2006 IOF+ANZBMS Combined Meeting

Combined Meeting of the
3rd IOF Asia-Pacific Regional Conference on Osteoporosis *and the*
16th Annual Meeting of the ANZ Bone & Mineral Society
~ 22-26 October 2006, Port Douglas, Australia ~
www.anzbms.org.au/asm/asm2006

SESSION TIME: 1030 - 1200, Monday 23 Oct 2006

Oral Abstracts

The Roger Mellick Young Investigator Award Entrants

O1

Cyclic GMP-dependent protein kinase II (cGKII) controls hypertrophic differentiation of chondrocytes through phosphorylation and inactivation of GSK-3β

Y. Kawasaki, F. Kugimiya, H. Chikuda, T. Ikeda, S. Kamekura, F. Yano, T. Saito, A. Higashikawa, M. Ushita, K. Nakamura, U.I. Chung and H. Kawaguchi
Orthopaedic Surgery & Tissue Engineering, University of Tokyo, Japan

cGKII is a kinase that lies downstream of the C-type natriuretic peptide/GC-B pathway which is essential for skeletal growth in both humans and rodents. A naturally occurring mutant rat KMI that lacks the kinase domain of cGKII is known to exhibit dwarfism due to the impaired hypertrophic differentiation of growth plate chondrocytes. To investigate the mechanism underlying the kinase activity of cGKII, the present study initially performed screening of its phosphorylation targets and found that glycogen synthase kinase-3β (GSK-3β) was strongly phosphorylated and inactivated by cGKII. In cultured mouse chondrogenic ATDC5 cells, type X collagen (COL10) mRNA level was induced by the cGKII overexpression and by an addition of lithium chloride, a selective inhibitor of GSK-3β. The cGKII-induced COL10 expression was attenuated by co-transfection of a constitutively active mutant GSK-3β, which was confirmed not to be phosphorylated by cGKII. We then examined the involvement of GSK-3β in the cGKII action in vivo. cGKII-deficient (–/–) mice exhibited skeletal growth retardation with an enlarged growth plate due to the impairment of chondrocyte hypertrophy. Although GSK-3β–/– mice were embryonically lethal, GSK-3β+/– mice developed and grew

normally. When we generated the compound-deficient mice (cGKII–/–;GSK-3β+/–), both the impaired growth and the enlarged growth plate seen in cGKII–/– mice were significantly restored, indicating that sufficient GSK-3β is needed for suppression of the chondrocyte hypertrophy by the cGKII deficiency. We conclude that cGKII controls skeletal growth by hypertrophic differentiation of growth plate chondrocytes through phosphorylation and inactivation of GSK-3β.

O2

Increased mortality following major and minor osteoporotic fractures: a 15 year follow-up study

D. Bliuc, T.V. Nguyen, J.A. Eisman and J.R. Center
Bone and Mineral Research Program, Garvan Institute of Medical Research, St Vincent's Hospital and UNSW, Sydney, NSW Australia

Although, immediate post-fracture mortality risk has been documented, long-term mortality is unclear. This study examined mortality following all clinical osteoporotic fractures over 15 years.

Fracture and mortality from all subjects (60+) in Dubbo were collected from 1989-2005. Fractures were classified into hip, vertebral, major (proximal humerus, distal femur, proximal tibia, pelvis, multiple rib) and minor (all others excluding finger and toe). Age and sex-specific standardised mortality ratios (SMR) were calculated using population mortality rates from Australian Bureau of Statistics. Mortality over time was analysed according to fracture type and age.

There were 880 fractures in women and 329 in men, over 27,687 and 20,054 person-years, respectively, followed by 399 deaths in women, and 181 in men. SMRs were increased following hip [women: 2.5 (1.7- 3.6) and men: 3.6 (1.9- 6.8)], vertebral [women: 1.9 (1.4- 2.7) and men: 2.3 (1.5- 3.7)], major fractures [women: 1.8 (1.2- 2.7) and men: 1.7 (1.0- 3.2)], and minor fractures in the older age-groups [women 1.5 (1.1- 2.1) and men 2.1 (1.2- 3.6)]. Mortality was relatively higher for hip and vertebral fractures in younger age-group with 5-12 life years lost. Mortality risk was highest in the first 5 years, but hip and vertebral fractures were associated with increased risk up to 10 years. A subsequent fracture was associated with a further 1.5- 2.3-fold increased mortality risk.

