Oral Abstracts

3B (Basic) - Osteoblast formation and function

O23 Frog-1, a novel osteoblast membrane protein stimulating mineralization
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Screening of cDNA libraries using a signal-trap approach to define the “secretome” of bone identified a gene novel to osteoblasts that we have termed fragilis-related osteoblast gene 1 (frog-1). This gene had previously been assigned membership of the interferon inducible transmembrane protein (Ifitm)/fragilis family (Ifitm5 or fragilis4) through in-silico homology but no putative function has been assigned or extensive localisation undertaken.

Frog-1 encodes a 14.8 kDa 134 amino acid protein with two transmembrane domains. Northern blot analysis showed bone-specific expression with no expression in any other embryonic or adult organs or tissues. In-situ hybridisation in mouse embryos showed expression localised on the periosteum and in the developing trabecular bone. Immunohistochemistry in newborn and adult bone showed intense frog-1 expression in areas of high bone activity such as the developing primary spongiosa, adult vertebrae and mandibular bone.
Expression of frog-1 in MC3T3, UMR106 and primary rat osteoblasts further confirmed the osteoblastic nature of this gene. Importantly, expression was associated with the onset of mineralisation in these cells suggesting a role in bone formation which was further confirmed by frog-1 protein co-localisation with mineralised nodules and bone sialoprotein.

Functional evidence of a role in mineralisation was demonstrated by adenovirus-mediated frog-1 overexpression in UMR106 and primary osteoblasts. Elevated frog-1 resulted in dose-dependent increases in mineralisation up to 40% and 70% respectively.

Thus, we have identified frog-1 as a novel osteoblast protein and demonstrated a stimulatory effect on mineralisation possibly identifying a new regulatory pathway in bone and novel potential therapeutic approaches.

O24
Activity of 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1) in human osteoblasts mediates a pro-osteogenic response
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The activation of vitamin D by 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1) in osteoblasts has been demonstrated in bone cells. However, the role for local synthesis of calcitriol (1,25D) is unclear. We have examined vitamin D3 metabolism in primary normal human osteoblasts (NHBC) and in human osteosarcoma (HOS) cells. These cells express CYP27B1 mRNA and when treated with 25-hydroxyvitamin D (25D) secreted picomolar levels of 1,25D into the culture supernatant. HOS cells secreted the most 1,25D (~250 pM/1x10^6 cells/3 ml medium) which correlated with the highest level of CYP27B1 mRNA. NHBC exposed to physiological levels of 25D (20-100nM) in the presence or absence of serum, exhibit up-regulated transcription of the downstream genes receptor activator of RANKL, OCN and osteopontin. In addition, induction of the negative regulator of 1,25D, the 25-hydroxyvitamin D-24-hydroxylase (CYP24), was associated with increased synthesis of 1,25D. Both 25D and 1,25D exhibited anti-proliferative effects, as determined by fluorescent labelling of cells with CFSE and measuring the decrease in fluorescence with cell doubling. Likewise, both metabolites promoted in vitro mineralisation by normal osteoblasts. Finally, when the the mRNA for CYP27B1 was reduced by 80% in HOS cells by siRNA, the production of 1,25D was markedly reduced when treated with 25D (100, 200 and 400nM) and the mRNA for CYP24 and OCN were reduced to levels of the untreated controls. Together, our results suggest that autocrine and/or paracrine pathways of vitamin D3 metabolism in osteoblasts may regulate key functions independently of circulating 1,25D such as signalling for a pro-osteogenic response.

O25
The role of neuropeptide Y in the central control of cortical bone homeostasis
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Neuropeptide Y (NPY) is a neural signalling molecule implicated in the central regulation of bone mass. NPY is a ligand for the Y2 receptor and loss of hypothalamic Y2 function increases cortical bone mass. NPY is elevated in the hypothalamus of leptin-deficient ob/ob mice, which have reduced cortical bone mass. The effect of hypothalamic NPY on cortical bone mass and formation was therefore investigated.

Bone mass and cortical osteoblast activity was determined in NPYKO mice and following viral-mediated NPY overexpression in the hypothalamus of wildtype [vNPY-wt] and Y2KO [vNPY-Y2KO] mice. These models were compared to non-treated Y2KO and ob/ob mice. Bone mineral content was determined using dual X-ray absorptiometry and osteoblast activity by histomorphometry.
Elevated central NPY increased fat mass in ob/ob and viral-treated mice. Cortical osteoblast activity was increased in Y2KO [35%] and NPYKO [55%] compared to controls. By contrast, and similar to ob/ob mice, elevated hypothalamic NPY reduced tibial BMC in wildtype and Y2KO, supporting the central nature of NPY signaling and the negative action being independent of the Y2.

Hypothalamic NPY expression has a marked role in cortical bone formation. Increased central NPY signaling is associated with reduced osteoblast activity and cortical bone mass.

