President's Poster Abstracts

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PP-MON-01

Effects of several kinds of retinoid X receptor (RXR) ligands on osteoporotic model mice

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Retinoic acid regulate many biological processes including cell growth and differentiation and embryonic development through retinoic acid receptor (RAR) and retinoid X receptor (RXR). In the present study, we examined how RXR ligands influenced bone metabolism using osteoporotic model mice. Several kinds of RXR ligands (HX531, HX630 and PA024) and were synthesized in one of our laboratories, and were orally administrated into osteoprotegerin (OPG)-deficient mice and ovariectomized (OVX) mice daily for 30 days using diets containing 0.03% or 0.003% of RXR ligands. OPG-deficient mice exhibited severe osteoporosis due to enhanced osteoclastic bone resorption. Treatment with HX531 (0.03%), an RXR antagonist, markedly increased vertebral trabecular bone volume and femoral cortical bone volume in OPG-deficient and OVX mice. The efficacy of HX531 administration in preventing the loss in bone volume was equivalent to that of bisphosphonate (resedronate) injection for 30 days in OPG-deficient mice. Histomorphometric analysis was performed in vertebrae and femora of mice treated with HX531 to examine bone tissues in more detail. Both bone resorption-related parameters and bone formation-related parameters were elevated in OPG-deficient and OVX mice. HX531 administration strongly suppressed both elevated parameters in those mice. Administration of HX630 and PA024 (0.003%), RXR agonist, markedly elevated bone volume in OPG-deficient and OVX mice. It is noteworthy that HX630 and PA024 administration decreased the number of adipocytes in tibiae, which was increased in OVX mice. These results suggest that RXR ligands can be good medicines for treatment of osteoporosis.

PP-MON-03

A novel protein regucalcin localizes nuclear and suppresses L-type Ca2+ channel and calcium sensing receptor mRNA expressions in cloned normal rat kidney proximal tubular epithelial NRK52E Cells

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The effect of regucalcin, a regulatory protein in intracellular signaling pathway, on the gene expression of various mineral ion transport-related proteins was investigated using the cloned normal rat kidney proximal tubular epithelial NRK52E cells overexpressing regucalcin. NRK52E cells (wild type) and stable regucalcin/pCXN2 transfectant with subconfluency were cultured 24-72 h in medium containing either vehicle, aldosterone (10^{-8} or 10^{-7} M), or parathyroid hormone (1-34) (10^{-8} or 10^{-7} M). Regucalcin was markedly

localized in the nucleus of transfectants. Overexpression of regucalcin caused a significant increase in rat outer medullary K^+ channel (ROMK) mRNA expression, while it caused a remarkable decrease in L-type Ca^{2+} channel and calcium-sensing receptor (CaR) mRNA expressions. Overexpression of regucalcin did not have an effect on epithelial sodium channel (ENaC), Na, K-ATPase (alpha-subunit), type II Na-Pi cotransporter (NaPi-IIa), angiotensinogen, and Na^+-Ca^{2+} exchanger mRNA expressions. Culture with aldosterone (10^{-8} or 10^{-7} M) caused a significant increase in ENaC, Na, K-ATPase, and ROMK mRNA expressions in the wild-type cells. Those increases were weakened in transfectants. Culture with PTH caused a significant decrease in NaPi-IIa mRNA, L-type Ca^{2+} channel and CaR mRNA expressions in the wild-type cells, and it significantly increased Na^+-Ca^{2+} exchanger mRNA expression. The hormonal effects on their mRNA expressions were also seen in transfectants. This study demonstrates that regucalcin is localized into the nucleus, and that it has suppressive effects on the gene expression of L-type Ca^{2+} channel or CaR that regulates intracellular Ca^{2+} signaling in NRK52E cells.

PP-MON-05

Human relaxin-2 administration increases rat trabecular bone volume by inhibiting remodeling

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The hormone relaxin (RLX) affects remodeling in a number of connective tissues, but to our knowledge there are no reports on its effect on bone, even though normal human osteoblasts have recently been shown to express mRNA encoding the RLX receptor LGR7[1]. Therefore the current study investigated whether RLX influenced bone remodeling.

