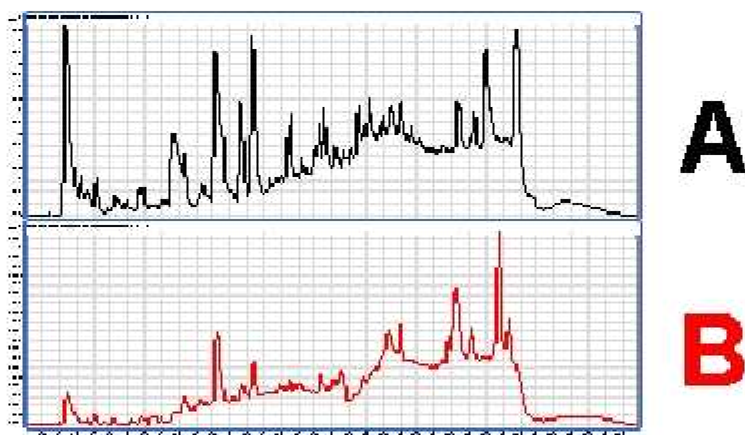

Figure 1. LC-MS Total Ion Chromatograms of two different urine samples A (black) and B (red).

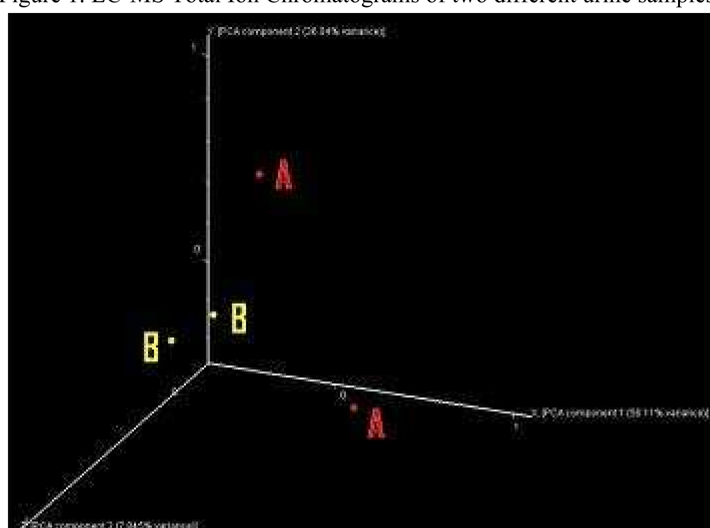

Figure 2. PCA scatter plot of urine samples A (red; n = 2) and B (yellow; n = 2) denoting the overall metabolic differences between the two samples.

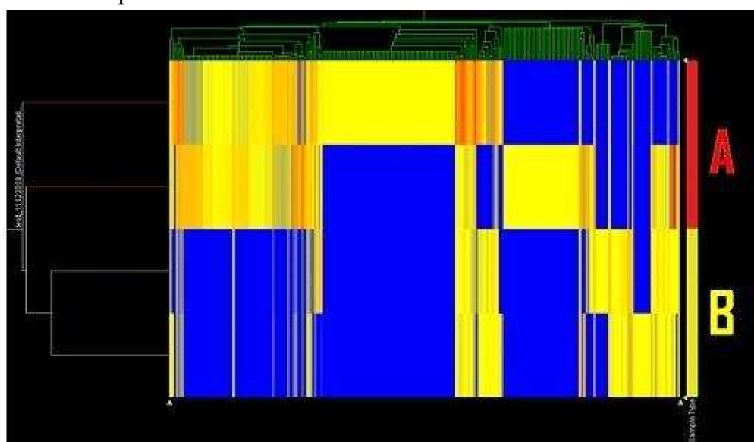

Figure 3. HCA of urine samples A (red; n = 2) and B (yellow; n = 2) illustrating metabolite differences between the samples on the basis of retention time and mass to charge ratio.

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EFFECT OF ANDROGEN ON AVIAN MEDULLARY BONE FORMATION

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Medullary bone is specific to female birds and plays an important role as a calcium reservoir for eggshell formation. The medullary bone appears just before the onset of egg-laying in bone marrow cavities of long bones, indicating that their formation is induced by

the increasing amounts of estrogen secreted from mature follicles. It has been demonstrated that estrogen directly induces the medullary bone formation mediated by estrogen receptor. Androgen is also essential for medullary bone formation, collaborating with estrogen, and the medullary bone formation is easily induced by the administration of estrogen to mature male birds. However, it is unknown whether androgen directly stimulates medullary bone formation. Therefore, we tried to detect the localization of androgen receptors in medullary bone, and to clarify the role of androgen receptor in medullary bone formation.

For immunohistochemistry, medullary bone was dissected from femurs of mature female chickens, and paraffin sections were prepared. After antigen retrieval method using an autoclave treatment (citrate buffer, 10 mM at pH6.0), the localization of androgen receptors was detected with the primary antibody (rabbi polyclonal IgG antibody against androgen receptor; PG-21; Upstate) and the avidin-biotin complex method. Next, medullary bone formation was induced by the treatment of male Japanese quails with a single injection of estradiol valerate (2 mg/100 g body weight) (control group), and concomitantly, bicalutamide (3 mg/100 g body weight), androgen receptor antagonist, was administered intramuscularly to the quails for 7 day (experimental group). Thereafter, their medullary bone sections were stained with alucian blue, and the proportion (%) of the medullary bone to bone marrow cavity was calculated with Image-pro Discovery software.

Androgen receptors are localized in osteoblasts on medullary bone surface. However, osteoclasts and bone marrow cells do not represent the localization of androgen receptors. Medullary bone formation is induced by a single injection of estradiol, and the proportion represents 14.30% of bone marrow cavity. On the other hand, bicalutamide significantly inhibits the estrogen-induced medullary bone formation (down to 0.94%).

In conclusion, androgen receptor is present in osteoblasts but not in osteoclasts, and androgen directly stimulates osteoblastic medullary bone formation.

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IMPACT OF IL-1BETA-INDUCED UPREGULATION OF CALCIUM SENSING RECEPTORS ON L-AMINO ACIDS SENSITIVITY IN HUMAN CALCITONIN-SECRETING CELLS

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We have previously demonstrated that L-amino acids allosterically activate extracellular Ca2+-sensing receptors (CaR) in CaR-expressing HEK293cells and normal human parathyroid cells. In normal parathyroid cells, L-amino acids activate intracellular Ca2+ mobilization and suppress PTH secretion. In the current study, we have investigated the impact of enhanced CaR activation on the intracellular Ca2+ mobilization and calcitonin secretion from human TT thyroid cells under either control conditions or following exposure to hIL-1β to promote CaR expression (1). TT cells were cultured in F12-K nutrient medium with 10% FBS in the absence or presence of 100 ng mL-1 human interleukin 1β (hIL-1β) for 48 h. For the analysis of intracellular Ca2+ mobilization, TT cells were cultured on coverslips, loaded with the Ca2+-sensitive dye fura-2 AM (5 μM; 1.5 h) and then analyzed for sensitivity to elevated Ca2+ concentration or L-amino acids including L-Phe or L-Trp. For analysis of calcitonin secretion, TT cells were cultured in 24-well plates in the absence or presence of hIL-1β as above and then incubated with Ca2+ (0.5 to 1.5 mM) in the absence or presence of 10 mM L-Phe for various times (0 - 12 min). In fura-2 loaded cells under control conditions, increasing the extracellular Ca2+ concentration from 0.5 to 2.5 mM had little or no effect on intracellular Ca2+ and, in the presence of 2.5 mM Ca2+, only around 10% of cells responded to 10 mM L-Phe. After 48 h exposure to hIL-1β, however, there was a marked increase in sensitivity to extracellular Ca2+ and L-Phe. Under these conditions, around 20-30% of cells responded to 2.5 mM Ca2+ alone and, in the presence of 2.5 mM Ca2+, greater than 90% of cells responded to 10 mM L-Phe. In preliminary experiments of calcitonin release, control cells exhibited enhanced secretion at Ca2+ concentrations at or above 1.5 mM but there was no response to 10 mM L-Phe. In TT cells that had been exposed to IL-1β, however, there was enhanced sensitivity to elevated Ca2+ concentration and a threshold level was identified at around 1.0-1.2 mM. In addition, in the presence of 1.0 - 1.1 mM Ca2+, 10 mM L-Phe markedly stimulated calcitonin secretion. In analysis of CaR protein expression by western blotting, hIL-1β enhanced the expression of the mature 160 kDa form. The data indicate that a recognized hypocalcemic agent, hIL-1β, upregulates CaR expression in TT cells and, in consequence, there is enhanced sensitivity to extracellular Ca2+ and amino acids with respect to intracellular Ca2+ mobilization and calcitonin secretion. 1. Canaff L & Hendy GN. J. Biol. Chem. (2005) 280:14177 – 14188

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ESTRADIOL REGULATES RENAL KLOTHO THROUGH ESTROGEN RECEPTOR ALPHA

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Background: Klotho is a glycoprotein predominantly expressed in the kidney, parathyroid gland, reproductive organs and choroids plexus in the brain. Up-regulation of klotho by vitamin-D has been reported. Overlap in the expression pattern of estrogen receptors and klotho raises the potential for estrogen regulation of klotho expression. Methods: The mouse kidney distal convoluted tubule cell line, DCT, was cultured in DMEM supplemented with either 10% regular FBS or charcoal stripped FBS with or without added estradiol. To determine the effects of estrogen on klotho expression in vivo, we used wild type (WT, n=4) and aromatase deficient mice (ArKO, n=4) treated with estrogen (20ug/mouse 3x/week) or vehicle for 3 weeks. We also compared klotho expression in

vehicle or estradiol treated ovariectomized estrogen receptor alpha (ERKOa) or beta (ERKOb) knockout mice to that of WT littermates. RNA and protein were prepared from cells or kidneys for real time PCR and WB analysis, respectively. Results: Klotho protein was significantly higher in cells grown in csFBS compared to 10%FBS. Addition of estradiol at 10(-8)M in 10% csFBS restored klotho expression to the same level as cells grown in 10%FBS. There was significantly higher expression of klotho both at the mRNA and protein levels in ArKO animals compared to WT; however, ArKO mice treated with estrogen had WT levels of klotho. WT castrated mice treated with estradiol showed decreased levels of Klotho. Protein extracts prepared from kidneys of estrogen receptor alpha mice had higher levels than wild-type littermates. The suppressive effect of estradiol was lost in ERKOa animals. Estrogen receptor beta loss had no effect on Klotho expression. Conclusion: Estradiol suppresses klotho expression in the murine kidney through estrogen receptor alpha.

LATE POSTER SUBMISSIONS

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A DELAY IN CONSOLIDATION IS OBSERVED IN A HETEROZYGOUS CONDITIONAL BMP2 DEFICIENT MOUSE MODEL OF DISTRACTION OSTEOGENESIS

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Distraction osteogenesis (DO) is a surgical technique used to treat limb length discrepancies, limb deformities, long bone non-unions and bone loss due to trauma, infection or malignancies. One of the main limitations of DO is the long consolidation period required for the bone to heal. Different methods have been researched to accelerate the consolidation phase of DO, including the exogenous application of bone morphogenetic proteins (BMPs). BMPs are growth factors that are required in the bone developmental pathway. Although numerous studies have tested pharmacological doses of BMPs during DO, the physiological role of BMPs during DO still remains unclear. In this study, we investigated the physiological role of BMP2 during DO in heterozygous conditional BMP2 knockout mice.

Distraction osteogenesis was performed on the right tibia of eighty wild-type BMP2 fl/+ and heterozygous BMP2 fl/+ cre mice using a miniature version of the Ilizarov fixator. Mice underwent a latency period of 5 days, a distraction period of 12 days (distraction rate of 0.2 mm every 12 hours) and a consolidation period of 34 days. Distracted samples were collected from four time points: 11 days (mid-distraction phase), 17 days (end of distraction phase), 34 days (mid-consolidation phase) and 51 days (end of consolidation phase). Samples were studied using µCT, Faxitron x-ray, immunohistochemistry, histology, Real Time-quantitative PCR and biomechanical testing.

Results from this study showed that mice with a gene-dosage dependant reduction of BMP2 expression may be contained a delay in consolidation. µCT analysis revealed a statistically significant decrease in trabecular number and increase in trabecular separation at 51 days in the heterozygous mice. Immunohistochemical studies demonstrated decreased BMP2, BMP7, BMPR1a, ACTR1, ACTR2b expression in the heterozygous mice at 34 days post-osteotomy; which can account for the poor bone formation patterns observed during the consolidation phase of DO. Biomechanical testing of 51 day samples revealed a decrease in stiffness and increase in ultimate displacement in the heterozygous mice compared to the wild-type controls, corresponding to the the weaker consolidated bone of the heterozygous mice during this phase. Therefore, results from this study suggest that BMP2 exerts a significant physiological role during DO.

