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ABSTRACTS

Abstracts of the ECTS Congress 2019

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Plenary Oral Presentations 1: Rare musculoskeletal diseases

PLO01

A rare mutation in SMAD9 associated with high bone mass identifies the BMP signalling pathway as a potential osteo-anabolic target for osteoporosis

Celia Gregson[1], Dylan Bergen[2], Richard Sessions[2], Lawrie Wheeler[3], April Hartley[2], Scott Youlton[4], Peter Croucher[4], Aideen McInerney-Leo[3], William Fraser[5], Jonathan Tang[5], Lavinia Paternoster[2], George Davey Smith[2], Matthew Brown[3], Chrissy Hammond[2], John Kemp[6], Paul Leo[3], Jon Tobias[2], Emma Duncan[3]

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Novel anabolic drug targets are needed to treat osteoporosis. Having established a large national cohort with unexplained high bone mass (HBM), we aimed to identify a novel monogenic cause of HBM and provide insight into a regulatory pathway potentially amenable to therapeutic intervention.

We investigated a pedigree with unexplained HBM in whom previous sequencing had excluded known causes of monogenic HBM. The carrier phenotype includes BMD Z-Scores +3 to +5, mandible enlargement, a broad frame, torus palitinus, pes planus, increased shoe size, a tendency to sink when swimming, with increased volumetric cortical and trabecular BMD, bone cross-sectional area and cortical thickness (measured by peripheral quantitative computer tomography) and predicted bone strength, with low/normal bone turnover. Notably, fractures and nerve compression were not seen.

Whole exome sequencing identified a rare (minor allele frequency 0.0014), highly evolutionarily conserved (GERP 5.53), heterozygous missense mutation in *SMAD9* (c.65T > C, p.Leu22Pro) segregating with HBM across three generations. The same mutation was identified in another two unrelated individuals with HBM. In-silico protein modelling predicts the mutation severely disrupts the structure of the MH1 DNA-binding domain of SMAD9.

Gene-based association tests were performed on 362,924 UK-Biobank study subjects with estimated BMD measured at the heel. *SMAD9*-annotated genetic variants were strongly associated with heel eBMD (lead SNP rs12427846-C, β 0.02, $p = 5.5 \times 10^{-16}$). Functional investigation identified high expression of *Smad9* in murine osteocytes and immunofluorescence showed high expression of Smad9 in pre-osteoblastic cells in juvenile zebrafish.

We report *SMAD9* as a novel HBM gene. SMAD9 is thought to inhibit bone morphogenetic protein (BMP) dependent target gene transcription to reduce osteoblast activity. Thus, we hypothesise *SMAD9* c.65T > C is a loss-of-function mutation reducing BMP inhibition. Given the inverse relationship between wildtype *SMAD9* and HBM, our findings support lowering *SMAD9* as a potential novel anabolic mechanism for osteoporosis therapeutics which warrants further investigation.

Keywords: High-Bone-Mass/*SMAD9*/DXA/Next-generation-sequencing/monogenic

PLO02

Therapeutic targeting of ER exit site engulfment by lysosomes in osteogenesis imperfecta

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Accumulation of misfolded mutant procollagen in the Endoplasmic Reticulum (ER) causes osteoblast malfunction in severe autosomal dominant osteogenesis imperfecta (OI). We previously found that misfolded procollagen entered osteoblast ER exit sites (ERESs), which were then engulfed and degraded by lysosomes in a process reminiscent of microautophagy. Lipidated LC3 at these autophagic ERESs appeared to be involved in the lysosomal engulfment. We therefore investigated how expression of ATG5, which is required for LC3 lipidation, affected lysosomal degradation of procollagen and OI severity in a G610C mouse model of OI. We found that ATG5 knockout only partially affected procollagen degradation, suggesting that lipidated LC3 is beneficial but not required for ERES engulfment by lysosomes. Nevertheless, $\sim$ 3-fold, tissue-nonspecific reduction in ATG5 expression suppressed the growth of WT and G610C animals and caused over 40% perinatal lethality in G610C animals ($P < 0.001$). ATG5 knockout in mature osteoblasts by osteocalcin-promoter-driven Cre recombinase reduced the mineral apposition rate ($P < 0.05$), amount of trabecular and cortical bone ($P < 0.05$), and femur strength ($P < 0.01$) in G610C but not WT animals. ATG5 knockout at an earlier stage in osteoblast differentiation by osterix-Cre produced a more severe OI phenotype with frequent spontaneous femur fractures in G610C mice, which were not observed at normal ATG5 expression. Tissue-nonspecific rescue of ATG5 by a transgene restored animal growth and prevented perinatal lethality ($P < 0.001$). Osteoblast-specific overexpression of ATG5 reduced OI severity, rescuing the growth deficiency of G610C mice and improving trabecular and cortical bone formation. Overall, our findings support the idea of enhancing autophagic degradation of procollagen as a therapeutic strategy in OI. Because the autophagic machinery based on ATG proteins has only a limited role in this process, we are currently investigating other approaches to targeting of ERES engulfment by lysosomes.

Keywords: Osteogenesis imprefecta, Procollagen, Autophagy, Osteoblast malfunction

PLO03

Role of lipocalin-2 in muscle failure-induced bone loss in the MDX mouse model of duchenne muscle dystrophy

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Lipocalin-2 (Lcn2) is an adipokine linked to bone and energy metabolism. Its serum levels directly correlate with mechanical unloading and inflammation. Both conditions are present in Duchenne Muscular Dystrophy (DMD) patients. We therefore investigated the role of Lcn2 in muscle failure-induced bone loss, using the MDX mouse model of DMD. We found increased Lcn2 serum levels in MDX mice at 1 (1.72-fold, p = 0.006) and 6 (1.42-fold, p = 0.002) months of age versus WT. Consistently, Lcn2 mRNA was higher in MDX versus WT diaphragm (2.93-fold, p = 0.014), quadricep (2.8-fold, p = 0.024), soleus (2.38-fold, p = 0.042) and extensor digitorum longus (5.7-fold, p = 0.005) muscles. This was confirmed by immunohistochemistry, which also showed that Lcn2 was mainly expressed by mononuclear cells in diaphragm and quadricep. Based on these results, we ablated Lcn2 in MDX mice by cross-breeding them with Lcn2KO mice (MDXxLcn2KO). Six-month-old MDXxLcn2KO mice had higher muscle strength, evaluated by grip force test (1.23-fold, p = 0.03), likely due to reduced muscle fibrosis (− 39%, p = 0.0078) and increased intact muscle fibres (1.45-fold, p < 0.0001) compared to MDX. Consistently, creatine kinase levels were normal (< 1302 U/L) in 57% of MDXxLcn2KO mice, and in only 25% of MDX. Moreover, microCT showed higher Trabecular Bone Volume/Tissue Volume (Tb.BV/TV) % (1.41-fold, p = 0.023) and Number (1.33-fold, p = 0.022) in MDXxLcn2KO mice compared to MDX. Similar results were observed in 3-month-old MDXxLcn2KO mice. To strengthen these results, we neutralised Lcn2 treating 2-month-old MDX mice with a Lcn2-blocking antibody (Lcn2-mAb), which increased Tb.BV/TV (1.22-fold, p = 0.01) and reduced osteoclast surface/bone surface (0.58-fold, p = 0.02) compared to MDX treated with irrelevant IgG. Consistently, grip force was increased (1.2-fold, p = 0.006) and diaphragm fibrosis was reduced (− 38%, p = 0.0378) by Lcn2-mAb. Increased Tb.BV/TV was also observed treating 2-week-old MDX mice (+15%, p = 0.0095) to mimic a preventive treatment. Together, these results point to Lcn2 as an important determinant of bone and muscle impairment in the MDX mouse model of DMD.

PLO04

Burosumab showed greater improvement in phosphate metabolism, rickets, and bowing than continuation with conventional therapy in children with X-linked hypophosphatemia (XLH)

Ola Nilsson[1], Erik Imel[2], Michael Whyte[3], Craig Munns[4], Anthony Portale[5], Leanne Ward[6], Jill Hickman Simmons[7], Raja Padidela[8], Noriyuki Namba[9], Hae Il Cheong[10], Wolfgang Högler[11], Meng Mao[12], Alison Skrinar[12], Chao-Yin Chen[12], Javier San Martin[12], Francis Glorieux[13]

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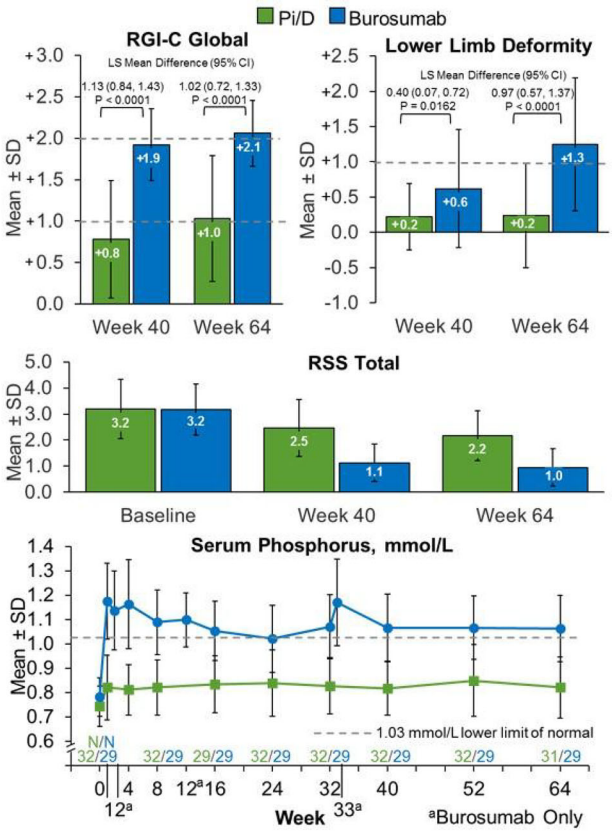
XLH is characterized by excess FGF23, hypophosphatemia, and skeletal deformities. Conventional therapy for XLH consists of multiple daily doses of oral phosphate and active vitamin D (Pi/D). Burosumab is a fully human monoclonal antibody to FGF23 approved for XLH.

In this Phase 3 trial (NCT02915705), 61 children with XLH (1–12 years old) were randomized 1:1 after a 7-day Pi/D washout to receive burosumab starting at 0.8 mg/kg SC Q2 W or reinitiate Pi/D titrated by investigators for 64 weeks. Eligibility criteria included a Rickets Severity Score (RSS) ≥ 2.0 despite prior Pi/D treatment. Healing of rickets was assessed by radiologists blinded to treatment using the Radiographic Global Impression of Change (RGI-C).

Compliance in both arms was high (> 95% based on dosing days). Compared with Pi/D, burosumab demonstrated greater improvement in serum phosphorus, global RGI-C (primary endpoint), RSS, alkaline phosphatase, and lower limb deformity (Figure). At Week 64, nephrocalcinosis score remained unchanged in 25 (78%) Pi/D and 26 (90%) burosumab subjects; decreased 1 in five (6%) and two (7%) subjects, respectively; and decreased 2 and 3 in one (3%) subject each with Pi/D. Adverse events (AEs) of interest were more frequent with burosumab, including hypersensitivity (38% vs 19% of subjects) and injection site reactions (52% vs N/A), and were mild to moderate in severity overall. Three serious AEs occurred per group, all unrelated to treatment and resolved. No subject discontinued study drug.

Burosumab resulted in significantly greater improvements in phosphate metabolism, rickets, and bowing than continued Pi/D in 1–12 year-old children with XLH.



[Changes in Serum Phosphorus, Rickets, and Lower Limb Deformity]

PLO05

Global natural history studies of generalized arterial calcification of infancy (GACI) and autosomal recessive hypophosphatemic rickets type 2 (ARHR2) due to ENPP1 or ABCC6 deficiencies

Frank Rutsch[1], Mary Hackbarth[2], Ulrke Botschen[1], Kristina Kintzinger[1], Sisi Wang[3], Rachel Gafni[4], Kerstin Mueller[3], Ruhi Ahmed[5], Eric Yuen[5], William Gahl[6], Carlos Ferreira[6]

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Background: ENPP1 Deficiency causes GACI type 1, characterized clinically by arterial calcifications and stenosis. Death, either intrauterine or in early infancy, is frequent, often due to myocardial infarction. The mortality rate decreases substantially in those surviving past 6 months. Majority of survivors develop ARHR2, characterized clinically by short stature, bone deformities and pain. ABCC6 Deficiency also causes GACI type 2, which is phenotypically identical to GACI type 1 but has a different genetic etiology. Animal data suggest that enzyme replacement therapy with ENPP1-Fc may prevent the mortality of GACI and morbidity of ARHR2.
Objective: Collect data on 100 patients to define the natural history of disease in order to optimally design ENPP1-Fc clinical trials.
Design: Combined data from two global retrospective Natural History studies conducted at academic centers of excellence.
Results: Of 42 patients enrolled, 37 had GACI (median age at diagnosis 1.2mo), 17 had ARHR2 (median age at diagnosis 72mo). For 38 patients with genetic analyses, 29 had *ENPP1* mutations and 7 had *ABCC6* mutations. In the 37 GACI patients, 14/37 patients died at a median age of 1.3mo; initial symptoms included dyspnea (65%) and cyanosis (16%); with 74% ventilated. Eighty-seven percent had arterial calcification presenting at 0.7–0.9 mo. Thirty had cardiac dysfunction, with cardiac failure in 17 and myocardial infarction in 6. Joint and organ calcification was present in 43% and 62%, respectively. In patients with ARHR2, 71% had pain, 53% had bowing, and 29% had short stature. Patients $>$ 1-year old with ENPP1 deficiency developed rickets in 72% (13/18).
Conclusions: Morbidity and mortality are high in GACI and ARHR2 patients and currently there are no approved therapies for treatment. The data obtained in these global retrospective natural history studies extends the knowledge on disease progression and burden for these two rare and serious calcification disorders in children.

PLO06

The iron-sensing receptor Tfr2 regulates osteoclastogenesis and the differentiation ability of hematopoietic stem cells

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Iron is indispensable for the organism. However, iron overload is toxic for cells and leads to tissue damage. The transferrin receptor 2 (Tfr2) is a critical regulator of iron homeostasis as mutations in the Tfr2 gene in humans and mice result in iron overload. We recently identified Tfr2 as an iron-independent regulator of bone homeostasis that inhibits bone turnover by interacting with BMP signaling. Here, we characterized the role of Tfr2 in osteoclastogenesis and the hematopoietic stem cell (HSC) compartment.

FACS analysis of bone marrow revealed that $Tfr2^{-/-}$ mice display 66% ($p < 0.01$) more osteoclast precursors (OCP) in the bone marrow than WT mice, independent of the iron-status of the niche. Seeding equal amounts of OCP, $Tfr2^{-/-}$ osteoclasts differentiated faster than WT cells and the number of TRAP-positive cells was twofold higher in $Tfr2^{-/-}$ cultures. Despite increased differentiation capacity, protein and gene expression of the osteoclast regulators NFATc1 and NF-kB were downregulated in $Tfr2^{-/-}$ osteoclasts (protein levels: 15-fold and 2-fold, respectively). However, analysis of $Tfr2^{-/-}$ and WT osteoclasts at early time points of differentiation (day 0–2) revealed an increase of viability of $Tfr2^{-/-}$ osteoclasts (1.5 fold, $p < 0.05$) and less apoptotic events ($-$ 25%, $p < 0.05$), suggesting an increased life span.

Furthermore, short-term (LSK $CD34^+CD150^-CD135^+$) ($-$ 25%) and long-term (LSK $CD34^-CD150^+CD135^-$) HSCs ($-$ 30%) were reduced in $Tfr2^{-/-}$ mice. Their capacity to differentiate into the granulocytic-monocytic lineage was impaired as indicated ex vivo by Methocult-based CFU assays. Finally, multi-lineage reconstitution of hematopoiesis of $Tfr2^{-/-}$ HSCs was tested in vivo by competitive HSC transplantations using the CD45.1/CD45.2 congenic system (CD45.1 WT + CD45.2 WT/KO à CD45.1 WT). After 16 weeks, $Tfr2^{-/-}$ showed markedly reduced repopulation ability ($-$ 60%) and a reduced ability for multi-lineage engraftment (CD3: $-$ 60%; CD19: $-$ 75%; CD11b: $-$ 25%).

Thus, Tfr2 is a novel regulator of HSC, myeloid differentiation, and osteoclastogenesis.

Plenary Oral Presentations 2: Bone pathophysiology

PLO07

Absence of the fractalkine receptor CX3CR1 decreases inflammatory bone resorption but increases the inflammatory phenotype of inflammatory osteoclasts

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In chronic inflammation, deregulated immune responses are related to bone destruction as in ovariectomy-associated (OVX) osteoporosis. Recently, our team showed that besides bone resorption, OCLs are immune cells inducing T cell responses through antigen-presentation. In steady-state, OCLs activate regulatory $CD4^+$ T-cells (tolerogenic OCLs/t-OCLs), while during inflammation OCLs induce TNFα-producing $CD4^+$ T-cells (inflammatory OCLs/i-OCLs). However, the exact role of i-OCLs and underlying molecular mechanisms leading to inflammatory bone loss remain unknown.

Transcriptomic profiling of the two OCL subsets and our previous findings identified the fractalkine receptor CX3CR1 to be upregulated in $\sim$ 25% of i-OCLs ($CX3CR1^+$) while $\sim$ 75% are negative

(CX3CR1^{neg}) (p = 0.001; log2FC = 3.2). To investigate the role of CX3CR1 in i-OCLs, we used transgenic CX3CR1$^{GFP/+}$ (WT) and CX3CR1$^{GFP/GFP}$ (KO) mice. OVX-KO mice showed higher BV/TV (p = 0.0037) and cortical thickness (p = 0.0003) than OVX-WT mice. In vitro studies demonstrated reduced i-OCL differentiation in KO mice compared to controls (p = 0.027). Further investigations of the immune function revealed that WT-CX3CR1^{neg} i-OCLs had lower capacity to engulf antigens (p = 0.0026) but higher expression of factors involved in antigen-presentation (e.g. MHC-II, p = 0.024). Moreover, presence of CX3CR1 in i-OCLs reduced their ability to induce TNFα-producing CD4^{+} T-cells (p = 0.0007) and conferred them to decrease the inflammatory effect of WT-CX3CR1^{neg} i-OCLs. This suggests a negative feedback effect of CX3CR1-expressing i-OCLs on inflammation (Figure 1). Ongoing experiments including RNA-Seq analysis between WT-CX3CR1^{+}, WT-CX3CR1^{neg} and KO-CX3CR1 i-OCLs will identify the role of CX3CR1 and molecular pathways involved in OCL-immunomodulation. These insights will help to elucidate mechanisms leading to inflammatory bone destruction.

Keywords: Osteoimmunology, Osteoclasts, Inflammation

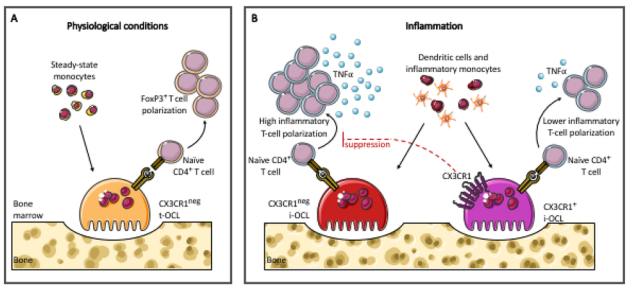

Figure 1: Characterisation of osteoclast subsets according to their CX3CR1 expression. (A) Under physiological conditions, tolerogenic osteoclasts (t-OCLs) originate from steady-state monocytes and induce regulatory CD4^{+}FoxP3^{+} T cells. These t-OCLs do not express CX3CR1 (CX3CR1^{neg} t-OCLs). (B) During inflammation, dendritic cells and inflammatory monocytes differentiate into inflammatory OCLs (i-OCLs) that induce TNFα-producing CD4^{+} T cells. Among these i-OCLs, approx. 75% are CX3CR1^{neg} and 25% CX3CR1^{+}. Here we show that CX3CR1^{+} i-OCLs are less inflammatory and have immunosuppressive effects on CX3CR1^{neg} i-OCLs, reducing their ability to induce TNFα-producing CD4^{+} T cells.

Fig. 1 Characterisation of OCL subsets according to their CX3CR1 expression.

PLO08

Tumour-derived extracellular vesicles affect the molecular profile of osteoblasts and stimulate osteoclast and endothelial functions

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Extracellular vesicles (EVs) are membrane-derived cargoes of biologically active molecules shed by cells, which are emerging as mediators of several pathological processes. However, their role in bone metastasis has been poorly explored. We investigated the effect of EVs isolated from the human breast cancer cell line MDA-MB-231(MDA-EVs) on osteoblasts, osteoclasts and endothelial cells. Osteoblast treatment with MDA-EVs inhibited *Cyclin D1* (− 60%, p = 0.001), *Runx2* (− 80%, p < 0.001) and *Osx* (− 80%, p < 0.001) mRNAs, while enhancing *Nos2* (15-fold, p = 0.015). An increase of mRNA and protein expression of IL-1β (twofold, p = 0.08), IL-6 (900-fold, p = 0.002) and Lcn2 (3.8-fold, p = 0.03) was also observed in MDA-EVs-treated osteoblasts compared to control. We next performed a cytokine array on supernatants from osteoblasts treated with MDA-EVs, finding an increase of the inflammatory cytokines CCL3 (sixfold, p = 0.008), CXCL2 (eightfold, p = 0.004), Reg3G (3.5-fold, p = 0.001) and VEGF (threefold, p = 0.005), while OPG and WNT1 were downregulated (− 58% and − 55%, respectively, p < 0.05). MDA-EVs contained mRNAs of genes involved in bone metabolism, as well as a secretome pattern, which included PDGF-BB, CCL3, CCL27, VEGF and Angiopoietin 2. In line with these observations, MDA-EVs significantly increased osteoclastogenesis (fivefold, p = 0.002) and angiogenesis, the latter evaluated both in vitro (% endothelial tube branching, 1.7-fold, p = 0.02) and in vivo (matrigel-plug assay, 15-fold, p = 0.045). Finally, we investigated whether naïve osteoblast EVs could directly mediate the effects on osteoclast and endothelial functions, finding a pro-angiogenic effect in vivo (2.6-fold, p = 0.002) while osteoclastogenesis was not affected. However, when EVs were isolated from osteoblasts educated with conditioned medium of MDA cells, they acquired a pro-osteoclastogenic (2.8-fold, p = 0.024) as well as a pro-angiogenic ability, both in vitro (1.5-fold, p = 0.046) and in vivo (eightfold, p = 0.01), compared to controls. In conclusion, these data demonstrate a role for osteoblast- and tumour cell-derived EVs in the deregulation of bone and endothelial cell physiology, fuelling the vicious cycle induced by bone tumours by means of EV cargoes.

PLO09

Mendelian randomization analysis reveals a causal influence of circulating sclerostin levels on bone mineral density and fractures

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In bone, sclerostin is mainly osteocyte-derived and plays an important role in adaptive responses to mechanical loading. In contrast, whether circulating levels of sclerostin also play a functional

role is currently unclear, which we aimed to examined by two sample Mendelian Randomisation (MR) here.

A genome wide association study (GWAS) meta-analysis of plasma sclerostin was performed in 10,584 European-descent individuals, and genetic instruments for sclerostin subsequently derived. Causal relationships were examined between circulating sclerostin and femoral neck bone mineral density (FN_BMD; n = 32,744, GEFOS), estimated BMD by heel ultrasound (eBMD; n = 426,824, UKBiobank), and fracture risk (n = 426,795, UKBiobank). Inverse variance weighted method was used to obtain MR effect estimates via MR-Base (www.mrbase.org).

Our GWAS meta-analysis found that common variants explained 16.3% of the phenotypic variance of sclerostin ($H^2 = 0.163$, P = 0.0017), and identified two novel plasma sclerostin loci, *B4GALNT3* (standard deviation (SD) change in sclerostin per A allele (b = 0.20, $P = 4.6 \times 10^{-49}$) and *GALNT1* (b = 0.11 per G allele, $P = 4.4x10^{-11}$). *B4GALNT3* is an N-acetyl-galactosaminyltransferase, adding a terminal LacdiNAc disaccharide to target glycocoproteins, found to be predominantly expressed in kidney, whereas *GALNT1* is an enzyme causing mucin-type O-linked glycosylation. Using these two SNPs as genetic instruments, MR revealed negative causal relationships for plasma sclerostin on FN_BMD (b = − 0.123, P = 0.0007) and eBMD (b = − 0.122, $P = 1.29x10^{-38}$), and a positive relationship with fracture risk (b = 1.117, P = 0.03) (SD change in eBMD (or log odds ratio of fracture risk) per SD increase in sclerostin). Genetic colocalization analysis demonstrated that common genetic signals within the *B4GALNT3* locus share the same causal variant with sclerostin, eBMD, and *B4GALNT3* expression in arterial tissue (Probability > 99%).

Our findings suggest that higher sclerostin levels are causally related to lower BMD and greater fracture risk. Hence, strategies for reducing circulating sclerostin, for example by targeting glycosylation enzymes as suggested by our GWAS results, may prove valuable in treating osteoporosis.

PLO10

Skin autofluorescence, a non-invasive biomarker for advanced glycation end-products, is associated with prevalent vertebral fractures independent of BMD: The Rotterdam Study

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Background: Advanced glycation end-products (AGEs) accumulation in bone has been implicated in reduced bone quality. Skin autofluorescence (SAF) is a marker of long-term AGEs accumulation in tissues, wherein skin AGEs have a half-life of 14.8 years. No studies have so far investigated the association of SAF with bone mineral density (BMD), trabecular bone score (TBS) and prevalent vertebral fractures (VFs) in the general population.

Methods: We studied 2853 individuals (mean age 74.1 years; 55.8% females) with available SAF, measured by an AGE reader™. VFs were coded using Genant's classification and we included only moderate and severe fractures. They were assessed on average 4–5 years before SAF. Linear and logistic regression analyses were conducted adjusted for age, sex, BMI, creatinine, smoking, type 2 diabetes status (T2DM) and additionally for lumbar spine BMD (LS-BMD) and TBS. Interaction terms were included to test for effect modification by sex, smoking and T2DM.

Results: Individuals with VFs (n = 193) had higher SAF levels (2.48 ± 0.44 vs. 2.39 ± 0.49 ; p = 0.01). Both LS-BMD (β = 0.013; p = 0.60) and TBS (β = − 0.026; p = 0.20) were not significantly related to SAF. SAF had a curvilinear association with VFs. Therefore, fractures were analyzed in age-adjusted, sex stratified SAF quartiles. The odds ratio (OR) (95% confidence interval) of the second, third and highest quartiles of SAF for VFs were 1.68(1.07–2.64, p = 0.02), 1.77 (1.13–2.77, p = 0.01) and 1.77 (1.13–2.77, p = 0.01) respectively, when the bottom quartile was used as reference. When we compared the top three quartiles combined with the bottom quartile, the OR(95% CI) for VFs was 1.74 (1.18–2.56, p = 0.005) which persisted after adjustment for LS-BMD 1.79 (1.20–2.65) and LS-TBS 1.73 (1.17–2.55). There was no interaction with sex, smoking or T2DM.

Conclusions: Increased skin AGEs are associated with higher prevalence of VFs but not with BMD or TBS. Longitudinal analysis is needed to confirm our cross-sectional findings.

Keywords: Advanced glycation end-products, Vertebral fractures

PLO11

Keratinocyte-derived S100A9 is important for psoriatic arthritis, but not psoriasis

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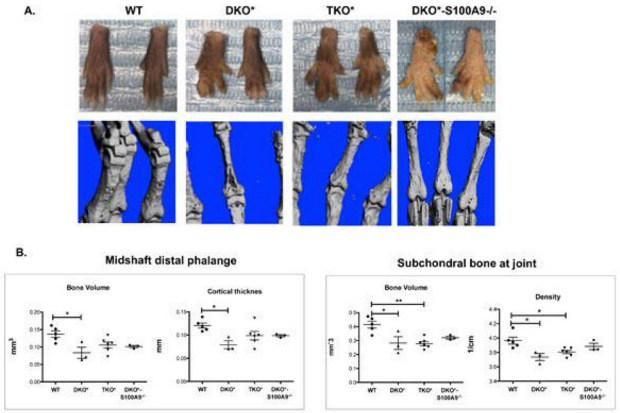
Psoriatic Arthritis (PsA) is a debilitating inflammatory joint disease that often develops in psoriatic patients. How organ cross-talk between skin and bone/cartilage can lead to this pathological condition is poorly understood. We previously generated a genetic mouse model (GEMM) that develops both psoriasis-like skin inflammation PsA-like joint disease upon inducible double epidermal deletion of cJun and JunB (DKO*). We found that the S100A8/A9 complex is highly elevated in the DKO* model and human psoriatic skin samples. In psoriasis, the main S100A9-expressing cells are keratinocytes and neutrophils. To address the role of keratinocyte-derived S100A9 in psoriasis and PsA and to identify therapeutic targets to reduce skin and/or joint inflammation, psoriasis-like GEMMs with inducible epidermal deletion of S100A9 (TKO*) or global deletion of S100A9 (DKO*-S100A9$^{-/-}$) were generated.

DKO*-S100A9$^{-/-}$ mice developed more severe inflammatory skin disease compared to DKO* and TKO* mice, suggesting that neutrophil-derived S100A9 plays a protective role in preventing skin inflammation. Although skin inflammation was aggravated in DKO*-S100A9$^{-/-}$, the joints were protected. MicroCT analyses revealed a less drastic PsA-like disease in DKO*-S100A9$^{-/-}$ but also in TKO* when compared to DKO*, suggesting that keratinocyte-derived S100A9 mediates PsA and inflammation-associated bone loss (Figure 1). Skin inflammation, cytokine profile and epidermal gene expression was comparable in DKO* and TKO*, while global deletion of S100A9 led to significant decrease in defensins and CXCL1 expression, likely contributing to the phenotype.

Our study shows that neutrophil-derived S100A9 may play a protective role in psoriasis-like disease, while keratinocyte-derived S100A9 is detrimental to synovial joints and bone health.



*[Psoriatic-arthritic-like phenotype and bone quantification (*p>0.05; **p>0.01)]*

PLO12

22 New osteoarthritis susceptibility genes identified using novel rapid-throughput joint phenotyping methods

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Very few osteoarthritis (OA) susceptibility loci have been identified, despite genetics comprising ~ 50% of OA risk, and new OA genes are urgently needed. Joint phenotyping in mice is hampered by small size, and need for cartilage hydration. The gold-standard histology is destructive and low-throughput. Accordingly, we developed new techniques to rapidly phenotype the mouse knee and identify novel OA susceptibility genes. Major signs of OA include articular cartilage damage and sclerosis of the underlying subchondral bone. We phenotyped articular cartilage by producing durable, nanometer-resolution joint surface replicas and quantifying damage by scanning electron microscopy. Decreased cartilage volume/thickness was then revealed by contrast-enhanced micro-computed tomography (uCT). Concurrently, we quantified subchondral bone sclerosis by uCT (increased bone mineral density, altered trabecular morphology) and X-ray microradiography (increased bone mineral content; BMC). Techniques were validated by OA provocation surgery to destabilise the medial meniscus (DMM). BMC was increased (1.67 standard deviations, SD), trabeculae were thicker (1.66 SD), cartilage volume decreased (2.55 SD) and surface damage increased (53.57 SD) after DMM surgery compared to sham ($P < 0.05$, *t*-tests, n = 16).

We performed rapid-throughput joint phenotyping of 50 mouse knockout lines generated by the International Mouse Phenotyping Consortium. Using reference data generated from 100 isogenic 16-week wild-type mice, we could identify outlier phenotypes that deviated > 2SD from the reference range with 80% power with samples from 3 knockout mice. We identified novel outlier joint phenotypes in 23 knockout lines (46%). 19 displayed OA phenotypes, suggesting that decreased gene expression causes OA. Of these, 1/19 deleted genes was known to be associated with OA, thus validating the phenotyping pipeline. Four knockout lines displayed phenotypes suggesting that decreased gene expression protects against OA.

In summary, we developed and validated three novel rapid-throughput techniques and phenotyped knee joints from 50 knockout mice. These studies identified 22 novel OA susceptibility genes.

?A3B2 tpb = 8.5mm? >

Plenary Oral Presentations 3: Osteoporosis and bone loss

PLO13

Loss of Dkk-1 in osteocytes prevents alveolar bone loss in mice subjected to experimental periodontitis

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Periodontitis is an infect-inflammatory highly prevalent disease that results in loss of connective tissue and bone support and can progress to bone destruction, tooth mobility and finally, tooth loss. Persistent inflammation causes alveolar bone loss not only by stimulating osteoclast activity, but also by directly suppressing bone formation via increasing the expression of Dickkopf-1 (Dkk-1), an inhibitor of Wnt signaling. In this study, we tested the hypothesis that Dkk-1 is a main contributor to periodontitis-induced alveolar bone loss (ABL). Therefore we subjected 12-week-old male and female mice with a specific deletion of Dkk-1 in osteocytes (Dkk-1; Dmp1-Cre mice) to experimental periodontitis. Their age- and sex-matched Cre-negative littermates served as controls. Periodontitis was induced by ligature around the upper 2nd left molar, the contralateral side was used as control. Mice were killed after eleven days and maxillae were removed for macroscopic, micro-CT, and histological analyses. Blood samples were collected for CTx and P1NP measurement by ELISA. The data show that the deletion of Dkk-1 on osteocytes prevented bone loss compared to cre negative mice with periodontitis (control). Micro-CT analysis showed that the distance between cementum enamel junction and alveolar bone crest (CEJ-ABC) was significantly reduced (0.28 ± 0.01 µm) in transgenic mice compared to their littermate controls (0.33 ± 0.02 µm; $p < 0.05$). Furthermore, the histological analysis displayed a significant reduction in osteoclast number as well as an increase in osteoblast and osteocyte numbers when compared to control. There was no change of P1NP serum levels between the groups, however, a significant reduction of CTx serum levels was observed in Dkk-1; Dmp1-Cre mice (35.6 ± 3.3 ng/ml) compared to control (57.0 ± 4.4 ng/ml). In summary, Dkk1 derived from osteocytes plays a crucial role on alveolar bone loss in periodontitis. Thus, blocking Dkk-1 may represent a promising therapeutic strategy to treat periodontitis-induced bone loss.

PLO14

Can the circulating Wnt-antagonists sclerostin and DKK1 alter bone mass by endocrine signalling?

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Osteocytes respond to local mechanical inputs by modulating secretion of the Wnt-antagonists sclerostin (SOST) and DKK1. A fraction ends up in the circulation, where both have been correlated with BMD. Whether circulating Wnt-antagonists contribute to bone anabolism at distant sites or are biomarkers of localised effects is yet to be determined. This study employed conditional mouse models of *Sost* and *Dkk1* to delineate their local and endocrine effects on bone tissue.

Limb-specific *Sost* or *Dkk1* null mice were generated using Prrx-1 Cre mice; retaining expression and secretion in the axial skeleton. These were compared to Cre controls and germline knockout mice. Lumbar vertebrae and femora were assessed at 16 weeks of age by DXA and microCT.

In SostPrrx1-Cre mutants, BMD was greater only in the hindlimbs [mean(SE), mg/cm^2], control [50(0.7)] vs SostPrrx1 [69(2), $p < 0.0002$], not in the lumbar spine, control [51(0.4)] vs SostPrrx1 [50(0.7), ns]. SostKO mice showed greater BMD in both the limb and spine.

By microCT, femoral cancellous bone volume (BV/TV, %) was similarly elevated in conditional and germline mutants: control [6.8(0.7)] vs SostPrrx1 [11.2(1.2), $p < 0.003$], SostKO [11.2(0.4), ns vs SostPrrx1]. In contrast, vertebral BV/TV was similar in Sost Prxx1 and control: control [12.6(0.8)] vs SostPrrx1 [11.6(1.2), ns], but elevated in *Sost*-KO [21.6(2.2), $p < 0.0001$].

Similarly, in DKK1Prxx1-Cre mutants, greater bone accrual was observed in the limbs, but no change in the vertebrae. Femur BV/TV: control [7.8(0.7)] vs DKK1Prrx1 [13.0(0.6), $p < 0.0001$], Vertebral BV/TV: control [14.0(0.6)] vs DKK1Prrx1 [13.2(0.6), ns]. Again, *Dkk1*-KO mice had greater BV/TV than wild type at both sites.

In conclusion, our results indicate that local sclerostin and DKK1 production is fundamental to the stimulation of bone accrual. However, our data do not support the endocrine regulation of bone accrual by either Wnt-antagonist.

PLO15

T cell-derived Dkk1 controls bone homeostasis and contributes to the pathogenesis of estrogen deficiency-induced bone loss

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Postmenopausal osteoporosis is the most common form of osteoporosis and results from reduced estrogen levels leading to increased bone turnover. Furthermore, activation of T cells contributes to bone loss. In this study, we show that Dkk1 is expressed in T cells and that surprisingly, T cell-derived Dkk1, rather than osteoblast-derived Dkk1, mediates estrogen deficiency-induced bone loss.

Global Dkk1 $^{fl/fl}$; Rosa26-CreERT2 conditional knockout mice were protected from ovariectomy (OVX)-induced bone loss, while Cre-negative littermates that served as controls, lost 25% of vertebral bone volume and showed an twofold increased bone formation four weeks after OVX. of note, osteoblast (Osx-Cre)-as well as osteocyte (Dmp1-Cre)-specific deletion of Dkk1 did not protect from OVX-induced bone loss. Bone volume was similarly reduced in Dkk1 $^{fl/fl}$; Osx-Cre (− 19%) and Dkk1 $^{fl/fl}$; Dmp1-Cre (− 15%) mice, compared to their respective control groups. As OVX was associated with an increased activation of CD69-positive CD4 and CD8 T cells, we assessed Dkk1 expression in T cells. Indeed, purified T cells expressed Dkk1 at mRNA and protein level. Dkk1 amounts secreted by T cells were sufficient to reduce the expression of osteogenic markers and Wnt target genes in osteoblasts in vitro. T cell-specific Dkk1-deficiency using Lck-Cre mice led to a twofold increase in bone volume in male and female mice. Histomorphometric analyses underlined the μCT results showing elevated mineral apposition and bone formation rates. T cell transfer from wildtype mice into Dkk1 $^{fl/fl}$; Lc k-Cre completely rescued their bone phenotype. Finally, T cell-specific Dkk1 deletion mitigated the negative effect of estrogen withdrawal on bone, as only ovariectomized Cre- controls exhibited a significant decrease of bone volume.

In summary, Dkk1 derived from T cells plays a crucial role in osteoblast biology and in the pathogenesis of estrogen deficiency-induced bone loss. Thus, blocking T cell activation and production of Dkk1 may represent promising therapeutic strategies to treat postmenopausal osteoporosis.

PLO16

Childhood exposure to passive smoking and bone health in adulthood

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Context: Passive smoke exposure has been linked with the risk of osteoporosis in adults.
Objectives: We aimed to examine the independent effects of exposure to passive smoking in childhood on adult bone health.
Design and Setting: Longitudinal, the Cardiovascular Risk in Young Finns Study

Participants: Study cohort included 1422 individuals followed up for 28 years since baseline in 1980. The study was approved by the institutional ethics committees, and written informed consent was obtained from all the study participants or their parents. Exposure to passive smoking was determined in childhood. In adulthood, peripheral bone traits were assessed with quantitative computed tomography (pQCT) at the radius and tibia, and calcaneal mineral density was estimated with quantitative ultrasound. Fractures were gathered by questionnaires.

Results: Parental smoking in childhood was associated with lower pQCT derived bone sum index in adulthood (Table 1, $\beta \pm SE -0.064 \pm 0.023$ per smoking parent, $P = 0.004$) in multivariate models adjusted for age, sex, active smoking, BMI, physical activity, serum 25-OH vitamin D concentration and parental socioeconomic position. Similarly, parental smoking was associated with lower heel ultrasound estimated bone mineral density in adulthood ($\beta \pm SE -0.097 \pm 0.041$ per smoking parent, $P = 0.02$). Parental smoking was also related with the incidence of low-energy fractures (OR 1.28, 95% CI 1.01–1.62). Individuals with elevated cotinine levels (3–20 ng/ml) in childhood had lower bone sum index with pQCT ($\beta \pm SE -0.206 \pm 0.057$, $P = 0.0003$). Children with high cotinine levels and whose parents smoked had significantly lower pQCT derived bone sum index compared to those with low cotinine levels (< 3 ng/ml) and smoking parents ($\beta \pm SE -0.192 \pm 0.072$, $P = 0.008$).

Conclusions: Children of parents who smoke have evidence of impaired bone health in adulthood.

Keywords: Passive Smoking, Cotinine, peripheral bones

Table 1. The effects of parental smoking in childhood on bone sum indices measured by pQCT in adulthood]

β ± SE	Bone area, mm2	Bone mass, mg	Bone density, mg/cm3	Bone strength, z-score	Bone sum index, z-score
Parental smoking	− 11.1 ± 7.4	− 17.1 ± 6.4	− 1.27 ± 0.63	− 0.042 ± 0.020	− 0.064 ± 0.023

PLO17

Comparison of the predictive ability of quantitative and qualitative scoring methods of osteoporotic vertebral fractures using operational skeletal fragility outcomes

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True osteoporosis vertebral fractures (OVF) are strong predictors of future non-vertebral fractures and mortality. We previously showed OVF prevalence differs significantly across the quantitative morphometry (QM) and algorithm-based qualitative (ABQ) definitions. In absence of a gold standard, we aimed comparing the ability of the two OVF definitions to predict skeletal fragility outcomes, including future non-vertebral (NVF), osteoporotic fracture (OV) and mortality. In a population-based cohort of men and women 55 years and older we defined OVF on lateral radiographs at baseline, based on ABQ and QM (height reduction > 26%) definitions. Mean follow-up time was 22.6 years for fracture and 26 years for mortality outcomes. Cox regression models, adjusted for age, sex, BMI, cohort effect, osteoporotic treatment, corticosteroid use and smoking status, were ran to estimate the risk (hazard ratio) of incident fractures or mortality across OVF definition. Among 8964 individuals, 6.2% had an OVF according to QM (Grade > = 2) and 3.8% according to ABQ. Furthermore, 4% had OVF defined by only QM, 2.1% only by ABQ, 1.6% by both QM and ABQ. Prevalent ABQ OVF were associated with Incident NVF (HR = 2.17;95%CI 1.83:2.58) and OF (HR = 1.37;95%CI 1.13:1.67) as well as mortality (HR = 1.18, 95%CI 1.02:1.36), whereas QM-defined only with incident OF (HR = 1.66;95%CI 1.41:1.95). OVF defined only by ABQ were superior when compared directly to QM in predicting incident OVF (HR = 1.18;95%CI 1.02:1.36) and NVF (HR = 2.05;95%CI 1.15:3.65) but only in men. OVF defined by both ABQ and QM were superior to predict OF compared to OVF defined only by QM (HR = 1.61;95%CI 1.03:2.47) and to predict mortality (HR = 1.42;95%CI 1.06:1.89) but not when compared to those identified only by ABQ (HR = 1.17; 95%CI 085:1.60 for NVF) (HR = 1.01:95%CI 0.66:1.56 for OF) and (HR = 1.17: 95%CI 0.85:1.60 for mortality). In conclusion, ABQ-defined OVFs, seem to pick up operational characteristics of bone fragility better than QM

PLO18

Low levels of sex steroids are associated with accelerated deterioration of cortical microarchitecture, in older men

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Backgrounds: In older men, low estrogen levels ***(total estradiol [17βE2], bioavailable estradiol [bio-17βE2]) are associated with poor bone microarchitecture, but data on androgens (total testosterone [tT], Apparent Free Testosterone Concentration [AFTC]) are discordant.

Materials and methods: We studied the link between baseline sex steroid levels and prospectively assessed bone microarchitecture in a cohort of 820 older men followed up for 8 years. AFTC and bio-17βE2 were calculated. Bone microarchitecture was assessed by HR-pQCT (XtremeCT, SCANCO) at baseline, after 4 and 8 years.

Results: Men in the lowest quartile of tT had more rapid decrease in cortical area (Ct.Ar), thickness (Ct.Th) and volumetric bone mineral density (Ct.vBMD) at the radius and the tibia ($p < 0.05$) vs. men in the highest quartile. Findings for 17βE2, AFTC and bio-17βE2 were similar. At the distal radius, men in the lowest quartile of each of the three above hormones had more rapid decrease in Ct.Ar, Ct.Th and Ct.vBMD vs. the highest respective quartile ($p < 0.05$). At the distal tibia, total vBMD and bone mineral content decreased, whereas trabecular area increased, more rapidly in the lowest bio-17βE2 quartile vs. the highest one ($p < 0.005$, $p < 0.01$ and $p < 0.005$, respectively). The findings were similar for AFTC. Finally, men having both AFTC (< 190 pmol/L) and bio-17βE2 (< 28 pmol/L) in the lowest quartile ("high risk group") had faster bone loss compared to men with levels of each hormone in the three upper quartiles jointly ("reference group"). For instance for Ct.Th: 2.61 ± 0.25 vs. 1.35 ± 0.44%/year, $p < 0.01$ at the radius and 1.46 ± 0.20 vs. 0.49 ± 0.35%/year, $p < 0.005$ at the tibia.

Conclusions: In a cohort of older men followed up prospectively for 8 years, low levels of AFTC and bio-17βE2, and less importantly tT and 17βE2, are associated with accelerated deterioration of cortical bone.

Keywords: Bone microarchitecture; Men; Aging; HR-pQCT; Sex steroids

Concurrent Oral Presentations 1—Basic/Translational: Osteoblasts and bone formation

COP07

The bone anabolic effect of Wnt1 is quickly reversed

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We previously described Wnt1 as a potent bone anabolic Wnt ligand that rapidly increases bone mass in young, adult and aging mice. Here we analyze the potency of Wnt1 to serve as a therapeutic target for treatment of osteoporosis. By using a doxycycline-inducible transgenic mouse model, where Wnt1 is specifically overexpressed in osteoblasts when omitting doxycycline, we could already show that increasing Wnt1 expression in aging 1 year-old mice increases bone mass after 9 weeks of induction. This effect was caused by an increased osteoblast number and bone forming activity. Now, we show that the bone anabolic effect of Wnt1 in aged mice is already seen after 3 weeks of induction of the transgene (BV/TV: control: 4.79 ± 1.12%, Wnt1Tg: 30.29 ± 3.23%, $p < 0.0001$). To address the stability of the bone mass gain, we added back doxycycline-containing diet in order to switch off the transgene. Surprisingly, the bone formation rate was rapidly normalized to the level of the control mice by adding back doxycycline for 1 week (BFR: control: 198.8 ± 29.83 $\mu m^3/\mu m^2/y$, Wnt1Tg: 236.6 ± 45.12 $\mu m^3/\mu m^2/y$). Moreover, this increased bone mass was largely reversed by adding back doxycycline-containing diet for 6 weeks as shown by histological analysis of vertebral bodies and by µCT of femora (BV/TV: control: 8.26 ± 1.25%, Wnt1Tg: 16.05 ± 2.49%, $p = 0.0203$). Therefore, an increased osteoclast activity is expected to reduce bone mass in these mice.

These results demonstrate that the fast and robust bone anabolic effect of Wnt1 can rapidly be abolished, showing the beneficial tight control of bone tissue by Wnt1. However, the increase in bone mass is quickly reversed most likely due to active bone resorption. Thus, a secondary antiresorptive treatment has to be taken in consideration when evaluating the therapeutic potential of Wnt1 for osteoporosis.

Keywords: Wnt1, Osteoblast, Osteoporosis

COP08

Altered lipid/cholesterol metabolism in mesenchymal stromal cells following low-serum adaptation accelerates osteogenesis in vitro and in vivo by modifying secretome compartments

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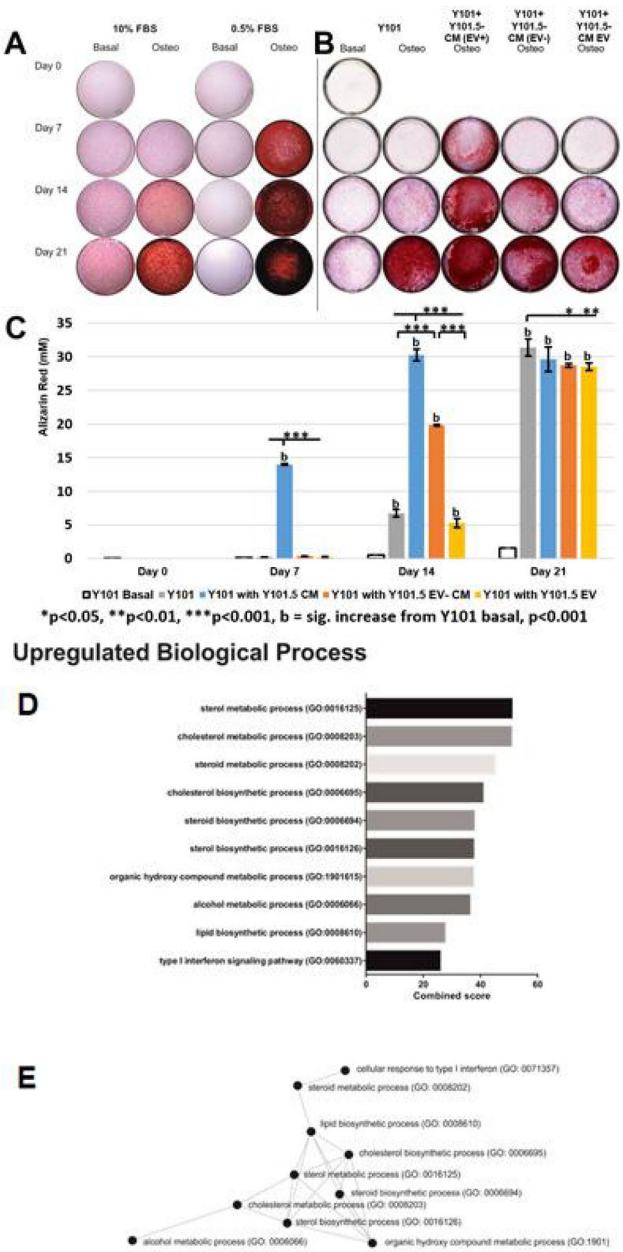
Osteogenic differentiation of mesenchymal stromal cells (hMSCs) is determined by their signalling inputs. Mechanistic investigations in vitro are complicated by undefined serum-derived bioactives. We engineered an immortalised clonal hMSC line (termed Y101) from which we generated low-serum (0.5%) adapted hMSCs (Y101.5).

Y101.5 demonstrated accelerated osteogenesis with 7 day calcium deposition equivalent to parental Y101 at day 21 (Fig. 1A). Results were unaffected by applying 0.5% or 10% serum, supporting an adapted Y101.5 pro-osteogenic phenotype. Y101.5 conditioned medium conveyed this pro-osteogenic phenotype to Y101 MSCs (Fig. 1B); an effect reduced with extracellular vesicle (EV)-depleted conditioned medium suggesting synergy between secreted and EV fractions (Fig. 1C).



[Figure 1: Accelerated osteogenesis in MSC due to altered lipid/cholesterol metabolism]

Using RNA-Seq we identified 510 significantly differentially regulated transcripts between undifferentiated Y101 and Y101.5. Bioinformatic analysis revealed significantly upregulated pathways in Y101.5 relating to fatty acid/lipid/cholesterol metabolism (Fig. 1D and E). Functional validation with a free fatty acid uptake assay demonstrated reduced lipid dependency in Y101.5. Cellular component terms enhanced in Y101.5 included "EV exosome"; "extracellular space" and "extracellular matrix (ECM)" collectively pointing to a modified secretome. Mass spectrometry identified differences ($p < 0.05$) in the secretome (100 down-, 194 up-regulated

peptides) and EVome (14 down-, 92 up-regulated peptides) in Y101.5 versus Y101, notably ECM-related, particularly collagen VI, shifted from Y101.5 secretome to EVome.

Implants in immunocompromised mice (ethically approved) indicated advanced osteogenesis (H&E) in Y101.5 compared to Y101 MSCs at 3 and 8 weeks post-implantation. Our findings demonstrate metabolic alterations favouring lipid/cholesterol biosynthesis determine secreted outputs of MSCs to accelerate osteogenesis.

Keywords: Osteogenesis, Bone, Regeneration

COP09

Deletion of PKA regulatory subunit 1A in osteoblasts causes dramatic bone turnover with an expansion of trabecular area at the expense of cortical bone

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Parathyroid hormone (PTH) plays a central role in regulation of calcium metabolism and is an osteoanabolic treatment for osteoporosis. We hypothesized that similar to PTH (1–34) treatment, an increase in PKA activity in osteoblasts will cause an increase in bone accrual. Our study aims to elucidate the effects of increased PKA activity in bone. Weekly injections of tamoxifen were administered to 1 month-old or 5 month-old C57Bl/6 male and female *col1*CRE^{ERT}/*Prkar1a*$^{fl/fl}$ mice, or *Prkar1a*$^{fl/fl}$ mice as controls, for 3–4 weeks to delete the PKA regulatory subunit 1A and increase PKA activity. This resulted in a decrease of whole body (− 6%), femoral (− 24%), and tibial BMD (− 22%) in 2 month-old *col1*CRE^{ERT}/*Prkar1a*$^{fl/fl}$ mice. µCT showed dramatic excess trabecular area and disappearance of cortical bone in vertebrae and femurs. Surprisingly, 6 month-old mice developed tumors in their tails. By µCT, at both ages, *col1*CRE^{ERT}/*Prkar1a*$^{fl/fl}$ mice showed decreases in cortical thickness and cortical BMD and increases in cortical porosity. Only 2 month-old *col1*CRE^{ERT}/*Prkar1a*$^{fl/fl}$ mice showed drastically decreased BV/TV (− 64%), Tb.N (− 49%) and trabecular BMD (− 30%). At both ages, deletion of Prkar1a dramatically increased bone turnover with a huge increase in osteoblast activity shown by serum-P1NP levels (6.5- to 13-fold) only single fluorescent labeling and a substantial increase in osteoclast activity shown by CTX levels (4.4- to 12-fold) and TRAP staining. In both age groups, cortical and trabecular bone showed a substantial increase in bone sialoprotein mRNA levels (3- to 6-fold) with a shutdown in osteocalcin mRNA (0.2- to 0.4-fold). Furthermore, PTH-regulated genes were also significantly changed: SOST expression (0.1- to 0.2-fold), RANKL (2- to 3-fold) and MMP13 (> 3 fold). The overall data show a great increase in trabecular bone mass with breakdown of cortical bone. In conclusion, high PKA activity in osteoblasts appears to be involved in increasing immature trabecular bone and resorbing cortical bone and mimics hyperparathyroidism.

Keywords: PKA, Osteoblasts

COP10

FGFR3: a key regulator of zebrafish cranial vault development

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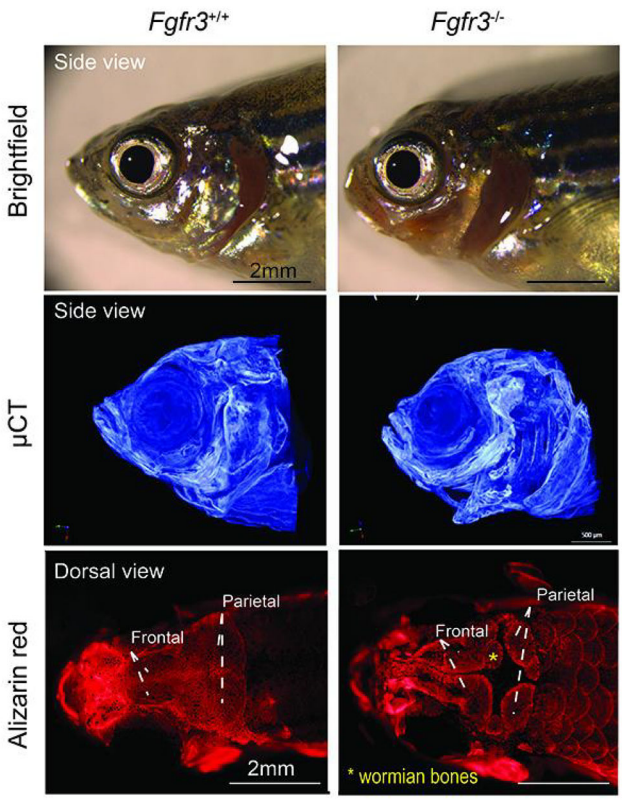
FGFR3 (Fibroblast Growth factor Receptor 3) gain or loss-of-function mutations result in craniofacial defects (craniosynostosis, macrocephaly or microcephaly and wormian bones formation). Considering the absence of craniofacial phenotype in *Fgfr3* mouse models and the relevance of zebrafish model expressing strongly *fgfr3* during skull development, we generated *fgfr3*$^{-/-}$ zebrafish to decipher the role of FGFR3 during cranial vault (CV) development.

First, to study the role of FGFR3, a receptor tyrosine kinase, on CV development, we inhibited its activity with the FGFR inhibitor NVP-BGJ398. Larvae expressing mCherry in osteoblasts (Tg(osx:mCherry)) were injected with NVP-BGJ398 during15 days every 2 days. Treated fish exhibited large fontanel area (7138 ± 213 µm^2 vs untreated 5299 ± 376 µm^2; $p < 0{,}001$). These data indicate that FGFR inhibition strongly disturbs the CV formation.

Secondly, we established the *fgfr3*$^{-/-}$ zebrafish line. Macroscopic analyses revealed that 3 month-old *fgfr3*$^{-/-}$ fish are smaller (1,850 ± 0,05 vs *fgfr3*$^{+/+}$127 ± 0,067 cm, $p < 0{,}01$). They have craniofacial defects with flat face and microcephaly. Skeleton analyses of *fgfr3*$^{-/-}$ fish using alizarin-red staining and µCT showed impressive modification of craniofacial skeleton shape with impairment of nasal, frontal and parietal bones and wormian bones(Fig 1). Studies of Tg(osx:mCherry; *fgfr3*$^{-/-}$) CV development revealed that absence of *fgfr3* prevented osteoblast expansion leading to drastic delay of CV formation.

In conclusion, we obtained the first animal model mimicking *Fgfr3*$^{-/-}$ related diseases (Microcephalia, Wormian bones) and we highlighted that FGFR3 is necessary to CV formation and to maintain osteoblasts homeostasis in cranial vault.

FGFR3, Cranial vault development, Zebrafish, Osteoblast



[fgfr3 mutants present drastic craniofacial defects]

COP11

Osteoblast lineage-specific deletion of PDGFR signaling potentiates the bone anabolic effect of anti-sclerostin antibody treatment in mice

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Sclerostin inhibits bone formation mostly by antagonizing LRP5/6, thus inhibiting Wnt signaling. However, we have recently found that sclerostin can activate platelet-derived growth factor receptor (PDGFR) signaling in vitro and that PDGFR activation in this context could be related to inhibition of osteoblast differentiation. To test the physiological relevance of a crosstalk between sclerostin and PDGFR signaling in osteoblasts, we treated 4-month-old mice lacking PDGFRα and PDGFRβ in osteoblast lineage cells (*Osx-Cre; Pdgfraf/f; Pdgfrbf/f*) and their control littermates (*Pdgfraf/f; Pdgfrbf/f*) with an anti-sclerostin antibody (anti-SOST Ab, 25 mg/kg twice a week) or its vehicle for 2 weeks. We took advantage of the inducible *Cre* expression in *Osx-Cre* mice to suppress PDGFR signaling at the onset of anti-SOST Ab treatment. Increase of trabecular bone volume after anti-SOST Ab treatment was more elevated in *Osx-Cre; Pdgfraf/f; Pdgfrbf/f* mice (+142% vs veh, $p \leq 0.001$) than in control mice (+81% vs veh, $p \leq 0.001$), whereas cortical bone volume increased equally in both mice (+19% vs veh, $p \leq 0.002$). Although osteoclast number was significantly reduced by the osteoblast-specific suppression of PDGFRs (− 38% vs veh, $p \leq 0.001$) or the anti-SOST Ab treatment (− 50% vs veh, $p \leq 0.001$) alone, it was not further inhibited by the combination of both conditions. Moreover, bone formation rate at trabecular surfaces was more augmented by anti-SOST Ab treatment in *Osx-Cre; Pdgfraf/f; Pdgfrbf/f* mice (2.9-fold vs veh, $p \leq 0.0001$) than in control mice (1.9-fold vs veh, $p \leq 0.001$). Interestingly, *Wisp1*, a Wnt target gene that inhibits osteoclastogenesis and stimulates osteoblast differentiation, was more expressed in response to anti-SOST Ab in tibiae of *Osx-Cre; Pdgfraf/f; Pdgfrbf/f* mice (1.8-fold vs veh, $p \leq 0.008$) than in those of control mice (1.2-fold vs veh, $p \leq 0.1$). Finally, PDGFR inhibition in osteoblasts increased Wnt3a-induced β-catenin transcriptional activity in vitro. In conclusion, our results show that osteoblast lineage-specific deletion of PDGFR signaling potentiates the osteo-anabolic action of anti-SOST Ab.

Keywords: Osteoblasts, Sclerostin, PDGFR, Wnt, Anabolic therapy

COP12

Role of Dlx5 and Dlx6 in the commitment of osteoblastic differentiation: application to osteoporosis

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Introduction: Osteoporosis is the consequence of imbalance of low anabolism to high catabolism. Impaired anabolism involves reduced osteoblast differentiation, mediated by transcription factors, including Dlx5 and Dlx6. In this project we study the role of Dlx5/6 in bone remodeling.

Methods: We analyzed the kinetic expression of Dlx5, Dlx6 and osteoblastic markers during osteoblastic differentiation from murine osteoblastic progenitor derived from calvaria and bone marrow. Analysis was carried out from control cells, cells with Dlx5/6 ex vivo or in vivo recombination in parallel to human bone marrow cells. We analyzed the bone phenotype of mutated mice in the absence of Dlx5/6 expression under Osx promoter.

Results: Dlx5 and Dlx6 expression increases at D7 during osteoblastic differentiation in the murine bone marrow and was stable to D21. The absence of Dlx5/6 in progenitor cells induced decreased levels of osteocalcin and alkaline phosphatase. Dlx5/6 $^{fl/fl}$Osx-Cre genotype was lethal. Dlx5/6 $^{fl/+}$Osx-Cre mice had normal cortical and trabecular parameters at 6 weeks, but had a lower cortical thickness (p: 0.027/p: 0.001 for females and males), BV/TV and Tb.Th (p: 0.006/p: 0.003 for females and males) with a lower BMD at 3 months in both sexes. Moreover, periosteal volume was lower (p: 0.024/p: 0.020 for females and males). The skulls revealed unclosed sutures and dental abnormalities.

Discussion: Dlx5/Dlx6 promote osteoblastic differentiation with an effect on late bone markers, in favor of a role in terminal differentiation. The deletion of these transcription factors under the action of the Osterix promoter generates lethality, in favor of an essential role in bone development. Heterozygous mutation show impaired bone acquisition during growth. To obtain Dlx5/Dlx6 deletion in osteoblastic precursor cells, a murine model of conditional induced deletion is generated.

Keywords: Dlx5, Dlx6, Osteoporosis, Osteoblast differentiation, Cortical bone

Concurrent Oral Presentations 1—Clinical/Public Health: Rare bone diseases and early onset osteoporosis

COP01

Persistent and recurrent cases in tumor-induced osteomalacia: a retrospective study

Xiang Li[1,2], Zinan Yin[1,2], Juan Feng[1], Juan Du[1], Xi Zhou[2], Yan Jiang[2], Ou Wang[2], Mei Li[2], Xiaoping Xing[2], Yong Liu[2], Weibo Xia[2]

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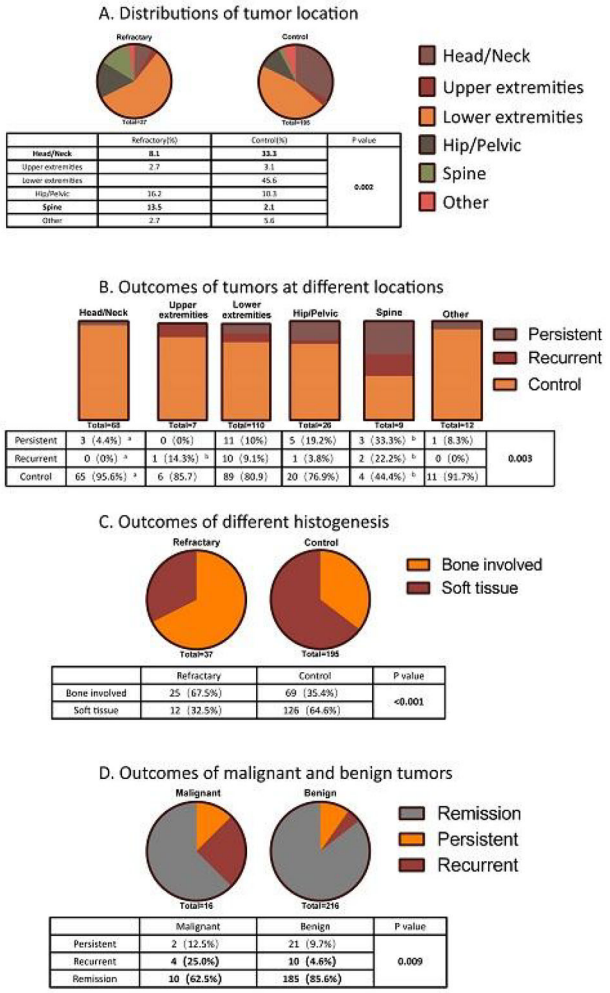
Objectives: Whereas most tumor-induced osteomalacia(TIO) cure after removing causative tumors, a few tumors can be persistent or recurrent. We aimed to clarify the incidences of these cases in TIO patients treated by surgery and their clinical characteristics.

Methods: We reviewed all TIO cases which received operation before December 31, 2017 in our hospital. TIO was established base on pathology and recovery of hypophosphatemia. Patients were divided into control group or refractory(persistent or recurrent) group.

Results: A total of 253 cases were confirmed as TIO, among which 23(9.1%) were persistent, 14(5.5%) were recurrent. The overall refractory rate is 14.6%. The gender distributions of refractory group were even with younger onset age(34.4 ± 12.9 *vs.* 38.8 ± 11.8, $p = 0.038$) when compared with control. The mean recurrent time was 33.8 (3–120) months. The location of refractory tumors consisted of neck/head(n = 3, 8.1%), upper extremities(1, 2.7%), lower extremities(21, 56.8%), hip/pelvic(6, 16.2%), spine(5, 13.5) and other(1, 2.7%). Refractory group had less tumors in head but more in spine than control group(8.1% *vs.* 33.3% and 13.5% *vs.* 2.1%, $p = 0.002$). Refractory rate was lowest in head/neck(4.4% persistent, 0% recurrent), and highest in spine(33.3% persistent, 22.2% recurrent). Reffering to histogenesis, refractory rate was significantly higher in bone involved tumors than soft tissue(67.5% *vs.* 32.5%, $p < 0.001$). The persistent rate was similar between malignant tumors and benign ones, whereas recurrent rate was higher in malignant tumors(25.0% *vs.* 4.6%, $p = 0.009$).

Conclusions: We first report the persistent and recurrent rate of postoperation TIO. Tumor located in spine or involved in bone may have worse outcomes.

Keywords: TIO, Persistent, Recurrent, Surgery



	Refractory(%)	Control(%)	P value
Head/Neck	8.1	33.3	0.002
Upper extremities	2.7	3.1	
Lower extremities		45.6	
Hip/Pelvic	16.2	10.3	
Spine	33.5	2.1	
Other	2.7	5.6	

	Head/Neck	Upper extremities	Lower extremities	Hip/Pelvic	Spine	Other	
	Total=68	Total=7	Total=110	Total=26	Total=9	Total=12	
Persistent	3 (4.4%) [a]	0 (0%)	11 (10%)	5 (19.2%)	3 (33.3%) [b]	1 (8.3%)	0.003
Recurrent	0 (0%) [a]	1 (14.3%) [b]	10 (9.1%)	1 (3.8%)	2 (22.2%) [b]	0 (0%)	
Control	65 (95.6%) [a]	6 (85.7)	89 (80.9)	20 (76.9%)	4 (44.4%) [b]	11 (91.7%)	

	Refractary	Control	P value
	Total=37	Total=195	
Bone involved	25 (67.5%)	69 (35.4%)	<0.001
Soft tissue	12 (32.5%)	126 (64.6%)	

	Malignant	Benign	P value
	Total=16	Total=216	
Persistent	2 (12.5%)	21 (9.7%)	0.009
Recurrent	4 (25.0%)	10 (4.6%)	
Remission	10 (62.5%)	185 (85.6%)	

[Figure 1. Persistent and recurrent TIO in postoperation patients]

COP02

Biomarker profiles in PLS3 and WNT1 osteoporosis—elevated serum DKK1 in abnormal PLS3 function

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Objectives: Pathogenic mutations in *PLS3* and *WNT1* lead to severe early-onset and progressive osteoporosis with peripheral and spinal fractures. Due to X-linked inheritance, *PLS3* mutation-positive males are more severely affected than females. *WNT1* mutations affect both genders equally. Despite the severe skeletal manifestations, conventional bone turnover markers are normal in mutation-positive patients. We analyzed novel biomarkers in families with *PLS3* and *WNT1* mutations to identify clinically usable markers distinguishing mutation-positive and -negative individuals.

Subjects and Methods: This cross-sectional cohort study involved 14 *PLS3* (median age 41 years, range 8–76 years) and 17 *WNT1* (52 years, 11–76 years) mutation-positive subjects from five Finnish families and 17 *PLS3* and 17 *WNT1*mutation-negative age- and sex-matched subjects from the same families. Fasting serum samples, collected at 8–9 a.m., were assessed for concentrations of dickkopf-1 (DKK1), sclerostin, intact and C-terminal fibroblast growth factor 23 (FGF23), and intact aminoterminal propeptide of type I procollagen (PINP).

Results: The results revealed significantly elevated DKK1 in *PLS3* mutation-positive subjects compared with *PLS3* mutation-negative ($p = 0.002$) or with *WNT1* mutation-positive ($p < 0.001$) subjects. Compared to mutation-negative subjects, the DKK1 difference was even more prominent in female *PLS3*mutation-positive subjects ($p = 0.009$) than in males ($p = 0.100$). Similar differences were not seen in WNT1 families. Sclerostin concentrations did not differ between any groups. Intact, but not C-terminal, FGF23 was significantly elevated in *WNT1* mutation-positive subjects ($p = 0.041$) and normal in *PLS3* subjects. PINP concentrations were normal in both groups.

Conclusions: Intriguingly, our results indicate increased DKK1 concentration in PLS3 osteoporosis and suggest a link between PLS3 and DKK1 in bone metabolism. Sclerostin concentrations are normal in WNT1 and PLS3 osteoporosis but FGF23 may be impacted by abnormal WNT1 signaling. These findings provide novel information on the molecular communications in bone. DKK1 may be a clinically useful biomarker for PLS3 osteoporosis.

Keywords: PLS3, WNT1, DKK1, Sclerostin, Osteoporosis

COP03

The relationship of radiographic rickets severity score with clinical outcomes in children with x-linked hypophosphatemia

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Background: The Rickets Severity Score (RSS) can be used to measure radiographic severity in X-linked hypophosphatemic rickets (XLH), a genetic disorder mediated by increased circulating FGF23.

Methods: The reliability of the RSS in XLH was assessed using data from a randomized, phase 2 clinical trial of burosumab, a fully human anti-FGF23 monoclonal antibody, in 52 children with XLH ages 5 to 12 years. Bilateral knee and wrist radiographs were obtained at baseline, week 40, and week 64. We evaluated the relationships of the RSS to the Radiographic Global Impression of Change (RGI-C), serum alkaline phosphatase (ALP), height Z–score, 6–minute walk test (6MWT), and the Pediatric Orthopedic Society of North America Pediatric Outcomes Data Collection Instrument (POSNA-PODCI).

Results: The RSS showed moderate-to-substantial inter-rater reliability between four raters (weighted kappa, 0.45–0.65; Pearson correlation coefficient (r), 0.83–0.89) and substantial intra-rater reliability (weighted kappa, 0.66; r = 0.91). Baseline RSS correlated with serum ALP (r = 0.47). Baseline RSS identified two subgroups (higher [RSS $\geq$ 1.5] and lower RSS [RSS $<$ 1.5]) that discriminated between

subjects with greater and lesser rachitic disease. Compared with lower RSS, higher RSS was associated with more severe clinical features, including impaired growth (Z–score, –2.12 vs − 1.44) and walking ability (6MWT percent predicted, 77% vs 86%), more severe self–reported pain (29.9 [more severe] vs 45.3 [less severe]) and impairment of physical function (29.6 [more severe] vs 40.9 [less severe]). During burosumab treatment, greater reductions in RSS corresponded to higher RGI-C global scores of change (r = –0.65). Improvements in RSS correlated with decreased serum ALP (r = 0.47).
Conclusions: The RSS is a reliable indicator of disease severity in XLH. Higher RSS values are associated with greater biochemical, clinical, and functional impairments in children with XLH.
Keywords: Rickets, Clinical trial, Radiology, Burosumab, XLH

COP04

Early onset osteoporosis due to heterozygous ENPP1 loss of function mutations in humans is recapitulated by homozygous ENPP1 loss of function mutations in mice

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Biallelic ENPP1 deficiency in humans induces hypophosphatemic rickets characterized by increased circulating FGF23 levels and renal phosphate wasting ('Autosomal Recessive Hypophosphatemic Rickets Type 2′, or ARHR2) but osteopenia or osteoporosis has not been described. Here, we describe three adult male patients (ages 43, 59, and 62) suffering from early-onset-osteoporosis who presented to the Institute of Osteology and Biomechanics at the University Hospital Hamburg, Germany. Two patients suffered from fractures in their thoracic spine and one a radial fracture. All patients exhibited elevated FGF23 levels and phosphate wasting. DXA scans of hip demonstrated T-score below − 2.5 and HRpQCT demonstrated significantly reduced trabecular and cortical thickness of the tibia and radius. Next generation sequencing revealed that all 3 patients had heterozygous missense mutations in ENPP1: Y471C (1412A > G) (2 patients) and H777R (2330A > G) (1 patient).

Bones from *Enpp1*$^{asj/asj}$ mice, a model of Enpp1 deficiency, were studied to understand the bone pathophysiology. Similar to what was observed in patients, 10 week-old male *Enpp1*$^{asj/asj}$ mice exhibited elevations in FGF23 (350% of WT), hypophosphatemia (77% of WT), and osteopenia with reduced trabecular and cortical bone by uCT (trabecular BV/TV was 62% WT), cortical thickness (78% of WT), increased osteoid width (130% of WT), and increased mineralization lag time (MLT) (214% of WT). Osteopenia persisted in 23 week-old male *Enpp1*$^{asj/asj}$ mice (BV/TV 40% of WT), along with reduced bone formation rates/bone surface (33% of WT), reduced mineral apposition rate (MAR 41% of WT), and increased MLT (196% of WT). Together these changes in bone parameters describe the bone pathophysiology of ENPP1 deficiency where decreased bone formation along with excess unmineralized matrix results in skeletons of low bone mass and undermineralized bone matrix. The murine data supports the human findings that ENPP1 deficiency increases fracture risk and that human heterozygous ENPP1 deficiency may be clinically significant.