Twelve male Sprague-Dawley rats, aged 18 weeks, were allocated into a treatment (n = 6) or control group (n = 6). Treated animals received 30 ng/ml of human RLX via subcutaneously implanted mini-osmotic pumps at a flow rate of 0.5 µl/h for two weeks. Controls received the equivalent volume of isotonic saline. Distal femora and proximal tibiae were processed for histomorphometric analysis. The region of metaphyseal trabecular bone analysed was a rectangle 1 mm x 2 mm and approximately 3 mm below the epiphyseal plate.

RLX treatment resulted in a 132% increase in trabecular bone volume ($p < 0.05$), a 42% increase in trabecular thickness ($p < 0.01$), a 79% increase in trabecular number ($p < 0.06$) and reduced bone turnover including the following parameters: osteoclastic number per bone perimeter (28% reduction; $p < 0.05$), mineralizing surface (48% reduction; $p < 0.01$), mineral apposition rate (25% reduction; $p < 0.01$) and bone formation rate (62% reduction; $p < 0.01$). These data show that the RLX treatment regimen inhibited trabecular bone remodeling and with the resultant increased volume of trabecular bone imply that this dose of RLX inhibited bone resorption to a greater extent than bone formation.

This effect of RLX on bone remodeling could be direct via actions on bone cells such as osteoblasts, or indirect via effects on tissues other than bone (e.g. blood vessels) to produce factors such as nitric oxide, which has been shown to be increased by RLX treatment and which in high levels is known to inhibit both bone resorption and bone formation.

Kristiansson P *et al.* (2005). Ann. N.Y. Acad. Sci. 1041: 317-319.

Human relaxin was kindly provided by BAS Medical, San Mateo, CA, USA.

PP-MON-07

1alpha, 25-dihydroxyvitamin D(3) inhibits RANKL-induced osteoclastogenesis of human bone marrow cells via suppression of protein expression as well as nuclear localization of C-Fos

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Vitamin D plays a critical role in calcium and bone metabolism through its action in the intestine, bone, kidney, and parathyroid gland. In cocultures of the bone marrow and osteoblastic cells, 1alpha, 25-dihydroxyvitamin D(3) (1,25D) induces the expression of the RANKL in osteoblasts, thereby stimulating osteoclastogenesis in vitro, led to the widespread belief that vitamin D is a bone-resorbing hormone. However, in ovariectomized animals, it was reported that 1,25D recovered the bone mineral density after estrogen deficiency. The aim of this study is to elucidate the direct effect of 1,25D on human osteoclast (OCL) differentiation *in vitro*. CFU-GM from human bone marrow were used for this study. The formation of OCLs was stimulated with 30 ng/mL M-CSF and 30 ng/mL sRANKL for 6 days. OCLs were identified as the tartrate-resistant acid phosphatase-positive multinucleated cells. 1,25D treatment significantly and dose-dependently inhibited OCL formation. And treatment in day 1-2 was most effective. Based on this result, we investigated the expression and subcellular localization of c-Fos, the essential transcription factor for differentiation of OCL, by western blot analysis and immunostaining. The expression of c-Fos protein was increased 12 and 24 hours after starting culture with M-CSF and sRANKL, and moreover, c-Fos localized in the nuclei after 18 hours. On the other hand, 1,25D treatment reduced the c-Fos protein and accumulation in the nuclei. These results suggest that 1,25D suppresses M-CSF and sRANKL-induced osteoclastogenesis in human bone marrow cells through the reduction in protein expression and nuclear localization of c-Fos.

PP-MON-09

Parathyroid hormone and parathyroid hormone-related protein stimulates multiple signalling pathways in osteoblasts

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PTH regulates bone and mineral metabolism, with its action on the osteoblast predominantly mediated by the cAMP/PKA pathway. However, not all actions of PTH can be attributed to this pathway. We have determined the effects of PTH (1-34), N- and C-terminal peptides and full length PTHrP on various signalling pathways using osteoblast (UMR106.01) cells transfected with pathway-specific luciferase reporter constructs.

