W.L. and Forwood, M.R. (Australia)

**P-MON-69** **Anti-catabolic treatment augments anabolic therapy in a rat critical femoral defect model.**
Godfrey, C.B., Mikulec, K., McDonald, M. and Little, D.G. (Australia)189

**P-MON-71** **The osteogenic potential of muscle cells**
Liu, R., Schindeler, A. and Little, D.G. (Australia)

## *CLINICAL METABOLIC BONE DISEASES*

**P-MON-73** **Absence of somatic SQSTM1 mutations in pagetic tissue**
Bava, U., Naot, D., Matthews, B.G., Callon, K.E., Pitto, R., McCowan, S., Cundy, T., Cornish, J. and Reid, I.R. (New Zealand)

**P-MON-75** **Intestinal radiocalcium absorption is not affected by treatment with raloxifene in women with postmenopausal osteoporosis**
Wilson, H., Zhou, H.Q., Yao, R., Vasikaran, S., Kent, G.N. and Will, R.K. (Australia)

## *CLINICAL OSTEOPOROSIS*

**P-MON-77** **Comparison of anti-vertebral fracture efficacy among available therapies: a Bayesian analysis**
Nguyen, N.D., Eisman, J.A. and Nguyen, T.V. (Australia)

**P-MON-79** **Trunk muscle response to perturbation is affected by osteoporotic vertebral fracture and thoracic kyphosis**
Greig, A.M., Bennell, K.L., Briggs, A.M. and Hodges, P.W. (Australia)

**P-MON-81** **Study of bone mineral density and effect of various environmental factors on it, in resident doctors working at a teaching hospital in India**
Bandgar, T., Kumar, M.S., Shivane, V., Mathew, J., Prashanth, M., Anjana, K., Harshavardhan, P., Joshi, S.R., Menon, P.S., Shah, N.S. and Seth, G.S. (India)

**P-MON-83** **Urine C-terminal PTHrP levels do not change over the menopause transition**
Ebeling, P.R., Ho, P., Guthrie, J.R., Dennerstein, L. and Martin, T.J. (Australia)

**P-MON-85** **Osteoporosis: a comprehensive risk assessment of an elderly cohort with the consideration of gender**
Howell, L., Bowman, J.A., Attia, J. and McEvoy, M. (Australia)

**P-MON-87** **Chronic anti-epileptic drug treatment is associated with impairment in balance function. A twin and matched sibling pair pilot study.**
Petty, S.J., Paton, L.M., El Haber, N., O'Brien, T.J., Bennell, K., Berkovic, S.F. and Wark, J.D. (Australia)

**P-MON-89** **Alendronate treatment increased bone mineral density at multiple skeletal sites in postmenopausal Chinese women with fast bone loss**
Qin, L., Choy, W.Y., Au, S.K., Hung, W.Y., Leung, K.S., Dambacher, M.A. and Leung, P.C. (Hong Kong, PR China)

**P-MON-91** **Improving the management of patients with osteoporotic fractures: the OMEN Program**
Lee, P., Yap, C., Lih, A., Nickolls C. and Seibel, M.J. (Australia)

**P-MON-93** **Low BMD in steroid-dependent nephrotic children**
Kano, K., Yamada, Y., Nishikura, K., Kojima, E. and Arisaka, O. (Japan)

**P-MON-96** **Differences of bone mineral density between spine and hip in osteoporotic patients**
Kong, G.M., Moon, S.H., Kim, D.J., Seo, B.H. and Yoon, T.H. (Korea)

## *PAEDIATRICS*

**P-MON-98** **Bisphosphonate treatement in chronic recurrent multifocalosteomyelitis: a case series**
Simm, P.J., Allen, R.C. and Zacharin, M.R. (Australia)

## *EPIDEMIOLOGY, PUBLIC HEALTH AND GENETICS*

**P-MON-100** **Age-related macular degeneration and risk of falls and fractures: Geelong Osteoporosis Study**
Hayles, A.L., Henry, M.J., Hunt, N., Kotowicz, M.A., Nicholson, G.C., Guymer, R. and Pasco, J.A. (Australia)

**P-MON-102** **Why is smoking a risk factor for fractures? A new study.**
Bradbeer, M., Day, L., Kantor, S., Segan, C., Osborne, R., Nowson, C., Gill, S., Sambrook, P., Fedorova, T. and Wark, J.D. (Australia)

**P-MON-104** **The Hong Kong Adolescent Bone Health Cohort Study (ABC Study): an analysis of cross-sectional data**
Woo, J., Cheung, C.S.K., Lau, E.M.C. and Leung, P.C. (Hong Kong, PR China)

**P-MON-106** **Prediction of bone loss in elderly men by biochemical markers**
Frost, S.A., Nguyen, N.D., Center, J.R., Eisman, J.A. and Nguyen, T.V. (Australia)

**P-MON-108** **Older women have lower 25-hydroxyvitamin D levels and a higher prevalence of vitamin D deficiency and insufficiency than middle-aged and older men**
Bolland, M.J., Grey, A., Ames, R.W., Horne, A.M., Mason, B.H., Gamble, G.D. and Reid, I.R. (New Zealand)

**P-MON-110** **Is The Message Getting Through? An Analysis Of DXA Use In Australia 1995 To 2005.**
Zammit, T. and Pocock, N. (Australia)

**P-MON-112** **Genetic linkage and genomic imprinting effects on osteoporosis**
Guo, Y.F., Wang, W., Xiao, P., Shen, H., Deng, H., Drees, B., Recker, R.R. and Deng, H-W. (PR China and USA)

**P-MON-113** **Prostate cancer and BMD: Geelong Osteoporosis Study**
Sarah, A.J., Pasco, J.A., Henry, M.J., Nicholson, G.C., Kotowicz, M.A. (Australia)

**P-MON-115** **Knowledge of osteoporosis in Iranian female adolescents**
Mirzaaghaee, F., Moinfar, Z., Eftekhari, S., Khezri, M.K., Sedaghat, M., Mazidi, M. and Aliramezani, M. (Iran)

**P-MON-117** **Vitamin K intake is lower in knee osteoarthritis patients - A general population-based cohort study - The ROAD project**
Oka, H., Yoshimura, N., Muraki, S., Mabuchi, A., En-yo, Y., Yoshida, M., Saiga, A., Kawaguchi, H. and Nakamura, K. (Japan)

### *DENSITOMETRY, IN VIVO ANALYSIS*

**P-MON-121** **Structural geometrical accuracy using a custom built anthropometric phantom of the proximal femur**
Khoo, B.C.C., Beck, T.J., Wearmouth, J.W., Singer, K.P. and Price, R.I. (Australia and USA)

**P-MON-123** **Ultrasonic measurement of human cancellous bone utilizing Biot's theory and its clinical application**
Yamamoto, T., Hagino, H., Katagiri, H., Okano, T. and Teshima, R. (Japan)

**P-MON-125** **Peripheral quantitative cumputed tomography in patients receiving long term glucocorticoid therapy**
Kantor, S., Gong, F.F., Wong, P., McColl, G. and Wark, J.D. (Australia)

**P-MON-128** **Subregional BMD is a better predictor of vertebral morphology than standard DXA-derived BMD measures**
Briggs, A., Kantor, S., Greig, A., Fazzalari, N. and Wark, J. (Australia)

**P-MON-130** **Determination of precision error for low bone mass in DXA by Phantom Study**
Ghasemzadeh, A., Larijani, B., Sarkar, S., Bajoor, A. and Salimzadeh, Z. (Australia and Iran)

**P-MON-132** **Bone mineral density in young men patients with Ankylosing Spondylitis**
Tam, M.T.M. and Thuy, V.T.T. (Vietnam)

**P-MON-134** **Establishing a normal range of bone and tissue in sheep**
Schultz, C.G., Dier, J., Chatterton, B.E., Kuchel, T.R. and Moore, R.J.

### *BONE QUALITY AND MECHANICAL PROPERTIES*

**P-MON-136** **Effect of screw insertion torque level on cortical bone pullout strength**
Cleek, T.M., Reynolds, K.J. and Hearn, T.C. (Australia)

**P-MON-138** **Three-dimensional simulation of trabecular bone remodelling**
Reynolds, K.J., Cleek, T.M., Burrow, L.M., Fazzalari, N.L. (Australia)

**P-MON-140** **Analysis of the microstructural properties and bone strength of the human femoral head**
Baek, M-H., Won, Y-Y., Cui, W-Q. and Yun, T-B. (Korea)

## *ANIMAL MODELS OSTEOPOROSIS*

**P-MON-01**

### Comparison of effects of gamma-linolenic, eicosapentaenoic and docosahexaenoic acids on bone post-ovariectomy in rats

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Several studies have reported a beneficial effect of essential fatty acid (EFA) consumption on bone mass post-ovariectomy. However little is known about the effects of individual EFAs on bone.

**Aim:** To determine the effects of three EFAs on bone in ovariectomised (OVX) rats.

**Methods:** OVX rats were supplemented for 16 weeks with 0.5g/kg bodyweight/day of gamma-linolenic (GLA), eicosapentaenoic (EPA) or docosahexaenoic (DHA) ethyl esters or a mixture of all three (MIX, ratio 2:1:4). Longitudinal femur (F) and lumbar spine (LS) DEXA measurements and week 16 tibial pQCT measurements, serum vitamin D and PTH concentrations were compared to those of non-supplemented sham and OVX (OVXC) controls.

**Results:** At week 16, LS BMC was significantly higher in the DHA than OVXC group (p=0.05). There were no significant differences between DHA and sham groups in either F or LS BMC. Tibial periosteal circumference was greater in DHA than OVXC (p=0.02). Trabecular BMC in the tibial metaphysis was not significantly different between DHA and sham groups but was lower in OVXC than sham (p=0.06). 25-hydroxyvitamin D3 was lower in DHA (p=0.06) and MIX (p=0.07) compared to sham. The MIX supplement had a much weaker effect on BMC compared to DHA alone.

The OVX-induced decrease in F BMD was larger in GLA compared to OVXC. PTH was higher in GLA compared to all other groups (p=0.05).

EPA had no significant effect on bone.

**Conclusions:** DHA protected against ovariectomy-induced decreases in BMC. GLA exacerbated ovariectomy-induced bone mineral loss possibly via a PTH-mediated mechanism.

**P-MON-03**

## Modified goat milk raises peak bone mass and reduces OVX induced bone loss in the female rat

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Goat milk has been shown to promote the uptake and retention of nutrients important for bone health. We investigated, whether long term intake of various goat milk powdered formulations could raise peak bone mass (PBM) thereby reducing bone loss induced by ovariectomy in rats. Seventy five weanling female Sprague-Dawley rats were fed a semi-synthetic base diet with soy as the protein source for 4 weeks and then randomised into 5 groups (n=15). Three groups were fed goat milk diets, while two control groups were kept on the soy protein diet for 10 weeks. The goat milk formulae tested were a Goat Growing-up milk product with added pre- and pro-biotics (Formula 1), and two experimental goat products. Both contained added prebiotic, but differed with respect to added medium chain fatty acids (Formula 2, unsupplemented; Formula 3, supplemented). At week 11, rats fed the test diets and one group of control rats were ovariectomised (OVX) and fed for a further 21 weeks. Bone mineral density (BMD) was measured intermittently every 4-6 weeks.

PBM was higher in rats fed goat milk formulae than in control rats fed soy diet. However, OVX resulted in a similar rate of bone loss in all rats. At the end of the trial, rats fed formula 1 still had a significantly higher BMD in the lumbar spine and significantly higher femur mineral content compared to OVX control rats. Formula 1 also had a significant effect on bone strength. Goat milk specific nutrients may be beneficial to bone health.

**P-MON-04A**

## Epimedium-derived flavonoids ameliorated trabecular bone of proximal tibia through a dual mode of action in established ovariectomy-induced osteoporotic rats

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**Aim:** To systematically investigate the treatment effect of Epimedium-derived Flavonoids (EF) [1] on trabecular bone at material, architecture and remodeling level in established ovariectomy-induced osteoporotic rats.

**Methods:** Forty 12-month-old female Wistar rats were averagely randomized into SHAM-8 group, OVX-8 group, SHAM-24 group, OVX-24 group and OVX-EF-24 group. They were experienced either sham-operation or ovariectomy. Eight weeks after operation, the SHAM-8 rats and OVX-8 rats were all sacrificed as baseline for confirmation of trabeculae loss in proximal tibia. Then, daily oral administration of EF (10mg/kg/day) started in OVX-EF-24 group, whereas daily oral vehicle started in both SHAM-24 group and OVX-24 group. It lasted for sixteen weeks. At sacrifice, the right proximal tibiae were dissected for trabecular BMD by pQCT, trabecular architecture by micro-CT, and trabecular remodeling by histomorphometry. Blood samples were collected for bone biochemical markers such as DPD and OC.

**Results:** Trabecular BMD in OVX-8 group was significantly lower than that in SHAM-8 group. A significantly higher trabecular BMD was found in OVX-EF-24 group than OVX-24 group. The micro-CT parameters except trabecular number showed much more improvement of trabecular architecture in OVX-EF-24 group compared to OVX-24 group (Fig1). The histomorphometry presented significantly higher bone formation variables in OVX-EF-24 group than SHAM-24 group, whereas significantly lower bone resorption variables were found in OVX-EF-24 group than OVX-24 group (Fig2). The fashion of changes in biochemical markers was consistent with histomorphometry.

**Conclusion:** EF ameliorated trabecular bone of proximal tibia through a dual mode of action rebalancing bone turnover in favor of bone formation.

**Acknowledgement:** The experimental study was supported by Hong Kong RGC (CUHK/4097M). Technical assistance from Dr. Sun Y and Mr. Lau CP were gratefully acknowledged.

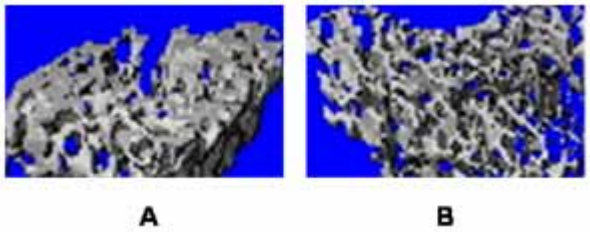


**Figure 1.** Representative MicroCT images of trabelcular architecture of proximal tibia in each group.
A: OVX-EF-24 group; B: OVX-24 group

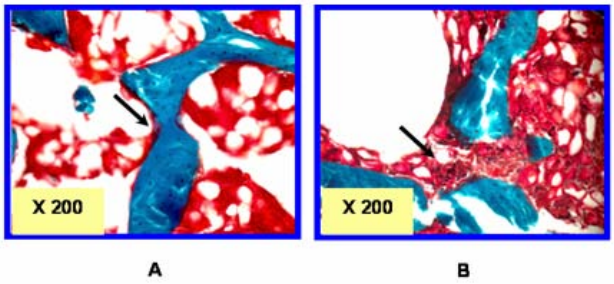


**Figure 2-1.** Representative image from un-decalcified section with Goldner's staining to show the eroded surface in each group.
A: OVX-EF-24 group; B: OVX-24 group.

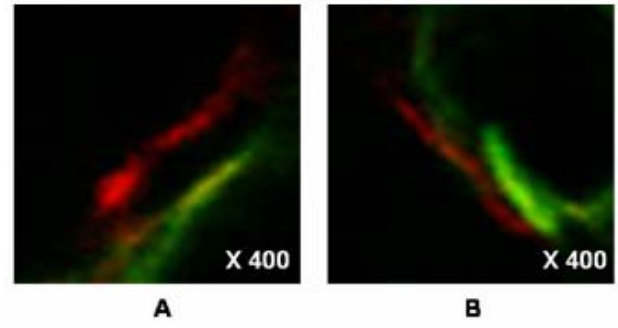


**Figure 2-2.** Representative images from un-decalcified section using fluorescence scope to show the mineral apposition in each group.
A: OVX-EF-24 group; B: SHAM-24 group.

**Reference:**

Zhang G, Qin L, Hung WY, et al. Flavonoids derived from herbal Epimedium Brevicornum Maxim prevent OVX-induced osteoporosis in rats independent of its enhancement in intestinal calcium absorption. Bone. 2006; 38:818-825.