COP05

Exome sequencing with functional follow-up identifies KIF26B as a novel genetic determinant of familial osteoporosis

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Objectives: Genetic factors play an important role in osteoporosis pathogenesis. The study aimed to identify the genetic determinants of primary osteoporosis in a Maltese family, and investigate functionality of novel genes and variants using in vitro and in vivo methods.
Methods: A 2-generation family of 12 relatives (including 7 siblings) aged 34–77 years was recruited. Osteoporosis was defined using DXA scans of the lumbar spine (LS) and hip. The proband had a LS T-score of − 4.0. Exome sequencing was performed on 6 relatives and replication of shortlisted variants was sought in the Malta Osteoporotic Fracture Study (MOFS, n = 1012). In vitro protein expression was analysed by transfecting in COS-7 cells and western blotting, whereas gene knockout consequences in zebrafish were assessed through measurements of BMD by micro-computed tomography, mineralisation rate using Alizarin red staining of the skeleton, and resorption activity by TRAcP staining of regenerating scales.
Results: Exome variant filtering following dominant inheritance identified a novel nonsense variant p.Q287X within the Kinesin family member 26B (*KIF26B*) gene in affected relatives. Heterozygosity was detected in two women from the MOFS (MAF < 1%) exhibiting low LS BMD (average T-score: − 2.3). Western blotting analysis resulted in complete protein ablation in the presence of the alternate allele. At 1 month post-fertilisation (mpf), *kif26b* knockout fish displayed reduced numbers of mineralised vertebrae relative to wild-type fish matched for age and length. Notorious decreases in BMD (− 23%, $p = 0.01$) and bone volume/tissue volume (− 40%, $p = 0.01$) were observed at 3mpf, whereas at 4mpf, a higher osteoclast activity, in regenerating scales at 6-day ($p = 0.04$) and 14-day ($p = 0.03$) recovery set-points, was seen.
Conclusions: Findings postulate *KIF26B* to be a novel determinant of osteoporosis. KIF26B is involved in intracellular transport and cell division, and controls morphogenesis via Wnt5a-Ror signalling. Further elucidation of its role in bone physiology may identify novel targets for osteoporosis treatment.

COP06

Blood as a proxy for bone tissue—An epigenome-wide pilot study in bone-blood pairs

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Currently known genetic variants only explain a small proportion of the phenotypic variance in skeletal strength. Part of this "missing" data may be found through studying epigenetics. Since obtaining human bone is difficult, this pilot study investigated the feasibility of using blood as a substitute for studying DNA methylation in bone.

Paired bone-blood samples were collected from elderly females during hip replacement surgery (n = 12; mean age 76y). The study was approved by Lund University Ethics Committee (LU 957-03). DNA methylation across 850 k CpG sites in the genome was measured using the Infinium MethylationEPIC beadchip.

The data was analyzed to identify positions that had similar methylation levels (SMPs) in both bone and blood, applying a two-step selection criterion:

i. t-test followed by FDR analysis [adjusted p-value < 0.05], to test the association of Pearson's correlation coefficients between methylation levels (M-values) of the paired bone-blood samples;
ii. filtering based on the difference between methylation levels (β-values) of the paired samples [$\max(\Delta\beta) < 0.2$].

Based on these, 29,292 SMPs across over 7000 genes were identified. Most of the SMPs were located in CpG islands (35%), and gene body (24%) regions. Among these, we identified SMPs within genes which have previously been identified in large GWAS, including ESR1, EN1, and WNT16. Besides, more than 12,000 of the SMPs were located within ± 200 kb of SNPs associated with bone phenotypes, including BMD, fracture and osteoarthritis. Shortlisting of genes for follow-up is ongoing.

The results indicate that a considerable number of sites are similarly methylated in both bone and blood, demonstrating that blood can be a surrogate for bone tissue. Potentially, in large cohort studies methylation analysis is feasible, as a way to unravel the regulation of skeletal strength.

Concurrent Oral Presentations 2—Basic/Translational: Local and systemic regulators of bone turnover

COP19

Loss of mechanosensitivity in a mouse model for gerodermia osteodysplastica due to an altered lacuno-canalicular osteocyte network

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With increasing age bone tissue loses its capacity to respond to mechanical loading. But whether this is also true for premature aging disorders is unknown. We investigated the effect of a strain matched (1200 με) two week in vivo tibia loading protocol in the *Gorab*^{Prx1} mouse model for the progeroid rare bone disorder gerodermia osteodysplastica. The bone formation response to loading was investigated by microCT and histomorphometry, while the osteocyte lacuno-canalicular network was investigated by rhodamine staining and confocal microscopy. After loading, control mice showed a robust increase of the cortical mineral apposition rate (MAR 0.88 vs. 2.56 μm/d; $p < 0.05$), which resulted in a greater cortical area (0.55 vs. 0.61 mm^2; $p < 0.05$) and in a doubling of the trabecular bone volume fraction (7% vs. 14%; $p < 0.05$). Unexpectedly, this anabolic response to loading was completely abolished in the *Gorab*^{Prx1} mutants (MAR 0.72 vs. 0,72 μm/d; n.s.). Instead, time-lapse microCT imaging revealed an elevated and disorganized mineralizing surface at basal level, which did not change after mechanical loading. In search for an explanation for this loss of mechanoresponsiveness we focused our attention to the osteocytes, whose density was roughly doubled in number in the *Gorab*^{Prx1} mutants (86,547 vs. 179,107 1/mm^3; $p < 0.001$). However, mutant osteocytes displayed an abnormal morphology. A quantification of the lacuno-canalicular network revealed a reduction of the number of canaliculi per lacuna (78 vs. 38; $p < 0.001$) resulting in a lower canalicular density and connectivity. We previously demonstrated abnormal proteoglycan glycosylation leading to a disorganized ECM in *Gorab*^{Prx1} mutants. These current data suggest that the altered lacuno-canalicular network preventing proper strain amplification and an anabolic response to loading importantly contributes to bone fragility in gerodermia osteodysplastica.

COP20

Weekly shifts in light–dark cycle disrupt circadian clock gene expression in bone and reduce bone turnover

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The past decade, epidemiological studies have associated disturbances of the biological clock, as occurs in shift work, with low bone mineral density and increased fracture risk. As a large part of the working population participates in shift work (e.g. almost 30% of workers in the U.S.), this demonstrates the need for further research on the risk of skeletal disorders associated with circadian disturbances. In this study, we aimed to investigate to what extent rhythmicity exists in bone, and whether circadian disruption by weekly shifts in light–dark cycle affects bone turnover and structure in mice.To evaluate whether gene expression in bone is rhythmic, tibiae were collected from mice every 6 h over a 24 h period (n = 9/timepoint). In these bones, we found diurnal expression patterns of clock genes (*Rev-erbα*, *Bmal1*, *Per1*, *Per2*, *Cry1*, *Clock*), as well as genes involved in osteoclastogenesis, osteoclast proliferation and function (*Rankl*, *Opg*, *Ctsk*) and osteocyte function (*c-Fos*).To

study the importance of this rhythm for bone health, mice were subjected to either normal light–dark cycles or weekly 12 h shifts in light–dark cycle for 16 weeks (n = 8/group). Weekly shifts resulted in a disruption of clock gene expression in bone (i.e. reversed rhythm of *Rev-erbα*($P < 0.001$) and *Cry1*($P < 0.01$), and attenuated rhythm of *Bmal1*($P < 0.001$) and *Clock*($P < 0.05$) three days after a shift), and a reduction in plasma levels of procollagen type 1 amino-terminal propeptide (P1NP, − 22.4%; $P < 0.05$) and tartrate-resistant acidic phosphatase (TRAP; − 19.9%; $P < 0.01$), suggestive of reduced bone formation and bone resorption, respectively. Moreover, shifts in light–dark cycle significantly altered trabecular bone structure as determined by micro-CT, and seemed to reduce bone length and weight, consistent with impaired bone growth. Collectively, these results suggest that circadian rhythm is important for bone health, and that circadian disruption negatively affects bone turnover markers and bone structure.

Keywords: Circadian Rhythm, Clock genes

COP21

The microbiome plays a crucial role in mediating negative effects of chronic psychosocial stress on bone growth

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Chronic psychosocial stress is a risk factor for the development of physical and mental disorders which are often accompanied by an over-reactive immune system. Given that stress-induced inflammation is lacking in germ-free and antibiotics-treated mice, a causal role of the gut microbiome is likely. We showed previously that chronic psychosocial stress disturbed long-bone growth. Here, we address the hypothesis that fecal transplantation from non-stressed mice to stressed mice attenuate the negative effects of stress on bone, involving the immune system.

7-weeks-old male C57BL/6 N mice were subjected to the chronic-subordinate-colony-housing (CSC) paradigm, a model for chronic psychosocial stress, for 19 days. Single-housed (SHC) mice were used as controls. We infused SHC and CSC-recipient mice rectally with SHC-feces at days 4/11 and assessed immunological and bone parameters on day 20. Furthermore, SHC and CSC-recipient mice were infused with CSC-feces or saline. n = 6–8/group. Mann–Whitney-U/Kruskal–Wallis-test + Bonferroni, $p < 0.05$.

KC, IL-6 as well as MCP-1 levels were increased in the plasma of saline-infused CSC (KC: 7.7 vs. 12.8 pg/ml p = 0.016; IL-6: 3.6 vs. 8.0 pg/ml p = 0.004; MCP-1: 20.0 vs. 40.1 pg/ml p = 0.028) and CSC-recipient CSC mice (KC: 7.1 vs. 14.2 pg/ml p = 0.008; IL-6: 8.4 vs. 20.0 pg/ml p = 0.048; MCP-1: 16.8 vs. 61.0 pg/ml p = 0.004) vs. respective SHC-animals, indicating increased immune activation by chronic stress. SHC-recipient CSC animals only showed increased MCP-1 (p = 0.029), but not KC and IL-6 levels, indicating stress-protective effects of fecal transplantation. Regarding bone parameters, CSC mice displayed reduced tibia length and increased growth plate thickness and disorganization compared to control animals, indicating disturbed long-bone growth. The effects of stress on bone parameters were abolished when transplanting CSC mice with feces from non-stressed mice.

In conclusion, our data provide evidence for a role of the microbiome in adverse consequences of chronic psychosocial stress on bone. One mechanism of stress protection might be the reduced immune activation under fecal transplantation treatment.

COP22

Role of matricellular protein periostin (Postn) on bone fragility in two models of type 2 diabetes

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Type2 diabetes (T2D) has emerged as a novel risk for fragility fractures independently of BMD. T2D exhibit low bone turnover and high sclerostin levels. We previously reported that periostin expressed by osteocytes and lining cells are required to decrease sclerostin and stimulate bone formation, hence Postn-/- exhibit bone fragility.

We hypothesized that T2D decrease bone qualities/strength by a downregulation of periostin. For this purpose, female Postn-/- at 12 weeks of age, received either a high fat or chow diet (HFD 60% vs CD 10% of fat) for 5 weeks; and female Db/Db mice, i.e. diabetic model were treated by a Postn adenovirus collagen 2.3 Kb promotor (Ad-Postn) or by Ad-null. Bone structure, formation and strength were evaluated by microCT, histomorphometry, and three point pending. Using primary OB exposed to high glucose (25 mM), Postn mRNA expression decrease at Day9 and 14 (− 61%, − 77% vs 5.5 mM, $p < 0.001$) associated with a decreased in ALP protein levels. In vivo, we confirm a lower Postn mRNA in HFD (− 37% vs CD, $p < 0.05$) and in Db/Db mice (− 41% vs C57Bl6j, $p < 0.01$). In WT, HFD significantly decreased BV/TV, TbTh, CtTV and CtBV (− 26%, − 11%, − 12%, − 17% vs CD, all $p < 0.05$). Hence ultimate force and stiffness are significantly decreased (− 11 and − 12% vs CD, $p < 0.05$). In contrast, in Postn-/-, HFD did not induce any change in microstructure and strength. In Db/Db mice, Ad-Postn significantly increased CtTV, CtBV, PssLPm, EcsLPm (+6.2%, +16.6, 173 and 534%, all $p < 0.05$). As a consequence, ultimate force and stiffness were improved by Ad-Postn (+14.5 and +17.7% vs Ad-null, $p < 0.05$). In conclusion, T2D decrease periostin expression. In absence of periostin, deleterious effects of HFD on bone structure and strength are inhibited. In opposite, over expression of periostin in Db/Db mice rescue both structure and strength by increasing bone formation.

COP23

Evidence that the intestinal microbiome regulates susceptibility to osteoarthritis in mice

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Background: In recent years, increasing interest has focussed on the role of the microbiome in the pathogenesis of various diseases including inflammatory disease, osteoporosis, and osteoarthritis. Here we examined the role of intestinal microbiome in the development of osteoarthritis in mice subjected to destabilisation of medical meniscus (DMM) and assessed the effect of probiotics on osteoarthritis development over and above normal microbiome.

Methods: The intestinal microbiome was depleted by broad-spectrum antibiotics from one week before birth until the age of 6 weeks when the mice were subjected to DMM following microbiome

reconstitution or sham reconstitution. Mice with reconstituted microbiome were also treated with a mixture of probiotic strains or vehicle (glycerol). The severity of arthritis was evaluated by microCT and histological analyses and differences between treatments were compared by one-way ANOVA. All experiments had local ethical approval by the University of Edinburgh.

Results: Probiotic treatment in mice with reconstituted microbiome significantly inhibited osteoarthritis progression at the femoral condyle with (mean ± SEM) 28.1% ± 5.8 less cartilage damage ($p = 0.018$) compared to mice with sham reconstitution and vehicle treatment. Particularly, the medial femoral condyle had 36.3% ± 4.3 less cartilage damage compared to controls ($p = 0.003$). Microbiome reconstitution alone only moderately reduced cartilage damage at the femoral condyle by 19.7% ± 7.7 but this was not statistically significant ($p = 0.147$). There was no significant difference in osteoarthritis progression in the tibial plateau between different treatment groups. MicroCT analysis showed that although microbiome reconstitution alone did not have a significant impact on any subchondral bone indices, additional probiotic administration significantly increased BV/TV (6.5% ± 1.6; $p = 0.032$) and Tb.Th (8.1% ± 2.0; $p = 0.026$).

Conclusions: We conclude that treatment with probiotics prevents DMM-induced cartilage damage progression at the femoral condyle. These findings suggest that the intestinal microbiome plays a role in this model of osteoarthritis. Further studies on the role of probiotics in humans with osteoarthritis are warranted.

COP24

Skeletal muscle-specific ablation of Slc20a1/Pit1 andSlc20a2/Pit2 causes early postnatal lethality in mice

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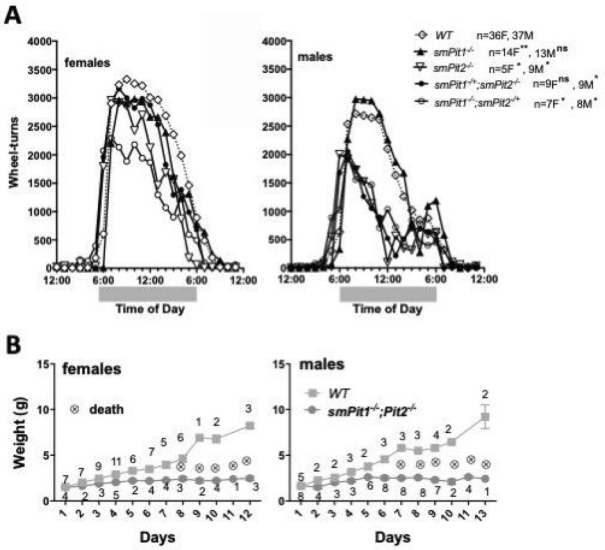
Low blood phosphate (Pi) reduces muscle function in hypophosphatemic disorders. When severe it can result in respiratory-, heart-failure and death. It is unknown, whether homeostatic hormonal changes in response to low blood Pi or reduced supply of Pi for myocellular metabolism cause reduced muscle function. Furthermore, Pi may activate signaling pathways important for normal muscle function Pi transport-independently.

To reduce Pi supply without causing hypophosphatemia and hormonal changes, we generated conditional knockout mice lacking one or two copies of the house-keeping Pi transporters *Pit1* and *Pit2* in skeletal muscle, using the postnatally expressed *human skeletal actin (HSA)*-cre. These mice show a gene-dose dependent reduction in running activity resembling the impaired endurance seen in hypophosphatemic (*hyp*) mice (Fig. 1A). In contrast to *hyp*mice, grip strength is preserved.

Simultaneous conditional deletion of both transporters is lethal by postnatal day (P) 13 (Fig. 1B). Already at P1, mutant animals exhibit skeletal muscle hypoplasia, which progresses to marked atrophy and myofiber degeneration by P10. This is accompanied by growth retardation evident at P2 ($p < 0.01$). By P10, mutant pups have severely impaired mobility, inconsistent gastric filling and depleted adipose stores, resulting in death due to hyponutrition, skeletal muscle/respiratory-failure and hypothermia by P13. Skeletal development is qualitatively comparable to WT littermates, but quantitatively delayed. Cardiac muscle is histologically normal.

Our findings indicate that *Pit1* and *Pit2* are essential for normal myofiber development and/or survival, and that the reduced endurance that accompanies hypophosphatemia may be largely independent of homeostatic hormonal changes.



[Spontaneous running wheel activity and weight development of smPit1/2 mutant mice]

Concurrent Oral Presentations 2—Clinical/Public Health: Osteoporosis and risk predictors

COP13

A machine learning approach to predict non-vertebral fracture risk from dual X-ray absorptiometry images

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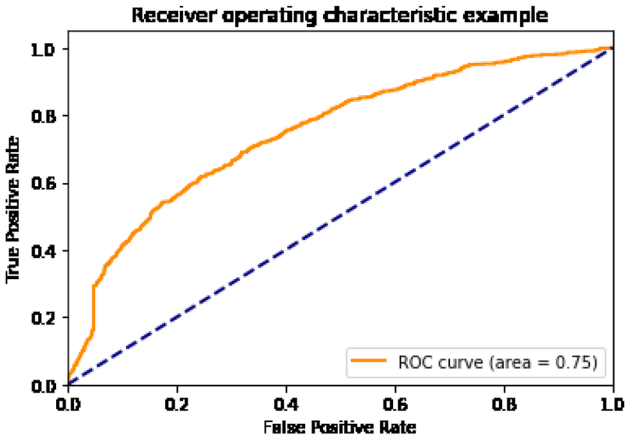
Background: The assessment of osteoporotic fracture risk by bone mineral density (BMD) with dual X-ray absorptiometry (DXA), or by using fracture risk assessment models still performs poorly: only 44% of non-vertebral fractures in women above 55 years occur in the subjects with osteoporotic range BMD (Schuit, Bone 2014). The positive predictive values of fracture risk assessment models are between 7.6% and 12.0% (Dagan, BMJ 2017).

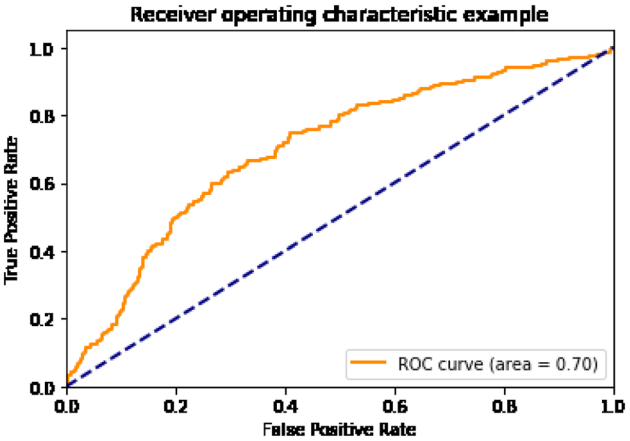
Methods: We used a machine learning approach to predict non-vertebral fracture risk from DXA images. This study included femoral neck DXA images of 3824 participants from Rotterdam study. The model was trained to predict the probability of future non-vertebral fracture from the DXA image alone.

Results: The model showed AUC 0.75 (Figure 1) on the training set and 0.70 (Figure 2) on the test set. The Pearson coefficient of correlation between the fracture risk prediction from our model and femoral neck BMD t-score was − 0.50. The model is now undergoing further validation on 2 additional independent cohorts.

Conclusion: We used machine learning methods to develop a model that performs similarly or better than BMD or fracture risk prediction models.



[Figure 1]



[Figure 2]

COP14

Does screening for high hip fracture risk impact on falls risk? A post hoc analysis from the SCOOP study

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SCOOP was a randomised controlled trial comparing a hip fracture risk screening programme, using FRAX® with or without dual X-ray absorptiometry (DXA), with usual GP management. Screening reduced the incidence of hip fractures by 28%, an effect likely mediated by osteoporosis treatment in high risk patients. An alternative explanation, that knowledge of fracture risk might modify falls risk, was tested in this analysis.

SCOOP recruited 12,483 women aged 70–85 years who were randomly assigned to the intervention arm (n = 6233) or control (n = 6250). We analysed fall risk factors at entry, including any potential differences between the groups. We then determined whether there were differences in falls incidence between the randomisation groups, especially in those identified at high fracture risk.

Several risk factors for incident falls were identified. As expected, women sustaining one or more falls were slightly but statistically significantly older at baseline than those remaining fall free during follow up (75.8 ± 4.2 vs 75.1 ± 4.0, $p < 0.001$). Higher BMI, prior fracture, glucocorticoid use, secondary osteoporosis, and a fall in the year prior to entry were consistently associated with an increased risk of falling. In addition, a higher FRAX 10 year probability of hip fracture was also associated with increased likelihood of falling with increases in risk of 1–2% for every 1% increase in hip fracture probability. However, the risk factors were well balanced between the study arms and, importantly, there were no statistically significant differences in the incidence of falls between the two study groups. In particular, there was no significant interaction ($p = 0.18$) between screening and falls risk when compared across the range of baseline FRAX hip fracture probabilities.

Screening for 10-year fracture risk does not modulate fall risk, supporting the conclusion that the reduction in hip fracture is mediated by anti-osteoporosis therapy.

Keywords: Fracture risk, FRAX, Falls

COP15

T-score as an Indicator of fracture risk on therapy: evidence from romosozumab vs alendronate treatment in the ARCH trial

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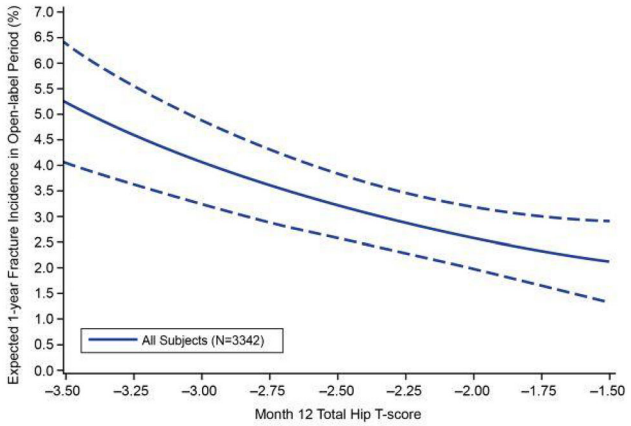
Evidence suggests that bone mineral density (BMD) achieved during treatment reflects fracture risk. We explored the relationship between T-scores achieved after 12 months (m) with romosozumab or alendronate and subsequent fracture risk in the ARCH trial (NCT01631214).

Postmenopausal women with osteoporosis and prior fragility fracture were randomized 1:1 to romosozumab 210 mg SC QM or alendronate 70 mg PO QW for 12 m, followed by open-label (OL) alendronate 70 mg PO QW for ≥ 12 m, with an event-driven primary analysis. We examined change from baseline in BMD and T-scores at 12 m, relationship between total hip (TH) T-scores at 12 m and subsequent nonvertebral fracture rates, and fractures in the OL period (new vertebral fractures 12–24 m based on 24 m spine radiographs, and clinical, nonvertebral, and hip fractures in the full OL period).

4093 patients were enrolled: 2046 romosozumab/2047 alendronate, mean baseline T-scores: − 2.96 at lumbar spine, − 2.80 at TH. 3465 patients (1739 romosozumab/1726 aledronate) received ≥ 1 alendronate dose during OL (median follow-up = 1.9 years). Mean TH BMD increased by 6.2% (romosozumab)/2.8% (alendronate) at 12 m (T-score increases of 0.31/0.15). 12 m TH T-score was associated with 1-year nonvertebral fracture rate during OL (Figure), independent of initial treatment. During OL, romosozumab-to-alendronate patients had a 75% lower relative risk of new vertebral fracture ($p < 0.001$) and reductions in clinical (32%, $p = 0.001$), nonvertebral (19%, $p = 0.120$), and hip (40%, $p = 0.041$) fractures vs alendronate-to-alendronate patients.

Higher TH T-scores achieved on-therapy at 12 m resulted in subsequent lower fracture risk regardless of treatment received, supporting the concept of T-score targets in osteoporosis.

Keywords: Osteoporosis; Fracture risk; Postmenopausal



The dashed lines indicate upper and lower 95% confidence intervals. Likelihood ratio test P<0.001.

[Figure: Month 12 total hip T-score and nonvertebral fracture rate during the open-label period]

COP16

Added value of bone biopsy in the assessment of skeletal status within a year of renal transplantation?

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Renal transplantation (Tx) largely corrects CKD-associated disturbances in bone and mineral metabolism, but fracture risk remains high. The 2017 CKD-MBD guideline suggests it "reasonable" to consider a bone biopsy in the first 12 M post-Tx to "guide treatment", but evidence for this is lacking. The objective of this study was to explore the added value of a bone biopsy in the evaluation of skeletal status within a year post-Tx. 33 consecutive non-diabetic renal transplant recipients (14 women), median age 50 years (22–67 years), with stable creatinine < 150umol/L, agreed to have a double-tetracycline labelled trans-iliac bone biopsy 12–18 M post-Tx, concomitantly to standard practice evaluation of skeletal status (BTMs, DXA and spine radiographs). Undecalcified biopsies were evaluated qualitatively (TMV classification) and quantitatively (standard histomorphometry). Intact PTH was increased in 26/33 patients, but total ALP and bone ALP only in respectively 14/33 and 15/31 patients. Ten patients had T-score 2.5 at LS and/or FN, 25 had FN osteopenia. Eight patients had 28 vertebral fractures(VF). There was no significant correlation between age, sex, mode and years on dialysis, any biochemical parameter, BMD measurements and prevalent VF, which were observed with high or low turnover and with high, low or normal BMD. 87% of biopsies qualitatively showed low bone volume, 49% high turnover & 22% low turnover. Disturbed mineralization was diagnosed in 22%, independently of D levels or prevalent VF. Quantitatively, mean BV/TV was low (17.4% ± 4.9) and predominant high turnover confirmed (BFR/BS:2.5 ± 2 mm^2/mm^3/d, ES/BS:12 ± 4%). Similar to the case with BTMs and BMD, there was no association between qualitative and quantitative histological parameters and prevalent VF at 12–18 M post-Tx. However, bone histology at this time-point did detect otherwise unsuspected delayed mineralization and was predictive of increased risk for new vertebral fractures on preliminary analysis of longer-term 5-year follow-up data.

Keywords: Bone histology, Bone turnover, BMD, Vertebral fractures

COP17

Fracture risk assessment in women treated with aromatase inhibitors: effect of oral bisphosphonates

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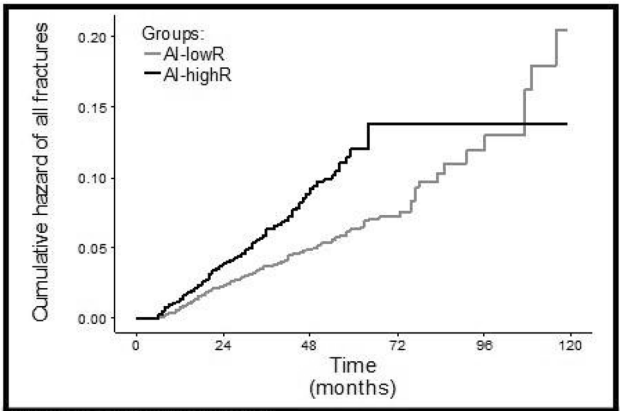
Adjuvant treatment for estrogen-receptor-positive breast cancer are aromatase inhibitors (AI) which have been associated with increased bone loss and excess fracture risk. Bisphosphonates (BP) are recommended to minimize bone loss. We conducted an observational cohort study to evaluate fracture risk in AI-treated patients.

Data on 15,052 AI-treated women were obtained from primary care records in Catalonia between 2006 and 2015. Participants were separated according to their fracture risk: patients without osteoporosis diagnosis and without BPs (low-risk patients: AI-lowR), and patients with osteoporosis diagnosis and/or BP candidates (high-risk participants: AI-highR). Patients were included from the first day of AI-treatment until the earliest of: AI or BP cessation, death or migration, end of data availability, or fracture event. Survival analysis were performed, including Kaplan–Meier to estimate cumulative probability plots; and Cox accounting for competing risk of death, adjusted for propensity score, to estimate subdistribution hazard ratio/s (SHR [95%CI]).

A total of 10,899 AI-lowR and 4153 AI-highR records were included, with fracture incidence rates [95%CI] = 12.32/1000 person-years [11.15 to 13.57] and 20.06 [17.18 to 23.30], respectively (Fig 1). AI-highR patients had 49% of increased risk (SHR: 1.49 [1.21 to 1.84]) compared to AI-lowR. Within AI-highR, the incidence rate was lower in BP-treated patients than non-BP-users: 18.69 [15.18 to 22.66] vs 26.12 [19.54 to 34.07], respectively. Cox analysis shows a fracture reduction in BP-users compared to non-users (SHR: 0.69 [0.49 to 0.99]).

In summary, BP-users during AI-therapy undergo lower fracture incidence than non-users. Monitoring fracture risk is advisable for improving the life quality of AI-patients.



Number of patients at risk (n):							
Groups	Al-lowR	10,899	7,099	3,580	328	91	17
	Al-highR	4153	1794	611	25	7	1
	Time (months)	0	24	48	72	96	119
Cumulative number of events (n):							
Groups	Al-lowR	0	209	343	387	397	401
	Al-highR	0	100	155	166	166	166
	Time (months)	0	24	48	72	96	119

[**Fig. 1** Cumulative hazard plot of fracture incidence. Abbreviations: highR, high-risk patients; lowR, low-risk patients.]

COP18

Genome-wide association meta-analysis identifies four loci for osteocalcin levels

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Osteocalcin is an important bone protein involved in bone mineralization and bone turnover. It's encoded by the BGLAP gene, however, there are no data on the influence of common genetic variation on osteocalcin levels. Here, we performed a genome-wide association study for osteocalcin levels including 7448 European individuals above the age of 45. In total, 7,856,905 (HRC1.1 reference panel; minor allele frequency $> 1\%$) SNPs were tested in association with the natural log transformed osteocalcin levels adjusting for age, sex and four genomic principal components. Next, we carry-out inverse-variance weighted meta-analysis and used LD-score regression to estimated heritability and genetic correlation (shared heritability) with other clinically related traits. SNP-based heritability was estimated as 0.34 (se:0.06). Variants at four loci reached genome-wide significance (GWS): 1q22 (SMG5, $P = 4.5 \times 10^{-9}$), 5q31.3 (HMHB1, $P = 1.7 \times 10^{-8}$), 8q24.12 (COLEC10, $P = 1.7 \times 10^{-8}$) and 13q14.11 (TNFSF11, $P = 2.3 \times 10^{-14}$). The latter two loci are known bone mineral density [BMD] loci which harbour genes relevant for the RANKL/RANK/OPG signalling pathway. The GWS SNP (rs2246476) mapping to 1q22 was significantly associated with SMG5 expression in human bone tissues rich in ostecyte content ($P = 4.2 \times 10^{-7}$). Osteocalcin had modest negative genetic correlations with BMD (femoral neck BMD rg: -0.22, se:0.08; lumbar spine BMD rg: -0.30, se:0.09), type 2 diabetes (rg: -0.37, se:0.09) and several metabolic traits such as waist-to-hip ratio (rg: -0.30, se:0.07), obesity (rg: -0.22, se:0.06), and triglycerides (rg: -0.18, se:0.06). Next, using a Mendelian randomization approach we found no robust evidence (low power) for causal association between genetically determined osteocalcin levels and femoral neck or lumbar spine BMD. Overall, genetic factors substantially explain the phenotypic variance of serum osteocalcin levels with modest proportion of the genetic factors shared with different metabolic and bone traits (i.e. pleiotropy). Notably, our findings provide new insights into the genetic variation of osteocalcin levels with genetic variants mapping to important bone pathways. A large meta-analysis is underway.

Concurrent Oral Presentations 3—Basic/Translational: Molecular basis of bone pathology

COP31

TGFβ inhibition with chemotherapy heals murine myeloma bone disease and improves fracture resistance

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Multiple myeloma causes a destructive bone disease in $\sim$ 90% of patients and current therapies do little to repair existing bone damage. In patients, bone repair agents will be administered with chemotherapy. This study aimed to determine if bone recovers after chemotherapy and if this is enhanced by bone anabolic therapy.

Human U266-GFP-luc myeloma cells were i.v. injected into NSG mice (n = 7/group). After tumour and osteolytic lesion development, mice were administered first-line chemotherapeutics (bortezomib + lenalidomide) ± a bone anabolic (SD208; transforming growth factor (TGF)β receptor I kinase inhibitor) or vehicles for 2 weeks. Tumour and bone lesions were monitored in vivo by bioluminescent imaging, serum paraprotein and μCT. Flow cytometry, histomorphometry, μCT, TRAP and P1NP ELISAs, QPCR, Raman spectroscopy and 3-point bending were performed at endpoint. Myeloma and healthy patient bone marrow stromal cells (BMSCs) were treated with TGFβ ± SD-208, osteogenic differentiation was assessed by alkaline phosphatase staining and QPCR.

Osteolytic lesions developed 8 weeks after tumour inoculation. Vehicle-treated mice exhibited progressive lesion development and

virtually no trabecular bone at endpoint. Total lesion area was unchanged after 1 week of chemotherapy, but after 2 weeks lesions began to repair, with reduced TRAP + osteoclasts and increased osteoblasts ($p < 0.05$). Mice treated with chemotherapy + SD-208 exhibited enhanced repair of bone lesions, with partial repair of perforating lesions within 1 week and complete repair within 2 weeks, with improvement over chemotherapy alone ($p < 0.05$). SD-208 significantly increased trabecular bone volume after 2 weeks ($p < 0.05$). Analysis of bone material and mechanical properties found enhanced matrix maturation and fracture resistance with SD-208 ($p < 0.05$). SD-208 promoted osteoblastic differentiation of patient BMSCs.

In conclusion, SD-208 enhanced healing of myeloma bone disease and fracture resistance when administered with first-line chemotherapeutics and promoted osteoblastic differentiation of patient BMSCs, providing incentive for clinical translation to improve patient bone outcomes.

COP32

Absence of Dipeptidyl Peptidase 3 leads to increased oxidative stress and bone pathology

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Oxidative stress and antioxidant pathways are crucial in bone homeostasis; impairments of these mechanisms contribute to the pathogenesis of skeletal diseases. We focused on the dipeptidyl peptidase 3 (DPP3), an exopeptidase activating the Keap1-Nrf2 antioxidant pathway. This latter is widely investigated in bone, but no data were reported regarding DPP3. We demonstrated DPP3 expression by bone cells, and generated a DPP3 KO mouse model. DPP3 KO mice showed a mild growth defect, an increased BM cellularity (WT $5.70 \pm 0.43 \times 10^7$, KO $7.46 \pm 0.3 \times 10^7$ cells; $p < 0.01$), decreased BV/TV (WT 17.26 ± 1.19, KO $10.99 \pm 1.27\%$; $p < 0.01$) and Tb·Th (WT 0.0523 ± 0.0012, KO 0.0430 ± 0.0014 mm; $p < 0.01$), increased BS/BV (WT 66.72 ± 1.26, KO 82.62 ± 2.67 1/mm; $p < 0.01$) and Tb.Sp (WT 0.0226 ± 0.009, KO 0.0283 ± 0.017 mm; $p < 0.05$). Oc.S/BS was increased (WT 6.6 ± 0.7, KO $9.9 \pm 0.9\%$; $p < 0.05$), as well as serum CTx (WT 0.249 ± 0.072, KO 0.704 ± 0.152 ng/ml; $p < 0.05$) and TRAP activity (WT 4.35 ± 0.21, KO 5.32 ± 0.39 U/L; $p < 0.05$). OCs function was sustained by an inflammatory milieu: the expression of inflammatory proosteoclastogenic cytokines was higher in the DPP3 KO bone tissue as compared to WT (IL6, twofold, $p < 0.05$; IL1b, twofold, $p < 0.05$; TNFa, 3.5-fold, $p < 0.01$). Moreover, oxidative stress was enhanced in DPP3 KO *versus* WT mice (4-HNE^+ vertebral area: WT 3.57 ± 0.38, KO $6.50 \pm 0.43\%$; $p < 0.001$). In vitro studies confirmed higher OCs activity and ROS production in the DPP3 KO (OCs n: WT 210 ± 30, KO 132 ± 18; $p < 0.05$; resorbed dentin area %: WT 47.59 ± 4.90, KO 48.70 ± 5.55; resorbed dentin volume %: WT 27.19 ± 2, KO 35.07 ± 1.62; $p < 0.05$; MFI ROS: WT 4394 ± 258, KO 6233 ± 953; $p < 0.05$); of note, neutrophils and macrophages had a higher ROS production, too. Moreover, absence of DPP3 exacerbated bone loss in an OVX model. These data suggest DPP3 might be a new osteoimmunological player involved in human diseases with increased bone resorption.

Keywords: Oxidative stress, Osteoclasts, Osteoporosis

COP33

Correlation between bone marrow adiposity and bone resorption in modeled osteoporosis

Vagelis Rinotas[1], Efthymios Tzortzis[2], Panagiota Siatra[2], Kostas Kritikos[1,2], Panagiotis Nikolaou[1,2], Maria Papadaki[1,2], Eleni Douni[1,2]

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Bone Marrow Adiposity (BMA) increases during aging and is further enhanced in osteoporosis, whereas its pathophysiological significance remains insufficiently understood. We have recently established two novel genetic osteoporosis models, Tg5516 and Tg5519, by expression of human RANKL (Receptor activator of nuclear factor-κB ligand), the main inducer of osteoclastogenesis, in transgenic mice (TgRANKL). A common characteristic in both transgenic lines is the progressive development of BMA.

To understand the mechanisms that promote BMA expansion in osteoporosis, we analyzed its progression in both TgRANKL osteoporosis models and explored the adipogenic potential of bone marrow mesenchymal stromal cells (BMSCs). Histological analysis and micro-computed tomography demonstrated that BMA development in the Tg5516 line was restricted at the distal femural metaphysis, while in the Tg5519 model, BMA expanded progressively throughout the marrow cavity and correlated with increased bone resorption, indicating a close interaction between BMA and osteoclastogenesis. *Ex vivo* analysis of BMSCs from TgRANKL mice showed a dramatically altered adipogenic differentiation profile as shown by oil-red staining (WT: 0.12 ± 0.005 vs Tg5519: 0.72 ± 0.20, $p < 0.05$) that strongly correlated with increased expression of adipogenic markers, including PPARγ (WT: 1.29 ± 0.26 vs Tg5519: 7.70 ± 0.70, $p < 0.01$) and C/EBPα (WT: 0.78 ± 0.19 vs Tg5519: 9.71 ± 1.23, $p < 0.01$) as well as RANKL (WT: 0.91 ± 0.10 vs Tg5519: 46.56 ± 8.44, $p < 0.01$) as shown by qPCR. Furthermore, the effectiveness of an anti-osteoporosis treatment in BMA development was investigated upon treatment of TgRANKL models with the bisphosphonate alendronate. Notably, alendronate effectively improved bone mass and attenuated BMA expansion at metaphysis (WT: 0.31 ± 0.22 vs Tg5519: 44.41 ± 2.52 vs Tg5519 + ALN: 8.27 ± 1.75, $p < 0.001$), indicating a possible involvement of osteoclasts and bone resorption in BMA development.

Our results demonstrate that TgRANKL mice constitute unique genetic models for investigating the pathogenic mechanisms that regulate development and expansion of BMA in osteoporosis.

Keywords: RANKL, Osteoporosis, Bone marrow adiposity, Transgenic mice, Alendronate

COP34

Acvr2b ligand trap (luspatercept) improves bone mass in myelodysplastic mice and mice with ovariectomy-induced bone loss

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Luspatercept is a novel erythroid-stimulating agent and acts by trapping activin receptor type IIb ligands. It has been shown to improve erythropoiesis in humans and mice with myelodysplastic syndromes (MDS), a hematological disorder characterized by ineffective hematopoiesis. Recently, we have found that these patients also have an increased risk for osteoporosis. In this study, we tested whether the mouse analog of luspatercept, RAP-536 (Acceleron Pharma, Cambridge MA), can also improve the bone phenotype of myelodysplastic mice (NUP98-HOXD13 transgenic mice, NHD13) and protect from bone loss induced by estrogen withdrawal.

Ten-week-old wild-type (WT) mice were ovariectomized (OVX) and treated with 10 mg/kg RAP-536 2 ×/week for 4 weeks. Twenty-week-old NHD13 mice and WT littermate controls received a dose of 15 mg/kg RAP-536 for 3 weeks.

RAP-536 treatment improved erythropoiesis in WT and NHD13 mice by increasing the red blood cell number [+ 20%; $P < 0.05$ and +13%; $P < 0.05$, respectively]. In addition, the reduced bone volume fraction in OVX mice [− 31%; $P < 0.05$] and the lower trabecular number in NHD13 mice [− 16%; $P < 0.05$] were normalized back to WT levels. While RAP-536 had no effect on histological osteoclast parameters in NHD13 mice, it decreased the number of osteoclasts in OVX mice [− 46%; $P < 0.05$]. In OVX mice, RAP-536 increased the bone formation rate as determined with dual calcein labeling and P1NP serum levels [+ 95% and +37%; $P < 0.05$]. Interestingly, RAP-536 did not further increase the already increased bone formation rate and P1NP serum levels in NHD13 mice [2.8-fold; $P < 0.001$].

Taken together, luspatercept not only improves erythropoiesis in MDS mice, but also their bone phenotype. In addition, our study suggests that RAP-536 may also be useful to treat postmenopausal osteoporosis, the most frequent form of primary osteoporosis.

COP35

Transcriptomic approach to gain insights on multiorgan alterations affecting a mouse model of autosomal dominant osteopetrosis type2 (ADO2)

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ADO2 is a rare genetic disorder characterised by dense yet fragile bones. The disease is induced by dominant negative mutations of the *Clcn7* gene, encoding the chloride/proton antiporter type 7, which is expressed in several organs in addition to bone. We observed extra-skeletal alterations in a small cohort (n = 8) of *Clcn7*-ADO2 patients. Therefore, to better dissect the pathogenesis of extra-skeletal alterations, we performed an un-biased RNA deep sequencing (RNA-dSeq) analysis in lungs, kidneys and muscles of an ADO2 mouse model carrying the $Clcn7^{G213R}$ heterozygous mutation. Bioinformatic analyses revealed enrichment in genes involved in the onset of fibrosis in ADO2 lungs, kidneys and muscles (NES = 1.27; q = 0.1; NES = 1; q = 0.4; NES = 1.38, q = 0.05; respectively). Further analyses demonstrated an enrichment in these organs of macrophage-specific genes (lungs NES = 1.08, q = 0.1; kidneys NES = 0.91, q = 0.8; muscles NES = 1.25, $q < 0.001$). Moreover, KEEG pathway analysis revealed the presence of 10 altered molecular signals shared by all organs analysed, which included the pro-fibrotic TGFβ pathway ($p < 0.05$). The results from the RNA-dSeq were confirmed by histopathology showing perivascular fibrosis (Masson's collagen staining: lung + 1.5; kidney +1.7; muscle +3fold $p < 0.04$) and increase of F4/80-positive macrophage number (lung + 2; kidney + 1.6; muscle + 12 fold; $p < 0.05$). Finally, real-time RT-PCR and immunofluorescence confirmed the upregulation in ADO2 organs of Tgfβ1/Tgfβ2, and the phosphorylation/nuclear translocation of the downstream transcription factor Smad2/3 ($p < 0.05$). Along with the alteration in visceral organs, ADO2 mice showed an increased anxiety and depression compared to the WT ($p < 0.05$). In line with this, RNA-dSeq of ADO2 brains revealed enrichment in genes involved in long-term depression, long-term potentiation and neuroactive ligand-receptor interaction ($p < 0.05$). Finally, in vitro ADO2 osteoclasts subjected to RNA-dSeq exhibited 387 over- and 63 under-expressed transcripts vs WT ($p < 0.05$) involved in hyper-activation of cytokine–cytokine receptor ($p < 0.001$), Jak/Stat signal ($p = 0.001$) and ECM receptor ($p < 0.001$) pathways, further confirming the complexity and the multifaceted features of the disease.

COP36

Paradoxical effects of JZL184, an inhibitor of monoacylglycerol lipase, on bone remodelling in healthy and cancer-bearing mice

Silvia Marino[1,2], Daniëlle de Ridder[1], Ryan T. Bishop[1], Nathalie Renema[3], Marco Ponzetti[4], Antonia Sophocleous[5], Mattia Capulli[4], Abdullah Aljeffery[1], Giovana Carrasco[1], Marianela Dalghi Gens[6], Asim Khogeer[1,7], Stuart H. Ralston[5], Jürg Gertsch[6], Francois Lamoureux[3], Dominique Heymann[1,8], Nadia Rucci[4], Aymen I. Idris[1,7]

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Cancer-induced bone disease (CIBD) is a serious complication in bone sarcomas and metastatic carcinomas of breast and prostate origin. Monoacylglycerol lipase (MAGL) is an enzyme of the endocannabinoid system, responsible for the degradation of the most abundant endocannabinoid in bone 2-arachidonoyl glycerol. Cannabinoids and their receptors (Cnr) are implicated in cancer and bone remodelling, but the role of MAGL in CIBD remains unknown. Here, we used in vitro, ex vivo and animal models of primary sarcoma and secondary prostate and breast cancer in bone to test the effects of pharmacological inhibition and knockdown of MAGL on the development of CIBD.Knockdown and treatment with the verified MAGL inhibitor JZL184 (10uM) inhibited the in vitro migration and invasion of a panel of human osteosarcoma, and osteotropic prostate and breast cancer cell-lines (40–50% reduction), and suppressed the ability of these cells to stimulate osteoclast formation (50% reduction ($p < 0.01$). In vivo, administration of JZL184 (16 mg/kg/thrice/week) reduced osteolytic bone metastasis (50% reduction, $p < 0.05$) and skeletal tumour growth (30% reduction, $p < 0.05$) in human breast MDA-MB-231 and prostate PC3 models, and reduced lung metastasis (80% reduction, $p < 0.05$) and ectopic bone formation (45% reduction, $p < 0.05$) in human KHOS osteosarcoma models. In human KHOS and PC3 models, JZL184 suppressed cachexia and prolonged survival ($p < 0.05$). Functional and histological analysis revealed that the osteoprotective action of JZL184 in cancer-bearing mice is predominately due to inhibition of tumour growth and metastasis without changes in osteoblast and osteoclast numbers ($p > 0.05$). Paradoxically, JZL184 reduced bone volume in non-cancer bearing wild-type mice (30% reduction, $p < 0.05$) and this effect was abolished in mice deficient in Cnr1 and Cnr2 receptors.Thus, targeting MAGL is of potential therapeutic efficacy in primary bone

cancer and bone metastasis, however, activation of the skeletal endocannabinoid system may limit the usefulness of MAGL inhibitors as osteoprotective agents.

Concurrent Oral Presentations 3—Clinical/Public Health: Metabolism, diabetes and bone quality

COP25

Bone quality analyses in cases with type 2 diabetes mellitus reflect patterns of femoral cortical bone reorganization along with high porosity

Eva Maria Wölfel[1], Katharina Jähn[1], Felix N. Schmidt[1], Birgit Wulff[2], Herbert Mushumba[2], Klaus Püschel[2], Michael Amling[1], Petar Milovanovic[1,3], Graeme M. Campbell[1], Björn Busse[1]

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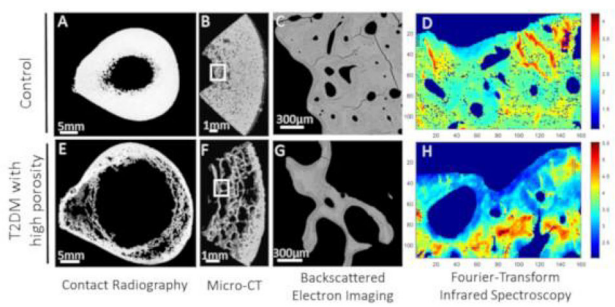
Diabetes mellitus is associated with an increased fracture risk, yet the underlying mechanisms are not identified. Patients with Type 2 Diabetes Mellitus (T2DM) present a normal to high bone mineral density making it particularly challenging to identify patients at risk. Here we study femoral cortical bone to characterize the microstructure of diabetic bone. During autopsy we collected 12 mid-diaphyseal femoral cortices of T2DM-diagnosed cases and 11 age-matched controls following IRB approval. We performed micro-CT on the anterior quadrant of the cross-section. Next, we applied quantitative backscattered electron imaging and nanoindentation. Complementary to that, we performed Fourier-transform infrared spectroscopy for compositional analysis. With 3D micro-CT we identified a subgroup within the T2DM group with exceptionally high cortical porosity compared to control and T2DM cases (44.36 ± 16.2 vs. 9.96 ± 5.61 vs. 10.93%, $p < 0.05$). In the endocortical region of the T2DM group with high porosity we found a higher amount of areas with low calcium content (Calcium low: 9.82 ± 1.73 wt % and 4.99 ± 0.87 wt %, $p = 0.007$) and a higher heterogeneity (Calcium width: 2.65 ± 0.19 wt % vs. 2.43 ± 0.1 wt %, $p = 0.011$) compared to controls. Additionally, the Young's modulus was lowest in the endocortical region of the T2DM group with high porosity compared to control (18.11 ± 1.99 GPa and 21.3 ± 2.98 GPa, $p = 0.038$). Taken together, our results do not only suggest the presence of two morphological patterns of bone reorganization in T2DM cases, they also provide new insights into cortical bone microstructure of individuals with T2DM to identify the ongoing changes in the bone matrix leading to an increased fracture risk.



[Multi length scale analysis of cadaveric human femoral cortical bone of control and T2DM cases]

COP26

Changes in bone quality after Roux-en-Y gastric bypass: a prospective cohort study in subjects with and without type 2 diabetes

Ingvild Krsitine Blom-Hogestol[1], Tom Mala[1], Jon A Kristinsson[1], Cathrine Brunborg[2], Hanne L Gulseth[1], Erik Fink Eriksen[1]

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Obesity and type 2 diabetes (T2D) are associated with an increased risk of skeletal fractures despite a normal areal bone mass density (aBMD), possibly due to reduced bone material strength. Roux-en-Y gastric bypass (RYGB) enables a substantial and persistent weight loss, and resolution of obesity related comorbidities such as T2D. However, the procedure induces a decrease in aBMD and increased bone turnover and fracture rate. To our knowledge, changes bone material strength after RYGB have not been explored. We examined 34 participants before and one year after RYGB, of whom 13 had T2D. Bone material strength index (BMSi) was evaluated by impact microindentation. Except for glycosylated hemoglobin (HbA_{1c}), participants with and without T2D were comparable before surgery. After RYGB the participants had lost a mean ± SD of 33.9 kg ± 10.9 kg, had increased physical activity, had unchanged vitamin D levels, and all but one of the 13 participants with T2D were in diabetes remission. BMSi increased from 78.1 ± 8.5 preoperatively to 82.0 ± 6.4 after RYGB, corresponding to an increase of 4.0 ± 9.8 in absolute units or 6.3% ± 14.0, $p = 0.037$. The increase was comparable in participants with and without T2D. In subjects with T2D a larger decrease in HbA_{1c} was, associated with a larger increase in BMSi, β − 9.2 (− 16.5 to − 1.9), $p = 0.019$. Bone turnover markers (CTX-1 and PINP) increased by 195.1% ± 133.5 and 109.5% ± 70.6, respectively. aBMD decreased by 4.3% ± 5.6 in the lumbar spine, 8.2% ± 4.6 in the femoral neck, 11.6% ± 4.9 in total hip and 9.4% ± 3.8 in total body. Our findings indicate that bone material strength improves despite an increase in bone turnover and a decrease in aBMD one year after RYGB. Trends were statistically comparable in participants with and without T2D. Improved glucose control was associated with improved bone material strength in participants with T2D.

COP27

The effect of the glucagon-like peptide-1 receptor agonist Liraglutide on bone turnover and bone mass in type 2 diabetes patients, a randomized controlled trial

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Liraglutide is a GLP-1 receptor (GLP-1) agonist used in the treatment of type 2 diabetes (T2D). Animal studies indicate that Liraglutide may increase bone mineral density (BMD) by suppressing bone resorption. The aims of the study were to investigate the effect of Liraglutide in patients with T2D on bone turnover and bone mass.

We conducted a randomized, double-blinded, clinical trial with Liraglutide/placebo for 26 weeks. The relevant ethics committee approved the study. Inclusion criteria: T2D and age > 30 years.

Exclusion criteria: HbA1c > 75 mmol/mol, treatment with insulin, GLP-1 analogues, DPP-4 inhibitors or glitazones, body weight > 140 kg, or conditions affecting bone metabolism or incompatible with Liraglutide treatment. The primary endpoint was change in collagen I cross-linked C-terminal telopeptide (CTX). We used the linear mixed model in statistical analyses.

A total of 56 participants completed all study visits. Plasma (P) procollagen type 1 N-terminal propeptide (P1NP) decreased significantly in the Liraglutide group from baseline to week four ($p < 0.01$), subsequently increased to week 13 ($p = 0.03$), and was persistently elevated until end of study. P-P1NP in the placebo group did not change significantly throughout the study. For P-CTX, there were no significant changes in either group nor differences between groups. Total hip BMD decreased in the placebo group throughout the study compared with no change in the Liraglutide group ($p = 0.02$ for difference between groups). The body weight decreased in the Liraglutide group from baseline to week 4 ($p < 0.01$) and remained significantly lower throughout the study compared with baseline. The weight did not change significantly in the placebo group.

Liraglutide affects bone formation in patients with T2D, possibly partly mediated by changes in body weight. Liraglutide treatment showed no effect on bone resorption, but may preserve hip BMD. Liraglutide treatment is safe regarding bone health.

Keywords: Diabetes, Bone turnover markers

COP28

Genetic evidence for a causal role of serum phosphate in coronary artery calcification: the Rotterdam study

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Background: Coronary artery calcification (CAC) is a pathologic process specific of atherosclerosis and able to predict cardiovascular events. Hyperphosphatemia (hyperP) has been associated with CAC in kidney disease, but the relation in normal phosphate (P) setting is unclear.

Objectives: To evaluate the association between P and CAC in a population-based setting, assessing evidence of causality through Mendelian Randomization (MR).

Methods: Serum P and CT- CAC were assessed in Rotterdam Study I. Phenotypic associations through generalized models were adjusted for age, BMI, smoking, 25OHD, CRP, glucose and cholesterol:HDL ratio. MR was implemented through an allele score including eight P single nucleotide polymorphisms (SNPs) reported by the GWAS catalogue. MR assumptions were assessed through SNP strength; regression with potential confounders and testing or allowance of pleiotropic effects. Methods included two-stage least square, adaptive lasso and variational Bayes.

Results: In phenotypic analyses, serum P was related to CAC with sex interaction ($p_{interaction} = 0.005$) [men β: 0.93 (0.62–1.23), $p = 6x10^{-9}$, n = 816; women β: 0.41(0.09–0.74), p = 0.013, n = 940]; exclusion of hyperP and chronic kidney disease (CKD: eGFR < 60 mL/min) yielded [men β: 0.90(0.54–1.26), $p = 2x10^{-6}$, n = 711; women β: 0.43 (0.04–0.82), p = 0.032, n = 790]. In MR analyses, instrumented P through the unweighted allele score was related to CAC (sex-combined β: 1.83(0.06–3.60), p = 0.043, n = 1693)—also after exclusion of hyperP and CKD (sex-combined β: 2.79(0.69–4.89), p = 0.009, n = 1446). No evidence of invalid instruments was found. Allowance for pleiotropy yielded [sex-combined β: 1.91 (Cr I 0.47–3.80)].

Conclusions: P is related to CAC in the general population with a stronger effect in men. MR findings support causality; also for P and CAC in subjects without hyperP and CKD. Further research into mechanisms and sex differences is needed. Our findings might have an impact in public health.

Keywords: Phosphate, Coronary calcification

	n	B (95% CI)	p	n	B (95% CI)	p
Whole cohort	1693	1.83(0.06–3.60) unweighted	0.043	1693	1.81(0.08–3.55) weighted	0.041

[Mendelian Randomization results for serum P and CAC in RS-I: allelic score method]

COP29

Periosteal expansion in response to endosteal resorption through periostin and RANKL interaction is conserved in humans

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We previously reported that endosteal bone loss induced by high levels of RANKL is accompanied by an increased periosteal bone formation and diameter that is mechanically driven, but limited by cathepsin K-dependent periostin degradation in mice.

Here we evaluate whether these mechanisms are playing a role in humans. Trabecular (Tb) and cortical (Ct) parameters were measured at the distal radius and tibia by HRpQCT, in 695 post-menopausal women from the Geneva Retirees Cohort (GERICO) (mean age 65.0 ± 1.4 years). Circulating levels of cathepsin K-digested periostin fragments (K-Postn) were measured by a specific ELISA. We investigated the relationships between the percentage changes of medulary area (Me.Ar), cross section area (Tt.Ar), cortical area (Ct.Ar) and K-Postn over a follow-up of 5.7 ± 1.9 years in the whole cohort, and in a subgroup with denosumab (Dmab, n = 19), bisphosphonates (BPs, n = 20), and 49 controls matched for age, BMI, BMD and fracture.

Changes in Ct.Ar was negatively, and those of Tt.Ar positively associated with changes of Me.Ar ($r = -0.60$ and 0.83, both $p < 0.0001$). K-Postn was negatively associated with changes of Tt.Ar ($r = -0.08$, $P < 0.05$), without significant association with Ct.Ar.

Dmab and BPs significantly reduced Ct.Ar decrease (respectively −5.4% and −3.6% vs −13.8% in Ctl, $p < 0.01$). In contrast, only Dmab limited the decrease of Tt.Ar (+0.1 vs −0.5% in BPs and −0.3% in Ctl, p = 0.09). In addition, K-Postn decreased in Dmab (−1.5%) vs BPs and Ctl (+5.9 and +5.2%). Moreover, Dmab cancelled the correlation between K-Postn and Tt.Ar.

In conclusions, the cross section area increases with the medullary area is also true in aging women. This increased periosteal diameter is inversely related to the level of periostin degradation. In turn RANKL inhibition by DMab improves outer diameters by reducing periostin degration. Hence the molecular mechanisms that couple bone remodeling/modeling in mice is also true in humans.

COP30

No progression in the bone phenotype with age in patients with the LRP5-High bone mass phenotype

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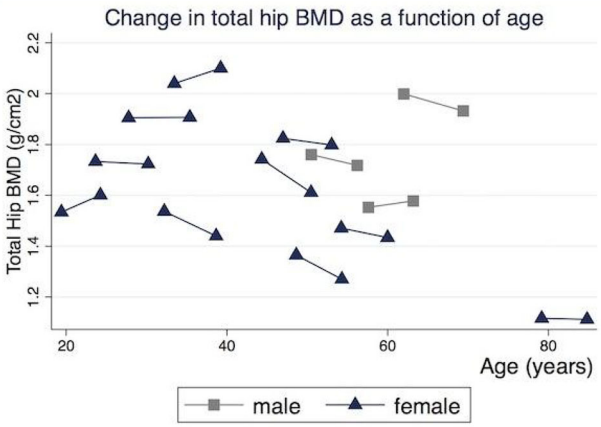
Objectives: Wnt signalling is involved in bone formation. Gain-of-function mutations in the Wnt co-receptor LRP5 cause the high bone mass phenotype (LRP5-HBM). It is unknown if the bone phenotype progresses with age. We aimed to determine if the LRP5-HBM phenotype progresses with age.

Methods: Whole body, total hip and lumbar spine BMD and volumetric BMD, bone geometry and structure were measured by DXA and HR-pQCT at two time points in LRP5-HBM (T253I) patients. Changes (second-first scan) in bone-related outcomes were assessed in participants aged > 25 years using Wilcoxon signed-rank and regression models (adj. for sex). The ethics committee approved the investigation.

Results: 15 cases (11 women) aged (median (quartiles)) 44.3 (23.6–54.2) years were recruited, and scans were repeated after 5.8 (5.7–6.5) years. A plot of changes in $BMD_{total\ hip}$ is presented



[Total Hip BMD measured at two different times (median 5.8 years). Change not significant (p=0.10)]

No overall difference in BMD_{lumbar}, $BMD_{total\ hip}$, or BMD_{WB} or any HR-pQCT derived parameters were observed at the different time points except for increased trabecular vBMD and BV/VT (p = 0.02). Changes in tibial but not radial cortical vBMD correlated negatively with age (p = 0.02), and radial but not tibial trabecular vBMD correlated with age (p = 0.01). BV/VT correlated with age in radius only (p < 0.01). There was no association between age and changes in radial and tibial bone perimeters or cortical thickness.

Conclusions: Successive expansion of bone was not observed. In adulthood, the phenotype seems to stabilize with deceleration of bone mass accrual at certain bone sites.

Concurrent Oral Poster Presentations 1—Basic/Translational

P030

Extracellular vesicle protein and miRNA cargo reflect functional heterogeneity of mesenchymal stromal cells

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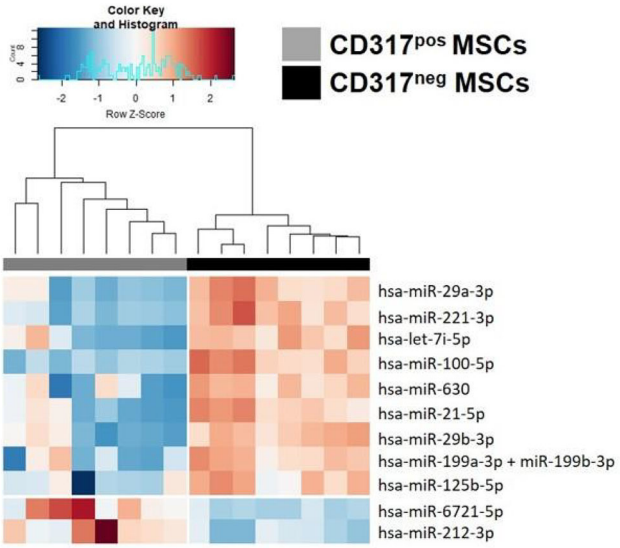
Identifying regenerative interventions using mesenchymal stromal cells (MSCs) is a priority in musculoskeletal research. MSCs are a heterogeneous population with paracrine signalling functions conveyed through cargoes of extracellular vesicles (EVs). Therefore, EVs may offer an acellular alternative to cell-based therapies. We have identified a biomarker (CD317) that discriminates human MSC subpopulations with regenerative properties ($CD317^{neg}$) from those with enhanced pro-inflammatory profiles ($CD317^{pos}$). We investigated the protein and microRNA composition of EVs isolated from $CD317^{neg}$ and $CD317^{pos}$ MSC lines to determine the impact of MSC heterogeneity on EV-mediated cell–cell signalling and help define appropriate EV-based therapeutic routes.

EVs were isolated by ultracentrifugation and characterised by Transmission Electron Microscopy and Nanoparticle Tracking Analysis. Using LC–MS/MS and bioinformatics we identified a significantly enhanced EVome in $CD317^{neg}$ compared to $CD317^{pos}$ MSCs (68 versus 2 upregulated proteins, p < 0.01) that was functionally linked to cell migration and matrix interactions. NanoString technology was used to quantify the expression of ~ 800 EV miRNAs and highlighted differential expression of 11 miRNAs between $CD317^{neg}$ and $CD317^{pos}$ MSCs (fold-change range − 2.52 to 66.25, q < 0.001–0.018) (Fig. 1).



[Fig.1 miRNAs significantly differentially expressed between CD317+ and CD317- MSC line EVs]

Of these, 9 were upregulated in $CD317^{neg}$ MSCs including miRNAs previously implicated in the positive regulation of osteogenesis (miR-125b-5p, let-7i-5p, miR-29a-3p, miR-29b-3p and combined mir-199a-3p/miR-199b-3p) and negative regulation of inflammation (miR-125b-5p, miR-100-5p, miR-221-3p and miR-21-5p). Therefore, EV cargoes may identify biomarker signatures for MSC subsets that determine MSC function. These findings also highlight the potential to develop novel acellular approaches for tissue regeneration by exploiting the secreted outputs of defined MSC subsets.

Keywords: EVs, MSC, miRNA

P039

Differential response of trabecular and cortical bone following recovery from microgravity-induced bone loss

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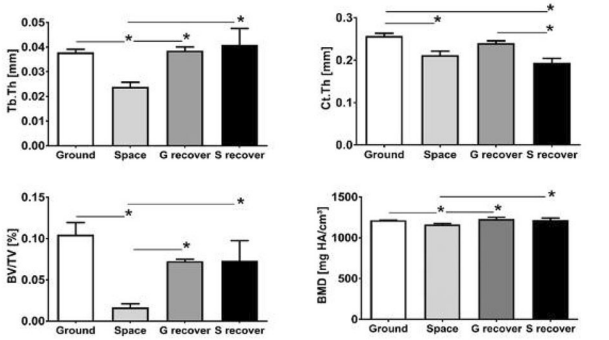
Space missions are important to understand how skeletal health is influenced by atmospheric parameters. Several pathophysiological effects occurring in space hinder long-term missions. Bone loss is one of the major microgravity-induced effects that impairs the astronaut's health, which is however partially reversible. Vigorous exercise regimes are thus a standard program to date but further pharmacological treatments are sought.

Wild type C57BL/6 mice were sent to space on the BION-M biosatellite mission for 4 weeks (n = 5) and compared to ground mice (n = 6). A third group of mice were left to recover on the ground for 7 days post space mission (n = 5) and similarly compared to a ground control group (n = 6). The tibiae from all mice were extracted and imaged with micro-computed tomography (μ-CT40, Scanco).

Space mice displayed a significant reduction of both bone compartments, with a lower trabecular thickness (Tb·Th, 37.9 ± 3.1 mm vs. 23.9 ± 4.2 mm, p = 0.0027), as well as a cortical thickness (Ct.Th, 257.1 ± 15.4 mm vs. 211.6 ± 21.8 mm, p = 0.0033). While the trabecular phenotype recovered compared to ground level (40.9 ± 11.5 mm), the Ct.Th did not recover after 7 days at ground and stayed constant on space level (194.1 ± 23.0 mm). Moreover, the trabecular bone volume fraction recovered (BV/TV, 16.6 ± 10.0% vs 73.2 ± 42.0%), as did the Tb.Th. Interestingly, the cortical bone mineral density recovered (BMD, 1163.7 ± 29.7 mg HA/cm^2 vs. 1218.0 ± 24.0 mg HA/cm^2), while the Ct.Th did not.

We determined a compartment-dependent recovery from microgravity-induced bone loss and a rapid regain in cortical BMD potentialy guided by osteocytes with a lack of osteoclast-osteoblast-induced cortical thickness regeneration.

Keywords: Microgravity, μCT, Bone structure, Recovery, Mice



[Results from the groups 'Ground', 'Space', 'Ground recovery' and 'Space recovery' (G, S recover)]

P058

Novel role of syndecan 3 in regulating osteoblastogenesis through the wnt signalling pathway

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Syndecan3 (Sdc3) is a transmembrane heparan sulphate proteoglycan co-receptor, expressed during skeletogenesis, however, its role in pre- and postnatal bone development and homeostasis is unclear. We showed that adult Sdc3-knockout (Sdc3KO) mice have low bone volume, increased bone fragility and a blunted anabolic response to mechanical loading, compared to wild-type (WT). Here we investigated the role of Sdc3 in prenatal bone accrual and studied the Wnt-signalling pathway, essential for normal osteoblastogenesis postnatally.

We measured bone volume of tibias of 2-day-old Sdc3KO (N = 15) and WT (N = 17) pups by μCT. The canonical Wnt-signalling pathway was analysed by qPCR and Western Blot (WB) in bone marrow stromal cells (BMSCs), under osteogenic- and non-osteogenic conditions, and in bone-chips-derived osteoblasts of Sdc3KO and WT young adult mice (N = 3–4).

Bone volume was increased by 40% ($p < 0.001$) in Sdc3KO vs WT pups, in contrast to the adult phenotype. However, qPCR analysis of BMSCs under osteogenic conditions revealed a 35% decrease in the Wnt receptor LRP5 gene expression in Sdc3KO BMSCs vs WT ($p < 0.05$), but no difference in LRP6. Expression of the Wnt regulated gene Axin2 was reduced in Sdc3KO by 45% ($p < 0.001$). Both, the Frizzled1 (co-receptor of LRP5/LRP6) and β-catenin (downstream transcription factor) levels were reduced by 65% on WB ($p < 0.001$). However, Frizzled1 or β-catenin levels in BMSC under non-osteogenic conditions were unaffected by Sdc3 deletion.

In Sdc3KO bone chip-derived osteoblasts Frizzled1 and β-catenin protein levels were also reduced by 30% ($p < 0.05$ and $p < 0.01$ respectively) and the RNA expression of the Wnt-regulated transcription factor RUNX2 by 75% ($p < 0.01$) vs WT.

Our results indicate an important role for Sdc3 in osteogenesis, with differential effect on prenatal (attenuating) and postnatal (enhancing) bone accrual. Postnatally, our data suggest that Sdc3 enhances Wnt-signalling through the stabilisation of the Frizzled1 receptor in committed osteoblasts.

Keywords: Syndecan3, Osteogenesis, Osteoblasts, Wnt

P040

Cortical shell thickness in thoracic vertebrae is age- and region-dependent

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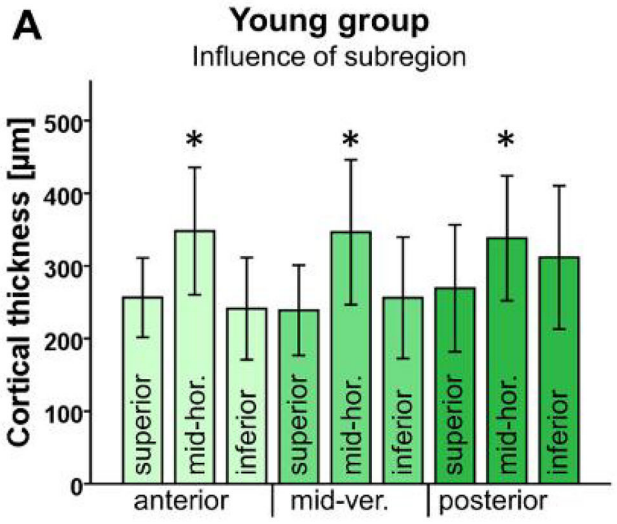
Fracture risk increasing bone loss in the spine is mostly described as loss of trabecular bone. However, the cortical shell contributes approximately 40% of the maximum vertebral load and becomes more relevant with aging. While trabecular bone quality is heterogeneous and age-dependent, these aspects are understudied for the cortical shell. Therefore, our aim was to assess cortical shell thickness in subregions and evaluate aging and osteophyte influences.

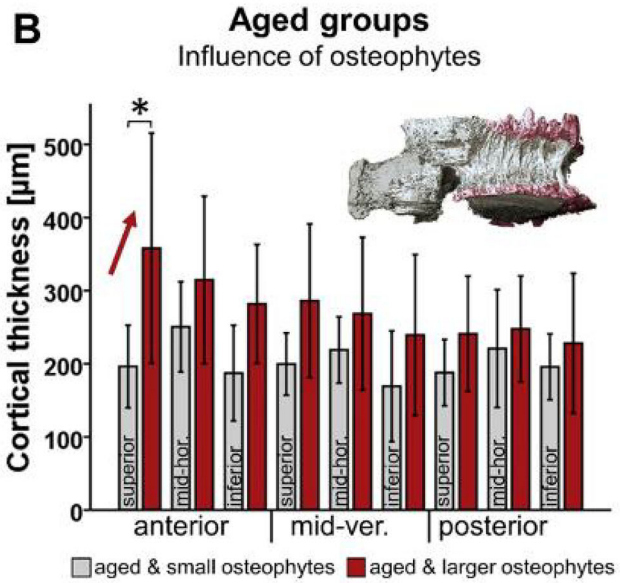
We obtained T12 vertebrae from 10 young (31 ± 6y) and 13 aged (71 ± 5y) women and performed HRpQCT scans (voxel 41 μm). After reconstruction, datasets were segmented using a fixed threshold. The cortical shell was identified and its thickness analyzed using custom-written algorithms for 9 subregions of left vertebral bodies (anterior/mid-vertical/posterior vs. superior/mid-horizontal/inferior). Osteophytes were characterized based on Zukowski et al.

The young group revealed higher values in mid-horizontal subregions compared to neighboring superior or inferior regions (Figure 1A, $p < 0.05$) but not the aged group. Aged individuals with small osteophytes showed reduced cortical thickness compared to young in five regions. Aged individuals with larger osteophytes presented higher thickness values than those with small, but only significant in anterior–superior (Figure 1B, p = 0.022).

In young women, a thicker cortex in subregions where trabecular volume is typically reduced indicates a compensatory function. With aging, cortical thickness is reduced, which might be partially reversed by osteophyte growth. This highlights the necessity to include cortical shells in whole vertebra finite element models and further investigate influences of cortical vertebral bone loss on fracture risk.

Keywords: Vertebrae, Cortical shell, Aging, Osteophytes





[A: Cortical thickness varies in young. B: Osteophyte occurrence increases thickness in aged.]

P056

Delayed bone healing in HFD-induced obesity model: evidence for impaired differentiation capacity of osteoblasts and osteoclasts progenitors

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The cellular mechanisms of obesity and type 2 diabetes (T2D)-associated impaired fracture healing are poorly studied.

Thus, we examined the cellular and molecular changes in bone progenitor cells and bone marrow micro-environment during bone fracture healing in 8-week old C57BL/6 obese male mice fed high fat diet (HFD) (60% fat) for 3 months and compared to control mice on normal diet (ND). The dynamics of fracture healing were studied using mCT-scanning and quantitative histology. MSC and hematopoietic cell (HC) populations were examined using cellular and molecular assays.

A delayed bone healing evaluated by µCT-scanning was observed in HFD compared to ND (BV/TV: − 31.6% at day (D)14 and − 38.7% at D21). Histomorphometric analysis revealed decreased callus newly formed bone (7.03% ± 1.6 vs 14.44% ± 2.8 HFD vs ND; $p < 0.05$ n = 6) and decreased bone resorptive surfaces (10.71% ± 1.37 vs 15.72% ± 0.8 HFD vs ND; $p < 0.01$ n = 5). The number of osteoblast SCA1 + , and osteoclast TRAP + progenitors within the fracture area were increased in HFD condition (94.76 ± 13.0 vs 54.16 ± 8.9 cells/mm^2 in ND; $p < 0.05$ n = 6 and 254.1 ± 39.9 vs 147.9 ± 21.6 cells/mm^2 in ND; $p < 0.05$ n = 6, respectively). In addition, we observed accumulation of adipocyte in the callus area and the formed bone was inversely correlated with callus adiposity ($R^2 = 0.49$; $p < 0.02$). Cultured MSC and HC (CD45 +/CD31 +/Ter119 +) from HFD mice exhibited impaired osteoblast but enhanced adipocyte differentiation and impaired osteoclastogenesis. Interestingly we also found impaired LPS responsiveness of the HC from HFD mice suggesting a protective mechanism against bone marrow inflammation in obesity.

Changes in MSC cellular composition and marrow environment in obesity leads to delayed fracture healing caused by differentiation defect of bone progenitor cells. These mechanisms are relevant for understanding the pathophysiology of impaired fracture healing in T2D.