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OSTEONECROSIS OF JAWBONES IN TWO OSTEOPOROSIS PATIENTS TREATED WITH A NITROGEN-CONTAINING BISPHOSPHONATE (NBP): ATTEMPTS AT OSTEONECROSIS REDUCTION BY REPLACING NBP WITH ETIDRONATE (A NON-NBP)

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Among the bisphosphonates (BPs), the nitrogen-containing BPs (NBPs, such as zoledronate, risedronate, and alendronate) have anti-bone-resorptive effects (ABREs) that are much more powerful than those of the non-nitrogen-containing BPs (non-NBPs, such as etidronate and clodronate). In the last few years, a thousand or so cases of osteonecrosis of the jawbones (ONJ) have been suspected of being associated with the administration of NBPs. However, the mechanism underlying the osteonecrosis remains unclear, and there are no effective therapeutic methods. Since NBPs accumulate in the hydroxyapatite within bone, our fear is that more cases will come to light if NBPs continue to be used as they are now. The situation is made more complex by (a) NBP-associated ONJ developing even after a pause in the NBP-treatment, and (b) ONJ sometimes appearing long after discontinuation of NBP-therapy (in some cases, as long as 12 months after). Interestingly, and importantly, few ONJ cases have been reported in patients treated with

non-NBPs such as etidronate and clodronate. We previously reported that in mice: (i) etidronate (a non-NBP), when intraperitoneally co-administered with alendronate (an NBP), competes against the NBP for binding to bone hydroxyapatite and (ii) etidronate can reduce the inflammatory effect of alendronate (Funayama et al. Calcif Tissue Int 76:448-457, 2005). These findings led us to expect that etidronate might eliminate an NBP that had already accumulated within bone, and that etidronate might therefore be useful as a substitution drug in NBP-treated patients at risk of ONJ. Here, we describe the apparent effectiveness of such etidronate-replacement therapy in two NBP-treated patients with ONJ and/or osteomyelitis. They had been receiving oral risedronate (for 55 or 66 months) as treatment for osteoporosis. Bone scintigraphy of the mandible revealed a marked accumulation of 99mTc-HMDP. The risedronate treatment was discontinued, and treatment with oral etidronate (200 mg/day) was begun according to the standard prescription (i.e., two weeks• f ingestion and three weeks• f rest). Within 3 weeks of the start of the treatment, pain and pus had disappeared. In case-1, bone-scintigraphy images revealed shrinkage of the inflamed area of the mandible. We discuss the rationale for this therapeutic strategy. (bone-scintigraphy images in case-2?)

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CHANGES OF GENE EXPRESSION PROFILING AND PATHWAYS RELATED FAT METABOLISIM IN FEMORAL HEAD OF STEROID-INDUCED OSTEONECROTIC RATS

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Objective :To understand the changes of gene expression profiling and pathways related fat metabolism in femoral head of steroid-induced osteonecrotic rats in the contribution to pathgenesis of steroid-induced osteonecrosis.

Methods : We applied lippolysccharide(LPS) and methylprednisolone(MPSL) to prepare a rat model of steroid-induced osteonecrosis(SIO).Rats were divided into two groups: normal(N) group and model(M) group. All rats sacrificed after 6 weeks. Then we applied genome wide cDNA microarry technology to analyze genes expressed in femoral head of two groups and also serum levers of cholesterol(TC) were measured in two groups.

Results: (1) There were total 111 expession genes which changed by a minimum of two-fold and also it was found that 18 pathways had significant changes in these differential genes by molecule annotation system molecule annotation system (MAS) analysis in femore head of model group compared with normal rats.

(2) There were 6 upregulated genes related fatty acid metabolism(FAM) pathway(Acadl,Cpt2,Acaa2,Acat1,Acsl1,Hadhb), 2 upregulated genes related fatty acid elongation in mitochondria(FAEM)(Acaa2,Hadhb),3 upregulated genes related adipocytokine signaling pathway (ACK)(Cpt2,Prkaa2, Acsl1)et al in femore head of model group compared with normal rats.

(3) Serum levels of cholesterol significantly increased 21.2% in model group compared with normal group(1.754 ± 0.264 mol/L vs 1.447 ± 0.142 mol/L ,p<0.00 1).

Conclusions :The data suggest that the upregulated genes related fat metabolism in their pathways of femoral head of steroid-induced osteonecrotic rats might paly an important role in promoting the synthesis of fatty acid and cholesterol and fat metabolism could be mediated by FAM, FAEM and ACK pathways. The changes of multiple gene expression and that numerous pathways could play major roles in steroid-induced osteonecrosis pathogenesis.

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ANALYSIS OF CIRCULATORY FGF23 PROTEIN

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FGF23 is a physiological humoral factor regulating phosphate and vitamin D metabolism. Previous in vitro studies indicated that a part of FGF23 protein is proteolytically cleaved between 179Arg and 180Ser by subtilisin-like proteases, and only full-length FGF23 has a biological activity to reduce serum phosphate and 1,25-dihydroxyvitamin D levels. However, it is currently unknown how much of circulatory FGF23 protein is present in intact and processed forms in vivo and whether this ratio changes with impairment of renal function. There are several assay methods for FGF23. Intact FGF23 assay (Kainos) detects only full-length uncleaved FGF23. In contrast, C-terminal assay (Immutopics) recognizes both full-length and processed C-terminal fragment of FGF23. Therefore, we compared plasma FGF23 levels measured by these two assays in patients with tumor-induced osteomalacia (TIO) and end stage renal disease (ESRD) undergoing hemodialysis. In addition, we estimated the quantity of full-length and processed C-terminal fragment of FGF23 by immunoprecipitation and Western blotting using an antibody against the C-terminal portion of FGF23.

All cases showed very high FGF23 levels by both assays. In 30 patients with ESRD, FGF23 was 5917.8 +/- 1350.6 pg/ml (mean +/- SE, reference range 10 - 50 pg/ml) and 6525.2 +/- 1296.3 RU/ml (reference range < 150 RU/ml), by full-length and C-terminal assay, respectively. In 3 TIO patients, it was 2555.7 +/- 1782.4 pg/ml and 4277.8 +/- 3346.5 RU/ml. There was a strong positive correlation between FGF23 levels measured by these two assays. In addition, the relationships between FGF23 levels measured by these two assays were similar in patients with TIO and ESRD. Western blotting indicated that about 30% of FGF23 is present in the processed form and this ratio is not different between patients with TIO and ESRD. These results indicate that a certain amount of circulatory

FGF23 is present in the processed form and this processing is not affected by the impairment of renal function. Therefore, the high level of FGF23 by C-terminal assay in patients with ESRD is not derived from the accumulation of processed C-terminal fragment of FGF23.

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COMPARISON OF CORTICAL BONE THICKNESS, POROSITY, AND DENSITY IN PRE- AND POSTMENOPAUSAL WOMEN MEASURED BY HR-PQCT AT THE DISTAL RADIUS AND TIBIA

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Reduced cortical thickness (Ct.Th) and increased cortical porosity (Ct.Po) have been documented in menopause [1] and linked to increased risk of fracture. The purpose of this study was to use high resolution peripheral quantitative computed tomography (HR-pQCT) on a population-based sample to compare Ct.Th, Ct.Po and cortical density (Dcort) between premenopausal women with normal bone mineral density (DXA FN T-score > -1) and postmenopausal women with normal, osteopenic, and osteoporotic bone mineral density. Automated cortical segmentation procedures [2], validated with µCT measurements, were used to obtain direct measurements of Ct.Th and Ct.Po. The analysis was applied to distal radius and tibia HR-pQCT scans from the Calgary cohort of the Canadian Multicentre Osteoporosis Study (CaMos). We used analysis of variance to compare cortical bone outcomes between 51 premenopausal normal, 72 postmenopausal normal, 101 postmenopausal osteopenic, and 11 postmenopausal osteoporotic women. At both the radius and tibia we found that postmenopausal women (all groups) had higher Ct.Po (3.6 to 13.2%, p<0.001) and lower Ct.Th (-4.8 to -32.6%, p<0.001) than premenopausal women. Dcort was also significantly lower in all postmenopausal women compared with premenopausal women (-5.7 to -22.4%, p<0.001). Osteopenic and osteoporotic postmenopausal women both had higher Ct.Po (2.0 to 7.6%, p<0.001), lower Ct.Th (-12.2 to -29.2%, p<0.001), and lower Dcort (-6.2 to -15.7%, p<0.001) than the normal postmenopausal women. Postmenopausal osteoporotic women had 4.7% higher Ct.Po (p=0.005), -17.8% lower Ct.Th (p=0.006), and -8.5% lower Dcort (p<0.001) than the osteopenic women at the distal tibia. At the distal radius, only Ct.Po was significantly greater in osteoporotic women (3.9%, p=0.007). The morphological results in our population-based sample are consistent with previous HR-pQCT studies [3], and these results provide new data about the cortical porosity of the bone in pre- and postmenopausal women. The functional importance of these differences in cortical bone microstructure, as they relate to HR-pQCT estimates of bone strength, is currently under investigation.

(1) Seeman E (2002) Lancet 359:1841-50

(2) Buie HR, Campbell GM, Klinck RJ, MacNeil JA, Boyd SK (2007) Bone 41:505-15

(3) Boutroy S, Bouxsein ML, Munoz F, Delmas PD (2005) J Clin Endocri Metab 90:6508-15

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ALENDRONATE + VITAMIN D THERAPY OR REFERRED CARE IN OSTEOPOROTIC WOMEN: RATIONALE AND DESIGN, INCLUDING MEASUREMENT OF PHYSICAL FUNCTIONS, FALLS, AND POSSIBLE GENETIC MARKERS

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AIM: Vitamin D is required for bone strength and also acts on muscle function. Vitamin D insufficiency is prevalent, and often overlooked by physicians. A planned study and its extension will examine the effects of a single tablet containing the bisphosphonate alendronate 70 mg plus vitamin D_3 5600 IU (ALN+D) compared with referred care on serum vitamin D and BMD. Falls physical function, and genomic sampling will be exploratory endpoints.

METHODS: In an upcoming international, randomized, controlled trial of 6 months with a 6-month extension (under the same treatment assignments), approximately 800 women (>=65 years of age, osteoporotic, at increased risk of falls, with baseline 25(OH)-vitamin D of 8 to 20 ng/mL) will either receive ALN+D weekly or be referred to their primary care physicians (who are not investigators in the trial) for one of the usual osteoporosis therapies. Women in the ALN+D group with <=1000 mg daily calcium intake at baseline will receive 500 mg elemental calcium/day. The primary endpoint will be proportion of patients with serum 25(OH)-vitamin D<20 ng/mL. Secondary endpoints will include bone turnover markers. Exploratory endpoints will include fall event rate, the Short Physical Performance Battery (SPPB), and the relationships among genotype, RNA expression, total body composition, and SPPB. Falls will be reported by patients to their study site. Fall case report forms will include 15 questions concerning detailed description, location, and outcome of the fall. Falls due to fragility, but not due to a syncopic event or external force, will be included for data analysis. Fall case report forms will be adjudicated by an independent committee, blinded to patient-treatment group. Safety will be monitored.

CONCLUSION: This study may demonstrate relationships among osteoporosis/vitamin D therapy, falls, physical function, and molecular/genetic information.

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BASELINE BMC EXPLAINS ONLY 38 PERCENT OF GAIN IN BMC BETWEEN 5 AND 7 YEARS OF AGE IN NEW ZEALAND CHILDREN

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Children with large skeletons might be expected to gain more bone mineral than those with smaller bones. The present study was undertaken to determine to what extent baseline bone mass influences subsequent gain in BMC in healthy prepubertal children. Heights and weights were determined and total body BMC and body composition were measured using DXA (Lunar DPX-L) in 150 children participating in the FLAME birth cohort study. At 5 years of age girls (n=57) had 614.4 (86.5)g and boys (n=93) 658.2 (90.9)g of bone mineral content. Mean (SD) 2-year gains in the girls and boys respectively were: BMC (g) 205 (41) and 220 (49), bone area (cm2) 206 (40) and 212 (46), height (cm) 12.2 (1.3) and 12.1 (1.4), weight (kg) 5.4 (1.9) and 4.7 (1.4), lean mass (kg) 3.67 (0.73) and 3.95 (0.86), and fat mass (kg) 1.24 (1.64) and 0.39 (0.84). Deviations from the expected values for BMC gain were seen throughout the range of baseline bone mass at 5 years of age. Baseline BMC explained 38% of the 2-year BMC gain with a further 14.8% (girls) and 29.0% (boys) being explained by deviations from expected gains in height and weight. Changes in body composition also influenced BMC increments. Deviations from expected gain in lean mass explained 12.0% (girls) and 22.6% (boys) while deviations from expected gain in fat mass explained only 7.8% (girls) and 1.6% (boys) of the 2-year BMC change. We conclude that although baseline skeletal mass influences the magnitude of gain in bone mineral, variations in anthropometric growth and body composition change (particularly lean mass) also make important contributions to BMC gains at this young age.

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ANALYSIS OF THE BONE MARROW STROMA IN AN IN VIVO MODEL OF HIGH BONE TURNOVER

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Expansion of a population of fibroblastoid cells in the bone marrow (BM) is typical of hyperparathyroidism. An expansion of similar cells was also observed in the BM of transgenic mice expressing a constitutively active PTH/PTHrP (PPR*Tg) in mature osteoblasts, and was fully abrogated by treatment with osteoprotegerin. The goal of this study was to characterize these fibroblastoid cells in PPR*Tg mice, and to investigate whether they shared any feature with cells of the inflammatory stroma.

For this purpose, PPR*Tg mice were crossed with a transgenic mouse line which expresses Green Fluorescent Protein (GFP) in mature osteoblasts (Col1GFP) in order to generate PPR*Tg/Col1GFP mice. Majority of the fibroblastoid cells in PPR*Tg/Col1GFP mice did not express GFP, which indicates that they were not mature osteoblasts. In situ hybridization analysis revealed that a variety of osteoblast markers were heterogeneously expressed by these cells. Collectively, these data indicate that the fibroblastoid population in the BM of PPR*Tg mice was mainly contributed by immature cells at early stages of osteoblast differentiation. Flow cytometry analysis of BM cells isolated from PPR*Tg/Col1GFP and Col1GFP mice, respectively, revealed the presence of a population that expressed both GFP and CD45, a pan hematopoietic cell-surface marker, and was thus phenotypically similar to “fibrocytes”. Fibrocytes are collagen-producing cells of hematopoietic origin, and have been identified in wounds and pathological fibrosis. Notably, number of these fibrocyte-like cells was significantly increased in the BM of PPR*Tg mice.

In conclusion, our findings indicate that: 1) expression of a constitutively active PTH/PTHrP receptor in mature osteoblasts leads to expansion of immature cells of the osteoblast lineage with mechanisms that are likely to be indirect and require osteoclast activity; 2) fibrocyte-like cells, which are typical of the inflammatory stroma, are also present in the normal BM and their number is increased in PPR*Tg mice.