PTH or PTHrP did not stimulate NFAT, NFkB or AP-1 dependent reporter constructs. In contrast, PTH 1-34, PTHrP 1-34 and PTHrP 1-141 increased Tcf (T cell factor), CRE (cyclic-AMP response element) and 6xOSE2 (osteoblast-specific cis-acting element) enhancer element-induced luciferase production in a time and dose-dependent manner, with maximal stimulation at 4 hours, 10-50nM, respectively. There was no significant difference between PTH1-34 and PTHrP 1-141 stimulation of these reporters (0.08-50nM). Furthermore, pre-treatment with cycloheximide did not ablate PTH 1-34 or PTHrP 1-141 effects, suggesting de novo protein synthesis is not required.

PTH or PTHrP stimulation of Tcf, CRE, and 6xOSE2 was ablated by pre-treatment with the PKA inhibitor H89, but unchanged by PKC or NFkB inhibitors, suggesting that the cAMP/PKA pathway was the predominant mediator of PTH effects on osteoblasts. Supporting this idea, PGE2 and Forskolin also stimulated Tcf, CRE, and 6xOSE2 reporters. Blocking MAPK signalling inhibited PTH-mediated stimulation of CRE, but not other reporter constructs.

These data show that PTH and PTHrP have pleiotropic effects on osteoblasts including stimulation of CRE (PTH1R signalling target) and the Wnt pathway target (Tcf), highlighting the potential for crosstalk between these two anabolic pathways.

PP-MON-12

Akt1 in osteoblasts and osteoclasts contributes to the maintenance of bone mass and turnover

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The serine-threonine protein kinase Akt has been proposed to be involved in bone metabolism in vitro. This study initially confirmed that Akt was phosphorylated in mouse calvarial primary osteoblasts (POB) by stimulation of IGF-I or BMP-2, and in M-CSF-dependent bone marrow macrophages (BMMϕ) and mature osteoclasts by stimulation of M-CSF. Among three Akt isoforms, Akt1 was most strongly expressed in these cells. We then investigated Akt1-deficient (-/-) mice to elucidate the role of Akt1 in bone. Akt1-/- mice exhibited osteopenia by bone densitometry, 3D-μCT and pQCT. Histomorphometric analysis revealed that parameters of both bone formation (Ob.S/BS, MAR & BFR/BS) and resorption (N.Oc/B.Pm & ES/BS) were lower in -/- mice than in wild type (+/+) mice. Cultured -/- POB showed reduced differentiation, matrix synthesis and mRNA levels of osteogenesis markers. After serum deprivation, cell survival rate was lower, and caspase-3 activity was higher in -/- POB than in +/+ POB; both were restored by Akt1 and Bcl-XL introduction. In addition, nuclear localization of FoxO3a was enhanced in -/- POB. Osteoclastogenesis in co-culture were reduced when either POB or bone marrow cells were derived from -/- mice. Furthermore, RANKL mRNA level in -/- POB, osteoclastogenesis from -/- BMMϕ inducted by soluble RANKL, and survival of -/- BMMϕ-derived osteoclasts were decreased. We conclude that Akt1 deficiency leads to low turnover osteopenia through dysfunctions of osteoblasts and osteoclasts: the former through increased susceptibility to mitochondria-dependent apoptosis; and the latter through suppressed RANKL expression in osteoblasts and defects in differentiation / survival of osteoclastic cells.

PP-MON-14

Bone-associated macrophages (BAMs) regulate osteoblastic mineralization

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The presence of F4/80$^+$ macrophage-like cells (referred to here as Bone-Associated Macrophages, BAMs) on the surface of both periosteal and endosteal bone is not commonly recognised and the role of these cells in physiologic bone remodelling remains unknown. The aim of this study was to investigate the influence of BAMs on the in vitro differentiation and mineralization of osteoblast cells. We have demonstrated that the traditional method of primary osteoblast isolation from neonatal murine calvaria also isolates a population of BAMs that expand during osteoblast differentiation *in vitro*. Microarray analysis of differentiating calvarial cultures demonstrated that a large number of macrophage-associated genes were expressed during the time course. To delineate the distinct functional roles and interdependence of BAMs and osteoblasts, we have used magnetic assisted cell sorting to generate a pure population of osteoblast cells by removing hematopoietic cells from digested calvarial preparations. The highly pure osteoblast cultures did not contain BAMs at any stage of the 21 day *in vitro* differentiation assay and the removal of BAMs increased the extent of *in vitro* mineralization as assessed by Von Kossa staining. We have developed a protocol to isolate pure BAMs, involving enzymatic digestion of adult mouse bones and FACS sorting F4/80$^+$/CD11b$^+$ BAMs. This pure population of BAMs will be used to further delineate the role of these cells in regulating osteoblast differentiation. Demonstration of the novel role of macrophages in bone mineralization may provide novel therapeutic strategies for the treatment of bone diseases.