P057

Glucocorticoid receptor dimerization is deleterious in trauma-induced compromised fracture healing

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After severe trauma, fracture healing is impaired due to overwhelming inflammation. Glucocorticoids (GCs), acting via the glucocorticoid receptor (GR), influence fracture healing by modulating the trauma-induced immune response. Our previous study revealed an important role of the GR in endochondral ossification by promoting cartilage-to-bone transition. GR dimerization dependent gene regulation is essential for the anti-inflammatory effects of GCs. Thus, we investigated in a murine model of combined femur fracture

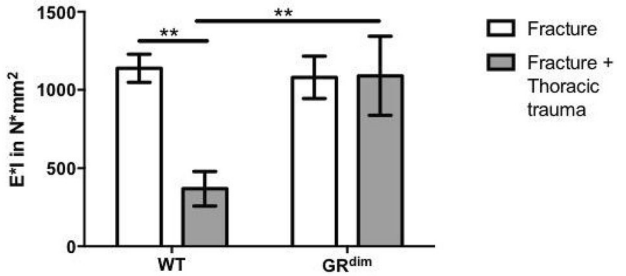
and thoracic trauma, if GR dimerization is involved in the pathomechanisms of trauma-induced compromised fracture healing.

We used male mice with a point mutation impairing GR dimerization (GR^{dim}) and wildtype littermate controls (WT). With ethical approval, femur osteotomy was performed and stabilized by an external fixator. The mice were also challenged with an additional thoracic trauma. Fracture healing was analysed by histomorphometry, μCT, and biomechanical-testing.

As expected, in WT mice the thoracic trauma compromised fracture healing. 23 days after fracture, bone formation in the fracture callus was strongly reduced in comparison to WT mice with isolated fracture (− 47%, p = 0.003), resulting in a reduced bending stiffness of the healed bone (− 68%, p = 0.001). In contrast, fracture healing was not affected in GR^{dim} mice by the additional thoracic trauma. Furthermore, residual cartilage was increased in calli of WT compared to GR^{dim} after thorax trauma (+167%, p = 0.0009) indicating delayed cartilage-to-bone transformation.

Collectively, our data show that intact GR dimerization critically contributes to trauma-induced compromised bone repair, suggesting that GR dimer disruptive ligands could improve fracture healing in this context.

Keywords: Glucocorticoid receptor, Fracture, Trauma.



[Bending stiffness of fracture calli on day 23 post-fracture and thoracic trauma.]

P074

Metastatic breast cancer burden is reduced in a Tgif1-deficient bone microenvironment

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Osteoclast activation is a hallmark of breast cancer bone metastasis. However, a key role of osteoblasts is suggested by the finding that osteoblast-conditioned medium (CM) stimulates breast cancer cell (BCC) migration ($p < 0.001$). We identified the homeodomain protein TG-interacting factor-1 (Tgif1) as strongly expressed in osteoblasts upon BCC stimulation, indicating a potential role of Tgif1 in the osteoblast-BCC interaction. Indeed, CM from osteoblasts of mice bearing a germline deletion of Tgif1 ($Tgif1^{-/-}$) failed to induce BCC migration compared to CM from wild-type osteoblasts ($p < 0.001$), suggesting that Tgif1 in osteoblasts supports BCC motility. To test if Tgif1 contributes to metastatic bone disease, we employed a syngeneic metastasis model ($n \geq 9$). The presence of single BCCs or micro-metastases in tibiae was reduced by 25% in $Tgif1^{-/-}$ mice determined by confocal microscopy. Bioluminescence imaging revealed a decreased number of bone metastases compared to control littermates (24% vs. 50%) 7 days after intracardiac injection of 4T1-*GFP-Luc* BCCs, leading to a prolonged survival (12 vs. 9.5 days). BCCs localized in close proximity to Endomucin-positive vascular cells and osteoblasts. While Tgif1-deficiency did not affect the vessel number or size in the bone marrow, osteoblast number ($p < 0.001$) and activity ($p < 0.01$) was reduced compared to control. This suggests that the protective effect on bone metastases might be mediated by osteoblasts and not by the bone marrow vasculature. To determine the underlying molecular mechanism, we performed unbiased SILAC and RNA-seq analyses and identified Semaphorin 3E (Sema3E) as abundantly expressed and secreted by $Tgif1^{-/-}$ osteoblasts. Recombinant Sema3E dose-dependently impaired BCC migration, suggesting that Tgif1 supports BCC migration by suppressing Sema3E expression in osteoblasts. In summary, we propose that lack of Tgif1 in osteoblasts attenuates BCC migration and metastases formation, possibly through suppression of Sema3E, thereby establishing osteoblasts as regulators of bone metastases.

Keywords: Breast cancer, Bone metastasis, Osteoblast, Tgif1

P321

New mouse model for atypical type VI osteogenesis imperfecta caused by IFITM5 S40L mutation

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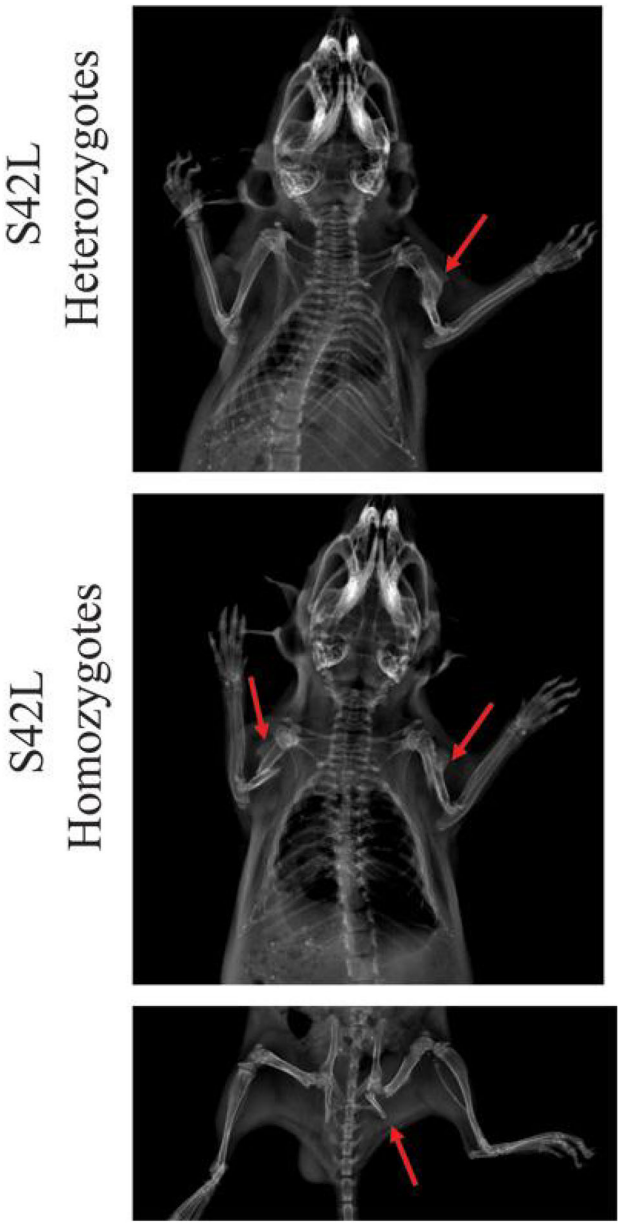
Osteogenesis Imperfecta (OI), a well-known bone dysplasia, is now understood as a collagen-related disorder caused by mutations in *COL1A1* and *COL1A2* and 14 other genes. Type V OI, caused by a recurrent dominant mutation in the plasma membrane protein *IFITM5*/BRIL, and type VI OI, due to recessive null mutations in the anti-angiogenic factor PEDF, have distinct severity and bone histology. *IFITM5* S40L, reported in six patients, causes severe dominant OI with phenotype and bone histology similar to type VI OI, and without Type V OI distinctive features.

We generated a conditional knock-in mouse model to investigate atypical type VI OI. The mutation, located at BRIL S42L in mice, was activated using E2A-CRE matings. S42L is non-lethal in both heterozygous and homozygous mice. Newborn heterozygous S42L pups have flared rib cage and shoulders and knees dislocations. In radiographs, S42L heterozygote mice exhibit » 50% humeral fractures in 1- (19/30 HETS) and 2-month-old (11/21 HETS) mice, while homozygotes incur fractures in 95% (20/21 HOMZ) of upper limbs, as well as femora and pelvis. Whole body BMD was significantly decreased at 1-month in male and female heterozygotes ($p < 0.01$). Micro-CT showed elevated trabecular BV/TV and TbN ($p < 0.05$). In vitro mineralization by alizarin red staining ($p < 0.05$) was increased as were transcription levels of osteoblast genes throughout differentiation in heterozygote mice ($p < 0.05$). Taken together these results suggest that the *IFITM5* S42L mouse model is a suitable model to further investigate the mutation mechanism of and the metabolic pathways connecting BRIL and PEDF.



[Radiographs of IFITM5 S42L heterozygous and homozygous mice]

P322

Development of lentiviral vector gene therapy for the treatment of autosomal recessive osteopetrosis

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Autosomal Recessive Osteopetrosis (ARO) is a severe inherited disease characterized by dysfunctional osteoclasts. Fifty-five percent of ARO patients have mutations in *TCIRG1* gene, encoding for the a3 subunit of V-ATPase proton pump, necessary for bone resorption. Patients are characterized by high bone density and fragility, neurological defects and bone marrow fibrosis leading to extramedullary hematopoiesis with increased number of circulating $CD34^+$ cells. Allogenic haematopoietic stem cells transplantation (HSCT) is the treatment of choice for osteopetrotic patients, but the high incidence of adverse outcomes after HSCT and the low availability of compatible donors, pave the way for the development of a gene therapy (GT) approach to cure ARO. To develop a novel GT strategy, we analyzed circulating ARO $CD34^+$ cells in terms of frequencies of hematopoietic lineages, taking advantage of a novel flow cytometry protocol. In parallel, we generated two lentiviral vectors, driving *TCIRG1* expression under the control of the phosphoglycerate kinase promoter, with or without the *dNGFR* marker gene. We tested our GT protocol on the *oc/oc* spontaneous murine model, closely resembling the human disease. The *oc/oc* mice have a life expectancy of two weeks, conversely GT mice reached up to four months of age. Of note, we observed an amelioration of bone architecture and reduced parathyroid hormone level in the serum. Furthermore, we observed the reorganization of white and red pulp of the spleen by histological analysis. In parallel, $CD34^+$ cells isolated from a small volume of blood of ten ARO patients, were transduced and expanded applying a protocol that allows stemness maintenance. We performed in vitro assays to evaluate resorption capacity of patient-derived osteoclasts and we evaluated the long-term multi-lineage repopulating potential of expanded $CD34^+$ by primary and secondary transplant into NSG mice. In conclusion, our results suggest that GT represents a feasible alternative treatment for TCIRG1-dependent osteopetrosis.

P324

Enzyme replacement therapy corrects bone remodeling pathologies, but not chondrocyte pathologies, in a mouse model for mucopolysaccharidosis type VI (MPS-VI)

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Skeletal pathologies are frequently observed in lysosomal storage disorders, yet the relevance of specific lysosomal enzymes in bone growth and remodeling is poorly defined. Mucopolysaccharidosis type VI (MPS-VI) is such a lysosomal storage disorder caused by mutations in *ARSB*, coding for the glycosaminoglycan-degrading lysosomal enzyme arylsulfatase B, predominantly affecting the skeleton. By deep skeletal phenotyping of *Arsb*-deficient mice, we previously characterized their skeletal phenotype as osteoclast-rich osteopetrosis with lysosomal storage accumulation in all skeletal cell types. Since the influence of enzyme replacement therapy (ERT) on skeletal homeostasis in MPS-VI is still unknown, we treated *Arsb*-deficient mice by weekly injection of 1 mg/kg human recombinant ARSB. Treatment of *Arsb*-deficient mice from 12 to 24 weeks of age demonstrated that the high bone mass phenotype and the underlying bone cell deficits could be fully corrected by ERT in the trabecular compartment, but not in the cortical bone compartment. Next, to investigate whether the defects in skeletal growth and remodeling can be prevented by early start of the treatment, *Arsb*-deficient mice were

treated from 4 to 12 and from 4 to 24 weeks of age. Interestingly, the high bone mass phenotype was generally prevented by early treatment, whereas skeletal growth deficits still persisted. Electron microscopy demonstrated that lysosomal storage accumulation was still present in different chondrocyte populations (growth plate, articular) after treatment, and not in the bone remodeling cell types. In line with these findings, cellular uptake experiments additionally showed that all skeletal cell types could take up and activate the human recombinant form of ARSB, whereas chondrocytes could not. Taken together, our results provide an important proof-of-principle for the feasibility of ERT to correct bone remodeling pathologies, but possibly not chondrocyte-related pathologies, in lysosomal storage disorders like MPS-VI.

Keywords: Arylsulfatase B, Lysosomal storage disorders, Mucopolysaccharidosis type VI, Enzyme replacement therapy

P323

Mutations in the neuroblastoma amplified sequence (*NBAS*) gene in acrofrontofacionasal dysostosis type 1

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Acrofrontofacionasal Dysostosis type 1 (AFFND1) is an extremely rare, autosomal recessive syndrome, comprising facial and skeletal abnormalities, short stature and intellectual disability. We analyzed an Indian family with two affected siblings by exome sequencing and identified a novel homozygous truncating mutation (c.6237-3C > G) in the Neuroblastoma-Amplified Sequence (*NBAS*) gene in the patients' genome. The NBAS protein acts in the non-sense mediated decay (NMD) and the Golgi-to-ER retrograde traffic. Retrograde transport was impaired in HEK293T cells overexpressing the truncated NBAS protein, as assessed by co-immunofluorescence analysis for NBAS and Calreticulin (Manders´ coefficient 0.5453 ± 0.03697 and 0.4087 ± 0.02865 in the presence of WT or truncated NBAS respectively, $p < 0.01$). The NMD function was analyzed by qPCR, Western blot and co-immunoprecipitation analysis, resulting impaired in the presence of truncated NBAS protein. We examined NBAS expression in mouse embryos at various developmental stages by immunohistochemistry, and detected the protein in developing chondrogenic and osteogenic structures of the skeleton as well as in the cortex, hippocampus and cerebellum, which is compatible with a role in bone and brain development. Z-nbas knock-down in fish embryos resulted in significant splanchnocranial alterations of the angle between the Meckel´s cartilage and the palatoquadrate/ceratohyale cartilages; and between the right and left ceratohyale cartilages (> 37 embryos injected with scrambled, ssMO or TB-MO, or not-injected; $p < 0.01$). Overall, these data indicated a conserved function of NBAS in skeletal morphogenesis and supported the hypothesis of a causative role of mutant *NBAS* in the pathogenesis of AFFND1. To investigate further the elicited pathogenetic mechanisms, we generated a knock-in (KI) murine model carrying the intronic AFFND1 mutation. Preliminary analysis on the homozygous KI mouse cDNA showed a lower production of the aberrant transcript as compared to humans, even after NMD inhibition with cycloheximide, possibly due to a species-specific regulation of the splicing process. Further analyses are ongoing.

Concurrent Oral Poster Presentations 1—Clinical/ Public Health

P003

The effect of vitamin D supplementation on its metabolism in a randomized controlled trial

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Background: Vitamin D is essential for maintaining calcium and phosphate homeostasis. 25-hydroxy vitamin D (25(OH)D) is most commonly measured to assess vitamin D status. Other vitamin D metabolites such as 24,25-dihydroxy vitamin D ($24,25(OH)_2D$) provide additional insights into vitamin D status or metabolism. Earlier studies found that the vitamin D metabolite ratio (VMR), calculated as $24,25(OH)_2D/25(OH)D$, could predict the increase after vitamin D supplementation. However, studies have been inconsistent in whether this is a better predictor than baseline 25(OH)D. Therefore, the aim of our study was to assess whether the increase in 25(OH)D after supplementation was predicted by VMR better than baseline 25(OH)D.
Methods: Plasma samples of 108 vitamin D insufficient (25(OH)D < 75 nmol/L) individuals with hypertension who completed the Styrian Vitamin D Hypertension Trial (NCT02136771) were used. Participants received either vitamin D (2800 IE daily) or placebo for 8 weeks. We used ANCOVA analyses to calculate the treatment effect (ANCOVA) and used Pearson correlation analysis between changes in 25(OH)D concentrations and baseline parameters.
Results: The treatment effect after vitamin D supplementation for 25(OH)D was 32.4 nmol/L (95% CI 25.9–38.9; $p < 0.001$). Treatment effects for $24,25(OH)_2D$ and VMR were 3.32 nmol/L (2.71–3.94; $p < 0.001$) and 1.5 (1.0–2.0; $p < 0.001$), respectively. Correlation between baseline 25(OH)D and its change after supplementation was $r = -0.561$, $p < 0.001$. The change in 25(OH)D after vitamin D supplementation also correlated with $24,25(OH)_2D$ ($r = -0.527$, $p < 0.001$) but not with VMR ($r = -0.096$, $p = 0.499$).
Conclusions: In our cohort, the vitamin D metabolite ratio did not predict the change in 25(OH)D. The concentration of $24,25(OH)_2D$ at baseline did predict the change, but to similar extent as baseline 25(OH)D. Therefore, our results suggest that measuring $24,25(OH)_2D$ is of no added value in predicting the 25(OH)D concentration after supplementation. Our data also suggest that the activity of 24-hydroxylase increased after vitamin D supplementation.

P001

Low bone turnover is associated with functional hypoparathyroidism in type 2 diabetes

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Bone turnover is reduced in patients with type 2 diabetes (T2DM), however its determinants remain unclear. Within a cohort of patients with T2DM we examined the relationships between markers of bone turnover and glycaemic control, disease duration and calciotropic hormones.

This case–control study included 110 patients with T2DM (mean ± SD; age 63.7 ± 6.0 years; BMI 29.8 ± 4.3 kg/m^2, HbA1c 7.5 ± 1.2%; median disease duration 13.5 years [IQR 8–20]) and 92 non-diabetic controls (age 60.5 ± 6.3 years, BMI 24.9 ± 4.5 kg/m^2, HbA1c 5.5 ± 0.3%) who are prospectively followed in the DiabOS-Study (an ongoing observational cohort study evaluating skeletal health in T2DM). Biochemical markers of bone formation (PINP, bone-specific alkaline phosphatase [BAP]) and resorption (CTX), as well as measures of calcium homeostasis (iPTH, 25OHVD, calcium, magnesium), IGF-1 and HbA1c were assessed at baseline.

After adjustments for age, gender and BMI, patients with T2DM had lower serum levels of PINP ($p < 0.001$), CTX ($p = 0.03$), iPTH ($p = 0.03$), magnesium ($p < 0.001$) and higher HOMA-Index ($p = 0.03$) as compared to non-diabetic controls. Serum calcium, creatinine, 25-OHVD, IGF-1 and nutritional calcium intake did not differ between groups. Intact PTH was positively correlated with magnesium levels ($r = 0.21$, $p = 0.03$). In multivariate logistic regression analyses, only serum iPTH remained an independent predictor of bone markers in T2DM ($p = 0.006$ for PINP, $p = 0.002$ for BAP and $p < 0.001$ for CTX). In contrast, HbA1c, disease duration, age, HOMA-Index and BMI were not associated with bone turnover markers.

We conclude that functional hypoparathyroidism is an important regulator of low bone turnover in T2DM. Whether low bone turnover is mediated by hypomagnesemia-induced inhibition of PTH secretion or by direct effects of circulating sclerostin needs to be investigated.

Keywords: Diabetes mellitus, Bone turnover, PTH, Glycaemic control

P005

The association between osteocalcin, adiposity and bone health: results from a population based cohort

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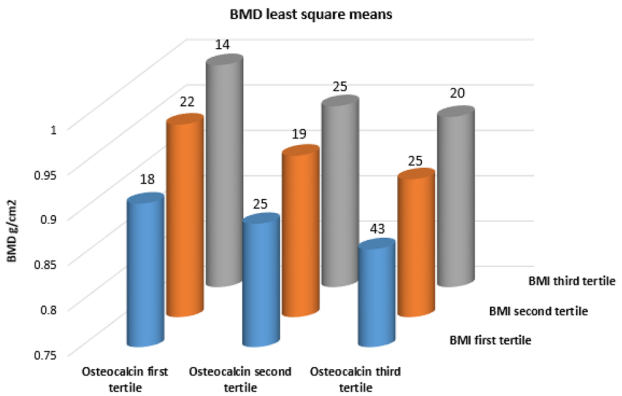
Osteocalcin, an indicator of osteoblast differentiation, is an emerging bone turnover marker. Recently, there has been a growing interest in the potential extra-skeletal effects of osteocalcin including fat mass regulation; important predictor of bone mineral density (BMD). Therefore, we aimed to investigate the association between osteocalcin, adiposity and BMD. We included 6661 participants (56.8% females) from a prospective population-based cohort (age:62.6 ± 9.1). All participants underwent Dual Energy X-Ray Absorptiometry (DXA) to measure femoral neck BMD. Incident fractures were identified and collected from medical registry data. Body mass index (BMI, kg/m2) was used as proxy for adiposity. Serum osteocalcin was measured by electrochemiluminescence immunoassay (ECLIA) and was transformed to obtain a normal distribution using an inverse normal transformation. Linear regression analyses were performed adjusting for age, sex, and cohort effect. In addition, we modeled time-to-event to estimate hazard ratios of sustaining future fracture. Linear regression analyses showed strong inverse association between osteocalcin and FN-BMD (β: − 0.03; 95% CIs − 0.04 to − 0.02) and BMI (β: − 0.92; 95% CIs: − 1.02 to − 0.82). However, individuals with low BMI and high osteocalcin levels had significantly lower BMD and higher number of wrist fractures (mean = 0.86 ± 0.14; 43 fractures) compared to individuals with high BMI and low osteocalcin levels (mean = 0.99 ± 0.15; 14 fractures) ($P < 0.0001$) [Figure 1]. In addition, higher levels of osteocalcin were associated with increased risk of wrist fractures (HR = 1.30; 95% CIs = 1.13 to 1.49) but not hip fractures (HR = 0.95; 95% CIs = 0.82 to 1.10). In conclusion, osteocalcin may be an important predictor of osteoporosis and fracture risk in the elderly.



[Figure 1 shows least square means of FN-BMD across age and sex specific BMI and osteocalcin tertiles]

P041

Alterations of bone geometry, bone volumetric density, and microarchitecture but not areal bone mineral density in Chinese patients with primary hypertrophic osteoarthropathy

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Objectives: Primary hypertrophic osteoarthropathy (PHO) is a genetic disorder related to failures in prostaglandin metabolism. Disturbed prostaglandin E2 (PGE2) catabolism resulting in increased PGE2 level is suggested in the pathogenesis. Plain radiographs of PHO patients in previous studies showed periostosis and acro-osteolysis, indicating altered bone structures in PHO patients. However, previous studies did not provide any information on bone structure in PHO patients. This study aimed to use for the first time a high resolution peripheral quantitative computed tomography (HR-pQCT) and evaluate bone microarchitecture in PHO patients.

Methods: In this study, HR-pQCT was performed in 20 PHO patients and 40 healthy controls. Areal bone mineral density (aBMD) and biochemical tests were also conducted in PHO patients.

Results: The current study has first demonstrated bone microstructure changes in PHO patients using HR-pQCT. In comparison with healthy controls, both PHOAR1 and PHOAR2 patients had larger bone areas at radius and tibia whereas volumetric BMD (vBMD) of all the PHO

patients were significantly lower than controls. At the radius, all indices of trabecular microstructure were inferior in PHOAR1 patients. Cortical thickness in both PHOAR1 and PHOAR2 patients were increased at the radius and tibia, respectively ($p = 0.010$ and $p < 0.0001$). Bone stiffness (S) and failure load (F.ult) in PHOAR1 and PHOAR2 patients showed an opposite trend, which was a decrease in PHOAR1 ($p = 0.024$ and $p = 0.038$ respectively) and increased in PHOAR2 patients ($p = 0.007$ and $p = 0.006$ respectively). Subgroups analysis revealed that PHOAR1 had inferior bone microstructure at the tibia when compared with PHOAR2 patients. Correlation analysis revealed that PGE2 correlated negatively with vBMD, the trabecular and cortical thickness at the tibia rather than the radius.

Conclusions: This study demonstrated bone structural changes in PHO patients using HR-pQCT, provided new insight into the bone quality assessment of PHO patients.

Keywords: Primary hypertrophic osteoarthropathy; PGE2; HR-pQCT;

P042

Secondary dislocation of distal radius fractures is associated with lower total and cortical volumetric bone mineral density and lower cortical thickness at the distal radius

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Objectives: Distal radius fractures (DRFs) with an acceptable position can be managed conservatively. By being able to anticipate early stage instability, unnecessary manipulation can be prevented and timely surgical treatment achieved. The aim of this study was to investigate the associations of patient characteristics, bone mineral density (BMD), bone microarchitecture and calculated bone strength with secondary dislocation of a DRF based on radiographic alignment parameters.

Methods: Dorsal angulation, radial inclination and ulnar variance were assessed on all conventional radiographs of 251 patients, 38 men and 213 women, to determine the anatomic position of the DRF at presentation (primary position) and during follow-up. Secondary dislocation was assessed in all 154 conservatively treated patients with an acceptable position, preceded (N = 97) or not preceded (N = 57) by primary reduction (baseline position). Additionally, bone microarchitecture and calculated bone strength at the contralateral distal radius and tibia were assessed by HR-pQCT in a subset of respectively 63 and 71 patients.

Results: Characteristics of patients with and without secondary dislocation did not differ. Total (OR 0.27 [95%CI 0.10–0.73], p = 0.010) and cortical (OR 0.31 [95%CI 0.12–0.80]) volumetric BMD and cortical thickness (OR 0.32 [95%CI 0.13–0.80]) at the distal radius were associated with secondary DRF dislocation and remained significantly associated in multivariate analysis.

Conclusions: In conclusion, our data demonstrate that lower total and cortical vBMD and lower cortical thickness at the distal radius are independently associated with secondary dislocation of a DRF, suggesting that the cortical, rather than trabecular bone, is responsible for retaining an adequate fracture position.

Keywords: Distal radius fracture, Dislocation, Bone microarchitecture, HR-pQCT.

P133

A 'NGS-digital PCR' method gives new evidences on the molecular mechanism responsible for osteochondromas' growth in mo disease

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Osteochondroma (OC) is a benign bone tumor which occur as several neoplasms in the Multiple Osteochondromas syndrome (MO). The most severe complication is its malignant transformation into peripheral secondary chondrosarcoma (PSC). Although MO has been linked to heterozygous defects in EXT1/EXT2 genes, there are contradictory results about the requirement of their biallelic inactivation.

Using a combined NGS-DigitalPCR approach enabling the detection of low percent mutations, we evaluated the EXT1–2 somatic status in 40 cartilaginous samples (22 OCs and 18 PSCs). Ten healthy cartilaginous tissues have been also analyzed.

A second somatic point mutation was detected in 2 OCs and 1 PSC. In all other samples the allelic ratio in somatic DNA was different by what observed in the corresponding constitutional DNA with an increase of the mutated allele. Digital PCR characterized the second somatic hit as a copy number variation (CNV) involving the EXT gene carrying the germline variant (23 deletions and 12 amplifications, depending on the sample); of note, 17/40 samples (12 OCs and 5 PSCs) have somatic CNVs in both EXT genes. All healthy samples show the presence of CNVs—always amplifications—in both genes.

The high sensitivity of the combined 'NGS-DigitalPCR' method highlighted a molecular mechanism—never assumed before—responsible for lesions' growth in MO. The requirement of a second somatic hit has been confirmed and it is mainly characterized by a mosaic impairment of EXT copies caused by somatic CNVs (deletion or duplication) responsible for a quantitative increase of the defective allele. The presence of CNVs in both EXT genes in both pathologic and healthy cartilage suggests the presence of a tissue-specific molecular mechanism responsible for a constitutional randomized CNV events in EXT genes responsible for a casual 'over-representation' of the defective allele.

Keywords: Multiple Osteochondromas, EXT1-EXT2, NGS-DigitalPCR, CNV, Cartilage

P161

Changes in bone marrow adipose tissue one year after Roux-en-Y gastric bypass: a prospective cohort study

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Bone marrow adipose tissue (BMAT) has been postulated to mediate skeletal fragility in type 2 diabetes (T2D) and obesity. Roux-en-Y gastric bypass (RYGB) induces a substantial weight loss, and resolution of comorbidities. However, the procedure induces reduction in aBMD, increased bone turnover and fracture rates. No previous study has evaluated bone marrow biopsy measured BMAT before and after RYGB. We examined 30 participants preoperatively and one year after RYGB. Preoperative BMAT was positively associated with HbA1c, β 4.4 (0.41 to 8.3) and negatively associated with lumbar spine and femoral neck aBMD, β − 31.1 (− 55.1 to − 7.1) and β − 30.8 (− 58.4 to − 3.2), p = < 0.05 for all. After adjustment for age, BMI and gender only the association between lumbar spine aBMD and BMAT remained significant. One year after RYGB, participants had lost 32.6 kg ± 10.8 or 27.2% ± 8.7 of total weight. BMAT decreased from 40.4% ± 1.7 preoperatively to 35.6% ± 12.8, p 0.042. Participants who lost more BMI units or decreased more in total body fat mass exhibited more pronounced reductions in BMAT β 1.3 (0.31 to 2.4) and β 0.5 (0.027 to 0.98), p = < 0.05. This remained significant after adjusting for gender, preoperative BMI and BMAT. Females reduced more in BMAT than males, − 9.3% ± 8.6 and +1.8% ± 14.7, p = 0.014, the difference remained significant after adjusting for age, preoperative BMAT and BMI. In males change in serum estradiol was associated with change in BMAT, this was not observed for females. Participants with preoperative T2D had similar decrease in BMAT to participants without T2D, − 3.0% ± 14.8 and − 5.9% ± 11.2. Our findings indicate that BMAT is associated with lower aBMD and poorer glycemic control in subjects with morbid obesity. One year after RYGB we observed a 10.7% decrease in BMAT. The BMAT reduction was comparable in participants with and without T2D and appeared gender specific.

P162

Maternal citamin D status in 2nd and 3rd trimester and offspring bone mass at age 8–9 years: an observational study

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Objectives: Studies on the associations between maternal 25(OH)D concentrations and offspring bone mass show diverging results. We investigated the association between maternal 25(OH)D and offspring bone mineral density (BMD) and spine trabecular bone score (TBS), a measure of bone quality, at 8–9 years of age.

Methods: Forty-seven mother- and offspring pairs were included. Blood samples were collected at pregnancy week 18–22 and 32–36 for analyses of serum 25(OH)D. Offspring BMD at lumbar spine (L1-L4), distal femur and whole body, and spine TBS were measured by dual x-ray absorptiometry at the age of 8–9 years (55% females).

Results: The proportion of women with serum 25(OH)D < 50 nmol/L was 26% and 30% in second and third trimester, respectively, and 68% and 60% had serum 25(OH)D < 75 nmol/L. Mean BMD Z-scores in offspring were 0.066 ± 0.953 for spine and 0.369 ± 1.021 for whole body. After adjustment for confounders (maternal age, education, smoking and body mass index), a positive association between maternal 25(OH)D in second trimester and offspring lumbar spine BMD was observed (mean change 9.76 mg/cm^2 (CI: 0.53 to 18.98) per 10 nmol/L 25(OH)D increase). The same relationship was observed in late pregnancy after adjustment (7.63 mg/cm^2 (CI: 0.21 to 15.06) per 10 nmol/L 25(OH)D increase). No associations were found between maternal serum 25(OH)D and offspring BMD at other sites or spine TBS.

Conclusions: After adjustment for maternal confounders, we found a positive association between maternal 25(OH)D and spine BMD in offspring at 8–9 years of age. These findings may imply increased future risk for osteoporosis in offspring whose mothers are vitamin D deficient, underscoring the significance of optimal maternal vitamin D status during pregnancy.

Keywords: Offspring BMD, Maternal vitamin D, Pregnancy

P175

Functional assays of two non-coding variants in *WNT16*

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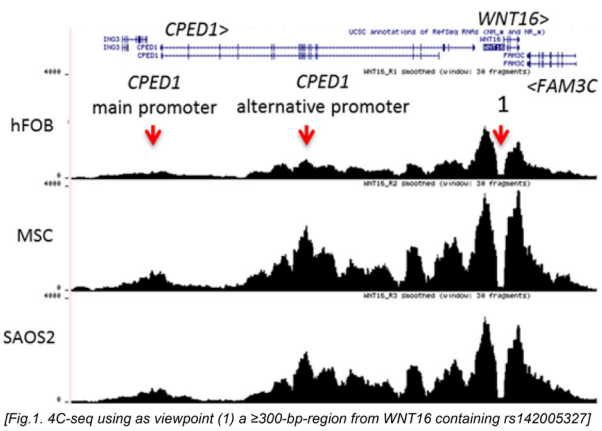
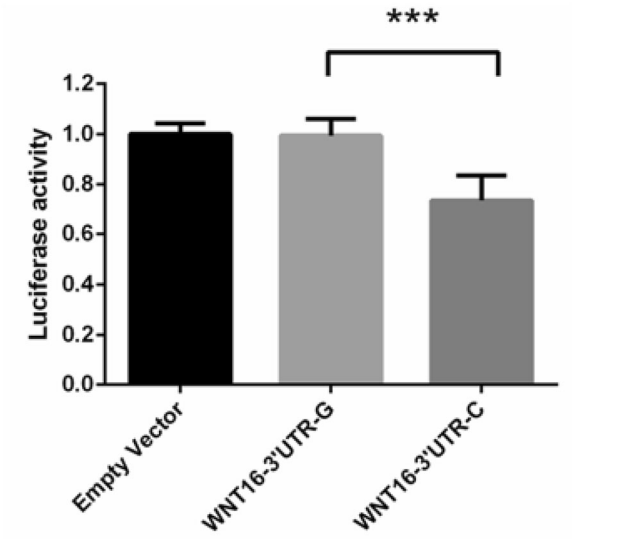
Numerous studies have highlighted the role of *WNT16* in bone mineral density (BMD) determination. However, little is known about the implication of its neighbours *CPED1* and *FAM3C*. Here we present new evidences, through in vitro functional experiments, of two variants in *WNT16*: a common intronic CT insertion (rs142005327) associated with BMD and a rare 3´UTR variant (rs190011371) found in 3 women with BMD below the mean of the BARCOS cohort (Martínez-Gil et al., SciRep 8:10951, 2018). The intronic variant rs142005327 is located in a region containing chromatin enhancer or promoter marks in osteoblast cell lines (ENCODE). To detect possible interactions of this region with other parts of the genome we have performed 4C experiments in 3 bone cell lines (SAOS2, hFOB and MSC). We have found that it interacts with the *CPED1* promoter and an alternative promoter of the same gene, upstream of exon 12, active in differentiating osteoblasts (Maynard et al., 674:127–133, 2018) (Fig. 1). Related to the rare 3´UTR variant rs190011371, we performed luciferase assays and observed significant allelic differences, where the minor allele presented reduced expression (Fig. 2). This work provides evidence of regulatory roles for the two tested variants and suggests a role for *CPED1* in BMD determination.



[Fig.1. 4C-seq using as viewpoint (1) a ≥300-bp-region from WNT16 containing rs142005327]



[Fig.2. Effect of rs190011371 on gene expression.*** P<0.001.]

P176

Genome-wide association study identifies one novel locus associated with thickness and mineralization of metacarpal bones

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Objectives: Bone health index (BHI), embedded in BoneXpert software, is a measurement of metacarpal cortical thickness and mineralization. BHI has been shown earlier to be correlated with lumbar (LU-) and total body (TB-) bone mineral density (BMD) and postulated to aid the assessment of bone health in children. In the current study, we aimed to perform a genome-wide association study (GWAS) to identify genetic determinants of BHI.
Methods: GWAS of BHI was performed in a multiethnic population-based cohort. We included 2633 children with a mean age of 9.8 (SD = 0.29) years. BHI was determined on hand DXA scans using BoneXpert software. Participants were genotyped using Illumina HumanHap 610 or 660 Quad chips (Illumina Inc., San Diego, USA) platforms and imputed to HRC1.1 reference panel. Single nucleotide polymorphisms (SNPs) exceeding minor allele frequency (MAF) greater than 5% and imputation quality greater than 30% were included in subsequent analyses. Standardized residuals of BHI corrected for sex, age and 10 genomic principal components (PCs) were created and tested for the association with genotypes. Genome-wide significance (GWS) threshold was set at $P < 5x10^{-8}$.
Results: We identified signals in 7q31.31 locus associated at GWS level with BHI. Top signal was mapping to *CPED1* (intron variant; rs798943; beta = 0.22; $P = 1.30x10^{-14}$).
Conclusions: We report variants mapping to the *CEPD1* locus associated with BHI. The locus has been associated with BMD and fracture risk (in adults). These findings provide additional evidence postulating bone health index as a good proxy for the assessment of pediatric bone health.

P177

Variants within the ZNF384 and COL1A1 genes influence bone mineral density and fracture risk in maltese postmenopausal women

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Objectives: Osteoporosis is a skeletal disease having a strong genetic component. We recently identified the splice variant *ZNF384* rs146089604 as a possible causal variant underlying primary osteoporosis in an extended Maltese family. ZNF384 transactivates *COL1A1*, which can be altered in the presence of promoter and intronic *COL1A1* variants. The study aimed to evaluate the effect of the *ZNF384* rs146089604, *COL1A1* rs1107946 (G − 1997T) and *COL1A1* rs1800012 (G + 1245T) variants, alone or in combination, on BMD and fracture risk in Malta.
Methods: Genotyping was performed in the Malta Osteoporotic Fracture Study comprising 1045 Maltese postmenopausal women aged 41–79 years. Testing was performed by Kompetitive Allele Specific PCR (*ZNF384* rs146089604), TaqMan allelic discrimination (*COL1A1* rs1107946) and PCR followed by restriction enzyme digest (*COL1A1* rs1800012). Odds ratios (OR) with 95% confidence intervals (CI) were computed using logistic regression analysis adjusted for confounders.
Results: Genotyping of the *ZNF384* rs146089604, *COL1A1* rs1107946 and *COL1A1* rs1800012 was successful in 1018 (MAF = 2%), 1033 (MAF = 19%) and 1041 (MAF = 27%) samples respectively. Women with the TT genotype for the *COL1A1* rs1107946 variant had a twofold increased risk of osteoporosis at the lumbar spine (adjusted OR: 2.1 [95% CI 1.0–4.5]) relative to women with a normal BMD. Gene–gene interactions revealed that carriers for the *ZNF384* rs146089604 and *COL1A1* rs1107946 had a threefold increased risk of osteopenia at the femoral neck (3.0 [1.0–9.5]). A similar but weaker association was seen for women with the *COL1A1* rs1800012 TT genotype (1.8 [1.0–3.2]). Heterozygosity for the *ZNF384* rs146089604 was associated with site-specific fracture risk (hip: 2.6 [1.0–6.4] and wrist: 2.2 [1.1–4.2]) irrespective of BMD.
Conclusions: Results suggest that *ZNF384* and *COL1A1* variants predispose to reduced BMD and increased fracture risk in a site-selective manner in Maltese women. Functional studies are warranted to understand the combined effects of these variants in osteoporosis pathogenesis to identify potential targets for treatment.

P220

Birthweight, limb muscle mass and abdominal adiposity in middle age: findings from the UK biobank imaging enhancement

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Objectives: Low birthweight has been associated with poorer musculoskeletal health in later life. We investigated relationships between birthweight and magnetic resonance (MR) measures of muscle and fat volume in UK Biobank.

Methods: UK Biobank is a large prospective cohort of men and women aged 40–69 years, including a detailed baseline assessment in which birthweight was collected by self-report. A subset underwent MRI examination with the dual-echo Dixon Vibe protocol, from neck to knees. Automated analysis was performed using the AMRA Profiler™ system, to segment and quantify total thigh muscle volume, visceral adipose tissue (VAT) and abdominal subcutaneous adipose tissue (ASAT). Total trunk fat (VAT plus ASAT) volume was calculated. Associations between birthweight and body composition outcomes (expressed as Fisher-Yates z-scores) were assessed using multivariate linear regression analysis. This study was conducted under generic approval from the NHS National Research Ethics Service (17th June 2011, Ref 11/NW/0382).

Results: 3457 participants (1401 men, mean (SD) age 61.0 (7.6) years and 2056 women, age 60.1 (7.4) years) underwent MRI body composition analysis and were able to recall their birthweight.

In both men and women, higher birthweight was associated with greater thigh muscle volume (adjusted for age and body mass index (BMI)): men, β (95% CI): 0.229 (0.156, 0.301) z-score, $p < 0.001$; women, β (95% CI): 0.284 (0.221, 0.346) z-score, $p < 0.001$. These associations persisted after additional adjustment for current smoking and physical activity.

Birthweight was neither associated with total trunk fat nor ASAT in either men or women after adjustment for age and BMI.

Conclusions: These findings provide unique evidence of associations between birthweight and volumetric measures of muscle size but not adiposity, and support the developmental programming hypothesis. Interventions to improve obstetric health and optimise birthweight may help to prevent sarcopenia in future generations.

Concurrent Oral Poster Presentations 2—Basic/Translational

P118

Pleckstrin homology domain containing protein family member 1 (PLEKHM1) regulates bone resorption through sealing zone dynamics and lysosomal targeting in osteoclasts

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Loss-of-function mutations in Pleckstrin homology domain containing protein family member 1 (PLEKHM1) cause osteopetrosis in the *ia/ia* rat and in humans. Mouse strains with global or lineage-specific deletion of Plekhm1 have highlighted roles for Plekhm1 in the lysosomal fusion machinery in autophagic and endocytic pathways, but effects on bone have been variable. Here, we examined osteoclasts from a new global PLEKHM1 loss-of-function mouse model containing a premature STOP codon at amino acid R714 (R714 STOP), resulting in significantly depleted levels of PLEKHM1 protein. R714 STOP +/+ mice had normal ruffled borders (RB) in vivo but showed a 135% increase in bone mass (BV/TV %) at 3 weeks compared to R714 STOP ± or wildtype mice, with no difference between heterozygotes and wildtype. This was associated with a 98% reduction in resorption in vitro in R714 STOP +/+ osteoclasts, but no defects in autophagy. We then used live-cell imaging to investigate the basis for the reduced resorption in cultures of osteoclasts on dentine. Osteoclasts cultures containing an actin probe (SiR actin), a lysosomal probe (LysoTracker), and a fluorescent bisphosphonate stain to detect resorption, were imaged for up to 2 h using a spinning disc confocal microscope. R714 STOP +/+ osteoclasts were more likely to display dynamic sealing zones (SZs), less associated with resorption pits compared to R714 STOP ± osteoclasts (49% vs 33%), while 34% of R714 STOP +/+ osteoclasts, compared to 53% of R714 STOP ± osteoclasts were able to target LysoTracker-positive lysosomes to the RB. We conclude that PLEKHM1 deficiency in the R714 STOP mouse leads to osteopetrosis through a decrease in the formation of stable SZs and appropriate targeting of lysosomes. We demonstrate that dynamic analysis of osteoclastic resorption is possible and an essential tool to help understand mechanisms leading to osteoclast dysfunction.

P124

Three-dimensional mechanical loading activates YAP/TAZ pathway and chemokines expression in osteocytes

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Osteocytes are mechanosensitive cells that control bone remodeling in response to mechanical loading. We hypothesized that the Hippo signaling may be involved in osteocytes mechanosensing since YAP/TAZ, the mains effectors of the Hippo pathway known to play a role in mechanotransduction. YAP/TAZ has been also reported to interact with several signaling pathways regulated during osteoblastogenesis. The objective of our study is to define the response of osteocytes in response to mechanical loading and identify the implication of the Hippo pathway.

MLO-Y4 osteocyte cell lines were cultured in concentrated collagen hydrogel. Osteocytes were submitted to mechanical loading using the Flexcell Compression System. RNA sequencing was performed to determine gene expression modifications. Immunofluorescence and Western blot analysis were carried on, respectively, from paraffin embedded hydrogel sections and total protein lysate.

Mechanical loading enhanced the expression of mechanosensitive target genes (E11GP38 and COX2, × 4 fold increase $p < 0.05$). RNA

sequencing revealed that a majority of YAP/TAZ target genes modulated by compression in osteocytes were up-regulated, this being confirmed by RTqPCR regarding gene expression analysis of YAP/TAZ target genes (ANKRD1 (x2) and TEAD4 (x4 fold) increase $p < 0.05$). We also observed that mechanical compression promoted nuclear translocation of YAP/TAZ, and increased total protein level of YAP/TAZ. In addition, mechanical loading induced an increase of a large panel of chemokines such as Cxcl3, Cxcl5 and Cxcl10. Further, YAP/TAZ mediates the up-regulation of Cxcl3 gene expression since YAP/TAZ knockdown partially blunted the increase of Cxcl3 in response to osteocytes compression.

In conclusion, our model highlights the activation of YAP/TAZ pathway in osteocytes in response to mechanical loading. Our results show the contribution of YAP/TAZ signaling in osteocyte mechanotransduction and in the regulation of chemokines expression. Therefore, the signaling pathway could represent a new target to promote bone remodeling and anabolism.

Keywords: Osteocyte, YAP/TAZ, Mechanotransduction

P132

Theobroma cacao extracts as a treatment for FGFR3-related disorders

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Fibroblast growth factor receptor 3 (FGFR3) gain-of-function mutations cause osteochondrodysplasia, including achondroplasia, thanatophoric dysplasia and Muenke syndrome with abnormal activation of FGFR3 and downstream signaling pathways.

To identify new drugs able to interact with the FGFR3 signaling pathways, we screened natural compounds. Among the natural compounds studied (*Hibiscus sabdariffa*, *Curcumin*, *Diosmin*, *Theobroma cacao*), we showed in cells expressing *Fgfr3*gain-of-function mutations a decreased of the activation of the FGFR3 signaling pathways. We found that *Theobroma cacao* was the most effective compound. We purified *Theobroma cacao*and isolated and characterized by HPLC-ESI-TOF–MS a flavanol, epicatechin as the most relevant compound.

Then, we evaluated the efficacy of epicatechin in human chondrocytes and mouse cells expressing normal or mutant *Fgfr3*. Inhibitions of the FGFR3 downstream signaling pathways (Erk1-2, p38, STAT…) were observed in chondrocytes. We confirmed western blotting data by immunohistological analyses.*Ex vivo*femur cultures isolated from $Fgfr3^{Y367C/+}$ mice showed that the length of the epicatechin-treated$Fgfr3^{Y367C/+}$ femurs (598 ± 134 µm; n = 15) were increased by 48% comparing to untreated $Fgfr3^{Y367C/+}$ femurs (405 ± 97 µm; n = 15) (Figure 1). We also showed no modification of the collagen type X expression in femurs treated with epicatechin ($1.74 \pm 0.24.10^5 \mu m^2$; n = 8*vs*$1.53 \pm 0.37.10^5 \mu m^2$; n = 7), suggesting that the epicatechin acts mostly on proliferative chondrocytes. The decreased expressions of FGFR3 and SOX9 are also observed in the proliferative zone.

In conclusion, epicatechin isolated from *Theobroma cacao*, could be used as a valuable compound in the treatment of FGFR3-related disorders.

Keywords: Chondrodysplasia, FGFR3, Drug treatment, Epicatechin

P138

Thyroid hormones augment osteoblast differentiation via the BMP-Smad1/5 signaling pathway

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Thyroid hormones (TH) are key regulators of bone homeostasis and hyperthyroid mice exhibit bone loss. As osteoblasts and osteoclasts are tightly linked and bone morphogenetic protein (BMP) signaling is crucial for osteogenesis, we analyzed whether TH exert their effects via the BMP pathway.

Osteoblasts were treated with 3,5,3′-L-triiodothyronine (T3) and BMP signaling was blocked either at the receptor level using LDN193189 (LDN) or at the ligand level using noggin. Expression of Ocn, Alp, Osx and Runx2 was measured by real-time PCR. Activation of Smad1/5 was quantified using Western blot analysis. ALP activity assay and Alizarin Red staining were performed. Further, 12-week-old male C57BL/6 mice were rendered hyperthyroid (hyper) or remained euthyroid and received injections of 3 mg/kg BW LDN or PBS (5x/week) over 4 weeks. BV/TV as well as P1NP and CTX serum concentrations were analyzed by µCT and ELISA, respectively.

T3 treatment enhanced expression of Ocn, Alp, Osx, and Runx2 (7-/1.6-/2-/1.4-fold; $P < 0.05$) as well as ALP and mineralization capacity (1.4-/1.8–fold; $P < 0.05/P < 0.01$). T3 activated Smad1/5-phosphorylation after 40 min (1.9-fold, $P < 0.001$ vs. T0). LDN and noggin both reversed T3-mediated upregulation of osteogenic markers and activity. LDN treatment reduced T3-activated Smad1/5 phosphorylation. In vivo, hyperthyroid mice displayed reduced trabecular bone volume at the femur (− 41%, $P < 0.05$) and spine (− 35%, $P < 0.01$) as compared to euthyroid mice. LDN treatment tended to increase BV/TV of hyperthyroid mice at the femur and spine (1.3-fold both, ns. vs hyper). Serum P1NP concentrations were elevated in all treatment groups while increased CTX levels of hyperthyroid mice (1.7-fold, $P < 0.001$) were reduced by 19% ($P < 0.05$) with LDN treatment.

Our data indicate that T3 enhances osteoblastogenesis via BMP-Smad1/5 signaling. Furthermore, blockade of BMP signaling can mitigate hyperthyroidism-induced bone loss in mice. Still, investigations are required to further unravel underlying mechanisms and translational applications.

P152

PIT2 deficiency results in skeletal phenotype associated with alteration of bone marrow adipose tissue

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A growing interest in the Bone Marrow Adipose Tissue (BMAT) and its intimate relationship with skeletal health has lately emerged. Our recent work identifies the sodium-phosphate co-transporter PiT2 as a regulator of endochondral and intramembranous ossification and a major determinant for bone quality and strength. Thus, given the strong relationship between bone and BMAT, we characterized the BMAT phenotype in PiT2-deficient mice (PiT2KO) (French Ethical approval n°02286.01).

We examined BMAT volume in PiT2WT and PiT2KO tibias by immunohistochemistry and Contrast-Enhanced high-resolution μCT (CE-CT). In P16 PiT2KO mice, we observed a threefold increase in the number of perilipin + adipocytes in the proximal and distal BMAT (n = 6–8 per genotype, $p < 0.0036$ and $p < 0.0076$ respectively). At P21, CE-CT analysis showed a dramatic BMAT volume increase within the proximal tibia of PiT2KO mice compared to WT (n = 6 per genotype, p = 0.0050).

Consistent with this, Real-Time qPCR analyses revealed an increased expression of Adiponectin (p = 0.0061) and FABP4 (p = 0.0424) in PiT2KO mice (n = 4) compared to WT mice (n = 7).

The inverse correlation between BMAT and bone volumes could originate from the ability of the Bone marrow Mesenchymal Stromal Cells (BMSCs) to differentiate either into adipocytes or osteoblasts. We are currently investigating the reciprocal relationship between these two populations by analyzing the effect of PiT2 deficiency on BMSCs cultured in a co-differentiation medium. In line with the in vivo phenotype, our first observations revealed an increased number of adipocytes in the absence of PiT2 (+70.8%, p = 0.0286). We are now using this in vitro model to identify the molecular mechanism underlying the action of PiT2 in the regulation of osteo-adipogenesis.

Altogether, our results suggest PiT2 as a potential new player in the communication between bone and adipose tissues and reveal the PiT2KO mice as a new model to better understand the role and regulation of BMAT in bone physiology.

P153

Synergistic effects of chronic nicotine exposure and diabetes mellitus (DM) on deterioration in bone microstructure and geometry: an experimental study in male rats

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Despite interest in increased fracture risk in DM, diabetic bony complications are still insufficiently understood. Although DM patients often smoke, it is unknown whether interaction exists between DM and nicotine on bone. Our aim was to analyze individual and combined effects of experimentally induced chronic hyperglycemia and nicotine exposure on the femoral microarchitecture and geometry. The micro-CT based bone assessment was performed on 35 three-month-old male Wistar rats belonging to four groups: chronic hyperglycemia, chronic nicotine exposure, combination of hyperglycemia and nicotine exposure, and control group. Chronic hyperglycemia caused mild trabecular deterioration compared to controls (lower trabecular number, more rod-like trabeculae—$p < 0.05$). The combination DM + nicotine showed more deleterious effects on trabecular bone (more rod-like trabeculae, higher trabecular separation, lower BV/TV, Tb.N and connectivity than in control group—$p < 0.05$). Nicotine alone showed no significant changes in microarchitecture compared to the control group. DM and DM + nicotine groups had lower cortical porosity than control and nicotine groups ($p < 0.05$) along with reduced periosteal-endocortical differences (1.4% and 0.7% vs. 2.8% and 2.2%, respectively; $p < 0.001$). More than 60% and 50% of pores were smaller than 18 μm in DM + nicotine and DM groups, respectively, compared to less than 40% in controls. Cortical thickness showed no significant intergroup differences (p = 0.89), whereas polar moment of inertia was notably reduced in DM + nicotine group compared to nicotine (p = 0.02) and control groups (p = 0.05), revealing lower bone strength. In summary, effects of chronic hyperglycemia on bone microarchitecture and geometry were accentuated by chronic nicotine exposure, although nicotine alone caused no significant bone changes and did not change hyperglycemic phenotype. Considering that nicotine exposure accelerates bony complications of DM, thereby increasing the fracture risk, it will be very useful to restrict nicotine exposure in diabetic patients in order to limit the extent of diabetic degradation of bone structure.

P154

Osteocalcin administration improves glucose metabolism in a lean model of type 2 diabetes

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Bone, an organ primarily regarded as a dynamic scaffold, has key endocrine actions to regulate glucose metabolism. Through osteoblast-secreted osteocalcin on its uncarboxylated form (uOCN), bone controls glycaemia not only by stimulating insulin secretion by the pancreas, but also by increasing insulin sensitivity in peripheral tissues. Consequently, hindered uOCN levels are associated to insulin resistance, linking bone to type 2 diabetes (T2D).

In Goto-Kakizaki (GK) rats, a lean model of spontaneous T2D, we consistently observed diminished uOCN levels compared to age-matched healthy animals. Therefore, this study aimed to evaluate the chronic effects of uOCN administration on insulin sensitivity, and ultimately on the metabolic outcome of T2D, in both GK rats and their healthy control strain (Wistar Han).

For 7 weeks, 12-week old male diabetic and control rats were daily injected either vehicle or uOCN at a physiological dose (n = 10 animals/group), while their blood glucose levels, body weight, and food intake were weekly monitored. At the end of the treatment, intraperitoneal glucose tolerance and insulin sensitivity tests were performed, and the area under the curve (AUC) determined; blood samples and tissues were collected for analyses.

From 3-weeks of treatment, T2D animals receiving uOCN had a 30% reduction in their blood glucose levels compared to vehicle (p = 0.03), while healthy animals presented no effects of the treatment. Neither body weight nor food intake was altered by the treatment, but T2D animals receiving uOCN had significantly lower amount of visceral adipose tissue (p = 0.04). Improved insulin sensitivity was observed in T2D treated group, not only by the significant reduction of AUC on insulin sensitivity tests (p = 0.02), but also on HOMA-IR index, namely due to the reduction in plasmatic levels of insulin.

Our results support the importance of uOCN as an insulin-sensitizing hormone, and ultimately indicate the potential of bone as a therapeutic target in T2D.

P173

Far upstream element binding protein 3 expression is associated with bone formation

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Genome-wide association studies (GWAS) are one of the most powerful approaches to identify genetic loci that are associated with bone mineral density (BMD). GWAS have identified hundreds of associations with BMD; however, only few have been functionally evaluated, and functional characterization remains a challenge. One of the loci significantly associated with femoral neck BMD at genome-wide level ($p = 3.4 \times 10^{-22}$) is SNP rs7851693 from the intron of far upstream element binding protein 3 (FUBP3) gene. Here, we investigated a functional role of FUBP3 in bone remodelling. Variants mapping to FUBP3 were prioritized using GCTA and FINEMAP. Expression of FUBP3 in 47 osteoporotic and osteoarthritic human bone tissue samples was compared to healthy controls. The expression of FUBP3 was significantly decreased (p = 0004) in bone tissues from osteoporotic patients as compared to healthy controls. Furthermore, we examined FUBP3 expression in whole fish during zebrafish development and adulthood, and fin regeneration, by in situ hybridisation and Q-PCR. Two fold increase in FUBP3 expression (p = 0,003) in the newly formed zebrafish fins suggests that FUBP3 is involved in tissue regeneration and formation of bone tissues. Moreover, we also investigated expression of FUBP3 during osteogenic, adipogenic and myogenic differentiation of human mesenchymal stem cells. Indeed, silencing of FUBP3 inhibited osteogenic differentiation confirming the involvement of FUBP3 in the formation of osteoblasts. Altogether, our results suggest that FUBP3 plays an important role in bone biology and osteoporosis susceptibility in humans.

P174

Leveraging unconfounded genetic risk scores to stratify fracture risk by age at onset

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Aim: Increasing age is the strongest and most recognized risk factor of osteoporosis and fracture risk in both sexes. GWAS have identified up to 1103 independent genetic variants associated with bone mineral density estimated from heel ultrasound (eBMD). Under the principle of Mendelian Randomization, estimates derived from genetic risk scores (GRS) are robust against confounding across strata. We investigated in a population-based setting whether a GRS constructed from eBMD variants is a robust predictor of incident fractures and their age at onset.

Methods: We included in this study 11,351 participants of the Rotterdam Study with GWAS data and up to 20 years of fracture follow-up. Incident non-vertebral fractures were confirmed in 2153 (19%) individuals using GP records and hospital registries. GRS was constructed as a weighted sum of the number of eBMD-decreasing alleles at 1031 genetic variants. Cox regression and Weibull survival analyses were implemented to determine the association of the GRS and fracture risk and event time ratio (ETR) adjusting for sex.

Results: Every GRS standard deviation (SD) increment was associated with 20% increased risk (HR 1.20 95%CI 1.15–1.25; $P < 2x10^{-16}$) of fracture. When analyzed across GRS quintiles, non-skeletal anthropometric characteristics were randomized across strata. As compared to individuals in the middle GRS quintile who hold the mean population BMD and fracture risk, individuals in the highest GRS quintile had 1.5 increased risk of fracture (HR 1.52 95%CI 1.09–2.11; P = 0.01). Also, fractures occurred significantly earlier in individuals with the largest number of BMD-decreasing alleles (ETR = 0.95, 95%CI 0.91–0.99).

Conclusions: GRS are increasingly accessible tools providing robust and interpretable fracture risk estimates that are not prone to confounding and allowing risk stratification capturing age at fracture. We show how a sub-optimal epidemiological tool for fracture prediction (heel ultrasound) is leveraged by using a genetic framework.

Keywords: Genetic risk scores; BMD; Fracture

P192

Mid1 is a novel mediator of subchondral bone resorption in antigen-induced arthritis

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Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune joint disease characterized by subchondral bone destruction not reversible by currently available therapeutics. We have shown that mice deficient for Fas gene (Fas-/-) are protected from local bone resorption in antigen-induced arthritis (AIA), a murine model of RA, and have a lower frequency of synovial myeloid cells, which down-regulate *Mid1* gene. The objective of the study was to evaluate the role of *Mid1* in bone resorption in AIA.

Materials and methods: After receiving ethical approval, arthritis was induced by immunization of mice with methylated bovine serum albumin (mBSA) with subsequent intra-articular injection of mBSA. Synovial myeloid (CD11b + Gr− 1 +) cell transcriptome was analyzed by Affymetrix ST 2.0 arrays. Bioinformatics analysis was performed using Bioconductor. Differences in gene expression were confirmed by qRT-PCR. WT-AIA mice were treated in vivowith metformin, which inhibits proinflammatory effect of Mid1, at daily dose 1 g/kg, to assess effects on arthritis development.

Results: *Mid1* gene was up-regulated in myeloid cells (logFC = 2.01, p(BH-adjusted) = 0.0003, limma + BH-adjustment) and bulk joint tissue (logFC = 8.74, p = 0.02, Welch-test) of WT mice in comparison to Fas-/- mice with non-resorptive arthritis. Despite its position on X chromosome *Mid1* expression in joints was not sexually dimorphic and was up-regulated in WT-AIA in both male (logFC = 1.92, p = 0.006, T-test) and female mice (logFC = 8.74, p = 0.02, Welch-test). Furthermore, expression positively correlated with knee

diameter (r = 0.68, p = 0.03, Spearman´s rank correlation) and levels of pro-inflammatory cytokines in arthritis joints (IL-1: r = 0.78, p = 0.008; IL-6: r = 0.70, p = 0.025; TNF:r = 78, p = 0.008, Spearman´s rank correlation). Metformin treatment of WT-AIA mice ameliorated the severity of arthritis assessed by knee diameter (3.69 ± 0.21 mm WT-AIA vs. 3.36 ± 0.12 mm WT-AIA + metformin, p = 0.008, T-test).

Conclusions: Mid1 is a novel mediator of subchondral bone destruction in arthritis and its inhibition might present a new therapeutic target for inflammation-mediated joint destruction.

Keywords: Mid1, Bone resorption, Arthritis

P207

Impaired muscle fiber morphology in the Chihuahua zebrafish model of classical dominant osteogenesis imperfecta

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Muscle and bone build a powerful unit to facilitate movement, to provide structure and to protect organs. In disease states, both types of tissue may be compromised individually. While the genetic disorder of classical dominant osteogenesis imperfecta (OI) leads to brittle bones and increased fracture risk, patients may also present lower muscle mass and function. Although positive bone-muscle effects following exercise are likely, the exact link between bone quality and muscle function remains understudied. Elucidating the specific mechanisms in the bone-muscle unit is thus essential to pave the way for new treatment strategies of OI. The Chihuahua zebrafish (*Chi/+*) carrying a heterozygous glycine substitution in collagen I is an interesting model of OI showing drastically impaired bone structure, mechanical properties, and cellular indices. The aim of this study is to assess whether muscle tissue in the *Chi/+* model is similarly affected as the compromised bone.

Chi/+ and wild-type (WT) zebrafish (2 months old) were used for muscle characterization (N = 6/group). Histomorphometry was performed on 4 μm-thin transversal whole-body sections stained with H&E. Fiber morphology was assessed in the trunk muscles where fast- and slow-twitch fibers are located in the central and lateral sides, respectively. Two regions of interest per section were evaluated.

Results clearly point to smaller muscle fiber cross-sectional area in *Chi/+* mutants with 204 ± 37 μm^2 vs. 280 ± 18 μm^2 (p = 0.05) and higher fiber density in *Chi/+* with 464 ± 70 fibers/mm^2 vs. 360 ± 22 fibers/mm^2 (p = 0.05). Similar to human OI, *Chi/+* zebrafish show substantially altered muscle function besides impaired mineralized hard tissue indices.

This study highlights that understanding the bone-muscle interaction in physiological and pathological conditions is important for improving OI disease management and further underlines the use of biomechanical loading regimes for zebrafish to investigate the response of the whole musculoskeletal system to both conventional and pharmaceutical treatment.

Keywords: Bone-muscle axis, Osteogenesis imperfecta, Zebrafish

P282

The bone biomechanical response during sclerostin-neutralizing antibodies treatment is maintained in periostin knockout mice

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Sclerostin neutralizing Ab (Scl-Ab) potently stimulates bone formation but these effects are not sustained. Periostin (Postn), a matricellular protein, mediates the anabolic response to mechanical loading and is a Sost inhibitor[1]. We previously observed that Postn expression is repressed by Scl-Ab in mice but that mechanical loading is able to reactivate bone formation in these conditions, concomitant to increased Postn expression. We hypothesized that Postn expression levels play a direct role in the bone biomechanical response during treatment with Scl-Ab.

Sixty-five four month-old $Postn^{-/-}$ and $Postn^{+/+}$ mice received Scl-Ab (50 mg/kg/w) or veh for 4 to 6 weeks. At week 4, axial compression was applied on the left tibia 3 days per week for 2 weeks at 16 N. The right tibia served as non-loaded control. P1NP level was monitored every 2 weeks to assess bone formation and bone microarchitecture was evaluated by μCT on femur, tibiae and L3 vertebra.

With Scl-Ab, P1NP levels peaked at week 2 and declined thereafter in both genotypes. Scl-Ab increased Tb.BV/TV at femur and vertebrae as well as Ct.Th similarly in $Postn^{+/+}$ (+145%, +204%, +37%, respectively vs Veh, $p < 0.001$) and $Postn^{-/-}$ (+252%, +306%, +40%, respectively vs Veh; $p < 0.001$). Loading during Scl-Ab treatment further increased tibia Tb.BV/TV and diaphysis Ct.Th, without interaction by genotypes ($Postn^{+/+}$ +22% and +7%; $Postn^{-/-}$ +12% and +8% vs nonloaded tibia, $p < 0.01$). However a significant interaction between loading and genotype was noted for Ct.Th at tibia metaphysis ($Postn^{+/+}$ +15%, $Postn^{-/-}$ +4%, p Int < 0.05).

In conclusions, periostin is not required for the overall bone anabolic response to Scl-Ab nor its reactivation by mechanical loading. These results suggest that increasing strain could sustain Scl-Ab bone forming effects by further suppressing Sost expression, rather than stimulating Postn expression, allowing for an improved Scl-Ab/target ratio.

Reference: [1]Bonnet N. et al., JBC, 2009.

Concurrent Oral Poster Presentations 2—Clinical/Public Health

P236

The fracture predictive ability of lumbar spine BMD and TBS as calculated based on different combinations of the lumbar spine vertebrae

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Trabecular bone score (TBS), a surrogate of bone microarchitecture, is an independent predictor of osteoporotic fractures. It is measured in the lumbar spine (LS) DXA scans in the same regions of interest as BMD, L1-L4. We aimed to study whether different combinations of the lumbar vertebrae in the calculations of LS TBS and BMD perform differently in major osteoporotic fractures (MOF) prediction.

Study comprised 1475 postmenopausal women (mean age = 64.5y) of the Swiss population-based study, who had undergone questionnaires, vertebral fracture assessments, LS DXA scans (Hologic) and TBS (Medimaps, v4.0) measurements. T-tests were ran to test the differences in general characteristics between women who fractured and those who did not; binary logistic regressions adjusted for age + BMI or for age + BMI + LS BMD were performed to

study the odds ratios per one SD decrease in LS BMD or TBS, respectively; the areas under the curve (AUC) were then calculated for each model.

During the 5 years of follow-up, 125 women had a fracture. Fractured women were older, had higher BMI, and lower LS BMD and TBS. TBS was an independent predictor of MOF. The best lumbar vertebrae combination for TBS was L1–L2 (OR(95%CI): 1.81(1.35–2.44); AUC (95%CI): 0.716(0.654–0.778)), whereas the poorest combination was L3-L4 (OR(95%CI): 1.24(0.92–1.68); AUC (95%CI): 0.686(0.621–0.751)). Similarly, for BMD the L1-L2 combination also had the best performance in MOF prediction (OR(95%CI): 1.47(1.21–1.78); AUC (95%CI): 0.689(0.625–0.753)), whereas the L2-L4 had the poorest performance (OR(95%CI): 1.27(1.10–1.46); AUC (95%CI): 0.673(0.608–0.738)). Compared to the current used combination (L1-L4), L1-L2 and L1-L3 performed better for both BMD and TBS.

In conclusion, excluding L4 seemed to improve the fracture risk prediction in overall. L4 might be affected by spine lordosis, thus its accuracy is questionable. Nevertheless, further investigation is needed to clarify the possible causes for the differences in performance among the LS vertebrae combinations.

Keywords: Osteoporosis, Women health

P237

Balance function impairment and muscles strength deficiency in patients with osteoporotic vertebral fractures

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Background and aims: The aim of the study was to estimate the change of static and dynamic balance function and the lack of trunk muscle strength in osteoporotic patient with vertebral fractures (VFs).

Materials and methods: 90 patients aged 43–80 with primary osteoporosis (bone mineral density T-score in lumbar spine or femoral neck < -2.5 measured by DXA) were enrolled. Study group comprised of 60 subjects (56 women, 4 men) with at least 1 VF confirmed by X-ray. Control group included 30 subjects (28 women, 2 men) without any osteoporotic fracture. The examination program consist of stabilometry, back muscles tenzodynamometry, balance tests (Fukuda-Unterberger and One-leg-standing tests), functional tests (Up-and-go test, 10-meters-walk test, back and abdomen static and dynamic endurance tests).

Results: According to stabilometry study group was characterized by lower balance coefficient (BC) vs control group (77.0% vs 85.65%, p = 0.002), greater pressure center media-lateral (PC ML) deviation (1.2 vs − 1.2 mm, p = 0.025) and PC ML displacement (6.8 vs 4.8 mm, p = 0.01). Patients with VFs lose their balance faster during one-leg-standing test with open eyes (5.0 vs 7.5 s in control group, p = 0.05) and with closed eyes (2.0 vs 3.5 s, p = 0.05). Fukuda-Unterberger test showed greater side dislocation in study group (40°) vs controls (30°, p = 0.02). Muscle strength deficiency was estimated in study group in trunk flexors (TF) − 40.93% and in trunk extensors (TE) − 18.12% with an adequate function of the left lateral flexors (LLF) and in right lateral flexors (RLF). Patients with VFs had the lower muscle strength vs controls of TF (− 27.73 kg, p = 0.000), TE (− 21.28 kg, p = 0.000), LLF (− 24.06 kg, p = 0.005) and RLF (− 24.26 kg, p = 0.000). No significant difference between results of functional tests were registered ($p > 0.05$).

Conclusions: VFs in osteoporotic patients negatively affect static and dynamic balance function and are associated with reduction of trunk muscles strength.

P260

Men with COPD have increased risk of vertebral fractures independent of smoking: a prospective cohort study

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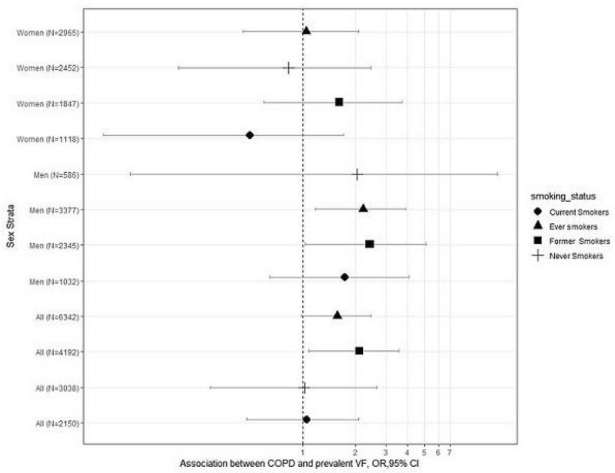
Individuals suffering from chronic obstructive pulmonary disease (COPD) have increased risk of osteoporosis and osteoporotic vertebral fractures (OVF). Nevertheless, the role that smoking plays in this relationship remains unknown. We aimed to estimate within a population-based cohort of elderly men and women the risk of OVF in subjects with COPD and the role thereof of smoking. OVF were diagnosed based on presence of endplate depression according to the algorithm based qualitative method (ABQ) and COPD was diagnosed based on an obstructive spirometry and in absence of spirometry, based on GP records. Sex-specific logistic regression models were used, to correct for age, BMI, FN-BMD, anti-osteoporotic, anti-diabetes treatment, corticosteroid use and smoking status. Analyses were additionally stratified for smoking status into: never, current, former and ever smokers. The prevalence of OVF was 3.1% among 5417 men and 5.2% among 3963 women; whereas of COPD 5.6% and 3.6%, respectively. Individuals with COPD had lower FN-BMD (0.902 vs. 0.870, p-value < 0.001) compared to individuals without. Smoking categories included never (32.4%), current (22.9%) and former (44.7%) smokers. Overall, COPD was associated with increased risk of OVF among men (OR = 2.05, 95% CI = 1.16; 3.62) but not among women (OR = 1.02, 95% CI = 0.66; 1.93). Correction for smoking did not essentially modify these associations. Further, in ever smoking men, COPD was associated with increased risk of OVF (OR = 2.49, 95% CI = 1.13; 5.46) (Figure). In conclusion, COPD is associated in men with increased risk of OVF independent of smoking status with other underlying mechanisms remaining to be elucidated.



[Association between COPD and prevalent OVF]

P261

Gut microbiota analysis and its skeletal effects in a sample of elderly subjects from a prospective population-based study

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A shift in gut microbiome composition is involved in many metabolic disorders. Recently, experimental data suggested that gut microbiome plays a relevant role in triggering inflammatory pathways that are critical for osteoclast activation and bone resorption. However this remains to be demonstrated in humans. Thus we performed a preliminary analysis of gut microbiome in a restricted sample of postmenopausal women and elderly men from an ongoing prospective population-based study. The study was approved by the local Ethical Committee. We selected 12 patients with osteoporosis and 12 age and sex matched controls with normal BMD. Briefly, the V4 region of the 16S ribosomal RNA gene was amplified from total faecal DNA samples and sequenced on an Illumina MiSeq instrument to characterize microbial communities. Starting from taxon relative abundance, within- and between-sample diversity (alpha and beta diversity, respectively) was analysed, using unsupervised learning techniques such as clustering and principal coordinates analysis (PCoA). A total of 23 genera and 13 families with proportions above 1% were detected in the overall sample with consistent interindividual variability and without major differences across genders. The OTU level rarefraction curves reached plateau phase, indicating that most bacterial species had been captured in all samples. There was no evidence of differences in the Shannon index (index of species richness and evenness) between osteoportics and controls. At the family taxa level, we observed trends for different proportions of Bacteroidaceae and Prevotellaceae between osteoporotic patients and controls, while a statistically significant increase in Enterobacteriaceae was observed in osteoporotics ($p < 0.01$). With regard to beta-diversity, PCoA analysis evidenced a substantial overlap between osteoporotics and controls while demonstrated a better discriminative capacity concerning patients with increased bone turnover that underwent substantial bone loss from baseline to follow-up (9–12 years), suggesting that microbiome may be a critical factor in the pathogenesis of high-turnover osteoporosis.

P283

Medical management of atypical femur fractures: a systematic literature review

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Atypical femur fractures (AFFs) are considered serious side effects of bisphosphonates and often show delayed or non-healing. After an AFF, bisphosphonates are usually discontinued. It is unclear if certain osteoporosis drugs can promote healing and how patients at high risk of fragility fractures should be treated after AFF. We performed a systematic literature review to evaluate the effect of teriparatide, raloxifene and denosumab both on healing and occurrence of AFF in order to advise on medical treatment after AFF. We retrieved 910 references and reviewed 66 papers, including 31 case reports, eight retrospective cohort studies and three prospective studies that reported the effect of teriparatide, but no randomized controlled trials. We pooled data on reported fracture union from these articles and found that radiological healing occurred within six months of teriparatide use in 13 of 29 conservatively treated incomplete AFFs (45%), nine of 10 incomplete AFFs with surgical intervention (90%) and 41 of 55 complete AFFs (75%). In nine of 29 incomplete AFFs on conservative treatment (31%) no union was achieved after 12 months of teriparatide and progression to complete fractures occurred in 4 patients. New AFFs occurred in six patients during or after teriparatide, but always after prior use of antiresorptives. AFF on denosumab was reported in 21 patients, including 11 patients treated for metastatic bone disease and seven without prior bisphosphonate exposure. Continuation or initiation of denosumab after AFF was associated with recurrent incomplete AFFs in one patient and two cases of contralateral complete AFF. Eight patients had used raloxifene prior to AFF, including one patient without prior bisphosphonate use. We conclude that teriparatide may lead to faster healing of surgically treated AFFs, but not of conservatively managed incomplete AFFs and we present options for treatment of osteoporosis after AFF in different scenarios.