(1) Calvi LM et al, J Clin Invest. 2001 Feb;107(3):277-86
(2) Bucala R et al, Mol Med. 1994 Nov;1(1):71-81

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ASSOCIATION OF BONE MINERAL DENSITY AND BIOCHEMICAL MARKERS IN HEALTHY YOUNG VOLUNTEERS FROM NORTH INDIA

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Background. Since Indians have highest prevalence of low bone mass and lower bone mineral contents at hip and lumbar spine as determined by dual X-ray energy absorptiometry, which is a surrogate marker of treatment efficacy that has been widely used in clinical trial. The purpose of this study was to investigate the association of four biochemical markers of bone turnover with whole body BMD and at all three sites. High levels of bone resorption markers are associated with increased risk of osteoporotic fractures.

A very high level of the bone turnover marker (T score >3) is suggestive of other metabolic bone disease. The combination of BMD and bone turnover measurement allows the identification of young subjects at a much higher risk for fracture.

Methods. Fifty one healthy subjects in the age group of 20 to 35 were taken. BMD was measured at the lumbar spine (LS) and femoral neck (FN) and forearm using DXA (GE Lunar, WI, and USA). Anthropometric measurements included height, weight, BMI and waist hip ratio (WHR). Estimation of Bone turnover markers included the levels of serum bone-specific alkaline phosphatase (sBAP), serum type I collagen cross-linked C-terminal telopeptide (sCTx), serum Osteocalcin and Parathyroid hormone (PTH)using standard ELISA kits.

Results. Out of fifty-one healthy volunteers only eight (15.68%) had normal BMD (6 males & 2 females), Seven (13.73%) were osteoporotic (5 women & 2 men) and thirty-six (70.59%) were osteopenic (20 males & 16 females).PTH negatively correlated with Total BMD and BMC, r=0.290, P<0.039 and r= 0.292, P< 0.038 respectively. Serum Osteocalcin had a positive corrlelation with BMI r=0.283, P<0.007.Serum Crosslaps showed a positive corrlelation with Total bone area (r= 0.373,P<0.007),Hip BMD(r= 0.333,P<0.017) and Forearm BMD(r= 0.357,P<0.010).There was a significant increase in serum PTH, levels in normal versus osteoporotic groups (P<0.004) and normal vs osteopenic groups.(P<0.030).We found no significant correlations with (sCTx), (sBAP) and sOsteocalcin fracture risks.

Conclusion. Patients with low BMD or high bone turnover marker values would be at risk for osteoporosis and warrant preventive measures with antiresorptive agents. High levels of bone resorption markers are associated with an increased risk of osteoporotic fractures.

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BMD AND BONE VOLUME FRACTION OF ENTIRE HUMAN VERTEBRAL BODIES EXAMINED BY DXA AND MICRO-CT

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The evaluation of fracture risk of patients is usually done using dual-energy X-ray absorptiometry (DXA) of the lumbar spine. Anterior-posterior (AP) projections are performed, with areal bone mineral density (BMD) measurements calculated within the whole vertebral body (L2, L3). However, the bone distribution and microstructure, and thus bone strength, might vary within the vertebra. Thus, subregional BMD measurements using lateral DXA scanning modality might be informative about fracture risk. Nowadays, X-ray micro-computed tomography (micro-CT) allows three-dimensional structural characterization of entire bone segments, non destructively and at high resolution. Aim of this study was to measure the BMD by lateral DXA in three subregions of the L2 human vertebra, and to compare it with measurements of bone volume fraction (BV/TV) in analogous subregions obtained by micro-CT.

Eight human cadaver spines (age range 61-91 years) immersed in a water bath were scanned by DXA in the AP and in the lateral projections. Then, subregional areal BMD analysis was done in the lateral projection of the L2 vertebra, with the examination area divided into three subregions (superior, central, inferior). The L2 vertebrae were then dissected and entirely scanned by micro-CT (18 µm pixel size). The micro-CT volume of interest comprised the trabecular bone of the entire vertebral body, and was divided via software into three equal subregions (superior, central, inferior), over which then analysis of the BV/TV was done.

Significant differences were found between the subregions (one way ANOVA for repeated measures), with BMD and BV/TV having higher average values in the inferior subregions than in the superior subregions ($p<0.05$). In the central subregion, the linear regression “BV/TV vs. BMD” had a high coefficient of determination ($R2=0.80$, $p<0.01$). While the BMD measured laterally over the whole vertebrae was significantly related to the total BV/TV ($R2=0.59$, $p<0.05$), the BMD measured in the AP direction was not ($p=0.33$).

These preliminary results suggest that, in contrast to AP BMD measurements, lateral BMD measurements in the L2 vertebra are significantly related to trabecular BV/TV. In particular, subregional BMD measurements are highly related to trabecular bone volume fraction in the central part of the vertebral body. These findings support lateral DXA examination as a valuable modality for improving the evaluation of vertebral fracture risk.

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VALIDITY OF USING A LINEAR MICRO-FINITE ELEMENT MODEL TO PREDICT TRABECULAR BONE APPARENT MECHANICAL PROPERTIES: COMPARISON WITH A NON-LINEAR MODEL AND EXPERIMENTAL DATA

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INTRODUCTION Micro-finite element (µFE) analysis is a popular tool for determining trabecular bone mechanical properties. Linear models have been shown to accurately predict areas of micro-damage and micro-fracture [1] ; with recent models attempting to predict apparent level yield stress and strain by incorporating material nonlinearity, but at the cost of increased computation time.

The objective of this study was to assess the capability of both linear and non-linear material models to predict apparent level mechanical properties. Trabecular bone specimens (1 cm3), taken from vertebrae of 12 human cadavers (mean (SD) age 69.25 (11.2) years), were imaged in a µCT scanner at 15µm resolution prior to undergoing uniaxial compression testing to 10% apparent strain.

METHODS Baseline image data were used to construct µFE models. Image data were resampled to 40µm resolution to ensure sufficient numerical convergence & minimise computation time; resultant FE meshes comprised on average 390,000 nodes.

µFE models were subjected to quasi-static loading to 5% apparent strain using both linear and non-linear material models. The linear analysis assumed a homogenous linear isotropic material model. The effective tissue modulus was back-calculated to match the apparent modulus obtained during testing. Non-linear analyses used a bilinear constitutive model with asymmetric yield strain and tangent modulus set to 5% of initial tissue modulus [2] .

RESULTS Due to the assumed fully-elastic behaviour failure stress could not be predicted for linear models; however at 3% apparent strain, average localised tissue strains were 2.84%, which is significantly larger than reported strain levels for trabecular tissue. For the nonlinear model, yield stress was underestimated; however regions of high stress or failed elements correlated with the same regions identified using the linear model.

DISCUSSION Whilst linear models are unable to predict apparent yield stress, they are useful for determining regions at risk of fracture. Incorporating non-linear material properties allows for a more realistic model, however at the cost of significantly increased computation time. Further investigation is required to determine non-linear behaviour of trabecular bone at the tissue level.

(1) Nagaraja, S. et al., J Biomech, 2005. 38(4): p. 707-716.

(2) Niebur, G.L. et al., J Biomech Eng, 2002. 124: p. 699-705.

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COMPLIANCE WITH WEEKLY ALENDRONATE AN OUTPATIENT BONE CLINIC STUDY

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The treatment of osteoporosis with alendronate requires long term adherence to be effective. The purpose of this study was to determine weekly adherence to alendronate in patients with osteoporosis attending a specialist outpatient metabolic bone clinic. Our study included 112 consecutive patients treated for osteoporosis with alendronate that attended the clinic between June 2005 and June 2006. 85% of patients were female and the average age was 74 years old (range 43 to 90). All patients were given written instructions on how to take alendronate. Compliance at twelve months following the commencement of therapy was assessed at subsequent clinic visits or from other hospital visits or admissions included in the patient's clinical records. 67 (59.8%) of patients were taking alendronate for at least twelve months. 13 (10.7%) patients discontinued alendronate within twelve months and there was no available follow up data for 32 (28.6%) of patients. Of those that discontinued within twelve months, 8 (61.5%) experienced adverse events, 2 (15.4%) were deceased and 3 (23.1%) discontinued for other reasons. Of the patients that discontinued due to adverse events, 4 (50%) experienced gastrointestinal symptoms and there were 1 of each of bone pain, jaw pain and migraine. Limitations of the study include the relatively large number of patients with insufficient follow up data (28.6%), and the use of patient hospital records as a mechanism of determining compliance. In this study the worst case adherence rate with alendronate at twelve months is 59.8%, and the best case is 83.8% compliance. The main reason for discontinuation was the development of adverse events, particularly gastrointestinal symptoms. This study has highlighted the importance of following up patients on long-term alendronate therapy and providing clear written instructions to ensure optimal compliance.

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ASSOCIATION STUDY OF CYP3A5 GENOTYPE WITH BONE MASS IN KOREANS

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Osteoporosis is a disease that is strongly influenced by genetic factors. Low bone mass and high bone turnover are highly related to sex hormones. Polymorphism of the CYP3A5 gene is known to influence the functionality of the CYP3A5, the second CYP3A

family member in the adult human liver , which can metabolize sex hormones. We, therefore, analyzed t he full extent of the CYP3A5 genetic polymorphism in 194 unrelated Koreans and evaluated the association of the CYP3A5 genotype with bone mineral density (BMD) in 2,178 women aged 40-79 years old. The most frequent single nucleotide polymorphism (SNP) was 6986A>G, which is responsible for CYP3A5*3. The next most frequent SNP was 31611C>T. Haplotype analysis using detected SNPs revealed that the most frequent haplotype was *3A (frequency: 0.724), followed by *1E (0.211), *3C (0.034) and *1A (0.023). CYP3AP1, CYP3A5*6, or *7 were not detected in our study. In 2,173 subjects, 62.7 % possessed the CYP3A5*3/*3 genotype which causes an aberrantly spliced mRNA with a premature stop codon and thus produces a non-functioning protein . A BMD difference, however, was not observed in women having whole CYP3A5 activity compared to women having deficient CYP3A5 activity, indicating no significant influence of CYP3A5 on bone metabolism. Our data suggest that the metabolic difference of CYP3A5 genotype may be counterbalanced by the major CYP3A such as CYP3A4.

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ANALYSIS OF BONE MINERAL DENSITY AND OSTEOPOROSIS RISK IN MANAWATU, NEW ZEALAND

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The 2006/07 New Zealand Health Survey estimates ~2.9% (or 90,000 people) of the NZ adult population are diagnosed with osteoporosis, with the incidence increasing with age. In our study we aimed to describe the population of the Manawatu region only.

Data was obtained from the only service provider for DXA (Lunar DPX-IQ Densitometer) within the Manawatu region. Baseline data from initial scans for females and males (>18 years of age), between the period of November 1996 to January 2008, were used for this study. The results, stratified for age and gender, are summarised in Table 1, and highlight the loss of BMD with age through deceasing T- and Z-scores, and increasing prevalence of osteoporosis and osteopenia.

Within the study population 2219 females reported a total 2717 fractures at the time of the baseline scan. Forearm fractures were the most common with 1265 fractures, followed by spine (685), and femur (340). 251 males reported a total of 304 fractures, with forearm fractures also the most common fracture (122), then spine (104) and femur (45).

Certain disease states and medications are known to increase risk of bone loss. Medication was reported when reporting for DXA scan. Corticosteroid use was the most common, with 954 females and 161 males reporting usage. The prevalence of other conditions reported included hyperthyroidism (343 females and 23 males) and renal disease (214 females and 28 males).

The incidence of osteoporosis in our population was much higher than previously reported for New Zealand, but this could be a reflection of the small and confined population studied. Further work is being done to assess patterns of bone loss and successfulness of intervention or treatment.

Table 1. Data are shown as mean ± SD.

	Females			Males		
	18-29yrs	30-54yrs	55+yrs	18-49yrs	50-69yrs	70+yrs
BMI (kgm^{-2})	23.0 ± 4.1	25.6 ± 5.2	26.1 ± 5.0	25.0 ± 5.5	26.5 ± 4.9	25.6 ± 4.6
Spine (n)	125	1961	5154	114	363	332
L2-L4						
T-Score	-0.22 ± 1.41	-0.05 ± 1.50	-1.20 ± 1.73	-0.11 ± 1.46	-0.64 ± 1.65	-1.00 ± 1.97
Z-Score	-0.18 ± 1.26	0.20 ± 1.48	0.48 ± 1.67	-0.18 ± 1.53	0.16 ± 1.57	0.24 ± 1.75
Osteoporosis %*	10.1	4.5	22.0	4.8	10.7	21.1
Osteopenia %*	22.5	21.2	34.0	27.2	34.7	27.7
Femur (n)	138	1967	5066	115	354	327
Total Femur						
T-Score	-0.46 ± 1.61	-0.16 ± 1.27	-1.31 ± 1.43	0.00 ± 1.31	-0.58 ± 1.31	-1.53 ± 1.57
Z-Score	-0.28 ± 1.31	0.05 ± 1.17	-0.14 ± 1.14	-0.12 ± 1.27	-0.08 ± 1.13	-0.49 ± 1.30
Osteoporosis %*	4.0	2.6	20.9	2.6	6.8	28.1
Osteopenia %*	17.5	22.8	38.3	18.3	31.1	37.3

*Osteoporosis = T-score ≤ -2.5; Osteopenia = T-score < -1 and < -1 and > -2.5

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FURTHER UNDERSTANDING FOR THE CAUSE OF BDA1 (BRACHYDACTYLY TYPE A1), THE CENTURY PUZZLE IN GENETIC HISTORY

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Brachydactyly type A-1 (BDA-1; MIM 112500), characterized by shortening or missing of the middle phalanges reported by Farabee in 1903, is the first recorded example of a human anomaly with Mendelian autosomal dominant inheritance and has been quoted in many genetic or biological textbooks. Fortunately, in our studies we successfully map the BDA-1 locus within an 8.1cM interval on chromosome 2q35-36 and then find three mutations of IHH (Indian hedgehog) are the cause for BDA-1.