PP-MON-16

Paclitaxel inhibits osteoclast formation and bone resorption via the modulation of MAPK and G2/M cell cycle arrest

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Many cancers have a propensity to metastasize to bone, leading to the formation of a vicious cycle of extensive bone destruction and tumor cell expansion. Remedies are critically needed for effective treatment that enables the disruption of this vicious cycle. Paclitaxel has been widely employed for the treatment of several malignant tumours, however, the mechanisms by which paclitaxel influences osteoclast formation, cellular apoptosis and RANKL signaling pathway of MAPKs and NF-κB remains ill-defined. In this study, we demonstrate that low concentrations of paclitaxel (<50 nM) dose dependently inhibited RANKL-induced osteoclastogenesis in both $RAW_{264.7}$ cells and bone marrow macrophage cultures. At high concentrations (>50 nM), paclitaxel induced apoptosis in osteoclast-like cells (OLC). Consistent with these findings, OLCs treated with paclitaxel displayed characteristic cytoskeletal aberrations including the disruption of F-actin and microtubule filaments. The inhibition of osteoclastogenesis and onset of apoptosis corresponds with mitotic arrest of the OLCs and their precursors. Pre-treatment of cells with paclitaxel (10 nM, 2 hrs) altered both basal and RANKL-induced activation of NF-kB and mitogen-activated protein kinase (MAPK) signaling molecules p38, ERK and AP-1. Furthermore, we have shown that paclitaxel (5 mg/kg) inhibits Lipopolysaccharide (LPS) induced osteolysis *in vivo* and also inhibits bone resorptive activity of the Giant multinucleated cells that were isolated from patients presenting Giant cell tumour of bone. Given its dual anti-cancer and anti-osteoclastogenic properties, paclitaxel may offer unique benefits for the treatment of cancers concomitant with osteolytic conditions.

PP-MON-18

Expression of ENC1, a Wnt target gene, in chondrocytic and osteoblastic cells

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The canonical Wnt pathway has been implicated in bone mass regulation; however, few Wnt target genes affecting this process have been elucidated. Therefore, this study was undertaken to establish a role for ENC1, a reported Wnt target gene, in bone biology. Transcript profiles of fracture callus tissues collected at early stages in a rabbit model of tibial distraction osteogenesis were generated using human ResGen cDNA arrays. ENC1 expression was up-regulated 40-fold between 2 and 4 or 6 weeks post distraction, correlating with a marked change in callus cellular composition. In 2-week callus, fibroblastic and cartilaginous cells predominated. At 4- and 6-weeks, mature osteoblasts and osteocytes were more abundant, coinciding with a notable increase in bone forming activity. ENC1 transcripts were localised to osteoblastic cells and remnant chondrocytes by *in situ* hybridisation of rabbit 4-week fracture callus sections. Furthermore, in normal mouse bone, ENC1 was expressed in growth plate and articular chondrocytes and in mature periosteal osteoblasts. Expression of ENC1 in cultured primary osteoblasts, and osteoblastic and osteosarcoma cell lines, was further confirmed by RT-PCR analysis. Polyclonal ENC1 antibodies have been generated and used to immunoprecipitate the over-expressed protein. Cellular expression of epitope tagged ENC1 in MG63 and SaOS2 osteosarcoma cells showed ENC1 to have a cytoplasmic distribution with staining of a subset of nuclei. Sequence analysis of the ENC1 promoter identified putative TCF/LEF binding sites, the Wnt responsive transcriptional target sequence. These results indicate that ENC1 is expressed in chondrocytic and osteoblastic cells and support its regulation by the Wnt pathway.