P284

Impact of renal function on BMD response to bisphosphonate treatment: real world observational data using linkage to national registers

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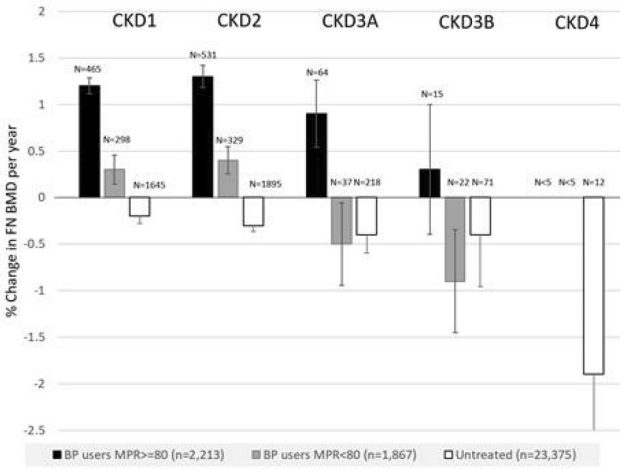
Background: Oral and intravenous bisphosphonates (BP) are contraindicated in severely compromised renal function (crea clearance < 30–35 ml/min) and a large proportion of patients referred for DXA scans have reduced renal function. The impact of milder degrees of renal function impairment on BMD gains in a real-world population is unknown.

Methods: The study population consisted of 4080 men/women who began oral or i.v. BP treatment following DXA, divided into high adherence (MPR >=80%, N = 2,213) and suboptimal adherence (MPR < 80%, N = 1,867). FN BMD change was calculated in patients who had an eGFR measurement and a DXA scan in the last year before initiating treatment, and where a follow-up DXA was available 2 to 3 years after treatment start (N = 1,761). BMD changes could be similarly tracked in 3,841 untreated persons. To reduce bias, the same person could be followed as untreated until osteoporosis treatment.

Results: Differences in characteristics included PPIs (22% for MPR >=80%, 28% for MPR < 80%), glucocorticoid use (29.2% vs 38.7%), diabetes (7.8 vs 5.6%). Though femoral neck BMD improvements were smaller in patients with eGFR < 60 (stage 3A) and largely absent with eGFR < 45 (stage 3B), the negative BMD change seen without treatment or with suboptimal adherence was avoided. In accordance with the contraindication, BP use in persons with baseline eGFR < 30 was practically non-existent.

Discussion: While femoral neck BMD improvement on BPs was smaller in stage 3A or worse, a benefit for adherent BP users in terms of preservation of femoral BMD was observed even in stage 3B (eGFR 30–44).



[Fig 1]

P304

Lumbosacral transitional vertebrae and the association with low back pain and spine degeneration: the Rotterdam study

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Purpose: Lumbosacral transitional vertebrae (LSTV) are congenital spinal anomalies, in which an elongated transverse process of the last lumbar vertebra fuses to the "first" sacral segment. The presence of LSTV may alter spine biomechanics. Consensus whether an LSTV is related to symptoms and degeneration is lacking. We determined the prevalence of LSTV in a population-based study and assessed the relationship with chronic low back pain and lumbar disc degeneration (LDD).

Methods: On anteroposterior pelvic radiographs, presence and type of LSTV was scored using Castellvi's classification, distinguishing non-fused LSTV (type I and II) and fused LSTV (types III and IV). Chronic low back pain was defined as reported low back pain lasting more than 3 months in conformance with the WHO definition. LDD (L1-L4) was radiographically defined as presence of osteophytes and disc space narrowing, using a semi quantitative scoring method. Logistic regression was adjusted for age, sex and BMI to analyze the association between presence of an LSTV and its subtypes and chronic low back pain and LDD.

Results: Among 4896 individuals, the prevalence of LSTV was 13.8% and higher in males compared to females (56.9% vs. 43.1%, $P < 0.001$). The presence of LSTV (all types) did not show a significant association neither with chronic low back pain (OR: 1.25; 95% CI: 0.99 to 1.59) nor with LDD (OR: 1.20; 95% CI: 0.92 to 1.52). Nevertheless, non-fused LSTV (type I and II) were associated with chronic low back pain (OR: 1.40; 95% CI: 1.03 to 1.90) but not the fused LSTV (OR: 1.04; 95% CI: 0.72 to 1.50).

Conclusions: The presence of an LSTV was relatively common in this study in the general population and more frequent in men compared to women. Furthermore, we found that non-fused types LSTV are associated with chronic low back pain, suggesting unfavorable biomechanics.

P307

Clinical, biochemical and radiological profile of normocalcaemic hyperparathyroidism: a multicentric cross-sectional evaluation

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Background: The normocalcaemic hyperparathyroidism(NHPT) has been defined as a condition with persistently normal total and ionized calcium levels associated with high levels of PTH. Various studies presented clinical aspects of NHPT, but the interpretation of these findings is confounded by differing methods used to exclude secondary hyperparathyroidism and by the small number of NHPT subjects enrolled.

Objectives: To assess the clinical, biochemical and radiological profile of NHPT in comparison with PHPT and control subjects.

Methods: We enrolled in a multicentric cross-sectional study patients with NHPT and primary hyperparathyroidism(PHPT) diagnosed according to recent guidelines. Body mass index(BMI) and age matched control subjects were consecutively recruited. All patients underwent a biochemical examination including calcium-phosphorus metabolism and bone turnover markers. We assess the lumbar spine(L1-L4), total hip, femoral neck, and non-dominant forearm bone mineral density(BMD) and the trabecular bone score(TBS). Morphometric vertebral fractures(VF) were assessed by DXA-scan.

Results: from December 2016 to July 2018, we identified 47 patients with NHPT, 41 with PHPT and 39 control subjects. All study groups presented similar age, BMI and kidney function. NHPT and PHPT patients had significantly higher PTH and 25(OH)-Vitamin D levels($p < 0.001$) and lower Ca*P($p < 0.001$) than controls. NHPT has lower CTX levels confronted with PHPT($p = 0.039$). NHPT($p = 0.035$) and PHPT($p = 0.003$) group have lower total hip BMD than controls; NHPT showed higher non-dominant forearm BMD than PHPT subjects($p = 0.017$), while compared to controls presented similar values. All study groups presented no significant differences in TBS. After adjustment for confounding factors, only PHPT group had an increased risk of VF than controls(OR:5.10, 95%CI:1.34 to 21.58). 31% of NHPT and 12% of PHPT patients fulfilled the criteria for asymptomatic hyperparathyroidism.

Conclusions: Until now, our study described the biochemical and radiological profile of the largest cohort of NHPT subjects. Our findings suggest that the profile of NHPT subjects is closer to control one.

Keywords: Normocalcaemic hyperparathyroidism, PTH

P308

Vitamin D supplementation in pregnancy: a randomized double-blind controlled trial

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To investigate the efficacy and safety of vitamin D (VD) supplementation, in the doses of 600, 1000, 2000, and 4000 IU per day on maternal (pregnancy weight gain, pre-eclampsia, gestational diabetes), fetal growth and newborn (newborn head circumference, birth weight, length and VD level) parameters.

This randomized double blind active controlled clinical trial was carried out in pregnant subjects, aged 18–40 years with gestational age between 12 and 16 weeks. Subjects randomized into four groups (Group 1—active control group received 600 units of VD per day; Group 2—1000 units/day; Group 3—2000 units/day; Group 4—4000 units per day). All groups received 1000 mg of elemental calcium. Primary outcome was changes in VD status of mother and newborn.

Total 243 subjects who completed the study were analyzed. High prevalence of VD deficiency was seen in study population (93.6%). S.VitD level improved significantly in all four groups except group 1. Cord blood S.VitD levels were significantly higher in Gr 2, 3 and 4 in comparison to Gr 1. The highest cord blood S.VitD level was seen in Gr 4 (41.38 ± 14.71 ng/ml) while lowest was in Gr 1. No significant difference was observed among all four groups in any other maternal, fetal and newborn parameters including insulin resistance in mother as well as in cord blood. No adverse effect observed.

Our study shows that supplementation of VD in mother started at 16 weeks, improves VD status of newborn. However, VD supplementation during pregnancy did not show effect on any other maternal, fetal and newborn parameter (maternal wt gain, pre-eclampsia, gestational diabetes, fetal growth, newborn head circumference, length, and weight and insulin resistance in mother at the time of delivery). Supplementation of VD at this dose is found to be safe without any hypercalcemia or hypercalciuria.

P313

-308G/A Tumor Necrosis Factor Gene Polymorphism May Influence Bone Mineral Density in Pediatric Inflammatory Bowel Diseases

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Disturbances of bone metabolism in inflammatory bowel diseases (IBD) can include osteoporosis, increased risk of low-energy fractures and linear growth failure and have multifactorial nature: systemic inflammation, malabsorption, vitamin D deficiency, corticosteroid (CS) therapy and some genetic factors.

The aim of our study was to evaluate differences in bone metabolism related to the presence of the polymorphic genotypes in children with IBD.

Materials and methods: 83 children with IBD (45 M and 38F) were included in the resent study. Lumbar spine BMD (DEXA), serum osteocalcin (OC), C-terminal telopeptides (CTT), 25-$OHD_3$and fecal calprotectin (CP), cumulative corticosteroid dosage were measured. The -308G/A tumor necrosis factor (TNF) gene polymorphism was detected with restriction fragment length polymorphism assay. Due to rare frequency of mm genotypes the patients were divided in 2 groups: carriers of nn and carriers of nm + mm (m-allele carriers). Data presented in the median and interquartile range (25%; 75%).

Results: Patients with "m" allele had more active IBD course (higher level of PIABC, CRP, cumulative doses of corticosteroids), exclude calprotectine level. Data about bone metabolism are in the table. No differences in the levels of calcium, phosphorus and alkaline phosphatase.

Conclusions: Patients with "m" allele of -308G/A TNF gene associated with lower bone mineralization and may be a marker of poor prognosis of IBD. Further trials are necessary.

Parameter	nn (n = 64)	nm + mm (n = 19)	p
Age, y	13.0 (10.0;15.0)	14.0 (12.0;16.0)	0.10
BMD, Z score, SD	− 1.2 (− 2.0; − 0.5)	− 1.9 (− 3.1; − 1.3)	0.016
BMD deficiency, %	14.0 (0.0; 24.0)	21.0 (15.0; 35.0)	0.03
PTH	39.7 (28.9; 48.0)	51.4 (35.2; 90.0)	0.15
Osteocalcin	62.7 (23.1; 134.5)	77.8 (32.3; 226.0)	0.42
25(OH)D	17.6 (14.3; 21.6)	14.8 (10.3; 17.6)	0.04
CTT	1.1 (0.8; 1.5)	1.2 (0.76; 2.0)	0.46

[Bone metabolism differences due to TNF-gene polymorphism]

Keywords: Osteoporosis, IBD

P337

Long-term bone mineral density changes after surgical cure in patients with tumor Induced osteomalacia

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No systematic data are available regarding long-term bone mineral density changes after surgical cure, in patients with tumor induced osteomalacia.

We studied ten consecutive cases (mean age ± SD: 45.5 ± 13.8 years; 5 males and 5 females). Basal BMD and corresponding Z-score values measured by DXA (Hologic QDR 4500A) were: L1-L4 = 0.692 ± 0.15 g/cm^2, Z-score = − 2.80 ± 1.60; femoral neck = 0.447 ± 0.10 g/cm^2, Z-score = − 2.66 ± 0.93; total femur = 0.450 ± 0.08 g/cm^2, Z-score = − 3.04 ± 0.85). Furthermore, Trabecular Bone Score (TBS) was evaluated in three patients (basal values: 0.990 ± 0.32).

Seven patients were intermittently followed after surgical excision of the tumor while supplemented with cholecalciferol and calcium salts; the remaining three were lost at follow-up. There was a striking increase of BMD values that peaked at 26.7 ± 6.50 months: L1-L4 = 1.289 ± 0.247 g/cm^2, $p < 0.001$, Z = score +1.75 ± 1.42; femoral neck = 0.890 ± 0.235 g/cm^2, p = 0.028, Z-score = + 0.50 ± 1.40; total femur = 0.834 ± 0.150 g/cm^2, p = 0.005, Z-score = − 0.74 ± 1.14). In patients with the greatest bone involvement at lumbar site, there was a striking increase of an average 1.5% ($p < 0.01$) in respect to baseline Z-score value for each additional month of observation during the first 2–3 years' post-surgery. An improvement of trabecular microarchitecture was also documented (TBS: 1.255 ± 0.16).

To the best of our knowledge, this is the first case series documenting an impressive increase of BMD at both lumbar and femoral sites, together with an improvement of trabecular microarchitecture as documented by TBS. This is the consequence of huge mineralization of the large amount of osteoid tissue after resolution of the disease.
Keywords: Tumor Induced Osteomalacia.

Poster SNAPs Presentations 1—Basic/Translational

P031

Osteoblast-derived extracellular vesicles drive mineral nucleation in stem cell cultures

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Extracellular vesicles (EVs) are lipid-enclosed nanoparticles (30–500 μm) with important roles in early mineralisation. We sought to determine the regenerative capacity of osteoblast-derived EVs we to develop an acellular yet biological approach for hard tissue regeneration. Secreted EVs were isolated from the culture medium of mineralising osteoblasts (16/SS/0172) over 3 weeks. EV size and concentration was defined using nanoparticle tracking analysis, transmission electron microscopy (TEM) and CD63 ELISA. Temporal variations in the EV proteome were analysed using liquid chromatography tandem-mass spectrometry (LC–MS/MS). The capacity of 5 μg/mL EVs to induce in vitro mineralisation in stem cell cultures was assessed against a clinical gold-standard, BMP-2. Differentiation was assessed by alizarin red calcium staining and alkaline phosphatase (ALP) quantification. Mineral phase was analysed using X-ray fluorescence (XRF) and infrared spectroscopy (IR). EVs ($Alix^+$/$TSG101^+$/$Annexin\text{-}2^+$/$CD63^+$) significantly ($P < 0.05$) enhanced ALP levels, mineralisation rate and mineral volume beyond BMP-2. XRF elemental mapping showed enriched areas of calcium and phosphorus co-localisation in EV supplemented cultures. IR analysis of the mineral phase confirmed the presence of octacalcium phosphate (OCP). Principal component analysis and accompanying TEM-coupled energy dispersive X-ray spectroscopy (EDX) localised mineralisation to the EV phospholipid membrane, implicating EVs as sites of nucleation. EV concentration, protein content and size correlated with osteoblast differentiation status. All EVs displayed a bimodal size distribution (week 1 and 2, 44 nm and 164 nm; week 3, 59 nm and 220 nm) with significantly fewer EVs generated as mineralisation advanced (week 3). Proteomic analysis of EVs revealed the presence of bridging collagens, calcium chelating proteins and extracellular binding proteins. The relative intensity of these proteins related to these biological processes was significantly ($P < 0.05$) upregulated at week 3. Our data suggests that EVs function as sites of mineral nucleation within stem cell cultures and hold considerable potential as an acellular yet biological approach to regenerative medicine.

P043

A novel algorithm to predict bone changes based on physiological loading in a preclinical murine model

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Understanding how bone adapts to mechanical stimuli is fundamental for optimising treatments against musculoskeletal diseases. Current algorithms focus on trabecular bone remodelling at the tissue level. This study proposes a novel algorithm to predict cortical bone changes based on physiological loading in a preclinical mouse model.

In vivo micro computed tomography (microCT) scans (10.4 μm/voxel) of the whole right tibia of five C57Bl6/J female mice were performed at weeks 14 and 16 of age [1]. The experiments were approved by the ethics committee at University of Sheffield. Linear isotropic homogeneous microCT-based finite element (microFE) models were created from the bone mineral density (BMD) calibrated microCT images. Physiological loading was applied. The surface elements of bone and adjacent background were permitted to undergo resorption or apposition, based on different local mechanical stimuli (strain energy density, SED, or maximum principal strain, ε_{p1}) with or without a lazy zone by assuming a linear relationship between the change in BMD and the mechanical stimulus. The parameters of the algorithm were tuned for each mouse. The predicted bone volume and spatial distribution of remodelling were compared with the experimental measurements.

The total predicted bone volume was similar (85%) for both stimuli (Fig. 1). SED performed better than ε_{p1} as the spatial distribution of bone densification matched at $98.2 \pm 0.9\%$ and $86.6 \pm 13.3\%$ respectively. The tuned threshold and rates reflected intra-specimen variation (SED threshold:0.486 ± 0.583 MPa; rate:0.154 ± 0.088 mg/cc/week). This algorithm shows potential for further use in pre-clinical studies, where prediction of bone growth and adaptation is important.

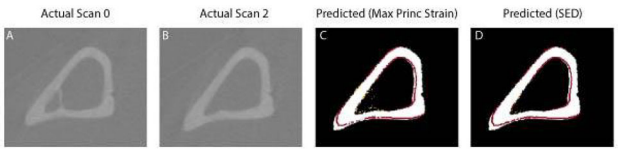

Fig.1 Difference between using maximum principal strain and SED as stimulus to predict bone formation for a slice at 60% of the bone length. (A-B) Actual slice obtained at week 14 (Scan 0) or 16 (Scan 2) of age. (C-D) Predicted bone remodelling overlaid on the binary image of actual scan 2. Red: bone apposition. Yellow: bone resorption.

References: [1]Lu et al. J Biomech 49:2095-9,2016

P076

Osteosarcoma cell-derived exosomes affect tumor microenvironment by specific packaging of microRNAs

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Bone microenvironment provides growth and survival signals essential for osteosarcoma (OS) initiation and progression. OS cells regulate communications inside tumor niche through different ways and, among all, tumor-derived exosomes have been described for their support to tumor cell dissemination and early events in metastasis. To define the contribution of OS cell-derived exosomes inside the tumor microenvironment, we investigated the effects induced in bone remodelling mechanism and tumor angiogenesis. Indeed, we demonstrated that exosomes induced osteoclasts differentiation by qRT-PCR (*Trap*, *Ctsk* and *Mmp9*; $p < 0.0005$) and by TRAP staining assay ($p < 0.0005$) and sustained bone resorption activity by dentine discs assay ($p < 0.0005$). In addition, we provide evidences that exosomes potentiated tumor angiogenesis via tube formation assay of Huvec cells ($p < 0.0005$) and increased angiogenic markers expression by qRT-PCR analysis (VEGF-A, IL-8 and IL-6; $p < 0.0005$). We therefore investigated the miRNA cargo from OS cell-derived exosomes and their parental cells by performing small RNA sequencing through NGS Illumina platform. Hierarchical clustering highlighted a unique molecular profile of exosomal miRNA; moreover, bioinformatic analysis by DIANA-mirPath revealed that miRNAs identified take part with various biological processes and carcinogenesis. Among these miRNAs, some were already known for their involvement in the tumor microenvironment establishment, as miR-148a and miR-21-5p. Finally, we found that enforced expression of miR-148a and miR-21-5p in pre-osteoclastic Raw264.7 cells (by qRT-PCR, $p < 0.05$ and by dentine discs assay, $p < 0.0005$) and Huvec cells (by tube formation assay, $p < 0.0005$) induced similar effects to those observed after exosomes treatment. Overall, our study showed that OS-derived exosomes influence crucial mechanisms inside tumor niche, also by a specific packaging of miRNAs.

P103

hMSC-TERTS^{CAS9}: a versatile tool to study osteoblast gene function

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Primary human bone marrow stromal cells (BMSCs) have been considered as an ideal source for the use in regenerative medicine. However, studying these cells in vitro is challenging due to inter-donor variations, their limited expansion capacity and the induction of cellular senescence. Although gene knockdown studies using siRNA/shRNA have been applied successfully in BMSCs, it may result in residual gene activity with heterogeneous effects between different knockdown constructs and individual cells. The aim of the current study is to generate Cas9 expressing BMSCs that can be used to generate genomic gene disruptions using CRISPR-Cas and applied in functional studies of osteoblast differentiation.

Immortalized BMSCs (hMSC-TERTs) were transduced with a lentiviral construct expressing spCas9 in the absence of guideRNAs (gRNA). hMSC-TERTs with high spCas9 expression were selected with puromycin. For the generation of gene disruptions, hMSC-TERTs^{Cas9} were transiently transfected with 1 or more gRNA constructs that target the genomic region of interest. Transfected cells were single cell sorted, clonally expanded and gene disruptions were analysed by Sanger sequencing.

We have successfully applied hMSC-TERTs^{Cas9} to knockout several transcription factors and a cell surface receptor by generating indels within coding regions resulting in a premature stopcodon. Even larger genomic deletion (200 bp) were established by co-transfection of 2 different gRNA constructs. Complete removal of the vitamin D receptor illustrated that these cells do not respond anymore to vitamin D and were unable to differentiate into mineralizing osteoblasts. Additionally, we were able to generate homo- and heterozygous gene disruptions of FGFR2 and illustrate that the presence of 2 functionally intact alleles are essential for normal osteoblast differentiation in hMSC-TERTs^{Cas9}.

hMSC-TERTs^{Cas9} are an efficient tool to study the effects of gene disruptions during osteoblast differentiation. We are currently generating base-editing variants that allow single nucleotide modifications to generate hMSC-TERTs that mimic monogenetic bone diseases in vitro

P104

Syndecan-1 deficiency influences bone cell communication in vivo and in vitro in Mice

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Syndecan-1 is a cell surface heparansulfate-proteoglycan that is able to bind OPG at its extracellular glucosaminoglycan chains and influences bone destruction during multiple myeloma. We investigated Syndecan-1 as a regulator of bone cell communication under native conditions using a Syndcan-1 deficient mouse model.

Syndecan-1 is expressed in primary osteoblasts and osteoclasts in vitro and blood serum levels of soluble Syndecan-1 increased during aging in mice (4–18 month). mRNA expression of Syndecan-1 is upregulated during osteoclastogenesis or in presence of osteoclastogenesis inducing factors ($1\alpha,25(OH)_2$Vitamin-D3 + Prostaglandin-E2) in osteoblasts that are known to regulate RANKL and OPG expression. In cocultures of primary osteoblasts and osteoclasts Syndecan-1-deficient osteoblasts released high concentrations of OPG which prevent osteoclast development (see figure). Syndecan-1 deficient mice show no obvious differences in bone structure compared to aged matched wild type animals (female, 4–18 month), but significant

higher amount of osteoclasts on trabecular bone surface of aged mice (18 month WT:1,54cells/mm; sdc1-/-: 5,61cells/mm). Syndecan-1 deficient trabecular bone show less OPG positive osteocytes (cell number per mm^2, mean ± SD: 76 ± 99) or RANKL positive osteocytes (68 ± 38) at the age of 4 month compared to wildtype, but much more RANKL positive osteocytes in aged vertebra (238 ± 90), even more than OPG positive osteocytes (183 ± 58).

Under conditions of bone formation Syndecan-1 seems to have a minor function. Strikingly, under osteoclast inducing conditions (in vitro, aging) the presence of Syndecan-1 seems to influence the communication between osteoblasts/osteocytes and osteoclasts via the OPG/RANKL ratio.

Keywords: Syndecan-1, Osteoblast, Osteoclast, Osteoprotegerin, Receptor-Activator-of-NF-κB-Ligand

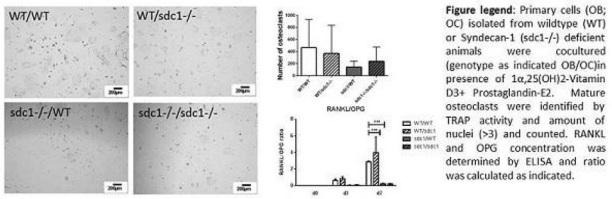

Figure legend: Primary cells (OB; OC) isolated from wildtype (WT) or Syndecan-1 (sdc1-/-) deficient animals were cocultured (genotype as indicated OB/OC)in presence of 1α,25(OH)2-Vitamin D3+ Prostaglandin-E2. Mature osteoclasts were identified by TRAP activity and amount of nuclei (>3) and counted. RANKL and OPG concentration was determined by ELISA and ratio was calculated as indicated.

[Syndecan-1 Deficiency Influences Bone Cells Communication]

P119

Macrophage-derived thymidine phosphorylase promotes osteoclastogenesis and bone resorption in inflammatory osteolysis

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Impairment of bone remodeling typified by increased activity of osteoclasts is the principal feature of destructive bone diseases, including rheumatoid arthritis, Paget´s *disease*, bone metastasis and periprosthetic osteolysis. In these diseases, immune cells produce multiple inflammatory mediators that aid in differentiation and activation of bone-resorbing osteoclasts, resulting in focal bone erosion or osteolysis. A better understanding molecular pathogenesis of osteolysis offers fundamental knowledge for developing potential therapeutic intervention. In the current study, thymidine phosphorylase (TYMP) was identified as common factor associated with osteoclastogenesis in vitro (Figure. 1). Interestingly, TYMP was dominantly expressed in the tissues surrounding loosened hip-implant, suggesting its role in osteolysis (Figure. 2). Consistently, murine calvarial osteolysis model showed that TYMP induced osteolytic lesions comparable with these induced by RANKL (Figure. 3). Together, our data highlight a new molecular target for therapeutic implications of bone diseases associated with inflammatory osteolysis.

Keywords: Inflammatory osteolysis, Osteoclast, Thymidine phosphorylase

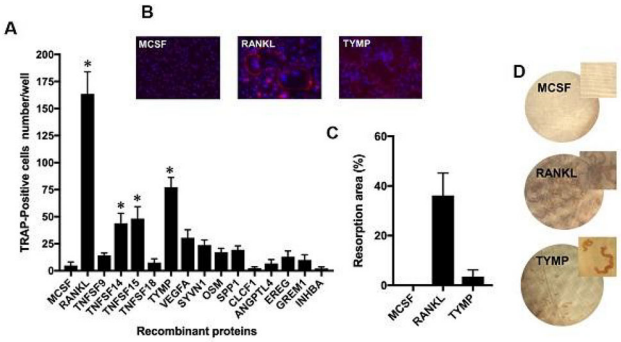

Figure 1. Identification of TYMP as osteoclastogenesis associated factor.
A) Effect of selected molecules on differentiation of monocytes into osteoclasts in vitro.
B) Actin ring staining for cells stimulated with growth factors including TYMP.
C) Quantification of bone resorption pits.
D) Representative images for bone resorption assay.

[Figure 1.]

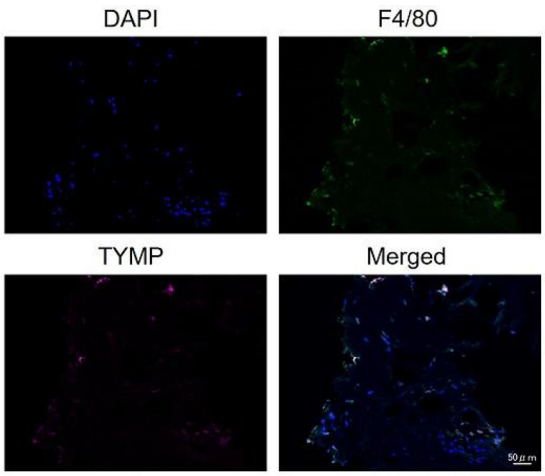

Figure 2. Fluorescence microscopic examination of synovial tissues from patient with hip-implant loosening. Sections were stained for F4/80 (green), TYMP (bright-far red), and cell nuclei (blue). Scale bars 50 μm.

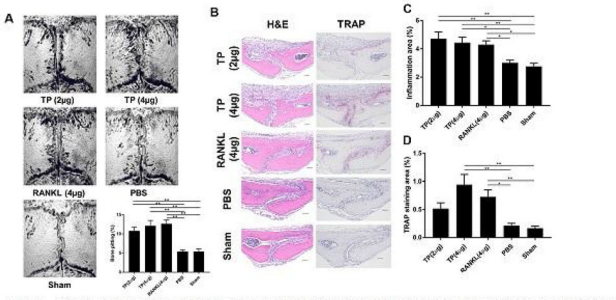

Figure 3. Effects of TYMP on bone resorption in calvarial osteoporosis murine model. A) Micro-computed tomography assessment of calvariae. Representative images for calvarial tissues and quantification of lytic areas. B) Representative images for histological observations of bone sections stained by H&E and TRAP. C & D) Quantification of inflammatory cells and TRAP-stained areas in calvarial bone sections. Scale bar 50 μm.

P120

miR-342-3p regulates the generation of osteoclasts

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Beyond their crucial role in bone remodeling, osteoclasts (OCL) are critical to immune responses. Heterogeneity of OCL precursors impacts on their capacity to polarize the immune adaptive response. It has been shown that monocytes (MN) and dendritic cells (DC) differentiate into OCL, which display tolerogenic or inflammatory properties, respectively. The molecular mechanisms driving OCL functional heterogeneity remain however unknown.

Objectives: To identify markers that discriminate MN-derived OCL and DC-derived OCL, and to investigate their role in OCL differentiation

Methods: MN-OCL and DC-OCL were generated from $CD11b^+$ and $CD11c^+$ cells isolated from mouse bone marrow, respectively (n = 10/group). OCL were also derived from the murine RAW264.7 cell line. Total RNAs were extracted and miRNA transcriptomic analysis performed. Markers were validated using RT-qPCR. Gain- and loss-of function studies of miRNAs were performed. RT-qPCR of OCL genes, TRAP staining, osteo-assay, apoptosis, mobility and acting ring formation were monitored during OCL differentiation.

Results: MiRNome analysis identified a 19-miRNA signature discriminating MN-OCL from DC-OCL (p-value < 0.05, FC > 2). Among these, miR-342-3p was fivefold expressed in DC-OCL than in MN-OCL (p = 0.018). RT-qPCR confirmed miR-342-3p as discriminating marker of DC-OCL and MN-OCL (p = 0.0013, AUC = 0.901). During OCL differentiation, miR-342-3p expression progressively decreased. Overexpression of miR-342-3p increased OCL differentiation as compared with controls, while miR-342-3p neutralization markedly reduced OCL differentiation. This later effect was due to increased apoptosis and reduced mobility of OCL progenitors.

Conclusions: Our study identified miRNAs able to discriminate OCL subsets and suggested that miR-342-3p might play a role in the inflammatory DC-OCL functions by promoting OCL differentiation [Lozano et al.]

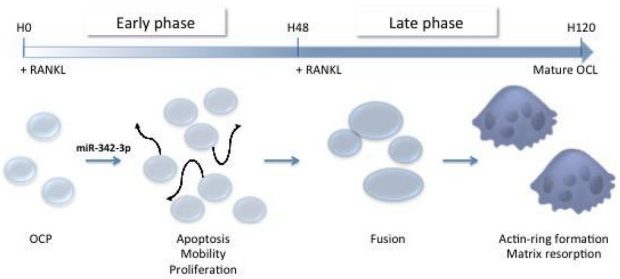

Figure 1: Schematic representation of the regulatory role of miR-342-3p during osteoclast differentiation. Our data suggest that high expression levels of miR-342-3p are necessary to promote osteoclast (OCL) differentiation, and that miR-342-3p controls early events including apoptosis and mobility of OCL progenitors (OCP).

P125

Relationship between the fluid flow pattern through the lacunocanalicular network and adaptive mechano-response in mouse tibia

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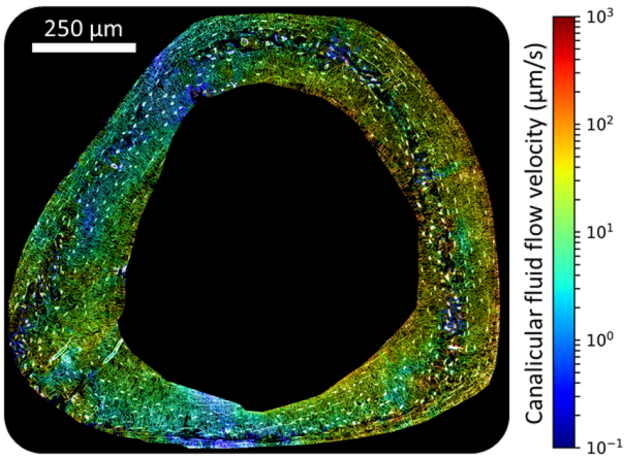
Osteocytes play an important role in bone mechano-sensation, a process crucial for bone maintenance and adaptation to changing mechanical demands. Their cell bodies and processes reside in a network of cavities and sub-micrometer wide canals called lacunae and canaliculi, respectively. According to the widely supported fluid flow hypothesis mechanical load induces interstitial flow through the lacunocanalicular network (LCN), resulting in shear force on the osteocytes. Time-lapse microCT combined with finite element modeling showed site-dependent mechano-response in mice subjected to controlled loading[1]. The aim of our study was to analyze and model LCN fluid flow to assess the relationship between LCN topology and local differences in mechano-response in mouse tibiae.

In vivo loading (left tibia loaded, right tibia control) of C57BL/6 mice (n = 5) was performed to elicit a bone formation/resorption response, detected via time-lapse microCT. Mice were sacrificed, tibiae were stained with rhodamine and the LCN in whole tibia cross-sections were imaged using confocal scanning laser microscopy. Circuit theory was used to model fluid flow through each canaliculus of the LCN (fig. 1). Our analysis shows that both the LCN topology and vascular porosity have a major influence on interstitial fluid flow patterns, which correlate with local bone formation/resorption data ($P < 0.001$, $R^2 = 0.6$). In conclusion, our results show that the LCN network topology could either block or amplify interstitial fluid flow, and by doing so considerably influence the local mechano-response in cortical bone.

References: 1. Birkhold et al. (2016) Scientific Reports 6:23480

Keywords: Fluid flow, Osteocyte, Lacunocanalicular network, Mechano-response



[Fig 1. Fluid flow pattern through the lacunocanalicular network of a mouse tibia.]

P134

Endogenous Lin28a protects osteoarthritic cartilage breakdown through HMGA2 expression

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Cartilage have poor regeneration abilities, for that his breakdown is irreversible. Previously we have shown that Lin28a overexpression protects cartilage degradation during osteoarthritis by inducing regeneration.

Here we asked if Lin28a has a physiological role and if HMGA2 a Lin28a target is implicated in the regeneration effect.

In vivo: Osteoarthritis was induced surgically (DMM), in inducible homozygous CreColII-Lin28aKO$^{fl/fl}$ KO male mice. WT and heterozygous littermates were used as control, and left knee was sham operated. Cartilage (OARSI score and immunohistochemistry) and bone (µtomography) were analyzed 8 weeks after surgery. Human cartilage sections were used for immunohistochemistry assay.

In vitro: Assays were performed using new born mice cartilage. HMGA2 downregulation by ShRNA or overexpression was induced by lentivirus infection. OA chondrocytes phenotype was induced by Wnt3a treatment. Samples were processed for Western Blot and Alcian Blue analysis.

Lin28a is detectable only in osteoarthritic human and murine cartilage and his expression is associated to HMGA2 expression. In mice after DMM, Lin28a KO worsening cartilage degradation (score 4 to 6, $p < 0.05$) and increases MMP13 expression (40% to 55%, $p < 0.05$), with no effect on subchondral bone.

Decreased HMGA2 expression in vitro result on a decrease of matrix production and anabolism gene expression (80%, $p < 0.05$). Moreover, an increase of catabolism gene expression was also observed (twofold, $p < 0.05$). On the opposite, HMGA2 overexpression increases matrix production (ninefold, $p < 0.01$). Furthermore, the overexpression protects chondrocyte from Wnt3a activation and mimics the effect of Lin28a overexpression.

We have demonstrated that Lin28a has a physiological effect on osteoarthritis progression. Moreover, HMGA2 factor which constitutes his main target, clearly orchestrates Lin28a effects on chondrocyte catabolism and anabolism activity.

Keywords: Osteoarthritis, Regeneration, Chondrocyte, Lin28a, HMGA2

Poster SNAPs Presentations 1—Clinical/Translational

P017

Sex steroids as determinants of Wnt-Signalling markers in men

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Background: Canonical Wnt-signalling is important for bone by regulating osteoblastogenesis and osteoblast function. Bone metabolism is also partly determined by sex steroid exposure and sex differences in serum sclerostin levels have been reported. However, it is unclear whether serum sclerostin and other circulating Wnt-signalling components are sex steroid dependent within healthy men.

Objectives: To determine whether serum sclerostin, osteoprotegerin (OPG) and Dickkopf-1 (DKK-1) levels associate with sex steroid exposure in men.

Methods: Cross-sectional data comprised 108 healthy males (34 ± 5 years) from the SIBLOS-cohort, and from the ENIGI-cohort 50 transgender women (TW) (35 ± 15 years) and 50 transgender men (TM) (23 ± 6 years) were evaluated before and 1 year after gender-affirming hormone treatment (cyproterone + estrogen and testosterone treatment, respectively). Sclerostin, OPG and DKK-1 were measured using a quantitative sandwich ELISA (Biomedica). Testosterone (T), estradiol (E2) were measured using LC–MS/MS, free fractions calculated.

Results: In SIBLOS, OPG was weakly inversely associated with E2 ($r = -2.8$; $p = 0.017$) and free T levels ($r = -2.4$, $p = 0.043$) and sclerostin with T ($r = -0.237$; $p = 0.045$), whereas no significant associations were found in the transgender groups. Sclerostin levels were non-significantly lower in TM than TW (29.67 pmol/l, 35.56 pmol/l, respectively; $p = 0.135$), but otherwise there were no between-group differences in Wnt-signalling markers. After hormonal treatment, sclerostin, OPG and DKK-1 levels were unchanged in TM (30.97 pmol/l). In TW, however, sclerostin levels decreased (28.04 pmol/l; $p < 0.001$), this difference being associated with changes in E2 levels ($r = -3.21$; $p = 0.025$).

Conclusions: Although circulating levels of Wnt-signalling components appear not strongly related to native sex steroid exposure in men, combined anti-androgen and estrogen treatment in TW reduced sclerostin levels. Contrastingly, no changes in sclerostin, OPG or DKK-1 were seen in TM receiving T treatment, suggesting that sclerostin production and secretion is regulated by estrogen but not androgen exposure.

Keywords: Wnt-Signalling, Sex steroids, Bone metabolism, Bone turnover markers

P044

Microcracks in subchondral bone plate is associated with cartilage preservation

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Introduction: Osteoarthritis (OA) is a disease of the whole joint characterized by cartilage loss, subchondral bone remodeling and thickening of subchondral bone plate. To date, the role of microcracks in cartilage integrity and subchondral bone homeostasis is not fully understood. The main goal of this work was to evaluate the link between cartilage degradation and presence of microcracks in calcified cartilage (CC) and subchondral bone plate (SBP). Furthermore, we also investigate the association between microcracks and osteocyte density in subchondral bone plate.

Methods: This study was performed using 18 bones cores obtained from cadaveric human knees. To measure microcracks, specimens were stained by the En-Bloc Basic Fuchsin staining. Trabecular bone parameters were assessed by micro-CT analysis. Microcracks and

osteocyte density were measured from thick sections. Cartilage lesions were assessed from toluidine blue staining.
Results: Microcracks were detected in both calcified cartilage and subchondral bone plate. Microcrack number in CC or SBP did not differ with age, and age and OA score were not correlated. Total numbers of microcracks was inversely correlated with cartilage lesions ($r = -0.47$ $p = 0.03$). Indeed, OA score was significantly lower with than without microcracks in SBP (2.14 ± 0.32 vs 3.67 ± 0.31, $p < 0.01$). In addition, we observed that osteocyte formed a dendrite network that abruptly stopped at the border of calcified cartilage. Osteocyte density in subchondral bone plate was higher with than without microcracks in CC (724 ± 60 vs 584 ± 32, $p = 0.02$) and was inversely correlated with trabecular subchondral bone volume ($r = -0.47$ $p = 0.04$).
Conclusions: Subchondral bone plate microcracks might be required for maintaining cartilage homeostasis. Microcracks in calcified cartilage may trigger osteocyte density in subchondral bone plate with subsequent regulation of subchondral bone remodeling to prevent cartilage damage.
Keywords: Microcracks, Osteocytes, Osteoarthritis, Bone, Calcified cartilage

P060

Bone health index for the assessment of pediatric fracture risk

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Objectives: Bone health index (BHI) is a measure of cortical thickens and mineralization across three metacarpal bones. BHI has been shown to correlate with DXA and pQCT derived bone mineral density (BMD) measurements. However, its ability to predict fracture risk has not been investigated. We aimed to investigate the association between BHI and prevalent fracture risk in a pediatric population of school age.
Methods: We included 2708 children (mean age 10 years) from a multiethnic cohort study with complete data for BHI, total body less head (TBLH-) BMD and fractures. BHI was measured using BoneXpert in hand DXA scans and analyzed across tertiles. Prevalent fractures were assessed questionnaire-based. Fracture risk estimates of BHI were modeled using logistic regression adjusted for sex, age, ethnicity, weight and lean mass fraction. Subsequently, the same model was additionally adjusted for TBLH-BMD.
Results: Prevalent fracture was observed in 402 (14.8%) children. Every SD decrease in TBLH-BMD was associated with 1.36 increased fracture risk (OR: 1.36, 95%CI 1.16–1.58, P = 0.0001). BHI was weakly correlated with TBLH-BMD ($\rho = 0.26$, $P < 0.0001$). Children in the lower BHI tertile showed $\sim$ 30% higher odds of prevalent fracture compared to those from the mid-tertile (OR: 1.26, 95% CI 0.97–1.63, P = 0.08) and third tertile (OR:1.36, 95% CI 1.04–1.76, P = 0.02). Further, inclusion of TBLH-BMD attenuated the associations by 6% (OR: 1.18, 95%CI 0.91–1.53, P = 0.22) with respect to the mid-tertile and by 14% (OR:1.17, 95% CI 0.89–1.55, P = 0.26) with the highest tertile.
Conclusions: This is the first study showing that cortical thickens and mineralization properties of the hand are associated with fracture risk in a pediatric population, although to some extent through correlation with BMD. Altogether, these findings postulate BHI as a novel proxy of whole-body BMD for the assessment of pediatric bone health and fracture risk in children.

P077

Myeloma-specific oncolytic adenovirus induces significant tumour oncolysis in vitro and in vivo and prevents cell line regrowth

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Myeloma is a largely incurable disease and despite current therapies achieving good initial responses, patients frequently relapse. Therefore, new approaches are required. One such approach is the use of oncolytic viruses. We developed an oncolytic adenovirus that utilizes transcriptional control of E1A under the myeloma-specific promoter CS1 (ADCE1A).

A panel of myeloma cell lines were treated with ADCE1A and cell death was monitored after 72 h using Flow Cytometry (FC) and propidium iodide (PI) staining. $CD138^+$ plasma cells from bone marrow aspirates were obtained from myeloma, plasma cell leukaemia patients and from healthy donors. The $CD138^+$ and $CD138^-$ populations from these samples were treated with ADCE1A and cell death was monitored after 4 days using FC and PI staining. Myeloma cell regrowth was assessed after bortezomib (2.5 nM) or bortezomib in combination with ADCE1A treatment using FC and PI staining. Viral efficacy was tested in a xenograft model of myeloma, where 5 weeks after tumour cell injection (10^6 U266 cells IV), mice were treated with ADCE1A ($1x10^7$ pfu, 2x/wk IV) or control (PBS) for 3 weeks. Tumour burden was measured ex vivo in bone marrow flushes of the long bones by FC.

ADCE1A caused oncolysis in all myeloma cell lines tested ($p = 0.0004$-$p = < 0.0001$). Notably, ADCE1A induced oncolysis in primary patient malignant $CD138^+$ plasma cells ($p = < 0.0001$) but not in $CD138^+$ cells from healthy donors. Additionally, ADCE1A had no effect on the non-malignant $CD138^-$ bone marrow mononuclear population from these patients and from healthy donors. ADCE1A prevented regrowth of myeloma cell lines following treatment with bortezomib in vitro over a 25 day period. In the U266 xenograft model, tumour load was significantly reduced ($p < 0.05$) compared to control treated mice.

In summary, ADCE1A has potential clinical efficacy as shown by preclinical models and patient tumour samples.

P080

Changes in trabecular bone score and bone mineral density following allogeneic hematopoietic stem cell transplantation

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Purpose: It has been demonstrated that bone mineral density (BMD) loss is substantial within the first 12 months after allogeneic hematopoietic stem cell transplantation (alloHSCT). However, the structural bone deficits after alloHSCT have not been well

characterized. The aim of this study was to evaluate the changes in BMD and TBS (trabecular bone score) in patients who received alloHSCT with follow-up of one year.
Methods: All patients 18 years and older who received alloHSCT between 2009 and 2015 were included. They evaluated for BMD at the time of alloHSCT and 12 months posttransplant.
Results: Twenty four subjects were included. Mean pretransplant z-score was normal before transplantation at all sites of measurement. However, 15, 17, and 8% of patients had a low BMD for age (i.e., z-score $\leq -$ 2DS) at the lumbar spine, femoral neck, and total hip, respectively. In addition, 29% of patients had a partially degraded lumbar spine TBS before alloHSCT. The average annualized rate of change in BMD at the lumbar spine, femoral neck, and total hip was $-$ 3.47%, $-$ 5.48%, and $-$ 6.84% per year, respectively. In addition, the average annualized rate of change in TBS was $-$ 1.92%. BMD at the femoral neck and total hip declined significantly from the baseline (all $p < 0.001$). In contrast, the reduced values of lumbar spine BMD ($p = 0.076$) and TBS ($p = 0.086$) were not significant.
Summary: To our knowledge, this is the first study to assess TBS in adult patients who received alloHSCT. During the first year of alloHSCT, the reduced value of TBS was not significant.
Keywords: Trabecular bone score; Bone mineral density; Hematopoietic stem cell transplantation

P163

Bone characteristics in elderly men with hypolactasia

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Objectives: In previous studies, we were able to show decreased bone mass in postmenopausal women with hypolactasia, based on genotyping of the LCT(-13910) locus. There are few data in elderly men. The aim of this study was therefore to investigate these findings in elderly men and correlate them to metabolic and cardiovascular parameters.
Methods: Biochemical and clinical parameters of 420 male volunteers of the BioPersMed cohort (Biomarkers in Personalised Medicine, Medical University Graz) were analysed to identify putative differences between lactose intolerant (genotype CC, hypolactasia) and lactose tolerant (genotypes TT, TC) men. Laboratory data in combination with DXA (dual X-ray absorptiometry)-derived measurements of bone density, dimensions and body composition as well as metabolic and cardiovascular parameters were analysed in relation to the genotyping results. Mann–Whitney-U-Test was used to check for statistical significance.
Results: Lactose intolerant (CC, 25%) men showed a significant overall decrease of bone parameters, consistently for Z-Scores ($p < 0.001$), T-Scores ($p = 0.003$), bone mineral density (BMD, $p = 0.004$), bone mineral content (BMC, $p = 0.045$) and bone mass ($p = 0.040$), compared to lactose tolerant (TT, 27%) men. Heterozygous men (TC, 48%) showed a gene-dose effect with lesser decreases than the CC group. Interestingly, we observed a small difference in adjusted urinary calcium level with borderline significance ($p = 0.05$). No differences in age, vitamin D levels or medication between the groups were registered.
Conclusions: In this study on elderly men, we have found a strong association between the A -13910 T/C dimorphism (LCT) genotype and bone properties, which underlines the importance of genetic variations in the context of bone strength. Genome-wide association studies (GWAS) in combination with food questionnaires will be linked to additional data for further combined effects in bone phenotypes.

P179

Identification of novel predisposing mutations in familial Paget´s disease of bone

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Paget's disease of bone (PDB) is a focal metabolic disease characterized by excessive bone resorption and formation, leading to bone deformity, pain and rarely neoplastic degeneration in osteosarcoma or giant cell tumor (GCT). Around 40% of hereditary and 10% of sporadic cases present mutations in *SQSTM1*, which encodes p62, involved in NFkB signaling and osteoclastogenesis. In the rest of the cases, the genetic background remains unknown. Moreover at least 7 candidate regions were identified by genome wide association studies in *SQSTM1* negative cases, even though the disease causing mutations within these loci have not been yet characterized. In order to better characterize the genetic background of the disease, we performed an extended amplicon sequencing targeting the whole genetic region of *SQSTM1* and other candidate genes in 35 patients negative for mutations in exon7 and 8 of *SQSTM1*. We found one case affected by P397R in *ZNF687*, a known mutation associated with GCT. Of note, this subject later developed the neoplastic degeneration, highlighting an important predictive value of such screening, since all GCTs occurring in PDB bear the same mutation. We also found a *ZNF687* mutation (G116R) affecting a highly conserved amino acid and new mutations in 2 genes. One patient presented with a variant on exon 6 of *SQSTM1* (S275 N), adjacent to the nuclear localization sequence and a new *TNFRSF11A* mutation (M566L) involving the intracellular tail of RANK was identified in one patient with early-onset polyostotic disease. Taken together these results provide further insights on the complex genetic predisposition of the disease.
Keywords: Genetics, Paget´s disease of bone

P327

COL1A1 C-propeptide mutations affect procollagen ER localization and reduce C-terminal procollagen processing

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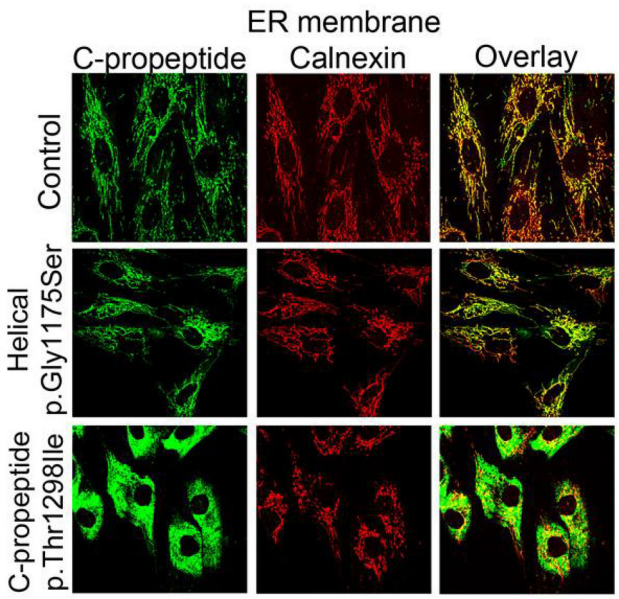
Approximately 6.5% of the type I collagen mutations that cause osteogenesis imperfecta (OI) are located in the C-propeptide. The C-propeptide is processed prior to chain assembly and is not present in fibrils in matrix, hence, the mechanism of procollagen C-propeptide mutations on bone quality remains unclear. Determination of the crystal structure of CPI homotrimers has renewed interest in genotype–phenotype correlations and mutation mechanism. We investigated skin and/or bone cells and tissues from seven *COL1A1* C-propeptide probands, versus cells from three probands with classical glycine helical substitutions and a normal control.

Our investigations produced two novel findings. First, immunofluorescence microscopy revealed that type I procollagen with C-propeptide defects mislocalized to the ER lumen in osteoblasts and fibroblasts, with an increased retention of procollagen in probands with substitutions in the petal region. In contrast, procollagen from control cells and from probands with helical substitutions localized normally to the ER membrane. Second, there was a striking delay in pericellular processing of procollagen with C-propeptide mutations, with increased pC-collagen and/or a reduction of mature collagen. In vitro cleavage assays confirmed this processing defect in procollagen with C-propeptide mutations, showing decreased cleavage with BMP-1 alone (28–33% of control cleavage) or BMP-1 + PCPE-1 (27–34% of control cleavage). The incorporation of pC-collagen into fibrils leads to altered dermal fibril diameters and disorganization of bone fibrils. The mislocalization of mutant procollagen in the ER together with reduced pericellular processing is expected to alter bone quality through abnormal osteoblast differentiation and matrix function, respectively.



[Mislocalization of procollagen with C-propeptide defects]

Keywords: Osteogenesis imperfecta, Collagen, BMP-1

P326

Interfamilial diversity in patients with collagen-related osteogenesis imperfecta

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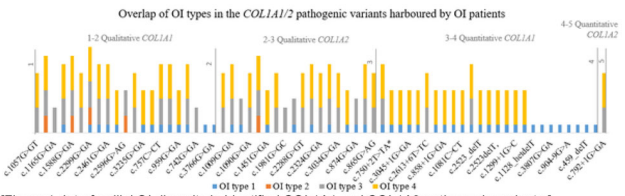
Osteogenesis Imperfecta (OI) is a spectrum of rare congenital bone fragility disorders, which ranges from mild osteopenia to lethality. Genotype–phenotype correlations remain elusive and patients harbouring the same pathogenic variant might develop phenotypes of different severity.

The main aim of current study was to evaluate associations between interfamilial diversity and genotype characteristics of collagen-related OI patients.

Data was recruited from previous Sanger sequencing mutational analysis. Total number of 65 patients who harboured *COL1A1/2* mutations, reported previously in the Dalgliesh OI variant database were included in the study. We have grouped pathogenic variants according to caused OI types, collagen I defect type and affected gene. Significance of correlations between interfamilial diversity and genotypes was tested with Fisher's test. The study was conducted in accordance with the Helsinki Declaration and authorized by the Ethical Review Committee.

We have noticed a pattern of interfamilial diversity, which correlated with the mutated gene (p-value = 0.0004) and defect type (p-value = 0.0002). (Table 1, Figure 1). Mutations without phenotype diversity were mainly OI1 and caused predominantly by the *COL1A1* quantitative defect. Quantitative defects in the *COL1A1* gene also caused diversity mainly between OI1 and 4. Qualitative defects predominantly represented interfamilial diversity of more severe phenotypes, i.e. OI3, 4.

OI interfamilial diversity potential is associated with genotype characteristics of the OI causing pathogenic variant. Presence of OI2 patients with the same mutation as non-lethal OI patients highlights need of additional awareness of OI phenotype modification factors and development of OI phenotype severity forecast biomarkers.



[Figure 1. Interfamilial OI diversity in identified COL1A1 and COL1A2 pathogenic variants.]

Keywords: Osteogenesis Imperfecta, Phenotype, *COL1A1/2*, Bone fragility

P338

The design of a long-acting PTH (TransCon PTH) phase 2 trial in patients with hypoparathyroidism, based on phase 1 results in healthy subjects

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Background: PTH deficiency in hypoparathyroidism (HP) leads to hypocalcemia and hyperphosphatemia. Standard of care (SoC), i.e., large doses of calcium (Ca) and active vitamin D, causes hypercalciuria and increased sCa x sP product. Daily Natpara® reduced doses of SoC but not 24-hr urinary calcium (uCa) excretion or incidence of hypo- and hypercalcemia due to its half-life of $\sim$ 3 h.
Studies have shown that continuous SC infusion of PTH (1–34) normalizes sCa, sP, sMg, uCa and bone turnover better than SOC or once- or twice-daily injections in HP patients.

TransCon PTH is a prodrug of PTH (1–34) for HP treatment. PTH (1–34) is transiently bound to a carrier via a linker. At physiological temperature/pH, linker auto-cleavage occurs, releasing active PTH at a controlled rate.
Phase 1 Results: TransCon PTH was well-tolerated. PK showed dose-dependent increases in Free PTH, with half-life of $\sim$ 60 h, and a flat, infusion-like profile within the normal range at steady-state. This resulted in sustained, dose-dependent increases in sCa. Despite mild hypercalcemia at higher doses, fractional excretion of Ca (FECa) remained normal. P1NP and BSAP showed no increase after 10 doses, supporting that an infusion-like profile of PTH within the normal range shows no anabolic activity.
Phase 2 Design: Forty adult patients with HP treated with SOC will be randomized to daily TransCon PTH 15, 18 or 21 μg PTH/d or daily blinded placebo via pen-injector for 4 weeks. The primary endpoint requires 1) normal sCa and 2) normal FECa (or $\geq$ 50% decrease from baseline), and 3) not taking active D, and 4) taking $\leq$ 1000 mg/d of Ca. All subjects enter a long-term extension where the TransCon PTH dose will be individually optimized.
Conclusions: The Phase 2 trial is designed to support the TransCon PTH target profile as a true PTH replacement therapy while confirming the starting dose in Phase 3.
Poster SNAPs Presentations 2—Basic/Translational

P139

The thyroid hormone transporter Mct8 is a negative regulator of trabecular bone mass in male mice

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Thyroid hormones (TH) are important regulators of bone metabolism. Their import into osteoblasts and osteoclasts is guided through specific transporter proteins such as the monocarboxylate transporter 8 (Mct8). Thus, we tested the hypothesis that Mct8 is a critical mediator of TH actions on bone.

Twelve-week old male mice with a global Mct8 deletion (M8, N = 12) or an osteoprogenitor-specific or osteoclast progenitor-specific Mct8-knockout (Mct8 $^{fl/fl}$; Osx:Cre, OsxCre +, N = 10; Mct8 $^{fl/fl}$; LysM:Cre, LysMCre +, N = 10) and their wildtype (WT, N = 8)/cre-negative littermate controls (OsxCre-, N = 10; LysMCre-, N = 10) were analyzed regarding their TH status, bone microarchitecture and bone turnover.

M8 mice had elevated serum triiodothyronine (+42%, $p < 0.001$), low thyroxine concentrations (− 52%, $p < 0.01$), and reduced trabecular bone volume at the femur (− 71% $p < 0.001$) and lumbar spine (–28%, $p < 0.01$) as compared to WT mice. M8 femora had thicker cortices (+5.4%, p = 0.05). Osteoblast and osteoclast numbers were increased in the femora (+31%/+ 33%, p = 0.09/$p < 0.05$) and vertebrae (+45%/+ 41%, $p < 0.05$) of M8 mice indicating an accelerated bone turnover due to high systemic T3. OsxCre + and LysMCre + mice were euthyroid, and displayed an augmented trabecular bone volume of the femur (+32%/28%, $p < 0.01$) and spine (+16% %/+ 20%, $p < 0.05$) as compared to respective Cre- mice. Cortical BMD was decreased in OsxCre + mice (− 2.2%, $p < 0.05$). Femur length and body weight of OsxCre + and LysMCre + mice did not differ from controls. At the tissue level, osteoblast numbers were increased 3.7-fold in OsxCre + ($p < 0.001$), whereas osteoclast parameters were unchanged. LysMCre + mice demonstrated lower osteoclast numbers (− 32%, $p < 0.05$) and enhanced osteoblast numbers (+25%, $p < 0.05$).

Our cell type-specific approaches identify Mct8 as negative regulator of bone mass in male mice. Moreover, Mct8 in osteoprogenitors distinctly affects cortical versus trabecular bone. Further investigations are required to unravel the underlying mechanisms.

P140

Anti-Müllerian Hormone deficiency: a new mouse model for bone changes following natural menopause?

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Background: Ovariectomised mice constitute a model for postmenopausal bone loss, but this model is limited by the abrupt loss of ovarian hormones compared to the natural human menopause. Mice deficient for the ovarian growth factor Anti-Müllerian Hormone (AMH) display gradual loss of ovarian function, thereby better resembling human menopause. Therefore, we investigated the effect of AMH and AMH type II receptor (AMHR2) deficiency on bone in mice.
Methods: Femurs of 4-, 10- and 16-month-old male and female wild type (WT), AMH knockout (AMHKO) and AMHR2 knockout (MRKI) mice (n = 9–12) were scanned using microCT. Data-analysis was performed using 'NRecon', 'Dataviewer' and 'CT analyzer' software. Statistical differences between the mice models were determined, using ANOVA and Tukey HSD post hoc tests.
Results: Female AMHKO (p = 0.151) and MRKI (p = 0.011) mice demonstrated a higher trabecular bone volume fraction (BV/TV) compared to WT mice at the age of 4 months. This was still present at the age of 10 months, mainly due to an increased trabecular thickness (p = 0.011 and $p < 0.001$ for AMHKO and MRKI, respectively). However, at the age of 16 months, these differences had disappeared. In male mice, there was no difference in trabecular bone parameters. Female MRKI mice had a greater cortical area compared to WT and AMHKO mice at 10 months (p = 0.009 and p = 0.061, respectively) and endocortical volume (p = 0.013 and $p < 0.001$, respectively), leading to an increased perimeter ($p < 0.001$ and $p < 0.001$, respectively). Male AMHKO mice had a smaller cortical area (p = 0.005), cortical thickness (p = 0.007) and MOI (p = 0.035) than MRKI mice at an age of 10 months.
Conclusions: AMH and AMHR2 deficiency seem to have a protective effect on trabecular and cortical bone in female mice, suggesting that AMH signaling may negatively impact on bone. Studies are underway to assess whether AMH has direct and/or indirect effects on bone.

P178

MiR-146a a key Player in bone metabolism

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Micro RNAs play a crucial role in the regulation of bone metabolism. MiR-146a, an important anti-inflammatory miRNA, was found to negatively impact osteogenesis and bone regeneration in vitro, by controlling the differentiation of mesenchymal stem cells. But to date its role in bone remodelling is not known.

Systemic bone of wt, miR-146a$^{-/-}$ and miR- 146a$^{-/-}$ TRAF6$^{+/-}$ animals was assessed histologically and via μCT analysis, over a period of 3 to 18 months of age. Serum cytokine levels were analysed by Elisa. MRNA expression levels were analysed by qPCR. To induce osteoporosis, ovariectomy (OVX) induced bone loss was performed.

Analysis of tibia using μCT analysis revealed significantly increased trabecular as well as cortical bone mass in miR-146a$^{-/-}$ compared to wt animals, starting at an age of 6 months ($n \geq 4$/group; μCT Tb. $p = 0.029$, Ct. $p = 0.036$). Doses reduction of TRAF6, a main target of miR-146a, using miR-146a$^{-/-}$ TRAF6$^{+/-}$ animals could not change the observed bone phenotype. Serum of aged miR-146a$^{-/-}$ animals displayed elevated activity of bone resorbing osteoclasts as amounts of CTX I in miR-146a$^{-/-}$ mice were significantly increased ($p = 0.007$). Increased expression of osteoclast and osteoblast marker genes in bones of aged miR-146a$^{-/-}$ mice leads to the suggestion of a regulatory role of miR-146a in osteoclasts and osteoblasts. Osteoporosis induction using the OVX disease model ($n = 7$), showed significant trabecular bone loss in ovariectomized wt mice (μCT $p < 0.001$). In contrast, we detected no trabecular bone loss in ovariectomized miR-146a$^{-/-}$ compared to control animals ($p = 0.512$), suggesting that loss of miR-146a protects bone loss induced by estrogen deficiency.

MiR-146a seems to control bone turnover and miR-146a$^{-/-}$ mice accrue bone over time. Moreover this miRNA has a negative influence on bone loss occurring during oestrogen loss induced osteoporosis. Therefore miR-146a could be possibly used as a therapeutic target in the treatment of osteoporosis.

Keywords: miRNA, Osteoporosis, TRAF6, OVX

P193

New role for RANKL as a proinflammatory modulator in modeled inflammatory arthritis

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Receptor activator of nuclear factor-κB ligand (RANKL), a member of the Tumor Necrosis Factor (TNF) superfamily, constitutes the master regulator of osteoclast formation and bone resorption, whereas its involvement in inflammatory diseases remains unclear. Here, we used the human TNF transgenic mouse model of erosive inflammatory arthritis to determine if the progression of inflammation is affected by either genetic inactivation or overexpression of RANKL in transgenic mouse models. TNF-mediated inflammatory arthritis was significantly attenuated in the absence of functional RANKL. Notably, TNF overexpression could not compensate for RANKL-mediated osteopetrosis, but promoted osteoclastogenesis between the pannus and bone interface, suggesting RANKL-independent mechanisms of osteoclastogenesis in inflamed joints. On the other hand, simultaneous overexpression of RANKL and TNF in double transgenic mice accelerated disease onset and led to severe arthritis characterized by significantly elevated clinical and histological scores as shown by aggressive pannus formation, extended bone resorption, and massive accumulation of inflammatory cells, mainly of myeloid origin. RANKL and TNF cooperated not only in local bone loss identified in the inflamed calcaneous bone, but also systemically in distal femurs as shown by microCT analysis. Proteomic analysis in inflamed ankles from double transgenic mice overexpressing human TNF and RANKL showed an abundance of proteins involved in osteoclastogenesis, proinflammatory processes, gene expression regulation, and cell proliferation, while proteins participating in basic metabolic processes were downregulated compared to TNF and RANKL single transgenic mice.

In conclusion, these results suggest that RANKL modulates modeled inflammatory arthritis not only as a mediator of osteoclastogenesis and bone resorption but also as a disease modifier affecting inflammation and immune activation.

Keywords: RANKL, TNF, Inflammatory arthritis, Transgenic models, Proteomics

P202

Tissue specific effects of glucocorticoids suggest inter-organ communication during fin regeneration

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Glucocorticoids are widely used as therapeutic agents to treat immune-mediated diseases in humans because of their anti-inflammatory and immunosuppressive effects. However, they have various side effects, in particular rapid and pronounced bone loss associated with fractures in glucocorticoid-induced osteoporosis. We have recently shown that treatment with the glucocorticoid prednisolone during zebrafish fin regeneration impacts on the number, activity and differentiation status of bone forming osteoblasts. However, the underlying cellular and molecular mechanisms of impaired bone regeneration remain incompletely understood. In order to further elucidate direct versus indirect effects of continued prednisolone exposure, we have performed transcriptome analysis in selected cell populations of the regenerate. Notably, prednisolone induced a partly ´inflammatory´ profile in osteoblasts while it exerted anti-inflammatory effects in innate immunity cells (macrophages) ($p < 0.05$, fold change $> 2/< -2$). In particular, chemokines were expressed in partly opposing manner in macrophages and osteoblasts, which illustrates the tissue specificity of glucocorticoid signaling during regeneration. Furthermore, mass spectrometry analysis indicates that fin amputation induces remote signals in organs such as the liver; these signals then appear to be employed in bone formation in the regenerate in a glucocorticoid-sensitive manner. This suggests that inter-organ communication is at work during fin regeneration and that this may be under control of steroid hormones such as glucocorticoids.

P208

Longitudinal evaluation of the musculoskeletal and frailty phenotype in a mouse model of accelerated aging

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Objectives: Frailty is a geriatric syndrome characterized by increased susceptibility to adverse health outcomes. One major symptom is the weakening of the musculoskeletal system (sarcopenia and osteoporosis). To better understand the underlying mechanisms, mouse models mimicking frailty and the associated phenotypes are needed. In this study, a model of accelerated aging (PolgA$^{(D257A/D257A)}$) was longitudinally monitored to determine its relevance as a model for age-related osteosarcopenia and frailty.
Method and Results: PolgA$^{(D257A/D257A)}$ (PolgA, n = 48) and wildtype-littermates (WT, n = 40) were aged in parallel to 46 weeks (approved by veterinary authorities). In agreement with previous studies, the cross-sectional comparison between genotypes at different ages resulted in significantly lower muscle cross-sectional area and weights in 40- and 46-week-old PolgA mice (figure 1A). Similarly, micro-CT analysis of caudal vertebrae and femora showed similar bone morphometric parameters at 20 weeks of age, which then diverged over time such that PolgA had significantly lower bone mass at 40 and 46 weeks (figure 1B). However, longitudinal micro-CT over 20 weeks revealed that this difference was not due to bone loss in PolgA, but rather to the continuous increase in bone mass in WT (figure 1C&E). Compared to WT, PolgA became frailer with age, shown by increased frailty index scores (*Whitehead 2014*) (figure 1D).
Conclusions: PolgA mice showed typical hallmarks of aging (frailty, sarcopenia, low bone mass). Nevertheless, they did not significantly lose bone up to the age of 46 weeks. Whether PolgA mice also recapitulate aging musculoskeletal phenotypes at the molecular level is currently under evaluation.

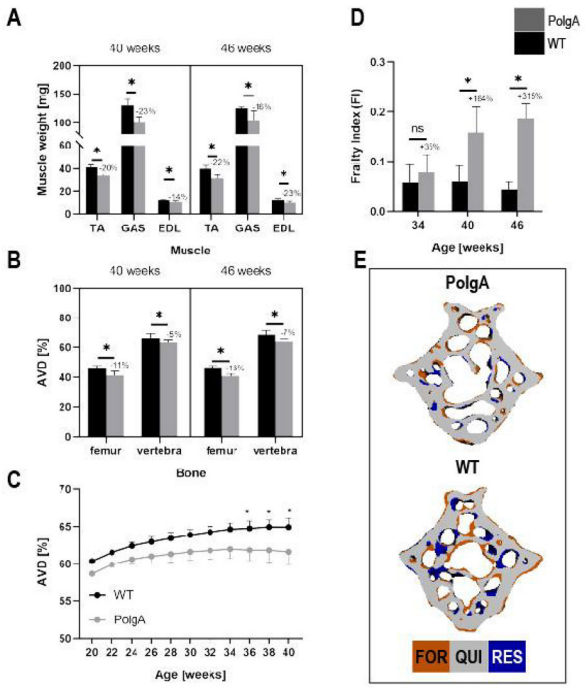

Figure 1: Cross-sectional comparison of muscle weights (TA=Tibialis Anterior, GAS=Gastrocnemius, EDL=Extensor Digitorum Longus) **(A)** and Apparent Volume Density (AVD) **(B)** at 40 and 46 weeks. Longitudinal monitoring of the 6th caudal vertebrae by time-lapsed micro-CT imaging **(C)** and frailty based on the clinical mouse frailty index (FI) **(D)** Representative overlays of bone cross-sections from week 20 and 40 displaying regions of formation (FOR), quiescence (QUI) and resorption (RES) **(E)**. (p<0.05 between PolgA (grey) and WT (black))

[Evaluation of the musculoskeletal and frailty phenotype in the PolgA mouse model]

P291

Humanin might protect against glucocorticoid induced osteoporosis in a mouse model of Duchenne muscular dystrophy

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Duchenne muscular dystrophy is a lethal muscle disease with onset in early childhood. The standard treatment for these children is long-term treatment with glucocorticoids like prednisolone or prednisone. The glucocorticoid treatment, combined with the lack of muscle load, makes the bone health in these children compromised. They often suffer from short stature and fractures are common. An analogue to the mitochondrial peptide humanin, HNG, has shown protective effects on glucocorticoid-induced growth retardation in normal mice. We therefore hypothesised that HNG also may protect bone health in a mouse model of Duchenne muscular dystrophy.
Methods: 5-week-old C57BL/10ScSn-*Dmd*^{mdx}/J (mdx) mice were subjected to subcutaneous implantation of a 2.5 mg slow-releasing prednisone or placebo pellet under anaesthesia with isoflurane. Intraperitoneal injections with 1 mg/kg/body weight HNG or vehicle were administered daily for 4 weeks. At termination, bones were dissected and after fixation in formaldehyde-solution they were preserved in 70% ethanol until analysis with peripheral quantitative computed tomography (pQCT).
Results: The mice treated with prednisone had 32% less cortical area than placebo treated mice ($p < 0.001$) The mice treated with a combination of prednisone and HNG had a 35% thicker cortical area than the mice treated with prednisone alone ($p = 0.040$). The cortical thickness was 27% thinner in the prednisone v.s placebo treated mice ($p = 0.002$). The mice treated with a combination of prednisone and HNG had 28% more cortical thickness than the animals treated with prednisone alone ($p = 0.047$). There were no significant differences in overall bone mineral density between the groups.
Conclusion: Our data indicate that HNG can protect against adverse effects of prednisone on bone health. More experiments are needed to clarify the mechanisms of action and rule out any interference with glucocorticoid efficacy.
Keywords: Duchenne, Osteoporosis, Glucocorticoids, Humanin

P292

Hepcidin-resistant ferroportin mutation causes low bone mass in mice

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Ferroportin (FPN) is the only known iron exporter and hence is a central regulator of iron homeostasis. Mutations conferring resistance of FPN due to hepcidin-mediated degradation are associated with hemochromatosis, a disease characterized by systemic iron overload. Even though iron overload is associated with low bone mass, the mechanisms involved are not completely understood. Here, we aimed to investigate whether the disruption in the hepcidin/FPN axis in *Fpn*^{C326S} mice and the subsequent systemic iron accumulation

impacts on bone tissue to a similar extent as in $Hfe^{-/-}$ mice, which are also iron overloaded.

Similar to *Hfe*, *Fpn* was highly expressed in primary murine osteoblasts and osteoclasts. In osteoclasts, *Hfe*, *Fpn*, and *Hamp* mRNA levels decreased during differentiation [10-, 25-, 2.5-fold; $p < 0.05$]. During osteoblast differentiation, *Fpn* and *Hamp* gene expression decreased [2.5-, 9.5-fold; $p < 0.05$], while *Hfe* increased by 1.5-fold ($p < 0.05$). In mice, BV/TV and Tb.Th were not different at the femur between 10 and 14-week-old male WT, $Hfe^{-/-}$ and Fpn^{C326S} mice. However, Tb.N was increased in both genotypes [+ 10%, $p < 0.05$], while Tb.Sp was decreased [− 11%, $p < 0.05$]. In contrast, both, $Hfe^{-/-}$ and Fpn^{C326S} mice exhibited lower BV/TV [$Hfe^{-/-}$, − 24%; Fpn^{C326S}, − 33%; $p < 0.05$] and Tb.Th [$Hfe^{-/-}$, − 10%; Fpn^{C326S}, − 15%; $p < 0.05$] in the fourth lumbar vertebra compared to WT mice. While BFR/BS was not different in the femur of both genotypes, it was reduced in the vertebral bone of Fpn^{C326S} [− 36%, $p < 0.05$] compared to WT mice. Serum levels of bone formation marker, PINP, were significantly reduced in both, $Hfe^{-/-}$ and Fpn^{C326S} compared with WT mice [$Hfe^{-/-}$, − 35%; Fpn^{C326S}, − 40%; $p < 0.05$].

The hepcidin-resistant FPN mutation in mice results in a more severe bone phenotype compared to $Hfe^{-/-}$ mice, which is characterized by low bone mass due to suppressed bone formation and site specificity showing bone loss only at the axial, but not the appendicular skeleton.

P305

Irisin in post-menopausal women with primary hyperparathyroidism: an interplay between Irisin and PTH

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Background: Irisin is a myokine able to ameliorate bone status, muscle atrophy and it influences also glucose and energy homeostasis. PTH is hormone able to exert several metabolic effects that may interact with irisin's ones. No studies have investigated the biological relation between Irisin and PTH.

Aim: To test the hypothesis that irisin and PTH mutually affect their biological action, we evaluated the FNDC5 mRNA expression in myotubes treated with PTH (1–34) and PTH-R mRNA expression in osteoblast treated with recombinant irisin. To confirm the in vivoimpact of PTH on irisin, we evaluated irisin serum concentration in post-menopausal women with primary hyperparathyroidism (PHPT) compared to age, sex and BMI matched control subjects with no impairment of calcium/phosphate metabolism.

Methods: C2C12 myotubes were treated with 100 nM of teriparatide for 3 and 8 h or with 100 nM of teriparatide for 6 days, refreshing medium every 48 h. MC3T3-E1 osteoblastswere treated with 100 ng/ml r-Irisin for 8 h. Teriparatide-treated myotubes, Irisin-treated osteoblasts and untreated controls were subjected to RNA extraction and qPCR analysis. In a cross-sectional, open-label trial, we enrolled 26 PHPT post-menopausal women and 31 age/BMI matched control subjects with no impairment of calcium/phosphate metabolism.

Results: Both short (p = 0.036) and continuous (p = 0.006) teriparatide treatment on myotubes significantly decreased FNDC5 mRNA expression respect to untreated control. r-Irisinled to a 50% down regulation of PTH-R mRNA expression compared to untreated cell (p = 0.029).

Irisin was significantly lower in PHPT group compared to age/BMI-matched controls (4.5 ± 1.1 vs 12 ± 5.2 μg/mL, $p < 0.001$). No significant correlation between Irisin and BMD or PTH was recorded in PHPT group.

Conclusions: For the first time, our pre-clinical findings suggest the existence of interplay between PTH and irisin metabolism that seems to be confirmed by the significant reduction of irisin concentration in post-menopausal women with PHPT.