In our following in vitro and in vivo studies, we use assays in cells and chick embryos, and gene targeted mice to show that a BDA1-E95K mutation in IHH (IHHE95K) impairs interaction between IHH with cell-surface transducers (PTC1) and modulators of HH signaling (HIP1), affecting the potency and range of signaling. In the mouse model that recapitulates the E95K mutation, homozygous IhhE95K mice display a classic BDA1 phenotype, with delayed endochondral ossification in the growth plate. As a result, we demonstrate the dominant E95K mutation in IHH causes digit abnormalities with occurrence of altering the capacity and range of signaling in vivo.

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VITAMIN D DEFICIENCY IN BREASTFED INFANTS

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Purpose: Vitamin D deficiency is a public health problem in many countries. There has been a reappearance of rickets from vitamin D deficiency in recent decades as a result of multiple factors. One of the factors is breast feeding. The purpose of this study was to describe the clinical presentation of rickets in breastfed infants.

Methods: Retrospective review of patients presenting to Ajou University hospital between 2003 and 2008 with rickets caused by vitamin D deficiency during breast feeding.

Results: Seventeen patients(10 boys and 7 girls) were diagnosed with vitamin D deficiency. There were six in the asymptomatic and eleven in the symptomatic patients. The mean age of the patients was 8.5 ± 0.5 months. The mean 25-hydroxycholecalciferol was 3.55 ± 1.88 ng/mL. 25-hydroxycholecalciferol levels were below 5 ng/mL in 13 patients. The mean serum alkaline phosphatase was 765.53 ± 563.9 IU/L, the mean intact parathyroid hormone was 231.6 ± 225.7 pg/mL. All except 3 patients were showed cupping and fraying of metaphysis.

Conclusion: Breast feeding is associated with increased risk of rickets. We recommend vitamin D supplementation of all breastfed infants to prevent rickets. Supplementation should begin within the first 2 months of life. Also, we hope to initiate further research and debate about guideline of vitamin D supplementation.

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THE EARLY ONSET OF CRANIOSYNOSTOSIS IN AN APERT MOUSE MODEL

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Activating mutations of FGFRs1-3 cause craniosynostosis (CS), the premature fusion of cranial bones, in man and mouse. Past analysis of a mouse model of the FGFR2 (Ser252Trp) Apert syndrome mutation suggested that increased apoptosis was the cause of suture fusion, beginning post-natally. We have reassessed coronal suture fusion in this mouse model, and provide the first detailed account of the process of embryonic coronal suture fusion in a mouse CS model. We find that the critical event of CS is the early (E13.5) loss of basal suture mesenchyme as the osteogenic fronts, expressing activated Fgfr2, unite to form a contiguous skeletogenic membrane. A mild increase in osteoprogenitor proliferation precedes but does not accompany this event, and apoptosis is insignificant. Bilateral coronal suture fusion occurs by E16.5. The more apical coronal suture initially forms appropriately but then undergoes fusion, at a slower rate, accompanied by a significant decrease in osteoprogenitor proliferation, and increased osteoblast maturation. Apoptosis now accompanies fusion, but is restricted to bone fronts coming into contact. During the process of suture fusion, we show that the progress of differentiation is accelerated, which correlates with the increased differentiation of mutant cells in vitro. Our studies suggest that the major determinant of Fgfr2-induced craniosynostosis is the failure to respond to signals that would halt the recruitment or the advancement of osteoprogenitor cells at the sites where sutures should normally form. The process of coronal suture fusion in this Apert syndrome mouse model resembles that in human Apert patients, suggesting the suitability of this model for developing effective therapies for the human condition.

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EFFECT OF CHRONIC POSTNATAL EXPOSURE TO HYPOXIC ENVIRONMENT ON CANAL NETWORK FORMATION IN INFANT RAT CORTICAL BONE

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Background: Chronic hypoxia retards skeletal growth. However, the morphological response of cortical bone microstructure (i.e., vascular canal network) to hypoxia is unknown. Using monochromatic synchrotron radiation CT, we investigated the structure of vascular canal network in infant rats exposed to chronic hypoxia.

Materials and Methods: Tibiae were harvested from 5- and 9-week-old male Wistar rats (hyp-5 and -9, n=8 each) housed in a hypoxic chamber (12-14%O2) after birth and from 4- and 5-week-old rats (cnt-4 and -5, n=8 each) maintained in ambient air. No difference was found in body weight and tibia length between hyp-5 and cnt-4 and between hyp-9 and cnt-5. The 2.5-mm-long diaphysis immediately proximal to the tibio-fibula junction was imaged with 20-keV X-ray energy and a voxel size of 3.1 μm at SPring-8 (Harima, Japan). The canal network was segmented by simple thresholding at a bone mineral density of 0.8 g/cm3 and the following indexes were computed: canal volume fraction (CaV/TV), mean density of canals penetrating transverse sections (CaN/TA), canal diameter (CaD), density of canal links (CaLn/TV), and densities of canal ends in the endocortical surface (CaN/ES) and the periosteal surface (CaN/PS).

Results: Bone and medullary volumes were similar in hyp-5 and cnt-4 and in hyp-9 and cnt-5. However, the canal network structure differed between hypoxic and control groups. In hyp-5, CaLn/TV was higher and CaN/ES and CaN/PS were lower than in cnt-4 (349±115 vs. 217±97 mm-3, 32±5 vs. 42±7 mm-2, and 28±4 vs. 37±7 mm-2, respectively) although CaV/TV, CaN/TA, and CaD did not differ between hyp-5 and cnt-4. Lower CaN/ES and CaN/PS in hyp-5 indicate less prominent invasion of microvessels into cortical bone, probably being unfavorable for trans-cortical perfusion. On the other hand, both hyp-9 and cnt-5 showed similar CaN/TA, CaLn/TV, CaN/ES, and CaN/PS, but CaV/TV and CaD were lower in hyp-9 (2.7±0.5 vs. 4.5±1.0% and 15.0±0.5 vs. 16.6±0.8 μm, respectively), implying higher vascular resistance than in cnt-5.

Conclusions: Chronic hypoxia changes the structure of vascular canal network in infant cortical bone, probably leading to a reduction in bone perfusion. Thus further reduction in O2 supply to bone will occur, thereby contributing to skeletal growth retardation.

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MYOCYTE ENHANCER FACTOR 2C IS REQUIRED FOR PROPER MC3T3-E1 OSTEOBLAST DIFFERENTIATION

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Myocyte Enhancer Factor 2c (Mef2c) is a MADS-box transcription factor required for muscle cell development. More recently, Mef2c has been demonstrated to participate in skeletal development by regulating chondrocyte differentiation. Additionally, its role in bone formation was further defined by tissue-specific inactivation in cells of the neural crest, which resulted in severe craniofacial defects. We identified Mef2c as a potential regulator of osteoblast differentiation through microarray gene expression analysis which revealed Mef2c transcript levels were significantly elevated in differentiating MC3T3-E1 cells (10.2 fold, p = 0.001). The levels of Mef2c expression was investigated through out an osteoblast differentiation time course. Mef2c displayed two distinct peaks in expression occurring at days 7 (22 fold) and 16 (16 fold) coinciding with the onset of ECM maturation and active mineralization phases respectively. The role of Mef2c in MC3T3-E1 differentiation was investigated via overexpression and knockdown studies. The overexpression of Mef2c resulted in the significant augmentation of alkaline phosphatase activity and accordingly, the osteoblast phenotypic genes bone sialoprotein (BSP) and osteocalcin (OSC) were also significantly elevated. Short hairpin (sh) RNA mediated knockdown of Mef2c transcripts resulted in significantly reduced alkaline phosphatase activity and decreased ECM mineralization. Consistently, significant decreases in BSP and OSC transcript levels were observed. The steady increase in Mef2c transcript levels observed during the initial phase of MC3T3-E1 osteoblast differentiation suggested a possible role for Mef2c in regulating cellular proliferation. To address this possibility, an MTT assay was implemented to investigate the effect of shRNA mediated knockdown of Mef2c on cellular proliferation. The assay revealed that cell numbers were significantly decreased as a consequence of Mef2c gene silencing suggesting the transcription factor participates in osteoblast proliferation. In conclusion, Mef2c gene expression is induced during the differentiation of MC3T3-E1 osteoblasts and is necessary for proper matrix mineralization.

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PERSONAL ULTRAVIOLET EXPOSURE AND VITAMIN D SYNTHESIS

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It is currently assumed that incidental UV exposure, particularly in a sunny climate should provide adequate vitamin D status for the population. This research was undertaken to test this assumption among healthy free-living adults aged 18 to 87 years, in southeast Queensland, Australia (27°S), during late February/early March 2007 (Australian summer). 42 adults (40 males, mean age 42±21 years) participated in this project, by having a blood sample taken to assess baseline serum 25(OH)D status and answering a self-reported questionnaire which sought demographic data and information about sun exposure. Participants were distributed UV dosimeters to assess personal sun exposure. 48 hours post baseline blood sample, a second sample was collected along with collection of the used UV dosimeters. The mean blood serum 25(OH)D at baseline was 70nm/L (s.d. 1.6 nm/L) and 48 hours post baseline was 78nm/L (s.d. 1.5nm/L). The median personal UV exposure in the final 24 hours the median personal UV exposure was 8.2 MED with a range of 0.2 MED to 7.4 MED. No significant correlation was found between personal UV exposure over the 48 hour period and change in 25(OH)D status between baseline and final collection. No relationships were also found between sunscreen use, age or gender. These results suggest that short term sun exposure does not impact on the 25(OH)D status, even though high exposures were of such intensity to promote the production of 25(OH)D.

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THE MC3T3E1 SUBCLONE 4 CELL-LINE AS AN IN VITRO MODEL IN THE STUDY OF THE ANABOLIC ACTION OF PARATHYROID HORMONE (PTH)

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The ability to recapitulate a PTH anabolic effect in cell culture has been a long desired system. Published studies of PTH increasing the mineralisation of extracellular matrix of osteoblastic cells in long-term culture have at times been difficult to reproduce or have required complex, intermittent treatment protocols, or both. More recently, Rey et al. (Bone, 2007) reported that an anabolic response could be obtained with continuous PTH (1-34) treatment of subclone 4 of the MC3T3-E1 cells (MC4 cells), with an increase in mineralisation by approximately 5-fold compared to untreated controls. We examined the effect of PTH 1-34 on mineralisation, ALP activity and ALP mRNA expression in the MC4 cell-line in our laboratory. MC4 cells were cultured in α-MEM and 10% FCS for 8 days, and from day 8 were treated continuously with 1nM, 10nM or 100nM PTH 1-34 and 10mM of β-glycerophosphate for up to 3 weeks. In contrast to the findings of Rey et al., a time course evaluating the effect of PTH 1-34 on mineralisation in the MC4 subclone demonstrated dose-dependent inhibition of matrix mineralisation with PTH treatments ($p<0.01$). To examine the effect of PTH on alkaline phosphatase activity and ALP mRNA, MC4 cells were cultured for 8 days then treated with 10-7 M PTH for 24 hours. PTH 1-34 increased alkaline phosphatase activity 9-fold ($p<0.05$) at 24 hours, in keeping with published results. A 10-fold increase in ALP mRNA expression was seen in MC4 cells treated with PTH 1-34 for 24 hours ($p<0.05$). Although cell culture is an invaluable tool in the study of bone biology, it has limitations in its capacity to reproduce the complexities of the in vivo system. Furthermore, reproducing in vitro conditions can be difficult, with the results of PTH treatment in MC4 cells in our laboratory resulting in inhibition of mineralisation in contrast to published data, and is consistent with findings in other stromal cell lines. Whilst all culture conditions were as described by Rey et al., we cannot exclude variations in fetal calf serum as a possible contributing factor for the different results obtained with this cell-line.

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COMPARISON OF 25-HYDROXY-VITAMIN D DETERMINATION THROUGH A COMMERCIAL PATHOLOGY LABORATORY AND A RESEARCH LABORATORY

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Vitamin D plays a major role in the regulation of calcium and phosphorus absorption from the small intestine and is therefore vital for skeletal development. Thus, vitamin D deficiency can, for example, lead to an increased risk of osteoporosis and bone fracture. Furthermore, low levels of vitamin D have been linked to decreased muscle strength as well as increased risk of colon, prostate and breast cancers.

Vitamin D is produced when 7-dehydrocholesterol, located in the epidermis of the skin, is irradiated with UV. After entering the bloodstream and being transported into the liver, vitamin D is hydroxylated to 25-hydroxy-vitamin D, which is the main circulating metabolite. A further hydroxylation reaction in the kidneys leads to the formation of 1,25-dihydroxy-vitamin D. This is the active steroid hormone which regulates calcium homeostasis.

Vitamin D is efficiently removed from the bloodstream through the first hydroxylation reaction in the liver, which happens within several hours after the release of the vitamin D from the skin, and is therefore no longer detectable. The second hydroxylation reaction that forms 1,25-dihydroxy-vitamin D is tightly regulated and the concentration of this metabolite does not necessarily reflect

an individual's vitamin D status. Therefore, in order to gain meaningful information about a person's vitamin D status, the concentration of 25-hydroxy-vitamin D needs to be measured.

Determination of 25-hydroxy-vitamin D is being offered from commercial pathology laboratories. We compared the results of 40 samples analysed in a commercial laboratory with our own research laboratory's measurements. Both methods used the same chemiluminescent immunoassay technology; however, in our laboratory we applied several QC/QA methods: internal quality controls were included in every batch of analysed samples and external quality was assessed by participating in DEQAS, the Vitamin D External Quality Assessment Scheme. Additionally, all samples were analysed by high-pressure liquid chromatography, performed in our laboratory. The results from these three methods were compared using different statistical methods and these analyses will be discussed, together with advantages and disadvantages of the methods.