## *NEURAL CONTROL*

**P-MON-05**

### DXA and PQCT techniques identify mouse strains with bone deficits due to sodium valproate treatment

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Background: The adverse effect of chronic anti-epileptic drug use, particularly valproate (VPA), on bone health is well recognised, but the mechanisms poorly understood. Previously we identified mouse strains sensitive (C3H/HeJ) and resistant (A/J) to VPA-induced bone disease by reductions in total bone mineral content (BMC).

**Aim:** Our aim was to replicate these findings using dual energy x-ray absorptiometry (DXA) and to determine cross-sectional measures of area and geometry, and volumetric density using peripheral quantitative computed tomography (pQCT).

**Methods:** Animals (7-8 weeks old) were placed on a 0 and 4 g/kg VPA diet for 16 weeks. In vivo total BMC (n=30 per diet) was assessed using DXA at 0, 8, and 16 weeks and ex vivo pQCT (n=12-16 per diet) was performed at 5, 10, 15, 20 and 50% proximal tibial sites and 10, 12.5, 15 and 20% distal femoral sites at week 16.

**Results:** DXA again identified C3H/HeJ as being sensitive ($p \leq 0.002$) to VPA-induced bone deficits and A/J as being resistant. pQCT revealed reduced total bone content (9%, $p \leq 0.01$), volumetric density (5%, $p \leq 0.01$) and cortical thickness (18%, $p \leq 0.003$) in VPA-treated C3H/HeJ animals.

**Conclusion:** Two independent methods (DXA and pQCT) have successfully identified mouse strains sensitive and resistant to chronic effects of VPA on bone mineral content, density and structure. The VPA-induced loss of cortical thickness could be explained by increased endosteal resorption and/or decreased periosteal apposition. Further studies in these strains have the potential to identify cellular, metabolic and genetic factors involved in AED-induced bone disease.

## *MINERAL METABOLISM*

**P-MON-07**

### In vitro actions of FGF23 on phosphate uptake and vitamin D metabolism

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Fibroblast growth factor 23 (FGF23) has recently been identified as the best candidate for the phosphate wasting hormone in tumour induced osteomalacia. In vivo experiments have previously demonstrated that FGF23 is associated with renal phosphate wasting and low serum 1,25 $(OH)_2D_3$ concentrations. In vitro studies of FGF23 actions have been inconsistent with respect to phosphate uptake in cultured kidney cells. We have studied FGF23 produced by stably transfected HEK293 cells in the form of conditioned media or protein prepared by affinity column purification. We also used a constitutively active FGF23 mutant (R176Q) originally identified as a cause of autosomal dominant hypophosphatemic rickets. Conditioned media from cells expressing wildtype FGF23 inhibited phosphate uptake by OK3B2 cells 5.7% (95%CI 2.55, 6.93, p=0.006) compared to control media, whereas the positive control PTH inhibited uptake 49.5% (p<0.001). Conditioned media from R176Q transfected cells inhibited phosphate uptake by 17.4% (95%CI 7.90, 24.9, p=0.006)). We next studied FGF23 effects on vitamin D metabolizing enzymes cyp24 (24-hydroxylase) and cyp27B1 (1$\alpha$-hydroxylase) by reporter assays in OK3B2 cells. R176Q FGF23 conditioned media stimulated the cyp24 reporter, but had no consistent effect on cyp27B1. R176Q conditioned media also stimulated activity on a

MAP kinase reporter. The wildtype FGF23 conditioned media also induced a similar, but less potent pattern of response. Purified mutant or wildtype FGF23 failed to show significant inhibition of phosphate uptake or induction of cyp24 or MAP kinase reporters. A recently identified putative cofactor for FGF23 actions, klotho, was shown to be expressed in our HEK293 cells. We therefore conclude that conditioned media contains biologic activity in the presence of both FGF23 and klotho, whereas purified FGF23 in the absence of klotho is biologically inactive.

**P-MON-09**

## Suspected Inherited Rickets in Corriedale Sheep

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Inherited forms of rickets have been well described in humans, but are rare in domestic animals. A skeletal disease with lesions typical of rickets and with a likely genetic basis has recently been identified in Corriedale sheep in New Zealand. Affected lambs are normal at birth but develop a range of skeletal deformities within the first few months of life. Many either die or require euthanasia due to increasing reluctance to walk.

Lesions include swollen costochondral junctions, thickened physes in long bones, and collapse of subchondral bone, particularly in the proximal humerus. Microscopically, irregular tongues of hypertrophic physeal cartilage extend into metaphyses. Metaphyseal trabeculae are thickened, disorganised and often separated by fibrous connective tissue. Thick layers of unmineralised osteoid line many metaphyseal trabeculae and secondary osteons in cortices. Inappropriate osteoclastic resorption of trabecular and cortical bone is prominent.

Affected sheep are hypocalcaemic, hypophosphataemic and have normal serum 25-OH vitamin D concentrations, but serum $1,25(OH)_2D_3$ concentrations are significantly greater than in controls, suggesting a defect in end-organ responsiveness. This skeletal disease of sheep may be analogous to Hereditary Vitamin D Resistant Rickets of human beings and could prove to be a valuable animal model for studying this disease. A breeding trial is currently under way at Massey University to confirm the mode of inheritance and generate further affected lambs for investigation of the disease mechanism.

**P-MON-11**

## Assessment of proliferative activity of glandular cells in hyper functioning parathyroid gland using flow cytometric analysis

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**Objective:** The distinction between malignant and benign paprathyroid neoplasms is often difficult based on the currently avalable diagnostic tools. The basis for the diagnostic use of flowcytometric DNA analysis is abnormal cellular DNA content (aneuploidy), which allow easy, objective, quantitative determination of malignancy. We report the flow cytometry DNA findings from parathyroid tumors to evaluate the role of this technique as supplementary to routine histological study.

**Material and methods:** In this study we measured nuclear DNA of parathyroid tumors by flowcytometry in fresh material including eleven histologically begnin adenomas, two atypical adenomas, four hyperplasisas and four carcinomas. Samples of all the patients were freezed in liquid nitrogen and stored at -70° until used. The Propidium iodide stained nuclear suspensions were analysed by using flow cytometer. Ploidy was expressed as DNA index and proliferation index calculated as (SPF+G2M/G0G1+SPF+G2M)X100(%)

**Results:** Unequivocal evidence of aneuploidy was found in all the parathyroid neoplasms. DI of aneuploid tumors ranged from 1.06 to 3.12. Higher extent of aneuploidy was obserevd in malignacy(18.92%) and hyperplasia (14.88%)as compared to adenomas(8.4). Higher S-phase fraction (61.4% and 179.6%) and PI was observed in malignacy and hyperplasia. DI value was higher in malignancy and hyperplasia. Cell cycle distribution analysis data did not provide any significant information beyond ploidy levels.

**Conclusion:** In the present study even the histologically benign adenomas showed evidence of aneuploidy suggesting clonal hetrogenity. Large, proliferative adenomas in our patients require close followup.

## *CYTOKINE, GROWTH FACTOR AND HORMONE MECHANISMS OF ACTION*

**P-MON-14**

### Patterns of $[Ca2+]_i$ oscillations in calcium-sensing receptor-expressing HEK293 cells exposed to agonists and allosteric activators

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Calcium-sensing receptors (CaRs) are class 3 G-protein coupled receptors activated not only by $Ca^{2+}$ and various polycations, including spermine, which act as agonists, but also allosterically, by L-amino acids. Previous comparisons have suggested that extracellular $Ca^{2+}$ and L-amino acids activated distinct signalling pathways, however, the significance of the $Ca^{2+}$ gradient across the plasma membrane was not considered[1]. We have now investigated the patterns of cytoplasmic free $Ca^{2+}$($[Ca^{2+}]_i$) oscillations induced by $Ca^{2+}$, spermine and L-Phe in fura-2 loaded HEK293 cells. All three activators induced slow oscillations in $[Ca^{2+}]_i$ with frequencies of 0.7 – 1.6 $min^{-1}$. In the case of high $Ca^{2+}$ (5 mM) induced oscillations, the resulting frequency was independent of the baseline $Ca^{2+}$ concentration, between 1.5 and 2.5 mM. In the case of L-Phe (10 mM), however, the resulting frequency rose from around 0.7 $min^{-1}$ at 1.5 mM $Ca^{2+}$ to 1.5 $min^{-1}$ at 2.5 mM $Ca^{2+}$. Thus the response to L-phe was highly dependent on the extracellular $Ca^{2+}$ concentration. In addition, in response to spermine (2 mM), the oscillation frequency rose from around 0.8 to 1.2 $min^{-1}$, as the extracellular $Ca^{2+}$ concentration rose from 1.5 to 2.5 mM indicating that the frequency of the response may be dependent on the $Ca^{2+}$ gradient. Consistent with this idea the frequencies of $Ca^{2+}$ oscillations for L-Phe and spermine correlated with increases in the baseline intracellular $Ca^{2+}$ level. Taken together, the data indicate that differences in the signalling pathways between $Ca^{2+}$ and other activators might arise from differences in the baseline level of $[Ca^{2+}]_i$.

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**P-MON-16**

### Direct control of osteoclastogenesis by β2 adrenergic signal

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We examined the role β2 adrenergic signal on osteoclastogensis using RAW 264.7 cells as a model of osteoclastogenesis. A β antagonist, ICI 118551 at $10^{-6}$ M decreased the number of osteoclasts induced by RANKL by 70%. The β antagonist inhibited the osteoclastogenesis in a dose-dependent manner and Crenbuterol, a β agonist, increased the number of osteoclasts. RT-PCR revealed the expression of β2, but not β1 and β3 adrenergic receptor in RAW cells. In addition, RT-PCR analyses indicated that ICI 118551 had no effects on the expression level of β2 receptor during osteoclastogenesis. The addition of ICI 118551 at the late stage of culture period exhibited full inhibitory effects on osteoclastogenesis, suggesting that β2 signal was necessary for the late stage of osteoclast differentiation. TRAP staining of osteoclasts formed on a plastic dish showed that the β antagonist disorganized the gross structure of fused osteoclasts, but it had no effects on the formation of TRAP-positive mononuclear cells. It suggested that β adrenergic signal affects fusion machinery in osteoclastogenesis. RT-PCR analyses indicated that β antagonist decreased the mRNA of DC-STAMP and AQP9. In addition, we found β adrenergic signal regulates both the transcription of c-src and phosphorylation of Y-139 of c-src. We assessed the role of c-src in β2 signal, because c-src reorganized the cytoskeleton of osteoclast in the later stage of differentiation. Our findings will provide a novel mechanism of bone metabolism regulation by the sympathetic nerve system.

**P-MON-18**

## Intermittent and continuous PTH treatments act on differentiated osteoblasts which induce bone formation and resorption through distinct mechanisms

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**Aim:** The present study examined the cellular and molecular mechanism underlying the PTH action.

**Methods and Results:** We initially found that the type I PTH receptor expression by RT-PCR and the accumulation of cAMP in response to PTH (1-34, 10 nM) treatment were increased as the mouse primary calvarial osteoblasts (OB) differentiated, indicating that the direct target of PTH is differentiated OB. We then compared the effects of continuous (48 h X 3) and intermittent (6/48 h X 3) PTH treatments in four cultures: OB only, bone marrow cells (BM) only, the monolayer co-culture, and the separated co-culture in a double chamber dish divided by a porous membrane. Osteoclast formation determined by TRAP staining was most strongly induced by continuous treatment in the monolayer co-culture but not in the separated co-culture. RANKL expression by the PTH continuous treatment in OB culture was increased as the osteoblasts differentiated. Bone formation determined by ALP staining and mRNA expressions of osteogenic markers was potently stimulated by the intermittent treatment in both co-cultures with the increase of IGF-I concentration in the medium. In the separated co-culture, the anabolic effect was only seen in the BM layer. The anabolic action was abolished when the BM was derived from IRS-1 (an essential adapter molecule for the IGF-I signaling) KO mice.

**Conclusion:** The direct targets of PTH are differentiated osteoblasts. Continuous treatment causes osteoclastogenesis by RANKL expression via the cell-to-cell contact, and intermittent treatment causes differentiation of osteoblast precursors through IRS-1 signaling.

**P-MON-20**

## Impact of L-amino acids on human calcitonin-secreting thyroid C-cells

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We have previously demonstrated that L-amino acids allosterically activate extracellular $Ca^{2+}$-sensing receptors in CaR-expressing HEK293 cells and normal human parathyroid cells. In parathyroid cells, L-amino acids activate intracellular $Ca^{2+}$ mobilization and suppress PTH secretion. In the current study, we have investigated whether CaR-active L-amino acids including L-Phe and L-Trp stimulate intracellular $Ca^{2+}$ mobilization in human calcitonin-secreting TT cells. TT cells were cultured in F12-K nutrient medium with 10% FBS in the absence or presence of 10 nM 1,25 dihydroxyvitamin D or 100 ng $mL^{-1}$ interleukin 1 beta for 24-48 h, which have been previously shown to promote CaR expression (1). Cultured TT cells were cultured on coverslips, loaded with the $Ca^{2+}$-sensitive dye fura-2 and then analyzed for sensitivity to elevated $Ca^{2+}$ concentration or L-amino acids. Under control conditions, increasing the $Ca^{2+}$ concentration from 0.5 to 2.5 mM had little or no effect on intracellular $Ca^{2+}$ mobilization and only around 10% of cells responded to 10 mM L-Phe. After 24-48 h exposure to 1,25 dihydroxyvitamin D there was little or no change in the response to either $Ca^{2+}$ or L-Phe. However, after 24-48 h exposure to IL-1beta there was a marked increased in sensitivity to L-Phe such that greater than 90% of cells responded. The data are consistent with the concepts that IL1beta,, which is a recognized hypocalcemic factor in vivo, powerfully upregulates CaR expression in calcitonin-secreting C-cells and that extracellular $Ca^{2+}$-induced $Ca^{2+}$ mobilization and calcitonin secretion from human C-cells is markedly stimulated by CaR-active L-amino acids.

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**P-MON-22**

## Effect of adiponectin on bone cells

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Adiponectin is an adipocytokine, which has been found to be negatively correlated with body mass index and fat mass and yet, paradoxically adiponectin has been reported to positively relate to bone mineral density. Adiponectin's effect on bone metabolism is not well understood. In this study we have investigated its effects on osteoclast development and activity as well as on osteoblast proliferation in primary cell cultures.

Adiponectin was assayed in an osteoclastogenesis system, using mouse bone marrow cultures (for 7 days in the presence of $1,25(OH)_2D_3$) and was tested for its effects on osteoclast activity using isolated rat mature osteoclast assays, in which the resorption pits were scored after treatment for 24 hours. [$^3$H]-thymidine incorporation into neonatal rat primary osteoblasts was assessed after treating with adiponectin for 24 hours and pulsing for 6 hours.

The results showed that adiponectin significantly stimulated osteoclastogenesis by 21% at 0.1 μg/mL, but markedly inhibited this process by 26% and 54% at 1 and 5 μg/mL, respectively. It had no effect on bone resorption in this concentration range in the isolated mature osteoclast assays. Adiponectin was mitogenic to osteoblasts with 15% increase rate in DNA formation at 1 μg/mL and 24% increase at 5 μg/mL.

In conclusion, these results indicate that adiponectin is anabolic to bone and joins the large number of other nutritional hormones that are involved in the regulation of bone mass.

**P-MON-24**

## Chondromoduline-1 directly suppresses proliferation of human cancer cells in vitro

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**Introduction:** Chondromodulin-1(ChM-1), an endogenous anti-angiogenic factor expressed in cartilage, has been suggested to inhibit invasion of endothelial cells into cartilage. Ectopic administration of ChM-1 has been reported previously to suppress tumorigenesis in vivo, indicating that it could serve as a promising anti-tumor agent generally. However, it is not clear whether the anti-tumor effect is due to its anti-vascularization effect or direct action against oncocytes. In the present study, therefore, we aimed to elucidate the mechanism and signaling pathway of anti-tumor effect of ChM-1 in vitro.

