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ORAL COMMUNICATIONS

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OC1.1
Wnt16 Promotes Osteoblastogenesis and is Negatively Regulated by Glucocorticoids *In Vitro* and *In Vivo*

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Glucocorticoids (GCs) are effective drugs to treat inflammatory diseases, but exert detrimental effects on bone when used over longer periods of time. One of the main mechanisms of GC-induced bone loss is the suppression of osteoblast activity. Osteoblast-derived Wnt16 has recently been shown to determine cortical bone mass by regulating osteoclast function. However, its role in osteoblastogenesis and its regulation by GCs remain unknown. Here, we assessed the role of Wnt16 during osteoblast differentiation and tested whether GCs regulate Wnt16 expression. Wnt16 was highly expressed in primary murine bone marrow stromal cells (BMSCs), promoted osteoblastogenesis and activated canonical Wnt signalling in MC3T3-E1 cells. GC treatment using dexamethasone (DEX) decreased Wnt16 mRNA expression levels by 50% *ex vivo* in BMSCs. Wnt16 suppression was dose-and time-dependent, reaching a maximum after 48 h at a concentration of 1 μM. Consistently, treatment of mice with GC-containing slow-release pellets for two weeks reduced vertebral bone mineral density by 13% and Wnt16 mRNA levels by 35% in bone tissue. The suppression of Wnt16 by GCs was strictly GC receptor (GR)-dependent. Co-treatment of BMSCs with DEX and the GR antagonist RU-486 completely abrogated the GC-mediated suppression of Wnt16. Likewise, DEX failed to suppress Wnt16 expression in BMSCs derived from GR knockout mice. Additionally, Wnt16 mRNA levels were unaltered after GC treatment in bone tissue of GR^{dim} mice, which lack the ability of GR dimerisation and therefore binding of the GR to DNA, suggesting that GCs suppress Wnt16 via direct DNA-binding mechanisms. In line with this, DEX treatment reduced Wnt16 promoter activity in MC3T3-E1 cells. Thus, this study underlines the pro-osteogenic effect of Wnt16 and identifies Wnt16 as a novel GC target. As the suppression of Wnt16 could define a mechanism of reduced osteoblast activity, Wnt16 may represent a novel target for therapeutic intervention in GC-induced bone loss.
Disclosure: The authors declared no competing interests.

OC1.2
Osteoblast N-Cadherin Restrains Wnt/β-Catenin Signalling and the Osteo-Anabolic Effect of Dkk1 Inhibition

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We and others have shown that N-cadherin (Ncad) physically interacts with low density lipoprotein receptor-related protein-5 or 6 (Lrp5/6) and Axin, resulting in negative regulation of canonical Wnt/β-catenin signalling in osteoblasts. We tested whether removal of the N-cadherin gene (*Cdh2*) alters bone mass accrual in response to Lrp5/6 signalling. We administered a Dickkopf-1 (Dkk1) neutralizing antibody (αDkk1) to activate Lrp5/6 in mice with conditional *Cdh2* ablation driven by the 2.3 *Col1A1* promoter (Col1-cKO). At the dose of 5 mg/kg body weight i.p., 3 times/week for 4 weeks, αDkk1 was ineffective in WT mice, but produced a 2-fold increase of BV/TV (0.496±0.085 $p<0.01$ vs. baseline) in *Col-Cdh2* cKO mice. A higher dose (20 mg/kg) was equally effective in both genotypes. At the molecular level, a single dose of αDkk1 produced accelerated accumulation of β-catenin in bone of Col1-cKO relative to WT mice, indicating direct Wnt/β-catenin signalling activation in bone by αDkk1 and enhanced responsiveness in the absence of Ncad. To corroborate this finding, we introduced one Dkk1-resistant $Lrp5^{A214V}$ allele associated with high bone mass (HBM), in conditionally *Cdh2* ablated mice driven by *Osx1-Cre* (Osx1-cKO). Although bone mass (by μCT) was lower in Osx1-cKO than in WT, the compound $Lrp5^{A214V}$;*Cdh2* cKO mutants were osteosclerotic and indistinguishable from $Lrp5^{A214V}$ HBM mice. Despite lower total β-catenin abundance in Osx1-cKO bone marrow stromal cells, steady state cytosolic β-catenin was not decreased. Upon Wnt3a stimulation, N-terminally un-phosphorylated β-catenin was more abundant in Osx1-cKO than in WT cells, suggesting higher levels of active β-catenin in the absence of N-cadherin in response to Wnt3a. In summary, mice lacking *Cdh2* in osteolineage cells are hyper-responsive to Wnt signaling activation and to its osteo-anabolic effect. These results provide *in vivo* proof of the concept that Ncad restrains anabolic Lrp5/6 signalling in bone forming cells.
Disclosure: The authors declared no competing interests.
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OC1.3

Mechanical Loading Reduces Inflammation-Induced Human Osteocyte-to-Osteoclast Signalling

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Multiple factors contribute to bone loss in inflammatory diseases such as rheumatoid arthritis (RA), but circulating inflammatory factors and immobilisation play a crucial role. Mechanical loading prevents bone loss in the general population, but the effects of mechanical loading in patients with RA are less clear. Therefore, we aimed to investigate whether mechanical stimuli can reverse the modulatory effects of circulatory inflammatory factors present in RA-serum on osteocyte-to-osteoclast signalling. We also investigated whether inflammatory factors present in RA-serum alter the response of osteocytes to mechanical stimuli. Human primary osteocytes from trabecular bone pieces were treated with 10% serum from active RA patients or healthy controls for 7 days. Then cells were subjected to 1 h mechanical loading by pulsating fluid flow (PFF; 0.7±0.7 Pa, 5 Hz) or static control culture, and medium NO and PGE2 concentrations were determined. Cells were post-incubated without PFF for 1h, and cytokine gene expression was quantified by qPCR. Osteoclast precursors were cultured for 21 days with PFF-conditioned medium (PFF-CM) or static-conditioned medium (stat-CM) collected after 1h post-incubation, and osteoclast formation was assessed. RA-serum did not affect IL-6, CYR61, COX2, MEPE, or SOST gene expression in osteocytes. However it enhanced the RANKL/OPG expression ratio by 3.4-fold, while PFF nullified this effect. PFF enhanced NO production in both control-serum and RA-serum–pretreated osteocytes, while PFF only enhanced PGE2 production in control-serum–pretreated osteocytes. Stat-CM from RA-serum–pretreated osteocytes enhanced osteoclastogenesis compared with stat-CM from control-serum–pretreated osteocytes, while PFF-CM from RA-serum–pretreated osteocytes nullified this stimulatory effect on osteoclastogenesis. PFF-CM from control-serum–pretreated osteocytes also inhibited osteoclastogenesis. In conclusion, RA-serum containing inflammatory factors did not alter the intrinsic capacity of osteocytes to sense mechanical stimuli, but induced osteocyte-to-osteoclast communication, while mechanical loading nullified this effect, suggesting that mechanical stimuli could contribute to the prevention of osteoporosis in RA.

Disclosure: The authors declared no competing interests.

OC1.4

RNA interfering Strategy to Cure Autosomal Dominant Osteopetrosis Type 2 (ADO2)

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Genetic autosomal dominant diseases are generally due to heterozygous missense mutations that could be eradicated by RNA interference. We hypothesised that this approach could cure ADO2 and tested this treatment in ADO2 mice carrying a G213R amino acid substitution in the CLC-7 protein, encoded by the Clcn7 gene. Using a systematic mutation-driven strategy, we designed and tested in-vitro various small interfering (si)RNAs against this mutation and found a Clcn7G213R-siRNA that silenced specifically the mutant transcript in transfected HEK293 cells (-85%,p=0.02), without affecting the WT gene and rescuing bone resorption in ADO2 osteoclasts (+2.6-fold, p=0.003). This siRNA was made "sticky" by 3'dAdT overhangs, conjugated with the delivery system 'in-vivo-JetPEI®' and injected i.p. in ADO2 mice. Time- and dose-dependent experiments evidenced 4 mg/Kg every 48 h to be the most effective treatment, decreasing the mutant mRNA in tibias (-80%, p=0.01). Two-weeks treatment of ADO2 mice, down-regulated Clcn7G213R mRNA expression in bone and other organs, increased the serum bone resorption marker CTX over the osteoclast marker TRAcP 5b (+1.8-fold, p=0.002), and decreased trabecular BV/TV (-19%, p=0.04) and Tb.N (-16%, p=0.05) vs scrambled-siRNA treated ADO2 mice. After 4 weeks, trabecular BV/TV (-21%, p=0.03) and trabecular variables (Tb.N -19%, Tb.Sp +1.2-fold, p<0.03) returned to WT level, with a full rescue of the bone phenotype. In the rescued ADO2 mice, serum CTX/TRAcP, osteoclast number and erosion surface/bone surface were normalised (+1.2-fold, -32%, +2.1-fold, p=0.03, p=0.01, p=0.02, respectively, vs. control ADO2 mice), while osteoblast and dynamic parameters were unremarkable. Treatment was well tolerated, with no adverse events, and with normalisation of liver aspartate aminotransferase. To the best of our knowledge, this is the first experimental curative treatment of ADO2, which rescued osteoclast function and returned the bone phenotype to normal by a systemic RNA interference strategy. The invention is protected by the patent application RM2014A000272, which could provide the means to develop this siRNA strategy for therapy in humans.