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BONE CELL AUTONOMOUS EFFECTS OF OSTEOACTIVIN/GPNMB IN VIVO AND EX VIVO

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Osteoactivin/Glycoprotein nmb (OA/gpnmb) is a transmembrane glycoprotein. The protein is synthesized, processed and heavily glycosylated by osteoblasts. Its expression is associated with increased osteoblast differentiation and matrix mineralization. We have previously shown that OA/gpnmb expression in osteoblasts is regulated by BMP-2 through the Smad-1 signaling pathway. In this study, we used a mouse model with a naturally occurring mutation in the OA/gpnmb gene resulting from a premature stop codon that leads to the production of a truncated OA/gpnmb protein with no biological functions. OA/gpnmb mutant mice develop osteoporosis with age when compared to normal, wild type (WT) littermates. Histological and micro-CT measurements of femurs in mutant mice revealed a decrease in bone volume (BV/TV), trabecular number (Tb.N), and trabecular thickness in OA/gpnmb mutants compared to WT controls. Primary osteoblasts were generated from newborn OA/gpnmb and WT mice and examined for their differentiation ex vivo. All markers for early (alkaline phosphatase activity and collagen type I expression) and late (nodule formation, matrix mineralization and osteocalcin production) osteoblast differentiation were significantly reduced in the OA/gpnmb mutant osteoblasts compared to controls. We also examined bone marrow stromal cells isolated from OA/gpnmb and WT mice and testing their ability to differentiate into osteoblasts. Colony forming unit-fibroblasts (CFU-F) and CFU-osteoblasts (OB) (determined by alkaline phosphatase staining) were significantly reduced in mutant compared to WT mice. These data suggest that OA acts as positive regulator of osteoblast differentiation and function in vivo. We next examined osteoclast differentiation using a co-culture system established using normal osteoblasts as feeder cells and bone marrow (monocyte/macrophage) obtained from either OA/gpnmb mutant or WT mice in the presence of 1,25(OH)2 vitamin D3 and PGE2. Osteoclast formation/differentiation was determined by TRAP-staining and actin ring formation. Co-culture of bone marrow cells isolated from OA/gpnmb mutant mice and WT osteoclasts showed marked increase in osteoclast numbers and size when compared to osteoclasts generated from normal bone marrow cells and normal osteoblasts. These data suggest the OA/gpnmb acts as a negative regulator of osteoclast formation in vivo. Collectively, these data suggest that OA/gpnmb acts to regulate bone remodeling by positively affecting osteoblastogenesis and negatively regulating osteoclastogenesis in vivo.

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ANALYSIS OF BONE IN POMC KNOCKOUT MICE

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The proopiomelanocortin (POMC) gene encodes numerous peptide hormones secreted by the CNS, the pituitary, and other tissues in the periphery. These hormones include α-, β- and γ-melanocyte stimulating hormones (MSH), adrenocorticotropin (ACTH), β-lipotrophin, and β-endorphin. Roles for these hormones have been demonstrated in pigmentation, body weight and metabolism regulation, steroid hormone production, and pain modulation. In the hypothalamus and in the peripheral circulation, α-MSH is secreted in response to elevated leptin levels.

Several types of bone cells express subsets of the melanocortin receptors as well as the ACTH receptor. In vitro , α-MSH has been shown to increase bone turnover, increasing both osteoblast proliferation and osteoclastogenesis, while systemic administration of α-MSH reduces bone volume in vivo. There are few recent studies of the direct effects of ACTH on bone cells, and its activities in vivo are often confounded by the numerous steroid hormones it stimulates. β-endorphin is one of several endogenous opioids and this family has generally been shown to be anabolic to bone.

POMC knockout mice have non-functional adrenal glands with reduction or loss of all adrenal hormones, show increased linear growth, are morbidly obese and develop pituitary tumors with age.

In this pilot study, we examined tibia from POMC null mutants for changes in their bone characteristics before the onset of obesity (aged 8-10 weeks, 3 females per group) using computer assisted microtomography. Cortical thickness was significantly increased in POMC null mice (0.18mm±0.009SEM vs. 0.13±0.003, p=0.0139) versus controls. Changes in trabecular bone in POMC null mice

did not reach significance in several measurements: trabecular thickness (0.046mm±0.002 vs. 0.044mm±0.001), trabecular separation (0.20mm±0.006 vs. 0.23mm±0.03) or bone surface (12.1mm2±2.1 vs. 10.83mm2±0.37). Average femur length (13.9mm±0.15 vs. 13.4mm±0.23) and growth plate thickness also did not reach significance in this small number of animals, although interesting trends were again seen in the POMC null animals.

This preliminary study shows that ablation of POMC signaling results in changes in bone morphology consistent with some but not all of the constituent POMC hormones. We suggest that the combined loss of α-MSH and reduction of steroid hormone signaling may be responsible for increasing the rate of osteoblast proliferation and/or reducing the rate of osteoclast formation or function in bones, potentially leading to increased linear length. Changes in POMC hormone signaling impact bone formation during mammalian development and warrants further investigation for possible links to central control of bone metabolism.

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THE CHARACTERISATION OF THREE TCF-LUCIFERASE CONSTRUCTS IN PTH AND WNT INDUCED CANONICAL PATHWAY SIGNALLING

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The observation that Wnt signalling was critical in bone metabolism has been a major development in the area of bone biology. However, many questions regarding Wnt signalling in the regulation of osteoblast differentiation remain unanswered, including the complex interactions between PTH and Wnt signalling pathways. The study of Wnt action has resulted the development of TCF-reporter plasmids capable of monitoring canonical signalling in vitro. We have characterised and determined the utility of different TCF-constructs and their negative controls as they pertain to lithium chloride, Wnt 3a and PTH induced changes TCF/LEF-responsive gene transcription in osteoblastic cells. Three different TCF-constructs were used, and these differ in the number of TCF target sequences or the minimal promoters driving expression of a luciferase gene: TOPflash contains one set of three copies of the TCF binding site upstream of a c-fos minimal promoter; Upstate Biotech TOPflash (UB TOPflash) contains two sets of three copies of the TCF binding site upstream of a Thymidine Kinase minimal promoter; 8xTOPflash has 8 TCF binding sites with a minimal TA viral promoter. UMR 106.01 were transfected with the TCF-constructs and treated with LiCl, Wnt 3a and PTH. Treatment with 40mM LiCl resulted in a 10-fold increase in TOPflash, a 700-fold increase in UB TOPflash and a 300-fold increase in 8xTOPflash luciferase response. When monitoring responses to Wnt 3a, 8xTOPflash was the most sensitive TCF-vector demonstrating an increase in luciferase response with concentrations greater than 10ng/ml and a 400-fold increase with Wnt 3a concentrations of 100ng/ml. In contrast, TOPflash was stimulated 2-3 fold by 100ng/ml of Wnt 3a and UB TOPflash was stimulated 8-fold by 100ng/ml of Wnt 3a. When comparing the response of the TCF-reporters to PTH and PTHrP, N-terminal PTH/PTHrP increased TOPflash and UB TOPflash luciferase activity in a dose-dependent manner, whilst 8xTOPflash was not responsive to PTH/PTHrP. The various TCF-reporter constructs varied greatly in their responsiveness to LiCl and Wnt 3a, but the absent response of 8xTOPflash to PTH/PTHrP raised questions regarding the true biological effect of PTH on Wnt signalling. Recent evidence however supports PTH activation of ß-catenin signalling in osteoblasts in vitro and in vivo.

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ODANACATIB INCREASES BONE STRENGTH AND MAINTAINS BONE QUALITY IN ESTROGEN DEFICIENT ADULT RHESUS MONKEYS

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Odanacatib (ODN), a selective inhibitor of Cathepsin K (CatK) is a promising agent to treat osteoporosis. We examined bone strength/quality in the adult skeleton. Ovariectomized (OVX) rhesus monkeys (13-19yrs, N=8-11) were given ODN (0, 6, or 30 mg/kg (qd, PO)) for 21 months. BMD was measured quarterly. Central femur (CF) was tested in three-point bending, femoral neck (FN) in shearing, and vertebral body (LV4) in compression. Load-deformation curves were used to calculate Ultimate Load (F.U), Stiffness, Toughness, and Ductility. Peripheral quantitative computed tomography was used to determine BMC and cortical thickness (CFCTh) at the site of failure. Baseline LVBMD differed little among groups. Final LVBMD was 11% and 17% higher; HBMD was 10% and 16% higher; and FNBMD in ODN treated animals was 11% and 12% higher than OVX+0. CFCTh was higher (both 16%), as were CFF.U (25-32%) and CFStiffness (23-33%) with ODN than OVX+0. FNF.U was 17-19% higher and LV4F.U was 18% higher with ODN. Toughness and ductility (LV4) did not differ among groups. CFF.U and CFStiffness correlated to CFBMC ($r=0.95$; $P<0.001$ and $r=0.82$; $P<0.001$; N=28). LV4F.U and LV4Stiffness correlated to LV4BMC ($r=0.79$; $P<0.001$ and $r=0.46$; $P<.02$; N=27). CFF.U and CF Stiffness correlated with CFCTh ($r=0.70$; $P<0.001$ and $r=0.46$; $P<0.02$; N=28). ODN-treated monkeys have higher bone mass in sites of human osteoporotic fracture, greater bone strength and stiffness in the central femur, and trends toward better bone strength at the femoral neck and spine. The relationship of bone mass to bone strength is normal in cortical and trabecular bone. ODN increases bone mass and strength, while maintaining normal bone quality in adult rhesus monkeys.

	OVX+0	OVX+6mg/kg ODN	OVX+30mg/kg ODN
CFCTh (mm)	1.82±0.26	2.10±0.14*	2.12±0.226**
CFF.U (N)	1203±195	1507±251**	1589±203***
CF Stiffness (N/mm)	875±127	1067±248*	1157±212***
FNF.U (N)	2026±444	2405±307	2379±467
L4F.U (N)	3398±797	4017±1012	4017±972
L4 Ductility (mm)	0.979±0.207	0.990±0.144	0.942±0.170
Mean±SD; *(P<0.05); **(P<0.01); ***(P<0.001) vs. OVX+0 (H)-hip, (LV)-spine, CF-central femur, FN-femoral neck			

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MUSCLE CONTRIBUTIONS TO THE HIP JOINT CONTACT FORCE IN NORMAL WALKING

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Hip fracture due to osteoporosis is a significant public health problem. Hip fractures can result from an impact with the ground, or they can occur during the performance of daily activities such as standing up from a chair or during gait. Identifying the loading conditions under which the proximal femur is likely to fail would improve our understanding of the biomechanical causes of hip fracture and may aid in the development of more targeted muscle-strengthening exercises for preventing these injuries. The overall goal of our on-going work is to develop non-invasive methods for accurately assessing hip fracture risk in individual elderly persons. The specific aim of this study was to describe and explain individual muscle contributions to hip-joint loading during normal gait.

A three-dimensional dynamic simulation of walking was used to examine the contributions of the lower-limb muscles to the hip contact force. The body was modeled as a 10-segment, 23-degree-of-freedom articulated linkage actuated by 54 Hill-type musculotendon actuators, with foot-ground interaction modeled using stiff springs distributed under each foot. The simulation of walking was obtained by solving a dynamic optimization problem for the muscle excitations that minimized the metabolic energy expenditure per unit distance walked.

Not surprisingly, the model simulation results showed that the muscles which contributed most to hip contact force were those that span the hip. Muscles that span the back, knee and ankle joints also contributed, but with much less influence. The muscles that contributed most to hip contact force were the two hip extensors, gluteus maximus and hamstrings, and the hip abductor, gluteus medius. We also perturbed muscle strength in the model to study the effect on hip-joint contact force. A modest reduction in gluteus medius strength led to a considerable increase in hip contact force. This appears to be directly attributed to increases in the compensating muscles of rectus femoris, hamstrings, and tensor fasciae latae. Other mild weakness conditions (of hip flexors, quadriceps, and hip extensors) showed no noticeable change in contact force. Further studies could evaluate the effect of hip muscle strength on the distribution of stresses and strains along the femoral neck.

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DETRIMENTAL BONE EFFECTS OF SMOKING AND A HIGH FAT DIET IN MICE

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Smoking, high-fat diet and obesity are important risk factors for many globally-prevalent diseases. While cigarette smoking has been shown to be adverse to bone density and fracture risk, greater body weight may be protective against fractures. The aim of this study was to examine the effect of cigarette smoking and a high fat diet (HFD) on bone mass, density, and estimated bone strength.

After acclimatization, 40 5 week-old male b alb/c mice were randomly divided into four equal groups with similar average body weight for 7 weeks intervention: sham fed chow (sham/chow), sham fed high-fat diet (sham/HFD), smoke exposure (2 cigarettes, twice/day, 6 days/week) fed chow (SE/chow), and SE fed HFD (SE/HFD). Bone densitometric and geometric parameters of the tibia were measured using pQCT.

Sham/HFD mice were heavier than chow-fed and SE groups after the intervention (p <.001). SE/chow mice were lightest. Bone results were adjusted for body weight to account for differences between groups. Cortical bone measures were obtained at the tibial shaft (50%). Control mice (sham/chow) had the greatest cortical content, cortical thickness and periosteal circumference. Compared with control mice (sham/chow), smoking alone (SE/chow) resulted in a more slender bone; SE/HFD preserved periosteal circumference but with a thinned cortex due to endosteal expansion; and HFD alone (sham/HFD) resulted in mild reductions in cortical content, thickness and periosteal circumference. Tibial length and bending strength were similar between groups, although SE/chow mice had lower y-axis bending strength possibly due to smaller periosteal circumference and cortical thickness. Trabecular bone measures at the 5% site did not differ between groups.