This study demonstrates increased mortality following all major fractures, and minor fractures in older people. The high mortality risk post-fracture decreased over time but re-fracture increased it again.

O3

Anabolic effect of PTH in young rats is attenuated by calcitonin

J.H. Gooi[1], J.M.W. Quinn[1], M.T. Gillespie[1], M.A. Karsdal[2], T.J. Martin[1] and N.A. Sims[1]
[1]St Vincent's Institute, Fitzroy VIC, Australia 3065; [2]Nordic Bioscience Inc, Copenhagen, Denmark

Parathyroid hormone (PTH), at continuously high levels is a catabolic agent in bone, yet when administered intermittently, PTH has an anabolic effect, and is used for treating osteoporosis. To investigate whether active osteoclasts contribute to the anabolic effect of PTH, rats were treated with anabolic doses of PTH (3μg/kg/day or 30μg/kg/day hPTH(1-34) for three weeks) in the presence and absence of salmon calcitonin (sCT), an acute blocker of osteoclast activation. Treatment with hPTH(1-34) at 3μg/kg/day significantly increasing femoral trabecular BMD (Tb.BMD) by 32%. This was prevented when sCT was administered concurrently with PTH. In a separate experiment, treatment with hPTH(1-34) at 30μg/kg/day elevated Tb.BMD by 108%, and raised tibial trabecular bone volume (BV/TV) by 255%. When sCT was administered concurrently with hPTH(1-34) this anabolic effect was significantly attenuated; there was no significant elevation in Tb.BMD, and the anabolic effect of PTH on BV/TV was halved. Administration of sCT alone had no effect on Tb.BMD or BV/TV. Administration of sCT 5 hours after PTH, or 1 hour before PTH administration did not block the anabolic effect of PTH on Tb.BMD, indicating that active osteoclasts are required within one hour of PTH administration for the full anabolic effect of PTH.

In conclusion, this data suggest that the anabolic effect of PTH is dependent on acute activation of osteoclasts and this can be blocked by co-administration of CT. Thus in young female rats osteoclast-osteoblast communication is critical for PTH anabolic effects on bone.

O4

Kruppel-like zinc-finger transcription factor 5 (KLF5) contributes to the last stage of endochondral ossification through transcriptional induction of MMP-9

Y. Shinoda, N. Ogata, I. Manabe, T. Yamada, N. Kugimiya, U.I. Chung, R. Nagai, K. Nakamura and H. Kawaguchi
Orthopaedic Surgery and Cardiovascular Medicine, University of Tokyo, Tokyo, Japan

Aim: Kruppel-like zinc-finger transcription factor 5 (KLF5) is a member of the Sp/KLF family of transcription factors like osterix. We investigated the involvement of KLF5 in skeletal tissue metabolism.

Methods and Results: By immunohistochemistry and RT-PCR, KLF5 was expressed mainly in hypertrophic chondrocytes and osteoblasts. To analyze the in vivo function, we generated KLF5-deficient mice and investigated the skeletal phenotype. Although homozygous KLF5-deficient mice (-/-) died before 8.5 dpc, the heterozygous deficient (+/–) mice developed and grew normally, except for a skeletal growth retardation during the perinatal period. Histological analyses of the long bones of KLF5+/– embryos revealed that matrix calcification of hypertrophic zone at 15.5 dpc and the following bone marrow cavity formation were delayed. In KLF5+/– neonates, the hypertrophic zone was enlarged with remaining cartilage matrix and insufficient ossification. In primary chondrocytes derived from KLF5+/– mice, the expression of MMP-9, but not COL10, MMP-13 or VEGF, was suppressed than that from WT littermates. When we overexpressed KLF5 in mouse chondrogenic cell line ATDC5, the expression of MMP-9 was stimulated, and the promoter activity of MMP9 was strongly promoted in ATDC5 transfected with MMP-9 promoter-luciferase construct. On the other hand, osteoclast formation and MMP9 expression were not affected in osteoclast precursors overexpressing KLF5.

Conclusion: KLF5 insufficiency causes impairment of endochondral ossification in skeletal development through transcriptional inhibition of MMP-9. Considering that MMP-9-deficient mice are reported to exhibit a similar skeletal phenotype, the KLF5/MMP-9 system may play a pivotal role in the last stage of endochondral ossification.