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OC1.5

Dominant Mouse Model with Uncleavable Type I Collagen C-propeptide Processing Site has Extremely Brittle Bones

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Classical osteogenesis imperfecta (OI) is caused by type I collagen mutations. Mutations in the C-propeptide cleavage site of both *COL1A1* and *COL1A2* cause high bone mass OI, characterised by bone hypermineralisation. To elucidate the role of type I procollagen C-propeptide processing in bone formation, we generated a mouse with a heterozygous C-propeptide cleavage site defect (high bone mass, HBM), substituting both COL1A1 cleavage site residues to prevent BMP1 cleavage. Western blots on long bone extracts revealed unprocessed pro- and pC-collagen and cleaved C-propeptide in HBM bone. At 2 months, male HBM mice are significantly smaller in weight (77%) and length (92%) and have shorter femurs (92%). All 2

month HBM mice have pelvic deformities; 40% have kyphosis. Femoral aBMD in HBM mice is decreased 25% ($p<0.001$), but vertebral BMD is normal. On µCT, HBM femora have thinner cortices with decreased cortical area. Four-point bending revealed significantly decreased HBM femoral stiffness, yield load, and ultimate load. HBM femora are also extremely brittle; post-yield displacement is only ~10% of WT (0.23 vs 0.03, $p<0.001$). Collagen from HBM calvarial osteoblasts had normal biochemistry with normal trimer incorporation, however, HBM osteoblasts deposited only about 50% of WT matrix. *Sost* transcripts in HBM femora are decreased ~40% and suggests C-propeptide processing may also influence cellular differentiation. Dermal fibril diameters were smaller and more homogeneous in HBM than WT, with loss of large fibrils. The HBM mouse phenotype is similar to that of the *Bmp1*$^{-/-}$/*Tll1*$^{-/-}$ mouse which also has small size, thin cortices, reduced maximum load and a dramatic decrease in post-yield displacement. The HBM mouse demonstrates that the essential elements of the broader enzyme deficiency are reproduced by a substrate defect in type I C-propeptide cleavage. These data show the importance of the type I procollagen C-propeptide to both collagenous and mineral properties of bone.
Disclosure: The authors declared no competing interests.

OC1.6
Combination Sclerostin Antibody and Zoledronic Acid Treatment Outperforms Either Treatment Alone in a Mouse Model of Osteogenesis Imperfecta
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Background: Osteogenesis Imperfecta (OI) is a genetic disorder featuring bone fragility and decreased bone mass. Bisphosphonates in children with OI reduce bone catabolism and rely on modelling to form new bone. An anabolic treatment, Anti-Sclerostin Antibody (Anti-SOST Ab), is being investigated in clinical trials. We hypothesised that combined treatment may produce superior outcomes.
Methods: Female Col1a2 G610C mice and their wild type (WT) littermates were treated from week 5 to week 9 of life to either saline (control), zoledronic acid (ZA) 0.025 mg/kg sc weekly, Anti-SOST Ab given 50 mg/kg IV weekly (Anti-SOST), or a combination of both (ZA Anti-SOST). Outcomes included weekly DEXA for areal bone mineral density (BMD) (GE Lunar PIXImus WI, USA), µCT (SkyScan 1174 Kontich, Belgium), mechanical testing of tibiae in 4 point bending (Instron 5944, Massachusetts, USA). Data were analysed with one-way ANOVA (SPSS v11).
Results: Increases in tibial BMD were seen over time in all groups. Anti-SOST treatment alone had no effect on tibial BMD, while ZA (16%) and ZA Anti-SOST (27%) treatments produced significant increases from weeks 1-4 ($P<0.05$). µCT analysis showed increases in Tissue Mineral Density and Cortical Thickness for combined treatment over respective controls. Tibial 4-point bending showed only combined ZA Anti-SOST yielded a significant increase in strength and energy to failure in OI mice, restoring bone strength to the values of untreated WT mice. In the spine, all treatments increased compression strength over control, Anti-SOST 30%, ZA 43% and ZA Anti-SOST 91% ($P<0.05$).
Conclusion: Anti-SOST Ab alone had effects on trabecular but not cortical sites in this study in Col1a2 G610C mice. Roschger *et al.* reported minimal effect in the Col1a1(Jrt)/+ mouse model treated with Anti SOST Ab, whereas large effects were noted with just 2 weeks treatment in 8 week-old Brtl/+ mice, leading to increase in bone size and strength. A combination of zoledronic acid and anti-sclerostin antibody is superior over either treatment alone in the Col1a2 G610C model of OI. Further studies are required in alternate mouse models of OI to confirm efficacy across different models, and thus to predict possible efficacy across the heterogeneous population of OI patients.
Disclosure: Little DG Research Contracts Novartis Pharma; Kneissel M, Kramer I Novartis Pharma employees; Anti-SOST Antibody supplied by Novartis Pharma.

OC2.1
Melatonin Improves Bone Mineral Density (BMD) at the Femoral Neck in Post-Menopausal Women with Osteopenia: a Randomised Controlled Trial
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Background: Melatonin is known for its regulation of circadian rhythm, however, over recent years, studies have shown that melatonin also has a positive effect on bone. With age, the melatonin levels decrease leading to further imbalanced bone remodelling. We aimed to investigate whether treatment with melatonin may improve bone parameters.
Method: In a double-blind placebo-controlled investigator initiated study, we randomised 81 healthy post-menopausal women with osteopenia to one-year of treatment with melatonin in a nightly dose of 1 mg (N=20), or 3 mg (N=20), or similar placebo (N=41). At baseline and after 12 months of treatment, DXA measurements of body composition, and BMD at the spine and hip were collected. Biochemical markers of calcium homeostasis were measured throughout the trial.
Results: Mean age was 63 (range 56-73) years. Compared with placebo, BMD at the femoral neck increased by 1.4% (95%CI:-2.7;-0.0, $p<0.05$) in response to melatonin. A dose-response relationship was present ($p<0.01$) as BMD at the femoral neck increased by 2.3% (95%CI:0.7;4.0, $p<0.01$) in the high dose (3mg/d) melatonin group compared with placebo. Compared with 1 mg/d of melatonin, BMD in the 3 mg/d group increased by 1.9% (95%CI:0.0;3.7, $p<0.05$). Treatment did not affect BMD at other skeletal sites or levels of bone turnover markers, however, there was a significant decrease in 24 h urinary calcium in the melatonin group (-3.7%, IQR:-2.9;57.0) compared with placebo (8.5%, IQR:-11.5;19.4, $p=0.02$). Moreover, compared with placebo, melatonin decreased fat mass significantly by 6.8% (95%CI: 1.3;12.3, $p=0.02$), while lean body mass increased by 2.2% (95%CI:-4.8;0.3,$p=0.08$).
Conclusion: One year of treatment with melatonin improved BMD dose-dependently at femoral neck and showed beneficial effects on body composition in terms of a reduced fat mass and borderline increased lean tissue. Further studies are needed to assess mechanisms of action and whether nighttime melatonin may protect against fractures.
Disclosure: The authors declared no competing interests.

OC2.2
Spontaneous Femoral Varization as a Risk Factor for Atypical Femoral Fractures

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Background: Several reports have linked bisphosphonates (BPs) with atypical femoral fractures (AFFs), but there is still debate regarding the real influence of these drugs on the development of such fractures. We speculated that AFFs could be related to lower limb geometry, specifically to spontaneous femoral varization, which would result in increased stress on the femoral cortices.
Methods: In order to test this hypothesis, we conducted a case-control study examining the geometric characteristics of the femur in patients who had suffered an AFF during treatment with BPs and in control patients taking BPs for a long time and not experiencing AFFs. A standing X-ray of the lower extremities was obtained. The following parameters were measured: curvature of the femur, distance from the femur to the load line, femoro-tibial angle and load angle. Eight women on BPs suffered 11 AFF (8 complete fractures and 3 incomplete fractures). Three patients had AFFs of both femora, the control group included 21 women with postmenopausal osteoporosis.
Results: The geometric features of patients with AFFs were very different from those of the control group, with a marked tendency to increased curvature of the femur in the patient's group (Table 1).
Conclusion: Our results suggest that patients with disturbed lower limb geometry are at higher risk of AFFs. Therefore, it may be worthwhile to obtain a standing X-ray of the lower legs in patients on long-term BPs in order to identify those individuals more susceptible to AFF.
Disclosure: The authors declared no competing interests.

Table 1 [OC2.2]: Geometric features

	CASES		CONTROLS		p
curvature of the femur (grade)	9	2-20	–4.4	(–10)-5	0.00004
distance from the femur to the load line (mm)	37.9	14-53	6.3	0-17	0.00004
femoro-tibial angle (grades)	4	1-9	–3.6	(–8)-17	0.0003
load angle (grades)	7.4	5-12	–7.6	(–11)-9	0.0002

OC2.3
Osteoprotegerin Autoantibodies are Independently Associated with Reduced Bone Density in Coeliac Disease

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Background: Autoantibodies neutralising the effect of the bone regulatory cytokine osteoprotegerin (OPG) have been described in a patient with severe osteoporosis and coeliac disease. This study aimed to determine the prevalence of autoantibodies to OPG in patients with coeliac disease, and correlate their presence with bone mineral density.
Methods: A direct enzyme linked immunosorbent assay using recombinant OPG as a capture antigen was developed and used to screen serum from 282 patients with coeliac disease for autoantibodies to OPG. Bone mineral density data was available in 254 patients. A threshold for the presence of OPG antibody was defined as the mean plus three standard deviations of values obtained from 102 healthy controls.
Results: OPG autoantibodies were found in 14/282 (5%) patients with coeliac disease. Bone mineral density results are summarised in table 1. The presence of OPG antibodies was associated with lower spine bone mineral density T and Z-scores on both univariate analysis, and multivariate analysis including age, sex, height and weight as covariates ($p<0.05$). This association was also seen when analysing the titre of OPG antibody as a continuous trait. A non-significant reduction in mean bone mineral density hip scores was seen in patients with OPG antibodies. (See table 1.)
Conclusion: Raised levels of OPG autoantibodies are found in 5% of patients with coeliac disease and are independently associated with reduced spine bone mineral density. Further work is required to establish the clinical utility of testing for OPG antibodies.
Disclosure: PLR and SHR are co-applicants on a patent application protecting the detection and/or treatment of diseases associated with autoantibodies to osteoprotegerin. This work was supported by the ECTS Amgen Bone Biology Fellowship (2010) and Coeliac UK/CORE charity (2013).