Site	Sham/Chow	Sham/HFD	Smoke/Chow	Smoke/HFD	p Value
Weight (g)	26.36 (1.26)	28.69 (2.64)	22.62 (1.33)	25.34 (1.28)	<0.001
Cortical Content (mg/mm)	0.905 (0.09)	0.860 (0.12)	0.831 (0.05)	0.795 (0.05)	0.047

Cortical Thickness (mm)	0.253 (0.02)	0.240 (0.02)	0.237 (0.01)	0.220 (0.01)	0.001
Endosteal Circumference (mm)	2.220 (0.09)	2.199 (0.09)	2.162 (0.10)	2.403 (0.27)	0.006
Periosteal Circumference (mm)	3.815 (0.13)	3.709 (0.19)	3.655 (0.12)	3.789 (0.25)	0.188
Bending Strength x-axis (mm*3)	0.121 (0.03)	0.110 (0.02)	0.116 (0.01)	0.110 (0.01)	0.597
Bending Strength y-axis (mm*3)	0.122 (0.03)	0.123 (0.04)	0.106 (0.02)	0.116 (0.03)	0.556

Results - Mean (SD), p Value for between group analysis (oneway ANOVA)

In this novel model of cigarette exposure, smoking was adverse for cortical bone, particularly cortical content, thickness and periosteal circumference resulting in a thinner bone that is likely to be fracture-prone. Smoking interacted with a high fat diet and the combination resulted in cortical thinning with preservation of periosteal circumference. This may also increase bone fragility.

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SHORT-TERM VS LONG-TERM DURATION OF AED (ANTI-EPILEPTIC DRUG) PHARMACOTHERAPY: EFFECTS ON BONE HEALTH PARAMETERS

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Anti-epileptic drug (AED) therapy is a major iatrogenic cause of bone loss and fracture.

Evidence suggests an association between duration of AED-treatment and the risk of bone disease. However, the characteristics of this relationship and the influence of other factors have not been fully elucidated.

We investigated the role of the duration of AED therapy and its impact on bone by assessing the difference in bone health parameters in two AED-treated populations, comparing newly-diagnosed epileptic patients taking AEDs for ≤6 months and longer-term AED-taking patients (>6 months therapy) using a cross-sectional study design. Mean differences between groups in dual energy xray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT) parameters were evaluated and the impact of several putative risk factors (age at AED commencement, gender, AED-type, polytherapy and dosage) was explored. The study sample consisted of 91 participants. Population 1 comprised 34 newly-diagnosed, short-term AED-taking patients (56% male, 44% female) with a mean age (±SD) of 42.08 ±14.0 y. Population 2 consisted of 58 longer-term, AED-taking patients (59% male, 41% female) with a mean age of 44.29 ± 17.32 y. Of the 91 participants, pQCT scans were available in 62. Data were normally distributed and adjusted for age, height and weight. Parametric independent t-tests were then utilised to assess population mean differences.

Total hip areal bone mineral density (aBMD) presented a highly-significant difference, with those on longer-term AEDs exhibiting lower values (0.97 ± 0.014 g/cm2,mean ±SEM) than short-term users (1.05 ± 0.018), p=0.002 (two tailed). Total body aBMD was marginally different between short-term (1.14 ± 0.018 g/cm2) and long-term users (1.10 ± 0.013), p=0.051 (two tailed).

pQCT parameters were assessed at the 4% and 38% non-dominant radial and tibial bone sites. The 4% tibial trabecular density was the only parameter to display a highly-significant difference between groups, with long-term users (244.36 ± 5.55 mg/cm3) exhibiting lower values than their short-term counterparts (583.71 ± 11.53), p<0.01 (two tailed).

Preliminary examination of clinically-relevant sub-groups revealed several significant mean differences; however, a larger sample is required to confirm these findings. Observations to date provide evidence of poorer bone health with increased AED therapy duration.

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OSTEOPOROSIS AND VIT B12 DEFICIENCY AMONG METFORMIN USERS

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Human skeleton is one of the largest organ systems in the body receiving about 10% of the cardiac output and bone is a dynamic tissue, remodeled continuously during whole life through bone remodeling units (BRUs).Deficiencies of Vit. D and Vit. K are well known for pathophysiology of metabolic bone disease including osteoporosis. Vit A toxicity has also been implicated clearly for bone metabolism disorders.However,role of Vit B12(cobalamin) is not very clear for etiology of osteoporosis except occasional report of suggesting its deficiency in osteoporosois.Vit B12 deficiency is common among vegetarians and its a major concern for them.Megaloblstic anemia and neuropsychiatric manifestations are commonly seen due to Vit B12 deficiency. Chronic use of metformin has been reported as one of the causes of Vit B12 deficiency. Here we report three cases of osteoporosis (spine and hips) among patients who have also been detected as Vit B12 deficient with chronic metformin usage. All the three adult males aged 49,54,&56 yrs were on metformin for the past two years for treatment of T2DM.Detailed clinical history including various drugs intake affecting the bone mineral metabolism and systemic examinations were recorded.These cases were eugonadal and

euthyroid.They were investigated and complete haemogram including peripheral blood smear, serum electrophoresis, liver function tests, renal function tests,glycemic status along with RBC folate levels, vit B12 levels, vit D status(25-hydroxy Vit D),calcium,phosphorus, PTH, osteocalcin, beta crosslaps, total P1NP,BMD (DEXA)of hips, spine and wrist were estimated.BMD (T Score) of the patients at spine and hips were:caseI -2.6 &-1.8, caseII -2.8 & -2.2, caseIII-3.2 & -1.6 and levels of Vit B12 (Chemiluminescence) were 134, 164, 87 pg/ml respectively(Vit B12 ref values 211.0-911.0 pg/ml).25-hydroxy Vit D and PTH levels were normal in all three cases.Observation of metformin induced Vit B12 deficiency in present cases highlights the importance of complex process of osteoporosis and its correlation with Vit B12 deficiency. We suggest that more clinical studies need to be done to explain the cause and effect relationship of metformin usage, B12 deficiency and osteoporosis.

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LOW DIETARY CALCIUM INTAKE THROUGH MIDDLE SCHOOL GRADES AND ITS CORRELATES IN A DIVERSE SUBURBAN US COMMUNITY

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BACKGROUND: Adequate calcium and vitamin D intake is crucial during adolescence to attain peak bone mass. There is data on inadequate consumption of these nutrients in different population but predictors for suboptimal intake are varied and are not well defined.

OBJECTIVE: To evaluate the effect of gender, lifestyle factors, taste preferences, personal health beliefs and meal patterns in healthy middle school students in New York to identify the students' stereotypes and misconceptions.

DESIGN: A total of 250 students aged 10-14 years were selected from a public school in suburban New York and information was collected on calcium and vitamin D intake through a semi-quantitative food frequency questionnaire. A 15–question survey was developed utilizing standard validated questionnaire and reviewed by the Middle School Director of Science Curriculum to assess areas of knowledge on Vitamin D, Calcium nutrition and bone health. Each item was a multiple choice question with one correct response.

RESULTS: A moderate level of knowledge on bone health among students was demonstrated; with high scores on knowledge of the effects of the calcium on bones, and knowledge of osteoporosis and bone development. Girls were clearly more knowledgeable than boys. About 90% of students reported inadequate consumption of milk. Average reported intake of milk was only 1.38 cups/day (less than 500 mg calcium daily). Calcium supplements were used by 41 % students. 57% students reported not taking part in any physical exercise daily. The commonest misconception was that the bones are not living organs [37%]. Only 31 % knew that bones grow significantly during middle school. The most common reasons for avoiding milk were poor taste [82%], lactose intolerance or restricted dietary habits.

CONCLUSION: Attitude change lags behind knowledge. Future school calcium interventions need to take into account students' attitudes and perspectives. They should aim at motivating attitude change and preventive behavior through consistent and repeated calcium education messages that are supported by a calcium conscious school environment. Our findings have important implications regarding institution of dietary health strategies to promote skeletal health among adolescents in our community.

(1) Surgeon General Report on Bone Health: Prevention is Key. Press release at http://www.hhs.gov/news/press/2004pres/20041014.html

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NORMAL-CALCIUM, LOW-PROTEIN, LOW-SALT DIET FOR THE CONTROL OF RECURRENT STONES IN IDIOPATHIC HYPERCALCIURIA IN WOMEN

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Idiopathic hypercalciuria (IHC) is a very common condition and the main cause of nephrolithiasis. The traditional low-calcium diet is often ineffective in preventing the recurrence of stones, and may cause bone loss.

We studied the effects of a normal-calcium, low-protein, low-salt dietary intake in 38 pre-menopausal women (39.8±7.4 years) with a history of calcium oxalate stones and IHC (at baseline: average calcium intake 480±90 mg/day; water intake 1.8±0.3 L/day). IHC was diagnosed upon the finding of urinary calcium excretion >250 mg/day in at least 3 different urine samples, after excluding other known causes of hypercalciuria.

The 38 women were followed up for 4 years and we collected also their clinical records of the 3 preceding years for comparison. At baseline, all women had a marked increase in urinary calcium (402±71 mg/day), and the strontium test showed increased intestinal calcium absorption in 21 of them (55.3%).

All women received detailed written information on a diet providing normal daily intake of calcium (1000 mg) and low intake of protein (70 g, mainly vegetal), salt (2.3 g), and oxalate-rich foods. They were also instructed to avoid the simultaneous intake of calcium and oxalate and to maintain a water intake of at least 2 liters/day.

Recurrent stones were observed in 6 (15.8%) women during the 4 years of follow-up, while in the preceding 3 years, stones had recurred in 17 (44.7%) women. The relative risk of stone recurrence with the new dietary regimen was 0.35 (95% CI 0.16 to 0.78, p<0.05).

At the end of the 4-year follow-up, urinary calcium was significantly decreased (223±59 mg/day, p <0.01), as well as urinary oxalate and urea.

Osteopenia, according to the WHO criteria, was present in 30 (78.9%) of patients at baseline (Z-score -1.7±0.3), and was unchanged at the end of follow-up (Z-score -1.5±0.2).

We conclude that a low-calcium diet to prevent calcium oxalate stones is not recommended in pre-menopausal women with IHC. A normal-calcium, low-salt, low-protein diet can reduce calcium excretion and the recurrence of renal stones, and will also be useful in preventing bone loss.

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MECHANISMS OF EROSIVE GOUT: MONOSODIUM URATE MONOHYDRATE CRYSTALS REDUCE OSTEOBLAST VIABILITY

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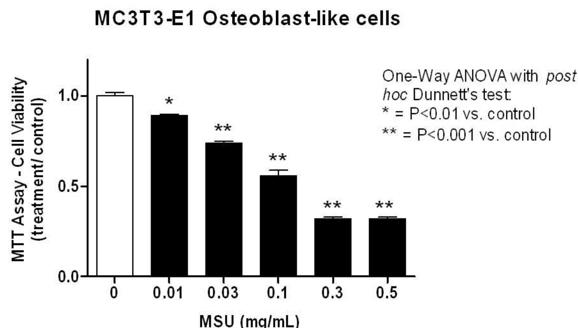
Gout is an inflammatory arthritis that is triggered by monosodium urate monohydrate (MSU) crystals within the joint. MSU crystals interact with surrounding cells and tissue within the joint causing inflammation. Bone erosion is a frequent manifestation of chronic gout, and leads to joint damage and deformity, with subsequent disability. We have recently shown that patients with erosive gout have disordered osteoclastogenesis and that MSU crystals indirectly promote osteoclast formation through interactions with stromal cells.

In this study we investigated the effect of MSU crystals on the proliferation of bone forming osteoblast cells. MSU crystals were prepared by recrystallisation of uric acid and added to MC3T3-E1 osteoblast-like and primary rat osteoblast cell cultures.

Cell proliferation was assessed following MSU treatment. MSU crystals decreased both MC3T3-E1 osteoblast-like and primary rat osteoblast cell viability in a dose-dependent manner. This inhibitory effect was maximal at the higher concentrations of MSU, 0.3 and 0.5mg/mL.

These results indicate that MSU crystals may lead to the development of bone erosion in gout both through promotion of osteoclast formation and reduction of osteoblast viability.



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OSTEOACTIVIN IN ALVEOLAR BONE REGENERATION IN EXTRACTION SOCKETS IN NORMAL AND DIABETIC RAT

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Delayed wound healing and impaired alveolar bone regeneration is associated with the metabolic abnormalities characteristic of poorly controlled diabetes mellitus. Osteoactivin (OA) is a novel bone anabolic factor that is known to play a critical role in osteoblasts differentiation and function. OA has been shown to be highly expressed at sites of active osteogenesis in vivo. In this study, we investigated the expression and localization of OA during healing and bone regeneration in teeth extraction sockets in normal and streptozotocin-induced diabetic rats. One week after induction of diabetes using Streptozotocin intraperitoneal injections, rats were examined for body weight, glucoseuria and glycosemia to confirm the diabetic condition during the study. Rats underwent extraction of first and second right maxillary molars. Animals were sacrificed at three, five, seven and ten days post-extraction. OA

localization was detected by immunohistochemistry staining. Expression of OA and other bone-related genes were determined by RT-qPCR analysis. An in vitro study was undertaken to support the results of the in vivo study. MC3T3-E1 osteoblasts like cells were isolated and cultured with different concentrations of D-glucose (15 and 25mM). Control cultures were treated with medium containing 5.5mM —glucose, standard glucose concentration in culture medium. Alkaline phosphates staining, calcium deposition and micro-array analysis of osteoblasts related genes were evaluated. Effect of OA peptide on both control and glucose treated cultures was determined by calcium deposition and expression of osteoblasts related genes (by RT-qPCR analysis). OA expression in differentiating osteoblasts in diabetic extraction sockets was significantly decreased when compared to normal animals. Expression of OA and other bone-related genes determined by RT-qPCR analysis showed a significant reduction in sockets of diabetic animals compared to controls. In vitro study showed inhibition of osteoblast differentiation and extracellular matrix maturation induced by glucose treatment. In addition treatment with OA peptide seemed to rescue the effect of glucose determined by alkaline phosphates staining, calcium deposition and expression of osteoblasts related genes in both control and glucose treated cultures. Osteoactivin plays a role in bone regeneration and formation in teeth extraction sockets. Reduction of OA expression level in extraction sockets in diabetic rats may play a role in the delayed healing and impaired alveolar bone regeneration in these animals. Future studies will explore the mechanism by which glucose effect on OA expression as an extracellular matrix protein that promotes osteoblast-mediated bone formation.