PP-MON-20

Cellular mechanisms for methotrexate chemotherapy-induced bone growth arrest and osteoporosis

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Chemotherapy-induced bone growth arrest and osteoporosis are significant problems in paediatric cancer patients and survivors; yet how chemotherapy affects bone growth remains unclear. This study characterised damage to bone growth mechanisms caused by a 5-day treatment with commonly used antimetabolite methotrexate (MTX) in young rats. Histological, cellular and molecular changes were examined in tibial growth plate cartilage and metaphyseal bone, two tissues responsible for bone lengthening. In growth plate, MTX didn't suppress proliferation, but induced chondrocyte apoptosis on days 6-11. Consequently, growth plate thickness was reduced on days 7-9 followed by a normal recovery by day 14. Suggestive of a possible recovery mechanism, expression of growth factors IGF-I and TGF-b1 in growth plate was increased on days 6-9. In metaphysis, heights of primary spongiosa were reduced on days 7-11, mirroring changes in growth plate thickness; and trabecular bone volume was reduced in the secondary spongiosa. While there was an initial increase in proliferation in osteoblasts and preosteoblasts (days 1-3), the densities of osteoblastic cells were not significantly affected after MTX treatment. However, mRNA expression of bone proteins collagen-1 and osteocalcin was decreased particularly on day 6. Suggestive of elevated resorption and accompanied by an increase in the ratio of expression of osteoclast differentiation factor RANKL and inhibitor OPG, density of osteoclasts on trabecular surface was increased on days 7-11. Therefore, MTX chemotherapy affects bone growth directly by inducing apoptosis and suppressing matrix production in growth plate, and by reducing matrix protein expression and increasing osteoclastic resorption in metaphyseal bone.

PP-MON-22

Strontium ranelate increases human osteoblast proliferation, differentiation and survival while decreasing their ability to promote osteoclastogenesis

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Strontium ranelate reduces the risk of vertebral and hip fractures in post-menopausal women. Previous studies on different models have shown that strontium ranelate increases bone formation and decreases bone resorption. The current study assessed the effects of strontium ranelate on proliferation, differentiation, osteoprotegerin (OPG) mRNA expression, and RANKL mRNA expression and cell survival after H_2O_2-induced oxidative stress in primary human osteoblasts (HOBs). HOBs were cultured in Dulbecco's modified Eagles medium with 10% foetal bovine serum, adapted to serum-free and calcium-free medium for 24h, and finally, treated with strontium ranelate in physiological Ca^{2+} (1mM). After a 24h- or a 48h-treatment, strontium ranelate increased human osteoblast proliferation, assessed by thymidine incorporation in a dose-dependent manner (0.01 to 2 mM Sr^{2+}), up to 3.8-fold with 2 mM Sr^{2+} (48h-treatment, $p<0.01$ vs. vehicle). Alkaline phosphatase activity was increased by 20 % after a 72h-treatment with strontium ranelate (1 and 2 mM Sr^{2+}) compared to vehicle ($p<0.001$). Concomitantly, strontium ranelate dose-dependently increased OPG mRNA expression, measured by quantitative RT-PCR, up to 1.6-fold with 1 mM Sr^{2+} ($p<0.01$), after a 24h-treatment. Under the same conditions, calcium chloride (1 mM Ca^{2+}) had no effect. Furthermore, after a 24h-treatment with strontium ranelate (0.01 to 2 mM Sr^{2+}), RANKL expression was dramatically decreased compared with RANKL expression observed in vehicle: remaining expression<10% with strontium ranelate concentrations $\geq$ 0.1mM, which corresponds to strontium blood concentration in human. Bone cell survival after oxidative stress was increased by strontium ranelate (0.1 mM, $p<0.05$ and 0.5-2mM, $p<0.01$). In conclusion, strontium ranelate, at concentrations close to the therapeutic levels, increases in human osteoblasts both replication and differentiation, thus promoting bone formation, and the ability to withstand oxidative stress. In parallel, human osteoblasts stimulated by strontium ranelate express more OPG and less RANKL, decreasing then their capability to stimulate osteoclastogenesis. Overall, these results support the dissociation of the strontium ranelate effects on bone formation and bone resorption.