Table 1 [OC2.3]: Bone mineral density of coeliac patients defined by OPG antibody status

Characteristic	OPG antibody present	OPG antibody absent	p value
Spine BMD T-score	-2.00 (±1.2)	-1.05 (±1.3)	0.02
Spine BMD Z-score	-1.12 (±1.39)	-0.10 (±1.2)	<0.01
Hip BMD T-score	-1.36 (±0.99)	-1.01 (±1.10)	0.29
Hip BMD Z-score	-0.38 (±0.84)	-0.03 (±0.97)	0.24

OC2.4

Mitochondrial DNA Point Mutation is Associated with Lower Bone Mineral Density and Altered Bone Structure in a Matched Case-Control Study

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Background: Mitochondrial dysfunction is associated with several clinical outcomes including diabetes, myopathy, hearing loss and is implicated in the human ageing process. Mitochondrial mutations cause osteoporosis in mouse models. The effect of mitochondrial dysfunction on bone has not been studied in humans.
Methods: We recruited 38 patients (24 female, 14 male) with the mtDNA3243A>G mutation aged 45.8 ±14.9 years. Twenty-three of the cases had diabetes mellitus. Cases were matched with respect to sex, age, height and menopausal status with healthy controls. All participants underwent DXA and HR-pQCT scans. Finite element analysis was used to assess bone strength.
Results: Cases and controls were matched with regard to age, sex and height, but cases had a lower body weight (63.3 vs. 75.7 kg) and higher calcium and vitamin D supplements. Based on DXA, cases had a lower total hip aBMD (0.82 vs. 0.95 g/cm^2, p<0.01), femoral neck aBMD (0.65 vs. 0.80 g/cm^2, p<0.01) and spine aBMD (0.91 vs. 0.98 g/cm^2, p=0.02). Compared to controls, cases had smaller cortical area (radius: 56.0 vs. 64.2 mm^2, p<0.01, tibia: 98.4 vs. 134.6 mm^2, p<0.01), thinner cortices (radius: 0.80 vs. 0.92 mm, p<0.01, tibia: 1.06 vs. 1.29 mm, p<0.01) and lower total bone vBMD (radius: 312.6 vs. 370.8 mg/cm^3, p<0.01, tibia: 275.8 vs. 316.2 mg/cm^3, p<0.01). In cases, cortical density was lower at the radius (888.8 vs. 913.9 mg/cm^3, p=0.02) and trabecular density was lower in tibia (154.1 vs. 176.8 mg/cm^3, p=0.02). In tibia, but not radius, estimated bone stiffness (165.8 vs. 209.4 kN/mm, p<0.01) and failure load (8.5 vs. 10.7 kN, p<0.01) was lower in cases. Hip BMD remained lower in cases after adjusting for weight.
Conclusion: Bone mass, microarchitecture and strength were compromised in patients with mitochondrial dysfunction. Further studies are needed to describe the effects of mitochondrial dysfunction on bone remodelling.
Disclosure: The authors declared no competing interests. Region of Southern Denmark; The A.P. Møller Foundation for the Advancement of Medical Science; Institute of Regional Health Services Research / University of Southern Denmark.

OC2.5

Bone Marrow Lesions Detected by Different Magnetic Resonance Sequences as Potential Biomarkers for Knee Osteoarthritis: Comprehensive Tissue Level Analysis

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Background: MRI-detected bone marrow lesions (BMLs) are associated with symptom severity and structural degeneration in knee osteoarthritis (OA). What BMLs represent at the tissue level is poorly described. The study aim was to characterise the cartilage-subchondral bone features corresponding to BMLs detected using two different MRI sequences for a knee OA cohort.
Methods: Whole tibial plateaus were retrieved from 54 patients (27-female, 27-male), aged 51-86 years, undergoing total knee replacement surgery for OA. To identify BMLs ex-vivo, 3T-MRI scans were performed using T1 and PDFS-weighted sequences. MRI images were used for cartilage volume measurement. Micro-CT was used to assess microstructure of subchondral bone plate (SBP) and trabecular bone (STB). Cartilage and subchondral bone were assessed by OARSI and histopathology. Bone turnover indices were quantitated.
Results: BMLs were detected in 78% of patients (remainder formed No-BML group). Of all BMLs, BML-1 group (BML detected by PDFS only) represented 62%; BML-2 group (BML detected by PDFS and T1) represented 38%. BML-2 had reduced cartilage volume (p=0.007) with increased OARSI degenerative changes (p=0.009) compared to No-BML. BML-2 SBP was thicker and had lower porosity compared with No-BML (p<0.0001). BML-2 STB had higher bone volume (p=0.003), thicker (p=0.002) and more plate-like trabeculae (p=0.0004). SBP and STB osteoid volume and thickness were increased for BML-2 compared to No-BML (p<0.0001). More bone marrow oedema, necrosis and fibrosis was present in BML-2 compared to BML-1 and No-BML (p=0.03).
Conclusion: Knee OA BMLs are associated with loss and degeneration of the overlying cartilage, together with more sclerotic bone morphology. These relationships are more significant for BML-2, suggesting that BML-2 type lesions represent a later stage of OA disease. BMLs detected with specific MRI sequences may act as potential MRI biomarkers for identification of individuals at high risk of progressive OA and inform development and monitoring of new therapies.
Disclosure: The authors declared no competing interests. This work was supported by the National Health and Medical Research Council of Australia (APP1042482).

OC2.6

Gender-Specific Effects of Bisphosphonates on Mortality among Austrian Hip Fracture Patients Aged ≥50 Years

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We retrospectively analysed effects of bisphosphonates (BPs) on mortality in Austrian hip fracture patients. For 31,668 patients ≥50 years sustaining a hip fracture in Austria between July 2008 and December 2010, information on survival with follow-up until June 2011 and on prescription of BPs between July 2007 and June 2011 was available. Using Cox and logistic regression analysis, cumulative all-cause mortality among patients who started treatment before or after fracture was compared with that among age- and sex-matched hip fracture patients without anti-osteoporotic medication. The minimum prescription interval was set at half a year, and matched subjects had to be alive during the prescription interval of his/her assigned treated subject. Compared with female patients receiving no anti-osteoporotic prescription, women who initiated BPs before first fracture (n=8,868) displayed unaltered short-term mortality (hazard ratio [HR] at 90 days after fracture: 0.91 [95%-CI: 0.76-1.09, p=0.30]) but decreased long-term mortality (odds ratios [ORs] at one year and three years' post-fracture, respectively: 0.70 [0.62-0.79, p<0.0001], 0.68 [0.61-0.76, p<0.0001]). Women starting BPs after first fracture (n=3,216) exhibited relative HRs of 0.29 (0.16-0.55, p<0.001) and 0.39 (0.29-0.52, p<0.0001) one year and three years' post-fracture, respectively. For males using BPs already before fracture (n=837), no statistically significant reduction in mortality emerged, however, lowered mortality at one year post-fracture was observed for men treated only after fracture (n=633) (HR 0.12 [0.02-0.88], p<0.05). Among hip fracture patients using BPs, mortality was reduced predominantly in females. The smaller effect of BPs on pre-fracture users' relative to post-fracture users' survival might reflect a selection bias inherent to this observational study with more co-morbidity among BP users than non-users. However, the high extent of mortality reduction found in post-fracture BP users portends a causal relationship with anti-resorptive treatment with BPs.

Disclosure: The authors declared no competing interests.

OC3.1

Identification of Chloride Intracellular Channel Protein 3 as a Novel Gene Affecting Bone Formation

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Osteoporosis is a common skeletal disorder characterised by low bone mass leading to increased bone fragility and fracture susceptibility. Identification of specific factors that stimulate osteoblast differentiation from human mesenchymal stromal cells (hMSCs) may deliver therapeutic targets to treat osteoporosis. The aim is to determine novel factors and mechanisms involved in human osteoblast differentiation. Gene expression profiling was performed on hMSCs differentiated towards osteoblasts or adipocytes using Illumina microarrays. We selected genes that were differentially (2-fold) regulated in the osteogenic versus the adipogenic condition, as well as up- or down-regulated (1.5-fold) versus time point zero. Based on bioinformatic analyses we identified the gene CLIC3 (Chloride Intracellular Channel Protein 3). Lentiviral overexpression of CLIC3 in hMSCs was used to assess the effect osteogenic differentiation. CLIC3 overexpression caused a 34% increase in both alkaline phosphatase activity (p=0.047) and mineralization (p=0.04). Next, we used an *in vivo* human bone formation model where hMSCs lentivirally transduced with the CLIC3 overexpression construct were loaded onto a scaffold (hydroxyapatite-tricalcium-phosphate) and implanted under the skin of NOD-SCID mice and analysed for bone formation after 8 weeks. CLIC3 overexpression led to a 15-fold increase in bone formation (0.33% vs. 5.05% bone area relative to scaffold, p=0.0007). Knockdown of CLIC3 in hMSCs using two short hairpin RNAs against CLIC3 resulted in 89-96% reduction in CLIC3 mRNA expression (p=0.0037 and 0.0026, respectively) and 70-90% less mineralisation (p<0.0001 for both) compared with scrambled control. In conclusion, we successfully identified CLIC3 to be a lineage-specific gene regulating osteoblast differentiation and bone formation. CLIC3 encodes a membrane transport protein that may function in cell growth, vesicle transport, and integrin trafficking. We are currently using pull down and proteomic analysis to investigate the molecular mechanism underlying the CLIC3 control of osteoblast differentiation.

Disclosure: The authors declared no competing interests.

OC3.2

Neuro-Protein CRMP4 Inhibits Bone Formation by Regulating BMP Signalling and Rhoa-FAK Network

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We employed a global gene expression profiling using DNA microarrays to characterise non-canonical osteogenic factors regulating the differentiation of bone marrow skeletal stem cells (marrow stromal stem cells, BMSCs) into osteoblastic cells. We identified CRMP4 (collapsing response mediator protein-4) that was the only member of CRMP1-5 family to be expressed by BMSCs. We found CRMP4, a cytosolic phosphoprotein that mediates Semaphorin-3A effects in neuronal differentiation to be expressed by proliferating chondrocytes and osteoblastic cells and its expression was detected in bone lining osteoblasts in postnatal and adult mouse bones. *In vitro* gain and loss of CRMP4 function in bone marrow stromal cell line ST2 revealed the inhibitory effect of CRMP4 on osteoblast differentiation. Consistently, mice lacking Crmp4 expression displayed significant increased bone mass by 40% compared with wild type controls due to increased trabecular and cortical bone

microarchitecture parameters as measured by micro-CT analysis. Histomorphometic analysis revealed significant increased osteoblast number/bone surface in Crmp4-/- bone with no effect on osteoclastic bone resorption parameters compared with WT controls. Mechanistic studies revealed that increased bone mass in Crmp4-/- mice was associated with upregulation of BMP2-induced osteogenesis in Crmp4-/- osteoblasts (OB) as evidenced by enhanced activation of canonical and non-canonical BMP2 signalling. Furthermore, Crmp4-/-OB exhibited enhanced activation of RhoA/focal adhesion kinase (FAK) signalling that led to cytoskeletal changes associated with increased rate of cell spreading as well as increased cell proliferation rate by increasing the percentage of Crmp4-/- OB in S/G2/M phases of the cell cycle compared with WT OB. The later effect was mediated via inhibiting p21Cip/Waf and upregulating cyclin D1 expression, two targets of RhoA pathway. These findings identify the neuro-protein CRMP4 as a novel negative regulator of bone formation by inhibiting BMP-induced osteogenesis and RhoA-stimulated OB proliferation. Thus, CRMP4 is a new therapeutic target for enhancing bone formation.