**Methods & Results:** HepG2 and Hela cells were cultured on plate or in soft agar. When cells were cultured on plate, ChM-1 inhibited proliferation of HepG2 cells but not of Hela. In contrast, when cultured in soft agar, ChM-1 inhibited colony formation of both HepG2 and Hela cells. Western blot analysis on cell cycle-related proteins demonstrated that ChM-1 slightly increased the level of p21 and decreased that of cyclin D1 without affecting the phosphorylation levels of Erk, AKT, and GSK3β. Luciferase reporter assays showed that ChM-1 inhibits activation of STATs, and their binding to GAS element but not to ISRE element.

**Discussion:** ChM-1 directly suppresses the proliferation of tumor cells in an anchorage-independent manner. Consistent with this, ChM-1 did not alter phosphorylation of Erk/AKT/GSK3β, the downstream molecules of extracellular matrix-integrin signaling pathway. Since one of the two signaling pathways through receptors for growth factors/cytokines converges to the anchorage-dependent pathway at phosphorylation of Erk/AKT, ChM-1 may exert the anti-tumor effect by the other pathway modulating STATs.

**P-MON-26**

## α1 Adrenergic agents modify RANKL/OPG expression and cell proliferation in osteoblastic cells

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Previous studies have shown that central control of bone mass is mediated via β2 receptors of the sympathetic nervous system [1]. Although the expression of α adrenergic receptors in human bone cells is controversial[1,2], Takeuchi *et al* [3] showed that epinephrine increased both RANKL and OPG mRNA expression in murine osteoblast-like cells via β and α adrenergic receptors. Human osteoblastic MG63 cells and primary human fetal bone cells (FBC) were cultured in DMEM with 10% FBS then adapted to serum-free medium for 24 h before treatments were added. Adrenergic receptors (α1b) were expressed in MG63 cells and FBC by RT-PCR and sequence analysis. As reported with murine cells, OPG mRNA expression was up-regulated 3-4-fold ($p<0.001$) after 24 h treatment with $10^{-3}$ M cirazoline (a specific α1 agonist) though lower concentrations had no effect. RANKL mRNA expression was increased dose-dependently up to 10-fold ($p<0.001$) after 24 h treatment with $10^{-6}$ M -$10^{-3}$ M cirazoline. Phenylephrine (α1 agonist) had a smaller effect on OPG (1.3 fold, $p<0.05$) and no effect on RANKL. Urapidil (α1 antagonist) also dose-dependently increased OPG expression 1.5 fold at $10^{-4}$ M ($p<0.001$) but decreased RANKL expression ($p<0.01$ at $10^{-4}$ M). Cell proliferation was dose-dependently increased after 24 h treatment with phenylephrine ($10^{-8}$ M-$10^{-5}$ M). The maximum effect at $10^{-6}$ M ($p<0.001$) was 1.6 fold greater than control and was partially suppressed by 2 x $10^{-4}$ M Urapidil. These data suggest that not only β2 but also α1 adrenergic receptors may play a role in modulation of bone turnover by the sympathetic nervous system in human bone cells.

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## *CELL BIOLOGY*

**P-MON-28**

## Decreased mesenchymal stem cells and increased adipogenic differentiation in stem cell pool – an extravascular event and marker in steroid-associated osteonecrosis

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**Objectives:** Recently, we systemically investigated both intra- and extravascular events in steroid-associated osteonecrosis (ON) in rabbits. The present study was designed to confirm our hypothesis that the histological extravascular events associated with both increased marrow fat size and disturbance of local blood perfusion was a result of steroid-associated adipogenic differentiation of bone marrow mesenchymal stem cells (MSCs).

**Methods:** Using our recent established steroid-associated ON rabbit model, bone marrow from proximal femurs were harvested for evaluation of quantity of MSCs. The osteogenic and adipogenic differentiation potential of MSCs were analyzed in terms of alkaline phosphatase activity and lipid droplets formation. In addition, the expression of core binding factor {alpha}1(Cbfa1) and peroxisome proliferator activated receptor {gamma} 2 (PPARγ2) were also evaluated.

**Results:** The results showed that there was a significant decrease in number of MSCs and an enhancement in differentiation into adipocytes in ON group. In addition, the MSCs of ON group also showed reduction in their differentiation potential into osteoblast lineage.

**Conclusions:** These findings support our hypothesis that one of the relevant extravascular events in pathogenesis of steroid-associated ON was alteration of MSCs differentiation. This finding shared the light on

potential effective prevention of steroid-associated ON using potent biological factors or chemical agents for inhibition of adipogenesis and promotion of osteogenesis of MSCs.

**P-MON-30**

## The histone deacetylase inhibitor, Suberoylanilide hydroxamic acid blocks osteoclastogenesis

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Histone deacetylase (HDAC) inhibitors are a series of agents that inhibit the growth of a range of transformed cells *in-vitro* and *in-vivo*, and are currently in Phase II/III clinical trials for the treatment of haematological and solid tumour malignancies.

In this study, we investigated the effect of the HDAC inhibitor, Suberoylanilide hydroxamic acid (SAHA) on osteoclast differentiation and bone resorption using three independent *in-vitro* model systems of osteoclastogenesis. When human peripheral blood mononuclear cells (PBMCs) and the RAW264.7 murine monocytic cells were cultured with the receptor activator of nuclear factor kappa B-ligand (RANKL), both formation of tartrate-resistant acid phosphatase (TRAP) positive multinucleated cells and bone resorption were increased compared with control cells that were cultured in the absence of RANKL. When added in combination with RANKL, SAHA dose- dependently inhibited RANKL-induced osteoclastic differentiation and bone resorption in both model systems. Similarly, the resorptive activity of mature osteoclasts that were isolated from human Giant Cell Tumours (GCT) of bone was also inhibited by SAHA treatment when cultured onto bone slices. The effects of SAHA on RANKL induced activation of NF-kB, which play a key role in osteoclastogenesis was investigated and shown to be modulated by SAHA treatment.

The ability of SAHA to inhibit osteoclast differentiation and activity provides a novel therapeutic approach for the treatment of cancer-induced bone disease.

**P-MON-32**

## TWEAK expression in tissues adjacent to pathogenic bone loss

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Tumour necrosis factor-like weak inducer of apoptosis (TWEAK) is a member of the tumour necrosis factor (TNF) superfamily. TWEAK possesses multiple biological activities and has been reported to induce osteoclasts from a macrophage cell line. Our aim was to determine TWEAK expression in tissues from rheumatoid arthritis (RA) and chronic periodontitis patients (PD) and to determine if TWEAK can induce osteoclastogenesis in peripheral blood mononuclear cells (PBMCs).

Inflammatory tissues were obtained from patients with active RA and PD. Osteoarthritic and normal gingival tissues were used as controls. Immunohistochemical analysis was performed using monoclonal antibodies against TWEAK and Fn14 (TWEAK receptor). Dual immunostaining determined the cell type expressing TWEAK. *In vitro* recombinant human TWEAK was added to PBMCs and cells were evaluated for TRAP expression and bone resorption.

TWEAK was highly expressed by leukocytes in RA synovial tissues, and in PD tissues adjacent to sites of periodontal bone loss. In comparison, only very weak TWEAK staining was seen in control tissues. TWEAK was strongly expressed by lymphocytes that appeared to be plasma cells expressing CD22 (B cell marker). Some CD68 expressing cells (macrophages) also weakly expressed TWEAK. *In vitro* studies showed that TWEAK can promote an osteoclastic phenotype, however, these TRAP positive multinucleated cells were unable to resorb mineralised tissue.

This study demonstrated that TWEAK is strongly expressed in tissues obtained from sites of pathological bone loss. The presence of TWEAK and the ability of TWEAK to stimulate an osteoclastic phenotype indicate that TWEAK may contribute to pathogenic bone loss.

**P-MON-34**

## OCIL regulates targets of the BMP and notch signaling pathways in osteoblasts

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Osteoclast Inhibitory Lectin (OCIL) is a type II membrane-bound C-type lectin known to inhibit osteoclast differentiation. We now identify its functions upon osteoblast and adipocyte differentiation. Treatment of cultured KUSA-O cells with recombinant soluble OCIL under osteoblastic conditions resulted in profound inhibition of mineralization with a corresponding decrease in mRNA expression of osteocalcin, BSP and osterix. OCIL also inhibited KUSA-O cell differentiation towards adipocytes, with a corresponding reduction in adiponectin and resistin mRNA expression. To elucidate further the role of OCIL in osteoblast function we investigated its effects to regulate potential differentiation targets such as noggin, HES-1 (a basic helix-loop-helix transcription factor), the HES-related repressor proteins (Herp-1, -2, -3), Delta-1 and Jagged-2 (both ligands for Notch) and the inhibitors of DNA binding-1 (Id-1,-2,-3). REAL TIME RT-PCR analysis demonstrated that OCIL increased noggin mRNA levels at 4, 5, 6 and 7 days of treatment (8-fold at day 4). In contrast, OCIL reduced HES-1 mRNA levels (by 70-80%) at 8hrs which was maintained through to day 4. Furthermore, OCIL suppressed HERP-2 mRNA levels (by approximately 50% throughout days 1-7). OCIL also suppressed Delta-1, Jagged-2, Id-2 and Id-3 mRNA levels at day 2 (by ~60-70%), but enhanced mRNA levels approximately 2-fold throughout days 4 and 5. These data provide further evidence to implicate OCIL in osteoblast and adipocyte differentiation and to modulate the expression of genes involved in BMP and Notch signaling, two very important signaling pathways that modulate bone formation.

**P-MON-36**

## IMATINIB mesylate inhibits osteoclastogenesis *in-vitro* and may promote bone formation *in-vitro* and *in-vivo*

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Imatinib mesylate (Glivec™) is an orally active tyrosine kinase inhibitor with activity against *c-abl*, *c-kit* and the PDGFR, currently used to treat chronic myeloid leukaemia (CML) and gastrointestinal stromal cell tumours. The molecular targets of imatinib are expressed in bone cells, and interruption of both *c-kit* and *c-abl* signalling induces osteopenia in mice, suggesting that imatinib might influence skeletal tissue. We have examined the effects of imatinib on bone cells in vitro, and bone turnover and BMD in a group of CML patients starting imatinib therapy. Imatinib dose-dependently (0.1-5uM) decreased the proliferation of, and modestly increased apoptosis of, pre-osteoblastic cells, while increasing osteoblast differentiation. In murine bone marrow cultures, osteoclastogenesis was dose-dependently inhibited by imatinib, an effect which was partly dependent on inhibition of the supporting stromal cell population and partly direct. Consistent with these findings, imatinib partially inhibited osteoclastogenesis in RAW-264.7 cells. In a group of 9 CML patients, serum osteocalcin increased at 3 and 6 months after commencing imatinib therapy, while ALP gene expression increased in bone marrow samples obtained from the same patients. Serum βCTX did not change, neither did BMD at hip or spine, after 6 months after imatinib therapy. These data demonstrate that, in vitro, imatinib promotes osteoblast differentiation at the expense of proliferation, and potently inhibits osteoclastogenesis. Limited prospective human data suggest that imatinib may increase bone formation. These data suggest a role for imatinib's molecular targets in bone cell function, and justify further examination of the skeletal actions of imatinib in vivo.

**P-MON-38**

## Granulocyte colony stimulating factor enhances human osteoclast formation and function

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Granulocyte Colony Stimulating Factor receptor was previously found to be 12-fold up-regulated on a 19,000 gene array comparing human Peripheral Blood Mononuclear Cell (PBMC) derived osteoclasts and macrophages [1]. G-CSF is a commonly used treatment for severe congenital neutropenia and pre-marrow transplant therapy with a potential adverse effect of mild to severe bone loss [2] – thus the effects of G-CSF on the *in vitro* PBMC model of osteoclastogenesis was investigated.

PBMCs were exposed to recombinant M-CSF and RANKL as well as optimised physiological doses of recombinant G-CSF for durations of 3, 7, 14 and 21 days respectively. Markers of osteoclast maturity such as dentine-resorption, multi-nucleation, as well as gene expression and protein presence of tartrate resistant acid phosphatase, cathepsin K and cytokine receptors, were analysed. A significant increase in the physical markers of cell-size, multinucleation and dentine-slice resorption was noted in both continuous and late-stage G-CSF treatment types. A refined gene-expression profile of selected osteoclast markers and chemokines at earlier time points (3, 5, 7 and 10 days) is being reviewed. The data suggest that G-CSF is one of the panoply of cytokines controlling on osteoclast formation.

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**P-MON-40**

## Osteoblast-adipose tissue interaction is involved in regulatory mechanisms of bone marrow homeostasis

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In bone marrow that consists of bone, adipose and hematosis tissues, the bone loss occurring with aging is related to a reduced osteoblastic bone formation and an increased adipose tissue mass. For the age-related osteoporosis, an interaction between the adipose tissue and osteoblasts lining surface of the bone seems critical; but this issue remains elucidated. As mesenchymal stem cells (MSCs) are suggested to exist in adipose tissues, bone marrow adipose tissue-derived MSCs may be involved in mechanisms of the marrow homeostasis. Here we showed an interaction between osteoblasts (MC3T3-E1) and human bone marrow adipose tissue fragments in their 3-dimensional collagen gel co-culture. As a reference, MC3T3-E1 cells or adipose tissue fragments alone were cultured in the gel. Cellular behaviors were analyzed by histochemistry, morphometry, immunohistochemistry. Cell growth was assessed by bromodeoxyuridine (BrdU)-uptake for 24 h. MC3T3-E1 osteoblasts decreased the number of adipose tissue-derived spindle cells that expressed markers of MSCs (CD105, c-kit and CD44). MC3T3-E1 cells decreased BrdU-uptake of the CD45-positive hematopoietic cells within adipose tissue, while the fragments decreased the BrdU-uptake of MC3T3-E1 cells. The data suggest firstly, that osteoblasts inhibit both generation of MSCs from bone marrow adipose tissue and growth of leukocyte-linked cells; and secondly, that the adipose tissue prohibits osteoblast growth. We conclude that osteoblast-bone marrow adipose tissue interaction may be involved in the regulatory mechanisms of bone marrow homeostasis.

**P-MON-42**

## Gene expression profiling of osteosarcomas provides insight into therapeutic response and the development of metastasis

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**Aims:** The aim of this study was to identify prognostic factors at the time of diagnosis of osteosarcoma that may predict patient outcome.

**Methods:** Gene expression profiling of 24 osteosarcoma diagnostic biopsy samples was performed using Agilent Whole Human Genome oligonucleotide microarrays. In addition, the gene profiles of matched patient samples (biopsy *vs.* tumour resection) were compared.

**Results: 1)** Approximately 50 genes were found to be differentially expressed ($p<0.05$) between good and poor responders. However, although these genes differentiated effectively between patients with extreme good (>95% necrosis) and poor responses (<25% necrosis), they failed to differentiate intermediate responders (30-90% necrosis). **2)** We found no evidence of a suite of chemotherapy-regulated genes in any patient samples. **3)** We identified profound changes in the expression profiles between the initial biopsy and the post-treatment resection sample in a patient who developed metastatic disease.

**Conclusion:** Our data indicate that chemotherapeutic sensitivity of osteosarcomas cannot be predicted by microarray profiling of patient biopsies at the time of presentation. In addition, chemotherapeutic sensitivity was not associated with changes in genes traditionally linked to chemoresistance (e.g. ABC transporters or drug metabolising enzymes). Significantly, our studies showed that the development of metastasis was likely to originate from the expansion of a population of tumour cells that comprise only a small fraction of the original tumour mass.

**P-MON-44**

## Microarray study of circulating monocytes in searching for functional genes of osteoporosis

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Peak bone mass (PBM) is an important determinant of osteoporosis. An imbalance between bone formation and bone resorption, favoring bone resorption by osteoclasts, is a major mechanism underlying osteoporosis. By either serving as the early precursors of osteoclsts or producing cytokines important to osteoclast differentiation, activation, and apoptosis, circulating monocytes actively participate in osteoclastogenesis. Gene expression study of blood monocytes from extremely high vs. low PBM individuals may identify genes functionally relevant to osteoclastogenesis and pathogenesis of osteoporosis.