Keywords: Irisin, PTH, Hyperparathyroidism, FNDC5, PTH receptor, Muscle

P325

Chronic recurrent multifocal osteomyelitis in children with hypophosphatasia explained by anti-inflammatory nucleotidase activity of tissue nonspecific alkaline phosphatase

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Hypophosphatasia (HPP) is a disease due to the deficiency of tissue nonspecific alkaline phosphatase (TNAP), and characterized by bone hypomineralization. TNAP's mineralizing function relies on the dephosphorylation of the mineralization inhibitor inorganic pyrophosphate (PPi), generated from adenosine triphosphate (ATP) by ectonucleotidase pyrophosphatase phosphodiesterase 1 (NPP1). In addition, symptoms of chronic recurrent multifocal osteomyelitis (CRMO), a sterile bone auto-inflammatory disease, have recently been described in childhood HPP. We hypothesized that CRMO in HPP is due to the lack of extracellular ATP dephosphorylation by TNAP. ATP is indeed a pro-inflammatory molecule that is released by virtually all cells in response to various danger signals to act in autocrine and paracrine manners. Extracellular ATP is dephosphorylated by CD39 into AMP, which is hydrolyzed by CD73 into the anti-inflammatory adenosine, which participates to the resolution of ATP-associated inflammation. RT-qPCR of bones of 7-day-old $Tnap^{+/-}$ mice revealed increased levels of *Il-1β* (60%) and *Il-6* (250%) and decreased levels of anti-inflammatory *Il-10* (50%) as compared with $Tnap^{+/+}$ mice, suggesting CRMO. In mature primary osteoblasts and growth plate chondrocytes, *Tnap* was more than tenfold more expressed than *Npp1*, *Cd39* and *Cd73*, and its inhibition with MLS-0038949 significantly decreased the dephosphorylation of exogenously added ATP and AMP (Pi quantification with malachite green) and led to higher levels (50%) of ATP released by the cells (luciferase activity). TNAP inhibition also increased the expression (twofold, RT-qPCR) and secretion (40%, ELISA) of IL-6. Finally, TNAP deficiency in $Tnap^{+/-}$ bones as well as IL-1β-decreased *Tnap* expression in osteoblasts and chondrocytes were not compensated by increased *Npp1* or *Cd39* levels. These results suggest the presence of

a vicious cycle in HPP bones, where TNAP deficiency triggers inflammation, which in return exacerbates TNAP loss of function.

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Poster SNAPs Presentations 2—Clinical/Public Health

P194

Mutations in osteoarthritis susceptibility genes cause joint shape variation detectable at larval stages in zebrafish

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Osteoarthritis (OA) is a joint degenerative disease and leading cause of pain and disability worldwide. The shape of synovial joint is a key component of its function and is commonly used to predict OA. GWAs have rapidly increased the number of genes associated with OA. Few genes have been linked to joint shape variation but for most of the them evidence for causality and functionality or if they affect joint shape is still lacking, moreover rapid and alternative screening platforms to test these genes in parallel must be developed. We use the zebrafish jaw joint (JJ) to investigate how shape varies during ageing and to test the impact of OA genes in joint shape. Through 3D morphometric analysis we show that joint shape abnormalities are detected in the aged zebrafish JJ and are accompanied by degenerative histological OA changes. Interestingly, mutations in OA associated genes (*chsy1*, *col9a1*, *col11a2*, and *wnt16*) lead to premature shape and cellular changes in young adults comparable with those found in aged zebrafish. We tested if shape changes would be detected during the JJ ontogeny in a broader list of OA mutants (*chsy1*, *col9a1*, *col11a2*, *gdf5*, *barx1*, *mcf2 l*, *dot1 l*, *wnt16* and *ncoa3*) using confocal imaging of larvae immunostained for collagen type 2 followed by 2D geometric analysis and 3D measurements. Our data show mutations in distinct classes of OA proteins leading to alterations in larval stages, affecting distinctive joint properties and cell behaviour changes. We tested whether the use of mosaic fish generated through CRISPR could be sufficient to detect shape and cell changes in larvae and adults. Surprisingly, wnt16mosaics showed comparable shape changes to the wnt16-/- stable mutant. Finally, we developed computational tools to analyse shape and cell behaviour in larvae zebrafish delivering a powerful and rapid screening system to test OA genes.

P238

Trabecular bone score (TBS) and life expectancy (LE) with and without osteoporotic fracture

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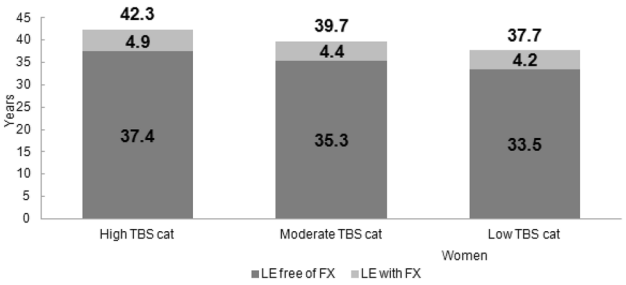
TBS, a texture analysis of lumbar spine (LS) DXA scans, predicts major osteoporotic fractures (MOF) independently of BMD. We aimed to study the association of TBS with overall LE, and when considering MOF.

Study comprised 6849 individuals(3982 women) with a mean age of 65 years from the population-based study. We developed a multistate life table to calculate LE for individuals who had low, moderate and high TBS values; considering difference in years lived with and without MOF. Calculations employed prevalence, incidence rate, and hazard ratios (HRs) for three transitions (healthy to MOF, healthy to death, and MOF to death), categorized by baseline TBS tertiles adjusted for BMI, LS-BMD and cohort.

During 7.2 years, 306 incident MOF and 522 deaths were observed in women; and 114 MOF and 558 deaths in men. Individuals with the lowest (HR: 1.25(0.92–1.71) in women and 1.91(1.08–3.40) in men) and mid (HR:1.12(0.82–1.52) in women and 1.75(1.01–3.02) in men) tertiles of TBS had a higher MOF risk as compared to the highest. Mortality of non-fractured individuals was also higher in the lowest and mid TBS tertiles. Fractured individuals with the lowest (HR:2.34(1.34–4.07) in women and 5.87(2.80–12.29) in men) and mid (HR: 1.81(1.02–3.22) in women and 2.80(1.33–5.88) in men) tertiles of TBS had higher risk of death as compared to the highest. Total LE was higher in women (39.7y) than in men (35.3y).

Women and men have an increased mortality risk after suffering a MOF. The risk is higher among those with lower TBS, and among men.



[Figure 1. Life expectancy with and without fracture in women of each TBS category]

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Peripheral trabecular bone microarchitecture is impaired in postmenopausal non-osteoporotic women with fragility fracture- the ChiVOS case–control study

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Introduction: Most fragility fractures occur in population with normal BMD or osteopenia by DXA ("non-osteoporotic" population). In these patients, BMD values are apparently normal or only mildly declined, and the current FRAX tool sometimes cannot be able to distinguish between patients at low and high risk.

We performed this study to compare HR-pQCT parameters of bone macro- and micro- architecture in non-osteoporotic postmenopausal women with and without fragility fracture. We also compared the HR-pQCT measurements in non-osteoporotic women with and without vertebral fractures.

Methods: We enrolled 153 postmenopausal women with BMD T-scores > -2.5 (mean age 70.7 years; 51 with fragility fractures

and 102 controls). By 1 onsite visit, an interview, DXA measurement, and thoracic/lumbar spine X-ray were performed to collect a set of data regarding socioeconomic/medical, vertebral fracture assessment, and specific densitometric information from the participant. Volumetric BMD and microarchitecture were measured at the distal radius and distal tibia with a three-dimensional HR-pQCT system.

Results: Groups had similar age, weight, height, BMI, BMD and FRAX scores. Fracture subjects had marked trabecular bone abnormalities, including lower trabecular vBMD at the radius, and lower trabecular number, higher trabecular separation and inhomogeneity of network at the radius and tibia ($p < 0.05$). The differences in trabecular number at the radius and trabecular separation and inhomogeneity of network at radius and tibia were independent of weight, height and total hip BMD, or FRAX major osteoporotic fracture scores. We also found the similar trabecular bone abnormalities in osteopenic women with osteoporotic fractures.

Non-osteoporotic women with and without vertebral fracture were discriminated by trabecular vBMD and trabecular BV/TV at the radius, and trabecular number, trabecular separation and inhomogeneity of network at radius and tibia beyond FRAX major osteoporotic fracture scores.

Keywords: Osteoporosis, HR-pQCT, Fracture, Trabecular

P263

The accuracy of self-reported fractures among a Belgian cohort of postmenopausal women: the FRISBEE study

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In large population-based epidemiological studies of osteoporotic fractures, self-report is an important way of obtaining information. This method is subject to errors of recall and may result in misclassification of fracture status. Surprisingly, this topic has rarely been assessed.

We aimed to assess the accuracy of self-report in the FRISBEE cohort (Brussels) of 3560 postmenopausal, aged 60–85 years, included between July 2007 and June 2013, followed yearly for the occurrence of incident fractures.

1016 fractures were reported between 2013 and 2018. We had access to 81.6% of the participant's radiological records, which gave us the possibility to validate 829 fractures.

85% (n = 705/829) fractures were confirmed by a radiological record.

No fracture was found on the radiological report at the indicated site in 119 cases, a rate of false positives (FP) of 14.4% (n = 119/829). Besides, 4 fractures were identified as a prior event and 1 was declared in wrong area.

The rates of FP varied by site: 4.4% (n = 3/68) at the hip, 16.8% at the spine (n = 21/125), 10.8% at the proximal humerus (n = 10/93) and 9.8% at the wrist (n = 14/143).

The global rates of FP were 11.2% (n = 48/429) for all 4 MOFs, 12.6% (n = 22/175) for "other major" (fractures of the pelvic bone, sacrum, elbow, upper leg, lower leg, tibial plateau/knee and ankle), and 22.3% (n = 49/220) for "minor" fractures (all other fractures).

Additionally, we analyzed several subject's baseline characteristics that could have influenced the rate of FP fractures. In a multivariate analysis, younger subjects, individuals with fractures on other sites than hip, with a lower education level and with a higher BMI (> 25) were more likely to report false positive fractures.

In summary, our data indicate that the inaccuracy of self-reported fractures can be significant for several major fractures, and influence any fracture risk prediction model.

Keywords: Osteoporosis, Fracture, Self-report

P262

Incidence of osteoporotic refractures and associated mortality in Korea using nationwide claims data

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Objectives: The purpose of this study was to investigate the incidence of osteoporotic refractures and fracture location, and to evaluate the associated mortality rate using nationwide claim data over a mean follow-up of 3 years.

Methods: The subjects were selected using the operational definition of osteoporotic

fractures in patients above 50 years of age. Refracture was defined as the event with the same operational definition occurring after 6 months of untreated period. The mortality rate was calculated using the Charlsons´ comorbidity index and statistically analyzed using Cox proportional hazards regression analysis.

Results: Between 2007 and 2012, a total of 18,956 first-time osteoporotic fracture cases were followed for a mean of 3 years (range, 1 to 7 yr). Of the 18,956 patients, 2941 patients experienced refracture, which accounted for 15.5% of all patients. The cumulative mortality rates of non-refracture and re-fracture groups at one year follow-up were 7.2% and 9.1%, respectively. After adjusting for age, gender, income, and comorbidity index, the mortality rate in patients with re-fracture was 1.2 times higher than for the patients without re-fracture over a mean 3-year follow-up (HR = 1.20, 95% CI; 1.08–1.34, $P < 0.001$).

Conclusions: In this nationwide study, the rate of osteoporotic re-fracture was 15.5% and the mortality rate in patients with re-fracture was 1.2 times higher than that in non-refracture over a mean 3-year follow-up.

Keywords: Incidence, Mortality, Nationwide claim data, Osteoporotic fracture, Refracture

P264

Clinical risk factors (CRFs) for osteoporotic fractures (OFs) are function of the fracture site: data from the FRISBEE study

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Introduction: Several CRFs predicting OFs have been described but their association with a particular site of fracture has not been extensively studied.
Objectives: We evaluated if some CRFs are specific for sites of OFs in the FRISBEE cohort ("Fracture RIsk Brussels Epidemiological Enquiry").
Methods: We analysed the association between CRFs included in the FRAX model or additional CRFs (falls, early untreated menopause, sedentary lifestyle and diabetes) and the first incident validated MOF (vertebral, hip, shoulder and wrist) or other major fracture (ankle, pelvis/sacrum, elbow, knee, long bones).
Results: 3560 postmenopausal women, aged 60 to 85 years (median 70), were recruited from 2007 to 2013, with a median follow up of 6.2 years. The first incident validated fractures (n = 436) were hip (52), vertebra (120), wrist (122), shoulder (68) or other major (74). For MOFs considered together, the risk of fracture was highly associated in uni- and multivariate analyses ($P < 0.01$) with BMD, osteoporosis (DXA), age, prior fracture and fall history (HR = 1.38;2.34;1.85;1.97 and 1.28, respectively). For each site analysed separately, in multivariate analyses, total hip BMD, osteoporosis, age, prior OF and smoking remained independent predictors for hip fractures (HR = 1.92;3.98;6.40;2.70 and 3.20, respectively); spine BMD, osteoporosis, age, prior OF and glucocorticoids for vertebral fractures (HR = 1.45;2.08;2.16;1.94 and 1.72, respectively); femoral neck BMD, osteoporosis and prior OF (HR = 1.56;1.81 and 1.67, respectively) for wrist fractures; spine BMD, osteoporosis and prior OF (HR = 1.31;2.48 and 1.75, respectively) for shoulder fractures; prior OF and diabetes (HR = 2.62 and 2.03) for other major fractures.
Conclusions: Our study confirms a strong association between the risk of fractures and BMD, osteoporosis, prior fragility fracture and age. Smoking for hip fracture, glucocorticoids for vertebral fracture, falls for MOFs and diabetes for other major fractures were also significant predictors. Our study indicates that the relative importance of CRFs is dependent on the fracture site.
Keywords: Osteoporosis, Risk factors, Fracture site, BMD, FRAX

P285

Motivations for eight years adherence to alendronate therapy

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Purpose: Researches have been mainly focused on reasons for discontinuation of osteoporosis therapy. No data are available on reasons for long term adherence.
Methods: We studied 204 long-term adherent new alendronate users: 65 postmenopausal outpatients still adherent (Group C, years on treatment = 8.70 ± 1.31) were compared to 139 patients age-matched who discontinued therapy (Group S, years on treatment = 8.64 ± 1.43).We evaluated lifestyle factors, main biochemical parameters, BMD values, fractures, Charlson comorbidity index (CCI), both at the beginning and end of the study. A questionnaire was administered to analyze the reasons for adherence, investigating areas of personal beliefs, fears, economic problems and side effects related to alendronate therapy.
Results: Main reason to start alendronate was represented by fragility fractures (S 91% vs C 86% of patients, p = 0.34). There were no significant differences between groups concerning baseline DXA values, number of major fractures and CCI. A higher education level was observed in Group C (C 54% vs S 35% of patients, p = 0.001). At the time of interview, there was a significant higher number of patients with a CCI of two in Group S compared to the beginning of treatment (56% vs 43%, p = 0.04), together with a higher number of patients taking more than 3 drugs (22% vs 11%, p = 0.01) compared to basal evaluation. Forty-seven percent of patients reported new diseases during the treatment as the main reason for stopping alendronate. A multivariate, stepwise logistic regression analysis showed that awareness of the disease was highly associated with adherence (OR = 0.20; 95% CI 0.045–0.93, p = 0.04) followed by a higher education (OR = 0.526, 95% CI 0.345–0.801, p = 0.003). Worsening of CCI was associated with discontinuation (OR = 2.75,95% CI 1.033–7.324, p = 0.04).
Conclusions: Education and disease awareness are associated with long-term alendronate adherence while competing health problems negatively impact on adherence to osteoporosis treatment.
Keywords: Osteoporosis, Long-term osteoporosis therapy, Bisphosphonates, Adherence, Alendronate

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Differentiation and functional activity of osteoclasts are impaired in CFTR-f508DEL patients

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Objectives: Cystic Fibrosis Bone Disease (CFBD) is one of the major comorbidities with diabetes affecting patients with cystic fibrosis (CF). Bone homeostasis is depending on effective coupling between osteoblast (OBs) activity and osteoclast (OCs) activity. OCs are differentiated from monocytic precursors under the action of several mediators (M-CSF, RANKL, sphingosine-1-phosphate (S1P)) whose expression is deregulated in CFBD (Velard F et al., AJRCCM, 2018). Our hypothesis is that the CFTR-F508del mutation could alter the differentiation and functional activity of OCs.
Results: CFTR-F508del mutation significantly (p = 0.017) reduced OCs number formed from monocytes of homozygous (n = 7) and heterozygous (n = 10) CF-F508 patients compared to non-CF healthy monocytes (n = 8). This reduction in differentiated OCs was associated with an increased OCs size (p = 0.007) and a decrease in their functional capacities to form resorption trenches, which is also found from healthy monocytes treated with the CFTR inhibitor Inh172 ($p < 0.05$). In CF-F508 patients (n = 5), S1P levels in serum were increased (+ 50%) compared with healthy subjects (n = 3). In contrast, S1P production by CF-F508 OCs was reduced (− 25%) as well as for healthy OCs treated with Inh172, compared to untreated healthy OCs.
Discussion: The S1P and its receptors play a major role in the circulation of OCs precursors from the serum to the bone compartment via a S1P concentration gradient. S1P stimulates OBs bone forming activity (Meshcheryakova A, 2017). Our results highlight the critical role of CFTR function in S1P production and the potential for differentiation and resorption activity of OCs. Our data suggest the existence of a disruption in the communication between OBs and OCs, via a reduction in the number of formed OCs and a decreased production of S1P. This may result in a reduction in osteoblast activity and consequently a decrease in bone formation in CF patients.
Keywords: Osteoclasts, Cystic fibrosis bone disease, S1P

P306

Hypophosphatemic osteomalacia induced by long-term low-dose adefovir Dipivoxil: clinical characteristics of 140 cases

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Objectives: To summarize the clinical characteristics of 140 cases with adefovir dipivoxil-induced hypophosphatemic osteomalacia.
Methods: The clinical data of 140 cases with hypophosphatemic osteomalacia caused by chronic hepatitis B in the treatment of adefovir dipivoxil were retrospectively analyzed.
Results: A total of 140 cases with hypophosphatemic osteomalacia who were treated with adefovir dipivoxil were included. The median age of the first visit was 58 years (48–64 years). The median duration of treatment was 12 years. Patients often showed muscle weakness, bone pain, activity limitation.The laboratory results showed that 130 cases (130/137) had hypophosphatemia [0.52 (0.41–0.62) mmol/L], and 127 cases (127/137) had high ALP levels [245 (171–303) U/L], 106 cases (106/117) had hypouricemia [122 (98–143) μmol/L], 22 cases (22/78) had hypokalemia, nondiabetic glycosuria occurred in 88 cases (88/125), proteinuria occurred in 105 cases (105/126), and 46 cases (46/117) showed metabolic acidosis. These results suggested renal tubular damage, Fanconi Syndrome. In addition, serum osteocalcin [26.8 (22.4–34.4) ng/ml] and beta C-terminal cross-linked telopeptides of type 1 collagen [991 (611–1344) ng/L] increased, suggesting that bone metabolism is active. 116 cases underwent imaging examination with "fracture, pseudo-fracture and osteoporosis" as the main performance. ADV administration was ceased immediately after diagnosis. Calcium carbonate and calcitriol were administered. 95 cases were followed up after treatment. The average follow-up time was 11.7 months (1–111 months). The results showed that the clinical symptoms were improved in 3 months and metabolic characteristics were recovered in 1 year.
Conclusions: Hypophosphatemic osteomalacia is prone to occur in patients with chronic hepatitis B treated with long-term adefovir dipivoxil at a therapeutic dose (10 mg/d). Its clinical manifestations are not specific, easily missed or misdiagnosed. After standard treatment, the prognosis is mostly good.
Keywords: Adefovir dipivoxil; Adverse drug reactions; Hypophosphatemic osteomalacia

P314

Effects of zoledronic acid on vertebral shape of children and adolescents with osteogenesis imperfecta

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Vertebral compression fracture (VCF) is a common and severe complication of osteogenesis imperfecta (OI). We aimed to prospectively observe the changes of vertebral shape during zoledronic acid (ZOL) treatment and assess influence factors of VCF in OI children. A total of 42 children and adolescents with OI received ZOL treatment for 6–36 months, who were classified into VCF and non-VCF (NVCF) groups matched for age and gender. Another 21 historical untreated control OI patients were also included who were matched for age, gender and clinical severity to OI patients with ZOL treatment. We performed quantitative vertebral morphometry on lateral spine radiographs and calculated concavity index (mh/ph), height-length ratio (ah/LL, mh/LL, ph/LL) and projection area (PA) of vertebrae from T4 to L4 before and after treatment. There were 31 OI patients with VCFs, who had significantly lower LS-BMD, mh/ph, ah/LL, mh/LL and ph/LL than patients without VCF at baseline. After ZOL treatment, the proportion of compressed vertebrae was decreased from 66.5% to 58.8% ($P < 0.001$), the average of mh/ph, ah/LL, mh/LL, ph/LL and PA elevated by 52.3, 35.0, 70.7, 12.5 and 50.8% in VCF group ($P < 0.01$). Compared to the historical untreated group, the compressed vertebrae proportion was less ($P < 0.001$) and the average of mh/ph, ah/LL and mh/LL were significantly higher ($P < 0.01$) in patients after ZOL treatment. LS-BMD and its increase was positively correlated to vertebral height and PA at baseline and the improvement of vertebral height and PA after ZOL treatment, respectively. In conclusion, ZOL could effectively reshape the compressed vertebrae in OI children. Low LS-BMD was an independent risk factor for VCF and the increase in LS-BMD was positively correlated to the improvement in vertebral shape after ZOL treatment.

NI Seminar

P190

Chronic inflammation in HLA-B27 transgenic rats, a model of spondyloarthritis, results in body and bone marrow fat depletion

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HLA-B27 transgenic rats (B27-Tg) are a well-established model of spondyloarthritis (SpA), a group of chronic inflammatory diseases that predominantly affects the axial joints. Nonetheless, systemic inflammation may also affect the eyes, skin, gut, and our studies previously showed that progressive inflammation also leads to bone loss. In this study, we analyzed the effect of chronic inflammation on bone marrow fat in B27-Tg rats.

Six-month-old male and female non-transgenic (NTG, N = 8) and SpA-prone B27-Tg (N = 6) rats of the 33–3 line (all on Fisher background) were examined. Chronic inflammation in B27-Tg rats was confirmed by a 2.7-fold increase of leukocytes and the development of anemia. Furthermore, the body weight of B27-Tg rats was reduced by 30% compared to NTG. Subcutaneous and gonadal fat were reduced by 70 and 65%, respectively in transgenic animals. Histologically, the fat pads of B27-Tg rats had smaller average size adipocytes (–55%), and the adipose tissue was highly infiltrated by immune cells. In addition, osmium staining revealed a threefold decrease in fat content in the bone marrow, which was confirmed by histological analysis counting adipocyte remnants. Loss of body and bone marrow fat resulted in a 2- to 3-fold decrease in the serum concentrations of the adipocyte-derived cytokines adiponectin and leptin as well as a twofold lower concentration of triglycerides. B27-Tg rats had increased serum levels of IL-17 and these correlated negatively with the amount of subcutaneous fat (-0.75, $p > 0.01$), gonadal fat (-0.80, $p > 0.001$), and bone marrow fat (-0.77, $p > 0.01$). Finally, treatment of 3T3 adipocytes with 50 ng/ml IL-17A during differentiation reduced adipogenesis by 30%. These data

show that chronic inflammation in B27-Tg rats severely decreases the amount of body and bone marrow fat and suggests that IL-17 may be implicated in this process.
Keywords: Inflammation, Bone marrow fat, Adipocytes

P136

Oxytocin-increase in bone, soleus muscle and hypothalamic paraventricular nuclei regulates the response to cold stress in mice exerting phenotype-dependent protective effects towards slow-twitch muscle

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Oxytocin (Oxt) is expressed in bone and required for muscle regeneration. Oxytocin receptor (Oxtr) regulates the response to cold stress (CS) through a feed-forward loop in brain. In this study we explored the physiological role of Oxt/Oxtr in bone, brain and soleus muscle(Sol) after CS in mice. The mRNA levels of *Oxt/Oxtr* in brain, bone, Sol after CS were measured. Immunohistochemistry for Oxtr was performed on the paraventricular nuclei (PVN) of the hypothalamus and on hippocampus as well as biochemical measurement of plasmatic Oxt. The expression of Myosin heavy-chain, *Mhc2b* (fast-glycolytic), *Mhc1* (slow-oxidative) was also investigated. Mice(n = 15) were divided into: controls maintained at room temperature (RT = 23 °C), exposed to CS at T = 4 °C for 6 h and 5-days(5d). *Oxt* mRNA was upregulated by fivefolds following 5d CS in bone ($p = 0.041$). *Oxtr* was up-regulated by onefold after 5d CS in Sol and by 0.5- and 0.3-fold respectively following 6 h and 5d CS in brain($p < 0.005$). *Mhc2b* was down-regulated by 0.96 and 0.88-folds after 6 h and 5d CS in Sol($p < 0.005$). Immunohistochemistry showed that Oxtr increases by twofolds in PVN and by 1.5-fold in hippocampus after CS($p < 0.005$). The plasmatic levels of Oxt are unaffected after 6 h but decreases by 0.2-fold after 5d CS ($p = 0.0141$). In sum, Oxy exerts phenotype-dependent protective effects towards slow-twitch muscle through up-regulation of Oxtr. CS induces a marked shift of Sol toward the slow-twitch phenotype. Oxy regulates the inter-organ communication between brain and Sol as shown by linear correlation analysis that was lost at 6 h ($R^2 = 0.17$) but improved at 5d ($R^2 = 0.67$) upon elimination of *Oxt* gene in brain. The plasmatic level of Oxt is low after 5d CS while the significant up-regulation of *Oxtr* found in Sol and PVN at 5d compounds the decrease of circulating Oxy. The increase of *Oxt* in bone may sustain its plasmatic level after CS.

P100

AKAP11 is a positive regulator of osteoblast extracellular matrix formation and mineralization

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Objectives: Bone matrix formation and mineralization are vital processes for skeletal health. Previous GWAS has identified *AKAP11*, in close proximity with *RANKL*, as a susceptibility locus of bone mineral density(BMD). *AKAP11* is a signaling-related molecule that facilitates signal compartmentation and transduction in various cells. However, its role in bone and relationship with RANKL is unknown. In this study, we investigated the role of *AKAP11* during osteoblast differentiation and matrix mineralization.
Methods: To evaluate the function of *AKAP11* in osteoblast, *AKAP11* knockout(KO) cells were generated using CRISPR/Cas9 gene editing in the mouse pre-osteoblastic MC3T3-E1 cells. Cell proliferation and osteogenic differentiation ability were evaluated. Expression of major bone signaling molecules was measured using qPCR. To better elucidate the molecular role of *AKAP11*, RNA-seq was performed to evaluate the transcriptome of *WT* and *AKAP11-KO* cells throughout differentiation.
Results: In the *AKAP11-KO* lines, Alizarin red S(ARS) staining of differentiated MC3T3-E1 revealed significant reduction of calcium mineral deposition (*AKAP11* ± : *− 44.1%, AKAP11*$^{-/-}$: *− 94.5%; P < 0.0001)*. Alkaline phosphatase(ALP) activity was significantly inhibited *(AKAP11-/-: − 41.1%; P < 0.001)* since differentiation phase(Day7). Furthermore, significant suppression of bone matrix genes expression were observed *(OC: − 58.9%; IBSP: − 27.9%; SPP1: − 85.1%; P < 0.001)* during the mineralization phase(Day16). In contrast, cell proliferation rate was not altered as demonstrated by MTT assay. There were also no significant expression alternations in major bone differentiation markers *(RUNX2; OSX)* and signaling molecules during osteogenesis. Notably, RANKL was not expressed in both WT and KO cells. Pathways enrichment analysis compared the transcriptomes of *AKAP11* KO lines line to *WT* controls revealed significant enrichment of differentially expressed genes in pathways associated with matrix organization, including collagen trimerizations *(P = 0.014)* and collagen fibrils assembly*(P = 0.025)* pathways.
Conclusions: This study suggested that AKAP11 plays a pivotal role in the bone matrix formation process, which is independent of RANKL and significantly contributes to proper matrix mineralization.
Keywords: RANKL AKAP11 Osteogenesis MC3T3-E1 mineralization

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Bone and the vasculature—a link between macro- and microvascular factors and bone metabolism?

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Background: Osteoporosis is a frequent disease, affecting up to 60% of elderly women and 30% of elderly men. Cross-sectional studies are repeatedly pointing to connections between bone metabolism and the vascular system, but these links are still poorly understood. In this study, we aim to connect parameters of bone metabolism, macro- and

microvascular parameters to further understand the mechanisms by which vascular impairment may alter bone structure and strength.
Methods: Data from the BioPersMed cohort (n = 1025, Biomarkers in Personalised Medicine a prospective follow-up cohort) were analysed, assembling asymptomatic patients with at least one cardiovascular risk factor. We performed bone density (BMD) and body composition measurements by dual-energy-X-ray-absorptiometry (iDXA scans). Macrovascular parameters include intima media thickness, blood pressure and pulse wave velocity (PWV). Microvasculature surrogates were provided by analysis of fundus photography and optical coherence tomography and included central retinal artery equivalent, central retinal vein equivalent, and arteriovenous ratio. Descriptive statistics was performed and Pearson' linear correlation coefficients were analysed.
Results: Total BMD correlates positively with PWV ($r = 0.084$, $p = 0.02$), systolic ($r = 0.134$, $p = < 0.001$) and diastolic ($r = 0.126$, $p < 0.001$) blood pressure, total body fat (TBF) ($r = 0.155$, $p < 0.001$) and microvascular status. BMD at the femoral neck correlates positively with TBF ($r = 0.174$, $p < 0.001$), and diastolic blood pressure ($r = 0.056$, $p = 0.038$). BMD at the femoral diaphysis correlates positively with TBF ($r = 0.246$, $p < 0.001$), systolic ($r = 0.065$, $p = 0.017$) and diastolic ($r = 0.066$, $p = 0.015$) blood pressure.
Conclusions: Correlations between BMD, pulse wave velocity and blood pressure as well as microvascular parameters suggest a link between bone metabolism and vasculature. Further investigation will elucidate potential mechanisms how impaired vasculature could lead to changes in bone metabolism, possibly adding more importance to these factors in conditions like diabetes, autoimmune diseases or smoking.
Keywords: Macrovasculature, microvasculature, bone, eye fundus

P016

Serum levels of Wnt-signalling parameters poorly reflect bone mass and metabolism in healthy boys and men

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Background: Bone turnover markers are used in research and clinical practice, but only in part reflect bone formation and resorption. Components of the Wnt-signalling pathway, regulating osteoblastogenesis and osteoblast function, can be measured in serum, however it is unclear whether they reflect underlying bone mass and metabolism.
Objectives: Determine whether serum levels of Wnt-signalling components reflect bone mass or metabolism in men during growth and after attaining peak bone mass age.
Methods: Sclerostin, DKK-1 and OPG were measured in 108 healthy males (34 ± 5 years) from the SIBLOS cohort and 122 peri-pubertal boys from the NINIOS cohort (13 ± 2 years) using the quantitative sandwich ELISA method developed by Biomedica. Procollagen type 1 N-terminal propeptide (P1NP), carboxy-terminal collagen cross-links (CTX) and osteocalcin were measured using ECLIA (Roche Diagnostics) in the SIBLOS cohort only. Dual-energy x-ray absorptiometry (Hologic) determined body composition and bone mineral content (BMC) at the whole-body and lumbar spine.
Results: In NINIOS, DKK-1 concentrations were higher than in SIBLOS (54.05 pmol/l, 33.45 pmol/l, respectively; $p < 0.001$), whereas OPG levels were non-significantly lower (3.50 pmol/l, 3.76 pmol/l, respectively; $p = 0.07$). No differences were found for sclerostin. No associations between lumbar or whole-body BMC and Wnt-signalling parameters were found. In SIBLOS, no associations were found between P1NP, CTX, and osteocalcin on the one hand and sclerostin, OPG and DKK-1 on the other hand.
Conclusions: Serum levels of sclerostin, OPG and DKK-1 were not related to bone mass in either peri-pubertal boys or adult men. Further, in adult men, they did not associate with P1NP, CTX or osteocalcin. However, as peri-pubertal boys showed higher levels of DKK-1 than adult men, DKK-1 being reflective for bone modelling cannot be ruled out. This will be addressed in the longitudinal part of the NINIOS study and by determining P1NP, CTX and osteocalcin in the NINIOS cohort.
Keywords: Wnt-signalling, Bone mass, Bone metabolism

P302

Vascular calcification relationship to bone microstructure in advanced chronic kidney disease

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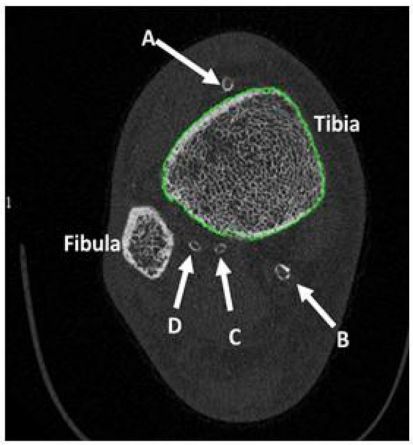
Introduction: Vascular calcification (VC) is highly prevalent (50–90%) in chronic kidney disease (CKD) and is associated with increased mortality. VC is also associated with 2–6 times increase in fracture risk. There is increasing evidence on the importance of bone microstructure assessment in CKD. This can be done using high resolution peripheral quantitative computed tomography (HR-pQCT) which can also be used to detect VC. Currently, abdominal aortic calcification (AAC) assessment using X-ray is the recommended test for VC in CKD. We aimed to assess VC relationship to bone microstructure in advanced CKD.
Methods: We recruited 69 CKD stages 4–5D patients who had HR-pQCT of distal radius and tibia, and dual energy X-ray absorptiometry (DXA) of lumbar spine, total hip and forearm. Ankle VC detected by distal tibia HR-pQCT was quantified in mgHA (Graph). AAC detected using vertebral fracture assessment images on DXA was measured using AAC-8 score. 43 CKD patients had trans-iliac bone biopsy evaluable for histomorphometry.
Results: Ankle VC had significant correlations with distal tibia cortical bone microstructure measured by HR-pQCT, negatively with cortical thickness (rho -0.35, $p < 0.01$) and positively with cortical porosity (rho 0.4, $p = 0.001$). No correlations were found with distal radius microstructure and DXA bone mineral density T-score. Amongst patients who had bone biopsy, ankle VC did not correlate with bone turnover, mineralization or volume. Ankle VC mass only weakly correlated with AAC score (rho 0.28, $p < 0.05$).
Conclusions: Ankle VC was associated with worse cortical bone microstructure of distal tibia.



This image was taken from one of our CKD participants. This image shows vascular calcification as circular hyperdensity shapes corresponding to anatomical territory of arteries in the ankle. Symbols: A, anterior tibial artery; B, posterior tibial artery; C, perforating branch of peroneal artery; D, peroneal artery.

Keywords: chronic kidney disease, vascular calcification

Poster Focus 1

P184

Microtomographic assessment of bone quality in murine joints affected by psoriasis arthritis

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Psoriatic arthritis (PA) is an inflammatory autoimmune-disease affecting the joint-cartilage-bone interface in the skeleton. In general, mid and end interphalangeal joints are predominantly affected by this disease. The immune reaction causes bone decay accompanied by pain and swelling of fingers. Quantitative assessment of the structural bone loss in the cartilage-bone-interface is needed to further unravel the cause and consequences of disease progression. We aimed to identify a sensitive µCT-protocol that reflects structural alterations in murine joints.

We used µCT-40 (Scanco) datasets (Figure 1) to quantify the bone quality of hind limbs mid phalangeal diaphysis and its proximal joint. Two PA models (control, study1 and study2 n = 5,3,6 respectively) were used.

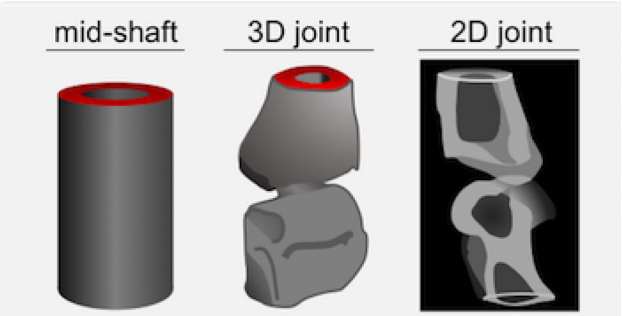

Figure 1: µCT datasets were used to compare the sensitivity of different parameters reflecting the structural decay of mid-shaft bone: bone volume (BV), cortical thickness (Ct.Th) and bone mineral density (M_{dens}). Quantitative joint analyses were performed by using parameters as joint bone volume (JBV) and bone density (TMD) in 3D, joint distances (JD) in 2 and 3D and subchondral bone thickness (ScBT) in x-ray images from µCT scans.

In both study groups mid-shaft BV and M_{dens} significantly differed from controls ($p \leq 0.05$), while Ct.Th was only different in the DKO group (Ctrl:0.121 ± 0.012 vs. DKO:0.0790 ± 0.0147 $p \leq 0.005$). M_{dens} indicated specific differences between the two study groups, while JBV reflected substantial cartilage problems in both study groups (Ctrl:0.414 ± 0.055, DKO:0.282 ± 0.078, TKO:0.277 ± 0.037, $p \leq 0.05$). No group changes were detected when JD was compared. ScBT reflected significant subchondral bone changes in Ctr, DKO, TKO groups ($p \leq 0.05$).

The tomographic assessment of cartilage-bone-interfaces signifies changes in health and disease of study groups. However, our data underlines that joint distances should be analyzed by spherical 3D measurements. Compositional changes in the bone-cartilage-interface can be quantified by density measurements. Assessment of subchondral bone thickness appears to be highly sensitive with respect to joint decay. Measurement of cortical indices should be combined with surface roughness information to characterize PA-induced bone decay.

P317

Defective O-glycosylation of FGF23 in a Chinese hyperphosphatemic familial tumoral calcinosis patient

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Objectives: Hyperphosphatemic familial tumoral calcinosis (HFTC) is a rare autosomal recessive disorder caused by deficiency or resistance of FGF23. Here, we collected a family with unreported FGF23 compound heterozygous mutations, aiming at investigating the clinical features, bone microarchitectures and the molecular mechanisms of the disease.

Methods: Clinical features and genetic analyses were collected from the patient. Bone microarchitectures were detected by high-resolution peripheral quantitative computed tomography (HR-pQCT). In vitro mutant and wild-type FGF23 expression and glycosylation were

analyzed by western blot and Wheat Germ Agglutinin affinity chromatography.

Results: The proband was a 25 year-old emaciated male who revealed lower extremities pain, tooth loss and multiple painless, subcutaneous calcified nodules in both lower extremities. Laboratory examinations revealed severe hyperphosphatemia (S-P: 3.01 mmol/L, normal: 0.81–1.45), significantly elevated c-FGF23,(304.7 pmol/L, normal: 4.51–36.09),high serum RANKL (621.45pml/L, normal: 297.04–381.64) and normal OPG (3.2 pmol/L, normal: 1.8–6.4). As for bone microarchitectures, both radius and tibia showed low total BMD and elevated total area. In radius, there were reduced cortical BMD, cortical thickness, trabecular number, BV/TV and thickness. The bone stiffness was significantly decreased by FEA. In tibia, the microarchitecture of cortical bone was poor like radius, while the trabecular bone revealed significantly elevated trabecular BMD, BV/TV and thickness. The bone strength of tibia was even stronger than healthy people. The patient carried c.413T > G (p.L138R) and c.491T > A (p.I164 N) compound homozygous mutations in FGF23 gene. Functional experiments in vitro revealed that two mutant FGF23 proteins had defective O-glycosylation and difficulties in secretion compared to wild type FGF23.

Conclusions: HFTC patient with FGF23 c.413T > G and c.491T > A mutations revealed hyperphosphatemia, elevated c-FGF23, increased bone resorption and poor bone microarchitecture. Defective O-glycosylation and secreting difficulties of FGF23 protein might be the molecular mechanisms of the disease.

Keywords: Hyperphosphatemic familial tumoral calcinosis, fibroblast growth factor 23, mutation, HR-pQCT, molecular mechanism

P089

Towards a nutraceutical-based complementary medicine in bone health: cruciferous vegetables extracts and derivates induce osteogenic differentiation of human mesenchymal stromal cells

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Introduction: The exponential increase in the geriatric population and the high prevalence of bone fragility highlights the need to investigate the potential role of active nutrients in the prevention of bone loss. Cruciferous vegetables are rich in glucosinolates (GLS) which are hydrolysed by plant or gut microbiota myrosinase in their biologically active form, isothiocyanates (ITC). Interestingly, ITC are a source of hydrogen sulfide (H_2S), a newly identified endogenous anabolic gasotransmitter with therapeutical potential in bone loss pathologies. Since ITC are less stable than GLS, we evaluated whether GLS can release H_2S before conversion to ITC and whether they retain the ability to induce osteogenic differentiation of human mesenchymal stromal cells (hMSCs), typical of H_2S donors.

Methods: Different cruciferous extracts and GLS were administered in vitro during osteogenic differentiation of hMSCs (3,3-100 μM). Alizarin red staining assay and Real-time PCR were, then, performed to evaluate mineral apposition and gene expression. Intracellular H_2S levels were detected by cytofluorimetric analyses while H_2S release by GLS in buffer was detected by amperometric measurements. Kruskall-Wallis test was performed for statistical analyses.

Results: GLS showed to release H_2S in buffer solution; moreover, H_2S intracellular levels rose under GLS stimulation. Among GLS, glucoraphanin significantly increased mineral apposition by hMSCs ($p < 0.01$) and up-regulated several osteogenesis reference genes such as BSP ($p < 0.05$) and SMAD1 ($p < 0.01$). Similarly, among extracts, lepidium sativum largely increased mineral matrix deposition (30 μM > 20%; $p < 0.0001$).

Conclusions: These preliminary results confirmed the biologically activity of GLS in hMSCs and suggest the use of cruciferous derivatives as natural alternatives to chemical H_2S donors; laid the ground for further studies aimed to correlate nutrients intake, H_2S blood levels and bone status and define preventive/clinical dietary protocols for patients with an increased risk of bone fragility.

Keywords: Hydrogen sulfide; osteogenesis; glucosinolates; nutraceuticals; bonel loss

P309

Progressive, immobilizing osteoporosis in a 11 year old, male, identical twin—an (unsolved) case report

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In children osteoporosis is a rare skeletal disorder. The muscle bone unit describes the interaction between muscle forces and resulting bone structure. Immobilization, primary muscle diseases and some medications can lead to secondary osteoporosis.

Here, we report on a 13 year-old boy with a healthy, identical twin with a 2-year history of waddling gait, followed by recurrent long bone fractures leading to complete immobility within 15 months. Otherwise, his past medical history was unrevealing.

DXA measurement showed decreased bone mineral density with an age-related z-score of − 4.0. A whole-body MRI and a lumbar puncture revealed unremarkable results. Pompes disease, Fabrys disease and spinal muscular atrophy, as well as most bone fragility syndromes were excluded by appropriate genetic and laboratory studies. Laboratory test showed no sign of a skeletal or rheumatic disease except for elevated, but unspecific, bone turnover markers.

At time of presentation in our clinic, the patient had already received two cycles of bisphosphonate treatment, without clinical improvement and was wheelchair bound.

The most prominent clinical sign was a peripheral weakness, with atrophy of the muscles of the upper extremities, as well as massive metaphyseal osteolysis of the extremities and the spine. Nerve conduction velocity and electromyogram were normal, as were results from a muscle biopsy. The bone biopsy showed a massive mineralization disorder, with no signs of increased bone resorption.

The underlying etiology in our patient remains unclear. Clinically it seems to be similar to classical hypophospatasia or rheumatological activation of osteoclasts. However, neither diagnoses could be confirmed by appropriate tests so far.

For symptomatic treatment, 8 cycles of osteoclast inhibition with denosumab (1 mg/kg s.c.) were applied. The clinical situation of the patient improved significantly, but without restitution ad integrum. Further diagnostics regarding the mineralization deficit and bone turnover are necessary.

Keywords: Osteoporosis pediatrics osteolysis mineralization denosumab

P298

Paget's disease or bone metastasis? A case report with diagnostic challenge

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We report, the case of a 68-year-old man, in whom radical retropubic prostatectomy was performed due to prostatic cancer. Few weeks after the operation a strong, diffuse pain occurred at the left tibia and lumbar spine. Considering the history, a search for metastatic bone changes was started.

On X-ray findings abnormal bone architecture, "blade-of-glass" change on the left tibia and an inhomogeneous lytic change one centimeter in diameter on the second lumbar vertebra were observed. MRI of the lumbar spine displayed multifocal untypical inhomogeneity on the concerned vertebra.

Also an isotope enrichment typically characterized by Paget's disease in the proximal tibia was detected by whole body bone scan (skeletal scintigraphy). Although the etiology of the enhancement found in the second lumbar vertebra was unclear.

Laboratory findings showed a mild increase of alkaline phosphatase level, while prostatic specific antigen level as well as other tumor factors were negative.

Malignant tumor could not be detected by oncological examinations. Zoledronate infusion therapy was administered with the diagnosis of Paget's disease. Shortly after the pain of the lower limb and lumbar spine was disappeared. The pelvic asymmetry and the deformity of the tibia caused by the disease were corrected by complex physiotherapy.

This case required a cautious and expert approach to differential diagnoses in order to deliver the most appropriate treatment with the most appropriate timing for any of the two diseases.

Keywords: Paget´s disease, prostate cancer, metastatic bone disease

P269

Short-term teriparatide treatment effect in patients with severe osteoporosis

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Among various osteoporosis medications, to date, bone forming agent such as parathyroid hormone is considered as one of the most potent medication for the increase of bone mineral density (BMD) and fracture prevention in patients with osteoporosis. However, its application is limited due to its discomfort for use and high cost. Accordingly, that was applied during a short period in real clinical field with a lack of evidence. The aim of Tthis study is to evaluated effects of short-term teriparatide on changes of BMD.

Total 116 patients (mean age: 74, female: 108) that underwent short-term teriparatide treatment (less than 12 months) in orthopedic department for severe osteoporosis were reviewed. Both spine and hip BMD were measured at baseline and 1 year after teriparatide treatment. Correlation between duration of teriparatide usage and BMD change was evaluated. According to duration of teriparatide treatment, changes of the BMD were also analyzed.

Mean duration of teriparatide treatment was 3.5 month (range 1–12). Mean spine and hip BMD were 0.638 ± 0.111 and 0.660 ± 0.104 g/cm^2 at the baseline, respectively. After the teriparatide treatment, mean total increments of spine and hip BMD were 8.1 ± 8.4 and $0.6 \pm 5.2\%$ at 1 year after the treatment, respectively. Although the increment of hip BMD was not correlated with duration of the teriparatide usage, the increments of spine BMD showed significant positive associations with the duration of teriparatide treatment ($r = 0.329$, $p = 0.002$). Amount of the spine BMD increment were 8.79 ± 8.07, 8.63 ± 8.48, 10.76 ± 9.09, 12.8 ± 9.4, $13.4 \pm 9.84\%$ when the teriparatide treatments were continued during more than 1, 2, 3, 4 and 5 months, respectively.

After short-term teriparatide treatment ($\leq$ 12 months), although hip BMD did not show any changes, significant increments were shown in spine BMD. The amounts of the BMD increments were proportional to longer duration of teriparatide usage.

Poster Focus 2

P070

Dissecting the role of CXCR4 in breast cancer cell-bone microenvironment interaction

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C-X-C chemokine receptor type 4 (CXCR4) is an α-chemokine receptor specific for the stromal-derived-factor-1. CXCR4 is involved in breast cancer (BrCa) homing to the bone and in hematopoietic stem cell (HSC) quiescence. We hypothesised that, mirroring its role in HSC, CXCR4 could also contribute to BrCa cell dormancy in bone. The disruption of dormancy of BrCa cells is responsible for the relapse after a prolonged disease-free period affecting 25% of patients. To test our hypothesis, we sorted human BrCa MDA cells expressing high CXCR4 (CXCR4^{HIGH}MDA), which represented 1–5% of the total MDA population. After stimulation with stem factors, in vitroCXCR4^{HIGH}MDA produced similar number and size of primary mammospheres compared to CXCR4^{LOW}MDA ($p > 0.3$), suggesting a comparable stemness and tumour-initiating ability. However, when CXCR4^{HIGH}MDA were injected in the tibia medullary cavity of immunocompromised CD1-nu/nu female mice, a significant lower number of overt tumours was observed by bioluminescence and X-Ray analyses over four weeks, compared to CXCR4^{LOW}MDA-injected mice (− 80% incidence; Chi square $z = 2.101$; $p = 0.001$). Accordingly, osteolysis measured at endpoint by microCT was lower in CXCR4^{HIGH}MDA injected mice (+1.3 fold cortical BV/TV % $p = 0.02$). Distant organ metastatic colonisation showed alike incidence but decreased size of liver micrometastases in CXCR4^{HIGH}MDA-injected mice (− 50% $p = 0.029$). These results evidenced that CXCR4^{HIGH}MDA-injected mice formed less tumours in bone and smaller metastases in liver, suggesting that the bone microenvironment mitigated CXCR4^{HIGH}MDA growth and metastatic spread. Accordingly, survival statistics run on public databases showed that BrCa patients who express high CXCR4 have a greater overall-survival compared to patients who express low CXCR4 (LogRank p-value:0.012). Altogether these results support the hypothesis that CXCR4 plays a key role in BrCa interaction with the bone microenvironment. More in detail, the bone microenvironment is likely to exert an inhibitory effect on the CXCR4^{HIGH}MDA, impairing proliferation and possibly contributing to cellular dormancy.

P014

Diagnostic accuracy of a novel TRAP5b assay in diagnosing renal osteodystrophy

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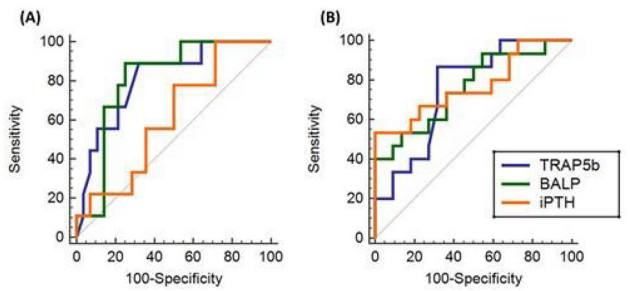
Introduction: Renal osteodystrophy (ROD) is common in advanced chronic kidney disease (CKD) and is associated with increased fracture and mortality. The gold standard diagnostic test for ROD is bone biopsy which is required before bone-specific treatment could be initiated to reduce fracture risk. Bone markers such as tartrate resistant acid phosphatase 5b (TRAP5b) may provide a non-invasive alternative to bone biopsy as it does not accumulate in CKD. We aimed to compare the diagnostic accuracy of a novel TRAP5b assay (Nittobo) against usual tests of bone alkaline phosphatase (BALP) and intact parathyroid hormone (iPTH) in diagnosing low and high bone turnover ROD.
Methods: We recruited 43 advanced CKD patients stages 4–5D. Fasting serum samples were analysed using Nittobo TRAP5b manual assay and Immunodiagnostic Systems automated assays for BALP and iPTH. Trans-iliac bone biopsy was performed for histomorphometry. Normal bone turnover was defined as bone formation rate/bone surface (BFR/BS) of 18–38 um^3/um^2/year. Receiver operating characteristics analysis was used to test diagnostic accuracy of the biomarkers to identify low and high bone turnover.
Results: Eleven patients had low, 15 had normal and 17 had high bone turnover. TRAP5b and BALP had similar diagnostic accuracy to diagnose low bone turnover ($p > 0.05$) (Graph A). Both markers were better than iPTH ($p < 0.05$). For diagnosing high bone turnover (Graph B), all markers had similar diagnostic accuracy ($p > 0.05$).
Conclusions: Diagnostic accuracy of the novel TRAP5b assay by Nittobo was as good as BALP in diagnosing low and high bone turnover.



[ROC curves for biomarkers to identify (A) low and (B) high bone turnover ROD.]

Keywords: Chronic kidney disease, renal osteodystrophy, biomarkers

P024

Dynamic deformation by fluid flow on hematopoietic progenitor cells alters osteoclast differentiation

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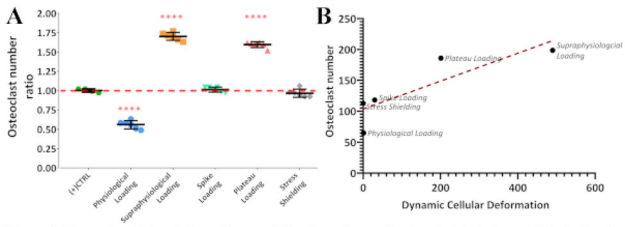
Bone undergoes constant remodeling to adapt to external mechanical loading. Under physiological conditions, mechanical loading causes bone formation to meet functional demands. In contrast, poor osseointegration of orthopedic implants changes the mechanical microenvironment in the peri-prosthetic interface, leading to bone degradation. How mechanosensitive cells shift from bone formation to degradation remains elusive. The goal was to determine the influence of different fluid flow regimes on cell deformation in an in vitro model for mechanical induced bone implant loosening.

Hematopoietic progenitor cells (1,0x10^5/cm2) were subjected to different loading regimes by pulsating fluid flow, using a parallel-plate flow chamber. Conditioned medium from mechanically loaded cells was added to a RANKL-induced osteoclastogenesis assay.

Soluble factors released after 2 min of supraphysiological loading (1.7-fold, $p < 0.001$) and plateau loading (1.6-fold, $p < 0.001$) induced osteoclast differentiation compared to the positive control, while physiological loading reduced osteoclast differentiation (0.4-fold, $p < 0.001$). Spike loading and stress shielding did not change osteoclast differentiation compared to the positive control (Figure 1A). The dynamic cellular deformation = ((Peak duration [s] × Peak wall shear stress [Pa]) + (Plateau duration [s] × Plateau wall shear stress [Pa])) × Peak wall shear stress rate [Pa/s] and had a positive correlation to the osteoclast number (Pearson R2 = 0.74, Figure 1B).

Our results suggest that the dynamic cellular deformation regulates the release of bone-modulating soluble factors by mechanosensitive cells. Understanding how mechanosensitive cells respond to changes in deformation leading to either suppression or induction of osteoclast differentiation could open up new treatment strategies to delay or stop prosthetic loosening.



[Figure 1: Dynamic cellular deformation regulates the release of osteoclast-inducing soluble factors.]

P219

SOST polymorphisms are associated with lean mass in Chinese male offspring

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Aims: The correlation between osteoporosis and lean mass has been established both genetically and phenotypically. In addition, genes associated with osteoporosis may also be candidates for lean mass. Human *SOST* gene inhibits osteoblastic bone formation by inhibiting the Wnt signaling pathway. This study aimed to investigate the effect of *SOST* gene polymorphisms on lean mass in young Chinese men.
Methods: We recruited a total of 1056 individuals from 353 Chinese nuclear families with male-offspring. The nuclear families were composed of one healthy male child between 18 and 44 years old.

Ten tagged single-nucleotide polymorphisms (SNPs) in *SOST* gene (rs1234612, rs1513670, rs1634330, rs1708635, rs2023794, rs7220711, rs74252774, rs851057, rs851058 and rs865429) were genotyped in all the above people. Lean mass was measured by dual-energy X-ray absorptiometry (DXA). The associations of the SNPs with lean mass were analyzed using the quantitative transmission disequilibrium test (QTDT). $P < 0.05$ was considered significant for all analyses.

Results: Using QTDT to detect within-family associations, rs1634330 was found to be significantly associated with the trunk lean mass ($P = 0.0049$). The 1000 permutations were in agreement with the within-family association result ($P = 0.041$).

Conclusions: Our results suggest that the genetic polymorphisms in *SOST* gene may contribute to variations in trunk lean mass of Chinese male offspring.

Keywords: *SOST*, lean mass, polymorphism, transmission disequilibrium test

P048

Skeletal maturation in relation to ethnic background in children of school age

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Objectives: Skeletal age is widely used to assess growth and development in children. Deviation of skeletal age from chronological age can influence health and disease. Our aim was to evaluate the influence of ethnic background on skeletal age in children.

Methods: We included children from a multiethnic population-based cohort, assessed at a mean age of 10 years. Ethnic background was described as geographic ancestry (questionnaire-based assessment of country of parents' birth/origin) (N = 5,325) and genetic ancestry (based on admixture analysis) (N = 3,364). Log-ratio transformation was used to determine whether the percentage of Asian or African ancestry as compared with that of Europeans (reference) influenced skeletal age. Skeletal age was assessed by a trained observer on hand DXA scans using the Greulich and Pyle atlas method. Associations between ethnic background and skeletal age were investigated using linear regression models adjusted for age, sex, height and BMI.

Results: Based on geographic ancestry, 84% of the children were classified as European, 6% as Asian and 10% as African. Children of European background had younger skeletal age than those of Asian background by $\sim$ 7 months (B = 0.55, 95% CI 0.43–0.67, $P < 0.001$) and African background by $\sim$ 4 months (B = 0.35, 95% CI 0.26–0.45, $P < 0.001$). A similar pattern was observed in the analysis of genetic ancestry. Furthermore, increase in the genomic percentage of Asian ($B_{(\text{per log[Asian/European]proportions})} = 0.08$, 95% CI 0.06–0.10, $P < 0.001$) or African ($B_{(\text{per log[African/European]proportions})} = 0.07$, 95% CI 0.06–0.09, $P < 0.001$) ancestry was associated with accelerated skeletal maturation.

Conclusions: Children of Asian and African background have higher skeletal age than children of European descent. The influence of ethnic background on skeletal age is not solely explained by anthropometric and cultural differences, with a genetic ancestral component playing a major role.

P052

Variation in mandibular cortical thickness in relation to bone mineral density in children of school age

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Objectives: Bone loss occurs throughout the skeleton including the mandible. Thinning of the mandibular cortex has been demonstrated in patients with osteoporosis and associated with poor oral health. We examined the association between mandibular cortical thickness and bone mineral density (BMD) measured at the total body (TBLH-) and skull- (SK-) in multiethnic children of school age.

Methods: We studied 1,798 children (48.9% males) at a mean age of 13.6 (SD = 0.28) years participating in a multiethnic population-based cohort. Cortical mandibular thickness was determined from panoramic radiographs (OPGs) using Panoramic Mandibular Index (PMI) and Mental index (MI). A subsample of 200 OPGs was assessed by two independent observers. TBLH-BMD was measured using iDXA. Linear regression models adjusted for age, sex and ethnicity were used to test association between MI and PMI with BMD.

Results: Both indices showed excellent intra-class correlation (ICC $\geq$ 90%, P $\geq$ 0.3) and good agreement between observers (inter-class correlation $\geq$ 78%, P $\geq$ 0.1). One-unit increase in BMD was associated with 0.13 (95% CI 0.10–0.15, $P < 0.0001$) higher PMI. Furthermore, the same increase in BMD resulted in 4.7 mm (95% CI 4.1–5.2 $P < 0.0001$) thicker cortices. Children of African background showed higher PMI and MI as compared to those of European background (B_{PMI}: 0.01, 95% CI 0.01–0.02, p = 0.001; B_{MI}: 0.45, 95% CI 0.29–0.62, $P < 0.0001$), while no significant differences were observed between children of Asian and European background (B_{PMI}: 0.003, 95% CI − 0.01 to 0.02, P = 0.55; B_{MI}: 0.20, 95% CI − 0.02 to 0.41, P = 0.08).

Conclusions: Thickening of the mandibular cortex differs by ethnicity (being greater in children of African descent) and is associated with higher BMD in children; relation already present during childhood. These findings are relevant for the assessment of oral and skeletal health during the life course (e.g. periodontitis, implant success, osteoporosis).

POSTER

P002

The significance of two serum markers in the diagnosis of rheumatoid arthritis

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Introduction: Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown etiology in the pathogenesis of which, the role of cyclic citrullinated peptides has not been fully elucidated, but their use as an early diagnostic marker is important.

Objectives: To determine the incidence of antibodies against cyclic citrullinated peptides (anti-CCP) and to collerate it with the presence

of rheumatoid factor (RF) in a group of patients with suspected rheumatoid arthritis.
Methods: 74 sera of patients with symptoms of mild to very severe rheumatoid arthritis were collected and tested for the presence of anti-CCP and IgM RF antibodies. The test for the anti CCP was performed with the MEDICON anti-RA/CCP enzyme immunoassay kit and for the IgM RF using the nephelometry method (Dade Behring).
Results: In 44 out of 74 patients the titers of both antibodies ranged within normal levels. Of the rest, 18 had elevated titers of both antibodies whereas 12 tested positive only for IgM RF. All patients with negative antibody titres and those with only RF antibodies positive, exhibited mild and non-specific symptoms whereas those 18 patients with elevated anti-CCP and IgM RF titers also had clinical features sufficient for the diagnosis of rheumatoid arthritis.
Conclusions: Anti-CCP antibodies are an important and specific indicator for the diagnosis of rheumatoid arthritis. Rheumatoid factor, although a sensitive marker for RA, lags behind in specificity because it is detected in small proportions not only in a part of healthy population but also in other autoimmune diseases.
Keywords: RF, anti-CCP, Rheumatoid arthritis.

P004

Presence of anti-nuclear antibodies in patients with positive anti-ccp antibodies

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Objectives: To study the incidence of positive antinuclear antibodies (ANA) in patients with diagnosed rheumatoid arthritis (RA) without any other coexisting collagen disease who had positive anti-CCP antibodies (antibodies to cyclic citrullinated peptide).
Methods: The population of our study consisted of 76 patients with diagnosed RA and positive anti-CCP antibodies. The presence of anti-nuclear antibodies (ANA) in serum was detected by indirect immunofluorescence using HEP-2 cells (BIOSNA). Sera with ANA titre $\geq$ 1/80 were considered positive.
Results: Of the 76 patients, 34 (44.7%) were ANA-positive by indirect immunofluorescence at $\geq$ 1/80. The type of immunofluorescence in the positive ANA cases was mainly homogeneous (82.3%). None of the positive ANA patients presented any positive specific auto antibody (anti-ds-DNA, anti-RNA, anti-Sm, anti-Ro, anti La, anti- Jo-1). The incidence of positive ANA was higher in patients with advanced RA than in patients with early disease.
Conclusions: The presence in sera of patients with RA of anti-nuclear antibodies (as well as other autoantibodies) appears to be non-specific and possibly implies polyclonal activation of B lymphocytes.
Keywords: ANA, anti-CCP, Rheumatoid arthritis.

P006

Serum 25-hydroxyvitamin D levels of healthy men in greece

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Objectives: The objective of this observational cross-sectional study is to identify the prevalence of vitamin D deficiency in healthy adult men in Greece, as reflected by the levels of 25-hydroxyvitamin D (25(OH)D), since recent data indicate that vitamin D deficiency can be common in countries previously considered as low risk (e.g. Mediterranean countries).
Materials and methods: A population of 134 community dwelling men was recruited at the health promotion events carried out by the Hellenic Society for the Support of Patients with Osteoporosis in rural and urban areas throughout Greece. Serum total calcium (Ca), phosphorus (P), creatinine, parathyroid hormone (PTH) and 25(OH)D were measured. The study was approved by the Ethics Committee of Harokopio University.
Results: The mean age of the population was 44.34 years (range 18–78 years) while 88.7% were18–65 years old. Mean serum 25(OH)D was 21.94 ng/mL, mean PTH was 40.01 pg/mL and mean Ca, P and creatinine were 9.21, 3.22 and 0.88 mg/dL respectively. Concerning the vitamin D levels, 42.5% of the subjects had deficient (0–19.9 ng/mL), 35.8% had insufficient (20–29.9 ng/mL) and only 21.6% had adequate (30–150 ng/mL) levels, while 8.2% had vitamin D levels $\leq$ 10 ng/mL. PTH was at normal range (15–65 pg/mL) for 89.3% of the population and 8.4% had high PTH (> 65 pg/mL). The levels of serum Ca, P, and creatinine, were within the normal range.
Conclusions: Approximately the half of Greek adult men (45.2%) in this study had vitamin D levels below 20 ng/mL. Given that low levels of 25(OH)D are associated with increased risk for fractures and exacerbate bone loss, this study highlights the emerging issue of 25(OH)D insufficiency in Greek men population and the need for targeted interventions even in age groups not previously considered as at risk.
Keywords: 25-hydroxyvitamin D, Greece, epidemiology, men, 25(OH)D deficiency, parathyroid hormone

P007

A pilot study of a-KLOTHO serum concentrations in patients with kidney disease using a highly sensitive fluorescence immunoassay based on plasmonic microtiter plates

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Objectives: It is well established that a-KLOTHO, being an essential co-receptor for FGF23, plays an important role in kidney disease (KD) and thus also in bone biology. A large number of studies tried to determine the value of a-KLOTHO levels as a biomarker in KD. (1). However data are very inconsistent and partly contradictory. Therefore we decided to use our recently developed highly sensitive and reliable fluorescence immunoassay platform to provide a new tool for a-KLOTHO serum measurements.
Methods: Antibodies recognizing human a-KLOTHO raised in rats and rabbits were used to setup a sandwich fluorescent immunoassay on plasmonic microtiter plates (2) with AlexFluor680 for detection. The assay procedure consists of incubating 50ul of fluorescent labeled detection antibody together with 10ul of serum sample over night in the dark followed by measuring at Em/Ex = 680/720 nm with a conventional fluorescent microplate reader. This assay prototype was used to determine a-KLOTHO in a collection of 13 samples from patients with mild to severe kidney disease according to ICD-10-CM coding.

Results: Intra- and Interassay CVs of this assay prototype were determined as 6% and 12%, LLOQ was 12,5 pmol/L and LLD 4,8 pmol/L. a-KLOTHO serum levels clearly increased with disease severity from 15.8 ± 7.2 (mild KD) over 46,0 ± 18.1 (moderate KD) to 150.0 ± 72,6 pmol/L (severe KD).
Conclusions: These results are in line with some studies that showed an increase of a-KLOTHO serum with deterioration of kidney functions while others found decreasing serum concentrations of this biomarker in such collectives. More studies and head to head comparisons are needed to clarify the influence of assay methods and of collective characteristics.
Literature:
1) Wang Q et al., Biomed Res Int. 2018 Aug 12
2) Hawa G et al. Anal Biochem. 2018 May 15;549: 39–44.

P008

Association between bone turnover marker and bone density in middle aged women

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Objectives: Recently, elevated serum homocysteine levels have been reported to be associated with increased fracture risk and low broadband ultrasound attenuation of heel in elderly men and women. We investigated associations between serum homocysteine levels and bone mineral density (BMD) in healthy Korean women.
Methods: The study population was comprised of 610 healthy premenopausal and postmenopausal Korean women. BMD at the lumbar spine and femoral neck were measured by dual energy x-ray absorptiometry. Serum concentrations of homocysteine were measured with a competitive immunoassay using direct, chemiluminescent technology.
Results: Serum concentrations of homocysteine were significantly higher in postmenopausal than in premenopausal women ($p < 0.001$). After the adjustment for confounding variables, we did not observe a significant correlation between serum bomocysteine levels and BMD in total subjects($\beta = -0.023$, $p = 0.506$ for lumbar spine; $\beta = -0.034$, $p = 0.493$ for femoral neck). With the analyses by menopausal status, there were still no significant associations between serum homocysteine levels and BMD in both premenopausal and postmenopausal women.
Conclusions: Measurement of serum homocysteine may not be useful for the risk assessment of osteoporosis.

P009

C-reactive protein for early detection of postoperative systemic infections in intertrochanteric femoral fractures

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This retrospective cohort study investigated perioperative C-reactive protein (CRP) for early detection of postoperative systemic infective complications in elderly patients with intertrochanteric femoral fractures. A total of 250 patients aged ≥ 65 years, with intertrochanteric femoral fractures that were surgically treated between January 2011 and December 2015 were included. CRP value was measured preoperatively and on postoperative day (POD) 3, 5, and 10, and analyzed with regard to postoperative systemic infections, delirium, and death within 1 year. The patients were divided into two groups according to postoperative systemic infection, and perioperative CRP responses between the two groups were compared. The CRP threshold that maximized sensitivity and specificity for the detection of patients with systemic infections was identified. Systemic infections were reclassified as pulmonary and extra-pulmonary infections. The mean CRP values preoperatively and on POD 3, 5, and 10 were 2.82, 10.10, 3.74, and 1.89 mg/dL, respectively. Postoperative systemic infections, delirium, and death within 1 year were noted in 35 (14.0%), 30 (12.0%), and 45 (18.0%) patients, respectively. The CRP value in patients with postoperative systemic infections significantly elevated on POD 5 and 10 ($p < 0.001$, $p < 0.001$), and cut-off values were 4.71 and 1.59 mg/dL on POD 5 and 10, respectively. Postoperative delirium and death within 1 year were observed more often in the group with postoperative systemic infections ($p = 0.003$, $p = 0.014$). Although preoperatively elevated CRP values did not influence the postoperative CPR responses, they were significantly associated with delirium ($p = 0.015$). The CRP value on POD 5 is the earliest predictor of postoperative systemic infections in elderly patients with intertrochanteric femoral fractures that managed surgically. Moreover, when the CRP value on POD 5 is > 4.71 mg/dL, the possibility of postoperative systemic infections should be considered.
Keywords: Intertrochanteric femoral fracture, Systemic infection, CRP

P010

Development and characterization of an extraction-free human CGRP sandwich ELISA

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Objectives: CGRP (calcitonin gene related peptide) is a secreted neuropeptide with a length of 37 amino acids that belongs to the amylin family. It has an N-terminal disulfide bond and exists in two isoforms (α-CGRP, β-CGRP) which differ only in three amino acids. CGRP is a strong vasodilator released from perivascular nerves and it is involved in the regulation of the vascular tone. Further, it plays an important role in pain transmission. CGRP has an osteoanabolic effect as it is involved in bone formation and inhibition of bone resorption.
Methods: We selected two monoclonal anti-human CGRP antibodies for the development of a sandwich ELISA assay. Antibodies were characterized for isoform binding and epitope mapping with microarray technology. Assay parameters like specificity, dilution linearity and spike recovery were determined, and different sample matrices were tested (serum, heparin plasma, citrate plasma, EDTA plasma).
Results: The selected antibodies react with both CGRP isoforms. The monoclonal coating antibody binds to a conformational epitope in the N-terminal part of the peptide, and the monoclonal detection antibody binds to a linear epitope directly at the N-terminus. The developed sandwich ELISA assay is calibrated with α-CGRP and it detects CGRP without extraction and with short incubation in human serum, heparin plasma, citrate plasma and EDTA plasma. All assay characteristics (specificity, dilution linearity, spike recovery) meet the standards of acceptance.

Conclusions: The novel human CGRP ELISA assay is a quick and easy to handle, reliable and accurate tool to quantify human CGRP in human serum and plasma samples.
Keywords: CGRP, calcitonin gene related peptide, sandwich ELISA

P011

Association of telomere length with lifestyle factors in postmenopausal women

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Objectives: Telomeres are the protective end caps of chromosomes that are shortened during each cell division, eventually leading to cell senescence and apoptosis. Telomere length measures could serve as a biomarker of biological age and a risk factor for age-related diseases including osteoporosis. Various lifestyle factors could modify telomere length by influencing oxidative stress and inflammation. We aimed to study the association of leukocyte telomere length with smoking and physical activity in postmenopausal women.
Methods: DNA was isolated from whole blood of 275 women aged 67 ± 9.43 years who have been 16.8 ± 10.0 years since menopause. Leukocyte telomere length (LTL) was measured using quantitative real time polymerase chain reaction. Data about smoking and physical activity (walking, running, cycling, swimming and other activities) was obtained from self-report questionnaires. The study was approved by the ethics committee.
Results: There was no statistically significant difference in LTL between ever-smokers and never-smokers (0.925 ± 0.218 vs. 0.929 ± 0.217, p = 0.647), however there was a trend for longer LTL in current non-smokers compared to current smokers (0.935 ± 0.217 vs. 0.884 ± 0,207, p = 0.104) and longer LTL in previous smokers compared to current smokers (0.987 ± 0.226 vs. 0.884 ± 0.207, p = 0.059). We could not demonstrate significant difference in LTL between physically active group, exercising at least 2.5 h per week and physically inactive group, exercising less than 2.5 h per week (0.919 ± 0.204 vs. 0.947 ± 0.259, p = 0,566) and also when separate analyses were done by specific activity.
Conclusions: This was the first study of influence of lifestyle factors on LTL in Slovenian postmenopausal women. Smoking tends to decrease the LTL, which may increase the risk of age-related diseases such as osteoporosis. Further studies including the duration and intensity of smoking should be performed.

P012

FluoBolt™-PERIOSTIN, a new highly sensitive fluorescence immunoassay for this matricellular protein based on plasmonic microtiter plates

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Objectives: The extracellular matrix protein PERIOSTIN has been extensively studied in respiratory-, bone- and oncological diseases and several different assays methods are currently available for this protein. For some applications (e.g. detection in breath condensate (1) or gingival crevicular fluid (2)) a high sensitivity of the used assay is required. Therefore we decided to use our recently developed highly sensitive fluorescence immunoassay platform (3) to provide a new tool for PERIOSTIN measurements for all research fields where this marker is studied.
Methods: A monoclonal mouse antibody recognizing the C-terminus of PERIOSTIN and a goat polyclonal antibody raised in goat were used to setup a sandwich fluorescent immunoassay on plasmonic microtiter plates with FITC, Cy3, Cy5 and AlexFluor680 for detection. The assay procedure consists of incubating 40ul of fluorescent-labeled detection antibody together with 20ul of sample over night in the dark followed by measuring at suitable excitation/emission wavelengths with a conventional fluorescent microplate reader. This assay was used to determine PERIOSTIN concentrations in a collection of blood donors without documented diseases.
Results: The LOD of this assay was 2 pmol/L and its LLOQ 11 pmol/L. Intra-/Inter-assay CVs ranged from 3 to 7% and 9 to 13% respectively. In a collection of 41 samples from blood donors (21 f, 20 m) aged 19–76 year we no age- or sex- dependency of serum concentrations. All samples were measurable and the found concentrations ranged from 1,5 to 24,5 pmol/L.
Conclusions: The developed assay is sufficiently reliable and sensitive for the detection of PERIOSTIN in human serum. Further studies must be performed to evaluate its usefulness for applications where high sensitivity of the used assay is required.
Literature:
1) Carpagnano GE et al. Chest. 2018 Nov;154(5):1083–1090
2) Kumaresan et al. Eur J Dent. 2016 Oct-Dec;10(4):546–550
3) Hawa G et al. Anal Biochem. 2018 May 15;549: 39–44.

P013

Changes in protein profile in bone marrow extracts before and one year after gastric bypass surgery

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The long-term skeletal changes after bariatric surgery are largely unknown, and identifying molecular mechanisms related to reduced bone health after long-term weight loss may reveal novel approaches for reducing the burden of these changes.