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OSTEOCYTE LACUNAR DENSITY VARIATION IN THE MIDSHAFT FEMUR OF PEOPLE OF ANGLO-CELTIC AND BANTU ANCESTRY

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Mammalian osteocyte lacuna densities reflect the rate of osteoblast proliferation, transformation, and incorporation into bone as osteocytes during growth. While it is well researched that interspecific lacuna densities vary inversely with body size, little is known about within-species variability with body size.

OBJECTIVES: Our aim is to characterize osteocyte density variation around the cortex of human midshaft femur samples obtained from individuals of known life history from the Melbourne Femur Collection (MFC) derived from the Victorian Institute of Forensic Medicine, Melbourne, Australia, and the University of Malawi College of Medicine (UMCOM), Blantyre, Malawi.

METHODS: We performed real-time 3D circularly polarized light microscopy of 100-micrometer thick histological sections of secondary osteonal bone. Lacunae were visualized, counted, and extrapolated to numbers per cubic millimeter. Lacuna densities were determined from endosteal and periosteal locations from six MFC females, aged 38, 42, 49, 55, 62, and 88 and from three UMCOM females aged 28, 35, and 50 to date.

RESULTS (ages combined):

	MFC		UMCOM	
Sector:	Periosteal	Endosteal	Periosteal	Endosteal
Mean:	19614	22712	28008	23519

UMCOM osteocyte lacuna density is higher (avg. 26512, SD=4636) compared to that of the MFC (avg. 20444, SD=1426). When specific sectors are evaluated, there is no age-related decline in lateral periosteal sectors, whose lacuna densities, when linearly regressed against body mass (Least Squares Model), are described by a significant positive relationship ($r=0.90$, $p<0.02$); no statistical relationship between lacuna density and body height was found. By contrast, in our pilot study of UMCOM individuals, we found no relationship with body mass, but with body height it is negative and significant ($r=-1.00$, $p<0.01$; the strength of this relationship is a statistical coincidence and will surely moderate with larger sample sizes). This is much unexpected, and will need to be confirmed by further study and considered for its life history meaning.

CONCLUSION: Perhaps an explanation for relatively high osteocyte density in UMCOM compared to MFC individuals relates to a difference in the way mass and height are accrued in these two regional human populations. Improved assessments of lacuna density variation on larger samples by monochromatic synchrotron x-ray imaging are planned.

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DIFFERENTIAL EFFECTS OF ODANACATIB ON TRABECULAR VERSUS CORTICAL BONE FORMATION IN ESTROGEN-DEFICIENT RHESUS MONKEYS

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Odanacatib (ODN), a selective, reversible cathepsin K inhibitor, increases BMD and suppresses bone turnover in postmenopausal osteoporotic women. We demonstrated that ODN, at approximate clinical exposure, fully prevented BMD loss and maintained normal bone quality in newly ovariectomized (OVX) monkeys. Here, ODN effects on bone formation rate (BFR) in lumbar vertebrae (LV), proximal femur (PF), and femoral neck (FN) were further characterized. Rhesus monkeys (13-19 yrs) were assigned to four groups (n=8-11): intact, OVX + vehicle, and OVX + ODN (6 and 30mg/kg, q.d., p.o.). For histomorphometry, labels were given at 15-day intervals–calcein at 10 months and tetracycline prior to necropsy. Sections (parasagittal LV2, cross-sectional PF and FN) were evaluated. BFR was the distance between dual tetracycline labels, and long-term (LT)BFR was the distance between tetracycline and calcein labels. Surface-based mineralizing surface (MSBS), mineral apposition rate (MAR), and bone formation rate (BFRBS) were determined. Baseline LVBMD differed little among groups. 21-month ODN treatment resulted in gains of 10% and 18% LVBMD, 14% and 21% hip BMD, and 11% and 15% FNBMD in 6 and 30mg/kg ODN groups, respectively, vs. vehicle. ODN dose-dependently reduced LVBFR. LVBFRBS was unaffected with 6mg/kg, and reduced by 78% with 30mg/kg. Interestingly, ODN had no effect on endosteal BFR in PF and FN. Periosteal PFBFRBS was unaffected by 6mg/kg, and tended to be higher with 30mg/kg ODN. Periosteal FNBFRBS also tended to be higher with 30mg/kg ODN. Unlike bisphosphonates, ODN appeared to stimulate long-term periosteal bone formation vs. vehicle. LTMAR at the PF periosteum was 71% higher with 6mg/kg and was 3-fold higher ($p<0.005$) with 30mg/kg ODN. LTBFR at the PF periosteum was 2-fold higher with 6mg/kg and 5-fold higher ($p<0.003$) with 30mg/kg ODN. In FN periosteal bone formation, LTBFR was unaffected with 6mg/kg and 2-fold higher with 30mg/kg ODN. Taken together, ODN treatment at 6 and 30mg/kg prevented loss of bone mass in OVX-monkeys. Effects of ODN treatment on bone formation appeared to be bone-site specific. While dose-dependently decreasing bone formation in vertebral body trabecular bone, ODN treatment either left endosteal BFR unaffected or displayed long-term stimulation in hip periosteal bone formation.

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POTENTIAL PROTECTIVE ROLES OF METALLOTHIONEIN IN ACUTE METHOTREXATE CHEMOTHERAPY-INDUCED BONE DAMAGE

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Methotrexate (MTX), a dihydrofolate reductase inhibitor, is commonly used as a chemotherapeutic agent when administered in high doses. Although its usage has been implicated behind the observed bone growth defects seen in some childhood cancer survivors, much remains to be elucidated over the underlying cellular and molecular mechanisms. Metallothioneins (MT) are a family of zinc binding proteins and may act as intrinsic antioxidants and protect tissues from damage. Several studies have also shown that loss of MT expression enhances cellular susceptibility to apoptosis and increased oxidative stress. Here we investigated the role of MT-I and –II in a mouse model of MTX-induced bone damage. Male C57BL6 wildtype (WT) and MT-1/2 double knockout (KO) mice at 4-weeks old were injected subcutaneously with MTX (12.5 mg/kg) once daily for 3 consecutive days. Bone and growth plate specimens of tibia were collected on days 0, 5, 8 and 14 after MTX treatment. Histological analysis showed a decrease in total growth plate height with age across all groups. Interestingly, by day 14 after MTX treatment, the proliferative zone of the growth plate in WT mice was significantly reduced ($p<0.01$) compared to day 14 WT controls suggesting a reduction in endochondral bone formation. Furthermore, ex vivo culture of bone marrow samples showed a significant reduction in the number of alkaline phosphatase positive CFU-f colonies by day 14 of the WT MTX-treated group compared to the day 14 WT control group, suggesting a reduction of osteogenesis. To determine potential oxidative stress caused by MTX an in vitro glutathione enzymatic assay will be utilised. Future studies will involve testing effective zinc supplementation in protecting bone, via upregulation of MT in this model.

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CHONDROGENESIS USING MESENCHYMAL STEM CELLS WITH PCL-BASED SCAFFOLDS

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INTRODUCTION

In this study, we tested the in-vitro feasibility of PCL as a scaffold for MSC-based cartilage tissue engineering and the effects of scaffold modifications.

METHODS

Three modifications of porous PCL scaffolds were examined, i.e., 1) PCL / Pluronic F127, 2) PCL/collagen, and 3) PCL/Pluronic F127/collagen, in addition to 4) PCL-only. Circular scaffolds of dimension 7mm x 2 mm were prepared, and hMSCs at passage 3 cells were suspended in DMEM/F-12 medium at 5 x 105cell/20 µl . Scaffolds were then placed individually in the wells of a 48 well-

plate. The scaffolds were left to stabilize inside the wells for 2 hours. Cell-scaffold composites were then placed in 15ml conical tubes, and cultured under DMEM/F-12 supplemented with 1% ITS, 10-7 M dexamethasone, 50 μM ascorbate-2-phosphate, 50 μM L-proline, 1 mM sodium pyruvate, and 5 ng/ml of TGF-β2 . Finally, after culturing for 21 days DNA levels were quantified, and qRT-PCR and GAG histological analyses were performed. The above procedure was repeated 5 times for each of the five donors.

RESULTS

The three surface-treated scaffolds had higher DNA contents than PCL-only scaffolds, and GAG contents in PCL/collagen and PCL/F127/collagen scaffolds were 1.5 and 1.2 fold higher than in PCL only scaffolds. Real-time PCR revealed that Col1A1 mRNA levels were lower in the three modified PCL scaffolds, and lowest in PCL/F127/collagen scaffolds. Sox-9 mRNA levels were elevated by 1.7-fold in PCL/collagen scaffolds and by 3.3-fold in PCL/F127/collagen scaffolds versus PCL-only scaffolds. Furthermore, COL2A1 mRNA levels were elevated by 4.7, 15, and 23-fold in PCL/F127, PCL/collagen and PCL/F127/collagen scaffolds, respectively, versus PCL-only scaffolds. On the other hand, Col10A1 mRNA levels were diminished in the modified PCL scaffolds, and were lowest in PCL/F127/collagen scaffolds. Histological findings generally concurred with GAG and RT- PCR findings, and demonstrated the affinity of PCL-based scaffolds for MSCs and the potentials of these scaffold in terms of inducing chondrogenic differentiation .

CONCLUSION

Our findings suggest that PCL-based porous scaffolds may be useful carriers for MSC transplantation in the cartilage tissue engineering field, and that collagen-based surface modifications further enhance the chondrogenic differentiation of MSCs.

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INCREASED VEGF IN THE SKELETON ENHANCES BETA-CATENIN ACTIVITY AND LEADS TO EXCESSIVE BONE DURING DEVELOPMENT, GROWTH AND ADULT BONE REMODELING

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Vascular endothelial growth factor (VEGF) and the Wnt pathway transcriptional regulator β-catenin both act broadly in embryogenesis and adulthood, including in the skeletal and vascular systems. Increased or deregulated activity of these molecules has been linked to cancer and bone-related pathologies. By using novel mouse models to locally increase VEGF levels in the skeleton, we found that embryonic over-expression of the major isoform VEGF164 in osteo-chondroprogenitors caused bone malformations that largely pheno-copied the effects of constitutive β-catenin activation. Juvenile or adult induction of VEGF in these cell populations also dramatically increased bone mass within 2 weeks, associated with enhanced angiogenesis. However, the bone architecture was severely disrupted by disorganized and aberrant vascularization, excessive ossification obliterating the marrow cavity, and severe cortical bone remodeling. In addition, VEGF164 induction in the bone micro-environment of adult mice caused pronounced bone marrow fibrosis and hematological anomalies. Cellular analysis revealed that VEGF over-expression altered osteoblast proliferation, differentiation and activity in vivo, and led to region-specific alterations in osteoclast activity. Mechanistically, genetic and pharmacological interventions indicated that VEGF increased bone mass via a VEGFR-2-, PI3-kinase/GSK3β- mediated pathway inducing β-catenin transcriptional activity in endothelial and osteoblastic cells. Consequently, several β-catenin target genes were upregulated in bones of VEGF over-expressing mice, contributing to the high bone density and bone marrow niche disruption. These insights into the actions of VEGF in the bone and marrow environment underscore its power as pleiotropic bone anabolic agent but also warn for caution in its therapeutic use. Moreover, the finding that VEGF can modulate β-catenin activity may have widespread physiological and clinical ramifications.

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IMMUNOEXPRESSION OF C-JUN, C-FOS, C-MYC AND MSX2 DURING ENDOCHONDRAL OSSIFICATION IN MICE

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Matrix metalloproteinases are zinc-dependent endopeptidases that degrade all components of extracellular matrix. They are able to remodelate the ECM during normal developmental processes such as embryogenesis and organogenesis, as well as in pathological

processes such as tumoral invasion. The biological mineralization research looking for discovering the genes involved in the molecular mechanisms that control the endochondral ossification process. MMPs and their inhibitors (TIMPs and RECK) are responsable for bone matrix remodeling and, probably, determinate the level of its turnover. They are regulated by the same transcription factors, such as c-jun, c-fos and c-myc. MSX2 is a homeobox-contains gene important for a limb development. Our previous studies indicated a differential expression of MMPs -2 and -9, RECK, TIMPs -1 and -2 during bone formation. Thus, our aim was investigated the temporal-spatial expression these transcription factors during endochondral ossification in mice. Femurs (n=5/period) were collected from foetuses (E13-E20) and 1 day postnatal (PN1) and processed for immunohistochemistry . In early stages (E13-E15), c-fos and c-myc no were immunolabeled, but c-jun was localizated in osteoblast-like cells at the center of cartilaginous anlagen. In later stages (E18-PN1), c-myc, c-fos and c-jun were immunopositives, mainly, in osteoblasts of ossification front and in hyperthrophic chondrocytes. MSX2 was found in all periods evaluated in osteoblasts, showing intense immunostain at E15 and its expression were decreased throughout this process. Taken together, we suggested that these transcription factors maybe are important to regulate the transcription of MMPs and their inhibitors during bone development.