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OC3.3

Transgenic Over-Expression of Vitamin D Receptor in Mature Osteoblasts Enhances Catabolic Activities under Dietary Calcium and Phosphorus Restriction

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Osteoblast-specific over-expression of vitamin D receptor (VDR) in a transgenic mouse on a FVB/N genetic background (OSVDR) increases bone volume due to both reduced RANKL-mediated osteoclastic bone resorption and enhanced bone formation. These observations are in contrast to reports of 1,25-dihydroxyvitamin D (1,25D) enhancing osteoclastic bone resorption and inhibiting bone mineralisation. To address this conundrum, 3w female mice with Osteoblast-specific Over-expression of Vitamin D Receptor (VDR) (ObVDR-Tg), Osteoblast-specific VDRKO (ObVDR-KO) and littermate control mice (WT, $VDR^{fl/fl}$) all on a *C57/Bl6* genetic background were fed calcium/phosphorus restricted diet (0.03%Ca, 0.08%Phos; LowCa/P) for 17 weeks and compared with a normal diet (1%Ca, 0.625%Phos; NormCa/P). ObVDR-Tg mice fed the NormCa/P diet demonstrated increased trabecular (64% $P<0.01$) and cortical bone volumes (8%, $P= 0.056$) when compared with WT mice with increased periosteal circumference ($P<0.05$). All mice fed the LowCa/P diet resulted in marked osteopenia with almost total absence of metaphyseal trabecular bone. However, LowCa/P fed ObVDR-Tg mice maintained the increased periosteal circumference, whereas LowCa/P fed ObVDR-KO mice decreased periosteal circumference. Furthermore, LowCa/P fed ObVDR-Tg increased the endosteal circumference, whereas LowCa/P fed ObVDR-KO decreased the endosteal circumference. Interestingly, LowCa/P fed ObVDR-Tg mice exhibited marked intra-cortical porosity and a 22% reduction in cortical osteocyte density. While, serum calcium and phosphorus levels were unaltered in LowCa/P fed ObVDR-Tg mice, serum FGF23 levels were 2-fold lower and serum 1,25D levels were 2-fold higher when compared with WT mice. In addition, RANKL mRNA levels and RANKL:OPG ratio were markedly raised in LowCa/P fed ObVDR-Tg mice. Thus, while overexpression of VDR in osteoblasts can mediate anabolic activities, under conditions of limited dietary calcium and phosphorus, profound bone catabolism prevails possibly due to a lack of appropriate FGF23 feed-back on renal 1,25D synthesis and enhanced RANKL-mediated catabolism.

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OC3.4

Absence of VDR in Mature Osteoclasts Results in Enhanced Resorptive Activity

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Mature osteoclasts express the vitamin D receptor (VDR) and while we have shown that these cells respond to active vitamin D (1,25(OH)2D3), the role for direct activity of vitamin D in regulating osteoclast function is not well understood. To assess the role for VDR-mediated activity in osteoclasts, osteoclast-specific vitamin D receptor knockout mice ($OclVDR^{-/-}$) were generated by mating Cathepsin K^{Cre} with floxed VDR mice ($VDR^{fl/fl}$). Male and female $OclVDR^{-/-}$ and $VDR^{fl/fl}$ littermates were assessed at 6 and 12 weeks of age under normal dietary conditions. In addition, isolated splenocytes from global VDRKO mice or their wild-type (WT) littermates were assessed for osteoclast formation, resorption activity and gene expression under osteoclast-forming conditions. 6w old $OclVDR^{-/-}$ mice demonstrated increased osteoclast surface (Oc.S/BS) in L1 vertebra in both female (+20%, $P<0.05$) and male (+67%, $P<0.05$) mice when compared to $VDR^{fl/fl}$ mice. In $OclVDR^{-/-}$ mice, V-ATPase (V0 subunit) mRNA was increased ($P<0.05$) and Calcitonin Receptor (CTR) mRNA markedly decreased ($P<0.05$). Despite this, biomarkers such as serum X-laps and TRAP5b were not significantly different between $OclVDR^{-/-}$ and $VDR^{fl/fl}$ mice. Furthermore, only males demonstrated a trend for decreased vertebral BV/TV% due to increase trabecular spacing (Tb.Sp) ($P=0.05$). Interestingly, RANKL mRNA levels were significantly decreased suggesting reduced signalling for osteoclastogenesis. VDRKO splenocytes cultured under osteoclastogenic conditions resulted in 2-fold fewer TRAP-positive multinucleated cells ($P<0.05$) compared with WT cells. However, the resorption area on Osteologic™ slides was 3-fold greater per VDRKO osteoclast ($P<0.05$). VDRKO osteoclast CTR mRNA levels associated with reduced Bax/Bcl mRNA ratio were markedly decreased compared with WT suggesting resistance to apoptosis. Thus, while vitamin D

receptor expressed within haematopoietic precursor cells may not be required for differentiation of osteoclasts, the role for VDR in mature osteoclasts appears to be to attenuate resorptive activity.

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OC3.5

Mature Osteoblasts Regulate Vitamin D-Mediated Bone Resorption during Growth and Dietary Calcium/ Phosphorus Restriction

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Active vitamin D (1,25D), bound to the vitamin D receptor (VDR), can directly regulate osteoblast activity modulating bone resorption via induction of RANKL. However, it is somewhat unclear as to which cells of the osteoblast lineage are predominantly responsible for this activity. We have generated mature Osteoblast-VDR Knock Out (mOb-VDRKO) mice using an osteocalcin promoter-Cre to demonstrate the role of VDR-mediated bone resorption in mature osteoblasts during growth and under dietary calcium/phosphorus restriction. 6 week old female mOb-VDRKO mice displayed a pronounced reduction in RANKL mRNA expression, metaphyseal osteoclast surface (OcSur/BS) and serum X-laps. As a consequence, trabecular bone volume (BV/TV%) was increased in the femur (19%, $p<0.05$) and vertebra (21%, $p<0.05$) in comparison to littermate controls. The increase in trabecular bone in female mOb-VDRKO persisted at 12w of age but was absent by 26w of age. By comparison, 6 week old female Osteocyte-specific-VDRKO mice (deletion driven by Dmp1-Cre), exhibited no structural differences in femoral trabecular BV/TV%, and unchanged OcSur/BS. However, vertebral BV/TV% was modestly increased (8%, $p<0.05$) in Oy-VDRKO mice. When 3 week old female mOb-VDRKO mice were subjected to a low calcium (0.03%) and phosphorus diet (0.08%) (LowCa/P) for 3 weeks, serum PTH levels and X-laps levels were approximately 2-fold greater than LowCa/P fed control mice, resulting in the abrogation of the bone phenotype to levels comparable to control mice. When the LowCa/P was continued to 20 weeks of age, higher serum PTH and X-laps levels persisted in mOb-VDRKO mice resulting in deleterious effects on bone including significant intra-cortical porosity. Collectively, these data suggest that mature osteoblasts play a greater role in VDR-mediated bone resorption than osteocytes in young mice. Furthermore, the absence of VDR in mature osteoblasts during calcium/phosphorus restriction results in inappropriately high PTH-mediated bone resorption, possibly through lack of appropriate VDR-mediated bone resorption.

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OC4.1

Unintentional Weight Loss and Fracture: The Global Longitudinal Study of Osteoporosis in Women

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Background: The adverse effects of weight loss on bone mineral density in postmenopausal women are well documented, and increased risk of distal forearm and hip fractures has been reported in studies with average follow-up periods of around 6 years after weight loss. The aim of this study was to investigate the effects of unintentional weight loss in postmenopausal women on the incidence of clinical fractures at multiple sites in the year following weight loss.

Methods: GLOW is an observational longitudinal study of non-institutionalised women aged ≥55 years recruited from 723 primary physician practices in 10 countries. Self-administered questionnaires were mailed and data collected included demographics, medical history, fracture occurrence, medications and weight loss of 10 lb (4.5 kg) or more over the preceding year. Cox models treating weight loss as a time-varying covariate were used to predict fracture in the following survey year, adjusting for factors such as age, prior fracture, co-morbidities, and falls that we have previously shown to be associated with the specific fracture.[1]

Results: Unintentional weight loss of ≥10 lb during the previous 12 months was reported in Year 2 by 3405 (8.0%) of 42,756 and in Year 3 by 3322 (7.7%) of 43,004 women. After adjustment for clinically relevant variables, a significantly increased risk was seen for hip (HR 1.83, 95% CI 1.25–2.69, $p<0.01$) and spine fracture (HR 1.46, 95% CI 1.02–2.09, $p=0.04$) in the year following the unintentional weight loss.

Conclusions: Unintentional weight loss in postmenopausal women is associated with increased risk of hip and spine fracture within the year following weight loss. The rapid time course of this increase in risk has not previously been reported and emphasises the need for prompt fracture risk assessment and appropriate management in women with unintentional bone loss.

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Reference

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OC4.2

Heavy Cannabis use is Associated with Reduced Fat Mass and Increased Fracture Risk but Does Not Influence Bone Density

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Preclinical studies have shown that cannabinoid receptors and their ligands regulate bone metabolism but the clinical significance is unclear. Here we investigated the effects of recreational cannabis use on bone health in the Muirhouse study based in a socially deprived area in the North of Edinburgh. We recruited 263 subjects from the local community through advertisements. Bone density and fat mass were measured on a Hologic QDR4500 densitometer and relevant clinical variables were recorded. Of the 263 individuals recruited 163 (61.9%) were regular cannabis users with a median lifetime use of 20,805 joints (range 4-197,100). The average age of participants was 44±10.3 years and 58% of subjects were female. We divided the population into three groups based on lifetime amount of cannabis taken; none (n=102); light users (4-20,800 joints; n=81) and heavy users (21,000-197,100 joints; n=79). Heavy cannabis users were younger than controls (41.3±1.0 *vs.* 49.6±0.9 years; p<0.001), had a higher dietary calcium intake (1368±104 *vs.* 884±45mg/day; p<0.001); a lower BMI (25.5±0.6 *vs.* 29.3±0.7; p<0.001) and lower fat mass (27.0±9.5 vs 33.8±8.6; p<0.001). The data for moderate cannabis users were intermediate between heavy users and controls (data not shown). Heavy users were significantly more likely to use other illicit drugs (65.8% *vs.* 2.9% for controls; p<0.001). There was no difference in BMD values between cannabis users and controls after adjustment for age, BMI, gender and other relevant variables. Fractures were more common in cannabis users (58% *vs.* 46%; p=0.06), and multiple fractures were significantly more common (10.6% vs 1.9%, p-0.008). Heavy cannabis use is associated with reduced fat mass and an increased risk of fracture, but is not associated with BMD. The differences between mice and men may be due to the complex nature of cannabis, which contains not only THC, a CB1 agonist, but multiple other cannabinoids.