For better knowledge about bone status, we aimed to identify changes in the protein profile in bone marrow aspirates after weight loss due to gastric bypass surgical intervention.
Methods: Proteins in bone marrow aspirates from 9 patients before and 1 year after gastric bypass surgery (2013/1159/REK Sør-Øst B), were isolated using Trizol (Thermo Fisher Scientific). Total protein content was identified using the BCA Assay (Thermo Scientific). The amounts of specific proteins, such as cytokines (HCYP2MAG-62 K; Millipore Merck) and bone markers (HBNMAG-51 K) in various samples, were determined by the Luminex 200 system where acquired fluorescence data were analyzed using the 3.1 xPONENT software (Luminex).
Results: Bariatric surgery resulted in reduced bone mineral density (p = 0.018) and enhanced levels of osteocalcin (p = 0.001).
In the bone marrow aspirates a reduction in the level of vascular endothelial growth factor (VEGF) (p = 0.046) and enhanced levels of osteopontin (OPN) (p = 0.021) and interleukin-1β (IL-1β) (p = 0.025) were observed.
Conclusions: The bone marrow protein profile indicate an enhanced resorption and bone turnover following the loss of weight and reduction in BMD after surgery.

P015

Biocompatibility and osteogenic potential of calcium phosphate for cleft palate repair

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Cleft lip ± palate is a common congenital craniofacial deformity with average prevalence of 1 per 1000 live births. Majority of patients receive alveolar bone grafts (ABG) during eruption of permanent lateral incisor or canine. Autologous bone is gold standard, however, donor-site morbidity is the major drawback. Therefore, synthetic materials are emerging as possible alternative ABG substitutes.

The purpose of this in vitro study was to investigate biocompatibility and osteogenic potential of four novel calcium phosphate-based materials (CaP) by developing in vitro osteogenic progenitor cell-based model.

Human embryonic palatal mesenchymal cells (HEPM) and normal gingival fibroblasts (MM1) were seeded onto granules of Hydroxyapatite (HA), Tricalcium phosphate (TCP) and two Biphasic calcium phosphate (BCP) with varying HA/TCP ratios (Kuros Biosciences BV, The Netherlands) in Minimal Essential Medium supplemented with 10% foetal calf serum ± dexamethasone, ascorbic acid and β-glycerophosphate (DAG) ± bone morphogenic protein2 (BMP2). Cells were analysed at days 3, 7, 14 and 21 using MTT assay, methylene blue and immunofluorescence staining of RUNX2 expression for biocompatibility, osteoconduction (were materials allowing the cells to grow over them?) and osteoinduction potentials, respectively. Data were analysed using ImageJ software. Statistical analysis was carried out using Prism5 software.

CaP materials were found to be biocompatible and osteoconductive (providing good scaffolds for supporting HEPM and MM1 cells). HA granules supported cell proliferation more than other materials. HEPM expressed RUNX2 significantly (**$p < 0.01$) more than MM1 cells when cultured with materials in presence/absence of DAG and BMP2. RUNX2 was expressed by HEPM within 3 days and peaked on day 7. Material-wise, expression levels of RUNX2 were stimulated by BCP > TCP > HA. However, the presence of DAG + BMP2 significantly (*$p < 0.05$) enhanced RUNX2 expression.

These findings suggests that CaP scaffolds were biocompatible, osteoconductive and osteoinductive, hence, suitable for growing/osteogenic differentiation of HEPM and therefore, promising candidates as ABG alternatives for cleft alveolus repair.

P018

Glycoengineering as a tool for regulating behaviour of bone marrow-derived mesenchymal stromal cells in biofabrication

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Bioprinting is an emerging and promising technique in the field of biofabrication, which provides its user 3D cell constructs with high resolution and reproducible quality. Even though the focus is on soft tissue, bone and cartilage are also subject to the bioprinting research. With regard to bone regeneration, mesenchymal stromal cells (MSC) are of special interest due to their important role in the bone marrow niche featuring a broad differentiation potential and immunomodulatory properties. During the printing process, MSCs are exposed to shear stress, which might lead to a worse outcome in terms of viability, differentiation and adhesion after printing. Our project aims to transiently enhance the cell stability to protect MSCs from shear forces by altering the glycocalyx via glycoengineering and to control the adherence of MSCs in hydrogels modified by click chemistry as well. Initially a glycoengineering protocol for MSCs was established. Viability tests showed no negative effects of the click reagents like azido sugars or Cu ions on cell viability. A qualitative microscopic assessment delivered the proof-of-concept and revealed that after 48 h the clicked fluorescence dye was still detectable on the cell surface. To identify potential sugar motifs mediating cell adhesion a glycochip comprising different glycans, as well as the RGD peptide as positive control was designed and MSC adhesion was analysed. Initial microscopic experiments revealed variable MSC adherence depending on the presented sugar motifs. In summary, the glycoengineering of MSCs via click chemistry was successfully established and can now be used as a tool for manipulating the cell surface. The next step will focus on the identification and design of suitable glycoconjugates, which are supposed to increase the cell stability after incorporation into the glycocalyx. Once identified via the glycochip assay, adherence modulating sugar motifs can be used to modify suitable bioinks.

Keywords: Glycoengineering, biofabrication, MSC

P019

The impact of kaolinite on human periodontal cells

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The clay mineral kaolinite is successfully applied in a broad spectrum of medical fields. Kaolinite is used topically to achieve hemostasis and as dermatologic protector. Furthermore it has antibacterial purposes and can be utilized as carrier material for pharmaceuticals and growth factors. These properties make kaolinite a promising material for regenerative dentistry. However, the effect of kaolinite on human periodontal cells is unclear. The aim of this study was to reveal the response of human periodontal cells to kaolinite. We incubated human periodontal cells with kaolinite at 30 mg/ml–0.0005 mg/ml and with the kaolinite-depleted conditioned medium corresponding to these concentrations. Cell viability was evaluated based on resazurin-based toxicity assays, Live-Dead staining and MTT staining. Control experiments were performed with L-929 cells. The production of the pro-angiogenic factors vascular endothelial growth factor (VEGF) and interleukin (IL)-8 was measured using ELISA. The resazurin-based toxicity assay, MTT assay and Live-Dead staining showed that kaolinite in suspension can impair cell viability dose-dependently in human periodontal cells and L-929. The corresponding kaolinite-depleted conditioned medium did not modulate cell viability significantly. VEGF levels were decreased in human periodontal cells upon kaolinite exposure. Kaolinite-depleted conditioned medium did not have an effect on VEGF production. IL-8 levels were not modulated by kaolinite and the corresponding

kaolinite-depleted conditioned medium. In conclusion, our results show that kaolinite can decrease cell viability dose-dependently. Kaolinite-depleted conditioned medium has no cytotoxic impact. IL-8 production stayed unchanged while VEGF production was slightly decreased in human periodontal cells. Overall, this study provides first insights into the response of human periodontal cells to kaolinite.

P020

Stress-shielding-induced bone loss following total hip arthroplasty affects orientation of newly formed collagen in the proximal femur

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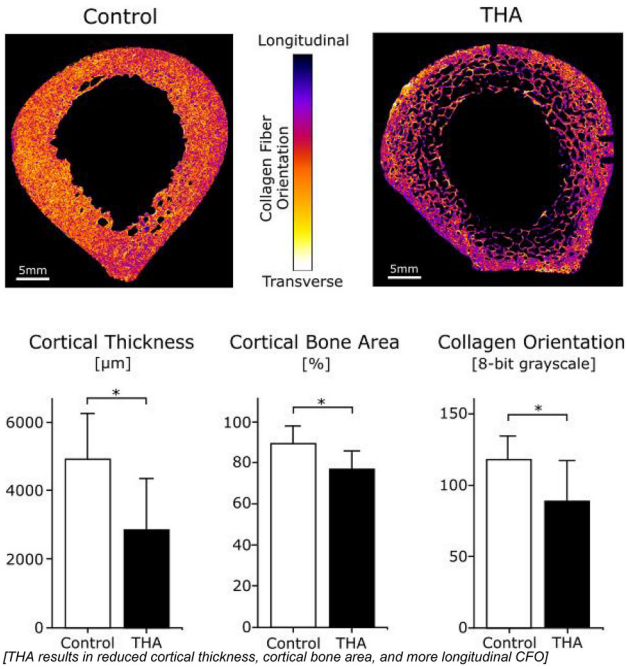
Total hip arthroplasty (THA) is one of the most successful orthopaedic procedures greatly improving the quality of life of the patients. However, the need for revision procedures remains a considerable problem: the total number of revision THA is projected to double by the year 2026. Aseptic loosening is a common reason for THA revision and is linked to peri-prosthetic mechanical relief of the bone (stress shielding). Here we investigate how stress-shielding-induced bone loss affects the bone matrix regarding its collagen fiber orientation (CFO).

Proximal femoral bone specimen were acquired after local ethics approval from seven female individuals (age:83.71 ± 11.7 years) who have undergone cemented THA (implantation duration:0.9–21 years) and compared to age- and gender-matched controls (n = 5). CFO properties were investigated in the cortex using circularly polarized light microscopy (CPLM) and expressed as a mean grayscale value (high values = transverse CFO; low values = longitudinal CFO). Non-parametric tests were employed to determine statistical significance ($p < 0.05$).

Cortical thickness was significantly lower in THA patients (2838 ± 1523 µm vs. 4894 ± 1368 µm) and the cortical bone area was significantly reduced compared to the control group (82.33 ± 5.31% vs. 89.52 ± 7.86%). In THA, significantly lower CPL grayvalues indicate more longitdudinal CFO (89 ± 29 vs. 119 ± 17).

Our study shows that CFO is strongly affected by stress-shielding-induced bone loss and characterized by a larger fraction of longitudinally orientated fibers. Stress shielding results in severe bone resorption coupled with simultaneous restructuring of newly formed collagen fibers oriented to better withstand the new loading scenario.

Keywords: Collagen Orientation, Bone Adaption, Total Hip Arthroplasty, Stress Shielding, Circularly Polarized Light



P021

Synergistic effect of hyperbaric oxygen therapy with parathyroid hormone [1–34] on calvarial bone graft in irradiated rat

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Purpose: To determine the synergistic effect of parathyroid hormone [1–34] in combination with hyperbaric oxygen on bone graft in rat calvarial bone defect model under impaired osteogenic condition.

Materials and methods: Twenty four rats were divided into 3 groups. Localized radiation with a single 12 Gy dose was administered to the calvarial. 4 weeks after radiation, calvarial circular defects were created in the parietal bones. All defects were filled with biphasic calcium phosphate. After grafting, parathyroid hormone was injected subcutaneously and hyperbaric oxygen therapy was administered. At 6 weeks after the bone graft, the rats were sacrificed and specimens were harvested.

Results: Histomorphometric evaluation showed the percent new bone area was higher in the PTH and PTH/HBO groups than in the Control group. Micro computed tomographic evaluation showed bone volume of new bone volume was higher PTH group than Control group. Bone surface in new bone volume was higher PTH/HBO group than Control group. In new bone volume, bone surface density was higher in the order of Control, PTH and PTH/HBO groups; all group was significant difference ($P < 0.017$).

Conclusions: Within the limitations of this study, our data indicate that parathyroid hormone with hyperbaric oxygen may reverse the impairment of bone healing by irradiation.

Keywords: Calvarial defect; bone graft; bone regeneration; parathyroid hormone; hyperbaric oxygen therapy

P022

The frequency of loading alters the bone healing response around implants

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When bone implants are loaded, they are inevitably subjected to displacement relative to bone. Such micromotion generates stress/strain states at the interface that can cause beneficial or detrimental sequels. The objective of this study is to better understand the mechanobiology of bone healing at the tissue-implant interface during repeated loading. Screw shaped Ti implants were placed in a hole slightly bigger than the implant diameter in two anatomical sites in rats, (i) the edentulous ridge in maxillae and (ii) the tibia. In both cases, specially-designed systems were developed to hold the implants, allow initial stabilization, protection from external forces, and controlled axial loading. Three loading regimens were applied, (a) no loading, (b) one daily session of 60 cycles with an axial force of 1.5 N/cycle for 7 days, and (c) two such daily sessions also for 7 days. The implants with surrounding interfacial tissue were harvested and processed for histological and histomorphometric analyses. Two-way ANOVA were performed to determine the differences in histomorphometric analysis between the groups. At both sites, histomorphometric analyses revealed that implants subjected to repeated loading sessions exhibited a significant decrease in bone-implant contact (35%) and increase in bone-implant distance (30%), as compared to unloaded implants and those subjected to only one loading session ($p < 0.05$). The results indicate that increasing the daily cyclic loading of implants induces deleterious changes in the bone healing response, most likely due to the accumulation of tissue damage at the bone-implant interface. They also suggest some commonality in mechanobiological factors influencing bone healing around implants in tibial and maxillary sites.

All animal procedures and experimental protocols were approved by the *Comité de déontologie de l'expérimentation sur les animaux of Université de Montréa*l.

Keywords: Bone; Implant; Loading; Histomorphometry; Tissue damage

P023

A decellularized human bone scaffold as an in vitro model of the skeletal stem cell niche

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Skeletal stem cells, usually referred to as mesenchymal stem or stromal cells (MSC), reside in a complex environment, the niche, which is critically involved in cell fate decisions. We here aimed to develop an in vitro model that mimics the skeletal stem cell niche.

Decellularized and decalcified bone slices were prepared from human femoral heads collected after patient's total hip arthroplasty with informed consent and agreement of the local ethics committee. Cylindrical-shaped scaffolds with 5 mm diameter were prepared and lyophilized. The efficiency of the decalcification and decellularization protocol was confirmed by x-ray imaging of scaffolds bone mineral density and qualitative histology confirmation of nuclei absence, respectively. The conservation of native bone ultra-structure was illustrated by histology of decellularized scaffolds and confirmed with m-CT quantitative analysis. Using the BoneJ plugin in Fiji the scaffold volume per total volume was calculated as $11.1 \pm 2.7\%$; trabecula thickness and space was 1.8 ± 0.5 µm and 23.9 ± 2.5 µm, respectively ($n = 8$). MSC were isolated from human femoral head-derived bone marrow, as previously described, and seeded in passage 2–5 into the scaffolds at different cell densities and their morphology and distribution assessed using CellTracker™ Green labelling. MSC readily attached to the porous surfaces after 24 h and homogeneous distribution in the scaffold volume was observed over time. Viability of MSC and their sustained metabolic activity was assessed through the reduction of resazurin in culture media, showing that viable MSC persisted over long culture times (14 days). The increase of metabolic activity over time was slower when compared with 2D monocultures. Our results indicate that meaningful human bone-derived 3D in vitro models could successfully be produced and support MSC viability. Future studies will further investigate the phenotype of MSC in the scaffolds as well as their response to microenvironmental cues.

Keywords: Skeletal niche, in vitro model, 3D scaffold, MSC

P025

Longitudinal monitoring of degradation processes of bioresorbable magnesium-alloy implants using micro-CT in vivo: the MgBone project

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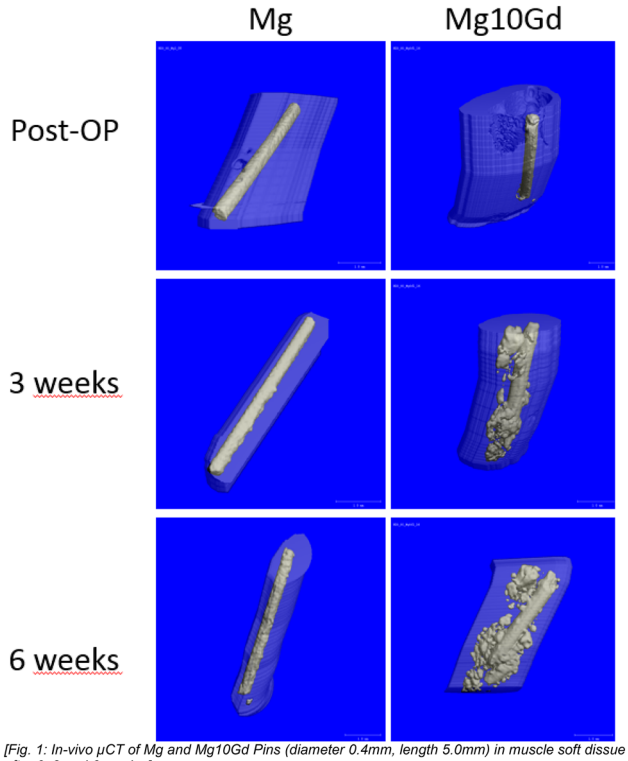
Bone implants made of various bioresorbable materials have medical advantages because a second surgery to remove the hardware after healing can potentially be avoided. However, degradation process tuning and in vivo biocompatibility are critical aspects. Degradation rates observed in vitro may differ from the in vivo situation. The optimal compound has mechanically stability during the initial healing process, then degrades fairly rapidly without negative side effect (like e.g. induction of gas) and, ideally, is osteoconductive and osteoinductive.

Pins made of pure magnesium (Mg; 99.95%) and magnesium alloyed with 10 wt % gadolinium (Mg10Gd) as well as Titanium as control were implanted in muscle soft tissue of mice ($n = 24$, (B6(C)/Rj-Tyrc/c (albino); age 10 weeks). Here we report on in vivo µCT data using a VivaCT 80 (Scanco, Switzerland) at 16 µm voxel size. Scans were acquired immidiately post-surgery, then weekly for six weeks.

In-vitro degradation measurement of Mg and Mg-10Gd revealed degradation rates of 0.75 ± 0.35 mm/a and 1.56 ± 0.90 mm/a, respectively. After six weeks, implants made of Mg-10Gd should have been largely resorbed. However, in vivo degradation rates are much smaller: Fig. 1 shows only partial degradation after six weeks. *In-vivo* degradation rates were (14.4 ± 1.7) % and (21.4 ± 5.1) % mass changes after six weeks, corresponding to approximately (0.25 ± 0.03)mm/a and (0.37 ± 0.09)mm/a.



[Fig. 1: In-vivo µCT of Mg and Mg10Gd Pins (diameter 0.4mm, length 5.0mm) in muscle soft dissue after 0, 3 and 6 weeks.]

In conclusion, monitoring the degradation processes in realistic conditions is essential. Furthermore, it will be tested whether the much slower in vivo degradation can still be observed if the implants are implanted into bone, a project currently ongoing in the context of the BMBF-funded "MgBone" study.

Keywords: Degradable Implants, Magnesium Alloy, µCT

P026

Single-dose local administration of teriparatide with octacalcium phosphate collagen composites achieved bony reconstruction after canine mandibular amputation

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Teriparatide (TPTD) is a bioactive recombinant form of parathyroid hormone (PTH), and approved for the treatment of osteoporosis. Octacalcium phosphate (OCP) and collagen (col) composite (OCPcol) which was developed in Japan demonstrated superior bone regeneration properties, and its commercialization appears to be forthcoming. Because bone repair of mandibular amputation is a crucial problem in maxillofacial surgery, the present study examined whether single-dose local administration of TPTD with OCPcol achieved bony reconstruction after canine mandibular amputation. OCPcol was prepared by mixing the granules of OCP and atelocollagen derived from porcine dermis, and commercially available hydroxyapatite and collagen composite (HAPcol) was purchased as a control. The amputation of critical sized (15 mm length) bone defect was made in the mandibular premolar region of male beagle dogs. The experimental animals were divided in four groups (n = 5 ∼ 6). OCPcol with dripped TPTD solution (56.5 µg), OCPcol, HAPcol with dripped TPTD solution (56.5 µg), or HAPcol was implanted into the defect and fixed with titanium plates. Intra-oral radiography was taken immediately and every month after implantation. After 6 months, the specimens were fixed and radiographed by a micro-CT. In OCPcol with TPTD group, the implanted area showed distinct radiolucency at immediately after implantation. After one through two months, lower radiopacity than original bone revealed throughout the defect. It was gradually increased, and 100% of the defect was bridged by newly formed bone, and the border between the original bone and the implanted area became indistinguishable at six months. Although 50% of the amputated mandible was bridged with newly bone in OCPcol group, no bone bridge (0%) was observed in both HAPcol with TPTD and HAPcol groups. These results suggest that OCPcol with the single local administration of TPTD enabled to reconstruct mandibular amputation with sufficient bone.

Keywords: Bone regeneration; Calcium phosphate; Collagen; Parathyroid hormone

P027

Promoted efficiency of alendronate-coated β-tricaclium phosphate in local delivery of bone morphogenetic protein-2

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β-tricaclium phosphate (TCP) is a clinically useful allograft to accelerate bone formation in the combination of recombinant human bone morphogenetic protein-2 (rhBMP-2) in the dental and orthopedic application. However, rhBMP-2 guides early resorption and abundant fatty marrow cavities at supraphysiological dose. To alleviate these adverse effects, we investigated the osteogenic effect of an anti-resorptive agent, alendronate (ALN) in β-TCP use for rhBMP-2 treatment. β-TCP coated with ALN (3 mM) (ALN-TCP) or non-coated β-TCP (TCP) was loaded with rhBMP-2 (1, 5, 40 µg/defect) immediately before implantation into critical-sized 8 mm calvarial defects of rats. In vitro BMP-2 release profile showed significantly reduced initial burst in ALN-TCP at increasing doses of rhBMP. ALN had no suppressive effect on in vivo initial gene expression of bone-forming/resorbing markers and osteoclasts activation increased by high dose rhBMP-2. However, ALN inhibited the high dose rhBMP-mediated upregulation of adipocyte differentiation markers, adipsin and PAPRγ by 33.3% ($p < 0.05$) and 35.3% ($p < 0.01$). This result was connected with 62.7% and 60.5% decrease (all, $p < 0.01$) of rhBMP-mediated fatty marrow cavities formation with ALN-TCP over TCP at 4 and 8 weeks. Micro-computed tomography and histology revealed that ALN-TCP at all doses of rhBMP led to greater bone volume (BV) and density at the central defect area over 4 and 8 weeks periods, compared to TCP. Notably, ALN-TCP-rhBMP 5 µg showed 47.4% greater BV ($p < 0.01$) over TCP-rhBMP 40 µg. These

results revealed that ALN-coating on β-TCP has several advantages in rhBMP-2 delivery having superior efficiency of low dose rhBMP to higher dose, and more compact structure at high dose rhBMP, probably mediated by suppression the formation of fatty marrow cavities, which highlight a feasible alternative of β-TCP to promote the bone-forming efficiency in BMP therapy.

Keywords: rhBMP-2, β-TCP, alendronate, fatty marrow cavities, bone formation

P028

Autologous blood coagulum as a carrier for BMP6 in posterolateral lumbar spine fusion

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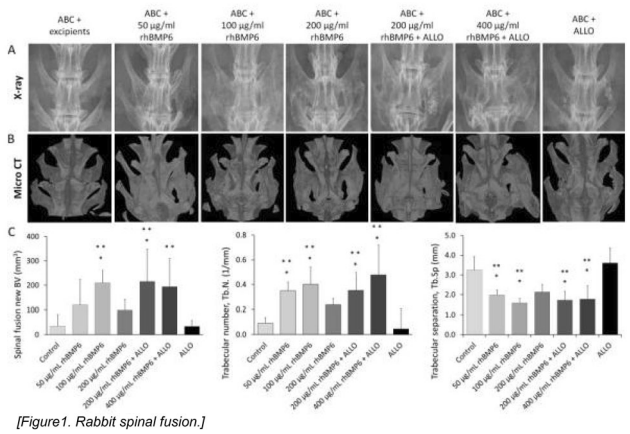
Posterolateral lumbar fusion (PLF) is one approach in which lumbar vertebrae are fused to relieve back pain caused by degenerative changes. We present rhBMP6 delivered in a carrier comprising of autologous blood coagulum (ABC) to induce bone formation and promote fusion in animal models.

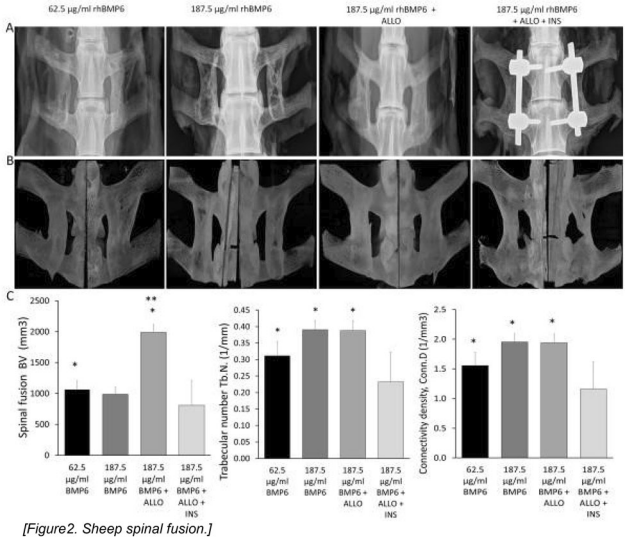
Rabbits and sheep underwent bilateral posterior fusion between lumbar vertebrae L4-L5 with ABC containing various doses of rhBMP6 and allograft, while in sheep, in one group metal pedicle screw system was implanted. Bone formation was assayed after 14 weeks in rabbits and 27 weeks in sheep. Results were tested using ANOVA with post hoc Tukey test. Ethical committee approval was obtained for all animal experiments.

In rabbit PLF model, rhBMP6 in the dose of 100 μg/ml resulted in the highest bone formation, while demonstrating a complete fusion in all rabbits. Addition of allograft had no influence on increasing bone volume. In sheep PLF model rhBMP6 facilitated spinal fusion while the addition of allograft stimulated bone volume. The use of metal instrumentation (INS) confined bone formation as compared to the group without instrumentation.

ABC may serve as a preferred carrier for rhBMP6 in mitigating premature resorption of the allograft while facilitating bone formation in PLF.



[Figure1. Rabbit spinal fusion.]



[Figure2. Sheep spinal fusion.]

P029

Improved proliferation and differentiation potential of aged adipose stem-progenitor cells

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Ageing impairs the function of stem-progenitor cells evidenced by increased senescence and decreasing regenerative ability. This results in restricted application and efficacy of aged stem-progenitor populations in regenerative medicine.

The potential to re-activate senescent stem-progenitor cells was investigated utilising adipose derived cells from elderly donors (≥ 60 years) with ethical approval (IRAS-223006). Cells were cultured in a three-dimensional system and treated with a small-molecule inhibitor of GSK3α/β, CHIR99021, which increased β-catenin nuclear localisation (15.46 fold $p \leq 0.01$) to activate Wnt-driven gene expression. Expression of hypoxia-inducible factor (HIF)-1α increased (3.47 fold $p \leq 0.01$) and B-Cell Lymphoma-2 (2.27 fold $p \leq 0.001$) indicating limited oxygen supply within the three-dimensional system, activated cell survival pathways. Furthermore, the percentage of cells undergoing proliferation increased (12.15% $p \leq 0.001$), evidenced by phospho-histone H3 staining. Proliferation related gene expression was upregulated including: cyclin D (2.37 fold $p \leq 0.01$), E2F transcription factor-1 (8.05 fold $p \leq 0.01$), proliferating cell nuclear antigen (1.77 fold $p \leq 0.01$) and Ki67 (16.41 fold $p \leq 0.01$). Critically, a decrease in senescence was demonstrated by β-galactosidase staining (55.21% to 3.72% ($p \leq 0.0001$) and down regulation of p16 and p21 genes (0.48 fold $p \leq 0.0001$ and 0.46 fold $p \leq 0.0001$ respectively).

Enhanced tri-lineage differentiation was demonstrated. After 21-day osteogenic induction, alkaline phosphatase activity increased with CHIR99021 treatment ($p \leq 0.05$) in the three-dimensional system. Runt-related transcription factor 2 (RunX2) gene expression increased fourfold ($p \leq 0.05$). Adipogenic differentiation, evidenced using Oil red O staining, increased almost three fold ($p \leq 0.0001$) with peroxisome proliferator-activated receptor-γ (PPAR-γ) gene

expression increasing 20 fold ($p \leq 0.05$). Following chondrogenic induction, sex-determining region Y-Box 9 (SOX9) and collagen type II gene expressions increased nine fold ($p \leq 0.001$) and 50-fold ($p \leq 0.01$) respectively.

The current studies demonstrate an innovative approach harnessing three-dimensional culture and a GSK3α/β small molecule inhibitor to reactivate previously senescent aged stem-progenitor cells, with significant implications for improved regenerative medicine approaches for elderly patients.

Keywords: Senescence, ageing, stem-progenitor, three-dimensional, Wnt pathway

P032

Application of pair distribution function studies to hydroxyapatite for assessment of physicochemical bone quality

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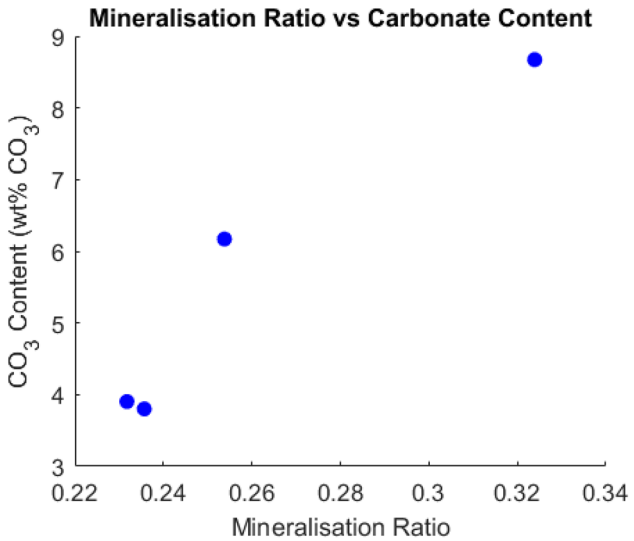
Carbonate occupies two non-equivalent sites within biological hydroxyapatite (HA) through substitution: phosphate ions (B-type) and hydroxyl ions (A type). Substitution levels and site alter the mechanical and physicochemical properties of HA; this is important for bone diseases such as osteoporosis, which is known to have atypical physicochemistry. Synthetic HA B-type substituions are produced at temperatures 25–100 °C whereas A-type are produced at temperatures ~ 1000 °C, making the presence of A-type substitutions in biological HA controversial. Differentiating substitutions is currently reliant on Fourier transform infra-red (FTIR) which exploits lattice site degeneracy of the $v_2CO_3^{2-}$ absorption band. This research aims to corroborate FTIR by exploiting pair distribution function (PDF) characteristics of such apatites. Measurements were collected of HA standards, synthetic HA with a range of carbonate substitution levels, and biological HA from four species with varying physicochemistry. Mineralisation Ratio (MR) was found to have a negative correlation with carbonate substitution for biological material (Figure 1).



[Figure 1: Samples of Mesoplodon densirostris rostrum, reed deer antler, bovine and porcine femur.]

The opposite trend was seen for synthetic HA, consistent with previous studies [1,2]. This suggests synthetic apatites may be a problematic model for studying biological apatites with regards to carbonate substitution. Comparison of X-ray diffraction coherence lengths as well as crystallite length in $< 00\ell >$ correlates well with MR for biological and synthetic samples ($p \ll 0.05$). Additionally, PDFs were examined to determine distinct features between A-type and B-type substitutions.

Keywords: Hydroxyapatite, Pair Distribution Function, Carbonate

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P033

Effects of manganese enhanced hydroxyapatite implants into tibia on strength of the mandible in rats

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The study involved 252 male rats with initial body weight of 135–145 g. The 1st group comprised intact animals, the 2nd group comprised animals with 2.2 mm defect in the tibia, and the groups 3 through 6 comprised the animals with the same 2.2 mm defects filled with biogenic hydroxyapatite enhanced with 0.1, 0.25, and 0.5% share of manganese. Upon expiration of observation terms (the 7th, the 15th, the 30th, the 60th, the 90th, and the 180th day), mandibles were excised and put to strength testing at bending. The data obtained were analyzed by means of variation statistics using standard applied software.

Fracture modeling results in decrease of strength of the mandible (breaking moment and minimum fracture energy were lower than those of controls by 7.22–17.37% and 9.82–14.24% respectively) from the 15th to the 90th days of observation. Implantation of pure hydroxyapatite into defect also affects mandible strength; alterations were observed on the 7th day of observation; from the 15th day of observation, strength of mandible restores faster than that of group without implantation. Manganese enhanced implants significantly reduce negative effects of fracture on strength parameters of the mandible; the implants with 0.25% of manganese showed the highest efficacy. With manganese concentration 0.25% restoration rate of strength of mandible appeared to be higher than that of the group without manganese enhancement beginning from the 15th day of observation. Implants with 0.5% of manganese cause manganese intoxication observed as decrease of breaking moment of mandible by the 60th days of observation by 5.88% and minimum fracture energy from 90th to 180th days of observation by 5.97% and 4.82% in comparison with those of group without manganese in implant.

Keywords: Mandible, bone defect, hydroxyapatite, strength, manganese

P035

Cortical quantitative ultrasound variables for the estimation of proximal femur BMD

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Total hip and femoral neck BMD measurements are used for the estimation of osteoporotic fracture risk. Additionally to these radiologic measurements, Quantitative Ultrasound (QUS) is useful for fracture risk prediction. Within a French-German research collaboration we investigated the feasibility of cortical QUS measurements at femur and tibia for femoral BMD prediction.

QUS through transmission measurements were performed using an inhouse scanner at the medio-anterior part of the tibia shaft and proximal femur. The fastest of the scanned signals was used to define times of flight for the femoral neck (TOF(Fem,N)), subtrochanter (TOF(Fem,ST)) and midtibial shaft (TOF(TIB)). Tibia and proximal femur samples were collected from 20 pairs of human limbs (7 M, 13F, age: 84 ± 8 years). DXA scans were performed with femur bones immersed in a water-bath to mimic surrounding soft tissue. Correlation coefficients and level of significance were calculated for regressions between QUS variables and BMD of neck and total hip.

QUS at femoral neck was not able to predict any BMD value. Subtrochanteric QUS as well as tibial QUS could predict total hip BMD. Only tibial QUS correlated significantly with femoral BMD. Results are summarized in table 1.

QUS at subtrochanter and tibia both correlated with total BMD, indicating a strong association between tibial and femoral bone parameters and a usefulness of tibia QUS, which is easier to be established as at the femur. In future, a potential benefit should be investigated with fractures as endpoints.

R^2	BMD(total)	BMD(neck)
TOF(Fem,ST)	0.35 $p < 0.01$	0.11 ns
TOF(Fem,N)	0.11 ns	0.03 ns
TOF(TIB)	0.42 $p < 0.01$	0.32 $p < 0.05$

P036

Bone tissue level changes in the human clavicle and ribs with aging

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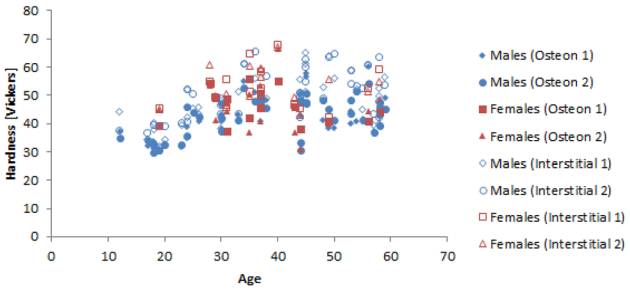
Increased mineralisation, porosity and degradation of bone structure exhibited in later life are well-entrenched in skeletal aging research. This investigation sought to quantify changes to mechanical properties of aspects of the skeleton frequently fractured by all, most often younger, ages at the bone tissue level. Examining the initial stages of the deterioration of mechanical competency and whether these changes parallel the well-established attainment and loss of bone mass which may highlight earlier opportunities in someone's life to intervene before changes in skeletal micro-architecture and mechanical properties are known to occur.

A human cadaveric sample (12–59 years) consisting of 55 right clavicles, 22 left and 27 right sixth ribs was utilised. All experiments conducted were approved by the university ethics committee. Standardised protocol was devised whereby a cross-section was removed from each specimen using a metallurgical saw under water irrigation, then embedded in resin and polished. The biomechanical properties of osteons and interstitial lamellae were quantified using a Nano-hardness tester. Statistical tests demonstrated a significant increase ($p < 0.05$) for hardness (Figure 1) and elastic modulus with age for individuals under 35 years, during the period where peak bone mass is reached. However, once skeletal maturity was exceeded, there ceased to be a significant relationship between age, and the mechanical properties measured. This change during adolescence and young adulthood occurred, similarly, for the ribs, though further tests also revealed statistically significant asymmetry ($p < 0.05$) between the left and right.

Keywords: Aging, biomechanics, nanoindentation



[Figure 1. Correlating Vickers Hardness with age for the clavicle]

P037

Assessment of bone loss in distal phalangeal joint in Chinese patients with primary hyperthrophic osteoarthropathy by high-resolution peripheral quantitative computed tomography

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Objectives: This study aimed to investigate bone loss in distal interphalangeal joint (DIP) in primary hypertrophic osteoarthropathy (PHO) patients by high resolution peripheral quantitative computed tomography (HR-pQCT), including for the first time the evaluation of bone geometry, volumetric density (vBMD), microstructure and bone erosions at the third DIP (3rd DIP). Also, to determine the clinical factors which influence the cortical and trabecular compartment of the 3rd DIP in PHO patients.

Methods: In this study, 15 PHO patients and 15 healthy controls were enrolled. The presence of bone erosion at DIPs was initially evaluated by X-ray. Then, the bone geometry, vBMD, microstructure parameters and size of individual bone erosion were measured at 3rd DIP by HR-pQCT. Blood biochemical markers were quantified for comparison between the two groups.

Results: Compared with X-ray, HR-pQCT assessment on bone erosions were more sensitive, with 14 PHO patients by HR-pQCT versus 10 PHO patients by X-ray judged at the 3rd DIP. The depth, width and volume of erosions in PHO patients were all small with the average erosion size 1.38 ± 0.80 mm, 0.79 ± 0.27 mm, and 1.71 ± 0.52mm3, respectively. The bone cross-areas at the defined region of interest of 3rd DIP were significantly increased than controls (all $p < 0.05$). Total vBMD was 11.9% lower in PHO patients than controls ($p < 0.05$). The bone microstructure was similar between the patients and controls, except for the trabecular separation, which was greater (+27.3%, $p < 0.05$) in PHO. Serum PGE2, high sensitive C-reactive protein (hsCRP) and erythrocyte sedimentation rate (ESR) levels were found negatively correlate with Total vBMD.

Conclusions: This study demonstrated for the first time that bone geometry, vBMD, bone microstructure and size of bone erosion at 3rd

DIP in PHO using HR-pQCT. The use of HR-pQCT would be of interests clinically to study bone deterioration in PHO patients.
Keywords: PHO, PGE2, DIP, HR-pQCT

P038

Osteocalcin is required for the alignment of biological apatite crystallites but not for the regulation of bone formation, glucose metabolism, testosterone synthesis, and muscle mass

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Introduction: Osteocalcin is the most abundant non-collagenous protein in bone. Osteocalcin has been shown to inhibit bone formation, because the bone mass is increased in osteocalcin-deficient ($Ocn^{-/-}$) mice due to the enhanced bone formation. Further, Glu osteocalcin has been shown to function as a hormone that regulates glucose metabolism, testosterone synthesis, and muscle mass.
Methods: *Bglap*and *Bglap2* were replaced withPGK-gb2-neo in E14 line of ES cells. $Ocn^{+/-}$mice were backcrossed with C57BL/6 8-10 times. Prior to the study, all experiments were reviewed and approved by the Animal Care and Use Committee of Nagasaki University Graduate School of Biomedical Sciences (No. 1403111129-23). Bone phenotypes were analyzed by m-CT, bone histomorphometry, serum markers, confocal Raman spectroscopy, XCT Research SA +, mXRD, microbeam X-ray diffractometer, and nanoindentation. Glucose tolerance tests, histological analyses of testis and muscle, and the assay of serum testosterone were done.
Results: The volume and mineralization of trabecular and cortical bone and parameters for bone formation and resorption were similar between wild-type and $Ocn^{-/-}$mice. Further, glucose metabolism, testosterone synthesis, or muscle mass in $Ocn^{-/-}$mice were similar to wild-type mice. The orientation degree of collagen fibrils, which is normally parallel to collagen fibrils, and the size of biological apatite (BAp) crystallites in the *c*-axis were normal, whereas the crystallographic orientation of the BAp*c*-axis, which is normally parallel to collagen fibrils, was severely disrupted. Moreover, Young's modulus along the bone longitudinal axis was significantly reduced in $Ocn^{-/-}$femurs ($p < 0.01$), and it was strongly corelated with the BAp *c*-axis orientation degree ($p = 4.7x10^{-4}$).
Conclusions: Osteocalcin is required for maintaining bone quality and strength by adjusting the alignment of BAp crystallites parallel to collagen fibrils, but that it is not physiologically involved in the regulation of bone quantity, glucose metabolism, testosterone synthesis, and muscle mass.

P045

Histomorphometric parameters and histological findings of the guided bone regeneration using biphasic calcium phosphate paste

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Introduction: Human bone has great potential for regeneration. In ideal conditions, biomaterials in dentistry should be resorbed gradually to allow the bone defect to be fully filled with newborn bone tissue. It has been shown that rapidly resorbable biomaterials may disappear even before osteoconduction of osteogenic cells elicits and causes bone formation, while non-resorbable biomaterials prevent primary osteogenesis as well as maturation of bone tissue.
Aim: Guided Bone Regeneration (GBR), which is one of the most frequently, used techniques in implant dentistry, with a predictable clinical outcome. In present study, we want to assess and describe histological indices of the bone sample, 6 months after GBR. We also wanted to quantify the percentage of the newly formed bone, percentage of the residual biomaterial and percentage of the soft tissue.
Materials and methods: Ten healthy patients were included in the study with at least one or two wall intrabony defects after tooth extraction. The intrabony defects were filled with biphasic calcium phosphate paste (Maxresorb inject, Botiss Dental GmbH) and covered with native collagen membrane (Jason membrane, Botiss Dental GmbH), to ensure the isolation of the bone defect against gingival connective tissue. Six months after healing, simultaneously with dental implant placement, bone biopsies were collected and values of the histomorphometric parameters were determined.
Results: Histological indices showed new bone formation at the peripheral border of the bone defect. The granules of Maxresorb inject are incorporated into bone tissue and there with no histological signs of inflammation. Histomorphometry reveals 18% of the newly formed bone, 30% of the residual biomaterial and 40% of the soft tissue.
Conclusions: In present study, we evaluated the regenerated bone by descriptive histological examination and histomorphometry. Maxresorb inject shows octeoconductive potential for bone regeneration.
Keywords: Guided bone regeneration, implant dentistry, Maxresorb inject, histology, histomorphometry

P046

Periostin accelerates bone healing mediated by human mesenchymal stem cell- embedded hydroxyapatite/tricalcium phosphate scaffold

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Periostin, an extracellular matrix protein, is expressed in bone, more specifically, the periosteum and periodontal ligaments, and plays a key role in formation and metabolism of bone tissues. Human adipose tissue-derived mesenchymal stem cells (hASCs) have been reported to differentiate into osteoblasts and stimulate bone repair. However, the role of periostin in hASC-mediated bone healing has not been clarified. In the current study, we examined the effect of periostin on bone healing capacity of hASCs in a critical size calvarial defect model. Recombinant periostin protein stimulated migration, adhesion, and proliferation of hASCs in vitro. Implantation of either hASCs or periostin resulted in slight, but not significant, stimulation of bone healing, whereas co-implantation of hASCs together with periostin further potentiated bone healing. In addition, the number of Ki67-positive proliferating cells was significantly increased in calvarial defects by co-implantation of both hASCs and periostin. Consistently, proliferation of administered hASCs was stimulated by

co-implantation with periostin in vivo. In addition, co-delivery of hASCs with periostin resulted in markedly increased numbers of CD31-positive endothelial cells and α-SMA-positive arterioles in calvarial defects. These results suggest that recombinant periostin potentiates hASC-mediated bone healing by stimulating proliferation of transplanted hASCs and angiogenesis in calvarial defects.
Keywords: Bone Healing, Periostin, Scaffold

P049

Autologous bone graft substitute containing autologous blood coagulum with rhBMP6 accelerates bone formation compared to commercial rhBMP2 on bovine collagen

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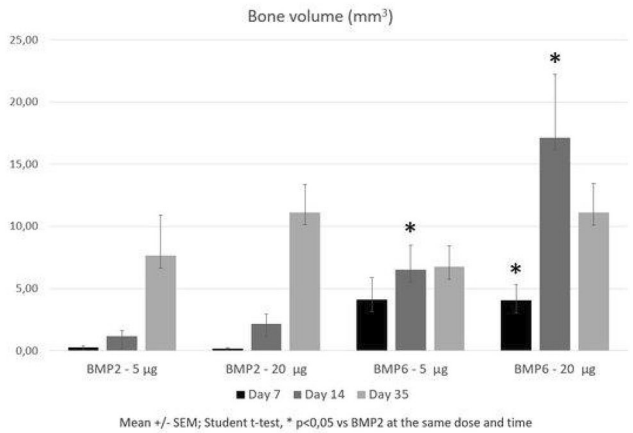
The aim of this study was to compare bone formation acceleration of a novel biocompatible carrier device for bone healing consisting of rhBMP6 in autologous blood coagulum (ABC) with bovine collagen and rhBMP2, currently the most commonly used bone device in clinics. To test bone formation efficacy of implant we used the axillary subcutaneous assay in rats. The rhBMP6 in ABC and rhBMP2 on bovine collagen were tested at doses of 5 and 20 μg per implant in 20 rats (4 implants per group and time point). Animals were killed and scanned using μCT device at day 7, 14 and 35 following implantation. Acquired images were reconstructed and the amount of new bone volume (BV) was analysed by μCT and on histology sections stained by von Kossa and Goldner. BV was higher in rhBMP6 + ABC then rhBMP2 + bovine collagen on day 7 and day 14 at both doses (Figure 1). On day 14, ABC + rhBMP6 (5 μg) implant had around 6 times more BV then implant with rhBMP2 + collagen, while with 20 μg rhBMP6 it increased 8 times. Both rhBMP6 and rhBMP2 had similar BV on day 35 suggesting that rhBMP6 in ABC significantly accelerated bone formation at early time points following implantation. The BV was equally increased as determined by histomorphometric analysis of histology sections. Therefore, ABC is a novel autologous carrier for rhBMP6 creating a permissive environment for rapid bone formation compared to commercially available bone repair device containing rhBMP2.



[Figure 1: Bone volume with various doses of rhBMP2 and rhBMP6 in rat SC assay]

P050

miR-31-5p and miR-33a-family promoters of human mesenchymal stem cells osteoblast differentiation by HIF-1α and EMT signaling modulation

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Epithelial to mesenchymal transition (EMT) and hypoxia signaling display a central role on osteoblast differentiation. Recent evidences suggested the role of miRNAs in the regulation of these pathways during bone regeneration, through modulation of balance between tissue formation and resorption mediated by osteoblast and osteoclast activities. In our recent in vitro studies, we demonstrated that miR-31-5p and miR-33a-family are involved in human mesenchymal stem cells (hMSCs) osteoblast differentiation by modulation of HIF-1α and EMT signaling. Starting from these evidences, we investigated the clinical significance of miRNAs modulation by miRNA microarrays on hMSCs-derived by discarded bone tissue from total hip replacement surgery. hMSCs were cultured in a normoxic or hypoxic environment. Microarray analysis revealed the important modulation of different miRNAs in female and male samples; in particular, the bioinformatic analysis of miR-targets, revealed a strongly modulation of EMT and HIF-1α proteins. The investigation on hMSCs, derived by bone tissue, maintained in normoxia and hypoxia conditions, revealed: *i)* a correlation between aging of hMSCs and miRNAs expression; *ii)* a modulation of HIF-1α and EMT genes in relationship with miRNAs presence. Gain and loss of functions in vitro normoxia experiment showed as miR-31-5p and miR-33a-family over-expression are able to promote the HIF-1α nuclear translocation and SNAIL/SLUG activation, improving osteoblast commitment of hMSCs as showed by cytofluorimetric analysis, in particular in younger hMSCs. These results suggest that hypoxia regulates the miR-31-5p and miR-33a family targets genes network to interfering with hMSCs osteoblast differentiation, representing a novel molecular mechanism for HIF-1-mediated EMT signaling. To sum up, a new possible therapeutic approach to bone regeneration was highlighted, which might be mediated by customizing delivery of miR-31-5p and miR-33a-family in line with the aging of the patients or osteoblast differentiate state of the patient target cells.
Keywords: Hypoxia, mesenchymal stem cells, miRNAs, osteoblast commitment, EMT.

P051

Distinct pre-osteoblastic expression of the novel high bone mass gene *SMAD9* in the zebrafish skeletal model

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Osteoporosis affects more than 27.5 million people in the European Union costing the member states ~ €37 million per year collectively. The incidence is rising due to an ageing society. Current osteoporosis treatment is incomplete and especially novel anabolic drug targets are needed to treat osteoporosis effectively. Recent advances in genetic data have contributed to better understanding of the biological cause of osteoporosis. As demonstrated previously with *SOST* and *LRP5*, high bone mass (HBM) cases provide a unique resource of osteoporosis drug targets. Using whole-exome sequencing, we have identified a novel and rare mutation in the DNA binding MH1 domain of *SMAD9* (c.65T > C, p.Leu22Pro, MAF 0.0014) in a genetically unexplained high bone mass (HBM) pedigree from a large national HBM cohort. This mutation is predicted to disrupt DNA binding of SMAD9 in protein modelling.

Recently it was shown that SMAD9 is a unique inhibitory transcription factor in the SMAD1/5/9 oligomeric transcription factor complex of the bone morphogenetic protein (BMP) signalling pathway. SMAD1/5/9 dependent BMP target gene transcription is key for maintaining bone formation and homeostasis. We show that SMAD9 and p.Leu22 is evolutionary conserved including in zebrafish.

Zebrafish have proven an effective model to study musculoskeletal diseases as this is well conserved over evolution which offers exciting avenues to study osteoblast activity, bone formation, and drug uptake in vivo. Here we present the first results of functional studies of *smad9* in zebrafish. We observed strong Smad9 expression in juvenile Sp7-negative pre-osteoblasts in growing bone elements. We also see low expression in adult fin ray bones however, Smad9 is dramatically upregulated in regenerating fin rays (forming new bone) and is specifically expressed in weak Sp7-positve and adjacent Sp7-negative cells. Currently we are analysing pharmacological targeting of osteoblast pathways, fracture repair, and CRISPR/Cas9 mutants of *smad9* to assess function of Smad9 in bone.

P054

Roles of a transcription factor 19A in the osteoblast development of sternum

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The sternum is a long flat bone lying at the most ventral midline of the thoracic skeleton. The sternum strengthens the thoracic cage, and protect internal organs such as heart and lungs, and this bone marrow is a major hematopoietic organ in birds and mammals. Despite the anatomical importance of the sternum, this development has not been qualified well.

Developmentally, the sternum arises from the thoracic lateral plate mesoderm (LPM). Some reports suggest the presence of specific mechanisms in thoracic LPM, which are different from those in somites; expression patterns of Hox-gene family and Scx gene. Identification of transcription factors, that regulate thoracic LPM-specific gene expressions, is pivotal for elucidating the sternal development.

Here, we report that a transcription factor, 19A is required for the sternum-osteoblast development. We found 19A is expressed in mesenchymal cells beneath each somite in zebrafish embryos. In order to know roles of 19A in mammals, we made conditional 19A KO mice($19A^{flox/flox}$). Whole-body 19A KO mice; CAG-cre $19A^{flox/flox}$ mice, exhibited significant ossification delays specifically in the sternum. Number of cells that express Runx2; an early stage-osteoblast maker, decreased in the thoracic LPM of this 19A KO mice, indicating that 19A regulates Runx2 (+) sternum-osteoblast progenitors.

To know where and when 19A is required for the sternum ossification, 19A was deleted in LPM-specific and stage-specific manners, by crossing Prx1-cre and Ubc-cre ER^{T2} mice with $19A^{flox/flox}$ mice. LPM-specific deletion of 19A resulted in same sternum phenotypes, suggesting that 19A is required in the LPM. Stage-specific 19A KO mice showed that the expression of 19A is critical for the sternum-bone formation around embryonic day(E) 10.5. To identify signaling that 19A regulate, we compared transcriptomes of the thoracic-LPM cells isolated from WT and 19A KO mice at E10.5. From these results, we will discuss how 19A regulates the sternum formation.

P055

Histopathologic hypoxia-like changes and decreased volume in fetal growth plates following gestational hypoglycaemia in rats

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Maternal hypoglycaemia causes foetal growth restriction. This study investigates the importance of timing and duration of continuous maternal hypoglycaemia on rat foetal growth plates (FGPs) during organogenesis and gestation.

To induce maternal hypoglycaemia, pregnant rats received infusion with human insulin throughout gestation until gestation day 20 (HI-GD20) or only until end of organogenesis (HI-GD17) followed by return to normoglycaemia. Controls received vehicle-infusion. Termination was on GD20. FGPs in the proximal tibia were evaluated using histopathology and stereology (H&E), immunohistochemistry (collagen X), and in situ hybridization (VEGF-A and HIF-1α). All procedures involving live animals were ethically approved.

In rats hypoglycaemic throughout gestation (group HI-GD20)—maternal plasma corticosterone levels increased compared to HI-GD17 and controls (337 vs 131 and vs 168 nM, $p < 0.001$ for both). Additionally, FGP volume was decreased (0.295 vs 0.4112 vs 0.479 mm^3, $p < 0.01$, $p < 0.001$) and severe histopathologic changes observed (incidence 100% vs 55% and vs 31%; $p = 0.0397$, $p = 0.0024$). The histopathologic changes (disarranged FGP zones (confirmed by Collagen X distribution) hypoplasia, hypertrophic chondrocytes) resembled those seen in FGPs following hypoxia; and increased mRNA staining of anti-hypoxic factors, VEGF-A and HIF-1α surrounded the affected areas. In rats hypoglycaemic only during organogenesis (group HI-GD17)—FGP volume was normal ($p = 0.139$) and incidence of histopathology was not increased ($p = 0.224$).

Conclusions: Maternal hypoglycaemia produces histopathologic changes in foetal tibial growth plates resembling those following hypoxia, likely through increased corticosterone levels. Sufficient materno-foetal glucose supply during gestation is critical, including during the period of organogenesis. By re-establishing normoglycaemia in late gestation it is possible to partially recover/prevent these effects.

Keywords: Skeletal development, gestational hypoglycaemia, rats, hypoxia

P061

Inhibition of breast cancer bone metastasis by KT

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MDA-MB-231 breast cancer cell line with triple negative was used for in vitro and in vivo studies. Cell migration was examined using simple scratch test and would healing assay, and the expression of HIF-1, Cathepsin K and Calcylin binding proteins (CYBP) was confirmed by western blotting. For identification of correlation between cancer cells and osteoclast, chemotactic assay and simple scratch test were performed using conditioned media from osteoclast (OcCM). For in vivo studies, 'Luc-MD' cell lines continuously expressed Luc-gene was established and was injected to mouse heart, and cancer signal was identified using IVIS system. KT was orally administrated from 3 weeks to 5 weeks after cancer cell injection. At 5 weeks, mice were scarified and bone conditions of mice was evaluated through Micro-CT analysis; Bone volume/Tissue volume (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N), trabecular bone mineral density (BMD).

KT significantly inhibited cell migration of MDA-MB-231. The expression of HIF-1 and Cathepsin K decreased, whereas that of CYBP increased in KT-treated cells. Transmigration and motility of MDA-MB-231 increased after exposure to OcCM but those were significantly decreased in KT-containing OcCM. After cardiac-injection of Luc-MD cells, cancer signals in mice mainly showed bone and brain at 3 weeks. At 5 weeks, the cancer signals in bone of KT-treated group were significantly weaker than those of control group. In addition, the values of BV/TV, Tb.Th, Tb.Sp, Tb.N and BMD in KT-group were similar to normal, whereas non-treated group (PC) exhibited severe bone loss.

In conclusion, KT inhibited cancer cell migration and cross talk between osteoclast and cancer cells. KT delayed secondary tumor growth in bone, and blocked bone loss by metastatic cancer. Taken together, KT have potential especially to treat bone cancer migrated from breast cancer cells.

P062

CD73/adenosine pathway involvement in bone metastases induced by non-small cell lung cancer stem cells

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In non-small cell lung cancer (NSCLC), CD133/CXCR4 + cancer stem cell (CSCs) can grow as spheres and initiate bone metastases. The extracellular release of CD73-generated adenosine (ADO) upregulates the expression of CXCR4 in cancer cells, thus promoting their ability to metastasize. We investigated the potential role of CD73-mediated adenosinergic activity in NSCLC CSCs-induced bone metastases.

We generated in vitro spheres from three NSCLC cell lines: A549 (osteotropic cell line), H2228 and H3122. We analysed CD73 expression on NSCLC adherent cell lines, spheres, primary tumors and lymphnodes by FACs and RT-PCR. We observed significantly higher CD73 expression in A549 spheres than in adherent cells ($81,3 \pm 3,8$ vs $14,3 \pm 4,1$ mean $\pm$ SE, $p < 0.001$), whereas it was similar in H3122 and H2228 adherent cells and spheres. CD133/CXCR4/CD73 + cells were higher in metastatic lymphnodes compared to primary tumors ($20,5 \pm 5,5$ vs $14,8 \pm 8,5$ respectively, $p < 0.05$).

We measured CD73-mediated ADO production after AMP treatment of spheres alone or co-cultured with osteoclast (OC) and osteoblast (OB), by HPLC. Interestingly, OCs alone rapidly transform all ADO in inosine, and when co-cultured with spheres ADO levels were reduced compared to ones produced by spheres alone (H2228 $11,2 \pm 0.19$ vs $27,3 \pm 0.7$; H3122 $24,7 \pm 9.1$ vs $64,6 \pm 2,6$; A549 $20,2 \pm 2$ vs $44,2 \pm 2$, mean area % of the peaks $\pm$ SD, $p < 0.05$). OBs alone and co-cultured with spheres release high level of ADO respectively (OB $32,2 \pm 1,8$; H2228 $29 \pm 0,1$; H3122 $25,17 \pm 3\ 5$; A549 $29,2 \pm 1,8$).

Our preliminary data suggest that CD73-mediated ADO release by NSCLC spheres is modulated by bone cells. OBs produce high ADO levels, which can contribute to create the immunosuppressive niche, at last leading to NSCLC sphere-induced bone metastasis.

Keywords: Bone metastases, non-small cell lung cancer, adenosine

P063

Effect of PDE4 inhibitors on osteosarcoma cells growth in vitro

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Phosphodiesterase type 4 (PDE4) inhibitors have been described as potential anti-cancer therapeutics (Akhtar W et al., 2016). But to date, no study on their effects on osteosarcoma has been performed. Actual therapies against osteosarcoma remain insufficient with 85% relapse rate and a 60% survival at 5-year (Mirabello L et al., 2009; Anninga JK et al., 2011). Previous studies carried out by the laboratory shown that pyridazinone based molecules (516, 532, 5016 and 5032) are able to inhibit PDE-4 (Barberot C et al., 2018), and exhibited cytotoxic effect in vitro, on human osteosarcoma cell line (Saos-2) (Moniot A et al., ECTS 2017, 2018). The here-presented work focuses on molecules effect on apoptosis, senescence and proteolytic activities on human osteosarcoma cell line. It also provides first results of cytotoxicity on murine osteosarcoma cell line.In our hands, osteosarcoma cells expressed PDE4-A/B/D, and cAMP production could be modulated by treatment with Zardaverine, a potent PDE4 inhibitor bearing a pyridazinone scaffold. After four days' incubation, 5032 induced apoptosis, increasing caspase-3 activity (50% vs non-treated cells, $n = 7$, $p < 0.05$) and no senescence was detected on Saos-2 cell lines. When looking at proteolytic activity, 516 and 532 induced a more marked decrease in MMP-2 and MMP-9 production than 5016 and 5032 (50% and 40% *vs* 17% and 6% respectively, $n = 4$). In addition, the cytotoxic effect of our drugs was confirmed on mouse osteosarcoma cell line with 65 μM and 45 μM IC50 on MOS-J for 5016 and 5032 respectively, consistent with values observed with Saos-2 (50 μM and 35 μM, $n = 7$, $p < 0.05$).Our data highlighted apoptosis as a potential mechanism of action of pyridazinone molecules to slow down osteosarcoma cells proliferation. They might also influence cancer cells migration

through proteolysis modulation. *In-vivo* assessment are in progress to determine if pyridazinones are real putative therapeutics to fight against osteosarcoma.

P064

Potentiated anti-tumor effects in human osteotropic breast cancer cells by combined inhibition of the mevalonate pathway and the p38 kinase

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Bone metastases are a major challenge in clinical breast cancer management. Statins are inhibitors of the mevalonate pathway, which induce apoptosis and activate the p38 kinase in different human tumor cells. Our study aimed to assess the potential role of p38 in resistance mechanisms against statins in breast cancer cells.

Human osteotropic MDA-MB-231 breast cancer cells were treated with statins and the p38 inhibitor SB202190. Cell Titer Blue, Caspase-3/7-Glo, and Cell Death Detection ELISA assays were used to assess vitality and apoptosis. The clonogenic potential was measured by treating 250 single cells for 9 days. The expression of Dickkopf-1 (DKK-1), a cancer cell-derived inhibitor of osteoblastogenesis, was assessed by real-time PCR and ELISA. Murine C2C12 cells were used to analyze the effects of tumor-derived DKK-1 on osteoblastic differentiation.

Atorvastatin (10 μM), simvastatin (10 μM), and rosuvastatin (100 μM) significantly reduced cell vitality by up to − 60%, activated caspases 3/7 by up to fivefolds ($p < 0.001$), and led to an accumulation of phosphorylated p38 in MDA-MB-231 cells. While low concentrations of statins or SB202190 did not exert anti-tumor effects alone, the combination resulted in a significant loss of vitality by 50%, induction of apoptosis (caspases 3/7 activation, DNA fragmentation by up to tenfolds; $p < 0.001$), as well as the significant suppression of colony forming units and gene expression of anti-apoptotic survivin (− 80% respectively; $p < 0.001$). The combinatory approach also significantly inhibited DKK-1 gene and protein expression (− 90% respectively; $p < 0.001$). MDA-MB-231 derived DKK-1 suppressed Wnt3a-driven osteoblastic differentiation of C2C12 cells by 70% ($p < 0.001$). However, this effect was significantly rescued when tumor cells were pretreated with rosuvastatin and SB202190 ($p < 0.05$). Our results suggest that p38 inhibition significantly enhances anti-tumor effects of statins in human osteotropic breast cancer cells. These observations warrant elucidation using preclinical murine models of osteolytic bone metastases.

P065

Bone microarchitecture and bone turnover in the irradiated human mandible

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Osteoradionecrosis of the mandible is a severe complication of radiotherapy The significance of bone remodeling in the pathogenesis of osteoradionecrosis is unknown. We thereforeaimed to assess the microarchitecture and turnover in irradiated cancellous mandibular bone in relation to radiation dose.

Mandibular bone biopsies were taken from 27 irradiated patients and 35 controls. Micro-CT scanning was performed to analyze microstructural parameters. Bone turnover was assessed by histomorphometry. Local radiation dose was calculated from radiotherapy plans to investigate a possible dose–effect relation. All participants signed an informed consent and the study was approved by the Medical Ethical Committee of the Amsterdam University Medical Centers (location VUmc), Amsterdam, The Netherlands (registration number 2011/220).

Osteoid volume, osteoid surface and osteoclasts number is decreased in irradiated mandibular bone. Trabecular number is lower and trabecular separation is higher in the irradiated group. Trabecular number increases and trabecular separation decreases with higher radiation dose. No differences in structural parameters were observed between the control group and the group with ≥ 50 Gy irradiation, but a difference was observed in bone mineral density (p: 0.03), connectivity density (p: 0.05), trabecular number (p: 0.002) and trabecular separation (p: 0.005) between the control group and the group irradiated with < 50 Gy.

Radiotherapy dramatically impairs bone turnover in the mandible, which leads to a deterioration in microarchitecture that only affects bone irradiated with a local dose of < 50 Gy. To better understand the mechanisms of irradiation damage and osteoradionecrosis, bone microarchitecture and cell activity should be further explored.

Keywords: Osteoradionecrosis, bone turnover, irradiation, mandibular bone, micro CT

measurement	Unit	Control (n = 35) (IQR)	Median	Irradiated (n = 27) (IQR)	Median	P
Trabecular number	Tb.N (1/mm^{-1})	1.94 (.67)		1.63 (0.63)		0.012
Osteoid Volume	OV/BV (%)	1.36 (5.71)		0.066 (0.168)		<0.0001
Osteoclast Number	NOc/BS (/mm2)	0.298 (0.562)		0.026 (0.123)		<0.0001

[bone micro-architecture and bone turnover in mandibular bone of irradiated patients]

P066

Role of autophagy in the crosstalk between osteosarcoma and the bone microenvironment

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Osteosarcoma (OS) is a bone cancer of osteoblastic origin which mainly affects children. Approximatively 15–20% of the patients exhibit a metastatic OS at diagnosis and 25–50% will develop metastasis. Survival for patients with metastatic osteosarcoma has remained virtually unchanged over the past 30 years, with an overall 5-year survival rate of about 20%. Surgery and chemotherapy are the standard treatment regimens but developping novel, effective-targeted therapies to treat this complex disease remains a challenge.

In the first part of the project, we analyzed the effect of an autophagy-deficient microenvironment on OS tumor development. To this aim, we used the AXT OS cell line in a syngeneic mouse model of autophagy deficiency in OB. Our results indicate that autophagy deficiency in primary OB modifies the bone matrix resulting in an increased OS cell proliferation. In vivo, autophagy deficiency in the bone microenvironment accelerates tumor growth and increases lung metastasis potential.

In the second part of the project, we plan to analyze the development of an autophagy-deficient OS tumor and to determine its effect on bone microenvironment. We generated an autophagy-deficient AXT OS cell line using the Crispr-Cas9 system. Autophagy deficiency in OS tumor cells decreases proliferation, migration and sphere-forming efficiency. In addition, our results suggest that autophagy-deficient tumor cells stimulate OC formation.

Taken together, these results suggest that autophagy in bone microenvironment limits OS growth while autophagy in OS tumor cells exerts a protumoral role. This work should allow a better understanding of autophagy participation in the crosstalk between osteosarcoma and bone microenvironment, and potentially lead to the identification of new therapeutic targets to improve the treatment of this pathology.

P067

Alpha-tocopheryl succinate suppresses osteolytic bone metastasis of breast cancer by inhibiting migration of cancer cells and RANKL expression of osteoblasts

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Once bone metastasis of cancer occurs, it has historically been incurable, and patients with bone metastasis of breast cancer have a reported 5-year survival rate of only 20%. Thus, it is urgent to develop therapeutic approaches to prevent and treat bone metastasis of breast cancer. Previously, we reported that alpha-tocopheryl succinate (αTS-suc) inhibits IL-1-induced RANKL expression in osteoblasts. Here we examined the effect of αTS-suc on osteolytic bone metastasis of breast cancer.

To examine the effect of αTS-suc on the metastatic capacity of breast cancer, MDA-MB-231-FL cells were injected into the left cardiac ventricle of BALB/c nude mice along with intraperitoneal injection of αTS-suc. Mice were analyzed by bioluminescence imaging. To investigate the effect of αTS-suc on osteolysis, 4T1 cells were directly injected into the femur of BALB/c mice along with intraperitoneal injection of αTS-suc. Micro-CT analysis and histomorphometric analysis of femora were performed.

αTS-suc inhibited cell migration and cell growth of 4T1 cells. In line with these results, bone metastasis of MDA-MB-231-FL cells was reduced in mice injected with αTS-suc. In addition, αTS-suc decreased osteoclastogenesis by inhibiting 4T1-induced RANKL expression in osteoblasts. Consistent with these results, 4T1-induced bone destruction was ameliorated by αTS-suc, and tumor burden and osteoclast numbers were reduced by αTS-suc in vivo.

Therefore, the results of the present study indicate that αTP-suc can be efficiently utilized to prevent and treat osteolytic bone metastasis of breast cancer with dual effects.

Keywords: Alpha-tocopheryl succinate, Breast cancer, Bone metastasis, Osteolysis, Vicious cycle

P068

Investigating the functional role of the ZNF687 transcription factor in aggressive forms of Paget's disease of bone

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Neoplastic transformation of Paget's disease (PDB) bones may rarely occur, conferring on the disease a poor prognosis. The genetic cause of Giant Cell Tumor associated with PDB (GCT/PDB) has been shown to relie on the P937R mutation in the ZNF687 gene, encoding a transcription factor involved in the DNA damage machinery to repair lesions by homologous recombination. It has been reported that all Italian GCT/PDB cases (15/15) described to date harbour the *ZNF687* mutation. To verify whether GCT/PDB patients of different ethnic origin were carriers of the same mutated gene, we sequenced *ZNF687* in a GCT/PDB biopsy of a deceased 45-yr-old black American woman. We found the P937R mutation also in this non-Caucasian patient, indicating that GCT/PDB pathogenesis is globally related to *ZNF687* mutations.

Moreover, we identified mutations in *ZNF687* in 1/28 pagetic osteosarcomas (R331 W) and 1/8 pagetic undifferentiated sarcomas (P937R), suggesting its rarer involvement also in other PDB neoplasms.

Despite the clear involvement of *ZNF687* in these severe forms of PDB, its biological function is still unknown. In order to gain insights into its role, we reprogrammed *ZNF687*-mutated and wild type fibroblasts to pluripotent stem cells (iPSCs). The iPSCs carrying the *ZNF687* mutation exhibited an early loss of stemness, as highlighted by the loss of expression of markers (Nanog, OCT3/4), thus suggesting a role for this transcription factor in the maintenance of pluripotency. Accordingly, we observed that the analogue *Zfp687* is actively expressed at early stages of mouse embryo development (ES; morula; blastocyst; E5,7; E6,5; E7,5; E10,5) with the highest expression at morula level.

In parallel, phenotypic characterization of the *Zfp687* knock-in mouse model is ongoing. Although μCT data suggest no significant differences among 3-month-old wild type, $P937R^{+/-}$ and $P937R^{+/+}$

mice, further analyses on older mice are in progress, considering the appearance of PDB as a late onset disorder.

P069

Disruption of prostate cancer cell—macrophage—osteoclast crosstalk by a novel TRAF6 inhibitor

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Tumour necrosis factor receptor-associated factor 6 (TRAF6) is a key regulator of the pro-inflammatory NFkB pathway and plays a key role in immunity and osteoclast formation through the regulation of RANK/CD40-mediated NFkB signalling. TRAF/NFkB signalling is implicated in cancer, but its role in the prostate cancer—immune cell crosstalk remains unknown. Here, we report that TRAF6 is highly expressed in a panel of androgen-independent, bone-seeking cell lines including C42B4, PC3 and DU145 cells compared to the androgen-sensitive LNCaP. Stable knockdown and the pharmacological inhibition of TRAF6 using the verified TRAF6 inhibitor 6877002 and its novel and more potent congener FSAS3 reduced PC3 cell viability (50% reduction, $p < 0.05$), migration (23% reduction, $p < 0.05$) and invasion (35% reduction, $p < 0.05$). Conditioned medium from M2 macrophage—but not M0 and M1 phenotype—enhanced human PC3 proliferation, migration and invasion and these effects were significantly inhibited by treatment with FSAS3 (0.3-1μM—proliferation, 22% reduction; migration, 35% reduction; invasion, 79% reduction) ($p < 0.005$). 6877002 and FSAS3 reduced the ability of M0 macrophage to form osteoclasts in the presence of RANKL and PC3 cells and/or their derived factors. Interestingly, FSAS has no effect on the ability of M1 macrophage to inhibit the proliferation of PC3 cells. Mechanistically, FSAS3 (1μM) reduced IκB phosphorylation induced by RANKL and macrophage-derived factors in PC3 cells, indicative of NFκB inhibition. We conclude that inhibition of TRAF6 in prostate cancer cells reduces their ability to influence the differentiation of bone marrow macrophage into osteoclast and tumour associated macrophage in vitro. In vivo studies to test the effects of FSAS, alone and in combination with chemotherapeutic agents, on mouse models of prostate cancer metastasis are ongoing.

Keywords: Osteoimmunology; inflammation; prostate cancer; osteoclast; macrophage.

P071

Targeting the CaSR in breast cancer cells brings down colonization and migratory response in a trabecular bone explant model, and reduces osteolytic lesions in vivo

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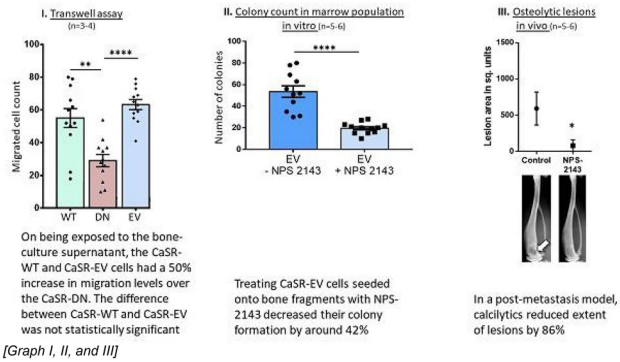
The calcium sensing receptor (CaSR) has emerged as a new target in the "vicious cycle" that amplifies the metastatic cascade in breast cancer bone metastases. Our aim was to study the involvement of CaSR in the metastasis of breast cancer cells to the bone using human femur tissue explants and to abate it with calcilytics (CaSR antagonists).

Methods: MDA-MB-231 cells over-expressing a full-length wild-type CaSR (CaSR-WT) or a dominant negative mutant (CaSR-DN), and transfected with an empty vector (CaSR-EV) for control were generated. NPS-2143 was used as a calcilytic. Trabecular bone fragments extracted from human femoral-heads, were used as explants to study the osteoinvasion in vitro. Mice were inoculated intra-tibially with MDA-B02 cells and received NPS-2143 (IP) for 7 days.

Results: Results of migration with bone-culture supernatant are presented in Graph-I. Interestingly, similar results were obtained when cells were allowed to colonize in the bone-fragment. The CaSR-WT and CaSR-EV had a threefold increase in colony formation over CaSR-DN ($p < 0.01$). Thus, we selected the CaSR-EV in further experiments (Graph-II), where NPS-2143 diminished migration toward bone supernatants ($p < 0.01$), and blunted proliferation sensitized by the conditioned media ($p < 0.01$). In vivo, calcilytics reduced incidence (4/5 vs. 1/6) and extent (Graph-III) of osteolytic lesions.



[Graph I, II, and III]

Perspectives: In a co-culture model, we demonstrate that the CaSR in breast cancer cells is involved in engraftment and chemoattraction to the bone. Further experiments are underway to reveal if calcilytics can be repurposed as a therapy for bone metastases from breast cancer.

Keywords: breast cancer, bone metastasis, CaSR

P072

RANKL and mammary gland pathophysiology in modeled osteoporosis

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Receptor activator of nuclear factor-κB ligand (RANKL) is one of the central regulators in bone remodeling by inducing osteoclast formation and bone resorption, while aberrant expression of RANKL has been correlated with osteolytic diseases, including osteoporosis. Apart from its fundamental role in the skeletal system, recent studies have highlighted the key role played by the RANKL protein in the formation of lactating mammary glands during pregnancy, as well as in the initiation of breast cancer. Given that RANKL mediates the paracrine effect of progesterone in the proliferation of mammary epithelial cells, RANKL is considered as a highly promising therapeutic target for the prevention and treatment of various breast cancer

subtypes. Our group has recently generated novel genetic models of osteoporosis by overexpression of human RANKL (hRANKL) in transgenic mice (TgRANKL). In the current study we investigated the expression pattern of RANKL in the mammary glands of TgRANKL mice, combining qPCR and immunocytochemistry at different time points. Our analysis demonstrated that hRANKL was expressed in the mammary glands of TgRANKL females and was further upregulated during pregnancy, indicating physiological regulation of transgene expression. Moreover, a specific localization of RANKL was identified at the luminal epithelial cells in ductal and alveolar structures. Both whole mount staining with carmine and histological analysis demonstrated a widespread epithelial expansion in TgRANKL mice as shown by quantification of ductal branches (WT: 10.1 ± 0.9 vs TgRANKL: 21.2 ± 2.2, $p < 0.01$) and terminal end buds (WT: 178.2 ± 11.1 vs TgRANKL: 315.5 ± 25.0, $p < 0.01$) at 4 months of age. Notably, immunocytochemical analysis revealed increased proliferation in epithelial cells, as shown by staining with the proliferative markers Ki67 and Cyclin D1.