PP-MON-24

Dynamic regulation of mesenchymal lineage transcription factors during osteoblast development in rat calvaria cell cultures

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Stem cells are assumed to reside in the so-called side population in several tissues including fetal rat calvaria (RC). Subpopulations of osteoprogenitor cells are derived from PPAR (peroxisome proliferator –activated receptor) γ–or Sox9-expressing precursors. Here we show that some of RC osteoblast-lineage cells can convert into adipocytes in the presence of a synthetic PPARγ ligand (BRL-49653). Together, these data may support the possibility of multi-cellular pathways for osteoblast-lineage determination. To further assess our finding, we also cultured RC osteoblast-lineage colonies at the single cell level. In regular RC cell cultures, we found that Sox9, MyoD and PPARγ were expressed through osteoblast development. These transcription factors and Runx2 were localized in the cytoplasm of proliferating cells. During osteoblast differentiation, the localization of Runx2 was shifted into the nucleus, while other transcription factors were retained in the cytoplasm. Some of osteoblast-lineage colonies after being subcultured with BRL-49653 converted into adipocytes. Concomitant with this, treatment of RC cells with BRL-49653 increased PPARγ expression and nuclear colocalization of Runx2 and PPARγ, while it did not affect Runx2, MyoD, and Sox9 expression. These results suggest that osteoblast-lineage cells may be in keeping with the expression of mesenchymal lineage transcription factors, but the nuclear import of these transcription factors except for Runx2 may be inhibited during osteoblast differentiation. It is reasonably considered that some osteoblastic cells may be able to convert into adipocytes in an active PPARγ -depedent manner, in agreement with recent evidence demonstrating that osteoblasts are relative to adipocytes amongst mesenchymal lineage cells.

PP-MON-26

Calcium balance in human lactation is correlated with biochemical indices of bone resorption

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Though a normal physiological phenomenon, human lactation places significant metabolic stress on the calcium (Ca) homeostatic metabolism of the mother, who provides 5-8 mmol/day of Ca for the infant. The (per-day) components of maternal Ca balance are; (i) gut Ca absorption (gutCa), determined by gut Ca absorption efficiency (F) and dietary Ca intake (dietCa); (ii) milk Ca excretion (milkCa); (iii) urinary Ca excretion (urineCa); (iv) endogenous faecal Ca excretion (endoCa); (v) skin Ca excretion (skinCa). The residual Ca balance (residCa [mmol/day] = gutCa-milkCa-urineCa-endoCa-skinCa) represents the Ca required from the maternal skeleton. This study examined whether residCa was elevated in Ca supplemented subjects, and whether residCa was correlated with biochemical indices of bone resorption.

From a randomized trial of effects on bone of 1000 mg/day Ca supplement in lactation, begun in pregnancy at 36 weeks, n=48 subjects (n=20 placebo; n=28 Ca) had measurements (i) – (iii) above, in the same 24-hr day, at 12 weeks established lactation, where gutCa=dietCa*Fα* tablet compliance. Fα measured using dual stable Ca isotopes; dietCa and milkCa by weighed records; (iv) and (v) estimated from the published literature (2.56 and 0.88 mmol/d). Fasting urinary hydroxyproline (UHyp), deoxypyridinoline (UDpd) and creatinine (UCr) were measured by standard methods.

At 12 weeks established lactation, residCa was –5.3 and 0.6mmol/d for placebo and Ca groups. UHyp/UCr and UDpd/UCr were both significantly correlated with residCa ($p<0.01$) in the combined group (n=48). UDpd/UCr ratio = -1.02*residCa (mmol/d) + 40.2 ($r=0.37$). This study provides biochemical evidence that Ca dietary supplementation suppresses bone resorption during lactation.

PP-MON-28

A comparison of bone mass and quality between peripubertal boys and girls - differential influence of physical activity and maturational status

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Background: Reports in the literature describe the benefits of physical activity for bone prior to puberty. Less attention has been given to peripubertal children. We investigated the relationships between bone mass, physical activity and maturational status in healthy peripubertal Caucasian boys and girls.