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OC4.3

Significantly Improved Muscle Strength, Running Speed, and Agility in Children with Hypophosphatasia Treated with Asfotase Alfa

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Hypophosphatasia (HPP) is the rare inherited metabolic bone disorder resulting from deficiency in tissue-nonspecific alkaline phosphatase (TNSALP). HPP can cause a spectrum of sequelae in children, including muscle weakness and compromised physical function. 5-12 year-old children treated ≥3 years with asfotase alfa, a recombinant bone-targeted human TNSALP, had improved skeletal mineralisation, growth, and physical function. Here, we report muscle strength and the individual subtests of the Bruininks Oseretsky Test of Motor Proficiency, 2nd Edition (BOT-2) in these children. This ongoing Phase II open-label extension study (6 mg/kg/wk subcutaneous asfotase alfa) assessed bilateral hip and knee extension and flexion, hip abduction, and grip strength by hand-held dynamometry (HHD), reported as percent predicted (%P; right side) for matched, healthy peers. Physical function was evaluated using the BOT-2 Strength subtest (e.g. long jumps, push-ups, etc.), and Running Speed/Agility subtest (e.g. shuttle runs, one-legged hop, etc.). 11/12 patients in the extension study participated in functional testing with last assessment (LA) at 3 years (n=7) or 3.5 years (n=4). Right-side strength (%P) ranged from median (min-max) 32 (9-53; hip extensor) to 60 (21-149; grip) at baseline. Strength in all right-side muscle groups improved at 3 months (P<0.05) except grip, and continued to improve to LA (median 59-98 %P, hip and knee extensor, respectively) (P<0.05). Left side results were similar. BOT-2 Strength scaled score (mean[SD] for healthy peers: 15[5]) improved from median (min-max) 4 (1-13) at baseline to 15 (10-24) at LA (P<0.0001). Median Running Speed/Agility scaled score improved from 3 (1-9) at baseline to 12 (7-19) at LA (P<0.0001). Performance on all BOT-2 subscales improved significantly. These children with HPP had substantial muscle weakness and impaired function at baseline. With asfotase alfa treatment, rapid and continued improvements in strength contributed to significant gains in physical function, which impact ability to perform activities of daily living.

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OC4.4

Odanacatib Anti-Fracture Efficacy And Safety in Postmenopausal Women with Osteoporosis: Results from the Phase III Long-Term Odanacatib Fracture Trial (LOFT)

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LOFT (NCT00529373) is a randomised, double-blind, placebo-controlled, event-driven trial of odanacatib (ODN), an oral selective inhibitor of cathepsin K. Postmenopausal women ≥65 years with bone mineral density (BMD) T-score ≤–2.5 at

total hip (TH) or femoral neck (FN), or with prior radiographic vertebral fracture (VFx) and T-score ≤–1.5 at TH or FN, were randomised to ODN 50 mg/week or placebo. Patients received vitamin D (5600 IU/week), plus calcium to achieve intake of 1200 mg/day. Primary endpoints were: new/worsening morphometric VFx; hip fractures; non-vertebral fractures. Secondary endpoints included clinical VFx; BMD; bone turnover markers. Safety/tolerability measures included adjudicated adverse events (AEs) of interest. 16,713 women were randomised; 16,071 were included in analysis. Mean age was 72.8 years; 46.5% had prior radiographic VFx. Mean BMD T-scores were: lumbar spine (LS) –2.7; TH –2.4; FN –2.7. Mean follow-up was 34.5 months. Versus placebo, ODN treatment resulted in relative risk reductions of: 54% for new/worsening morphometric VFx; 47% for hip fractures; 23% for non-vertebral fractures; 72% for clinical VFx (p<0.001). ODN treatment led to progressive increases over 5 years in BMD at LS and TH: 11.2% and 9.5%, respectively, versus placebo. The incidence of AEs and serious AEs overall did not differ meaningfully between groups. There were 271 deaths reported in the ODN group and 242 on placebo (hazard ratio 1.13 [95% CI: 0.95, 1.35]); this numeric imbalance in mortality did not appear related to a particular reported cause of death. Adjudicated morphea-like skin lesions and femoral shaft fractures (including those with atypical features) occurred in small numbers of patients, more commonly with ODN than placebo. No cases of osteonecrosis of the jaw were reported. Major cardiovascular events overall were generally balanced; however, there were numerically more adjudicated strokes with ODN than with placebo. Final blinded adjudication of major cardiovascular events is ongoing.

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OC4.5

Tracking of 25-Hydroxyvitamin D Status in Pregnant Women

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Background: When assessed in pregnancy studies, 25(OH)D is usually measured only once. However, it is unknown whether the ranking of an individual's 25(OH)D is maintained across pregnancy, which crosses several seasons. We therefore assessed the tracking of 25(OH)D from early to late pregnancy in a prospective mother-offspring study, the Southampton Women's Survey.

Methods: At 14 and 34 weeks gestation, serum 25(OH)D was measured, and diet and lifestyle questionnaires completed. We modelled seasonal variation in 25(OH)D separately for each time point using Fourier transformations, and then calculated the difference between actual 25(OH)D and the modelled value corresponding to the sampling date for each individual [denoted $25(OH)D_{dev}$]. We used Spearman's rank correlation to test tracking of $25(OH)D_{dev}$ from 14 to 34 weeks gestation. Multivariate linear regression was used to determine factors associated with alterations in an individual's $25(OH)D_{dev}$ ranking.

Results: 25(OH)D was available in 2060 and 2332 women at 14 and 34 weeks, respectively, with 1756 women included at both gestations. $25(OH)D_{dev}$ tracked moderately from 14 to 34 weeks (r=0.57, p<0.0001), although some women had marked changes in $25(OH)D_{dev}$ across pregnancy (median: -0.8; range: -150.1 to 129.6nmol/l). 25(OH)D tended to fall with greater pregnancy weight gain ($25(OH)D_{dev}$ β=-0.4nmol/l per kg, p=0.02), and to rise with greater strenuous activity in late pregnancy (β=1.0nmol/l per hour/week, p=0.03). Vitamin D supplementation was the strongest influence on tracking: compared with women who never used supplements, discontinuing supplementation after 14 weeks was associated with negative change in $25(OH)_{dev}$ (β=-7.2nmol/l, p<0.001), whereas commencing (β=12.2nmol/l, p<0.001) or continuing (β=8.0nmol/l, p<0.001) supplementation were positively associated.

Conclusion: Stability of an individual's gestational 25(OH)D relative to the population is modest, and affected by weight changes, activity levels and vitamin D supplementation. These findings may explain some of the observed heterogeneity in studies relating maternal vitamin D status to offspring health.

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OC4.6

Meta-Analysis of Observational Studies on the Effect of Incretin Treatment on Fracture Risk

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Background: In Europe, approximately 60 million patients suffer from type 2 diabetes mellitus (T2DM). T2DM patients are at increased risk of fracture. Incretin agents are used to treat T2DM, and they include two classes: glucagon-like-peptide 1 receptor agonists (GLP1-RAs) and dipeptidyl peptidase-4

inhibitors (DPP4-Is). Currently, there is no data available from electronic healthcare databases. The objective of this study was to evaluate the association between incretin agents and risk of fracture.
Methods: We used data from the UK Clinical Practice Research Datalink (CPRD), the world's largest primary care database, representative for the total UK population (2007-2012, n=13 million) and from the full country of Denmark (2007-2011, n=5.5 million). We used a cohort design and Cox regression analysis with CPRD data and a case-control study with conditional logistical regression in Denmark (which comprised all patients with a first fracture matched to controls). We compared current incretin use with non-use. A meta-analysis extracted hazard- (HRs) and odds ratios and their corresponding 95% confidence intervals (CIs) using generic inverse variance methods assuming a random effects model.
Results: Use of incretin agents was not associated with fracture risk in both countries (adj. pooled risk ratio DPP4-I and GLP1-ra: 1.01; 95% CI 0.92 – 1.12, 1.03; 95% CI 0.87 – 1.22, respectively). Increasing cumulative dose did not further decrease risk of fracture yielding adj. HRs of 1.07; 95% CI 0.90 – 1.27 (0-18.2 mg) adj. HR 0.84; 95% CI 0.67 – 1.06 (18.3-36.5 mg) adj. HR 1.05; 95% CI 0.81 – 1.37 (36.6-54.7 mg), adj. HR 0.97; 95% CI 0.78 – 1.20 (> 54.7 mg).
Discussion: Use of incretin agents was not associated with fracture risk in both countries, and higher cumulative dosages did not result in an inverse association. Our results do not support the conduct of further clinical research to study beneficial effects of incretin agents on fracture risk.
Disclosure: The authors declared no competing interests.