**O26**

Mechanical sensitivity of the tibia is increased in mice over-expressing the vitamin D receptor in osteoblasts

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**Introduction:** Transgenic mice overexpressing vitamin D receptor in mature osteoblasts (OSVDR) have normal body mass, but greater cortical area and periosteal mineral apposition rates in long bones, compared with controls (FVB/N). We hypothesised that this was explained by greater mechanosensitivity in OSVDR long bones. We used tibial 4-point bending to determine the response thresholds for mechanically-induced bone formation in OSVDR and FVB/N mice.

**Methods:** We applied 4-point bending to tibiae of 48 OSVDR and 48 FVB/N mice, 4 months old, and measured bone formation with fluorochrome labels. Mice were subjected to 5 magnitudes of applied bending calculated to engender strains of 600, 900, 1200, 1500 and 1800 microstrain (ue) at the periosteal surface (40 cycles/d for 10 days, at 2.0 Hz). One group of each line was subjected to sham loading (1800 ue).

**Results:** Tibial CSA was greater in OSVDR than FVB/N (p < 0.01). Both genotypes responded to loading with increased lamellar bone formation between 600 and 1200 ue, and increasing woven bone formation from 1200 to 1800 ue. Lamellar and woven bone responses were significantly greater in OSVDR, with MS and BFR/BS significantly greater than FVB/N between 600 and 1200 ue (p < 0.05). No difference between mouse lines was observed for MAR. Woven bone formation was initiated at lower strains and markedly enhanced in OSVDR. Sham loading did not affect bone formation.

**Conclusion:** These data support our hypothesis and suggest that increased mechanical responsiveness of the osteoblastic network may contribute to the OSVDR cortical phenotype.

**O27**

Adiponectin stimulates the proliferation, differentiation and mineralization of osteoblastic MC3T3-E1 cells via the adiponectin receptor type1 and AMPK signaling pathway

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Adiponectin is a key mediator of metabolic syndrome that is caused by visceral fat accumulation. Adiponectin and its receptor are known to be expressed in osteoblasts, but their actions on bone metabolism are still unclear. In this study, we investigated the effects of adiponectin on the proliferation, differentiation and mineralization of osteoblastic MC3T3-E1 cells. Adiponectin receptor type 1 (AdipoR1) mRNA was detected in
these cells by RT-PCR. The additions of adiponectin (0.01-1.0 µg/ml) dose-dependently promoted their proliferation by BrdU assay. The AMP-activated protein kinase (AMPK) was activated by both adiponectin and a pharmacological AMPK activator, 5-amino-imidazole-4-carboxamide-riboside (AICAR), in these cells. AdipoR1 siRNA transfection potently knocked down the receptor mRNA, and its effect sustained as long as 10 days after transfection. The transfected cells showed decreased expressions of type I collagen and osteocalcin mRNA by real time PCR and reduced mineralization by von Kossa and Alizarin Red stainings. In contrast, AMPK activation by AICAR (0.5mM) in wild-type MC3T3-E1 augmented their proliferation, differentiation and mineralization. Neither AdipoR1 siRNA transfection nor AICAR treatment affected Runx2 mRNA or protein expressions. Taken together, this study suggests that adiponectin might stimulate the proliferation and mineralization of osteoblasts via the AdipoR1 and AMPK signaling pathway in autocrine and/or paracrine fashions in bone microenvironment.

O28
Adiponectin knock-out mice have increased trabecular number and bone volume at 14 weeks of age
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Adiponectin, a hormone secreted by adipocytes, regulates energy homeostasis and glucose and lipid metabolism. Plasma levels of adiponectin are negatively correlated with body fat mass. Adiponectin inhibits the formation and activity of osteoclasts and increases the proliferation and differentiation of osteoblasts in vitro.

The aim of our study was to determine the bone phenotype of adiponectin knockout mice.

At 8 and 14-weeks of age, male adiponectin-deficient (Ad-KO) and wild-type (WT) C57BL/6J mice had similar body weight. We scanned the left proximal tibia using micro-CT at 5µm resolution and analysed bone microarchitecture by 3D analysis. We found significant increases in trabecular bone volume (BV/TV) (19.12±1.05% in Ad-KO vs 8.29±1.13% in WT, p=0.02) and trabecular number (3.61±0.13mm⁻¹ in Ad-KO vs 1.87±0.20mm⁻¹ in WT, p=0.02) as well as a significant decrease in trabecular separation (0.17±0.004mm in Ad-KO vs 0.25±0.005mm in WT, p=0.006) in 14-week old Ad-KO mice compared to controls. Similar differences between WT and Ad-KO were present in 8-week old animals, but these did not reach statistical significance.

Our results show that Ad-KO mice have increased number and volume of trabeculae, indicating that adiponectin is inhibitory to bone accrual in vivo. Although this is at variance with in vitro studies, our data concur with the observations from epidemiological studies in humans that adiponectin negatively correlates with both fat mass and bone mass. Therefore, adiponectin may be a contributor to the link between fat and bone mass.