Collectively, our results demonstrate that increased expression of RANKL in the mammary glands of osteoporotic TgRANKL mice is correlated with enhanced proliferation of mammary epithelial cells.

Keywords: RANKL, mammary gland, osteoporosis, transgenic mice

P073

Contribution of cancer stem cells to the metastatic capacities of osteosarcoma

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Lung metastases reduced the survival of patients with osteosarcoma. Cancer stem cells (CSC) were shown to contribute to tumor malignancy but the specific role of osteosarcoma stem cells during dissemination and metastasis development is not clear. We previously reported that calpain-6 expression identified CSC. We used K7M2 mouse and 143B human osteosarcoma cells expressing a GFP reporter gene under the control of calpain-6-promoter (Calp6-P-GFP) to track CSC during bone tumor and lung metastasis development in mice and to study migration capacities. The osteosarcoma cell lines contain 10-30% of Calp6-PGFP + cells. Seven days post-injection of K7M2 cells in the tibia, the developing tumors are mainly Calp6-P-GFP + proliferating cells, but after several weeks, large tumors contained heterogeneous amounts of CSC. High levels of calpain-6 in the primary bone tumors were associated with the development of numerous and large lung metastatic nodules. Sorted 143B Calp6-P-GFP-cells had strong migratory defects compared to sorted GFP + cells and cell that overexpress calpain-6(Calp6 +) as shown by scratch healing assays and time lapse microscopy. However, calp6-P-GFP- and GFP + cells had similar patterns of migration when cultured together. Conditioned serum-free medium (CM) on a 143B cell layer restored the migration of calp6-P-GFP- cells. This effect of CM was abolished when the 143B cells were treated with an inhibitor of exosome release. Together these results indicate that the metastatic potential of bone tumors depends on calpain-6-expressing CSC that communicate with the other tumor cells through exosomes to regulate migratory capacities.

Keywords: Osteosarcoma, calpain-6, stem cells, metastasis, migration

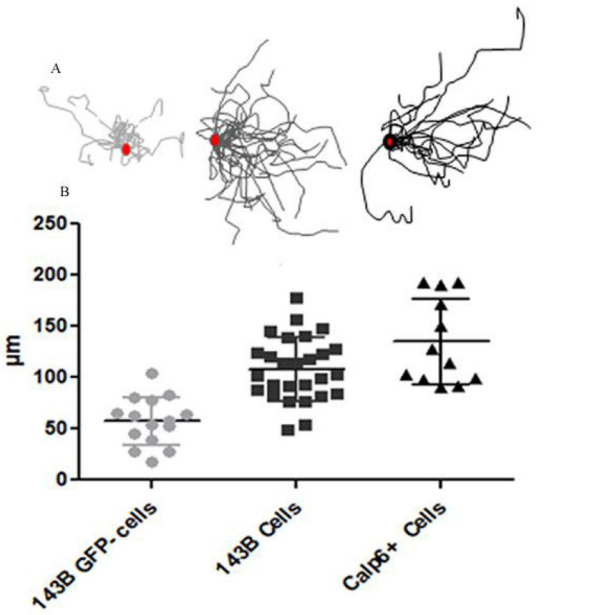

Figure: Time lapse microscopy to measure the migration of 143B Calp6-P-GFP- cells (n=14), 143B cells (n=27) and calpain-6-overexpressing cells (Calp6+) (n=12). The movement of the cells was determined taking 1 photo each 10min for 8 hours. A. the pathway of each cell was reconstituted. B. Distance travelled for each cell was measured. Anova test was performed ($p<0.0001$)

[Time Lapse microscopy for measurement of osteosarcoma cell migration]

P078

Vitamin D deficiency and increased risk of bladder and kidney carcinoma in mediterranean population

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Many epidemiological studies reported an inverse association between vitamin D and the incidence of various cancers. Studies indicate that vitamin D, measured as circulating 25-hydroxyvitamin D (25(OH)D), may be associated with a reduced risk of several types of cancer. It has also been suggested that the inverse relationship between vitamin D and the incidence of cancer is not uniform but U-shaped, indicating that after a certain point, higher-levels of vitamin D are positively associated with the cancer incidence. Our study investigates the association between different levels of vitamin D and the incidence rates of different types of bladder and kidney cancers. We also explore the shape of that association, using a sample of cancer patients residing in the northern coastal region of Croatia.

Total of 80 patients from Clinical Hospital Rijeka, Croatia, were included in this study. Patients were separated according to their diagnosis into bladder cancer (40 subjects) and kidney cancer (40 subjects) group. Serum 25-hydroxy vitamin D was measured with an enzyme immunoassay.

The incidence of both kidney and bladder cancer exhibits a clear nonlinear association with the 25-hydroxyvitamin D (25(OH)D) levels. Increasing 25(OH)D concentration levels are statistically associated with the decreasing risk of developing both kidney cancer and bladder cancer. In other words, categorgy2 and category3 concentrations are statistically associated with the decreasing risk of developing both kidney cancer and bladder cancer, relative to the lowest category1 (< 25 nmol/L) ($p < 0.01$). Highest category4

(> 75 nmol/L) concentrations, however, were not statistically differently associated with the risk of kidney or bladder cancer relative to the lowest category1, at 5% level ($p > 0.09$).

Our results suggest that higher serum 25-hydroxyvitamin D (25(OH)D) levels are associated with lower incidence rates of kidney and bladder cancer in the northern coastal region of Croatia.

Keywords: Vitamin D, bladder carcinoma, kidney carcinoma

P079

Bone mineral density of lumbar spine and femur in patients with gynaecologic cancer

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Objectives: To compare the bone mineral density (BMD) of the lumbar spine and femur in postmenopausal women with cervical and endometrial cancer without bone metastasis with that in normal control postmenopausal women

Methods: We retrospectively analysed the BMD of the lumbar spine and femur using dual-energy X-ray absorptiometry in 130 patients with cervical cancer, 68 patients with endometrial cancer, and 225 healthy controls.

Results: The serum levels of calcium, phosphorus, osteocalcin, and total alkaline phosphatase, and urine deoxypyridinoline were measured in all participants. Age, BMI, parity, and time since menopause were not significantly different between the three groups. The T-scores of basal BMD at the fourth lumbar vertebra (L4) were significantly lower in patients with cervical cancer (-0.68 ± 0.10) compared to those in the other two groups. Additionally, the incidence of osteoporosis at L4 according to the basal status of bone mass was significantly higher in patients with cervical cancer (10.0%) compared to that in controls (0.4%). Urine deoxypyridinoline levels were significantly higher in patients with cervical cancer compared to those in controls. No differences in basal BMD of the lumbar spine and femur were observed between patients with endometrial cancer and controls, and no significant differences in biochemical markers were detected between patients with endometrial cancer and controls.

Conclusions: Our results suggest that postmenopausal women with cervical cancer have a lower BMD and are at increased risk of osteoporosis in the lumbar spine before receiving anticancer treatment compared with postmenopausal women with endometrial cancer.

P081

Vitamin K role on the osteocalcin:GPRC6A axis: insights from non-mammalian models

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Beside its central role in skeleton structure, bone functions as an endocrine organ regulating glucose, insulin and testosterone metabolisms through osteocalcin (Oc) action. Osteocalcin is a bone matrix protein secreted by osteoblasts that has a high affinity for calcium ions thanks to the vitamin K-dependent g-carboxylation of the glutamate residues at positions 17, 21 and 24. During bone turnover osteoclasts create an acidic environment that provokes Oc decarboxylation, present in bone matrix and body fluids. The endocrine function of Oc has been associated with the uncarboxylated form (ucOc) through its binding to the receptor GPRC6A and the ratio between ucOc and total Oc is a proxy used to infer vitamin K status and intake. However, the mechanisms underlying ucOc action are still poorly understood, in particular those related to its binding to GPRC6A, while the action of the vitamin K on osteocalcin:GPRC6A interplay, and its effect on bone, glucose and insulin metabolism was never addressed in the same study. We have identified the zebrafish as a valuable model organism to study osteocalcin endocrine function through its interaction with GPRC6A receptor, and the role of vitamin K on ucOc availability, endocrine functions and bone quality. For that we have determined the expression of *gprc6a* and several marker genes for glucose, insulin and bone metabolism, evaluated the carboxylation status of osteocalcin and assessed bone phenotype in controls and in fish fed diets enriched with 1000 mg/kg of vitamin K1 using both synthetic and natural sources. A proper vitamin K status ensures Oc g-carboxylation and improved glucose, insulin and bone metabolism.

Keywords: Vitamin K; bone metabolism; osteocalcin; zebrafish

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P082

Low adhesive scaffold type I collagen prepared from porcine skin regulates the expression of genes relating to osteogenic differentiation

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Background: Collagen has biocompatibility and biodegradability with tissue or organ, therefore, collagen is the most promising material for tissue engineering. In general, the binding of rat marrow mesenchymal cells (rMMCs) to collagen scaffold is considered an effective to promote osteogenic differentiation. However, it is not clear why collagen is a suitable scaffold. At the ECTS2018 Congress, we showed that low adhesive scaffold type I collagen (LASCol) has marvellous ability to induce osteogenic differentiation in short term. In this study, we report the ability of LASCol scaffold by analyzing focused genes of osteogenesis.

Methods: Rat MMCs ($5x10^4$ cells/dish) were cultured on LASCol coated-dish with osteogenic basal medium. The alkaline phosphatase (ALP) activity of rMMCs cultured on the LASCol coated dish was observed. To evaluate osteogenic differentiation, mature RNA was isolated and reverse transcribed, subsequently the cDNA was analyzed on the real-time RT Profiler PCR Array. In addition, the upregulated genes were further validated by RT-qPCR with specific primers.

Results: Rat MMCs on the LASCol coated-dish formed spheroid bodies and most of them showed ALP activity. However, on the other scaffolds only 60%-70% of cells had the ALP activity. Thus, the contact with LASCol might increase the ratio of osteogenic differentiation. Interestingly, after three days culture only a few genes of 84 genes showed increasing or decreasing. Though the genes such as Col2a1, Bglap, and Phex upregulated, the threshold cycle of *Runx2* gene was similar. In particular, *Phex* might be an important gene because of the relative expression was high. Probably, LASCol would

have the ability to induce osteogenic differentiation of rMMCs by the different gene expression patterns.

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P083

Up-regulation of inhibitors of DNA binding/differentiation gene during alendronate-induced osteoblast differentiation

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Aim: Alendronate enhances bone morphogenetic proteins (BMP)-mediated osteoblast differentiation. A balanced regulation of inhibitors of DNA binding/differentiation (Ids) plays an important role in BMP-induced osteoblast differentiation. However, there are no studies on the possible roles of Idgenesin alendronate-induced osteoblast differentiation.This study investigated the effect of alendronate on the expression of Idgenesin osteoblast differentiation.

Methods: C2C12 cells were treated with alendronate for various concentrations and time periods. For evaluation of alendronate-induced osteoblast

differentiation in C2C12 cells, alkaline phosphatase (ALP) activity was measured. The expression of osteoblast differentiation markers such as ALP, type-1 collagen (Col 1), and osteocalcin (OCN), and the expression of Id-1 and Id-2 were measured by RT-PCR. In order to understand the mechanism underlying the regulation of Idgenes, the promoter region of the Id-1gene was identified. Database analysis of the promoter region for Id-1 using known consensus sequences identified several putative response elements, including CCAAT/enhancer-binding protein beta (C/EBPβ).

Results: Alendronate treatment significantly increased not only ALP activity but also expression of ALP, Col 1, and OCN, Id-1 and Id-2. C/EBPβ and alendronate cooperatively increased the promoter activity and expression of Id-1.

Conclusions: These results suggest that C/EBPβ-mediated Id-1 transcriptional activation may regulate alendronate-induced osteoblast differentiation of C2C12 cells.

P084

The effect of regulation of TRPC activation on periodontitis progression

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It has been reported that TRPC (transient receptor potential canonical) channels play an important role in the stimulation of osteoblast proliferation. However, the role of TRPC channels in periodontitis has not been clarified. Therefore, we investigated the effect on periodontitis-induced alveolar bone loss by regulation of TPRC activation.

In human periodontal ligament (PDL) cells cultured in osteoblast differentiation media, expressions of TRPC3/6, RUNX2, and osteocalcin were evaluated by real-time PCR on days 0, 1, 3, 5, 7, 14, 21, and 28. In periodontitis mice, expressions of RUNX2 and osteocalcin were determined in PDL area and osteoblasts of furcation of mandibular 1st molar on days 7, 14, and 28. Additionally, in periodontitis mice with treatment of TRPC3 inhibitor Pyr3, TRPC 6 inhibitor Sar7334, and TRPC6 activator flufenamic acid (FFA), alveolar bone loss, osteoclast formation, and expression of RUNX2 and osteocalcin were estimated on day 3 after periodontitis induction.

In PDL cells, TRPC6 expression peaked on day 7 compared with control. TRPC3 expression generally increased during differentiation. RUNX2 expression increased from day 3 to 7 compared with control and osteocalcin expression peaked on day 28. During periodontitis progression, expressions of RUNX2 and osteocalcin were elevated in PDL area and osteoblasts on days 7 and 28. Alveolar bone area was increased in periodontitis mice with Pyr3 treatment compared with periodontitis mice. The number of osteoclasts in periodontitis mice with FFA and Sar7334 treatment was less than periodontitis mice.

Expressions of TRPC6 and TRPC3 during differentiation of PDL cells into osteoblasts increased. Interestingly, in periodontitis mice, regulation of TRPC activation attenuated alveolar bone loss and osteoclast formation. These data suggest that the regulation of TRPC channels in periodontal tissue could affect the periodontitis progression.

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P085

Role of Msx2 in the osteoblast bone specificity

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It has long been assumed that all the osteoblasts were identical independently of their anatomical localization. However, growing published evidence suggest the existence of osteoblast specificity according to their bone anatomical localization. *Msx2* is a divergent homeobox gene with a different bone expression level according to the anatomical site and is considered as involved in the osteoblast specificity. Moreover, in Msx2 knock-in (Msx2 KI) mice, a regional osteopetrosis of the mandible has been previously reported (Aioub, 2007). In order to decipher the potential role of *Msx2* in the osteoblast specificity, we analyzed the axial and long bone phenotypes of the Msx2 KI mice. Because *Msx2* is widely expressed, we also generated a conditional Msx2 KO model invalidating *Msx2* expression specifically in mature osteoblasts.

The bone phenotype analysis in the Msx2 KI mice at 4 month of age interestingly revealed an osteoporotic phenotype. In femurs, we observed a bone mineral density decrease of 15% in KI *vs* WT associated with BV/TV decrease caused by a bone formation rate decreased without osteoclastic activity modification. However, in the vertebrae, the BV/TV is not significantly modified despite a clear bone formation decrease and a clear osteoclast activity increase.

The bone phenotype of the conditional KO Msx2 mice revealed no bone phenotype.

Our results confirm Msx2 as an actor of the osteoblast specifity and suggest an early role in the establishment of this specificity, as the lack of *Msx2* expression in mature osteoblasts does not affect bone development and growth. To confirm this conclusion invalidation of *Msx2* in preosteoblasts should be conducted. However, we cannot so far rule out that the observed bone phenotype in Msx2 KI mice might be explained by the invalidation of *Msx2* expression in other cells and/or other organs. Further studies are currently on going.

P086

Tuftelin, HIF-I and HIF-II in intramembranous osteogenesis

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Tuftelin was first identified as a constituent of the developing enamel, later found in odontoblasts and then even in several soft tissues, particularly those exposed to physiological hypoxia. Tuftelin has not yet been investigated during bone development, but its mRNA was elevated in peridental mesenchymal cells considered to contribute to mandibular bone formation in vivo.

To further precise this observation, the expression of tuftelin, HIF-I and HIF-II was investigated in the mouse mandibular intramembranous bone during prenatal development. Immunohistochemistry was applied to detect corresponding proteins in individual cells and RT-PCR to quantify the relative expression levels. The region of interest, the mandibular/alveolar bone associated with the first molar, was selected for its importance in the periodontium-mediated final tooth anchorage. In the mouse, the first mesenchymal condensation of this bone structure becomes apparent at the day 13 of pregnancy, while two days later, a complex vascularized bone containing osteoblasts, osteocytes and osteoclasts is present.

Within these 2 days, the relative expression of tuftelin (compared to actin) was within this time period around 8×10^{-4} times and presence of tuftelin was confirmed by immunohistochemistry in osteoblasts and osteocytes. The expression level of the gene encoding for HIF-II, known to be active in the mandibular bone, was around 10 times higher than that of tuftelin. Regarding the expression of HIF-I, not yet reported in the foetal intramembranous bone, it was even higher: around 50 times compared to tuftelin. While temporospatial distribution of HIF-II did not positively correlate with tuftelin positive cells, the most tuftelin positive cells displayed also presence of the HIF-I protein.

This investigation provides the first evidence for the expression of tuftelin during osteogenesis. In the alveolar/mandibular bone, tuftelin was demonstrated at the mRNA as well as protein level. Moreover, the expression of HIF-I in an intramembranous bone is shown here for the first time. Temporospatial correlations of tuftelin, HIF-I and HIF-II are subjected for further research.

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P087

Proposal of a new method to quantification of the mineralization process in vitro models

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Background: An important aspect to the long-lasting performance of a dental implant is osseointegration, which is the propriety of building new bone around it. This process can be measured by the amount of mineralization obtained when osteoblasts cells are cultured over the material. Confocal laser scanning microscopy (CLSM) has been largely used to analyze cell morphology, structures and functions. However, the use of confocal volume quantification to measure the amount of mineralization obtained by cultured osteoblasts has not yet been described in the biomedical literature.

Aims: Our objective was to develop a new protocol to quantify mineralization, based on 3D confocal microscopy.

Methods: Osteoblasts cells (MC3T3) were cultured on titanium and zirconia surface for 14 and 21 days, in modified osteogenic media. Basically, α-MEM media supplemented with 10% BFS, 50 μg/mL acid ascorbic and 10 mM β-glycerophosphate and 1 μg/mL of calcein (known to bind to Ca ions) was used. Afterwards, the cells were fixed and labeled with Rhodamine phalloidin and DAPI. The 3D image stacks were acquired on a Leica TCS SPE Confocal Laser Scanning Microscope. Mineralization volume was measured by software. Statistical analysis was performed in the Statistica software, by selecting the two-way Anova.

Results: Regarding the mineralization volume quantified, the amount of mineralization was increased over time for all groups. There was no significant difference between materials after 14 and 21 days of culture ($p > 0.05$). But the absolute values were much greater after 21 days of culture (69,729.55 μm^3 for titanium and 83,210.64 μm^3 for zirconia) in comparison with 14 days (2569.22 μm^3 and 3882.67 μm^3, respectively) ($p < 0.05$).

Conclusions: The volume quantification technique adopted was useful in providing information about the cellular status and mineralization.

Keywords: Osteoblasts. Bone Mineralization. Confocal Microscopy.

P088

Excessive O-GlcNAcylation-inducing conditions inhibit Wnt/beta-catenin signaling in mesenchynal stromal cells

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Hyperglycemia drives excessive chronic O-GlcNAcylation of proteins in diabetic patients. We have previously demonstrated that excessive O-GlcNAcylation inhibits BMP2-induced osteogenic differentiation of C2C12 cells via inhibiting transcriptional activity of Runx2. The aim of this study was to investigate whether excessive O-GlcNAcylation affects Wnt/β-catenin signaling under osteogenic conditions. To induce osteogenic differentiation, ST2 cells were cultured in the presence of Wnt3a. Protein O-GlcNAcylation was induced or inhibited by treating cells with N-acetylglucosamine (2.5 mM) or O-GlcNAc transferase inhibitor (1 μM STO45849), respectively. N-acetylglucosamine increased β-catenin O-GlcNAcylation as well as the total levels of O-GlcNAcylated proteins. N-acetylglucosamine downregulated Wnt3a-induced alkaline phosphatase activity and osteogenic marker gene expression in ST2 cells. N-acetylglucosamine suppressed Wnt3a-induced TOP-flash activity. In addition, N-acetylglucosamine destabilized β-catenin protein by increasing β-catenin ubiquitination in the presence of Wnt3a. These inhibitory effects of N-acetylglucosamine on Wnt3a/β-catenin signaling were rescued by addition of STO45849. These findings suggest that excessive protein O-GlcNAcylation conditions attenuate osteogenic differentiation partly via inhibiting Wnt/β-catenin signaling.

Keywords: O-GlcNAcylation, Wnt3a, beta-catenin, osteoblast differentiation

P090

SIRT-1 mediates zingerone-stimulated osteoblast differentiation

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Objectives: *Porphyromonas gingivalis* is a major causative periodontal pathogen which secretes many virulence factors leading to bone resorption. A key component of dry ginger, zingerone has various activity including anti-inflammatory effect. However, the effect of zingerone on osteoblast differentiation under *P. gingivalis* infection remains to be cleared. Thus, the purpose of this study was to investigate the effects of zingerone on osteoblast differentiation and the action mechanism of zingerone against *P. gingivalis* infection.
Methods: MTT assay was performed using murine osteblastic cell line, MC3T3-E1. To evaluate the effects of zingerone on cellular differentiation, the expression of RNX2 and osteocalcin was determined in MC3T3-E1 cells treated with and without SIRT 1 inhibitor, sirtinol using qPCR and western blot. The effect of zingerone on mineralization was examined by alkaline phosphatase (ALP) activity assay and alizarin red staining (ARS). For in vivo study, P. gingivalis was injected subcutaneously into calvariae of wild type and SIRT1 transgenic mice with or without zingerone. The mRNA expression of RUNX2 and osteocalcin in femurs was measured using qPCR.
Results: Zingerone increased osteoblast proliferation in a dose-dependent manner. Zingerone increased RUNX2 and osteocalcin expression and these increased expression was down-regulated by Sirtinol treatment. The degree of ALP activity and ARS stain was enhanced in MC3T3-E1 cells treated with zingerone compared to non-treated control group. Moreover, these effects were antagonized by sirtinol treatment. In mice, RUNX2 and osteocalcin expression by *P. gingivalis* infection significantly was decreased in wild type mice and these decreased expression was recovered in SIRT1 transgenic mice. In addition, zingerone alleviated the suppressed expression of these genes which were induced by *P. gingivalis* infection.
Conclusions: We showed that zingerone increased the expression of osteogenic genes through SIRT1 activation. Thus, zingerone might be suggested as a potential candidate for the treatment of periodontitis by stimulating bone formation.

P091

Osteoblastic differentiation is inhibited by autocrine effect of osteoblasts cocultured with adipocytes

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Adipocytes (ADs) inhibit osteoblastic differentiation through synthesis and secretion of TNF. Also, ADs increase the synthesis of *IL10* and *IL1β* by osteoblasts (OBs). In this context, the aim of the present study was to investigate the effect of conditioned medium (CM) by OBs cocultured with ADs on the expression of inflammatory cytokines and osteogenic and adipogenic markers in OBs cultured in non-conditioned medium. Mesenchymal stem cells derived from rat bone marrow and adipose tissue were cultured in osteogenic and adipogenic medium to allow differentiation into OBs and ADs, respectively. After that, the OBs and ADs were kept in co-culture for 3 days, and after 24 h the CM was collected. New OBs were cultured in osteogenic medium for 7 days, and then they were kept in CM for 3 days. The expression of inflammatory cytokines, osteogenic and adipogenic markers (Real-Time PCR) and alkaline phosphatase (ALP) activity were evaluated 3 days post-culture in CM. On day 7 after the beginning of culture in CM, the extracellular matrix mineralization was evaluated. OBs cultured in non-coditioned medium were used as control. The data were compared by Student's T test ($n = 3$, $p \leq 0.05$). All next parameters presented statistical significant difference for OBs cultured in CM compared to controls calibrated to 1.00. The results obtained were: *IL1b* 5.74; *IL6,* 2.96; *IL10,* 4.44; *Tnfa,* 2.00; *Pparg,* 1.42; *Ap2,* 1.36; *Adipoq*,

3.22; *Retn,* 2.18; *Runx2,* 1.17; *Osx,* 0.90; *Opn,* 0.94; *Oc,* 1.08; ALP activity, 0.39; Mineralization, 0.48. The results indicate that ADs affect the intracellular machinery of OBs. The conditioned medium by OBs that were co-cultured with ADs increased the expression of inflammatory cytokines of OBs cultured in non-conditioned medium, which could explain the reduced osteoblastic differentiation and the increase in adipogenic markers of the latter ones. (Ethical approval: 2017.1.604.58.3).
Keywords: Osteoblasts, Adipocytes, Coculture, Cytokines

P092

Identification of new players in phosphate signaling pathway in osteogenic cells

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Inorganic phosphate (Pi) is critical for development and homeostasis of all mineralizing tissues. The importance of Pi for these tissues is underscored by bone, cartilage, dentin and periodontal complex pathologies in genetic and acquired disorders affecting systemic Pi levels and local/cellular Pi availability. Pi regulates the mineralization process by acting as a signaling molecule affecting differentiation and function of cells in skeletal and dental tissues, however very little is known about Pi-induced signaling cascade in osteogenic cells. The goal of our studies is to decipher the signaling cascade that leads to the initiation of the mineralization process in response extracellular Pi. We used 17IIA11 (cranial neural crest origin) and MLO-A5 (lateral plate mesoderm origin) cell lines that have characteristics of committed osteogenic cells, as determined by high expression of key osteogenic transcription factors and enzymes required for mineralization and by rapid mineralization in osteogenic conditions. Activation of cellular response was evaluated by levels of activated Erk1/2 kinase (immunodetection), and expression of classic Pi-responsive genes: Dmp1 and Spp1/Opn (qRT-PCR). Activation and inhibition of specific molecules was accomplished either by genetic modification of model cells or pharmacologically. We determined that deficiency of mineralization-regulating transcription factor Trps1 results in loss of the mineralization potential of 17IIA11

cells, and loss of Erk1/2 activation in response to Pi. Cellular sensitivity to Pi was also abolished by depletion of Pth1r but inhibition of Fgfr had no effect on Pi-induced activation of Erk1/2 and gene expression. Furthermore, we demonstrated PKC but not PKA as Pth1r downstream effector in Pi signaling in osteogenic cells. In conclusion, we identified a new players in the Pi-induced signaling cascade in cells producing mineralized extracellular matrix.

Keywords: Mineralization, phosphate, signaling, osteoblasts

P093

Cyanobacteria as a source for bone anabolic compounds

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Bone is a dynamic tissue under constant remodelling throughout life and any dysfunction in this tightly controlled physiological process often results in severe skeletal disorders, osteoporosis being the most common. Current therapeutics for osteoporosis are limited and have issues related to costs, efficacy and long-term use. It is thus critical to continue searching for new treatments. Recently, the presence of osteoactive compounds were reported in extracts from several marine organisms with the ability to regulate bone homeostasis. This not only sets a new paradigm for bone therapeutics research but also highlights the importance of screening marine resources to identify compounds with bone anabolic potential toward the development of new medicines. In this study, 81 fractions derived from 8 cyanobacteria strains were tested for their in vivo osteogenic activity using zebrafish operculum system. A significant increase in opercular bone growth of zebrafish larvae exposed to 6 of these fractions was observed (up to 36%). To gain insights into cellular dynamics underlying the osteogenic effects observed, zebrafish transgenic lines of two osteoblast markers—*Tg(sp7:mCherry)* and *Tg(oc:EGFP)*—and one osteoclast marker *Tg(ctsk:DsRed)* were used to access compound effect on bone cell differentiation/maturation. Additionally, to validate the use of fish systems to accelerate the discovery of osteoactives for human health, in vitro proliferation and mineralization assays were conducted using mammalian bone-derived cell lines MC3T3-E1 and MG-63. This study highlights the potential of cyanobacteria as a source of natural compounds for bone therapeutic applications as well as the use of fish systems as an alternative to mammalian screening systems.

Keywords: Bone disorders, Cyanobacteria, osteoactives, Zebrafish, in vitro cell systems

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P094

Direct impact of glucocorticoid receptor on the RANKL promoter activity

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Glucocorticoid induced osteoporosis is the most common cause of secondary osteoporosis before 50 years of age. Glucocorticoid induced osteoporosis is defined by highly reduced bone formation and increased bone resorption caused through glucocorticoid receptor-mediated regulation of gene expression, which mainly intervenes in receptor activator of nuclear factor Kappa-B ligand (RANKL)/receptor activator of nuclear factor Kappa-B (RANK)/osteoprotegerin (OPG) signalling pathway. In glucocorticoid induced osteoporosis, osteoblasts signalling causes reduced osteoprotegerin (OPG) release and increased level of RANKL resulting in osteoclastogenesis. Chronic use of glucocorticoids thus results in lower bone mineral density and increased level of fragility fractures. The aim of our study was to examine whether glucocorticoid receptor can directly impact the expression of RANKL. For that purpose, we analysed the impact of overexpression glucocorticoid receptor on the activity of RANKL promoter in human osteosarcoma and human lung cancer cells. Overexpression of glucocorticoid receptor caused twofold higher ($p = 4\ 10^{-6}$) activity of RANKL promoter. Induction of RANKL activity by glucocorticoid receptor was alleviated when GRE (glucocorticoid response element) mutation was introduced in the RANKL promoter region suggesting that GR could directly activate RANKL promoter activity. This is the first study showing direct impact of glucocorticoid receptor on the RANKL promoter activity unveiling novel mechanisms behind development of glucocorticoid induced osteoporosis.

P095

Clinical results of bone marrow mesenchymal stem cell implantation for osteonecrosis of the femoral head

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Background: To date, several trials have reported the use of mesenchymal stem cell (MSC) implantation for osteonecrosis of the femoral head (ONFH). However, the clinical outcomes have not been conclusive. This study compared the clinical and radiological results of bone marrow mesenchymal stem cell (BMMSC) implantation with traditional simple core decompression (CD) using a matched pair case–control design.

Methods: We retrospectively reviewed 100 patients with ONFH (106 hips) who had been treated by CD alone and CD + BMMSC implantation.

Results: The mean follow-up period was 4.28 years. There was a difference in the THA conversion rate between the CD (49%) and CD + BMMSC groups (28.3%) ($p = 0.028$). ARCO stage progression was noted in 20 of 53 hips (37.7%) in the CD group and 19 of 53 hips (35.8%) in the CD + BMMSC group. Among collapsed cases (ARCO stages III and IV), there was no difference in clinical failure rate between the two groups. Conversely, in the pre-collapse cases (ARCO stages I and II), only 6 of 30 hips (20%) progressed to clinical failure in the CD + BMMSC group, whereas 15 of 30 hips (50%) progressed to clinical failure in the CD group ($p = 0.014$). Kaplan–Meier survival analysis showed a significant difference in the time to failure between the two groups up to 10-year follow-up (log-rank test $p = 0.031$).

Conclusions: These results suggest that implantation of MSCs into the femoral head at an early stage of ONFH lowers the THA conversion rate. However, ARCO stage progression is not affected by this treatment.

P096

Regulation of collagen proteostasis by FAM134B during bone growth

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Collagens are the most abundant proteins in animals. Type I and II collagens are the major components of bone and cartilage, respectively. Consistently, collagen-related diseases in humans are characterised by severe skeletal phenotypes. Collagens are synthesized and folded within the endoplasmic reticulum (ER) and transported via the Golgi apparatus to the plasma membrane for secretion. The ER quality control systems ensure that only properly folded collagens are secreted, the remaining fraction is degraded either by the ER associated degradation (ERAD) or (macro)autophagy. While ERAD has been extensively investigated, the mechanism by which autophagy degrades ER collagens is currently unknown.My laboratory has identified the ER transmembrane protein FAM134B and the chaperone Calnexin as bona fide autophagy receptors for misfolded collagens in the ER. Mechanistically, Calnexin mediates binding of ER-luminal misfolded collagen to FAM134B, which interacts with the autophagy protein LC3 in the cytosol and allows sequestration of collagen within the autophagosomes. Cells lacking FAM134B accumulates intracellular procollagen molecules, and in vivo, fish lacking FAM134B show impaired collagen secretion and defective bone mineralization, suggesting a physiological role of FAM134B-mediated autophagy during bone development and maintenance. Taken together our work sheds light on a collagen quality control machinery that can be potentially targeted to treat collagen-related disorders.

P098

Marine plants as valuable sources of antioxidant and anabolic bone compounds

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Discovery of new anabolic compounds with pharmaceutical value for treating low bone mineral disorders is of utmost importance. Recent studies indicate that natural resources contain potent bioactive compounds, like phytochemicals with antioxidant and antibacterial properties, recently found to stimulate osteoblast proliferation and differentiation.

Here we assessed the halophyte *Spartina alterniflora* as a potential source of natural osteoactive phytochemicals. Extracts prepared by liquid/liquid extraction (hydroalcoholic-extract, aqueous-fraction and ethyl-acetate fraction (EAF)) were assessed for antioxidant, mineralogenic and osteogenic bioactivities. EAF had the highest content of total phenolic compounds (± 120 mg gallic acid/g DW) and the highest antioxidant activity (by radical scavenging activity and reducing power assay). The mineralogenic potential of the three extracts was evaluated in vitro using marine fish cells. EAF significantly increased ECM mineralization in a dose dependent manner at concentrations ranging from 5 to 250 mg/mL ($p < 0.001$, one-way ANOVA). EAF was further evaluated in vivo using the zebrafish operculum mineralization system. Morphometric analysis of alizarin red-S stained opercula revealed that EAF at 1, 5 and 10 mg/mL have the capacity to significantly increase bone growth (up to 2.5 times) and, to outperform the effect of the positive control at 10 mg/mL. EAF also showed ORAC values close to those of the positive control (vitamin C; $2x10^6$ mmol T.E/kg) and antibacterial activities against terrestrial strains commonly targeted in biomedicine. Finally, EAF chemical signature was further determined by NMR, GC–MS and LC–MS.

Our results indicate that *S. alterniflora* ethyl-acetate fraction is enriched in phenolic compounds and exhibits remarkable biological activities, demonstrating the potential of marine plants as a sustainable source of molecules for therapeutic applications in bone disorders.

Keywords: Osteogenic-compounds; plant extracts; antioxidant
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P099

Zika virus infection perturbs osteoblast function

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Zika virus (ZIKV) infection is typically characterized by a mild self-limiting disease presenting with fever, rash, myalgia and arthralgia and severe fetal complications during pregnancy such as microcephaly, and arthrogyropsis. Virus-induced arthralgia due to perturbed osteoblast function has been described for other arboviruses. In this study, we investigated the role of osteoblasts in ZIKV infection and bone-related pathology. The effects of ZIKV infection on osteoblast differentiation, and function were monitored by quantifying activity and gene expression of key biomarkers, using human bone marrow-derived mesenchymal stromal cells (MSCs, osteoblast precursors). MSCs were induced to differentiate into osteoblasts and we found that osteoblasts were highly susceptible to ZIKV infection (> 10 million tissue culture infective dose ($TCID_{50}$/ml) within 2 days post infection). While infection did not cause a cytopathic effect, a significant reduction of key osteogenic markers such as *ALP* ($p < 0.05$) and *RUNX2* ($p < 0.05$) mRNA, ALP activity ($p < 0.01$), calcium content ($p < 0.05$) and increased expression of *IL6* ($p < 0.05$) in ZIKV-infected MSCs implicated a delay in osteoblast development and maturation, as compared to uninfected controls. Moreover, in order to identify key pathways affected due to ZIKV infection, we performed host transcriptomic response analysis using next-generation RNA sequencing. These data show that ZIKV infected osteoblasts mount a robust interferon response as expected, but these cells also exhibit modifications in cell cycle and lipid metabolism related pathways. In conclusion, we have developed and characterized a new in vitro model to study the role of bone

development in ZIKV pathogenesis, which will help to identify new targets for developing therapeutic and preventive measures.

P101

SSC-derived adipocytes induce the transdifferentiation of osteoblasts into pre-adipocytes

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Our preliminary findings lead us to propose bone marrow adipocyte secretions as new actors of bone loss. Indeed using an in vitro coculture model based on human skeletal stem cells (SSCs), we previously showed that soluble factors secreted by adipocytes induced the conversion of osteoblasts towards an adipocyte-like phenotype. The aim of this study is to better characterize the changes triggered by the co-culture and to strengthen our hypothesis of a transdifferentiation event.

A gene expression kinetics performed to analyze the temporal changes in the osteoblast phenotype revealed that the conversion of osteoblasts is quickly initiated (from 9 h of coculture) but still incomplete when compared to adipocytes. Transcriptomic analysis of 7 biological replicates demonstrated that osteoblasts presented different phenotypic changes according to the duration of coculture. In fact, 376 and 405 transcripts were significantly modified in cocultured osteoblasts compared with the monoculture controls upon 9 h and 48 h respectively. Gene ontology annotation analysis showed an enrichment in the adipocyte gene signature. In particular, we identified 24 genes which were associated with biological processes related to differentiation of adipocyte ($2{,}1 \leq FC \geq 16{,}82$).

Double immunofluorescence microscopic analyses were performed to demonstrate the co-expression of adipogenic and osteoblastic proteins on a single cell level. The results clearly showed that at least 12% of the osteoblastic cells expressed the adipogenic marker PPARy2, when osteoblasts cells were incubated during only 48 h with adipocyte conditioned medium. On molecular level, such conversion was associated with upregulated expression of specific reprogramming genes ($p \leq 0.05$). Moreover, whole genome methylation analyses showed that levels of 5-methylcytosine were strongly decreased ($p \leq 0.01$) in co-cultured osteoblasts, accompanied by an upregulation of TET1 gene expression ($p \leq 0.05$), an enzyme implicated in methylation.

Taken together, these data show that osteoblast transdifferentiation is initiated rapidly and then progresses in a multi-step process

P102

Adaptor protein CrkII negatively regulates osteoblast differentiation and function through JNK phosphorylation

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The adaptor protein CrkII is involved in several biological activities, such as mitogenesis, phagocytosis, and cytoskeleton reorganization. Previously, we demonstrated that CrkII plays an important role in osteoclast differentiation and function through Rac1 activation, both in vitro and in vivo. In this study, we investigated whether CrkII also regulates the differentiation and function of another type of bone cell, named osteoblast. Overexpression of CrkII in primary osteoblasts inhibited bone morphogenetic protein 2-induced osteoblast differentiation and function, whereas knockdown of CrkII expression exerted the opposite effect. Importantly, CrkII strongly enhanced c-Jun-N-terminal kinase (JNK) phosphorylation, and CrkII overexpression-mediated attenuation of osteoblast differentiation and function was recovered by JNK inhibitor treatment. Furthermore, transgenic mice overexpressing CrkII under the control of the alpha-1 type I collagen promoter exhibited reduced bone-mass phenotype. Together, these results indicate that CrkII negatively regulates osteoblast differentiation and function through JNK phosphorylation. Given that CrkII acts as a negative and positive regulator in osteoblast and osteoclast differentiation, respectively, regulation of CrkII expression in bone cells may help develop new strategies to enhance bone formation and inhibit bone resorption.

P105

Anti-inflammatory effect of Weissella cibaria on periodontal bone destruction mice model in vivo and Raw264.7 cell in vitro

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Purpose: The aim of this study was to evaluate the anti-inflammatory effects of Weissella cibaria on periodontal tissue destruction and regulation of inflammatory cytokines in mice and Raw264.7 cell in vitro.

Materials and methods: In vivo experiment: Ninety Mice were divided into six groups: negative control (Ctrl), positive control (LIP/Ctrl), PBS treated (LIP/PBS), W. cibaria-low (LIP/WC-L), W. cibaria-medium (LIP/WC-M), W. cibaria-high (LIP/WCH). The treating solutions were administered with a syringe for 14 days using a syringe. Micro-computed tomography scans were taken for the measurement of alveolar bone loss. The gingival tissue and serum were obtained from three groups (Ctrl, LIP/Ctrl, LIP/WC-H) and used for immunoassay. The data from the study were subjected to statistical analysis. In vitro experiment: Raw264.7 cells were treated Weissella cibaria for 12 h and treated LPS(lipopolysaccharide). After LPS treatment, we did NO(nitric oxide) assay and ELISA (IL-10, IL-6 and TNF alpha)

Results: The LIP/WC-H group showed significantly reduced alveolar bone loss at the distal aspect of the ligatured teeth compared to the LIP/Ctrl group. Although it was not statistically significant, there was a dose-dependent relationship between W. cibaria concentration and reduction in periodontal tissue destruction. In gingival tissues, the levels of both pro- and anti-inflammatory cytokines were significantly lower in the LIP/WC-H group than those in the LIP/Ctrl group.

In vitro, Weisswlla cibaria reduced NO and cytokine (IL-10, IL-6 and TNF alpha) secretion.
Conclusions: W. cibaria seems to be able to reduce periodontal destruction and inflammatory cytokine production in periodontal bone destruction mice model in vivo and cytokine secretion in Raw264.7 cell in vitro. Acknowledgement: This work (C0564353) was supported by project for Cooperative R&D between Industry, Academy, and Research Institute funded Korea Ministry of SMEs and Startups in 2017
Keywords: Alveolar bone loss, Periodontitis, Probiotic, Cytokine, Raw264.7 cell

P106

Impact of a caspase inhibitor on alveolar shape changes following tooth extraction, a pilot study

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Tooth extraction becomes a problem when the buccal alveolar bone is resorbing, making bone augmentation prior to implant installation mandatory. There is thus a great need to understand the molecular mechanisms of buccal bone resorption and, consequently, to develop strategies for socket preservation. Osteocytes have recently turned out to be master regulators of bone remodeling; particular dying osteocytes provoke a massive formation of osteoclasts and consequently bone resorption. We have raised the hypothesis that buccal bone resorption can be reduced by a pharmacological apoptosis inhibitor. To test this hypothesis, 16 inbred rats underwent bilateral tooth extraction of the first molars (M1) right side, and M2 left side. At the same time, one group systemically received cell the permeable fluoromethyl ketone-derivatized peptides for 10 days. The other group received the diluent. Differences in resorption were evaluated by geometric morphometrics (GMM). GMM reveals an increase of alveolar crest height compared to the control of 5% at the position of M1, and no changes in M2, both not reaching the level of significance ($p > 0.05$). These data suggest that depending on the anatomical localization, pharmacological apoptosis inhibitor can help to preserve the alveolar ridge. Further research should define if this phenomenon is a consequence of supporting osteocyte vs apoptosis inhibitory.

P107

Oleoylethanolamide, an endogenous GPR119 ligand, inhibits osteoclastic bone resorption and promotes apoptosis in mature osteoclasts

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Objectives: Oleoylethanolamide (OEA) is a bioactive lipid which is present in bone and known as an endogenous ligand for G protein-coupled receptor 119 (GPR119). Osteoclasts are bone-resorbing cells that cause bone-destructive diseases such as osteoporosis. In this study, we explored the impact of OEA on osteoclast differentiation, function, and survival of mature osteoclasts.
Methods: Osteoclast formation was assessed using mouse primary bone marrow-derived macrophages (BMMs). Actin ring formation and resorption pit assay was performed. Apoptosis assay was performed using ELISA Kit. Immunodetection of caspase-3 was assessed by immunoblotting. Short hairpin RNA (shRNA)-mediated knockdown of GPR119 was performed using lentiviral expression system.
Results: We found that activation of GPR119 with OEA did not affect osteoclast differentiation and fusion induced by RANKL. On the other hand, OEA inhibited osteoclast spreading at later stage of osteoclast development. Reflecting defective osteoclast spreading, OEA blocked actin ring formation, eventually attenuating bone resorptive activity of mature osteoclasts. Treatment of OEA in osteoclasts also reduced the expression of β3 integrin subunit protein, a pivotal regulator of osteoclast cytoskeletal function. Furthermore, OEA-mediated suppressive effect on osteoclast cytoskeletal organization was abrogated by knockdown of GPR119 using small hairpin RNA. In addition, activation of GPR119 with OEA accelerated apoptotic death in mature osteoclasts through the induction of caspase-3 and Bim expression and the suppression of M-CSF-mediated ERK activation.
Conclusions: Our results demonstrate that OEA has inhibitory effects on osteoclast bone resorptive activity and survival of mature osteoclasts. We therefore suggest that OEA may have potential therapeutic implications for the pathological bone disorders characterized by excessive osteoclastic bone resorption.
Keywords: Oleoylethanolamide, GPR119, Osteoclast, Cytoskeleton, Apoptosis

P108

Glucagon-like peptide 1 (GLP-1) increases bone resorptive activity of human osteoclasts in osteoclast/osteoblast co-cultures in vitro

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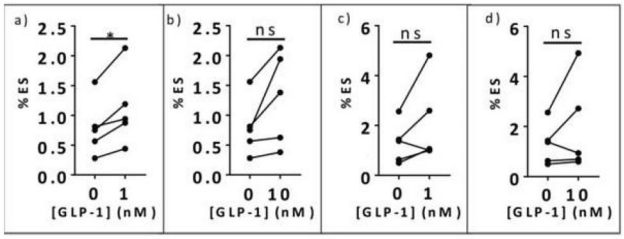
Objectives: Glucagon-like peptide 1 (GLP-1) is an intestinal hormone released in response to nutrients intake and promotes glucose-dependent insulin secretion. GLP-1 regulates bone remodelling in preclinical studies, but the impact on human bone cells is undetermined. We aimed to determine the effects of GLP-1 on human osteoclast activity and differentiation.
Methods: $CD14^+$ monocytes from human blood were used to generate osteoclasts and outgrowths from human bone specimens were used to generate osteoblasts (approved by the ethics committee). Human GLP-1 was added to the following cells reseeded on bone slices: 1) matured osteoclast cultures with serum and RANKL ($OC^{+serum+RANKL}$) and 2) osteoclast/osteoblast co-cultures without serum and RANKL ($OC + OB^{-serum-RANKL}$) for three days. Effects of GLP-1 on human osteoclasts were determined by blinded evaluation of eroded bone surface (%ES) using a microscope (100-point

grid). GLP-1 was added on day 6 and 8 during osteoclast differentiation and effects assessed by TRAcP activity on day 8 and 10.

Results: Five independent experiments on bone resorption and one on osteoclastogenesis were conducted. After five experimental repeats, GLP-1 reproducibly increased %ES in OC + $OB^{-serum-RANKL}$ cultures (Fig. 1 a: 1 nM $p = 0.018$, b: 10 nM $p = 0.072$), and a similar trend was observed in four of five experiments using $OC^{+serum+RANKL}$ (Fig. 1 c: 1 nM $p = 0.146$, d: 10 nM $p = 0.257$). GLP-1 (10 nM) stimulated osteoclastogenesis as determined by increased TRAcP activity ($p < 0.01$).

Conclusions: GLP-1 promotes human osteoclastic bone resorption in vitro in co-cultures with osteoblasts and may enhance osteoclastogenesis. This work is ongoing.

Keywords: Osteoclasts, GLP-1, osteoclast/osteoblast co-cultures



Effects of GLP-1 (1 and 10 nM) on osteoclastic bone resorption (eroded bone surface, %ES) in five independent experiments (each connected with a line). **a)** OC+OB without serum and RANKL (GLP-1, 1 nM), **b)** OC+OB without serum and RANKL (GLP-1, 10 nM), **c)** OC with serum and RANKL (GLP-1, 1 nM) and **d)** OC with serum and RANKL (GLP-1, 10 nM). Data are shown as the median from five bone slices per condition. Paired t-test, *=$p<0.05$, ns=not significant. OC=osteoclast, OB=osteoblast.

[Comparison of GLP-1 effects on %ES between five independent experiments on bone resorption]

P109

Effect of anti-Ly6G Ab on LPS-induced osteoclast formation in air pouch of mice

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Increase of neutrophils and osteoclasts is representative changes in periodontitis. The role of neutrophils in osteoclast formation has not been clarified. CXCL12 and RANKL is a chemotactic factor for osteoclast precursors and osteoclast differentiation factor, respectively. To estimate the role of neutrophils in osteoclast formation of periodontitis, we treated LPS-injected air pouch mice with antibody (Ab) against Ly6G, neutrophil marker for depletion of neutrophils.

C57BL/6 mice were subcutaneously injected with air into the back on days 0 and 3 and sacrificed on day 6. Anti-Ly6G Ab was intraperitoneally injected on days 5 and 6. Lipopolysaccharide (LPS), virulence factor of periodontopathogens, was injected into air pouch before 6 h of sacrifice on day 6. Number of neutrophils and osteoclast precursors and expression of CXCL12 and RANKL in neutrophils were estimated in blood or air pouch exudates by flow cytometry. Ly6G/CD11b and CD115/RANK were used as markers of neutrophils and osteoclast precursors, respectively.

Number of neutrophils in blood was lower in anti-Ly6G Ab-injected mice than non-injected mice. Number of neutrophils and osteoclast precursors were higher in air pouch exudate of LPS-injected mice than non-injected mice. In addition, $CXCL12^+/RANKL^-$, $CXCL12^-/RANKL^+$, and $CXCL12^+/RANKL^+$ neutrophils were higher in air pouch exudate of LPS-injected mice than non-injected mice. Interestingly, anti-Ly6G Ab injection reduced the number of osteoclast precursors as well as neutrophils in air pouch exudate of LPS-injected mice. Furthermore, anti-Ly6G Ab injection also reduced $CXCL12^+/RANKL^-$, $CXCL12^-/RANKL^+$, and $CXCL12^+/RANKL^+$ neutrophils in air pouch exudate of LPS-injected mice.

Anti-Ly6G Ab decreased migration of neutrophils and osteoclast precursors and CXCL12 and RANKL expression of neutrophils in air pouch induced by LPS, suggesting that neutrophils involve in osteoclast formation in periodontitis through CXCL12-induced osteoclast precursor migration and RANKL expression.

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2017R1A2B4002348)

P110

Effect of KP-A038, an imidazole derivative, on osteoclast differentiation and inflammatory bone loss through downregulation of PRDM1

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Excessive osteoclastic activity results in pathological bone-resorptive diseases, such as osteoporosis, periodontitis, and rheumatoid arthritis. As imidazole-containing compounds possess extensive therapeutic potential for the management of diverse diseases, we synthesized a series of imidazole derivatives and investigated their effects on osteoclast differentiation and function. In the present study, we found that a novel imidazole derivative, KP-A038, suppressed receptor activator of nuclear factor-κB ligand (RANKL)-mediated osteoclastogenesis and bone-resorbing activity in vitro and attenuated lipopolysaccharide (LPS)-induced bone destruction in vivo. KP-A038 significantly inhibited the induction of nuclear factor of activated T-cells cytoplasmic 1 and the expression of its target genes, including tartrate-resistant acid phosphatase (Acp5), cathepsin K (Ctsk), dendritic cell-specific transmembrane protein (Dcstamp), and matrix metallopeptidase 9 (Mmp9). KP-A038 treatment upregulated the expression of negative regulators of osteoclast differentiation, such as interferon regulatory factor-8 (Irf8) and B-cell lymphoma 6 (Bcl6), via repression of B lymphocyte-induced maturation protein-1 (Prdm1) induced by RANKL. Moreover, administration of KP-A038 reduced LPS-induced bone erosion by suppressing osteoclast formation in vivo ($p < 0.05$). Thus, our findings suggest that KP-A038 may serve as an effective therapeutic agent for the treatment and/or prevention of bone loss in pathological bone diseases, including osteoporosis and periodontitis.

P111

What are the peripheral blood determinants for increased osteoclast formation in the various inflammatory diseases associated with bone loss?

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Local priming of osteoclast precursors (OCp) has long been considered the main and obvious pathway that takes place in the human body, where local bone lining cells and RANKL-expressing osteocytes may facilitate the differentiation of OCp. However, priming of OCp away from bone, such as in inflammatory tissues, as revealed in peripheral blood, may represent a second pathway, particularly relevant in individuals who suffer from systemic bone loss such as prevalent in inflammatory diseases.

We used a systematic approach to review the literature on osteoclast formation in peripheral blood in patients with inflammatory diseases associated with bone loss. Only studies that compared inflammatory (bone) disease with healthy controls in the same study were included. Using this core collection, it becomes clear that experimental osteoclastogenesis using peripheral blood from patients with bone loss diseases in prevalent diseases such as rheumatoid arthritis, osteoporosis, periodontitis and cancer-related osteopenia unequivocally point towards an intrinsically increased osteoclast formation and activation (24/29 increased; 5/29 no difference compared to controls). In particular, such increased osteoclastogenesis already takes place without the addition of the classical osteoclastogenesis cytokines M-CSF and RANKL in vitro. T-cells and monocytes as OCp are the minimal demands for such unstimulated osteoclast formation. In search for common and disease-specific denominators of the diseases with inflammation-driven bone loss, we demonstrate that altered T-cell activity and a different composition—such as the CD14 + CD16 + versus CD14 + CD16- monocytes—and priming of OCp as seen in peripheral blood play a role in increased osteoclast formation and activity. The putative common denominators, skewness of monocyte subtypes, T-lymphocytes and the contribution of inflammatory cytokines such as TNF-α and M-CSF that are released in osteoclast cultures or that are present in peripheral blood are discussed.

Keywords: Osteoclastogenesis, inflammatory bone loss, rheumatoid arthritis, periodontis, CD14/CD16 osteoclast precursors

P112

Targeted inactivation of Rin3 increases trabecular bone mass in aged mice

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Background: Paget's disease of bone (PDB) is a multifocal disorder caused by increased bone turnover. The *RIN3* gene (Ras and Rab interactor protein 3) was associated with susceptibility to PDB by GWAS and also with bone mineral density in children. Our previous work showed that 3 month old mice with targeted inactivation of *Rin3* ($Rin3^{-/-}$) had an increased trabecular bone mass in the tibia and femur, and a reduction of active bone resorption surfaces. Here, we extended this study to older mice.

Methods: We conducted skeletal phenotyping of 10 $Rin3^{-/-}$ mice and 10 wild type (WT) female littermates on a C57BL/6 and 129/OlaHsd background. MicroCT was performed on 12 months old samples to assess trabecular changes in the femur, tibia and spine. Bone histomorphometry was performed on the femoral metaphysis following calcein double-labelling and TRAcP staining.

Results: Trabecular bone volume (BV/TV) was significantly higher in the femurs ($Rin3^{-/-}$ = [mean ± sem] 15.76 ± 2.13%; WT = 8.51 ± 0.89%; P = 0.002) and tibias ($Rin3^{-/-}$ = 14.03 ± 2.35%; WT = 6.81 ± 1.01%; P = 0.006) of $Rin3^{-/-}$ mice compared to WT. Similarly, trabecular number was increased in the femurs ($Rin3^{-/-}$ = 3.22 ± 0.40 mm^{-1}; WT = 1.89 ± 0.20 mm^{-1}; P = 0.004) and tibias ($Rin3^{-/-}$ = 2.71 ± 0.41 mm^{-1}; WT = 1.51 ± 0.22 mm^{-1}; P = 0.012). Analyses of bone histomorphometry showed no differences in indices of bone formation or bone resorption between genotype groups in the femur.

Conclusions: Although the reduced osteoclastic bone resorption that we observed in young mice has diminished with ageing, the microCT results are consistent with our previous results and show that *Rin3* as a negative regulator of trabecular bone mass throughout life confirming its role as a candidate gene for PDB.

Keywords: Paget´s disease of bone, *Rin3*, osteoclasts.

P113

Ablation of Stabilin-1 enhances bone resorbing activity in in vitro osteoclasts

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Stabilin-1 is a transmembrane receptor that regulates molecule recycling and cell homeostasis by controlling the intracellular trafficking and participates in cell–cell adhesion and transmigration. Its expression is observed in various organs, including the bone. Stabilin-1 has been detected in the synovial tissue of patients with rheumatoid arthritis and osteoarthritis and in bone marrow macrophages. However, its function and regulatory mechanisms in the bone remain unclear. In this study, to evaluate the physiological function of stabilin-1 in bone cells and tissue, we used a stabilin-1 knockout (Stab 1 KO) mouse model. In control mice, stabilin-1 was expressed in osteoblasts and osteoclasts. Specifically, stabilin-1 expression was maintained during osteoblast differentiation and significantly decreased after osteoclast differentiation ($p < 0.001$). To determine whether stabilin-1 affected osteoblast and osteoclast function, we established in vitro primary cell cultures. While osteoblast differentiation and function, and the expression of osteoblast differentiation markers were not changed between mesenchymal stem cells isolated from Stab 1 KO and wild-type (WT) mice, the expression of osteoclast differentiation markers was

slightly increased and the bone-resorbing activity was significantly increased in in vitro RANKL-induced osteoclasts of Stab 1-deficient bone marrow macrophages (BMMs) compared with those of WT BMMs. To observe the phenotypic alteration according to the in vitro results, we analyzed in Stab 1 KO mice. Analyses of the bone phenotype by microcomputed tomography and the morphology and arrangement of bone cells by histology showed a negligible difference between WT and Stab 1 KO mice. Stab 1-deficient bone showed an increased pattern but no significant difference in osteoclast surface and numbers. Finally, these results indicated that, although it may not have an essential role on in vivo bone development and bone cell function, Stab 1 affects in vitro osteoclast maturation and function for bone resorption.

Keywords: Stabilin-1 (Stab 1); deficiency; bone marrow macrophages; osteoclast maturation; bone resorption

P114

RANKL-specific increase in p38 phosphorylation enhances NFATc1 and *Atp6v0d2* causing increased osteoclastogenesis in ageing

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Osteoporosis is characterised by increased bone resorption due to highly active osteoclasts. Binding of RANKL to RANK activates MAPK and NF-κB pathways leading to osteoclastogenesis. Osteoclasts from aged mice are hypersensitive to RANKL, however the underlying mechanism is unknown. Here, we analysed the role of MAPK-NFATc1 pathway in age-related increased osteoclastogenesis.

M-CSF-dependent macrophages (MMFs) were grown out of bone marrow cells isolated from 3-month- to 12–14-month-old C57Bl6 mice. MMFs were stimulated with RANKL, or TNF-α in time response assays and MAPK-ases and IkB analysed by Western Blot. NFATc1 nuclear translocation and gene expression was assessed by confocal microscopy and qPCR respectively.

Western Blot of RANKL time-response (N = 4) showed a 78% increase ($p < 0.05$) in maximum phosphorylation of p38 in aged vs young MMFs at 15′. Area under the curve (AUC) of P-p38 and P-ERK in aged vs young MMFs was increased by 93% (N = 4, $p < 0.001$) and 34% (N = 3, $p < 0.05$) respectively over 30′. However, there was no difference in P-JNK or IκB. TNF-α did not cause differential p38 phosphorylation in aged vs young MMFs, indicating that the age-related increase in P-p38 is RANKL specific. RANKL stimulation of MMFs for > 48 h showed no effect on P-p38.

There was no difference in *NFATc1* RNA expression in aged vs young MMFs. However, 48 h of RANKL stimulation led to a threefold increase in NFATc1 nuclear translocation (N = 2) and a twofold increase in downstream *Atp6v0d2* gene expression (N = 3, $p < 0.01$) in aged vs young MMFs, which persisted in mature osteoclasts (RANKL for 120 h, $p < 0.05$).

Our data indicate that ageing leads to RANKL-specific increased phosphorylation of p38 and marginally of ERK in MMFs (but not pre-osteoclasts), which increases NFATc1 nuclear translocation followed by increased expression of *Atp6v0d2*, which likely contributes to enhanced age-related osteoclastogenesis.

Keywords: Osteoclastogenesis, ageing, p38, NFATc1, *Atp6v0d2*

P115

Regulation of the Cdc42-PAK2 axis by DCTN1 controls osteoclastogenesis

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Dynein, first identified as a heavy molecular weight microtubule-based motor, transports diverse cellular cargos such as proteins, vesicles, and organelles. Dynactin, an adaptor complex, is an indispensable component of the dynein's function. Here we show that DCTN1, the most important subunit of dynactin complex, has an important role in osteoclast differentiation. The expression level of DCTN1 was increased at early stage by RANKL and the elevated level was maintained during osteoclastogenesis. Knockdown of DCTN1 decreased osteoclast differentiation and suppressed the bone resorption activity. NFATc1 and c-Fos, important osteoclastogenic transcription factors, were also inhibited by DCTN1 knockdown. Consistent with in vitro results, DCTN1 siRNA injection onto mice calvariae disrupted osteoclast differentiation and bone-resorbing activity. The decrease in both NFATc1 and c-Fos by DCTN1 silencing could be attributed to the inhibition of Cdc42/PAK2 signaling pathway. Activation of Cdc42 by RANKL was inhibited when DCTN1 was silenced. Knockdown of PAK2, an effector molecule of Cdc42 signaling pathway, also suppressed osteoclastogenesis and NFATc1 induction. Forced expression of constitutively active Cdc42 in DCTN1-silenced bone marrow-derived macrophages rescued osteoclast differentiation. These results suggest that DCTN1 has a critical role in osteoclastogenesis by regulating the Cdc42-PAK2 axis.

Keywords: DCTN1, osteoclast, Cdc42, PAK21

P116

Comprehensive analysis of osteoclastogenic proteome reshaping reveals a novel regulator of osteoclast differentiation and function

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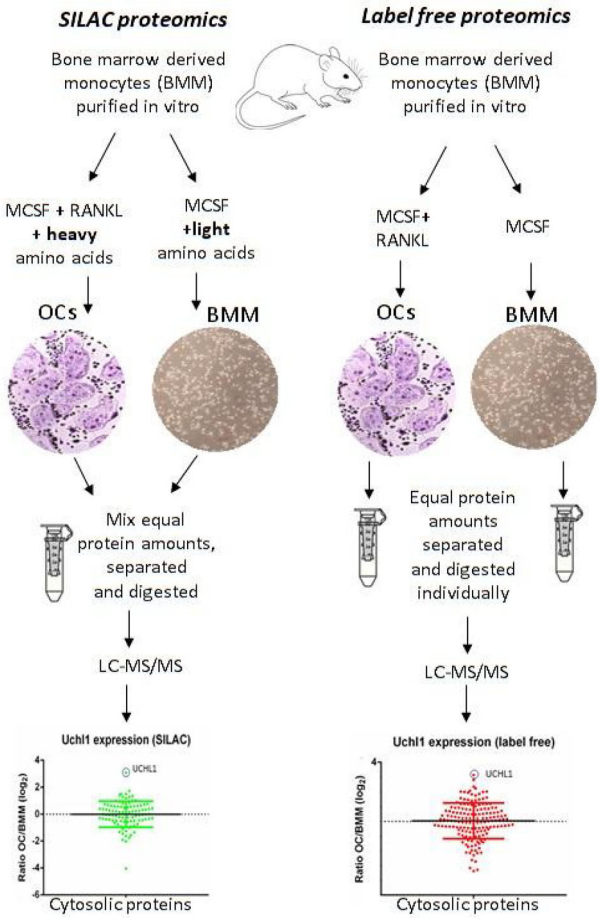
Osteoclasts (OCs) are bone marrow monocyte (BMM)-derived syncytia ensuring skeletal homeostasis through regulated bone resorption. We deployed two approaches to characterize the proteome reshaping of osteoclastogenesis in order to identify new regulators and putative targets for skeletal disorders. We compared the proteome of mouse purified BMMs and BMM-generated OCs through stable isotope labelling with amino acids in cell culture (SILAC)-based quantitative proteomics, subsequently validated by label-free mass spectrometry. Both approaches identified established OC markers (e.g. cathepsinK) among the proteins most upregulated in mature OCs, validating our strategy. Proteome analysis by cellular compartment demonstrated reorganization of specific organelles, including lysosomal and mitochondrial reshaping. Moreover, both proteomics identified proteins differentially expressed upon OCgenesis, previously not associated with OC function. These included ubiquitin (Ub) carboxy-terminal hydrolase L1 (UCHL1), a key component of the Ub proteasome system, previously implicated in

neurodegeneration. UCHL1 was increased ~ 9 times by SILAC in OCs compared to BMMs. Quantitative RT-PCR and immunoblotting analyses revealed a significant rise of UCHL1 expression (fourfold and > 10-fold, respectively) early in OCgenesis (72 h post RANKL). Attesting to a relevant role of UCHL1 in OCgenesis, pharmacologic and genetic inhibition of UCHL1 potently suppressed differentiation and resorptive activity in vitro. Overall, our work: i) offers a powerful strategy for the identification of novel regulators of OC activity; ii) uncovers the coordinated reorganization of organelle composition and metabolic activities upon OCgenesis; and iii) reveals a previously unrecognized key role of the UPS component UCHL1 in OC differentiation and function.

Keywords: Osteoclast; proteomics; UCHL1



[Proteomics]

P117

Increased resorption activity of osteoclasts observed in transient receptor potential channel TRPC6 deficient mice and cell lines can be attenuated by inhibition of TRPC3

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An impaired bone homeostasis with increased loss of bone can be caused by exceeded activation of osteoclasts. These bone-resorbing cells depend on calcium signaling during their multistep differentiation. A wide range of membrane channels, including the Transient Receptor Potential Channels (TRPC) controls the entry of calcium into cells.

Focusing the bone phenotype of TRPC6 knock out mice (n = 7) compared to wild type littermates (n = 10), we demonstrated by microCT analyses significantly decreased bone-volume per tissue-volume (− 37%, $p < 0.001$), trabecular thickness (− 24%, $p < 0.05$) and trabecular numbers (− 20%, $p < 0.005$) in their lumbar vertebrae. This impaired bone structure seems to be in accordance with an elevated number of osteoclasts (+29%, $p < 0.05$) and increased bone-resorption activity (+24%, $p < 0.05$). Primary bone marrow mononuclear cells (PBMMCs) isolated from TRPC6-deficient mice show elevated osteoclastogenic differentiation capacity (+33%, $p < 0.05$) compared to cells from wild type animals. In an in vitro model, established by CRISPR/Cas9 technology, we could demonstrate that osteoclasticly differentiated TRPC6-deficient RAW264.7 cells have elevated intra-cellular calcium levels (+10%, $p < 0.005$), which is in line with increased luciferase activity (+22%, $p < 0.05$) after transient transfection of a NFAT-luciferase reportergene plasmid.We could demonstrate for the first time that TRPC3 and TRPC6 both are expressed on osteoclasts, with an apparent functional linkage between them. TRPC6-deficient osteoclasticly differentiated PBMMCs show significantly increased expression of TRPC3 ($p < 0.05$), which can be addressed on the functional level. We could decrease intracellular calcium levels (− 20%, $p < 0.005$) and luciferaseactivity (− 56%, $p < 0.05$) by treating TRPC6-deficient cells with the specific TRPC3 inhibitor Pyr3. During osteoclastogenesis, co-stimulation with Pyr3 reduced number, size and resorptive capacity of these cells ($p < 0.01$).

This raises the question of an application in vivo and we conclude that modulating TRPC3 activity is an interesting target to improve bone structure.

Approved by LANUV (84-02.05.50.15.005).

Keywords: TRPC3, TRPC6, calcium, Pyr3
This research was supported by Elsbeth-Bonhoff-Foundation.

P121

Response to PTH in an osteocyte-rich ex vivo model

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Osteoporosis is caused by an imbalance between bone formation and bone resorption. Osteocytes comprise > 95% of bone cells and play a key role in the regulation of osteoblast and osteoclast activity. Thus, osteocytes are a potential drug target for osteoporosis therapy. Culturing osteocytes in vitro is challenging, as osteocytes in vivo are embedded within the bone matrix, and once removed from their natural environment their characteristics change. Here, we used an ex vivo culture system that retains the osteocyte microenvironment.

Objectives: To study osteocyte responsiveness to parathyroid hormone (PTH) in an osteocyte-rich ex vivo culture system.

The diaphysis of long bones was collected from 4 to 6 weeks old CD1 mice. Marrow was removed by centrifugation and clean bones were cultured for 24 h, followed by PTH treatment (250uM) for another

24 h. Changes in the expression of osteocyte marker genes were examined with qPCR. Secreted sclerostin in conditioned media was measured by ELISA. The presence of bone cells was determined histologically.

Histological analysis, and the abundant expression of osteocyte-specific markers, including *Sost* and *Dmp1*, confirmed our culture was osteocyte-rich. Treatment with PTH increased the expression of the *Tnfsf11* (encodes RANKL) by 1.6-fold in comparison to untreated control, while *Sost* (encodes Sclerostin) was 6.5-fold lower in the treated cells. Similar effects of PTH on gene expression in osteocytes have previously been shown in the literature. Osteocytes within the bone secreted sclerostin (584 pg/mg bone) into the culture media during the 24 h. PTH treatment did not alter the amount of sclerostin secretion.

This ex vivo culture system retains osteocytes within the bone matrix. The response of osteocytes to PTH was similar to those that had been previously shown in the literature. In future studies, this experimental model will be used to determine osteocyte responses to novel bone therapeutics.

Keywords: Osteocyte, ex vivo model, PTH, sclerostin, therapeutics

P122

Correlations between the osteocyte network and its surrounding matrix in newly formed bone

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The structure of bone is adapted at every hierarchical level to its mechanical needs, i.e. the extra cellular matrix (ECM) is subjected to a lifelong interplay between bone resorption by osteoclasts and bone formation by osteoblasts. Osteocytes are embedded in the bone matrix and orchestrate the remodeling process via fluid flow in the lacuno-canalicular network (LCN) and likely contribute directly to mineral homeostasis. Since the repair of bone fractures recapitulates skeletal development, healing mouse bone is an ideal model system to study correlations between the architecture of the LCN and the surrounding bone matrix in different tissue types.

To visualize correlations between LCN and ECM within the same bone volume we are using a combination of experimental techniques. Confocal laser scanning microscopy and high resolution μCT measurements are used to characterize the LCN, while the mineral content of the sample surfaces is measured by quantitative backscattered electron imaging (qBEI) and the mineral particle characteristics by scanning small/wide angle X-ray scattering (SAXS/WAXS).

Data about the LCN and ECM in healing bone does not only allow a distinction between cortex, cartilage and callus, but also between different bone types within the callus. Callus woven bone exhibited thicker and less organized mineral particles and a less dense LCN compared to newly formed callus lamellar bone. Overall, we identified correlations between osteocyte network architecture and mineral particle characteristics in all tissue types, which supports our hypothesis that osteocytes directly influence the mineralization process.

Keywords: Osteocyte, lacuno-canalicular network, mineral particles, healing bone, x-ray scattering

P123

Mineralization and autophagy in bone is suppressed by EphrinB2 through the RhoA/ROCK pathway

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Controlled mineralization of the collagen matrix is essential to maintain optimum bone strength. Previous work has shown that mice with EphrinB2 (*Efnb2*) knockdown in osteocytes develop brittle bones, and that this is associated, not with any change in bone mass, but with a high level of mineralization and abundant autophagosome formation within osteocytes. In this study, we sought to determine the mechanisms by which EphrinB2 acts in osteocytes to restrain the bone mineralization process.

To determine whether increased autophagy leads to increased mineralization, two osteocyte-like cell lines (OCY454 and Kusa 4b10) were treated under mineralizing conditions with 0.05, 0.1 and 1 nM of the autophagy inducer Rapamycin when first mineral crystals were detected. After 14 (OCY454) and 19 (Kusa4b10) days, when these cells express osteocyte markers, cells were fixed and mineral deposition was detected with Alizarin Red Staining (ARS). Both cell lines showed greater mineral deposition when autophagy was induced with Rapamycin. This suggests that stimulation of autophagy in osteocytes stimulates their mineral release.

We next sought to determine whether EphrinB2 inhibits autophagy, and, since EphrinB2 has been shown to signal through the RhoA/ROCK pathway, we tested whether the action was mediated by this pathway. We stimulated OCY454 cells with clustered EphrinB2-Fc to initiate intracellular signaling for 1 h, in the presence of Bafilomycin A1 to determine whether it modifies autophagosome formation. Cells were fixed and stained with anti- microtubule-associated protein 1 light chain 3 (LC3), and LC3 punctae were counted to determine changes in autophagy levels. EphrinB2-Fc treatment significantly suppressed Bafilomycin-induced accumulation of autophagosomes. Addition of the RhoA/ROCK inhibitor H1152 prevented this decrease, indicating that EphrinB2 reduces autophagosome formation, at least in part, through RhoA/ROCK signalling.

This reveals a novel pathway explaining how EphrinB2 signaling in osteocytes limits mineralization and maintains bone strength.

P126

Trichloroethylene-related compound *S*-(1, 2-dichlorovinyl)-L-cysteine localized on mouse cartilage

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Trichloroethylene (TCE) is generally used as synthetic material for alternative fluorocarbon or metal-degreasing agent. TCE is metabolized into *S*-(1, 2-dichlorovinyl)-*L*-cysteine (DCVC) in the body and DCVC is well known to cause renal-cellular injury after metabolic activation by cysteine conjugate β-lyase. We performed the in vivo disposition of [^{35}S] DCVC in mice after intraperitoneal administration at a dose of 30 mg/kg (6 mg/2.42 MBq/ml). DCVC was well absorbed rapidly, distributed highly in the kidneys and moderately in the pancreas, liver, and bone. Furthermore, we revealed by whole-body autoradiography that DCVC and its related substances were localized on the femoral epiphysis, vertebral endplates, and tracheal cartilage in mouse,

P127

Glucocorticoid treatment in TNF-alpha overexpressing mice impairs bone growth and humanin levels

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Background: Glucocorticoids (GCs) are widely used to treat a variety of inflammatory diseases in children. Both GC treatment and the inflammation itself are toxic to the growth plate chondrocytes and causes growth retardation in mice. We have previously shown that humanin, a small mitochondrial derived peptide, can rescue from growth retardation caused by GCs. However, these studies have been performed in normal mice and not in a disease model of chronic inflammation. In this study, we therefore investigated if GC treatment in an inflammatory animal model exerts any deleterious effects on growth plate chondrocytes, although GCs suppress inflammation. We also investigated effects of inflammation and GC treatment on systemic regulation of humanin.
Aims and Objectives: The aim of this study was to investigate the effects of GCs given under inflammatory conditions on longitudinal bone growth and systemic humanin serum levels.
Methods: We used four week old TNF-alpha overexpressing mice (TgTNF, n = 10–12/group), treated with dexamethasone (Dexa, 3 mg/kg body weight) or saline, for four weeks. At the end of the study, femur bone length was measured with digital caliper and humanin levels in serum were measured with ELISA.
Results: GC treatment in TgTNF mice for four weeks significantly decreased longitudinal bone growth compared to control ($p = 0.03$). Interestingly, we found that GC treatment also decreased systemic humanin levels when compared with control ($p = 0.03$).
Conclusions: Our results show that systemic treatment with GCs in a disease model of inflammation decreases longitudinal bone growth, an effect that has so far only been studied in healthy animals. We further showed that GC treatment suppresses serum humanin levels.