OC5.1
Inhibition of the Interleukin-6-Induced STAT3 Signalling Pathway is Chondroprotective
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Background: High levels of interleukin-6 (IL-6) have been found in the synovial fluid of patients with osteoarthritis (OA), suggesting that IL-6 may be involved in the pathogenesis of OA. The objectives were to investigate the effects of IL-6 in chondrocytes and to determine its main signalling pathways; and to study the impact of IL-6 inhibition in an experimental mice model of OA.
Methods: The effects of IL-6 (10-50-100 ng/mL) were determined in vitro (primary culture of mouse chondrocytes) and *ex vivo* (mouse femoral head articular cartilage). Proteoglycan content (Alcian blue and Safranin O staining, DMM blue assay), expression of catabolic factors (qPCR, Western Blot, immunostaining), NO and PGE2 production and apoptosis (TUNEL assay) were evaluated. IL-6-induced signalling pathways were determined by western blot. The impact of STAT3 blockade was investigated using a specific inhibitor – Stattic – ex vivo and in a mice model of OA induced by destabilisation of the medial meniscus (DMM).
Results: *In vitro* and *ex vivo*, IL-6 dose-dependently induced a dramatic loss of proteoglycan content through an increase in the expression of MMP3, MMP13, ADAMTS4 and ADAMTS5. By contrast, IL-6 had no effect on col2, aggrecan, col10 or VEGF. IL-6 induced chondrocytes apoptosis without increasing NO or PGE2 production. Inhibition of STAT3 by Stattic counteracted the catabolic and pro-apoptotic effects of IL-6 *ex vivo*. Finally, we orally administrated either Stattic (25 mg/kg/2d) or a saline for 6 weeks in C57/Bl6 mice (n=18) subjected to DMM. The severity of the OA lesions as assessed with the OARSI histological score was significantly lower in the Stattic group: 2.65 ± 1.44 vs. 4.5 ± 0.93 (p=0,004).
Conclusion: Our findings indicate that IL-6 has numerous catabolic effects in cartilage, mainly mediated by STAT3. STAT3 blockade protects against DMM-induced OA in mice, suggesting that IL-6 might be a promising therapeutic target in OA.
Disclosure: The authors declared no competing interests. This work was supported by the Société Française de Rhumatologie.

OC5.2
Genetic Variants in the SUPT3H-RUNX2 Locus Confer Susceptibility for Bone and Cartilage Related Disorders via Long-Range Regulation Of RUNX2
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Genome-wide association studies (GWAS) have identified in total 6 independent SNPs within the 5' region of the *RUNX2* gene to be robustly associated with 5 different cartilage and bone related phenotypes. We aim to elucidate the effect of the identified SNPs on the regulation and expression of *RUNX2* and how these confer susceptibility to cartilage and bone related disorders, such as osteoarthritis and osteoporosis. Independent GWAS signals and SNPs in LD with the GWAS loci were identified with GCTA conditional joint analysis, the SNAP tool, and HaploReg (V2.2, Broad Institute). GWAS SNPs and SNPs in high LD were analysed for enrichment in genomic regulatory regions, and co-location with DNA binding proteins using data from the ENCODE-Project, Roadmap epigenetics project, and the FANTOM5 database. In human cartilage explants we measured *RUNX2* expression by RNA sequencing, CTCF-DNA binding by ChIP-qPCR and preformed eQTL analysis to determine the effect of the SNPs on gene expression. We found 6 genetically independent GWAS signals to co-localise to regions with enrichment of active enhancer markers, H3K4me1, H3K27ac, DNase1 hypersensitivity enrichment and bi-directional CAGE reads, in osteoblast and chondrogenic cells. The BMD associated SNP located ~700 kb away from the *RUNX2* promoter, had a significant effect ($p<0.05$) on *RUNX2* gene expression in human cartilage. In addition, we observed that when we stimulated *RUNX2* expression in human chondrocytes by TGFβ stimulation, there is an increase in binding of the chromatin-loop mediating protein, CTCF, near the *RUNX2* promoter. We have found that variants in the *SUPT3H-RUNX2* locus associated to cartilage and bone phenotypes are located in gene regulatory regions, and affect *RUNX2* gene expression. We hypothesise that the SNPs are localised in long-range enhancers which, mediated by a CTCF

chromatin-loop to the *RUNX2* promoters, regulate *RUNX2* gene expression in bone and cartilage development.
Disclosure: The authors declared no competing interests. This work was supported by the Netherlands organisation for scientific research (NWO) VIDI-scheme.

OC5.3
MUC1 in Osteoblasts Balances Osteogenesis and Angiogenesis under Hypoxia
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It is critical that bone formation and angiogenesis are tightly coordinated during bone development and fracture healing. Oxygen tension impacts both processes. Previously we demonstrated that hypoxia limits osteoblast differentiation/mineralisation and strongly induces mucin1 (MUC1) expression in human osteoblasts. Expression of MUC1 is positively associated with hypoxia-driven angiogenesis. Thereby MUC1 is a likely candidate to control both osteogenesis and angiogenesis. We investigated MUC1 function in osteoblasts and its role in the interaction between bone formation and angiogenesis. Hypoxia (2% O_2)-induced inhibition of osteoblast differentiation (Alkaline phosphatase activity -64%) and mineralisation (-89%) was prevented by blocking MUC1 function using either a specific inhibitor (GO-201) or 2 shRNAs. This was supported by studies using osteoblasts cultured from bone marrow of *Muc1* knockout mice. Conditioned medium of osteoblasts cultured under hypoxia (HCM) stimulated endothelial migration (+80%) and angiogenesis (+150%), which was prevented by blocking MUC1 in osteoblasts using GO-201 or shRNA. Mass spectrometry analysis identified among others vascular endothelial growth factor (VEGF)-A and macrophage migration inhibitory factor (MIF) to be present in control HCM but not in HCM of osteoblasts treated with GO-201 and shRNA. VEGF neutralising antibody or MIF inhibitor 4-IPP prevented HCM-induced endothelial morphogenesis. HCM induced nitric oxide (NO) production (1.8 fold increase) in human endothelial cells and inhibition of NO production blocked the angiogenic effect of HCM. Finally, it was shown that nuclear translocation of the MUC1 cytoplasmic tail in osteoblasts is essential for the effects observed. In conclusion, we demonstrate that MUC1 in osteoblasts is at the crossroad of oxygen control of osteoblast differentiation/mineralisation and angiogenesis. The level and nuclear translocation of MUC1 in osteoblasts determines whether under hypoxia either bone formation or angiogenesis prevails. Thereby, these data contribute to the molecular understanding of the balance between osteogenesis and angiogenesis in bone development and fracture repair.
Disclosure: The authors declared no competing interests. This work was supported by the European Union (PIRSES-GA-2011-295181).

OC5.4
Osteoblast-Secreted Extracellular Vesicles Stimulate the Expansion of CD34⁺ Human Umbilical Cord Blood Cells
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Umbilical cord blood (UCB) is increasingly used in haematopoietic stem cell (HSC) transplantations; however, the low cell numbers are still remaining as a limiting factor for proper engraftment. Osteoblasts play important roles in regulating HSC self-renewal and differentiation. Recently, extracellular vesicles (EVs) have been implicated in stem cell fate regulation via horizontal transfer of proteins and nucleic acids between cells. In this study, we focused on the characterisation of osteoblast EVs and investigated their potential in *ex vivo* expansion of $CD34^+$ UCB cells for clinical use. We used human pre-osteoblasts (SV-HFO cells) to isolate EVs, and characterised EVs by electron microscopy, proteomics, and RNA sequencing, and investigated their functional effect on human $CD34^+$ UCB cells by qPCR and flow cytometry. Characterisation analyses demonstrated that osteoblast EVs are heterogenic in size, contain novel osteoblast EV proteins primarily linked to ribosomal activity and RNA processing, and are enriched with small RNAs. Treatment of $CD34^+$ UCB cells with osteoblast EVs led to donor-dependent 2-3-fold expansion ($p < 0.01$) of the $CD34^+$ expressing progenitors in 10 days. MicroRNA profiling demonstrated that osteoblast EVs contain abundant amounts of miR-29a, one of the key regulators of early haematopoiesis. Interestingly, EVs treatment led to the two-fold down-regulation ($p < 0.01$) of HBP1, a miR-29a target that has been shown to be a cell cycle inhibitor, in $CD34^+$ UCB cells. Consequently, cell cycle analysis showed that EVs stimulated progression from G0/G1 to S/G2 phase ($p < 0.05$), which may explain the mechanism by which EVs stimulate UCB cell expansion. Finally, EV-expanded $CD34^+$ UCB cells showed good clonogenicity and differentiation potential *in vitro* and successful engraftment in a NOD/SCID-IL2Rγ (NSG mice) xenograft model *in vivo*. In this study, we demonstrated that osteoblasts secrete EVs that expand UCB cells *ex vivo*, and uncovered the first clues that contributed to the understanding of EV function.
Disclosure: The authors declared no competing interests. Erasmus MC Stem Cell and Regenerative Medicine Institute.

OC5.5
Sclerostin Depletion and its Effect on Fracture Healing in the Mouse Model
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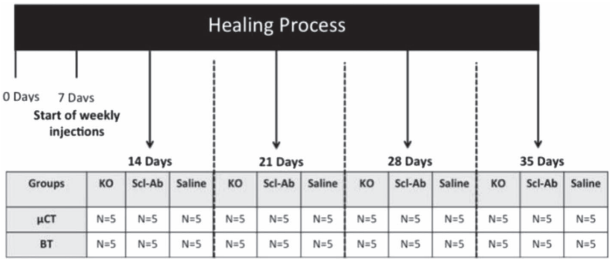
Background: Sclerostin is a secreted glycoprotein that interacts with LRP5 receptor on osteoblasts and inhibits the intracellular Wnt signalling pathway, leading to decreased bone formation. When sclerostin is inactivated bone formation is therefore stimulated. This stimulation has been proven in



Groups	KO	Scl-Ab	Saline	KO	Scl-Ab	Saline	KO	Scl-Ab	Saline	KO	Scl-Ab	Saline
µCT	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5
BT	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5

Figure 1. Fracture model protocol.

fracture studies, which showed that sclerostin deficient mice have larger and stronger calluses with accelerated fracture healing, both in sclerostin knockout and sclerostin antibody injection models. These observations suggest that sclerostin inhibition and depletion show improved and accelerated fracture healing, but the effect of these two mechanisms have not been compared to assess the accurate effect of the Scl-Ab injections. Therefore we designed a study to compare the effect of sclerostin depletion (sclerostin knockout) and inhibition (Scl-Ab injection).

Methods: Ten-week-old male SOST knockout (KO) (N=20) and wild-type (WT) (N=40) mice underwent insertion of a tibial intramedullary pin after which a mid-shaft tibial osteotomy was performed. The mice were divided into three groups: SOST KO (N=20), WT with Scl-Ab injection (N=20) and WT with saline injection (N=20). The Scl-Ab group received an intravenous dose of 100mg/kg weekly starting on day 7. Each group was managed and sacrificed according to the specified protocol (Figure 1). For data analysis, one-way ANOVA (Analysis Of Variance) was performed followed by Tukey's post hoc test at each time point. P values<0.05 were considered statistically significant.