We recruited 878 healthy pre-menopausal females of Han ethnicity, whose ages are from, 20-45 y i.e., the time when PBM is attained and maintained. PBM was measured by a Hologic QDR 4500 W dual-energy X-ray absorptiometry (DXA) scanner (Hologic corporation, Waltham, MA). For each study subject, the information on age, sex, medical history, family history, female history, physical activity, alcohol use, dietary habits and smoking history was obtained at the time of PBM measurement. From 10% upper and lower extremity of the PBM distribution in the population (n=878) by total hip Z score, we recruited 12 samples (Mean Z score ±SD = -1.72±0.60) with extremely low PBM and 13 samples (Mean Z score ±SD = 1.54±0.55) with extremely high PBM.

Affymetrix HG-U133 plus2.0 GeneChips were used to disclose the expression profiles of the circulating monocytes. Monocyte isolation was performed with a Monocyte Negative Isolation Kit (Dynal Biotech Inc.,

Lake Success, NY). Preparation of cRNA, hybridization, and scanning of the HG-U133 plus2.0 GeneChip was performed according to the manufacturer's protocol (Affymetrix, Santa Clara, CA). With the normalized expression signals, we performed t-test for equality of means of the expression signal in low vs. high BMD group, and detected 23 significant differentially expressed genes ($p<0.001$). There are 20 genes up-regulated in high BMD group and 3 genes up-regulated in low BMD group. These genes mainly are involved in cell proliferation, transcription regulation, protein folding etc. Four genes (STAT1, SERPING, GBP1, and CCR5) were selected to confirm their differential expressed genes by real-time reverse transcription PCR. These experiments are ongoing.

By collaborating with genetics studies at the DNA and protein levels, microarray will become a powerful and efficient approach to dissection of osteoporosis genes and their functions. This work represents the first in vivo microarray study of osteoporosis in Chinese. It offers clinically important perspectives and demonstrates the potential power of functional genomic approaches in genetic dissection of this complex disease

**P-MON-46**

## Role of caveolin in mineralization by murine osteoblastic MC3T3-E1 cells

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Caveolin is a major structure protein in caveolae, which are flask-shaped membrane invaginations and specialized lipid-microdomains of plasma membrane, and play important roles in signal transduction and molecular transport. There are three isoforms of caveolin (caveolin-1, 2, 3), and two of them (caveolin-1, –2) are abundantly expressed in osteoblasts. However, the role of caveolins in osteoblasts is unclear. We investigated the role of caveolin in matrix mineralization and the relationship between cavoelin and matrix vesicles which play an important role in matrix mineralization. Murine osteoblastic cell line, MC3T3-E1 cells were treated with 50μg/ml ascorbic acid and 2.5mM sodium phosphate for seven days to induce calcification, and the fractions of lipid-microdomain and matrix vesicle were isolated by detergent-free method and collagenase method, respectively. Caveolin-1 and –2 were localized in not only the lipid-microdomain fractions but also the isolated matrix vesicles. Immuno-electron microscopy analysis of murine tibia revealed that caveolin-1 was localized in both plasma membrane of osteoblast and matrix vesicles in bone matrix. We hypothesized that caveolin-1 had important roles in bone calcification because it was contained in matrix vesicles in which primary calcification is initiated. The role of caveolin-1 in matrix mineralization was examined by manipulating the expression of caveolin-1 in MC3T3-E1 cells. Over-expression of human caveolin-1 enhanced matrix mineralization. On the other hand, down-regulation of caveolin-1 expression by RNAi resulted to repression of matrix mineralization. These results indicate that caveolin would be an important factor of bone mineralization, at least in part, via matrix vesicle functions.

**P-MON-48**

## Retrovirus-mediated conditional immortalization and analysis of osteoclast precursor cell lines

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SV40 T antigen (T) has been widely adopted to establish cell lines because its expression immortalizes rodent primary cells. The forced expression of a temperature-sensitive mutant of T (tsT) has been shown to conditionally immortalize primary cells. In this study, we conditionally immortalized osteoclast precursor cells (OPCs) using retrovirus-mediated gene transfer of a derivative of tsLT, which expresses LT but not ST. The immortalized OPCs were designated MDBMT (M-CSF-dependent bone marrow cells immortalized by tsLT). MDBMTs continued to grow at 33.5°C, but stopped growing and even decreased in number at 39°C. We examined MDBMTs surface markers by flow cytometry, using three monoclonal antibodies. MDBMTs were positive for Mac-1 and F4/80, markers for macrophages, and were negative for CD25, markers for activated

macrophages. To determine whether MDBMTs can differentiate into osteoclasts, the cells were stimulated by RANKL. Differentiation into osteoclasts was monitored by TRAP (tartrate-resistant acid phosphatase) activity. MDBMTs differentiated into TRAP-positive multinucleated osteoclasts when cultured at 33.5°C, which was inefficient compared with RAW264 cells (An OPCs cell line). To further analyze MDBMTs, we cloned a couple of cell lines MBCT3 and MBCT5, from MDBMTs. While MBCT5 differentiated into TRAP-positive multinucleated osteoclasts, MBCT3 did become TRAP-positive but nonetheless remained as single cells. We measured migration activity of RANKL-treated MBCT3 and MBCT5 in response to M-CSF. However, they were not different. These results suggest that MDBMTs consists of cells with various differentiation capabilities. The cell lines are useful in analyzing a mechanism of differentiation, particularly multinucleated osteoclasts formation.

**P-MON-50**

## Establishment of a rat culture system for studying the morphological diversity of hypertrophic chondrocytes

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Hypertrophic "dark" and "light" chondrocytes have been reported in growth cartilage during endochondral ossification. We have recently observed that these cells undergo two different forms of non-apoptotic physiological cell death (PCD) in horse growth cartilage, and have established a culture system for studying hypertrophy and death of equine chondrocytes. The aim of the current study was to develop a culture system using rodent chondrocytes in order to study differences between dark and light cells and their modes of death. Chondrocytes were isolated from femoral epiphyseal cartilage from neonatal rats, cultured as pellets in different serum concentrations and treated with T3 or staurosporine. After 10 days, the pellets were examined by light and electron microscopy. Approximately equal numbers of dark and light cells were observed in pellets cultured in 10%FCS, but chondrocytes cultured in the presence of T3 were predominantly hypertrophic light cells. Staurosporine induces apoptosis in many cell types, but when staurosporine was added to pellets cultured in 10%FCS, apoptotic chondrocytes were rarely observed. Instead, there was an increase in the proportion of dark cells dying by the process observed *in vivo*. Staurosporine significantly down-regulated mRNA expression of type X collagen, type II collagen, runx2 and aggrecan. Fewer dark cells were seen in chondrocyte cultures from rats than in those from horses, which is probably due to the fact that very few dark cells are observed in neonatal growth cartilage from rats. This will be a useful system for studying molecular mechanisms of chondrocyte hypertrophy and death.

**P-MON-52**

## Physiological death of hypertrophic chondrocytes

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Death of hypertrophic chondrocytes is an important step in the endochondral ossification pathway, but how these cells die remains unclear. Although most studies have reported apoptosis as the fate of hypertrophic chondrocytes, recent publications have reported that chondrocytes die by non-apoptotic physiological cell death (PCD). The aims of the current study were to document the morphology of dying chondrocytes in equine growth cartilage and to study the regulation of their PCD *in vitro*. Growth cartilage from prenatal and postnatal horses was examined by electron microscopy. Hypertrophic chondrocytes exist in two forms, dark and light. Dying dark chondrocytes showed nuclear condensation and progressive extrusion of their cytoplasm into the extracellular matrix. Dying light chondrocytes showed digestion of the cytoplasm within the cell membrane. Chondrocytes isolated from equine foetal cartilage were cultured in pellets in 10% or 0.1% FCS, and treated with and without triiodothryonine (T3) or transforming growth factor-β1 (TGFβ-1). Staurosporine, which induced apoptosis in chondrocytes cultured in monolayer, was added to pellet cultures at day 20 for 24 hours. After 21 and 28 days, the pellets were examined histologically. Pellets cultured in 0.1% FCS showed a lower percentage of all forms of cell death than pellets grown in 10% FCS. Addition of T3 increased the percentage of dying light and dark cells, however, TGF-β increased the proportion of dying dark

cells. Staurosporine did not induce apoptosis in pellets but induced typical dark cell death. These observations demonstrate that light and dark hypertrophic chondrocytes die by distinctive-non apoptotic modes of PCD.

**P-MON-54**

*Special Note: This poster was selected as a President's Poster, a clerical error placed it amongst the General Posters in the program. It will be displayed and attended by the author as a President's Poster on Monday (No. PP-MON-34). A copy of the poster will also be displayed with the General Posters.*

## Extracellular inorganic phosphate alters gene expression in early chondrocytes through phosphorylation of ERK

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The concentration of extracellular inorganic phosphate (Pi) increases during chondrocyte differentiation. We hypothesized that the changes in the concentration of extracellular Pi might be sensed by chondrocytes from the early stage and transduce signals, leading to the altered gene expression. To identify the genes regulated by extracellular Pi in early chondrocytes, we first performed microarray analyses using ATDC5 cells, a cell model for chondrocyte differentiation. Utilizing RNA from ATDC5 cells of proliferating stage that were incubated in the presence of 1 mM or 10 mM Pi for 24 hours, we have found that increased extracellular Pi altered the expressions of multiple genes including alkaline phosphatase gene. Then we examined the effects of extracellular Pi on the phosphorylation state of intracellular proteins. Extracellular Pi was increased from 1 mM to 10 mM, and the cytoplasmic proteins were harvested 0–24 hours later and subjected to immunoblots to analyze the phosphorylation state at tyrosine (Tyr) or threonine (Thr) residues. High Pi (10 mM) led to an increased phosphorylation at Tyr in several proteins within 30 minutes, and 2-dimensional electrophoresis revealed that the major signals corresponded to ERK. Treatment with phosphonoformic acid (PFA), an inihibitor of type III sodium/phosphate co-transporter, cancelled the Pi-induced ERK phosphorylation. In addition, the Pi-induced alterations in gene expression were also abolished by PFA treatment. These results suggest that ATDC5 cells of proliferating stage sense the environmental Pi concentration via type III sodium/phosphate co-transporter, and that ERK is involved in the downstream signal transduction.

**P-MON-56**

## Expression of cytokine, chemokine and osteoclast differentiation-related genes in monocytes from Chinese females with high or low bone mineral density

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**Background:** Immune system and bone can influence each other, and monocytes also play important roles in immune function and formation of osteoclasts. Some cytokine and chemokine genes were found to be linked or associated with bone mineral density (BMD). However, very few studies were simultaneously performed on the relationship between gene expression and BMD.

**Aims:** The present study was to explore the relationship between the expression of cytokine, chemokine and osteoclast differentiation-related genes in monocytes and BMD variation.

**Methods:** 878 healthy Chinese Han females at age of 25-45 years were recruited and their areal BMDs at hip and spine were measured with a dual energy x-ray absorptiometry scanner (Hologic QDR4500W). 14 subjects with top hip BMD (1.03±0.05 g/cm$^2$) and 12 subjects with bottom hip BMD (0.70±0.06 g/cm$^2$) were selected from the above sample. Monocytes from peripheral blood were isolated from the 26 subjects with a negative isolation protocol. Total RNA in monocytes were then extracted and analyzed for gene expression with

Affymetrix HG-U133 plus 2.0 microchips. Differential expression analysis was conducted with statistical software dCHIP.

**Results:** Interleukin 1 beta (*IL1B*), interleukin 1 receptor antagonist (*IL1RN*), interleukin 8 (*IL8*), interleukin 15 (*IL15*), chemokine (C-X-C motif) ligand 10 (*CXCL10*), chemokine (C-C motif) ligand 4 (*CCL4*), chemokine (C-C motif) ligand 20 (*CCL20*), interferon-induced protein 44-like (*IFI44L*), interferon-induced protein 44 (*IFI44*), signal transducer and activator of transcription 1 (*STAT1*) and guanylate binding protein 1 (*GBP1*) genes were up-regulated in the low BMD group compared with high BMD group. Interleukin 6 (*IL6*) gene did not express but Interleukin 6 receptor (*IL6R*) gene expressed much in all subjects. Monocytes in peripheral blood did not express receptor activator of NF-κB ligand (*RANKL*), receptor activator of nuclear factor κB (*RANK*), osteoprotegerin (*OGP*), TNF receptor-associated factor 6 (*TRAF6*) genes.

**Conclusion:** These results showed that monocytes affected BMD mainly through cytokines or chemokines instead of RANKL/RANK/OPG and it was also suggested that interferon pathway might be strongly related to the BMD variation. These results highlight the potential use of the microarray data in identifying genes associated with BMD or osteoporosis in immune system.

## *BIOMATERIALS*

**P-MON-58**

### Calcium-silicate based ceramics for bone regeneration

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**Introduction:** Aseptic loosening of the prosthetic joint replacements due to the formation of thick fibrous tissue at the device/bone interface remains to be the major cause of implants failure. Novel micro-engineered surfaces are required to interlock implants with the surrounding skeletal tissue, thereby increasing the life of prostheses. We have previously shown that incorporation of trace elements, such as magnesium (Mg) and zinc (Zn); into commonly used orthopedic implants enhance the biological activity of biomaterials [1]. This study aims at 1) chemically modifying currently available calcium silicate (Ca-Si) based ceramics (Wollostonite, WT ($CaSiO_3$) by incorporating Mg and Zn into their structure for future applications as coatings onto currently used orthopaedic implants. 2) determining the biological activity of primary human osteoblast (OB) & osteoclast (OC) when cultured on the modified materials (Akermanite (AT)($Ca_2MgSiO_7$) and Hardystonite (HT)($Ca_2ZnSi_2O_7$), compared to the control WT.

**Methods:** MTS ((4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) and alkaline phosphatase assays were used to determine OB viability and differentiation when cultured on AT, HT and WT. The effect of the different ceramic-based materials on OC (differentiated from human blood monocytes) attachment and resorption was determined using scanning electron microscope and confocal microscopy.

**Results:** The ionic product released from AT, HT and WT ceramics did not exert any toxic effect on the OB. When cultured on AT and HT OB exhibited excellent attachment and spreading, indicating that modification of Ca-Si ceramics with Mg and Zn can modulate biological activity of bone cells OB & OC.

#### *References*

1 H.Zreiqat, et al. (2002) J Biomed Mater Res, 62 (1), 175-184

**P-MON-59**

## Analysis of *in vivo* responses to purified PHBV and hydroxyapatite/PHBV composite implanted in a murine tibial defect

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Effective bone biomaterials have properties that are suitable to provide structural support for bone regeneration and elicit minimal inflammatory and toxic effects when implanted. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) is a biodegradable polymer purified from bacteria, that possesses suitable mechanical strength for use as a bone biomaterial and has a slow rate of degradation in biological environments. The aim of this study was to evaluate the *in vivo* biocompatibility of purified PHBV and a hydroxyapatite/purified PHBV (HA/PHBV) composite. Cylindrical blocks of the materials were implanted into a murine tibial cortical defect, consisting of a hole drilled through the diameter of the tibial diaphysis. The defect was either filled with a material plug or left unfilled as a control. The animals were sacrificed at 1 week and 4 weeks after surgery and tibiae were decalcified and paraffin embedded. Bone sections were examined using standard histological staining as well as immunohistochemical staining to determine the extent of the cellular response. In the control samples, a callus repair response was found at 1 week and woven bone was present at 4 weeks. Both PHBV and HA/PHBV implants induced a mild inflammatory response 1 week after injury, which persisted at 4 weeks. New woven bone adjacent to the implant at 1 week was remodelled to restore the marrow cavity, although bone remodelling was not observed at the biomaterial interface at 4 weeks. Our data indicate that solid PHBV and HA/PHBV biomaterials induce a mild tissue reaction with no evidence of bone remodelling at their tissue interface.