O5

Measures of childhood fitness are associated with calcaneal quantitative ultrasound in adulthood: a 20 year prospective study

S. Foley, A. Venn, S. Quinn and G. Jones
Menzies Research Institute, University of Tasmania, Hobart, Australia

Aim: To describe the associations between childhood fitness measures and calcaneal quantitative ultrasound (QUS) parameters in adulthood.

Methods: A representative longitudinal sample of 1299 children (mean age 11yrs, range 7-15) was measured in the Australian Schools Health and Fitness Survey in 1985 and approximately 20 years later (mean age 32). Fitness measures in childhood included 1.6km run, 50m sprint, physical work capacity at 170 beats/min (PWC_{170}), lower limb muscle strength and standing long jump. We measured qualitative ultrasound index (QUI), broadband ultrasound attenuation (BUA), speed of sound (SOS), and estimated bone mineral density (eBMD) using a Sahara bone densitometer.

Results: In females, PWC_{170} in childhood was positively associated with all four QUS parameters ($p<0.001$-0.005). 1.6km run time was inversely associated with QUI ($p=0.029$), SOS ($p=0.030$) and eBMD ($p=0.029$). Standing long jump was positively associated with QUI ($p=0.016$), SOS ($p=0.004$) and eBMD ($p=0.016$). In males, 50m sprint time was positively associated with QUI, SOS and eBMD (all $p<0.05$). There was an interaction between age as a child and PWC_{170}, and all QUS parameters ($p<0.01$) with PWC_{170} measured in younger years having a greater influence on adult bone mass (7-10yr olds, $r=0.20$-0.25, all $p<0.05$; 11-15yr olds, all $r=0.06$, all $p>0.05$).

Conclusion: This is the first prospective study to demonstrate that childhood fitness levels, particularly in females and in the prepubertal years, are predictive of adult skeletal status as measured by QUS. These results suggest that interventions aimed at increasing fitness in early childhood may lead to an increase in peak bone mass.

O6

TRPV4 is involved in unloading induced suppression of bone formation and activation of bone resorption as a mechanonsensitive channel in bone

F. Mizoguchi[1,2,4], A. Mizuno[3], Y. Ezura[1,2], K. Nakashima[1,2], N. Miyasaka[2,4], M. Suzuki[3] and M. Noda[1,2]
[1]Molecular Pharmacology, Tokyo Medical and Dental University Medical Research Institute, Tokyo, Japan. [2]21st Century Center of Excellence Program for the Frontier Research on Molecular Destruction and Reconstruction of Tooth and Bone. [3]Department of Pharmacology, Jichi Medical School, Tochigi, Japan. [4]Department of Medicine and Rheumatology, Tokyo Medical and Dental University, Tokyo, Japan.

Transient receptor potential vanilloid 4 (TRPV4) is a Ca2+ permeable nonselective cation channel and it has been suggested to be a channel that plays a role to sense heat, hyposmolality and pressure in several tissues. In bone, TRPV4 is expressed in osteoblast and chondrocyte. But the function of TRPV4 in bone is not yet understood. Mechanical stress plays an important role in the maintenance of bone mass. Unloading in bed ridden patients induces rapid bone loss. However, it has not been fully understood how bone senses the mechanical stress. We examined whether TRPV4 is a sensor of mechanical stress in bone, and is involved in the bone loss due to unloading. TRPV4-/- mice were subjected to tail suspension, a model of unloading. In 3D-CT analysis, unloading for two weeks resulted in the reduction of the secondary trabecular bone mass and the height of the primary trabecular bone in wild type mice. TRPV4 deficiency blocked such reduction. In histomorphometric analysis, double calcein labeling showed the reduction in mineral apposition rate due to unloading in WT mice. But TRPV4 deficiency prevented such reduction. Osteoclast number in secondary trabecular bone was not significantly altered regardless of the genotype or the unloading conditions. But in primary trabecular bone, osteoclast number was increased by unloading in WT mice, and this increase was prevented by TRPV4 deficiency. These results indicate that TRPV4 is one of the mechanosensitive channels in bone and positively regulates bone formation in osteoblast and suppresses bone resorption under the mechanical stress.