Methods: Anthropometrics, muscle strength and power, flexibility, calcaneal broadband ultrasound attenuation (BUA) (QUS-2, Quidel) and bone mineral density (BMD) (XR-36, Norland) were evaluated in 99 peripubertal children (46M; 53F). A bone-specific physical activity rating (BSPAR) was calculated and dietary calcium estimated by questionnaire. Maturity was determined by Tanner stage and age of peak height velocity (PHV).

Results: Boys had greater age at PHV, weight, height, clothing sizes, arm and forearm girths, muscle strength and power, and dietary calcium than girls. Girls were more flexible than boys ($p < 0.05$). There was no sex difference in BSPAR or time spent in sedentary activities. Boys exhibited greater FN BMC (4.343 vs 3.862), TR BMC (9.394 vs 7.167), and TR BMD (0.748 vs 0.674), while girls had higher BUA (81.7 vs 75.9) and LS BMD (0.875 vs 0.791). BSPAR and vertical jump height predicted BMD and BUA most strongly for boys, whereas, years from PHV was the strongest predictor for girls.

Conclusions: Sex-specific factors influence bone development around puberty. We found that maturational status predicts variance in parameters of bone health in girls, while physical activity and muscle power are more influential for boys.

PP-MON-30

The use of MRI and freely accessible software in the calculation of apparent trabecular bone volume

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Microarchitectural parameters of bone can be quantified using 3D modalities such as QCT and MRI. There are many techniques, most using specific software and hardware that is currently unavailable outside of the centres in which these methods are being developed. Thus, the aim of this study was to quantify an apparent trabecular bone volume (appBV/TV) *in vivo* using MRI and freely available medical image viewing software.

Calcaneum scans were performed on male subjects enrolled in the Geelong Osteoporosis Study according to previously published methodology[1]. Identification of the most central scan in the sequence was performed and, using OsiriX 2, a circular region of interest (ROI) ($2cm^2$) was placed 2cm anteriorly from the posterior tuberosity and propagated throughout the scan sequences, giving uniformity of ROI placement in areas devoid of cortical bone.

OsiriX histogram function was used to plot signal intensities for each ROI. Using developed histogram based threshold binarisation techniques, the histogram was separated into bone and marrow phases, allowing the calculation of an apparent trabecular bone volume expressed as percentage of total ROI volume. CV for appBV/TV determined from 5 separate scan sequences in the same subject after repositioning was 4%. CV determined from the same scan sequence in one individual analysed 5 times was 1%.

With acceptable CV, appBV/TV calculated using MRI and freely accessible software may prove to be a complementary diagnostic tool to DXA.

[1]Boutry *et al.* (2003) *Radiology* 227:708-17

PP-MON-32

Regional variations in three-dimensional microstructural properties of proximal femoral trabeculae with aging

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Objectives: The purpose of this study was therefore to explore region-dependent changes in the 3D microstructure of trabecular bone in human proximal femur, with respect to aging.

Materials and Methods: A total of ninety trabecular bone cores were obtained from five regions (femoral head, superior and inferior region of the neck, and superior and inferior region of the trochanter) of eighteen normal femora of eighteen Korean male cadaver donors, aged 40-90 years. These specimens were scanned using high-resolution micro-computed tomography (micro-CT). The following 3D microstructural parameters were calculated: bone volume fraction (BV/TV), trabecular number (Tb.N), thickness (Tb.Th) and separation (Tb.Sp), structure model index (SMI), and degree of anisotropy (DOA).

Results: The results showed that the trabecular morphology changed significantly with age, as well as varied from different regions of the proximal femur. There was a significant decrease in bone volume fraction and an almost identical decrease in trabecular thickness associated with aging at any region. Regional analysis demonstrated a significant difference in BV/TV, Tb.Th, DOA, Tb.Sp, Tb.N between superior and inferior neck, as well as a significant difference in BV/TV, Tb.Sp, DOA, SMI, Tb.N between superior and inferior trochanter.

Conclusions: This investigation has demonstrated that the effects of age on trabecular bone microstructure in the proximal femur are mainly concentrated on decline in bone volume fraction and thinning in trabeculae in this cadaveric population. As a result of mechanical and age-related adaptation, significant regional variations in microstructural properties of trabecular bone are likely to important factors affecting the mechanical properties of the proximal femur.

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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.