## *ORTHOPAEDICS, RHEUMATOLOGY AND TISSUE BIOLOGY*

**P-MON-61**

## Zoledronic acid modulates the expression of S100A8 In adjuvant arthritis

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**Introduction:** Rheumatoid arthritis (RA) is a chronic inflammatory disease and anti-rheumatic therapies such as bisphosphonates (BPs) are aimed at minimizing bone damage. The effect of BP on inflammation and articular cartilage damage in RA are not well studied. S100A8, a calcium binding protein, is upregulated in RA. Recently, S100A8 was found to be expressed by chondrocytes. This study determined if the BP, zoledronic acid (ZA) 1) inhibits inflammation and joint destruction in adjuvant arthritis and 2) modulates the expression of S100A8 in synovial inflammation and in articular cartilage chondrocytes.

**Methods:** Adjuvant arthritis was produced in male Dark Agouti rats and ZA was administered s.c. to groups of 10 rats in a dose range of 0.3 – 10 mg/kg. Control animals received saline. The effect of ZA on S100A8 expression in rat ankle joints was determined using polyclonal antibodies to S100A8. All experiments were approved by the animal care and ethics committee of UNSW.

**Results:** ZA, dose-dependently reduced arthritic severity by as much as 75.6 $\pm$ 5.7% (mean $\pm$ se). S100A8 was predominately expressed in articular cartilage cells. ZA treated (1–10$\mu$g/kg) arthritic animals showed significantly higher levels of S100A8 expression in articular cartilage chondrocytes, compared to control animals. ZA treatment reduced the formation of pannus, whereby the expression of S100A8 was significantly reduced.

**Conclusion:** ZA is a potent anti-inflammatory agent that increased the expression of S100A8 in the articular cartilage chondrocytes, where a significant reduction in joint damage was observed. This may imply that S100A8 has a protective role against RA.

**P-MON-63**

## Characterisation of RING finger protein Praja1 in RANKL-induced osteoclastogenesis

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Osteoclasts are the primary bone resorbing cells and play a pathological role in many osteolytic conditions due to their excessive formation and activity. Therefore, identification of novel molecular targets or mechanisms involved in the regulation of osteoclast formation and function would provide a means of therapeutic benefit to sufferers of these diseases. We have performed a microarray analysis and successfully identified a number of differentially expressed genes during RANKL-induced osteoclastogenesis. Among these candidates is Praja1, a RING finger motif containing gene currently with an unknown function in osteoclasts. Remarkably, this study for the first time demonstrates that overexpression of wildtype Praja1 in COS-7 cells significantly inhibits the transcriptional activation of Nuclear Factor kappa B (NF-kB). Two RING finger domain mutants were generated to determine the manner in which Praja1 inhibits NF-kB transcription activity. Overexpression of the RING finger mutants demonstrates a less potent inhibitory effect of NF-kB transcriptional activity, suggesting inhibition by Praja1 is predominantly mediated via its RING finger domain. Overexpression of wildtype Praja1 decreases the steady state levels of TRAF6, a key adaptor protein in RANK signalling. These results suggest that Praja1 might possess a regulatory role in RANKL-mediated osteoclastogenesis via attenuating signalling pathway activities through modulation of TRAF6 stability. Regulation of osteoclasts is recognised as a means of treatment for osteolytic diseases and Praja1 is revealed as a potential candidate that could ultimately be used in the development of therapeutics in these morbid conditions.

**P-MON-65**

## Zoledronic acid enhances femoral head ossification and retards osteonecrotic collapse in a rat model of Perthes disease

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Perthes disease is a common form of spontaneous femoral head osteonecrosis. Resorption of osteonecrotic tissue causes femoral head deformation and collapse.

Previously, we showed improved outcome with the use of the bisphosphonate Zoledronic Acid (ZA) in a Perthes disease model, the spontaneously hypertensive rat (SHR). We further investigated this result during femoral head development in SHR and normal rats.

Spontaneous ischaemic femoral head osteonecrosis occurs in 50% of 9 week male SHR's. Monthly ZA (0.05mg/kg) or saline commenced at 3 or 9 weeks in SHR and Wistar rats. Harvests were at 3, 6, 9, 12 and 15 weeks of age.

Epiphyseal Quotient (EQ) measures femoral head sphericity. Significant increases in mean EQ occurred with ZA treatment at 6, 9 and 15 weeks of age in SHR's ($p<0.01$). ZA treatment from 9 weeks did not improve EQ.

Femoral head BMD was significantly increased 11%-29% at 6, 9, 12 and 15 weeks in SHR and Wistar rats with ZA ($p<0.01$). Again ZA from 9 weeks showed no improvement.

Histology of femoral heads revealed that percent epiphyseal ossification was increased at 6 and 12 weeks ($p<0.01$) in Wistar and at 12 and 15 weeks in SHR's with ZA ($p<0.05$).

This study suggests ZA treatment during femoral head development reduced the occurrence of femoral head collapse due to osteonecrotic insult in SHR's. ZA enhanced epiphyseal ossification in SHR and Wistar rats. By enhancing and preserving femoral head architecture ZA treatment improved resistance to deformation. Furthermore, early ZA treatment was necessary to achieve optimal effects.

**P-MON-67**

## Non-steroidal antinflammatory drugs can delay bone formation during healing of experimental stress fractures

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Despite their clinical importance, there is almost no histological information available on the mechanism of healing of stress fractures. Non-steroidal anti-inflammatory drugs are still the most widely used medication in the management of musculoskeletal injuries, yet their effect on the healing of stress fractures is unknown. Cyclooxygenase enzymes, particularly COX-2, are central in bone resorption, remodelling and bone formation. We have optimised the rat ulna-loading model of fatigue fracture to examine the histology and histomorphometry of stress fracture healing and to determine the effect of a variety of NSAIDs on this healing.

Stress fractures were created by cyclic loading of the ulna in female Wistar rats. Loading was stopped at a loss of stiffness of 10%. Ulnae were harvested 2, 4 or 6 weeks following loading. Rats were treated daily with Ibuprofen, DFU (a selective COX-2 inhibitor), paracetamol or placebo. Loading consistently produced a "saucer" stress fracture in the distal half of the ulna diaphysis that healed by direct remodelling along the fracture line as well as woven bone proliferation around the periosteum. There were no treatment effects of Ibuprofen on resorption area or length of fracture being remodelled. Ibuprofen treatment resulted in a marginally significant reduction in woven bone area and healing of the fracture line at 6 weeks ($P < 0.1$). We conclude that non-selective NSAIDs have the potential to delay stress fracture healing. Further analysis will examine the effects of selective COX-2 inhibition, novel NSAIDs and paracetamol, on stress fracture healing.

**Acknowledgements:** This work is funded by a NHMRC grant.

**P-MON-69**

## Anti-catabolic treatment augments anabolic therapy in a rat critical femoral defect model.

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**Introduction:** Bone repair following fracture involves the interplay of anabolic and catabolic events. Successful fracture repair requires an initial predominance of anabolism in order for bony callus formation. We hypothesised that the addition of a carefully timed anti-catabolic, Zoledronic Acid (ZA) could optimise the dose of an anabolic agent OP-1 (BMP-7).

**Methods:** Sixty male Wistar rats received a 6mm critical femoral defect and were randomly assigned to three dose groups of OP-1 (25µg, 10µg or 5µg). Half of the rats in each dose group received 0.1mg/kg ZA intravenously or saline two weeks post-surgery. Bone repair was assessed by radiography, qCT, histology/ histomorphometry 8 weeks post-surgery.

**Results:** There was a significant difference in BMC and callus volume between the ZA and saline groups in the 25µg OP-1 group. There was no significant difference between the 25µg OP-1/saline group and 10µg OP-1/ZA groups by qCT. While there was a slight trend in increased union rate in the ZA treated groups this was not significant.

**Conclusion:** ZA treatment significantly improved BMC and callus volume in the 25µg group and led to the lack of significant difference between the 25µg/saline and 10µg/ZA groups showing that the addition of the anti-catabolic can be used to optimise the dose of the anabolic. Further studies will examine improved ZA dosing regimens to improve fracture union rates between these groups.

**P-MON-71**

### The osteogenic potential of muscle cells

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Fracture healing is a complex physiological process involving many cell types. Periosteal cells and mesenchymal stem cells (MSCs) from the bone marrow are often viewed as the major contributors in this process. However, recent studies have indicated that cells originating from the adjacent muscle may also play a role. We hypothesise that a sub-population of muscle cells may be particularly important in the repair of high energy fractures where the periosteum is damaged and there is poor marrow cell access.

Previous *in vitro* studies have demonstrated that muscle cells activate an osteogenic gene program upon treatment with the potent bone forming agent, bone morphogenetic protein-2 (BMP-2). However, few studies have addressed the inherent osteogenic potential of myoblasts compared to other cell types. In this study, we compared the responsiveness of muscle cells (C2C12 myoblasts and primary myoblasts) to BMP-2 stimulation relative to cells with recognised osteogenic potential (MC3T3-E1 pre-osteoblasts and primary MSCs).

Our results indicate that C2C12 myoblasts are highly responsive to BMP-2, moreso than MC3T3-E1 pre-osteoblasts. Outcome measures included alkaline phosphatase (ALP) staining and activity, mineralisation, and osteogenic gene expression by quantitative PCR. Similar results were observed using primary cells.

In conclusion, our *in vitro* data indicates that myoblasts are capable of osteogenic differentiation with relatively low levels of osteogenic stimulation. These findings support our hypothesis that muscle-derived cells may play critical roles in fracture repair, however this concept will need to be further explored using *in vivo* models.

## *CLINICAL METABOLIC BONE DISEASES*

**P-MON-73**

### Absence of somatic SQSTM1 mutations in pagetic tissue

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Paget's disease of bone is a focal disorder characterised by increased bone resorption coupled to increased bone formation. The cause of the disease remains unknown, however genetics, slow viral infection and other environmental influences have all been suggested to play a role.

Mutations in the sequestosome 1 gene (SQSTM1) have been implicated in both familial and sporadic cases of Paget's disease, and have been found in osteoclasts and osteoblasts from pagetic tissue in all cases in a recent case series (CTI 76:478, 2005).

We have looked for mutations in the region corresponding to exons 7 and 8 in mRNA of SQSTM1 in the pagetic tissue of 23 patients, three with a family history of Paget's disease. RNA was extracted from pagetic osteoblasts grown from trabecular bone explants, and from pagetic stromal cells grown from bone marrow. Following production of cDNA from DNase treated RNA, the region of interest in the SQSTM1 gene was amplified using specific primers. Purified PCR fragments were sequenced and examined for any mutations.

Of these patients, only one possessed the P392L mutation. This patient does not have a known family history of Paget's disease. The mutation was found in the PCR product as well as in genomic DNA that was isolated from a blood sample of the patient, and is therefore a germ-line rather than a somatic mutation.

The present results indicate that somatic mutations of SQSTM1 are not common in Paget's disease, with no evidence of such a phenomenon in this series of 23 patients.

**P-MON-75**

## Intestinal radiocalcium absorption is not affected by treatment with raloxifene in women with postmenopausal osteoporosis

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**Aims:** Intestinal calcium absorption is a crucial factor in maintaining calcium homeostasis. Estrogen therapy helps correct the decline in calcium absorption that is typical with onset of menopause. Raloxifene is a selective estrogen receptor modulator that is a current treatment of osteoporosis. The aim of this study was to investigate the affect of raloxifene compared to alendronate on intestinal calcium absorption in women with postmenopausal osteoporosis.

**Method:** Calcium and vitamin D replete postmenopausal women with osteoporosis (n=102), at least 6 months beyond the menopause with a history of at least one past minimally traumatic fracture were studied. Patients were randomised to ALN 70mg/week, RXL 60mg/day or both, and bone turnover was measured at 6 and 24 months. Parathyroid hormone (PTH) and the biochemical markers of bone turnover osteocalcin, N-telopeptide and alkaline phosphatase were measured as well as serum 25-hydroxyvitamin D, calcium, phosphate, creatinine, albumin, urinary calcium excretion and phosphate. Z scores were calculated for PTH and osteocalcin to account for assay variations. Intestinal $^{45}Ca$ absorption was measured as previously described[1] .

**Results:** No difference in change in calcium absorption was observed between the treatment groups at either 6- or 24-months. Absolute change in calcium absorption was found to be significantly, negatively, correlated to baseline calcium absorption at both 6- ($r=-0.258$, $p=0.009$) and 24-months ($r=-0.46$, $p=0.001$)

**Conclusion:** Intestinal calcium absorption was not affected by treatment with raloxifene in the population of women studied.

1. Nordin BEC, et al. 1998. J Nucl Med. 39:108-13.

## *CLINICAL OSTEOPOROSIS*

**P-MON-77**

## Comparison of anti-vertebral fracture efficacy among available therapies: a Bayesian analysis

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In the absence of a comparative head-to-head clinical trial, the selection of an optimal treatment is difficult. The present analysis was undertaken to compare the anti-vertebral fracture efficacies among currently available therapies.

Published data from RCTs evaluating the anti-fracture efficacy of alendronate (n=6 trials), etidronate (n=9), risedronate (n=5), calcitonin (n=8), calcium (n=3), fluoride (n=6), raloxifene, (n=4), HRT (n=13), parathyroid hormone (PTH, n=10) and strontium renelate (n=5) were included in the analysis. A Bayesian analysis model was utilized to estimate the effect size for an individual therapy. The index of superiority, was estimated for each pair of drugs. This was defined as the probability that a drug's effect size is 20% greater than another drug.

Compared to placebo, most active therapies significantly reduced the risk of vertebral fracture, with variable magnitudes: alendronate (OR; 95% credible-interval: 0.51; 0.38-0.68), etidronate (0.50; 0.31-0.85), risedronate (0.58; 0.44-0.78), raloxifene (0.59; 0.44-0.84), HRT (0.62; 0.39-0.92), strontium (0.56; 0.46-0.76), and particularly PTH (0.31; 0.22-0.42). However, the evidence of effect was uncertain for calcitonin (0.77; 0.56-1.11), calcium (0.73; 0.40-1.24) and fluoride (0.75; 0.52-1.06). More importantly, pairwise comparison of active therapies by the index of superiority suggested superiority for PTH when compared to the others. The probabilities that PTH is superior to alendronate was 0.91, etidronate: 0.80; risedronate: 0.97, HRT: 0.96, and raloxifene: 0.98. Probabilities of equivalent boundary between two treatments are shown in the following table.

These results indicate that while PTH, bisphosphonates, HRT, raloxifene and strontium were efficacious in reducing vertebral fracture risk, their effect sizes were comparable. However, PTH appears to have higher anti-vertebral efficacy that all other treatments.

Probability (Pr) of equivalence in efficacy (within 20% difference) between any two treatments. Results were based on the Pr that $P(k_i \leq \log OR \leq k_j)$ where i=-0.2231 (or OR=0.8) and j=0.1823 (OR=1.2)

| | HRT | (6) | (5) | (4) | (3) | (2) |
|---|---|---|---|---|---|---|
| PTH | 0.04 | 0.02 | 0.03 | 0.02 | 0.09 | 0.20 |
| Etidronate (2) | 0.58 | 0.62 | 0.63 | 0.68 | 0.78 | |
| Alendronate (3) | 0.54 | 0.65 | 0.69 | 0.76 | | |
| Strontium (4) | 0.72 | 0.83 | 0.86 | | | |
| Risedronate (5) | 0.75 | 0.84 | | | | |
| Raloxifene (6) | 0.77 | | | | | |

**P-MON-79**

## Trunk muscle response to perturbation is affected by osteoporotic vertebral fracture and thoracic kyphosis

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**Aims:** Osteoporotic vertebral fractures are associated with an increased risk of sustaining further vertebral fractures, however, the mechanisms through which this occurs are unclear. Alterations in trunk muscle control may have implications for fracture risk through increased vertebral loading and impaired balance. The aim of this study was to explore the relationships between vertebral fracture, thoracic kyphosis, and trunk muscle control in a population with osteoporosis.

**Methods:** Electromyography (EMG) of the trunk flexor and extensor muscles was collected during the performance of rapid voluntary arm movements (forwards and backwards). Twenty-four individuals with osteoporosis were grouped according to presence of fracture (10 participants with vertebral fracture and 14 without fracture) and thoracic kyphosis (low and high). Normalised EMG activity was compared qualitatively between groups.