Results: Both Scl-Ab and KO groups showed significantly increased trabecular BV/TV (bone volume/ total volume) at the fracture site (mid-shaft of the tibia) compared to the saline group at all time points and also showed no significant difference between them at all time points (except at 28 days postoperative) (Figure 2). On biomechanical testing the Scl-Ab and KO groups showed significant increased strength in stiffness at days 14, 28 and 35 compared to the saline group (Figure 3A). Concerning ultimate force and work to failure the KO group showed significant increase in the force required compared to both the Scl-Ab and saline groups at 21,28 and 35 days. While the Scl-Ab group showed increased forced required to fracture the callus compared to the saline group at these time points, but this was only significant for work to failure at 28 days (Figure 3B, D).

Conclusion: Scl-Ab injections showed promising results, which were comparable to the complete depletion of sclerostin, especially at earlier stages of the healing process. In addition, our results indicate that sclerostin antibody exerts its greatest effect in the earlier stages of fracture healing (days 14 and 21), after which the healing process plateaus and thus completing this process at an earlier time point. Further re-

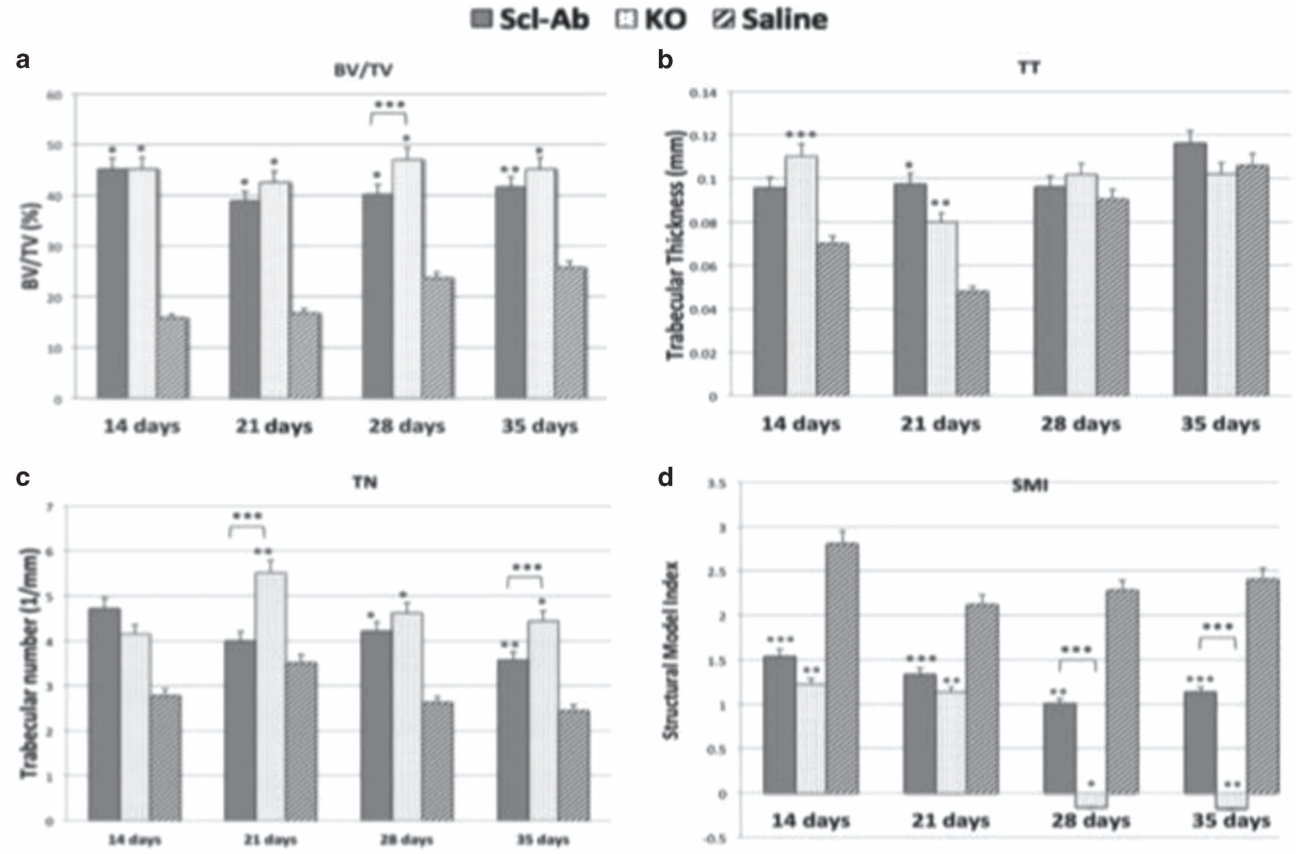


Figure 2: MicroCT results across all time points. Data presented as mean and standard error of the mean (*P<0.001, **P<0.01, ***p<0.05).
Abrev: BV/TV; bone volume/total volume, Tb.Th; trabecular thickness, Tb.N; trabecular number, SMI; structural model index).

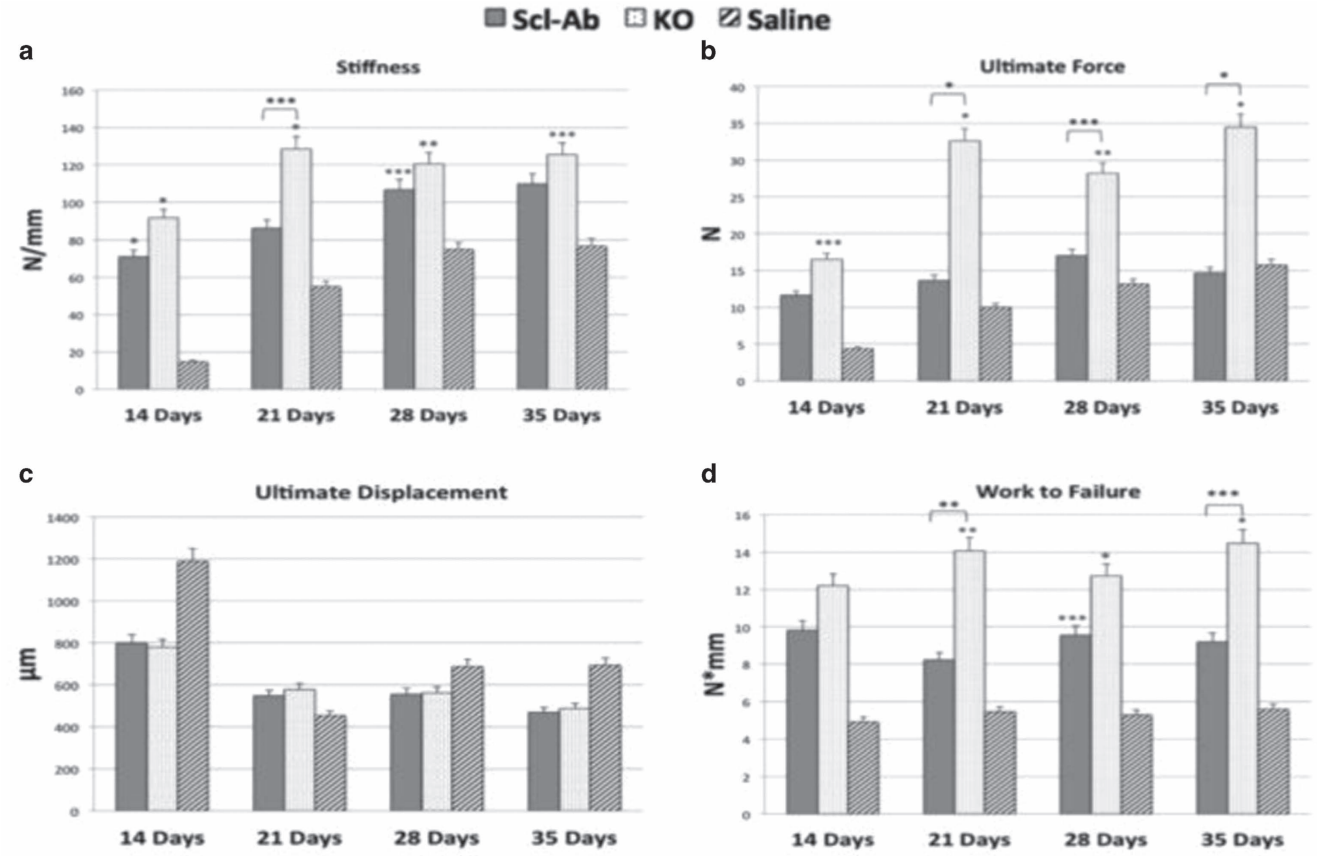


Figure 3: Biomechanical testing results across all time points. Data presented as mean and standard error of the mean (*P<0.001, **P<0.01, ***p<0.05).
Abrev: N; newton

search into accurate dosage and adequate timing of administration is required before these promising results can be implicated as a modality for accelerating fracture healing in humans and management of delayed / nonunion.
Disclosure: The authors declared no competing interests.

OC6.1
Secular Change in Fracture Incidence is Not Associated with Better Post-Fracture Outcomes: a Time-Trend Comparison between Two Birth Cohorts
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During the last decade, hip fracture incidence declined and life expectancy improved. However, it is unclear whether the outcomes following osteoporotic fracture have also changed. The aim of this study was to compare re-fracture risk and excess mortality following osteoporotic fracture between two birth cohorts and over 2 time intervals 1989-1999 and 2000-2010. Study participants comprised women and men 60+ participating into DOES1 (born before 1930) and DOES2 (born after 1930). All fractures excluding head, fingers and toes were recorded between 1989 and 2010. Age-standardised fracture incidence and mortality rates were calculated in two time intervals: 1989-1999 (for DOES1) and 2000-2010 (for DOES2). The difference in excess mortality between the 2 cohorts was assessed using standardised mortality ratios (SMR) calculated for each study cohort using time-specific population mortality rates. The prevalence of osteoporosis declined and the level of treatment increased significantly in DOES 2 compared to DOES 1. Fracture incidence declined by ~10% in both genders, however, not significantly. Interestingly, re-fracture risk was similar for DOES1 and DOES2 [women age-adjusted RR 2.0 (95% CI, 1.6-2.5) in DOES1 and 1.9 (95% CI, 1.7- 2.3) in DOES2 and men, 3.5 (95% CI, 2.7-4.8) in DOES1 and 3.4 (95% CI, 2.7- 4.5) in DOES2]. Crude mortality rates decreased during study follow-up. However, after taking into account the difference in general population life expectancy during the 2 study periods, the excess mortality post-fracture was similar [women, SMR 2.1 (95% CI, 1.7- 2.6) in DOES1 and 1.7 (95% CI, 1.2- 2.4) in DOES2, and men, 1.9 (95% CI, 1.5- 2.5) in DOES1 and 1.9 (95% CI, 1.3- 2.7) in DOES2]. Thus despite a reduction in the prevalence of osteoporosis and improvement in treatment uptake over the last 2 decades, re-fracture risk and fracture-associated excess mortality was similar. The reasons for this deserve urgent exploration.
Disclosure: The authors declared no competing interests. This work was supported by the National Health Medical Research Council Australia (NHMRC project ID; DB 1073430, JRC 1008219, TVN, JRC and JAE 1070187).