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CROSS-TALK BETWEEN CTGF AND TGF-BETA1 IN MESENCHYMAL STEM CELL CONDENSATION

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Condensation or the aggregation of mesenchymal stem cells (MSCs) precedes chondrocyte differentiation and is required for cartilage formation. CTGF is a matricellular protein that has been found to be expressed during MSC condensations in vivo. It has been shown that TGF-β1 regulates CTGF expression and that CTGF acts as a downstream mediator of TGF-β1 effects on extracellular matrix production. It has also been reported that CTGF has the ability to bind TGF-β1 and modulates its effects. Using C3H10T1/2 MSCs as a model for mesenchymal condensation, we have shown previously that TGF-β1 induces MSC condensation in vitro associated with increased matrix production, proliferation and migration and this induction is mediated by CTGF. In this study, we were interested to examine whether CTGF overexpression can mediate MSC condensation in the absence or presence of TGF-β1. C3H10T1/2 MSCs were infected with adenovirus over-expressing CTGF tagged with GFP achieving a 6-7 fold increase in CTGF mRNA and protein expression. Adenovirus expressing only GFP was used as control. Cells overexpressing CTGF did not show any MSC condensation. Surprisingly, TGF-β1 induced MSC condensation was inhibited in cells overexpressing CTGF. These results suggest that a fine equilibrium of CTGF expression is required for TGF-β1-induced MSC condensation. We next examined the effect of CTGF overexpression on MSC adhesion and spreading associated with vinculin localization at focal adhesion and actin cytoskeletal reorganization. Cells overexpressing CTGF spread more robustly with an increased punctuated signal of vinculin at sites of focal adhesion with the formation of lamelopodia when compared to cells infected with GFP virus. We next examined the signaling pathway associated with MAP Kinase family to evaluate differences between TGF-β1-induced MSC condensation and the inhibitory effect of CTGF overexpression on MSC condensation. Phosphorylated P38, Jnk and Erk were increased in the GFP-infected MSCs treated with TGF-β1. However, MSCs infected with GFP-CTGF and treated with TGF-β1 showed only an increase in phosphorylated Jnk and Erk but not P38. These findings indicate that p38 MAPK may mediate MSC condensation by TGF-β1. Further studies are warranted to modulate P38 expression to elucidate the interaction between CTGF and TGF-β1 in regulating MSC condensation.

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SKELETAL PHENOTYPE IN TRANSGENIC MICE OVER-EXPRESSING CTGF IN CELLS OF THE OSTEOBLAST LINEAGE

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CTGF has recently emerged as an important growth factor in osteogenesis, demonstrated by its ability to promote proliferation, matrix production and differentiation in cultures of osteoblasts. Since most of the data concerning the role of CTGF in osteogenesis has come from in vitro studies, in this study we generated transgenic mice in which CTGF is over-expressed under control of the truncated 3.6kb collagen type I (pOBCol3.6) promoter (CTGF pOBCol3.6 mice). This promoter was chosen because it is expressed early during osteoblast differentiation. The targeting vector used to generate transgenic mice also contained LacZ (to identify cells expressing the transgene) and an enhancer element to boost CTGF expression. The presence of the transgene was determined by PCR of tail DNA using transgene specific primers. Six lines were established by mating founder mice with C57/Blk6 wild type (WT) mice. Multiple tissues were used to examine specificity of transgene expression using PCR with transgene specific primers, followed by confirmation of CTGF mRNA expression levels by Northern blot analysis. Transgene expression was highest in long bone and calvaria, with lower levels of expression in other type I collagen producing tissues (lung and skin). Two of the transgenic lines with different CTGF expression levels were used for analysis of the skeletal phenotype. Mice from one line survive, however, mice from the other line die within a few days after birth. Line one showed a 3-4 fold (moderate expression) increase and line two showed a >7-8 fold (high expression) increase in CTGF protein levels in bone when compared to age matched WT mice. Histological and

morphometric examination of the distal femoral metaphysis from TG mice with moderate over-expression of CTGF exhibited significant increases in trabecular bone volume associated with increased osteoid thickness and osteoblast activity/numbers compared to WT mice. Increased thickness of the periosteum with increased numbers of osteoprogenitor cells was also observed in TG compared to WT bone. Primary cultures of osteoblasts derived from these TG mice also exhibited enhanced differentiation (ALP staining and mineralization) compared to WT cultures. Surprisingly, examination of bones from transgenic mice over-expressing CTGF at very high levels demonstrated an increase in osteoclast number and size. These data suggest that the precise effects of CTGF on bone cell differentiation and function depend on the magnitude of CTGF over-expression. Moderate levels of CTGF have a direct effect on osteoblasts to promote bone formation, while high levels favor the formation of osteoclasts, perhaps indirectly through a RANK-L dependent mechanism.

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DEVELOPMENT OF THREE-DIMENSIONAL CULTURES FOR ASSESSMENT OF CELL PROLIFERATION AND OSTEOGENIC DIFFERENTIATION IN VITRO

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Bone is a complex tissue that is three dimensional in nature and capable of bearing mechanical stress. It is very difficult to simulate the characteristics of this tissue in vitro in monolayer cell culture. Therefore, reliable and stable three-dimensional (3D) systems for in vitro osteogeneic assessment are critical to study the behaviour of this tissue under different treatments in order to develop potential therapeutic strategies. The aim of this study was to develop 3D systems for evaluation of cell proliferation and osteogenic differentiation in vitro. The following 3D scafflolds were investigated: collagen scaffolds with or without channels, collagen/hydroxyapatite scaffolds, coralline hydroxyapatite and hydroxyapatite microspheres. Human bone marrow mesenchymal stem cells (hMSCs) and a murine osteoblastic cell line (2T3) were used to establish cultures on these scaffolds. Cells were seeded on these scaffolds and cultured in alph-MEM supplemented with FBS, beta-GP and asc-2P) for differentiation. Lactoferrin, an anabolic glycoprotein extracted from milk, was used to treat the cells in order to evaluate its effect on the 3D cell cultures. The cultures were assessed for cell proliferation using Alamar Blue, cell viability using Live/Dead staining, osteogenic differentiation using alkaline phosphatase and mineralisation using Alizarin Red S or Calcein. The 3D cultures were imaged with various bioimaging technologies, including laser confocal microscopy, fluorescent microscopy with Z-scan and extended focus, scanning electron microscopy and image analysing software for quantitative analysis. Collagen scaffolds with multiple channels demonstrated better penetration of cells into the scaffolds. Both coralline hydroxyapatite scaffolds and the hydroxyaptite ceramic microspheres supported cell proliferation and osteogenic differentiation, and the latter scaffolds have potential for large scale 3D dynamic culture, in which microspheres are slowly rolled in a rock-and-roll system. Lactoferrin-treated osteoblastic cells in microsphere culture, demonstrated significant increases in proliferation (3 fold) of 2T3 cells at day 3 ($p<0.05$) in non-osteogenic culture medium compared to vehicle controls.

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ANABOLIC AND ANTI-CATABOLIC SYNERGY IN BONE TISSUE ENGINEERING

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Introduction

Recombinant human bone morphogenetic protein-7 (rhBMP-7) is an anabolic bone drug used clinically for the treatment of bone defects. We have previously published that when given systemically, anti-resorptive (anti-catabolic) bone drugs such as bisphosphonates can act synergistically in a critical defect model1 . Local delivery of bisphophonates has been proposed to be deleterious for bone tissue engineering applications, due to toxic effects2. We have explored the potential synergy between rhBMP-7 and the biphosphonate Pamidronate (PAM) when both are delivered locally via a polymer pellet surgically implanted in the hind limb of a mouse.

Methods

Poly-D,L-lactic acid polymer (PDLLA) pellets containing 25µg rhBMP-7 +/- increasing doses of PAM were produced under sterile conditions. These were implanted into the hind limb muscle of 11wk old fe male C57BL6 mice to induce ectopic bone over 3 weeks. Bone formation was assessed by radiography (27kV) and quantitative computed tomography (QCT).

Results

X-ray data confirmed that local PAM could augment rhBMP-7 induced bone formation (Fig 1B-D) compared to controls without PAM (Fig 1A). However, the highest dose of PAM at 2mg/mouse produced a negative effect on BMP-induced bone formation (Fig 1E), and was confirmed by QCT scanning.

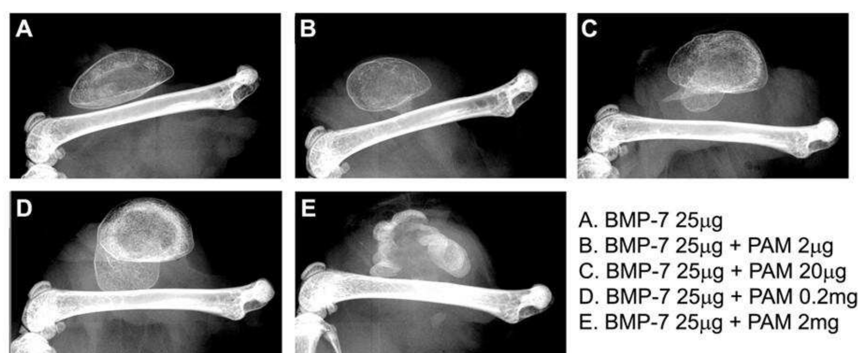

Figure 1. Radiographs of bone nodules

Discussion

The primary action of bisphosphonates (e.g. PAM) is to inhibit bone resorbing cells (osteoclasts). We speculated that PAM would antagonise the pro-osteoclastic effects of rhBMP-7 and thus maximise its pro-anabolic effect. However, at high doses, bisphosphonates can have non-specific effects that affect other cell types. Thus it was speculated that while low doses of PAM may augment rhBMP-7 induced bone, high doses may affect cells other than osteoclasts and suppress bone formation. This is what was observed in our model; PAM increased bone formation in a dose-dependent manner up until 0.2mg. Notably, the abnormal bone seen in the 2mg treatment group was comparable to the dose range used by Choi et al.2 who reported inhibited bone formation with local PAM treatment in a skull defect mode.

(1) Little et al. J Bone Miner Res. 20:2044-52 (2005)

(2) Choi et al. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 103:321-8 (2007)

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THE RELATIONSHIP BETWEEN PENTOSIDINE AND INDICES OF ARTEROSCLEROSIS IN PRE-MENOPAUSAL JAPANESE WOMEN

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Advanced glcation end products (AGE) in collagen have been reports to decrease the mechanical property of bone. Collagen cross-linking can divided into two types ; enzymatic and nonenzymatic. The enzymatic formation of cross-links may have beneficial effect on bone strength. On the other hand, nonenzymatic formation of cross-links may have effect on arterial stiffness and bone fragility .

The purpose of this study was to elucidate the relationships between pentosidine (one of the AGE) and indices of arterosclerosis (total cholesterol in serum and arterial stiffness) in pre-menopausal Japanese women.

The subjects studied were 66 pre-menopausal women at the mean age of 45.6years (37-52yrs). The height, body weight and BMI were measured as physical parameters. Previous disease, menstrual status and the age of menarche were determined using a questionnaire form. The bone mass of calcaneus was measured by using ultrasounds bone densitometer (AOS-100, ALOKA Co., Japan) . The arterial stiffness was measured by using cardio-ankle vascular index (CAVI : VaSera VS-1500N, Fukuda Denshi Co., Japan) . The CAVI has been recently reported as a new index of arterial stiffness, which is less influenced by blood pressure than pulse wave velocity (PWV). In addition, we investigated total cholesterol and calcium in serum, deoxypyridinoline in urine as a marker of bone resorption and bone alkaline phosphatase in plasma as a marker of bone integration.

The subjects were divided into two groups, a higher pentosidine group (n=27) and a lower pentosidine group (n=39). The average of total cholesterol and calcium in serum and deoxypyridinoline in the urine were higher in the higher pentosidine group than those in the lower group, while no difference was observed in bone mass of calcaneus and arterial stiffness (CAVI).

These results suggested that increased pentosidine level is a possible case of progression of arterosclerosis and bone resorption in pre-menopausal women.

VISCERAL ADIPOSITY IS INVERSELY ASSOCIATED WITH BONE MINERAL DENSITY

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Backgrounds: It has long been thought that obesity protects against osteoporosis. However, recently published epidemiologic studies challenged this thought, and showed that after control for body weight, obesity *per se* was inversely correlated with bone mineral density (BMD), and also associated with higher risk of non-spine fractures. Meanwhile, another clinical study showed that after adjusting for body mass index, metabolic syndrome was also associated with lower BMD, and higher incidence of osteoporotic non-vertebral fractures. Thus, we hypothesized that visceral obesity as well as body composition might be associated with BMD.

Methods: A total of 427 subjects (268 men and 159 women), who visited Severance hospital for medical checkup, were included in this study. The body composition was measured using the bioelectrical impedence analysis methods. The ultrasonography was performed to measure subcutaneous and visceral fat thickness. BMD was measured using dual energy x-ray absorptiometry.

Results : The mean age of participants was 55.5 ± 8.6 years for men, and 55.1 ± 9.8 for women. The mean BMI was 25.1 ± 2.6 for men, and 22.7 ± 3.1 for women. After adjusting for covariates including age, weight, regular alcohol consuming, regular exercise and postmenopausal state (in women), percent lean mass was positively associated with BMD at spine (r = 0.214, P =0.002 in men; r = 0.254, P =0.008 in women), femur neck (r = 0.203, P =0.002 in men; r = 0.166, P =0.077 in women), and total hip (r = 0.181, P =0.005 in men; r = 0.212, P =0.026 in women), whereas percent fat mass was inversely correlated with BMD at spine (r = -0.203, P =0.002 in men; r = -0.175, P =0.059 in women), femur neck (r = -0.195, P =0.002 in men; r = -0.172, P =0.061 in women), and total hip (r = -0.149, P =0.018 in men; r = -0.191, P =0.039 in women). Visceral fat thickness also showed negative relationship with BMD at spine (r = -0.159, P =0.017 in men; r = -0.275, P =0.004 in women), femur neck (r = -0.176, P =0.005 in men; r = -0.273, P =0.004 in women), and total hip (r = -0.139, P =0.028 in men; r = -0.255, P =0.008 in women), but subcutaneous fat thickness was not associated with BMD at those sites.

Conclusion: Lean mass may have a beneficial effect on BMD, whereas fat mass may affect BMD negatively. Not subcutaneous fat, but visceral fat also has a negative relationship with BMD.