**Results:** The elderly participants demonstrated co-contraction of the trunk flexor and extensor muscles during forwards arm movements which is different to the triphasic (alternating trunk flexor and extensor activity) response observed in young populations. Individuals with vertebral fractures demonstrated a more pronounced co-contraction response, while individuals with high thoracic kyphosis demonstrated a more pronounced triphasic response.

**Conclusions:** Results demonstrated that in this elderly population, arm flexion is associated with co-contraction of the trunk flexors and extensors. This stiffening strategy may have several consequences for individuals with vertebral fragility, including an altered balance response and the potential to create increased compressive loads through the spine. Both of these consequences have implications for fracture risk.

**P-MON-81**

## Study of bone mineral density and effect of various environmental factors on it, in resident doctors working at a teaching hospital in India

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**Aim**: To study Bone Mineral Density (BMD) and the impact of sunlight exposure, physical activity, dietary factors and Vitamin D status in healthy resident doctors.

**Materials & Methods:** 214 doctors (174 males and 40 females), 25-35yrs of age working at a teaching hospital in Mumbai underwent BMD by DXA. Detailed history regarding diet (protein, calcium, and phosphorus intakes), physical activity (type, duration) sunlight exposure (calculated to minutes/week) was obtained. Serum Calcium, inorganic phosphorus, alkaline phosphatase, Vitamin D & PTH levels were estimated.

**Results**: BMD in this healthy cohort was found to be significantly lower than that of Caucasian reference population and in earlier studied healthy Indian population. Bone density was classified as per WHO criteria (Table).

| | Osteopenia | osteoporosis | Normal BMD |
|---|---|---|---|
| **males** | 121(69.54%) | 32 (18.39%) | 21(12.07%) |
| **females** | 27 (67.5%) | 5 (12.5%) | 8 (20%) |

Serum calcium, phosphorus and alkaline phosphatase levels were normal in all the subjects. 175 (87.5%) were Vitamin D insufficient (< 20 ng/ml or <50nmol/L) and in 129(64.5%), PTH levels were elevated (>45pg/ml or >45ng/L). PTH showed steep rise at 25(OH) D3 value < 20ng/ml (50nmol/L). Positive correlation was found with BMI, physical activity (in males only) and dietary calcium phosphorus ratio with BMD at various sites.

**Conclusion:** BMD of healthy resident doctors was found to be significantly lower than that of Caucasian and earlier studied healthy Indian population. Prevalence of Vitamin D deficiency was high in spite of adequate sun exposure. BMI, Physical activity and dietary factors positively correlated with BMD.

**P-MON-83**

## Urine C-terminal PTHrP levels do not change over the menopause transition

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Osteoblast-derived PTHrP is a potent endogenous bone anabolic agent. In genetically manipulated mice, insufficiency of PTHrP accentuates the anabolic action of exogenous PTH on bone. Although PTHrP is not detectable in the serum of normal individuals, a urine radioimmunoassy can detect C-terminal (C-T) PTHrP fragments in normal individuals and in patients with humoral hypercalcaemia of malignancy.

We performed a cross-sectional study of urine C-T PTHrP levels in 165 women aged 45-57 years across the menopause transition. We examined relationships between urine C-T PTHrP and bone formation and resorption markers, and spinal BMD. The standard used in the C-T PTHrP assay was PTHrP (107-139). The antibody was a rabbit antibody vs. 107-139 with an epitope between 129-139. The antibody could identify PTHrP (1-141), (35-141) and (85-141) fragments. Inter- and intra-assay coefficients of variation were 13% and 5%, respectively.

C-T PTHrP concentrations did not differ according to menopausal status in 157 women for whom bone markers and BMD were available. Levels in pre- (n=45), peri- (n=66) and postmenopausal (n=46) women were (mean ± SEM) 120±23; 82±14 and 119±21 pmol/mmol Cr, respectively. C-T PTHrP levels were not related to markers of bone formation [osteocalcin, bone alkaline phosphatase or C-terminal type I procollagen propepetide (PICP)]. Nor were C-T PTHrP levels related to markers of bone resorption [total or free deoxypyridinoline (DPD) or N-telopeptide (NTX)] (r=0.12, p=0.13) or spinal BMD.

Conclusion: C-T PTHrP is detectable in urine and its urine excretion does not increase after menopause in women. Although there is evidence in the mouse that PTHrP is an endogenous bone anabolic agent, in this study urine C-T PTHrP fragments were not related to bone formation markers or spinal BMD in women.

**P-MON-85**

## Osteoporosis: A comprehensive risk assessment of an elderly cohort with the consideration of gender

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Current osteoporotic screening, diagnostic and treatment regimes appear to neglect the majority of cases thereby underestimating the true impact of this disease on the Australian public (Nguyen, Center, & Eisman, 2004). Even more critical is the lack of awareness of osteoporosis as a disease that affects men (Diamond & Lindenberg, 2004). The current study performed a comprehensive risk assessment on an elderly cohort of both males and females (55 – 85 years), which included self-reported survey information on lifestyle factors, self / family history of fracture, and ultrasound investigation of the heel. Of the 806 Hunter Cohort Study (HCS) participants, 394 (212 females, 182 males) completed both the survey and ultrasound conditions. Participants' estimated ultrasound results, combined with the clinical risk factor information, was utilised in categorising risk groupings (low / moderate-high). Based on these criteria, 49% of individuals were identified as moderate-high risk. Doctor related discussions and screening rates within this risk group were 31% and 29% respectively. Discussion / screening rates between gender in the moderate-high risk group found that males have significantly less clinician intervention than that of females. The presence of a late-onset fracture was found to be an indicator of clinician intervention in only 42% of cases.

It can be concluded that moderate-high risk individuals for osteoporosis may present more often in an elderly community than health professionals presently screen for. Males in particular appear to be neglected as far as appropriate screening is concerned.

**References:**

Diamond, T. & Lindenberg, M. (2004). Osteoporosis detection in the community. Australian Family Physician, 31, (8), 751-752.
Nguyen, T.V., Center, J.R., & Eisman, J.A. (2004). Osteoporosis: underrated, underdiagnosed and undertreated. Medical Journal of Australia, 180, S18-S22.

**P-MON-87**

## Chronic anti-epileptic drug treatment is associated with impairment in balance function. A twin and matched sibling pair pilot study.

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Rationale: Anti-epileptic medication (AED)-users have increased fracture rates. Potential causes for this association include bone disease and increased falls during seizure and at other times.

**Aim:** To assess chronic AED-users' muscle strength and balance function utilizing a twin and matched sibling AED-discordant pair approach.

**Methods:** 7 AED-discordant pairs (6 female, 1 male): 3 monozygous, 1 dizygous, 3 sibling pairs (within 3 years of age) with mean (SD) age 55.6 (17.6)years, height 161(6)cm, weight 67.9(13.6)kg were assessed. Mean duration (SD) of AED therapy =29(19) years. Validated tests of muscle strength, balance and gait, predictive of falls risk were performed:(i) Nicholas Manual Muscle Tester (NMMT), (ii) Kincom, (iii) Chattecx Balance System (CBS): Sway index(SI), anterior-posterior(AP) and lateral(LR) sway in 3 platform positions and (iv)Lord's balance test (LBT). Physical activity measured by Human Activity Profile (HAP). Paired t-tests utilized

to assess mean within-pair differences (AED-user vs. non-user). Relevant serum AED levels were not above therapeutic range at time of assessment.

**Results:** No significant within-pair difference in age, height, weight, muscle strength or activity level. Significant within-pair differences were seen with multiple measures of balance: LBT: eyes closed (+87mm, p=0.026); foam mat eyes open (+46mm, p=0.043); foam mat eyes closed (+193mm, p=0.067 n=5 pairs); CBS: Stable position+ distraction (SI+0.36cm, p=0.013, LR+1.28cm, p=0.00, AP+1.68cm, p=0.02); Anterior-posterior moving platform (SI+0.66cm, p=0.045, AP+2.66cm, p=0.022); Medial-lateral moving platform + distraction (AP +1.16cm, p=0.024).

**Conclusion:** There is significant within-pair difference (AED-user vs. non-user) in balance measures which may have important implications for AED-users indicating potential increased falls risk and therefore fracture risk.

**P-MON-89**

## Alendronate treatment increased bone mineral density at multiple skeletal sites in postmenopausal Chinese women with fast bone loss

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To identify the high-risk groups and provide pharmacological treatment is one of the effective approaches to prevent osteoporosis and osteoporotic fractures[1-3]. This study investigated the effect of 12-month Alendronate treatment on bone mineral density (BMD) and bone turnover in postmenopausal women with fast bone loss. A total of 148 healthy Hong Kong postmenopausal Chinese women aged 50 to 65 were recruited for BMD measurement using a multilayer pQCT (Densiscan 2000) at baseline and one year later at the non-dominant distal radius[2]. 43 women were subsequently identified as fast bone losers, who lost more than 3% trabecular bone in the past one year and then randomized into Alendronate and placebo control group for BMD measurement at spine and hip using DXA and bone turnover markers, including bone forming alkaline phosphatase (ALP) and bone resorbing urinary Deoxypyridinoline (DPD) before intervention. All measurements were repeated at 6 and 12 months. Results showed that Alendronate treatment significantly increased BMD, more in weight-bearing skeletons (4.1% at spine and 3.5% at hip) than in non-weigh-bearing skeleton (1.1% distal radius) after 12 months treatment. Alendronate treatment was able to stop fast bone loss states almost completely. The underlying mechanism of Alentronate treatment was explained by significant suppression of bone turnover. In conclusion, 12 months Alendronate treatment was effective in increase of BMD at multiple skeletal sites in postemenopausal women with fast bone loss.

**P-MON-91**

## Improving the management of patients with osteoporotic fractures: the OMEN Program

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Worldwide, osteoporosis is underdiagnosed and undertreated. Even patients with minimal trauma fractures (MTF) are often neither diagnosed nor treated for their underlying bone disease. Subsequent to a 'baseline study' into the status quo of management of patients presenting with MTF, we have implemented the 'Osteoporosis Management Enhancement (OMEN) Program' at Concord Hospital, Sydney, Australia. We here report initial results of this prospective interdisciplinary program.

**Methods:** For the 'baseline study', data from 537 patients presenting to Concord Hospital with a diagnostic code of fracture were analysed. Of those, 302 subjects were classified as having a MTF and entered the

analysis. 'Implementation': as of June 2005, all non-frail patients with a MTF were identified and referred to the OMEN program for further investigation and treatment.

**Results:** In the 'baseline study' 70% of MTF occurred in women. While 65% of patients were assessed for falls risk, only 20% were asked about risk factors for osteoporosis. An X-ray was obtained in 97% of patients, but only 4.1% had a BMD measurement. At discharge, 35% were on some form of osteotropic medication; only 8.6% received an anti-resorptive agent and 2.1% received combination therapy with anti-resorptive agents, vitamin D and calcium.

'Implementation': Between June 05 to July 06, 169 patients with incident MTF were referred to the OMEN program: 76% female; 58% <70 years of age; 98% living at home; 31% had at least one previous MTF; 17% had secondary causes of osteoporosis. In the entire group, osteopaenia was present in 40% (FN) and 22% (LS), and osteoporosis was seen on BMD in 15% (FN) and 32% (LS). In patients 70 years or younger, central fractures (pelvis, sacrum, hip) were more often associated with osteoporosis than with osteopaenia (6.9% vs. 1.7 of all patients), whereas peripheral fractures (humerus, radius, hands, femoral shaft, tibia, ankle, foot) showed equal BMD distribution (44 vs. 47%). The opposite pattern was observed in patients >70 years of age. At the time of fracture, serum levels of bone specific alkaline phosphatase (BAP) were normal in all patients, while urinary DPD was normal in 69% of patients. Serum BAP levels peaked at 2 weeks (>50% of patients > ULN) and normalized by 15 weeks. Urinary DPD peaked at 1 week after a fracture. Following investigations, 62% of patients were placed on bisphosphonates plus calcium and/or vitamin D supplementation. 22% received either calcium or vitamin D without anti-resorptive treatment. All patients received advice in regards to lifestyle, dietary factors and risk avoidance.

**In conclusion**, a specific clinical program dedicated to the care of patients presenting with MTF improves disease recognition and patient management. Younger patients with peripheral fractures (eg feet) should undergo similar osteoporosis screening as the older population with more classical osteoporotic fractures (eg hip).

**P-MON-93**

## Low BMD in steroid-dependent nephrotic children

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Aims and Methods: Glucocorticoid-induced osteoporosis and bone fractures are serious clinical problems with the rising use of steroids in children with steroid-dependent nephrotic syndrome (SDNS) and frequent relapsers (FRs) because of high doses of glucocorticoids over long periods. On the other hand, Leonard et al. [N Engl J Med 351:868,2004] reported that glucocorticoid-induced increase in body mass index (BMI) might serve to preserve the body mineral content of the spine and to increase whole-body bone mineral content in children with glucocorticoid-sensitive NS. We examined the relationships between BMI and bone mineral density (BMD) z-score in children with SDNS and FRs (Group 1, n=17), or infrequent relapsers (Group 2, n=13). Results: A BMI of more than 95% of that of healthy age and sex-matched controls was seen in 9 (53%) of 17 children in Group 1 and in 0 of 13 children in Group 2. A BMD z-score of less than -2.5 (osteoporosis) was seen in 11 (65%9 of 17 children in Group 1 and in 0 of 13 children in Group 2. All 3 children with vertebral compression fractures had a BMI of more than 95% that of the control values. Conclusion: There is a high incidence (65%) of steroid-induced osteoporosis in children with SDNS and FRs with no relation to BMI.

**P-MON-96**

## Differences of bone mineral density between spine and hip in osteoporotic patients

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**Purpose:** To evaluate differences and correlations of spine and hip region BMD in osteoporotic patients with or without spine fracture above 60 years old.

**Materials and Methods:** From January 1999 to December 2002, We measured and evaluated BMD of L3 and hip by DEXA in 52 patients with spine fracture(fracture group) and 96 osteoprotic patients without spine fracture(non-fracture group) above 60 years.

**Results:** The average age of patiens with spine fracture is 72.1 years and without spine fracture is 66.9 years. There were no statistical significant differences of BMD of spine, neck of femur and trochanteric area between 2 groups. But the BMD of Ward triangle of fracture group decreased significantly in statistics. The correlation coefficient between the lumbar spine and trochanteric area were 0.674 in fracture group and 0.794 in non-fracture group. They had statistical significance

**Conclusion:** The BMD of Ward triangle of fracture group had lower value, but the BMD of lumbar spine had no differences between 2 groups. Therefore in these persons who have decreased BMD of Ward triangle should be concerned about high risk of vertebral compression fracture.

## *PAEDIATRICS*

**P-MON-98**

### Bisphosphonate treatement in chronic recurrent multifocalosteomyelitis: a case series

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**Background:** Chronic recurrent multifocal osteomyelitis (CRMO) is a rare inflammatory disorder of the skeleton most often seen in children and adolescents and characterized by expansile lesions within bone. Many treatment modalities have been used, with varying clinical outcomes. Bisphosphonates have been proposed, due to their major action as osteoclast inhibitors.

**Methods:** We report the use of pamidronate in 4 patients with CRMO, 2 patients with predominately tibial involvement, 1 patient with clavicular disease and 1 patient with both lower limb and clavicular involvement. These patients all had ongoing pain and loss of function despite the use of conventional treatments such as non-steroidal anti-inflammatory agents. All 4 patients were treated with pamidronate 1mg/kg/dose every 2-4/12 with duration of treatment ranging from 12 - 42 months.

**Results:** All patients reported reduced pain following their first infusion. Three patients showed radiological changes varying from decreased oedema surrounding the lesion to dramatically reduced dimension of lesion on plain Xray.

No significant adverse effects of treatment were noted.

The treatment effect has been ongoing with no relapse following the cessation of regular pamidronate infusions.