OC6.2

Intronic Mutations in the *TCIRG1* Gene Cause Human Autosomal Recessive Osteopetrosis

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Autosomal Recessive Osteopetrosis (ARO) is a rare genetic bone disease with genotypic and phenotypic heterogeneity, sometimes translating into delayed diagnosis and treatment; in particular, intermediate cases often constitute a diagnostic challenge. Mutations in the TCIRG1 gene are responsible for more than 50% of ARO cases, and a wide range of molecular defects have been found. Here we describe the identification of 4 different single nucleotide changes in intron 15 in 5 patients from 4 unrelated families. These novel mutations were in the middle of a 368 nucleotide long intron, far from the canonical splice sites; therefore, they were missed by standard gene amplification and sequencing, focused on exons and exon-intron boundaries, and went ignored by exome sequencing. In 3 out of 5 patients, by cloning and sequencing a number of independent cDNA clones covering exons 14 to 17, we demonstrated a reduced splicing efficiency, which did not completely abrogate the production of the normal transcript. In conclusion, we identified an intronic region in the TCIRG1 gene which seems to be prone to splicing mutations. These molecular defects allow the production of a small amount of protein sufficient to dampen the severe phenotype usually associated to TCIRG1 mutations. Indeed, the patients bearing these variants displayed a different level of severity of the disease, with 3 out of 5 reaching adulthood with a mild presentation. On this basis, we suggest the analysis of the TCIRG1 gene is appropriate not only in the molecular work up of severe patients, but also of intermediate cases. In addition, our results demonstrate that standard protocols for gene testing are likely to be revised. In particular, intron 15 should be included in the routine sequencing of the TCIRG1 gene; more in general, the effect of intronic changes in genes associated with osteopetrosis should be carefully evaluated.

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OC6.3

The Effects of Daily Teriparatide on the Spine and Femoral Strength Assessed by a Finite Element Analysis of Clinical Computed Tomography Scans in Rheumatoid Arthritis Patients

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Background: To evaluate the quantitative effects in RA patients who are treated with TPTD after 12 months by several methods.

Methods: Twenty-seven RA patients were enrolled in this prospective study. All patients who were receiving TPTD were evaluated according to changes in two bone turnover markers (serum procollagen type 1 N-terminal propeptide [P1NP] and, tartrate-resistant acid phosphatase-5b [TRACP-5b]) from baseline to 1, 3, 6 and 12 months. They were assessed according to bone mineral density (BMD) by dual X-ray absorptiometry (DXA) and bone strength by quantitative computed tomography (CT) at baseline. They were re-evaluated after 12 months. Nonlinear finite element analysis (FEA) was performed on the CT scans to compute an estimate of spinal and femoral-fall configuration predicted bone strength (PBS) by FEA.

Results: Patients were aged 68.2 years and their duration of symptoms was 12.2 years. The majority of subjects had moderate disease activity (mean baseline 28-joint Disease Activity Score, 3.0±1.3). The mHAQ scores median were 0.8. On average, PINP (baseline, 1, 3 and 6 months) was 42, 141, 144 and 153μg/l, TRACP-5b was 423, 527, 583 and 601 mU/dl. Patients had significantly greater levels of serum PINP and TRACP-5b ($p<0.05$ compared with baseline) at all points measured. On average, BMD-spine (baseline and 12 months) was 0.89, 0.94 g/cm^2 ($p<0.01$) (median change 6.3%), BMD-femoral neck was 0.62, 0.62 g/cm^2 ($p=0.31$) (median change 1.4%), PBS-spine was 3508, 4070 N ($p<0.01$) (mean change 19.8%), and femoral PBS- fall was 1428, 1441 N ($p=0.2$) (mean change 1.6%).

Conclusions: Our results show that TPTD can increase BMD and FEA on RA patients, and indicate that bone loss can be prevented in patients with RA by TPTD. FEA should detect changes of TPTD effects more sensitive than DXA. We will have to follow these effects in longer term.

Disclosure: The authors declared no competing interests.

OC6.4

Hyponatraemia is Prevalent and Associated with 30-Day Mortality in Hip Fracture Patients

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Background: The relationship between bone metabolism and plasma sodium levels has lately gained increasing interest as hyponatraemia has been linked to both increased risk of osteoporosis and fractures. The aim of this study was examine the frequency of hypo- and hypernatraemia in patients admitted with a fractured hip and the association with 30-day mortality in these patients.

Methods: A database of all surgically treated hip fracture patients admitted to our hospital between January 1996 and November 2013 was searched for all patients aged 60 years or above. 7755 patients were identified and a search for plasma sodium levels for these patients was conducted in the hospitals laboratory system. 7644 (98.6%) had a preoperative admission plasma sodium measurement and composed the study cohort. Comorbidity was included in the form of Charlson Comorbidity Index, which was calculated based on data from the Danish National Patient Registry. Hyponatraemia was defined as $[Na^+]<135$ mmol/l and hypernatraemia as $[Na^+]>145$mmol/l.

Results: The patients had a mean age of 82.5 (SD 8.5) years and 76.5% (5845/7644) were female. 19.0% (1455/7644) were hyponatraemic, 1.6% (123/7644) were hypernatraemic and 79.4% (6066/7644) were normonatraemic on admission. There was an increased 30-day mortality rate for patients with hyponatraemia (12.1%, p=0.008 (chi-square)) and hypernatraemia (16.3%, p=0.02 (chi-square)) compared to normonatraemic patients (9.7%). The hazard ratios for 30-day mortality were 1.26 [1.06;1.49] (unadjusted) and 1.35 [1.14;1.60] (adjusted for sex, age and comorbidity) for hyponatraemic patients and 1.74 [1.12;2.72] (unadjusted) and 1.76 [1.13;2.78] (adjusted for sex, age and comorbidity) for hypernatraemic patients.

Conclusion: The study showed that the prevalence of hyponatraemia in hip fracture patients was high. Furthermore, patients with decreased or elevated plasma sodium levels had an increased mortality rate. Disturbances in plasma sodium levels may itself cause increased mortality but could also be a surrogate marker for frailty in these patients.

Disclosure: The authors declared no competing interests.

OC6.5

The Calcineurin Inhibitor Tacrolimus as a New Therapy in Severe Cherubism

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Cherubism is a rare genetic disorder characterised by extensive growth of a bilateral granuloma of the jaws, resulting in facial disfigurement. Cherubism is caused by gain-of-function mutations in the *SH3BP2* gene, leading to over-activation of NFATc1-dependent osteoclastogenesis. Recent findings in human and mouse cherubism suggested that calcineurin inhibitors might be drug candidates in cherubism medical treatment. A 4-year-old boy with aggressive cherubism was treated with the calcineurin inhibitor tacrolimus for one year, and clinical, radiological, and molecular data were obtained. Immunohistological analysis was performed to compare pre- and post-operative NFATc1 staining and TRAP activity. Real-time PCR was performed to analyse the relative expression levels of *OPG* and *RANKL*. After tacrolimus therapy, the patient showed significant clinical improvement, including stabilisation of jaw size and intra-osseous osteogenesis. Immunohistological analyses on granuloma showed that tacrolimus caused a significant reduction in the number of TRAP positive osteoclasts and NFATc1 nuclear staining in multinucleated giant-cells. Molecular analysis showed that tacrolimus treatment also resulted in increased *OPG* expression. We present the first case of effective medical therapy in cherubism. Tacrolimus enhanced bone formation by stimulating osteogenesis and inhibiting osteoclastogenesis.

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Blood Circulating miRNAs are Indicative of Skeletal Fractures in Postmenopausal Women with and Without Type 2 Diabetes and may be Promising Candidates for General Fracture Risk Prediction

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Fracture risk in type-2-diabetes (T2D) and postmenopausal osteoporosis is routinely assessed with FRAX or DXA although these methods show limitations especially in T2Diabetics. Novel, general applicable biomarkers are therefore desirable. MicroRNAs (miRNAs) are secreted into the circulation from cells of various tissues proportional to local disease severity and were recently found to be crucial to bone homeostasis ("osteomiRs") and T2D aetiology. The objective of this study was to analyse circulating miRNAs in a well-characterised study of postmenopausal and diabetic osteoporosis and to evaluate their utility for general fracture risk assessment. MiRNA-qPCR-arrays and differential-expression-analysis of 153 miRNAs were performed from 74 serum samples drawn from postmenopausal T2D women with (DMFX, n=19) and without fracture history (DM, n=19) as well as from non-diabetic women with (Fx, n=19) and without fracture history (Co, n=17). Group-wise non-parametric statistical comparisons were used with BH-adjustment of p-values for multiple testing. Circulating miRNAs exhibiting significant differences were then used for building multi-parametric models to differentiate fracture patients from controls. Cumulative ROC analyses yielded AUC-values of 0.978 for Fx/Co-comparisons (based on 4 miRNAs) and 0.933 for DMFX/DM-comparisons (based on 4 miRNAs). Interestingly, the 4 highly discriminative miRNAs of each comparison did not overlap. We found that some of them have been previously described as "osteomiRs", such as miR-155-5p, an initiator of osteoclastogenic differentiation, or miR 96-5p, an osteocyte negative marker. All remaining ones had not been previously characterised (e.g. miR-188-3p and miR-203a) yet. Therefore, additional *in vitro* tests were performed, to characterise their (anti)-osteogenic activity. Our data provide first evidence that certain circulating miRNA levels are indicative of fragility fractures in postmenopausal women with and without diabetes and may be novel candidates for general fracture risk screenings. Future studies will elucidate if this knowledge can be used to improve current diagnostic techniques to predict fracture risk and therapy response in elderly women.

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