**Conclusion:** Bisphosphonates appear to be a useful and safe adjunctive treatment in CRMO when simple therapies such as anti-inflammatory agents fail to control symptoms or where lesion expansion continues.

## *EPIDEMIOLOGY, PUBLIC HEALTH AND GENETICSs*

**P-MON-100**

### Age-related macular degeneration and risk of falls and fractures: Geelong Osteoporosis Study

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Age-related Macular Degeneration (AMD) is a debilitating disease affecting central vision. We aimed to determine if women with AMD were more likely to fall and/or fracture. The relationships between AMD and falls/fracture were assessed in women aged ≥60 yr enrolled in the Geelong Osteoporosis Study. Non mydriatic digital fundus images of both fundi were recorded and independently graded by two observers for signs of AMD. Due to small numbers, early and late signs of AMD were pooled. Self-reported falls were obtained by questionnaire and participants were classified as fallers if they had fallen to the ground ≥2 times in a 12-month period post AMD evaluation. Incident fractures (all causes) were identified from radiological reports for the 2-year period coincident with AMD testing. Of 452 eligible women, complete data were available for 295 (median age 70.5yr, range 60.0–90yr).

AMD was diagnosed in 77 women. A greater proportion of AMD women were fallers (10/77 vs. 13/218, p<0.0001) or sustained fractures (9/77 vs. 9/218, p<0.0001). AMD women were older (74.8 vs. 69.4yr, p<0.0001). Age-adjusted OR (95%CI) associated with AMD was 2.35 (0.95-5.83, p=0.065) for falls and 2.46 (0.90-6.75, p=0.080) for fracture.

These results suggest that women with AMD may be more likely to fall and may be at increased risk for fracture. Falls and fracture prevention strategies may be of benefit in this group.

**P-MON-102**

### Why is smoking a risk factor for fractures? A new study.

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Cigarette smoking appears to increase the lifetime risk of hip fractures by 30 – 40 %. Up to 10 % of all hip fractures may be attributable to smoking. Vertebral fracture risk also is increased in smokers. Smokers are reported to have lower bone mineral density (BMD) at multiple skeletal sites. However, the mechanism underlying smoking-associated bone loss and fractures is unknown and whether fracture risk is reversible with smoking cessation is unclear. This project aims to clarify these important issues.

Same-sex twin pairs, where one of each pair was a current smoker (at least 15 cigarettes/day), completed health and lifestyle questionnaires, had bone and soft tissue measurements by dual energy X-ray absorptiometry and gave fasting morning blood samples.

Areal BMD in 41 pairs (14 Males, 68 Females; age range 40-76 y) was lower at the lumbar spine (L2-4; z-score mean 0. 32 vs 0.80, p = .016) and total hip (z-score mean – 0.05 vs 0.67, p =.000) in smokers compared with non-smokers. Total bone mineral content was also reduced in smokers (2.065 kg) vs non-smokers (2.170 kg; p=.004). Smoking was also associated with lower body weight, BMI and total fat mass.

Our pre-planned investigation of bone turnover markers and relevant hormone levels in these twins with substantial smoking-associated BMD differences should help to explain how smoking adversely affects BMD and fracture risk.

**P-MON-104**

## The Hong Kong Adolescent Bone Health Cohort Study (ABC Study): an analysis of cross-sectional data

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**Aim of the study:** Osteoporosis is a health problem worldwide. Little is known about bone accretion in adolescents particularly in Asia. Cohort study on healthy adolescents can help us to delineate the relationship between BMD and anthropometric, growth parameters, and lifestyle factors during adolescence.

**Methods:** A total of 207 healthy girls (aged 10-11) and 208 healthy boys (aged 11-12) participated in this cohort study. At baseline, BMD, birth weight, body composition, anthropometry, pubertal development and lifestyle factors were measured and recorded. In this analysis, we focused on investigating the relationship of BMD to body composition and anthropmetric parameters.

**Results:** Significant correlation of age, pubertal development, body composition, anthropmetric parameters with BMC and BMD at various sites were observed in both sexes. Among all the parameters, lean mass was the major determinant for BMC (girl, r=0.85-0.89; boy, r=0.82-0.88) and BMD (girls, r=0.50-0.77; boy, r=0.39-0.65). After controlling for age or pubertal development, the results were similar.

**Conclusion:** To our knowledge, this is the first cohort study in Asia-Pacific. This cohort will provide important information on normative data of bone mass and changes in bone accrual during growth. In this cross-sectional analysis, BMD and BMC values showed highest correlation with lean mass than with weight and height, suggesting that muscle mass may be one of major determinants for bone mass during puberty. Further analyses taking into account other confounding factors such as physical activity, dietary intake, sexual maturation indices, as well as subsequent follow up of the cohort, are in progress.

**P-MON-106**

## Prediction of bone loss in elderly men by biochemical markers

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Bone mineral density (BMD) declines with advancing age with a high inter-subject variability. However, it is not known what factors account for the variability of bone loss. This study was designed to assess the contribution of common biochemical markers to the variability in bone loss in elderly men. This study included 378 men aged 60+ years as at 1989, who subsequently have had at least two BMD measurements (GE Lunar Corp, WI) during 1989 and 2005. Sex hormones (estradiol, testosterone, sex hormone binding globulin (SHBG), 25-hydroxyvitamin D, parathyroid hormone, insulin-like growth factor I) and osteocalcin (OC) were measured at baseline. A linear mixed-effects model was used to estimate the contribution of biochemical markers to bone loss. The annual mean percent change in BMD at the femoral neck was -0.6 (SD 2.5). Advancing age and higher BMD were significantly associated with bone loss. Furthermore, increased bone loss was found to be significantly associated with higher levels SHBG ($p = 0.02$) and OC ($p = 0.01$) after adjusting for baseline BMD and age. Both markers accounted for less than 5 percent of variance in bone loss. There was no association between bone loss and any other hormones.

These results suggest that although SHBG and OC were associated with bone loss in elderly men, the strength of association was very modest. Because of their low predictive value, it is unlikely that these markers can be used to predict change in BMD for an individual patient.

**P-MON-108**

## Older women have lower 25-hydroxyvitamin D levels and a higher prevalence of vitamin D deficiency and insufficiency than middle-aged and older men

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Studies from North America and Europe have reported high prevalence of vitamin D insufficiency, seasonal variation in 25OHD levels, and age- and gender-related differences in 25OHD levels in middle-aged and older subjects. Whether these findings occur in a subtropical location like northern New Zealand is not known.

1606 healthy older women (mean age 74y) and 378 healthy middle-aged and older men (mean age 57y) living in Auckland were recruited for 2 separate studies of calcium supplementation. 25OHD was measured at baseline in all participants.

There was significant monthly variation in 25OHD levels in men and women, but women had lower levels throughout the year, a finding that persisted after adjustment for potential confounders. The prevalence of vitamin D insufficiency (25OHD < 50nmol/L) in women was high (summer:28-58%, winter:56-74%) whereas in men it was much lower (summer:0-7%, winter:0-20%). 25OHD levels declined with age in women but not men. The major determinants of 25OHD in both genders were month of blood sampling, physical activity (both surrogates for UV-B exposure) and fat mass. Neither the gender-related differences in 25OHD levels nor the relationship between adiposity and 25OHD was explained by differences in vitamin D binding protein levels.

Vitamin D insufficiency is common in Auckland in healthy older women but not in healthy middle-aged and older men. The striking gender-related differences in 25OHD levels are not due to differences in the biological determinants of 25OHD but more likely due to gender-related differences in behavioural and cultural factors that influence sunlight exposure.

**P-MON-110**

## Is the message getting through? An Analysis of DXA use in Australia 1995 to 2005

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**Introduction:** In the last decade due to various educational programs the awareness of osteoporosis in Australia has greatly increased. This is mirrored in the significant increased use of DXA scans. There is however no published data on the success of educational efforts at targeting those most at risk.

**Method:** In the absence of any reliable Australia wide survey we have used claims against Medicare DXA item numbers as an index of osteoporosis awareness. Utility data for the various DXA item numbers were obtained for males and females, over a ten-year period from 1995 to 2005.

**Results:** For the age groups 55-64, 65-74 and 75-84 the number of DXA services has been steadily increasing over the last 10 years. However, for males and females >85yrs there has been a relatively smaller increase in DXA utilisation in both absolute number and scans per capita. The absolute number of males undergoing DXA is also low compared to females but when corrected for the prevalence of osteoporosis there are only small differences between the two sexes.

**Conclusion:** The increased use of DXA over time confirms significant success of the osteoporosis educational programmes to date. The programmes have however failed in addressing the very elderly. In view of the high prevalence of osteoporosis in subjects over 85 it may be cost effective to design educational programmes specifically targeting this section of the community. The results also question the common assumption that awareness of the risk of osteoporosis in males is inappropriately low compared to females.

**P-MON-112**

## Genetic linkage and genomic imprinting effects on osteoporosis

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**Introduction**: Osteoporosis is a common disease with strong genetic control. Genomic imprinting, an epigenetic phenomenon by which the expression of a gene is determined by its parental origin, could be a mechanism contributing to the inheritance of osteoporosis.

**Purposes:** 1) To identify osteoporosis-related genetic loci; 2) To test the hypothesis that genomic imprinting effects may influence the variation of osteoporosis.

**Methods:** We conducted a whole genome linkage scan in 451 extended families with a total of 4,498 subjects, genotyping 393 microsatellite markers for the 22 autosomes, employing a composite phenotype that combines osteoporotic fracture and low bone mineral density (BMD), i.e. Z score in the bottom 10% percentile. Furthermore, we performed genome-wide imprinted linkage analyses under an allele-sharing model, by allowing for parent-of-origin effects. In addition, extensive simulations were performed to identify the genome-wide significant and suggestive significant thresholds based on the studied sample.

**Results:** For conventional linkage analyses in the total sample, we identified suggestive linkage on chromosomes 14q32 (LOD=2.61), 7p14 (LOD=2.42), and 11q25 (LOD=2.09). Suggestive evidence of loci where imprinted genes might be acting was found with paternally derived alleles on chromosomes 1q42 (LOD= 2.12) and 9q34 (LOD=1.88). Tentative evidence of linkage to maternally derived alleles was found on chromosome 7q22 (LOD=1.67).

**Conclusion:** The current study further delineates the genetic basis of osteoporosis, and for the first time, suggests that genomic imprinting effects may play a role in the etiology of osteoporosis.

**P-MON-113**

## Prostate cancer and BMD: Geelong Osteoporosis Study

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Previous observational studies have shown that women with breast cancer have increased BMD attributed to high oestrogen exposure. Similarly, testosterone and oestrogen may be implicated in the pathogenesis of prostate cancer. To test the hypothesis that untreated men with prostate cancer have increased BMD, we measured BMD in a randomly-selected sample of 644 men aged ≥60yr with and without prostate cancer.

Twenty-nine men were excluded due to oral glucocorticoid, bisphosphonate, calcitriol or exogenous testosterone exposure (n=23), and self-reported orchiectomy or androgen deprivation therapy (n=6). Among the remaining 615 men eligible for analysis, there were 36 cases of prostate cancer. BMD was measured using DXA (Prodigy, Pro); self-reported prostate cancer, current medication use and smoking history were documented by questionnaire.

Men with prostate cancer were older (median (IQR), 79.9 (74.9-85.1) vs 73.9 (67.0-81.0)yr) than controls, however there were no differences in weight, height or smoking history (20/36 vs 342/579, P=0.7). Among non-smokers age- and weight-adjusted BMD was 9.8% lower at the spine (mean±SE, 1.196±0.060 vs 1.326±0.015g/cm$^2$, P=0.04) and 4.9% lower BMD for whole body (1.173±0.026 vs 1.234±0.007g/cm$^2$, P=0.02) for men with prostate cancer. Trends for lower BMD were found at the femoral neck (P=0.1), ultradistal-forearm (P=0.1) and mid-forearm (P=0.09). In contrast, no associations were detected among smokers.

Untreated men with prostate cancer may have reduced BMD and smoking appears to mask this association. The deficit in bone mass may reflect the complexity of the hormonal milieu in which prostate cancer arises.

**P-MON-115**

## Knowledge of osteoporosis in Iranian female adolescents

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**Purpose:** The purpose of this study was to explore female students' knowledge about osteoporosis efficient factors in Tehran, Iran

**Method:** A cross-sectional study with a 31-item questionnaire was used in this research. A convenience sample of 1000 adolescents in grade 1-3 who attended at 6 high schools participated in this study. The data was described by chi$^2$ test.

**Result:** According to 22 question about knowledge, suitable knowledge of students was estimated 40.8% .Of 9 familial factors ,only correlation between occupation of father and knowledge was significant statically($p<0.05$). Knowledge was higher in independent- medicine versus dependent- medicine occupation.

**Conclusion:** Overally, the knowledge of these adolescents who were in crucial period of their lives for accruing bone mineral, was limited and they didn't have enough knowledge of efficient factors of osteoporosis (complication of diseases, sex, race, smoking, sun exposure, exercise, calcium-rich foods and menopause).

**P-MON-117**

## Vitamin K intake is lower in knee osteoarthritis patients - A general population-based cohort study - the ROAD project

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**Aim:** Osteoarthritis (OA) is an illness that lowers the QOL of the elderly, shortens their healthy life expectancy. In order to clarify the relationship between knee OA and nutrition, the present study analyzed nutrition data from "Research on Osteoarthritis Against Disability"(ROAD).

**Methods:** Subjects were 855 residents of Hidakagawa-cho, one of the participating areas of the ROAD, who were older than 40 years of age (mean age: 69.3 years) and agreed to participate in the baseline survey. Using the brief-type self-administered diet history questionnaire (BDHQ), which ascertains average dietary habits in the last month, each subject was interviewed in order to calculate energy intake and daily intake of the following 29 nutrients: animal protein, plant protein, animal fat, plant fat, carbohydrate, Na, K, Mg, Ca, P, Fe, Zn, Cu, Mn, vitamin D, E, K, B1, B2, B6,B12, niacin, folic acid, pantothenic acid, cholesterol, soluble dietary fiber, insoluble dietary fiber, and salt. Knee OA was defined as ≥ grade 2 OA according to the Kellgren-Lawrence grading system

**Results:** Among 29 nutrients examined, mean intake of each nutrient was compared between subjects without or with knee OA. Significant differences were observed for the following 16 parameters: plant protein, plant fat, carbohydrate, K, Mg, Zn, Mn, vitamin B1, B2, B6, B12, C, E, K, niacin, pantothenic acid. Significant 16 subjects were divided into two groups with respect to the median value of these parameters, and the relationship of each parameter to knee OA was assessed by step-wise logistic regression analysis. The results showed that vitamin K intake is lower in knee osteoarthritis patients.

## *DENSITOMETRY, IN VIVO ANALYSIS*

**P-MON-121**

### Structural geometrical accuracy using a custom built anthropometric phantom of the proximal femur

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Material and structural properties influence bone strength. Structural strength may be determined through imaging methods, though there is no commercially available phantom to assess geometric accuracy. This paper describes an anthropometric femur geometry phantom and it's testing on DXA, pQCT and QCT scanners.

Phantom neck cortex is made in 6 segments with varying elliptical outer contours and fixed inner circular contour. Along major axis, cortical thickness ranges from 0.5mm to 4mm. Cortical shell is made from bone equivalent dental plaster formulation, "trabecular core" of commercially available trabecular substitute equivalent to mineral density of 200 mg/cm$^3$. Conical shaft cortex was filled with paraffin wax to simulate fatty marrow. Phantom was scanned with Hologic QDR4500A DXA, StratecXCT2000 pQCT and GE-LightSpeed 64 Multi-slice-CT scanners. Pixel spacing along projections was 1.35, 0.15 and 0.68mm respectively. Algorithms were written specifically to generate comparable properties across 3 modalities using projection principles.

Coefficients of determination, $R^2$ values were high in all methods, best in CT. Errors ranged to 16% in bone CSA and 20% in Z. CT errors were generally systematic and probably amenable to algorithm improvement. DXA errors were modest except at one segment.

Table 1: Average % errors and $R^2$ for DXA, QCT and pQCT against phantom dimensions (Column 1).

| | QDR4500A | QCT | pQCT |
|---|---|---|---|
| **Bone CSA** | | | |
| **$R^2$** | 0.958 | 0.997 | 1.000 |
| 1.718 | 0% | -15% | 16% |
| 1.954 | 2% | -15% | 12% |
| 3.367 | -14% | -2% | 4% |
| 4.545 | -2% | -3% | 1% |

**P-MON-123**

### Ultrasonic measurement of human cancellous bone utilizing Biot's theory and its clinical application

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**Aim:** To assess bone mass with quantitative ultrasound (QUS) utilizing the Biot's theory and compare that measured by the current bone mineral density measurement instruments.

**Methods:** In vitro study: Human femoral head was sectioned to 10 mm-thick slices perpendicularly to the femoral cervical axis. The samples were subjected to the measurement using PVDF transducers, and the bone mass and elastic constant of cancellous bone (ECCB) were calculated form the slow and fast waves. The bone volume ratio(BVR) was measured in the same sample by micro CT, and structural parameters were calculated from the micro CT data.

In vivo study: In adult subjects aged 20-80 years, BMD of distal radius (RBMD) was measured using LD100, QDR4500 (DXA), and XCT960 (pQCT) (BMD of the lumbar spine (LBMD) was also measured using DXA).

**Results:** In vitro study: BVR and attenuation of transmitted slow waves was negatively correlated (r=0.88). BVR was positively correlated with the sound velocity of fast waves (r=0.73), and with Mean Intercept Length (0,0) (r=0.80). A similar tendency was noted in ECCB.

In vivo study: The correlation coefficients of RBMD measured by LD100 and other instruments are shown below (Table). Concerning the presence or absence of vertebral insufficiency fracture (50 years of age or older), the RBMD was significantly lower in patients with fracture than in those without fracture in all measurements using LD100, XCT960, and QDR4500. ECCB was also lower in the fracture group, but no significant difference was noted in LBMD.

| correlation coefficient | XCT960 | QDR4500-UD | QDR4500-L |
|---|---|---|---|
| LD100 | r=0.744* | r=0.832* | r=0.549* |

* p<0.01

**Conclusion**: QUS utilizing the Biot's theory is useful for evaluation of fracture risk.

**P-MON-125**

## Peripheral quantitative cumputed tomography in patients receiving long term glucocorticoid therapy

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Glucocorticoid (GC)-induced osteoporosis (GIOP) is a common iatrogenic cause of bone fragility. Although 50% of patients receiving longterm GC suffer low-trauma fractures, those at higher fracture risk cannot be predicted confidently. We have established a cohort to evaluate novel approaches to the diagnosis and monitoring of GC-induced bone loss, including the potential role of peripheral quantitative computed tomography (pQCT). PQCT has been little studied in GIOP. Patients (n=22) aged 21-70 years (6 male, 16 female) taking prednisolone (median cumulative dose 14,866 mg) for rheumatic disorders were evaluated by dual energy Xray absorptiometry at the lumbar spine (PA and lateral) and hip, pQCT at the 4% and 33% tibial sites, and quantitative ultrasound at the calcaneus. Total volumetric density (Tot D) and trabecular volumetric density (Trab D) at the 4% site were highly correlated with femoral neck BMD ($r = 0.76$, $P < 0.01$; $r = 0.74$, $P < 0.01$, respectively). Tot D and Trab D at the 4% site also were correlated with L2 and L3 lateral BMD ($r = 0.60$, $P<0.005$; $r = 0.43$, $P<0.05$, respectively) and less consistently correlated with L2 and L3 PA BMD. Broadband ultrasound attenuation (BUA) in 41 GC-treated patients (21 – 70 years: 12 male, 29 female; median GC dose 11, 357 mg) was most highly correlated with L2 and L3 lateral BMD ( $r = 0.458 – 0.602$; $P < 0.01$ - $< 0.001$) and total hip BMD ($r = 0.411 – 0.575$; $P < 0.01$).

These preliminary findings strongly suggest potential roles for pQCT and BUA in the diagnosis and monitoring of GC-induced bone loss and indicate the need for further prospective evaluation of these techniques in GC-treated patients.

**P-MON-128**

## Subregional BMD is a better predictor of vertebral morphology than standard DXA-derived BMD measures

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Vertebral morphology is influenced by age, gender, race, vertebral level and BMD[1, 2]. The ability to predict vertebral morphology from BMD is important in a population with low bone mass since vertebral morphology affects spinal curvature and therefore spinal loads and fracture risk. Measurement of BMD within vertebral subregions is better able to differentiate between individuals with and without vertebral fractures than standard DXA parameters. The aim of this study was to determine whether lateral subregional BMD

measured with DXA was a better predictor of vertebral morphology than standard DXA measures of BMD. AP and lateral DXA scans of the lumbar spine and lateral spinal radiographs were performed on 22 postmenopausal women with osteoporosis, of which 7 had sustained a vertebral fracture. Standard DXA parameters were derived, and in addition, BMD was calculated in 6 vertebral subregions (srBMD) from the lateral scan[3]. Radiographs were used to measure maximal anterior vertebral height loss ($H_{Amax}$) from T1-T12. Simple linear regression determined the ability of the different measures of BMD to predict $H_{Amax}$. Standard measures of BMD were unable to account for a significant proportion of the variance observed in $H_{Amax}$ ($r^2$=0.06-0.08, $p$>0.05), other than the total lateral spine (L2-L4) measure ($r^2$=0.19, $p$<0.05). All srBMD measures, other than the subregion adjacent to the superior endplate, were able to account for a significant proportion of the observed variance in $H_{Amax}$ ($r^2$=0.18-0.28, $p$<0.05). Subregional BMD has a stronger association to vertebral morphology than standard DXA-derived BMD measures, providing further evidence that srBMD measures are more informative of vertebral fragility.

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**P-MON-130**

## Determination of precision error for low bone mass in DXA by Phantom Study

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**Background:** Precision error determines the ability of DXA systems to detect small changes in patient bone mineral density(BMD).Factors affecting precision include the scan region, consistency of scan acquisition and analysis, operator training, densitometry equipment, software, and short-and long –term variation in densitometry performance.

DXA systems perform best when scanning healthy young subjects of average size, when the optimal levels of x-rays reach the detectors .A variety of bone mass could be introduce to fluctuation of coefficient of variation (%CV).

**Aim:** The aim of this study is evaluation of %CV for Hologic (QDR-4500C), Lunar (DPX-MD) and Norland (XR-46) by very low bone mass created spine phantom.

**Material and Methodes:** A spine phantom was made by cooperation of Research Center of Science and Technology in Medicine (RCSTIM) and Endocrinology and Metabolism Research Center (EMRC).

This phantom consisted of $K_2HPO_4$ powder and Perspex that simulated osteoporotic spine.10 scan form phantom performed by each system without repositioning. Acquisition and analyses was performed by trained technologist. We used fast array mode and medium mode for Hologic and Lunar respectively, since the Norland system cannot get image in standard mode, we applied high precision mode for it . BMD precision error was calculated as the SD*100/Mean BMD.

**Results:** We found %CV equal 0.81, 1.46 and 0.88 for Hologic, Lunar and Norland respectively.

**The Mean of the BMD, SD and %CV of systems**

| Densitometer Model | Mode | Number of scan | Mean | SD | %CV |
|---|---|---|---|---|---|
| Hologic QDR-4500C | Fast array | 10 | 0.705 | 0.0057 | 0.81 |
| Lunar DPX-MD | Medium | 10 | 0.813 | 0.0119 | 1.46 |
| Norland XR-46 | High precision | 10 | 0.682 | 0.0060 | 0.88 |

**Conclusion:** We conclude that Hologic has good %CV for very low bone mass than others systems and calculation of BMD by Lunar is higher and by Norland is lower than Hologic.

**P-MON-132**

## Bone mineral density in young men patients with Ankylosing Spondylitis

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**Objective**: To determine bone mineral density (BMD) in young men patients with Ankylosing Spondylitis (AS).

**Subjects and Methods**: Twenty five men patients with AS aged 15-29 years were studied (Modified New York criteria 1984) compared with twenty age matched headthy men as controls.

BMD of the lumbar spine and femoral neck were measured by dual X-ray absoptiometry (DEXA).The activity of the disease were assessed by a visual analog scale (VAS), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), ESR

**Results:** Mean age 20.6 (4.2). Mean disease duration (year) 3.1 (1.8).

BMD of patients with AS were reduced in both the lumbar spine and femoral neck compared with controls group: (0.86 (0.16) g/cm$^2$ ($p< 0.001$); 0.71 (0.15) g/cm$^2$ ($p < 0.001$) respectvely.

**Conclusion:** These data suggest that a reduced BMD spinal and hip in patients with AS may cause relation disease activitiy.

**P-MON-134**

## Establishing a normal range of bone and tissue in sheep

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This study aimed to follow the changes in bone mass and tissue composition in sheep from 4 months to 4 years of age and to determine the age of peak bone mass and tissue proportions. Knowledge of the age of skeletal maturity is important in designing animal models of bone strength, remodelling and metabolism.

A cohort of 14 female Merino lambs had DXA measurements (mean±SD) of the lumbar spine and total body performed every 6 months from 4 months of age to 2 years of age. Three and four year old ewes were included to complete the data set. Measurements of anaesthetised animals were performed on a Hologic QDR2000 bone densitometer.

| | Bone | | Composition | | | |
|---|---|---|---|---|---|---|
| **Age (months)** | **Spine BMD (g/cm$^2$)** | **Total Body BMD (g/cm$^2$)** | **BMC (g)** | **Fat (kg)** | **Lean (kg)** | **Weight (kg)** |
| **4** | 0.519±0.045 | 0.679±0.024 | 298.5±49.4 | n/a | n/a | n/a |
| **10** | 0.525±0.039 | 0.823±0.046 | 457.6±68.2 | 1.4±0.6 | 24.7±1.9 | 26.6±2.4 |
| **16** | 0.869±0.057 | 1.052±0.037 | 1058.4±99.9 | 8.5±2.2 | 33.7±1.6 | 43.2±2.9 |
| **24** | 0.868±0.083 | 1.067±0.051 | 1181.3±92.2 | 7.2±2.4 | 39.3±2.4 | 47.6±3.5 |
| **36** | 0.819±0.073 | 1.079±0.058 | 1278.1±153.4 | 10.2±3.6 | 41.5±4.5 | 53.0±6.2 |
| **48** | 0.907±0.100 | 1.117±0.053 | 1270.1±184.7 | 7.3±3.9 | 51.4±6.7 | 51.4±6.6 |

BMC peaked by 36 months, with lean tissue plateauing by 24 months. Near peak BMD was observed in the lumbar spine and total body at 16 months of age (at approximately the mass at which Merino ewes reach sexual maturity). This peak BMD is 10% lower than the human lumbar spine.

We conclude that studies requiring peak bone density should use sheep at least 16 months old, whilst peak lean mass requires sheep at least 24 months old.

## *BONE QUALITY AND MECHANICAL PROPERTIES*

**P-MON-136**

### Effect of screw insertion torque level on cortical bone pullout strength

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During insertion, screw torque can reach levels at which bone failure and stripping occurs. Surgeons reportedly induce torque levels surprisingly close to this limit, tightening to an average of 86% of failure torque. Although high screw forces are desirable for stability, a trade-off must exist between screw force levels and cumulative damage from tightening affecting the strength of the surrounding bone. The objectives of this study were to: 1) measure the rotational characteristics of cortical bone screws inserted to clinical and failure torque levels, and 2) determine the effect of torque level on material strength as assessed by screw pullout. Ten pairs of ovine tibiae were used. One side of each pair was used for measuring ultimate failure torque (Tmax) at sites along the shaft. These Tmax and bone density values were used to predict Tmax at contralateral sites. Screws were inserted and tightened to 50%, 70% and 90% of predicted Tmax. Pullout tests were performed and maximum force values normalized by cortical thickness. Failure tests indicated tightening to 86%Tmax occurs after yield and leads to an average 51% loss in stiffness. A trend towards lowered pullout strength for screws tightened to 90%Tmax compared to 50%Tmax and 70%Tmax ($p<0.05$) was observed. A single test at 90%Tmax resulted in a pullout strength value 40% less than the average 70%Tmax pullout strength. Limitations include small sample size and inexact estimation of Tmax. We conclude screw tightening to clinical levels may lead to sufficient damage to compromise holding strength in surrounding cortical bone.

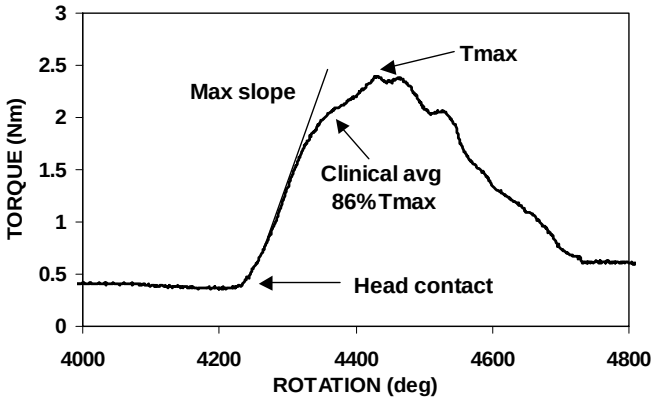


Figure 1. Typical test-to-failure torque curve from head contact through failure. Note the clinical average of 86%Tmax occurring after material has begun to yield.

**P-MON-138**

### Three-dimensional simulation of trabecular bone remodelling

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Computer simulations have been used to connect discrete physiological events of bone remodelling with the observed changes in mass and internal architecture. We have recently developed an algorithm simulating the 3D remodelling process in trabecular bone. Our aim has been to use *a priori* knowledge of dynamic remodelling variables to simulate trabecular architecture transformation over time. Similar to other published models, our algorithm is based on a stochastic approach, with each remodelling "cycle" consisting of a randomly selected surface voxel site where resorption (osteoclast activity) and formation (osteoblast activity) is simulated. The algorithm was applied to a limited number of micro-computed tomography datasets of

human vertebral trabecular specimens and tested for stability using repeated simulations of 500,000 cycles with a net formation deficit of 6.7% at each cycle. High repeatability (CV<0.5%) was observed in measured morphometric parameters including bone volume, bone surface area, trabecular thickness, trabecular separation, trabecular number, and degree of anisotropy. Comparison of simulated changes with a small cross-sectional human dataset indicated considerable population variability in parameters for a given bone volume fraction. Repeatable morphometric variability was produced through our algorithm by altering resorption and formation input variables and by setting a preferential direction for surface voxel selection. Given the anisotropic structure of bone, directional dependence is not unexpected and points to the importance of considering targeted remodelling in response to load. A longitudinal study using an animal model of bone loss is underway which will allow further algorithm refinement to simulate temporal changes in trabecular bone architecture.

**P-MON-140**

## Analysis of the microstructural properties and bone strength of the human femoral head

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**Objective:** The present study quantitatively investigated the trabecular bone microstructure and bone strength of the primary compressive trabecular system of the human femur with and without osteoporosis using the micro-CT, the finite element analysis (FEA) and the INSTRON material testing system.

**Materials and Methods:** Cylindrical trabecular bone cores were obtained from primary compressive trabecular system of human femoral head with and without osteoporosis under the fluroscope. The sample size was diameter of 19mm, height of 15mm. All samples were scanned using micro-CT. The microstructural parameters were determined by automatically software such as trabecular thickness, seperation, bone volume fraction, degree of anisotropy, structure model index and trabecular number. Based on 2-D slice micro-images, a finite element model was reconstructed. After micro-CT scanning, the compressive tests were performed for all samples using INSTRON material testing system. The indices reflecting bone strength including yield stress and Young's modulus were determined.

**Results:** Our data showed deterioration of trabecular microstructure and decrease in the bone strength in the compressive trabecular system with osteoporosis. Relationship between the microstructural and the mechanical properties were significantly consistent in the primary compressive trabecular system with and without ostoporosis.

**Conclusion:** Our results suggested that micro-image-based finite element analysis as a substitute of the bone strength test can be a useful tool to predict the fracture risk.