P128

Low adhesive scaffold collagen prepared from type I collagen induces the chondrogenic differentiation of human bone marrow stromal cells

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Objectives: Chondrocytes derive from multipotent skeletal progenitors and proceed to maturate through sequential differentiation steps. Since cartilage lacks the ability to repair itself, the regeneration of cartilage by chondrocytes is a very attractive research for tissue engineering. It has been well known that human mesenchymal stem cells (hMSCs) have the ability of chondrogenic differentiation. However, it is necessary to culture for 2–3 weeks to differentiate into chondrocytes. Recently, we succeeded in developing low adhesive scaffold type I collagen (LASCol) from porcine skin. In this study, we report that LASCol markedly facilitates chondrogenic differentiation of hMSCs.
Methods: Culture dish was coated with 10 mg/mL of LASCol (own preparation) or Atelocollagen (Cellmatrix Type I-C, Nitta Gelatin Inc.). Human MSCs ($4x10^4$ cells/dish) had been cultured on each coated-dish with chondrogenic basal medium for 14 days. The morphological changes of cells were observed by a phase-contrast microscope. To evaluate chondrogenic differentiation, we stained the secretions of acidic glycosaminoglycans with Alcian Blue solution. Moreover, the quantity of mRNA of chondrogenesis-related genes (*Acan*, *BMP2, Col10a1, Sox9*, and *Tgfb1*) was analysed by RT-qPCR.
Results: Human MSCs freely moved on the LASCol coated-dish, and then formed spheroids. The observation was good agreement with our previous results of osteogenic differentiation from hMSCs. On the LASCol coated-dish, acidic glycosaminoglycans were secreted earlier than usual. The mRNA expression of *Bmp2*, *Col10a1*, *Sox9*, and *Tgfb1* of cells cultured on the LASCol coated-dish increased more than twofold higher than control group.
Conclusions: We demonstrated, by Alcian Blue staining and RT-qPCR, that LASCol has the acceleration effects of chondrogenic differentiation of hMSCs.
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Keywords: Chondrocyte, collagen, differentiation, scaffold, RT-qPCR

P129

TFEB promotes chondrocyte ER remodelling via transcriptional regulation of ER-phagy

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Long bone growth strictly relies on chondrocytes cellular differentiation. The transition from proliferating to hypertrophic is associated to qualitative and quantitative changes in the proteome and secretome of growth plate chondrocytes. Hence the endoplasmic reticulum (ER) might undergo extensive remodeling to accommodate folding and secretion of distinct cargos. To date very little it is known about the mechanisms controlling ER-remodeling in chondrocytes. Here we show that chondrocyte´s ER remodeling occur by the activation of the ER-phagy process, a mechanism by which autophagy sequesters portions of ER and target them to the lysosomes for degradation. We show that this process is induced transcriptionally by the MITF family of transcription factors TFEB and TFE3, which activate the ER-phagy process by enhancing ER, lysosomes and autophagy gene expression in chondrocytes during hypertrophic differentiation. Notably, immortalized chondrocytes KO for TFEB and

TFE3 show lysosome dysfunction, altered ER and defective collagen secretion. Thus, our data show that a TFEB-mediated transcriptional induction of ER-phagy sustains chondrocytes differentiation process during bone growth.

Keywords: TFEB, lysosome, ER-phagy, ER, chondrocytes

P130

Parathyroid hormone treatment modulates cartilage quality

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Parathyroid hormone (PTH) has demonstrated bone anabolic effects and PTH receptors are present in chondrocytes. By acting on the entire osteochondral plate, PTH could prevent the development of osteoarthritis as suggested by preclinical studies.

The objective of this study is to demonstrate the modulatory effect of systemic parathyroid hormone administration on cartilage quality and subchondral bone, using an ovariectomized (OVX) mouse model with impaired cartilage quality and subchondral bone loss. PTH was injected at a dose of 80 mg/kg/day for 8 weeks. A bio-indentation test (PIUMA, OPTICS) was performed at both condyles. The elastic modulus (MPA) and the force (mN) were recorded by fixing a depth of indentation that affects the upper third of the cartilage. The cartilage thickness and bone microarchitecture were evaluated by computed tomography (Scanco 40) using an ionic contrast agent (Hexabrix).

	SHAM (n = 6)	OVX (n = 5)	OVX + PTH (n = 7)
Modulus (MPa)	3.88 ± 0.32	1.77 ± 0.27*°	4.00 ± 0.45
Force (uN)	828 ± 25	460 ± 37*°	848 ± 21
Hyalin Cartilage (mm)	0.017 ± 0.001	0.022 ± 0.006	0.017 ± 0.001
Mineralised Cartilage (mm	0.047 ± 0.002	0.050 ± 0.007	0.042 ± 0.003
BV/TV (%)	32.5 ± 3.0	20.1 ± 5.4*	32.4 ± 4.6

[* versus SHAM, ° versus OVX + PTH (ANOVA)]

PTH treatment fully prevented the decrease in modulus and indentation force induced by OVX at the level of the cartilage. Cartilage thickness did not change after OVX or after PTH administration. Administration of PTH prevented OVX induced alteration of the subchondral bone microarchitecture.

Ovariectomy induces an alteration in the cartilage quality (without modifying its mass) and in subchondral bone, both resembling early osteoarthritis. The administration of PTH totally prevents these deleterious effects on the osteochondral plate. PTH treatment could represent a potential therapeutic intervention for osteoarthritis.

P131

Investigating the role of the AP-1 transcription factor in osteoarthritis using genetically engineered mice

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Objectives: To determine the role of two members of the AP-1 transcription factor—c-Fos and JunB -in Osteo-arthritis (OA), the most frequent form of arthritis and to identify novel molecular mechanisms, which could be targeted therapeutically.

Methods: Genetically-engineered inducible and cartilage-specific c-Fos or JunB loss-of-function mouse models were generated by combining *c-fos or junb* floxed alleles with the Col2a1-CreERT transgene. c-Fos or JunB were inactivated by intraperitoneal Tamoxifen injection at 2.5 weeks of age. Experimental OA was induced surgically in mice at 6 weeks of age by destabilization of the medial meniscus (DMM). In the joints, protein expression was analyzed by immunohistochemistry and immunofluorescence (n = 3) and cartilage damage was evaluated histologically based on the OARSI grading system ($n \geqq 5$).

Results: Protein expression of JunB was increased in articular cartilage after DMM, while c-Fos was decreased. Importantly, c-Fos deletion in articular chondrocytes aggravated DMM-induced cartilage damage, while JunB inactivation had no effect ($n \geqq 5$). While DMM induced articular chondrocyte loss in wild-type mice, chondrocyte number and cartilage area in c-Fos-deficient littermates were further decreased in the damaged region. In addition, increased tidemark split was observed in the articular cartilage of c-Fos-deficient mice subjected to DMM. Finally, c-Fos inactivation in articular chondrocytes was sufficient to promote spontaneous, age-induced proteoglycan loss ($n \geqq 3$).

Conclusions: In cartilage c-Fos protects from naturally-occurring proteoglycan loss and DMM-induced OA, while JunB is dispensable. Thus, strategies preserving c-Fos expression in chondrocytes might be beneficial for OA prevention and treatment.

P135

The role of Dickkopf-1 in thyroid hormone-induced changes of bone remodeling

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Background: Thyroid hormones are critical regulators of bone homeostasis, but their mechanisms of action remain incompletely understood. Exogenously induced hyper- and hypothyroidism in mice was recently found to be associated with an altered expression of the Wnt inhibitor Dickkopf-1 (Dkk1), which is a determinant of bone mass. In this study, we assessed the role of Dkk1 in thyroid hormone-induced changes in bone using conditional Dkk1 knockout mice.

Methods: Male mice with a global (Dkk1 $^{fl/fl}$; Rosa26-CreERT2) or osteocyte-specific (Dkk1 $^{fl/fl}$; Dmp1:Cre) deletion of Dkk1 were pharmacologically rendered hypothyroid or hyperthyroid. The bone phenotype was analyzed using μCT analysis, dynamic histomorphometry, and serum concentrations of bone turnover markers.

Results: Hypo- and hyperthyroid Cre-negative mice of either Cre-line revealed the expected changes in bone volume with hypothyroid mice displaying a 40–60% increase in vertebral trabecular bone volume, while hyperthyroid mice lost 45–60% of bone volume. Similar

changes at the spine were observed when Cre-positive hypothyroid or hyperthyroid mice were compared to their euthyroid controls (+30 to 36% in hypothyroid mice and − 30 to 43% in hyperthyroid mice). Interestingly, Cre-positive mice of both lines did not gain or lose as much bone at the femur when rendered hypo- or hyperthyroid. While Cre-negative hypothyroid mice gained 80–100% bone volume, Cre-positive hypothyroid mice only increased their bone volume by 55–90%. Similarly, Cre-negative hyperthyroid mice lost 74–79% bone, while Cre-positive hyperthyroid mice merely lost 40–54%. Despite these site-specific differences, both, global and osteocyte-specific Dkk1 knockout mice displayed similar changes in bone turnover as their Cre-negative controls in the hypo- and hyperthyroid state. While osteoblast and osteoclast parameters were increased in hyperthyroidism, hypothyroidism potently suppressed bone cell activities.

Conclusions: Loss of Dkk1 is not sufficient to fully reverse thyroid hormone-induced changes in bone mass and bone turnover.

Keywords: Hypothyroidism, Hyperthyroidism, Dickkopf-1, Bone remodeling

P137

Intestinal calcium absorption upregulates during lactation without the vitamin D receptor (VDR) but is downregulated without calcitriol: evidence of an alternate receptor?

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Vdr null and *Cyp27b1* null [calcitriol-null] mice normalize calcium metabolism during pregnancy and recover bone mineral content (BMC) and strength post-weaning. However, *Cyp27b1* nulls lose twice-normal BMC during lactation and produce milk with low calcium.

Why does loss of VDR differ from loss of calcitriol during lactation? Do the high levels of calcitriol in *Vdr* nulls act on an alternate receptor to upregulate intestinal calcium absorption (CaAbs)? We compared *Vdr* null and *Cyp27b1* null mice.

WT and null sisters were raised on a calcium and lactose-enriched "rescue" diet. At pre-pregnancy, day 18.5 pregnancy, and day 10 lactation, mice received 0.5 microCi ^{45}Ca by gavage. After 10 min, blood was collected by cardiac puncture. Duodena were snap frozen for gene expression analysis.

Compared to pre-pregnancy, *Vdr* nulls had 2.5 ± 0.3-fold increased CaAbs ($p < 0.03$) during pregnancy and maintained 2.1 ± 0.6-fold during lactation; WT was similar throughout. In contrast, *Cyp27b1* nulls had 9.2 ± 1.5-fold increased CaAbs during pregnancy but returned to baseline (1.0 ± 0.4-fold) during lactation.

Duodenal expression of *Trpv6* and *S100 g* increased fourfold ($p < 0.01$) and twofold ($p < 0.01$) respectively in WT during pregnancy but did not change in *Vdr* nulls; *Pmca1* and *Ncx* showed no increases in WT or *Vdr* null during pregnancy or lactation. *Pdia3* is a putative alternate receptor for calcitriol but its baseline expression was equal between WT and *Vdr* null, and decreased 0.6-fold during pregnancy and lactation in both ($p < 0.03$).

In summary, CaAbs increased during pregnancy in *Vdr* and *Cyp27b1* nulls, but during lactation only in *Vdr* nulls. None of the changes in duodenal gene expression explained increased CaAbs in *Vdr* nulls.

In conclusion, CaAbs upregulates during pregnancy without calcitriol; neither absence of VDR or calcitriol prevented it. However, CaAbs requires calcitriol during lactation, with the high levels in *Vdr* nulls likely acting on an alternate duodenal receptor.

P141

Obesity and flat vertebra

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Background: Spine is a mechanical structure, in a young and healthy individual, disposes their vertebral bodies in harmony with their stature. It is postulated that the important obesity in the early stages of life, could modify the vertebral parameters by skeletal overloads, but the problem is that the current vertebral indexes do not measure a relation of the person height with his vertebra.

Materials and methods: We selected a population between 90 patients 20–55 years old, obese (BMI ≥ 35), both sexes, who was in bariatric surgery protocol, had a significant obesity in their childhood, and had not exclusion criteria of flat vertebra. As a control group, those wicht met the inclusion and exclusion criteria except obesity

As variables and Method see figure 1/2, and finally, we applied a comparative study of average of height and vertebral index (VI) results: IV = 10 × LVD8/(HVD8 x stature)

Results and conclusions: A patients was analyzed. See Figure 2
It is a small study, and according to height or the created index, it does not seem that obesity in development modifies the overall height or the vertebral parameters. In addition, the index gives a stable value regarding the sex of both populations in the VD8.

Figure 1. With a chest lateral plate, not rotated, and in the eighth dorsal vertebra (VD8), by proximity to the Scheuermann kyphosis, we calculate his length (LVD8) and his height (HVD8), measured in mm. Thus, from the horizontal one of his pedicle, taken at that height and parallel to the vertebral plate with superior disc contact, LVD8 is 35.50mm. Drawing a line perpendicular to the previous one (90.1º), and measured from the most sclerous area of the vertebral plate with disc contact superior to inferior, passing through the midpoint of its length (17.74mm), we obtain a HVD8 of 20.69mm.

Control Group				
Sex	Age (Years)	Height (m)	BMI	VI (m^{-1})
73,3% Male	45,15	1,694	26,9	10,1
76,1% Female	44,8	1,603	25,5	10,2
Obese group				
Sex	Age (Years)	Height (m)	BMI	VI (m^{-1})
26,7% Male	47,6	1,687	36,6	10.2
23,9% Female	46.9	1,597	40	10.3

Figure 2. A population of 90 patients was analyzed, with 20 patients in the study group (22.2%), compared to 70 patients in the control group (77.8%), according to the criterion of BMI≥35. The comparative analysis of means did not show significant differences in the indexes or in the size of the individual.

P142

Diabetes Mellitus: a synonym to functional hypoparathyroidism

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Background: Poor control of blood glucose levels in patients of Diabetes Mellitus often results in low bone mineral density. The reason for this decrease in bone mass is at present uncertain. We evaluated correlation of calcium metabolism with blood glucose levels and normal renal function.
Methods: A total of 130 diabetic patients (35 Type 1, 95 Type 2) were enrolled in this study. In all patients plasma calcium (Ca), serum phosphate (PO4), serum parathyroid hormone (PTH), and 24-h urinary calcium (uCa) were determined under both poor and improved control (for at least 7 days) as ascertained by four blood glucose determinations daily.
Results: Improvement of blood glucose level was associated with reduction of uCa both in Type 1 (6.7 ± 1 vs 5.0 ± 0.9 mmol/day) and in Type 2 patients (4.3 ± 0.4 vs 3.1 ± 0.4 mmol/day). It was also found that considerably more Type 1 patients (15 out of 35) had PTH values below the detection limit (1.5 pmol/l) during poor than during improved control (4 out of 35). Type 2 patients also showed this difference but to a lesser extent. 33 out of 95 type 2 patients had PTH level below detection limit during poor control as compared to only 5 patients during good control. Comparison between the two types of diabetes showed that in Type 1 under poor control, Ca and PTH were lower, while uCa was higher, and after improved control, only uCa continued to be higher.
Discussion and conclusions: Increased uCa excretion and decreased PTH levels are associated with uncontrolled blood glucose levels (especially in Type 1 diabetes). Therefore decreased serum PTH levels in uncontrolled Diabetes Mellitus may be one of the factors leading to reduced bone mass. Hence it is justified to call Diabetes Mellitus a synonym to functional hypoparathyroidism.

P143

The effectiveness of vitamin D supplementation in functional outcome and quality of life of lumbar spinal stenosis requiring surgery: randomized prospective controlled trial

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Objectives: To identify the prevalence of vitamin D deficiency in patients with lumbar spinal stenosis(LSS) requiring surgery, and to compare the differences between the cases whether vitamin D is supplemented and vitamin D is not supplemented in terms of a quality of life during postoperative one year.
Methods: All patients with LSS who underwent surgery from March 1, 2015 to August 31, 2016 were enrolled. Among them, 33 patients with vitamin D deficiency were randomly divided into two groups(-supplemented group(A) and non-supplemented group(B)). Functional outcomes using Oswestry disability index(ODI) and Rolland Morris Disability Index(RMDQ) and quality of life using SF-36 were evaluated at 6 month, and 12 month follow up periods. Differences in functional score and SF-36 between the vitamin D supplemented and non-supplemented group were compared using a Mann–Whitney test.
Results: Among the total 102 patients, 78 patients(76.5%) had vitamin D deficiency. Of the 78 patients, 33 patients were included, 16 patients were group A and 17 patients were group B. There was no difference in age and 25-OHD level between the two groups(all $0 > 0.05$). Group A were better functional outcomes at twelve months after surgery($p < 0.05$). On the quality of life, group A were higher score than group B from six month later after surgery($p < 0.05$).
Conclusions: Vitamin D deficiency was highly prevalent in LSS patients(76.5%). Assessment of serum 25-OHD are recommended in LSS needing surgical intervention and active treatment vitamin D supplementation and maintenance of normal range should be considered for better postoperative functional outcome and quality of life.
Keywords: Lumbar Spinal Stenosis, Surgery, Vitamin D, Functional Outcome, Quality of Life

P144

A hospital based study of bone mass in asian Indian new born subjects

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Aim: To study bone mass (whole body BMD, BMC and bone area) in Asian Indian new born subjects and to study the associated factors.
Methods: Apparently healthy pregnant women (< 16 weeks gestation) recruited from Department of Obstetrics & Gynaecology, AIIMS. Data captured on maternal antenatal events: dietary intake (3-day 24 h recalls), femoral volume (3D USG) at 19 and 34 weeks

gestation, placental weight and serum vitamin D levels at baseline and post delivery. Newborn parameters included birth weight, length, head circumference; cord blood vitamin D & IGF-1 levels and bone mass assessment by DXA (Hologic Discovery A) within 15 days after birth. Association was sought between bone mass parameters and newborn and maternal factors using one way ANOVA and linear regression.

Results: The study population included 150 term & AGA newborns from mothers with mean age at recruitment of 26.7 ± 3.4 years, mean gestational age 14 ± 1 weeks at recruitment. The bone mass was: BMC 44.94 ± 7.3 g, BMD 0.25 ± 0.21 g/cm^2 (median 0.21, 0.15–1.38) and bone area 218.6 ± 20 cm^2. The mean placental weight was 506.9 ± 77.4gm (n = 60) and mean femoral volume as assessed by 3D ultrasound was 0.77 ± 0.29 ml at 19 weeks and 4.49 ± 1.28 ml at 34 weeks (n = 30). The mean birth weight, birth length and head circumference was 2991.3 ± 403.5gm, 50.7 ± 2.2 cm and 34 ± 1.3 respectively. New born BMC, BMD and bone area were significantly associated with placental weight, newborn birth weight, birth length & head circumference (all $p < 0.001$) however newborn IGF -1 was also significantly associated with newborn BMC and bone area ($p < 0.05$). None of the newborn bone mass parameter significantly correlated with maternal dietary intake and maternal or newborn vitamin D status.

Conclusions: Placental weight and newborn IGF-1, birth weight, birth length & head circumference could be the probable factors associated with new born bone mass.

P145

Normocalcaemic hypoparathyroidism: study of the prevalence and natural history in a United Kingdom referral population

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Introduction: Normocalcaemic hypoparathyroidism (NHYPO) is characterised by persistently low levels of parathyroid hormone (PTH) with normal levels of calcium. There is little in current literature on this disease, with only two studies published on its prevalence whilst its natural history remains relatively unknown.

Objectives: To identify the prevalence of NHYPO in a UK referral population and to study the natural history of the disorder.

Methodology: We retrospectively evaluated data from 6280 patients that had been referred for a bone mineral density measurement and a laboratory evaluation between 2013 and 2017. Mahalanobis distance was used to identify subjects as 'normal' or 'abnormal' and the reference intervals for adjusted calcium and PTH were used to divide these patients in different categories. A random group from the normal population was used as a control.

Results: Based on lab results on the index day, 22 patients (0.35%) were identified as having NHYPO. Four patients were excluded (cancer/chemotherapy or unconfirmed data), thus the final number of NHYPO patients was 18 (prevalence 0.29%). When studying the natural history, only 56% of the included patients had persistent normal levels of adjusted calcium throughout their follow up period

Conclusions: Previous estimates of the prevalence have been higher, between 1.1 and 2.4%.This is the largest study to date to study NHYPO, including the longest follow up period (10 years). The next steps are to find out the cause of this biochemical abnormality and the consequences, if any.

Keywords: Normocalcaemic, Normocalcemic, Hypoparathyroidism

P146

Aggravation of trabecular bone defect in ovariectomized Goto-Kakizaki (GK) type-2 diabetic rats

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Estrogen deprivation has known to increase bone turnover in postmenopausal women, in part, by enhancing osteoclastic bone resorption. Type 2 diabetes (T2DM), which has high prevalence in postmenopausal women, also deteriorates bone microstructure; however, whether a combination of T2DM and estrogen deficiency can aggravate impairment of bone microstructure and bone growth has been elusive. We, therefore, investigate the relative contribution of estrogen deficiency and T2DM on bone growth and microstructure in non-obese Goto-Kakizaki (GK) T2DM rats compared with wild-type (WT) Wistar rats, some of them (9 months old) were subjected to ovariectomy (OVX) to induce estrogen deficiency or sham operation. This study has been approved by the institutional ethics committee. Rats were divided into 4 groups, i.e., WT/Sham, GK/Sham, WT/OVX, and GK/OVX. Four months after surgery, femoral and tibial lengths were determined. Cortical and trabecular bone mineral density (BMD) were measured by μ-CT. Bone histomorphometric analysis found that estrogen deprivation and T2DM did not affect longitudinal bone growth. Estrogen deficiency induced bone loss in both trabecular and cortical regions. Similar results were observed in GK/Sham as compared to WT/Sham. However, the combined T2DM and estrogen deficiency caused further loss of trabecular BMD. Bone histomorphometry showed significant bone loss in GK/Sham as indicated by the decreased trabecular thickness and number as well as an increase in trabecular separation ($P < 0.001$). In addition, trabecular number of WT/OVX and GK/OVX rats decreased, and trabecular separation further increased as compared with their respective control groups. In conclusion, the combination of T2DM and estrogen deprivation markedly disturbed bone turnover rather than longitudinal bone growth. The combined condition showed more bone deterioration than each condition alone. Thus, an early prevention of prediabetic condition is expected to benefit postmenopausal women.

Keywords: Bone mineral density; bone turnover; type 2 diabetes mellitus; estrogen deficiency; Goto-Kakizaki rats

P147

A reduced lactose yogurt containing galactooligosaccharides (GOS) as a tool for lactose intolerant cover calcium daily intake that ensures bone health

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The galactooligosaccharides (GOS), natural prebiotics of human milk could be incorporated in dairy products, such as yogurt, by enzymatic action on milk lactose. We previously demonstrated: 1st. functional characteristics of such reduced-lactose yogurt containing GOS during the experimental normal growth; 2nd. Ca absorption (CaAbs) was similar to that observed by feeding the recommended rodent diet.

Objective: To evaluate the beneficial effects of this reduced-lactose yogurt containing GOS in body composition and bone retention during normal growth. Male weaning Wistar rats (n = 10 per group) received during 30 days AIN´93-G control diet (CD) or the yogurt containing GOS diet (ED). Food consumption was evaluated three times per week; body weight (BW) weekly; Ca and phosphorus (P) Abs during the last 3 days of the experience; femur Ca and P content, total skeleton bone mineral content (BMC) and bone mineral density (BMD) at the end of the study. Body composition and BMD of lumbar spine (LS), total (TT) and proximal (PrT) tibia BMD, and TT BMC were also evaluated.

Result (mean ± SD): Food consumption; BW; body fat and lean tissue percentages were similar in both groups. BMC (1.29 ± 0.14 vs. 1.32 ± 0.24 g), BMD (0.320 ± 0.010 vs. 0.320 ± 0.005 g/cm^2), TT BMD (0.246 ± 0.022 vs. 0.246 ± 0.018 g/cm^2) and BMC (0.029 ± 0.004 vs. 0.030 ± 0.014 g); LS BMD (0.250 ± 0.017 vs. 0.251 ± 0.019 g/cm^2) and Ca and P content in femur showed no significant differences. CaAbs % (84.9 ± 2.2 vs. 80.0 ± 5.4%; $p = 0.062$) and PAbs % (86.6 ± 6.6 vs. 78.0 ± 7.1%; $p < 0.05$); PrT BMD (0.303 ± 0.056 g/cm^2 vs. 0.266 ± 0.018; $p < 0.05$) were higher in ED vs. CD. The results evidenced similar body composition, higher Ca and P Abs and similar bone mass and retention.

Conclusion: These results may suggest that the reduced lactose milk funcional product assayed here appears to an optimal tool for maintaining an adequate intake of Ca, which prevents secondary bone disease caused by a milk-restricted diet.

P148

The effects of 60-day sodium benzoate intake and ionizing radiation on chemical composition of the lower incisor dentin in rats

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The study is aimed at investigation of macroelemental composition of the lower incisor dentin (LID) in rats in readaptation period after 60-day application of sodium benzoate (SB) and exposure to ionizing radiation (IR), and finding possibility of medication with sea buckthorn oil (SBO).

The experiment involved 240 rats with body weight of 180–200 g. The animals were distributed into 8 groups as follows: intact animals, animals that received *per os* SB in dosage of 1500 mg/kg daily for 60 days, animals exposed to IR (total 4 Grey in 4 sessions), received SBO in dosage of 300 mg/kg, combined SB and IR, SB and SBO, IR and SBO, and all three agents simultaneously. The animals were withdrawn from the experiment by means of anaesthetized decapitation. Dentin taken from lower incisor was prepared for chemical analysis.

Upon SB discontinue, Ca share and Ca/P ratio decreased as compared to the controls by 5.26% and 7.05%; after IR discontinue same values decreased by 7.94% and 11.43%; after combined action of SB and IR those values decreased by 10.65% and 15.14% ($p < 0.05$ in all cases). Restoration of chemical composition also depended on influence: by the 30th day after SB discontinue some differences were still observed, and after cessation of combined action chemical composition of LID did not restore. Application of SBO reduced negative effects of experimental conditions on chemical composition of the LID. The best recovery outcome was observed in animals that received only SB and the worst—in rats under combined action of IR and SB.

Thus, 60-day application of SB in dosage of 1500 mg/kg, exposure to IR and combined action result in destabilization of chemical composition of LID that expands even to readaptation period and SBO is an adequate correction drug.

Keywords: Rats, lower incisor, dentin, macroelemental composition

P149

Macroelemental composition of bones in rats of various ages after excessive palm oil intake and administration of Garciniae cambogia extract

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The aim of the study is to analyze macroelemental composition of skeletal bones in rats of different ages after excessive palm oil (PO) intake and administration of *Garciniae cambogia extract (GE)* as medication.

The experiment involved 216 rats of three ages: immature, mature and senile. The animals were split into the groups as follows: the 1st group comprised control animals; the 2nd group comprised the animals that received intragastric PO in dosage of 30 g/kg, and the 3rd group—PO and intragastric *GE* in dosage of 0.25 g/kg. The animals were withdrawn from the experiment by the 1st, the 10th, the 30th and the 60th day after 6-week PO intake. Bones (tibiae, hipbones, and L3 vertebrae) were prepared for chemical analysis.

Excessive intake of PO resulted in destabilization of the macroelemental composition of tibia. The alterations began manifesting from the 1st day of observation and continued growing throughout the experiment. Ca share and Ca/P ratio in immature animals were lower than those of the controls by the 60th day by 5.99–6.85% and 5.31–6.29% ($p < 0.05$ in all cases). In mature animals the same values changed similarly—by 6.41–7.95% and 7.96–9.51% respectively. In senile rats the same values changed in the same way by 7.10–7.89% and 8.40–9.46% respectively. After *GE* administration, by the 60th day, in immature rats Ca share and Ca/P ratio increased as compared to the 2nd group by 4.74–7.15% and 3.76–6.72%, in mature rats by 4.69–7.29% and 6.29–9.03%, and in senile rats—by 5.66–6.22% and 3.76–7.01% respectively.

Thus, excessive intake of PO results in destabilization of the macroelemental composition of bones. Terms and intensity of alterations depend on age of animals. Administration of *GE* reduces adverse effects of PO on the macroelemental composition of bones.

Keywords: Bones, macroelemental composition, palm oil, *Garciniae cambogia extract.*

P150

The sensory and sympathetic innervation of rat femur with type 1 diabetes mellitus

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Background: Type 1 diabetes mellitus (DM1) increases the risk of osteoporosis and fragility fractures. Osteoporosis is a result of bone loss that occurs by uncoupled remodeling. This process is controlled by numerous genetic, hormonal and neurogenic mechanisms. A number of neuropeptides, such as substance P (SP), calcitonin gene-related peptide (CGRP) and tyrosine hydroxylase (TH) which are synthesized in sensory and sympathetic neurons are implicated in the control of bone remodeling.
Objectives: To investigate the distribution of sensory and sympathetic nerve fibers in the bones of type I diabetic rats.
Methods: Experimental DM1 was induced in adult Wistar rats by an intraperitoneal injection of streptozotocin 60 mg/kg BW. Ethical approval was granted by the animal research ethical committee at UAE University. Femurs from both diabetic (n = 10) and control rats (n = 10) were dissected out and fixed in 10% formalin prior to decalcification. Immunohistochemistry technique was used to identify the nerve fibers. Cryostat sections (30 μm) were incubated with primary antibodies; rabbit anti-NF200 (pan neuronal marker), rabbit anti-TH (sympathetic nerves marker), goat anti- CGRP and guinea pig anti-SP (sensory neuronal markers); followed by species-specific secondary antibodies conjugated to Alexa 488. Sections were viewed using fluorescence microscopy.
Results: Results showed that bones are densely innervated with sensory and sympathetic nerve fibers labeled with NF, TH, CGRP and SP. However, statistical analysis showed a decrease in the number of both sensory and autonomic immunoreactive nerve fibers in type I diabetic rats compared with control animals $P < 0.05$ (paired samples t- test with a probability value of 95%).
Conclusions: The reduction in the bone sensory and sympathetic innervation might be involved in the pathogenesis of skeletal fragility in DM1.
Keywords: Type 1 Diabetes Mellitus, Neuropeptides, Osteoporosis, Immunohistochemistry

P151

Growth hormone secretagogue receptor is necessary to food restriction-induced increases in bone marrow adiposity in female mice

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Rational and objectives: Food restriction (FR) induces bone loss and high bone marrow adiposity (BMA), but the underlying mechanisms remain poorly understood. Physical activity was shown to decrease BMA. Increased ghrelin levels in FR conditions may contribute to altered bone phenotype. Whether elevated ghrelin levels are associated with BMA expansion during FR remains unknown. Therefore, we sought to elucidate the role of ghrelin signaling on bone phenotype and BMA in ghrelin receptor knock-out mice (GHSR-/-) after 21 days of FR associated with voluntary wheel running.
Experimental groups: Ghsr-/- and Ghsr +/+ 8 week-old C57BL/6 female mice were assigned into 4 groups (n = 6 to 10/group): ad libitum group (AL), AL + wheel (ALW), FR (FR), and FR + wheel (FRW) group. All FR mice were subjected to 50% restriction for 3 weeks with 30% FR for the first 3 days. All animal experiments were approved by the Animal Experimentation Committee of Paris Descartes University (#03422.02).
Results: FR reduced body weight by 16–19% in FR and FRW mice regardless of genotype (diet effect $p < 0.001$). All FR mice displayed a lower physical activity level compared to all AL mice ($p < 0.05$). All FR mice displayed higher acyl and desacyl ghrelin level (diet effect $p \leq 0.05$ in +/+ mice, $p < 0.001$ in -/- mice) than AL mice. FR-induced decrease in IGF-1 levels was shown in +/+ and -/- mice. FR associated or not with physical activity resulted in reduced trabecular thickness in +/+ and -/- mice ($p < 0.001$). FR was associated with decreased trabecular bone volume in +/+ mice only ($p < 0.05$). Although BMA increased in FR associated or not with physical activity (diet effect, $p < 0.020$), GHSR deficiency blunted BMA formation in FR mice (diet effect, $p > 0.05$).
Conclusion: GHSR activity could be a key mediator of FR-associated BMA expansion, independently of GH/IGF-1 axis.

P155

The comparison of serum vitamin D, bone mineral density and nutrient intake according to obesity in young adult women

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Background and Purpose: In the life cycle, bone mineral density is the most optimal condition in the 20 s. In Korea, vitamin D deficiency status is very serious in Korean women due to recent lack of activity, weight polarization, and inadequate nutritional intake. The purpose of this study was to investigate serum vitamin D status and to compare the blood profile, dietary intakes and physical activity in Korean young women.
Methods: A total of 143 female college students participated in the research. The subjects were surveyed by a self-administered questionnaire about general characteristics, physical activities. Body fat and lean body status were analyzed using a body composition analyzer. Nutrient intakes of the subjects were assessed by 3-days food record method. The BMDs of the lumbar spine was measured by dual energy X-ray absorptiometry (DEXA). The subjects were divided into two groups on their BMI.
Results: Obese group showed significantly higher weight, body fat (%), and body fat (kg) than normal group and T-scores of lumbar spines were significantly lower. As a result of blood tests, obese group showed high triglyceride and LDL cholesterol levels and vitamin D levels were

significantly lower. Physical fitness and activity status showed that sit and reach and sit up were significantly lower in obese group. The intake of carbohydrates was higher in the obese group than in the normal group, and the intake of vitamin C and vitamin D was significantly lower. Factors affecting serum vitamin D were analyzed as percent body fat, bone mineral density, triglyceride, and carbohydrate intake.

Conclusions: Regular activities, balanced nutrition, and weight management are important for normal maintenance of vitamin D level in young adult women.

Keywords: vitamin D, bone mineral density, obesity, nutrition, young women

P156

Body mass index effect on total and undercarboxilated osteocalcin in normoglucemic adult men

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Obesity and osteoporosis, two of the main common chronic diseases are interconnected. Regarding, both pathologies come from the deregulation of a common mesenchymal precursor. Moreover, bone through osteocalcin (OCN) and fat tissue through leptin appear to contribute to glucose homeostasis. The objective of this study was to evaluate the effect of BMI on serum levels of total OCN and undercarboxilated OCN (ucOCN), in non-diabetic overweight and obese adult men who were divided according to quartiles of BMI (range 29.0 to 47 kg/m^2). We determined ucOCN, OCN, leptin and insulin, in 54 adult non-diabetic men, having normal levels of glucose (80 to 110 mg/dL) and hemoglobin A1c (HbA1c $< 5.7\%$). The presence of overweight (OW) or obesity (OB) types I, II or III degree was determined according to BMI index. Glucose, HbA1c levels were measured by standard laboratory methods; OCN (ng/mL), ucOCN (ng/mL), leptin (ng/mL), insulin (uUI/L) levels by ELISA and 25hydroxyvitamin D (25OHD) (ng/mL) levels by a competitive protein-binding method.

Results: The results (mean $\pm$ SD) are summarized in Table 1.

	Glucose	ucOCN	OCN	CTX	25OHD	Leptin	Insulin
Overweight	97 ± 9	3.98 ± 0.73	25.8 ± 15.4	355 ± 30	21.2 ± 2.4	21 ± 19	12.1 ± 3.2
T1 Obesity	99. ± 8	4.49 ± 1.09	18.8 ± 6.7	313 ± 101	20.7 ± 6.9	19 ± 11	12.4 ± 4.1
T2 Obesity	102 ± 7	4.81 ± 1.22	15.3 ± 4.2	318 ± 54	22.3 ± 7.9	27 ± 11	24.2 ± 8.8
T3 Obesity	106 ± 3	4.31 ± 0.59	8.3 ± 4.1	344 ± 8	16.8 ± 2.8	41 ± 22	23.4 ± 4.1

There was an increase in ucOCN and a decrease in CTX levels between OW and the three types of OB ($P < 0.01$). OCN levels decreased and Leptin increased with the increase in BMI ($p < 0.01$). Insulin increased in OBTII and OBTIII vs. OW and OBTI ($p < 0.05$). The lowest 25OHD levels were observed in TIII obesity.

Conclusions: The present results suggested that BMI through leptin affects OCN concentration, which in turn influences insulin levels and glucose homeostasis.

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Association between global cardiac calcification (GCCS) and osteoporosis

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Epidemiologic and clinical data have suggested the existence of a biologic linkage between bone and vascular system. Osteoporosis and atherosclerosis are two prevalent major healthcare concerns that frequently coexist. Several studies reported correlation between lower values of Bone Mineral Density (BMD) and cardiovascular events. Furthermore literature suggests that cardiac calcification (measured with Global Cardiac Calcium Score, GCCS) is associated with cardiovascular events and mortality.

This study aimed to evaluate if cardiac calcium deposit was correlated with BMD.

In 36 subjects assessed for bone fracture risk (mean age 72 $\pm$ 5,7 years) we measured Bone Mineral Density (BMD) at lumbar spine (BMD-LS) at femur (Neck: BMD-FN; Total: BMD-FT) and we assessed with echocardiography a global cardiac calcium score (GCCS).

GCCS is a semi-quantitative score, that was applied assigning points for calcification in the aortic root and valve, mitral annulus and valve and sub-mitral apparatus, and points for restricted leaflets mobility.

The results show that there is a significant inverse correlation between BMD-FN and BMD-FT with GCCS ($r = -0{,}285$, $p < 0{,}05$ and $r = -0{,}376$, $p < 0{,}05$ respectively). No significant correlation was found between BMD-LS and GCCS. Dividing patients into two groups based on presence of bone fragility fractures we observed that the value of GCCS was higher in patients with bone fragility fractures (2,54 $\pm$ 1,3 vs 2,30 $\pm$ 1,5) but not statistically significant. Moreover, dividing patients on the basis of presence of sarcopenia we found that the values of GCCS was higher in patients with sarcopenia (3,0 $\pm$ 1,4 vs 2,1 $\pm$ 1,4) even though the difference didn't reach statistical significance.

Our data suggest link between osteoporosis and cardiac calcification. The burden of cardiac calcium seems to be higher in patients with fragility fractures. These findings confirm that osteoporotic patients have an higher risk of cardiac and vascular calcification and this confirm the greater risk of cardiovascular events in osteoporotic patients.

P159

Cardio-metabolic health and bone mineral density in postmenopausal women: a Korean population-based study

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Objectives: It has been suggested that bone mineral density (BMD) and cardiovascular disease have overlapping risk factors and share pathophysiological mechanisms. The present study was conducted to investigate the association between BMD and metabolic syndrome (MetS), a cluster of major risk factors for cardiovascular diseases from the Korean population-based study.
Design: This study was based on the data from the Korea National Health and Nutrition Examination Survey (KNHANES), conducted by the Korean Ministry of Health and Welfare. This cross-sectional nationwide representative survey based on a stratified, multi-stage sampling with a probability proportional to size. BMD was measured during 2008–2011 in KNHANES and categorized into 3 groups according to the World Health Organization's criteria. MetS was diagnosed according to the criteria from a joint scientific statement endorsed by major organizations including National Heart, Lung, and Blood Institute. Finally, a total of 3394 postmenopausal women were included.
Results: Overall 1573 postmenopausal women (46.3%) had MetS and mean age of women with MetS were significant higher compared to non-MetS women (65.4 years vs 62.7 years, $P < 0.001$). Complex sample analysis showed revealed that MetS was not associated BMD at the lumbar spine and proximal femur with adjustment for potential confounders (age, years since menopause, body mass index and physical activity). Also the presence of MetS did not have an effect on the probability of having low BMD. Analysis between BMD and five components of MetS demonstrated significantly higher BMD at femur neck (FN) in women with waist circumference $\geq$ 80 cm, which is cut-off value for Asian women (0.622 g/cm^2 vs 0.612 g/cm^2, $P = 0.045$)
Conclusions: MetS is not associated with BMD in Korean postmenopausal women, but high waist circumference has positive relationship with FN BMD.
Funding: The present study was supported by grant no 04-2015-0920 from the SNUH Research Fund.

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Investigation of bone fragility in patients with type 2 diabetes mellitus by high resolution quantitative computed tomography of the femoral shaft: the DiabOS study

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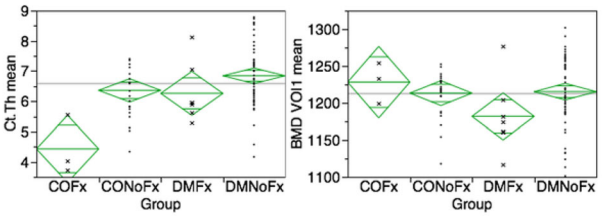
Bone fragility in patients with type 2 diabetes (T2DM) may be caused by impaired bone quality rather than loss of bone mass. Few clinical centres have HR-pQCT devices. We evaluated whether aspects of bone fragility could be measured using regular clinical CT. QCT at the proximal third of the femoral shaft was evaluated (QCTfs). We report first results on reliability and fracture discrimination.

Patients were recruited at the University Clinic Basel in the context of the ongoing DiabOS Study. QCTfs was acquired on a Siemens CT using HR-QCT protocol (140kVp, 200mAs, 0.6 mm slice thickness). We evaluated cortical thickness (Ct.Th), cortical BMD (Ct.BMD) and density weighted cortical thickness (wCt.Th). As reliability check, we correlated results of the left and the right femur.

Among 94 patients (51 m, 43f, age 51 to 75, mean 62y), 7 of 65 patients with T2DM and 4 of 29 controls had prior low trauma clinical fractures. Mean $\pm$ standard deviation for L/R femur, r2 and RMSE of a linear correlation were for Ct.Th: 6.62 $\pm$ 1.07 mm, 6.55 $\pm$ 1.10 mm, r2 = 0.85, RMSE = 0.42 mm; wCt.Th: 6.63 $\pm$ 1.13 mm, 6.69 $\pm$ 1.17 mm, r2 = 0.84, RMSE = 0.45 mm; Ct.BMD = 1201 $\pm$ 42.2 mg/cc, 1224 $\pm$ 44.2 mg/cc, r2 = 0.90, RMSE = 13.7 mg/cc. Fracture discrimination of mean L/R femur adjusted for age, gender, height and weight was $p < 0.10$, < 0.05, and < 0.06 for the 3 variables, with diabetes as confounder at $p < 0.11$, 0.11, and 0.24.

In conclusion, we demonstated the feasibility of HR-QCT data assessment at the cortical shaft with promising facture discrimination supporting the concept of different causes of fragility for T2DM versus osteoporosis.
Keywords: Diabetes mellitus, HR-QCT, cortical density, cortical porosity



[Fig. 1: Differences between fractures cases: Control (COFx, thin cortex) and T2DM (DMFx, low BMD)]

P164

SOX 6 does not regulate RANKL gene expression through its proximal promoter region

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RANKL is a regulator of bone turnover and its elevated expression is related to osteoporosis, a bone disease predominantly affecting postmenopausal women. There are many transcriptional regulators affecting RANKL's expression through its distal regulatory regions, such as parathyroid hormone, Vitamin D and different cytokines (1). However, its proximal promoter region remains poorly understood. Our previous data showed that SOX family of transcription factors takes part in regulation of RANKL expression. Based on results of the Genome-wide meta-analysis (1) we decided to test whether the transcription factor SOX 6, whose SNP rs7108738 was found to be associated with BMD variation, regulates the expression of RANKL gene through its proximal promoter region.

The functional evaluation of SOX6 included cloning of SOX 6 and testing its ability to activate or repress the RANKL gene proximal promoter using luciferase reporter assay. In addition we tested the impact of overexpression of SOX 6 on the expression of RANKL gene in HOS cell line using qPCR. We used western blot to confirm the transfection of SOX 6 protein.

Luciferase assays revealed that SOX 6 doesn't affect the activity of RANKL proximal promoter. Using qPCR we have also confirmed that SOX 6 doesn't regulate the expression of RANKL, regardless effective and confirmed transfection of SOX 6 into HOS cells.

In our study we have discovered that the transcription factor SOX 6, even though associated to BMD in GWAS study, does not regulate the expression of RANKL through its proximal promoter. The results implicate other Sox 6 pathways relate to BMD and osteoporosis and they should therefore be further studied.
References:
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P165

Screening for osteoarthritis candidate gene through TSA (Trial Sequential Analysis, TSA)

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Background: Osteoarthritis (OA) is a chronic disease of knee joint cartilage degeneration, with joint cavity stenosis, meniscus breakage and spur formation, and affects approximately 80% of those over 65 years of age. The risk factors for Osteoarthritis include genetic factors, and the heritability rate is about 50%. To clarify the pathological changes of degenerative joints, it is necessary to understand the susceptible gene loci.
Materials and methods: This study looked for a meta-analysis paper of all Pubmed, embase, cochrance databases, including all the keywords for osteoarthritis, genetic polymorphism, and meta-analysis. Trial Sequential Analysis (TSA) is used to determine "whether there is sufficient evidence to suggest that enough samples support the association of genotypes with disease." In addition, in order to confirm its functiona, this study also used the GTEx database to understand the relationship between each loci and mRNA expression.
Results: Through the keyword search, a total of 95 articles were collected. After the TSA were estimated for the 39 articles, 24-loci, It was found that SNPs such as ESR1, SMAD3, DVWA, GDF5, VDR and BMP14 were determined to be associated to OA. ESR1 and SMAD3 genes were protective effects of OA, DVWA, GDF5, VDR and BMP14 genes were risk factors. In another case of rs9340799 (XbaI) on ESR1, the number of samples is still insufficient to determine the result, and there is still research value.
Conclusions: This study confirmed that there are enough samples in 18 loci such as CYP19A1 to confirm that it is not associated to OA. It is no necessary to invest more resources for this 18 loci. In addition, genes have been identified (e.g. ESR1, SMAD3, DVWA, GDF5, VDR, BMP14 (6 loci) can be used as osteoarthritis screening kit.
Keywords: Polymorphism, Osteoarthritis, Trial Sequential Analysis

P166

Association of Collagen Type I Alpha1 Gene Polymorphism with Bone Mineral Density and Fractures in Patients with Chronic Kidney Disease Stage 5

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Objectives: The aim of the study was to analyze the influence of collagen type I alpha1 gene polymorphism on bone mineral density and risk of fractures in female patients with chronic kidney disease receiving hemodialysis.
Materials and methods: To test the relationship between COLIA1 gene polymorphism, bone mineral density (BMD) and fractures 86 patients (38 men and 48 women), middle age 38.7 ± 11.4 years, treated on hemodialysis for 4.1 ± 1.2 years, have been studied. Genotypes SS, Ss and ss have been considered. BMD was measured with dual energy absorptiometry at the lumbar spine, femoral neck and distal arm.
Results: The relative distribution of COLIA1 alleles was S—83.7%, and s—16.3%. The COLIA1 genotype SS was revealed in 67.4% patients, Ss—32.6%. No one studied patient had genotype ss. There were no remarkable differences in genotype SS and Ss groups in laboratory results, including intact parathyroid hormone, ionized calcium, phosphates and alkaline phosphatase. BMD assessed by Z criterion was lower in patients with genotype SS in every studied part of the skeleton, however the difference was not statistically significant ($p > 0.05$). Among 22 patients who had typical osteoporotic fractures, 28.6% had genotype Ss, and 24.1%—SS.
Conclusions: We did not find obvious association of COLIA1 alpha1gene polymorphism with bone mineral density in studied population of hemodialysis patients. However, we suggested the hypothesis that people with allele « s » could be more inclined to fractures.
Keywords: COLIA1 gene polymorphism, osteoporosis, fractures

P167

1,25-Dihydroxyvitamin D_3 partially restores the osteoblast and osteoclast functions of hemizygous β-globin knockout thalassemic mice

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β-thalassemia caused by loss-of-function mutation of β-globin gene has been shown to result in an impairment of intestinal calcium absorption, trabecular osteopenia and poor bone strength. However, it is unclear whether the thalassemia-associated bone loss is caused by certain humoral factors (e.g., proinflammatory cytokines or aberrant production of calciotropic hormone) or inherited defects of osteoblast and osteoclast functions. Herein, we collected long bones from hemizygous β-globin knockout thalassemia (BKO) mice and performed primary osteoblast and osteoclast culture. This study has been approved by the institutional ethics committee. The quantitative real-time PCR revealed that mRNA expression levels of several key osteoblast-related genes, such as Runx2, alkaline phosphatase (ALP) and osteocalcin, were apparently lower in primary osteoblasts of BKO mice compared to those of wild-type (WT) mice. The mRNA expression of divalent metal transporter-1, receptor activator of nuclear factor-κB ligand, macrophage colony-stimulating factor, and interleukin-1β did not change. Flow cytometry also revealed similar number of ALP-positive cells in BKO and WT groups. Interestingly, subcutaneous injection of 0.5 μg/kg 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] once-daily for 7 days could improve some characteristics of primary osteoblasts of BKO mice, e.g., the unregulated expression of Runx2, ALP and osteocalcin. In primary osteoclast

culture, the tartrate-resistant acid phosphatase (TRAP) staining of multinucleated cells showed less number of TRAP-positive cells in BKO group than WT group, whereas subcutaneous $1,25(OH)_2D_3$ treatment prior to bone tissue collection was able to increase the number of TRAP-positive cells as well as the expression of receptor activator of nuclear factor-κB and c-Fos. In conclusion, thalassemia is likely to impair both osteoblast and osteoclast functions, but some humoral factors may also contribute to cellular defects. Thus, subcutaneous $1,25(OH)_2D_3$ treatment is able to restore their functions although the primary bone cells are no longer exposed to $1,25(OH)_2D_3$.

Keywords: β-thalassemia, osteopenia, primary osteoblast, primary osteoclast, vitamin D

P168

Genetic variants in PRRG1 are associated with cardiovascular parameters according to diabetes stage, sex and BMI

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PRRG1 codes for a short transmembrane protein and belongs to the group of vitamin-K-dependent proteins. In a previous study we showed a decreased PRRG1 gene expression during intima thickening in atherosclerosis.

We investigated the association of PRRG1 SNPs with intima media thickness (IMT), left atrial volume index (LAVI), left ventricular mass index (LVMI), pulse wave velocity (PWV) and relative wall thickness (RWT) groups in three diabetes stages (no-, pre- and type 2 diabetes (T2DM)), in lean and overweight persons and males vs. females.

We investigated the BioPersMed cohort (n = 1025), containing asymptomatic patients with one cardiovascular risk factor. Determination of PRRG genotypes was done with GSA array (Illumina Inc., USA), pulse wave analysis with a SphygmoCor device (Atcor Medical, Australia) and echocardiograpy with the Vivid 9 device (GE Healthcare Austria GmbH & Co OG, Austria).

Diabetes stages were defined according to ADA criteria. A BMI < 25 was considered as lean, otherwise as overweight. RWT groups: concentric (> 0.42) and eccentric (≤ 0.42) hypertrophy. LAVI < 29 ml/m^2, LVMI $\geq 96/116$ (females/males) g/m^2, IMT $>$ 0.9 mm and PWV < 10 m/s were considered pathologic.

These associations might implicate a possible role of PRRG1 SNPs in the modulation of important cardiovascular parameters and thus contribution to cardiovascular risk.

SNP	No-diabetes		Pre-diabetes		T2DM	Lean			Overweight
ID	IMT	LVMI	LAVI	RWT	PWV	LAVI	LVMI	RWT	LVMI
rs113362444	0.034	0.002	–	0.028	0.020	0.059	0.076	–	0.003
rs5917507	0.063	0.017	0.015	0.009	0.059	0.046	–	0.025	0.027
kgp22829647	0.063	0.013	0.0.18	0.008	0.047	0.048	–	0.010	0.014
kgp22738694	0.094	0.028	–	0.050	–	0.041	–	0.002	0.012
rs5917212	0.038	0.083	–	0.049	0.067	0.073	0.035	0.002	0.099
rs6610168	0.037	0.085	–	0.048	0.075	0.066	0.038	0.004	0.087
kgp22730765	0.010	–	–	0.039	–	–	0.094	–	0.082

Associations of PRRG1 SNPs with cardiovascular parameters in various patient groups:]

P169

Validation of GWAS-derived novel regulators of bone homeostasis

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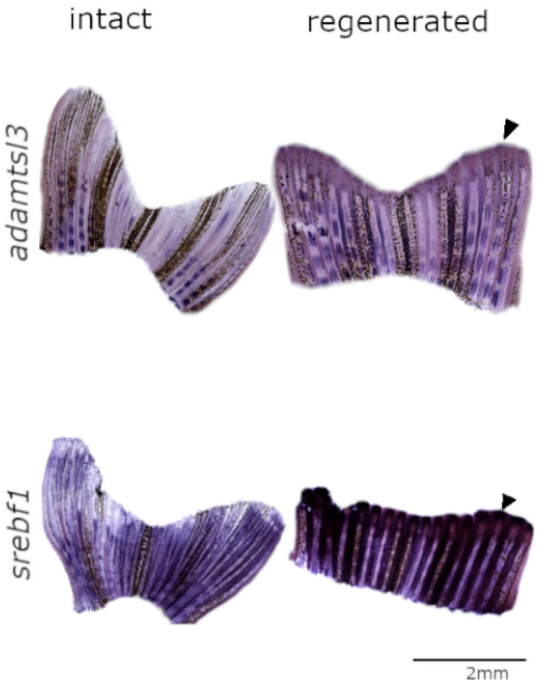
Objectives: Skeletal system is the first system in human organism which undergoes changes, by age-related processes. Together with increasing life expectancy the range of wide-spread skeletal disorders increases, such as osteoporosis, osteogenic imperfecta etc. Nowadays, genome-wide association study (GWAS) in humans for bone mineral density identified a lot of novel genes with unknown functions in bone. Here we aimed to validate the GWAS-derived novel genes, associated with BMD in zebrafish fins by screening the expression patterns.

Methods: We examined the expression patterns of selected genes (*adamtsl3* and *srebf1*) by in situ hybridization of specific RNA-probe to zebrafish both in intact and in regenerated 4 dpa (4 days post amputation) fins.



The qualitative expression of genes in zebrafish AB caudal intact and regenerated fins 4dpa (days post amputation) by in-situ hybridization of specific RNA-probe. The intensity of staining corresponds to expression of gene. Regenerated fins display the expression of adamtsl3 and srebf1 in blastema region (arrowheads). In intact fins the expression of these genes overlaps with bone tissue (lepidotrichia).

[The expression of candidate genes in zebrafish AB fins]

Results: We characterized the expression of adamtsl3 and srebf1 in zebrafish intact and regenerated caudal fins. The expression of these genes overlaps with the bony tissue (lepidotrichia) of the fin. The signal in blastemal region is observed in all specimens.

Summary and Significance: In this ongoing study, we investigate the spatial distribution of candidate genes from GWAS analysis in intact bone and regenerating tissue of using a fish model, as a validation strategy. Our results indicate of a possible involvement of *adamtsl3* and *srebf1* in bone homeostasis. Identification of novel bone-regulating genes by bioinformatics tools, and the validation of their function in vivo, can greatly enhance our understanding of bone biology, and in turn may lead to the development of new prevention approaches and treatments for bone-related disorders such as osteoporosis, osteogenesis imperfecta, etc.

P170

RNA-Sequencing analysis of peripheral blood monocytes from postmenopausal women with osteoporosis

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Osteoporosis is the most common bone metabolism disorder owing to an alteration between osteoclast and osteoblast activity. Monocytes are important cells in osteoporosis because they are osteoclast precursors and can secret osteoclast-specific cytokines relevant in bone metabolism. However, the role of monocytes in the pathophysiology of postmenopausal osteoporosis is still unclear. In this work, we used RNA-Sequencing to identify differentially expressed genes from peripheral blood monocytes between non-osteoporotic and osteoporotic postmenopausal Mexican women (n = 14), to elucidate new functional genes and pathways for postmenopausal osteoporosis at monocyte transcriptomic level. We identified 420 differentially expressed transcripts: 231 up-regulated and 189 down-regulated genes in osteoporotic women (Log2 FC = ± 1, $p < 0.05$). Biological pathway analysis showed WNT/β-catenin signaling cascades in tissue homeostasis, cell differentiation, and organogenesis as significant physiological pathways ($p < 0.05$). Wnt/β-catenin signaling is a key regulator of bone homeostasis since it drives osteoblast differentiation by promoting commitment and differentiation of mesenchymal stem cells towards the osteoblast lineage, while simultaneously decreases osteoclast differentiation by stimulating the production and secretion of osteoclast-specific cytokines important in bone metabolism and in osteoporosis. Consistently, disease ontology enrichment analysis pointed out "osteoporosis" as a disease associated with the data provided ($p < 0.01$). Additionally, by conducting an in silico analysis with a microarray independent dataset (n = 12), three genes (*ERAP1, STAT1,* and *SLITRK4*) were found to be down-regulated (Log2 FC = ± 1.5, $p < 0.05$). This RNA-Seq study in monocytes of postmenopausal osteoporosis, provided novel findings at gene and biological process levels and showed a landscape of the transcriptional regulation, which can be useful for the management and better understanding of postmenopausal osteoporosis in Mexican population. The Research Ethics Committees of the IMSS and the INMEGEN approved the study protocol and informed consent forms were signed by all women register in this study.

Keywords: monocyte, osteoporosis, RNA-Sequencing, genomics.

P171

A novel splicing mutation in LRP5 in idiopathic osteoporosis causes RANKL upregulation via noncanonical Wnt pathway

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Introduction: Primary or Idiopathic Osteoporosis (IO) in young adults is rare. Variants of several genes have been reported to be involved in the bone fragility.LRP5 is a co-receptor of the Wnt/beta-cateninof Wnt signaling pathway (also called canonical), and its disruptionalters this signaling pathway. We have found a novel splicing LRP5 variant, NM_002335.3: c.1413-2A > G at heterozygous level, in two independent patients, the significance of which is unknown.

Aim: Our aim was to validate the pathogenicity of c.1412-2A > T variant of Lrp5 and to study the impact of the variant on Wnt pathways.

Materials and methods: Targeted NGS sequencing panel of genes involved in osteoporosis was performed. Functional studies based on transcriptional and Western-blot analyses were realized from immortalized lymphocytes for the patient and controls. The experiments were performed with and without Wnt3a and Wnt5a ligands. A signed consent form was obtained for all experiments.

Results: Functional studies revealed a skip of the exon 7 and a creation of a stop codon at the end of exon 6. The variant c.1413-2A > G resulted in an increase of AXIN2 gene expression without wnt3a (42fold) and with wnt3a (153 fold) as compared to controls. The protein expression of AXIN2 was also increased (+50%). Also, RUNX2 mRNA level was increased in presence of Wnt5a (33fold) but its level was not modified in presence of Wnt3a, RANKL mRNA levels was decreased in presence of Wnt3a (- 80fold), but the high levels were maintained in presence of Wnt5a.

Conclusions: Intronic variant c.1412-2A > T in *LRP5*was responsible for a disequilibrium between Wnt canonical and non-canonical pathways. This likely led to an overproduction of RANKL via the activation of the non-canonical pathways which in turn promoted bone resorption. These data highlight the functional effect of LRP5 novel variant that involves bone resorption and formation.

P172

Zebrafish crispants recapitulate BMD decrease observed in lrp5 KO, indicating they may be used for screening of BMD candidate genes

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Objectives: In recent years, genome-wide association studies (GWAS) have revolutionized the understanding of the genetic architecture of common, complex diseases such as osteoporosis. This

approach reveals hundreds of candidate genes which may be involved in the mechanisms of disease. What is needed for post-GWAS exploration is a fast and reliable screen of candidate genes. One of the genes that came up in GWAS for bone mineral density (BMD) was LRP5, a co-receptor in the Wnt-signaling pathway, which controls differentiation and proliferation of osteoblasts. In humans, various LRP5 mutations were shown to affect bone mass. Our lab established a Zebrafish osteoporosis model by *lrp5* knockout (KO) and showed it had reduced notochord ossification at 7 days post fertilization (dpf) and lower BMD at adulthood. Here we aimed to evaluate a contribution to candidate gene screening strategy, based on zebrafish "crispants" (CRISPR-derived F0 mutants) of *lrp5*, a well-established bone effecting gene.
Methods: CRISPR-Cas9 was used to create *lrp5* crispants: one cell stage zebrafish were injected with Cas9 protein and *lrp5* gRNA. At 7 dpf crispants were stained with *alizarin red*. Notochord ossification in each crispant was analyzed using Fiji software, and the correlation between ossified area of the notochord and genotype (the latter is expected to be mosaic) was established.
Results: We found that *lrp5* crispants had the same notochord ossification level as injected control and WT fish (p-value > 0.05) at 7dpf.
Conclusions: In this study, we show that, unlike stable KO line, *lrp5* crispants do not demonstrate less ossification of the notochord compared to controls. These results are in accordance with the fact that crispants are mosaic- carrying various mutation which may have different effect on ossification. In addition, according sequence analysis, most of the crispants still possessed the intact WT allele, allowing the translation of WT lrp5 protein.

P180

Changes in the microstructural and mechanical properties in the medial condyle of human distal femur in advanced osteoarthritis

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Objectives: The purpose of this study is to analyze and compare the micro-structural and mechanical properties of subchondral trabecular bone of non-osteoarthritic and osteoarthritic distal femur using micro-images based on finite element analysis.
Materials and methods: Twenty distal femurs were harvested from 10 cadavers. The subchondral trabeculae were obtained from the middle of the articular surface of the medial femoral condyle of distal femurs. A total of 20 specimens were scanned using the micro-CT system. Micro-CT images were converted to micro-finite element model using the mesh technique, and micro-finite element analysis was then performed for assessment of the mechanical properties.
Results: According to the results, trabecular bone of osteoarthritic distal femur showed a decrease in trabecular thickness, bone volume fraction, structure model index, and yield stress and an increase in trabecular separation and structure model index.
Conclusions: Results of bone morphometry index and strength showed greater deterioration of microstructure and decreased mechanical strength in subchondral trabeculae of the osteoarthritic group.
Keywords: Trabecular bone, osteoarthritis, FE-model, Micro-CT

P181

Aloe-emodin inhibits osteogenic differentiation and calcification of mouse vascular smooth muscle cells

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Vascular calcification increased the risk of morbidity and mortality in patients with cardiovascular diseases, chronic kidney diseases, and diabetes. Aloe-emodin (AE), anthraquinone is a natural compound found in the leaves of Aloe-vera. Here, we examined the effects of AE on VSMCs calcification and the underlying mechanism by treating with calcification media (Ca^{2+}). AE repressed the phenotypes of Ca^{2+} induced calcification, as determined by calcium deposition level, Alizarin Red, and Von Kossa staining. Similarly, AE attenuated Ca^{2+} induced calcification by inhibiting osteoblast differentiation markers genes such as RUNX-2, SMAD4, OSX, collagen 1α and OPN. Likewise, AE suppressed Ca^{2+} induced protein expression of BMP-2, RUNX2, collagen 1α, OPN and SMA. Furthermore, AE significantly inhibited the calcification of ex vivo ring formation in murine thoracic aorta, and markedly inhibited vitamin D_3 induced medial aorta calcification in vivo. Altogether, our finding demonstrates that AE has important role in fine-tuning the vascular calcification program.

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The features and distribution of chronic non-bacterial osteomyelitis in Russian Federation

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Background: Data about incidence and prevalence of chronic non-bacterial osteomyelitis (CNO) in Russia is scarce. The aim of our study was to evaluate clinical features and prevalence of CNO in Russia.
Materials and methods: The diagnosis of CNO was made with criteria, proposed by Jansson (2007, 2009), after the exclusion of other causes of bone disease. Our cohort consists of three main subtypes: i) early-onset (< 5 years) CNO (n = 17); ii) CNO, associated (n = 20) and iii) not associated (n = 59) with rheumatic diseases (RD).
Results: The patients with early-onset (< 5 years) CNO characterized by 1) all children were initially diagnosed as having tuberculosis (TB) due to bone morphology findings (granulomatosus, e.g. tuberculosis-like inflammation), but had negative TB culture test; 2) initial treatment with combination of 3–4 anti-MBT drugs during 1–2 years was ineffective, patient continued to form new inflammatory bone foci; 3) patients had more severe clinical and radiological signs of disease, compare to others and 4) all patients have North Caucasus origin. Data in the table.

Parameter	EO-CNO (n = 17)	"CNO w/o RD" (n = 59)	CNO with RD (n = 20)	p
Onset age, years	3.0 (2.1–4.8)	7.3 (2.8–11.7)	10.3 (8.2–12.2)	0.0009
Gender, females	8 (47.1)	27 (45.8)	14 (70.0)	0.17
Foci number	5.0 (1.5–6.0)	3.0 (1.0–4.0)	2.0 (1.0–6.0)	0.048
Symptomatic arthritis	15/16 (93.8)	33 (55.9)	17 (85.0)	0.003
North Caucasus origin	17 (100.0)	0 (0.0)	0 (0.0)	<0.00001
Granulematosus inflammation (tuberculosis-like)	17 (100.0)	0 (0.0)	0 (0.0)	<0.00001
Prevalence of CNO	1: 55,000	1:450,000	1:1,375,000	<0.00001

[Characteristic of the patients with CNO.]

Conclusions: We have found the unique regional subtype of CNO in North Caucasus region with at least 9 times higher prevalence.

This work supported by the Russian Foundation for Basic Research (grant № 18-515-57001) and by Japan Medical Research Foundation (grant № 18jmrf001).

Keywords: Chronic non-bacterial osteomyelitits, children

P183

Description of a sandwich ELISA to quantify human leucine-rich α-2 glycoprotein 1 in serum and plasma

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Objectives: Leucine-rich α-2 glycoprotein 1 (LRG1) is a highly glycosylated 38 kDa protein. It belongs to the leucine-rich repeat protein family whose members are involved *e.g.* in protein–protein interactions, cell signaling, or cell adhesion. It is described as proangiogenic factor and increased levels are reported in various diseases where neovascularization is involved. For instance, it was shown that LRG1 induced by TNF-α promotes angiogenesis that then contributes to aberrant bone formation in the subchondral bone of osteoarthritis patients.

Methods: For the quantification of human LRG1 we developed a sandwich ELISA assay based on a peptide-specific coating antibody and a polyclonal detection antibody. Antibodies were characterized by epitope mapping of linear epitopes with microarray technology, and by the determination of binding kinetics with biolayer interferometry. Assay parameters like precision, specificity, dilution linearity, and spike recovery were assessed, and samples from apparently healthy subjects were measured.

Results: The peptide-specific coating antibody binds to a linear epitope in the N-terminal region of LRG1. Multiple linear epitopes recognized by the polyclonal detection antibody are distributed over the whole LRG1 sequence and are located in the N- and C-terminus, as well as within the leucine-rich repeats. Both antibodies bind to LRG1 with low dissociation rate constants. The assay detects LRG1 in human serum and plasma samples (heparin, EDTA, citrate). Assay parameters meet the international standards of acceptance.

Conclusions: This ELISA provides a reliable, accurate and well characterized tool for the quantification of LRG1 in healthy and diseased serum and plasma samples. It can be applied to investigate the role of this biomarker in bone disease.

Keywords: Leucine-rich α-2 glycoprotein 1, LRG1, ELISA, Biomarker

P185

Potential of freeze-dried human dental pulp derived stem cell conditioned media on the healing of inflammatory alveolar bone loss

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Freeze-dried form of stem cell-derived conditioned media (CM) provides therapeutical advantages including safety and long storage. However, there is little information regarding bio-activities and in vivo efficacy of freeze-dried CM. This study aimed to investigate the healing potential of freeze-dried CM on inflammatory bone disease. CM was obtained after culture of human dental pulp stem cells (hDPSC) on tri-dimensional (3D) collagen sponge or two-dimensional (2D) culture plate in serum-free media for two days. The inflammatory alveolar bone destruction was induced by weekly three times periodontal injection of lipopolysaccharide (LPS) for two weeks. Then, CM effect was evaluated by single topical injection of each CM at the same point of LPS injection in comparison with corresponding hDPSC injection. Total protein amounts showed 2.4 fold ($p < 0.01$) greater value of 3D-CM, compared to 2D-CM, but freeze drying method led to half yield of protein amounts in both CMs. Unique results were observed in the inhibition of both CMs on the inflammatory responses of macrophages. 3D-CM more effectively inhibited the expression of inflammatory cytokines (TNF-α and IL-1β) and inflammatory macrophage M1 marker (all, ($p < 0.01$) by in the presence of LPS while it increased anti-inflammatory macrophage M2 marker ($p < 0.01$). Healing efficiency on LPS- induced alveolar bone loss was in the order of 3D-CM, hDPSC, 2D-CM, among which 3D-CM alone showed a significant increase in bone mass ($p < 0.01$) and density ($p < 0.01$) over LPS group and even sham group. Immunohistochemical staining for M1 marker was reduced These results showed that the freeze-dried CM had anti-inflammatory capacity and was more effective than hDPSC injection in the healing of the inflammatory bone loss when prepared by 3D culture, suggesting that the freeze-dried 3D-CM would be a promising therapeutic treatment of stem cell therapies for inflammatory bone loss.

P186

Preclinical diabetes accelerates onset of Osteoarthritis—lessons from model system

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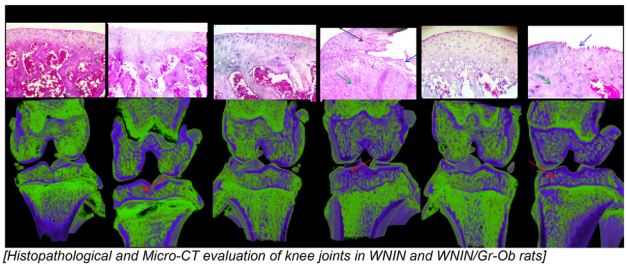
Objectives: Osteoarthritis (OA) is the most common joint disorder, affecting about half of the aged population worldwide. Multiple risk factors including obesity, ageing, elevated lipids, local/systemic inflammation predispose to OA. Metabolic syndrome—a congolmerate of interrelated metabolic risk factors has been implicated in the pathogenesis of OA. Interestingly, WNIN/Gr-Ob mutant rat strain (Muts) developed indigenously at our centre, exhibits preclinical diabetes-like alterations (Obesity, IR, IGT and higher BMI) with age.

We therefore aim to explore the potential application(s) of these Muts model in OA research to study metabolic and structural alterations (knee joints) with age akin to human OA.
Methods: Knee joints were harvested from female Muts aged 3, 6 and 9 months old and evaluated for OA-like changes using radiography, micro-CT and histopathology and compared against their age-matched Wistar controls (WNIN).
Results: Radiographic assessment showed ossification of soft tissues, osteophyte formation (6 months), subchondral sclerosis and bone cyst (9 months) in Muts. Micro-CT studies revealed significant reduction in subchondral trabecular bone porosity (red arrow) in Muts (6 and 9 months) implying subchondral sclerosis. Histopathological evaluation showed cartilage degeneration (blue arrow), subchondral sclerosis (green arrow), osteophyte (black arrow), bone cyst in Muts (6 and 9 months)—hallmark features of human OA (Ethical clearance obtained).
Conclusions: Our findings advocate for the potential application of these Muts as a befitting rat model in the field of OA research en route the 'natural progression' and spontaneous generation of OA-like changes correlating with human OA.
Obesity, Osteoarthritis, WNIN/Gr-Ob, Animal model, Metabolic Syndrome


[Histopathological and Micro-CT evaluation of knee joints in WNIN and WNIN/Gr-Ob rats]

P187

The SoxC family transcription factor Sox4 plays a role in osteoarthritis onset by up-regulating ADAMTS4 and ADAMTS5

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Objectives: Osteoarthritisis a common disease affecting joint cartilages. The molecular pathogenesis of osteoarthritis has not been fully understood. Therefore, early diagnostic markers and effective therapeutic agents for osteoarthritis have not been developed. In order to understand the molecular mechanisms of osteoarthritis onset, we attempted to identify transcription factors involved in osteoarthritis onset.
Methods: Superficial zone (SFZ) cells of articular cartilage were treated with retinoic acid (RA) exerting catabolism in cartilage, and subsequently microarray analysis was performed to identify the transcription factors. The identified transcription factors were introduced into C3H10T1/2 cells and SW1353 cells, and the effects on ADAMTS4 and ADAMTS5 expressions were analyzed by RT-qPCR, luciferase and ChIP analyses. Additionally, the effects of the transcription factors were assessed in mouse articular cartilage organ culture system. Finally, expressions of the identified transcription factors were determined in human OA cartilages by performing RT-qPCR..
Results: Microarray analysis revealed that Sox4, a SoxC transcription factor member, is increased about sixfold by RA treatment in SFZ cells. Overexpression of Sox4 or Sox11, another SoxC transcription family member, induced Adamts4 and Adamts5 expression. Luciferase and ChIP analyses also indicated that Sox4 bound to the ADAMTS4 and ADAMTS5 gene promoters and stimulated these gene promoter activities. Sox11, evoked similar effects. Furthermore, introduction of Sox4 adenovirus into the femoral head cartilages showed an increase in Adamts5 expression and articular cartilage destruction. Most importantly, in articular cartilages from human OA patients, mRNA expression of Sox4 and Sox11 was increased along with the degree of joint destruction severity of cartilage degeneration.
Discussion: Sox4 and Sox11 are involved in pathogenesis of osteoarthritis through regulating ADAMTS4 and ADAMTS5 expressions.

P188

Association of T-cell and B-cell aberrancies with disease activity in rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis

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Background: Despite the known autoimmune pathogenesis, the association of the specific T-cell and B-cell aberrancies for a particular rheumatic disease (rheumatoid arthritis (RA), ankylosing spondylitis (AS) and psoriatic arthritis (PsA)) have not yet been fully elucidated.
Methods: Mononuclear cells were isolated from peripheral blood of CTRL (n = 43), RA (n = 42), AS (n = 43) and PsA (n = 42) patients (upon Ethical approval and informed consent). Using flow-cytometry, we identified subpopulations of T-cells: Th1 (CD3 + CD4 + CCR4-CCR6-), Th2 (CD3 + CD4 + CCR4 + CCR6-), Th17 (CD3 + CD4 + CCR4 + CCR6 +), Tc (CD3 + CD8 +); B-cells: naïve (CD19 + IgD + CD27-), unswitched memory (UM, CD19 + IgD + CD27 +), class-switched memory (CSM, CD19 + IgD-CD27 +), double-negative memory (DNM, CD19 + IgD-CD27-) and plasmablasts (CD19 + IgD-CD27 + CD38 +); in addition to activation (CD86) and maturation (CD32) markers, and correlated their frequencies with the disease activity: DAS28 (Disease Activity Index including 28-joint count), ASDAS (AS Disease Activity Score), BASDAI (Bath AS Disease Activity Index). Functional tests included mitogen (PMA/ionomycin)-induced lymphocyte proliferation (Cell Proliferation Dye eFluor670) and activation (CD69). In addition, culture supernatants were used to test the osteoclastogenic response of peripheral monocytes stimulated by RANKL and M-CSF.
Results: Th1 were increased in RA in comparison to CTRL ($p = 0.03$). CD32 was increased on naïve ($p = 0.042$), UM ($p = 0.042$), DNM ($p = 0.07$) from AS, and plasmablasts from RA ($p = 0.026$). In RA, Th17 correlated with aCCP (rho = 0.898, $p = 0.002$) and RF (rho = 0.746, $p = 0.021$), DNM B-cell with RF (rho = 0.854, $p = 0.007$), and CD32 + naïve B-cell with DAS28 (rho = 0.899, $p = 0.015$). In AS, CD32 + DNM B-cells correlated with BASDAI (rho = 0.721, $p = 0.019$) and ASDAS (rho = 0.794,

p = 0.006). In PsA, CD86 + CSM B-cells correlated with DAPSA (rho = 0.786, p = 0.021) and DAS28 (rho = 0.757, p = 0.049). In AS, lymphocytes showed increased activation and proliferation. Finally, T-cell supernatant of RA patients enhanced osteoclastogenesis in vitro (84[52–90] in RA vs. 56[42–62] in CTRL).

Conclusions: The association of the specific lymphocyte subset with a particular rheumatic disease may indicate the role in arthritis pathogenesis and possible usage as a disease marker.

P189

Effects of a ketogenic diet on the progression of osteoarthritis in obese mice

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Osteoarthritis (OA) is prevalent among the obese population, where low-grade metabolic inflammation and a damaging adipokine profile accompany weight-related joint overload.

Histone deacetylase (HDAC) inhibitors have shown the ability to slow-down OA progression in vitro and in vivo, while downregulating protease expression.

beta-hydroxy-butyrate (BHB) is a ketone body whose plasma level increases following a low carb/high fat ketogenic diet (KD). BHB affects histone modifications and gene expression in vitro and in vivo, acting as an HDAC inhibitor.

As KD could simultaneously induce weight-loss, decrease metabolic inflammation and increase BHB circulating levels, it might be beneficial in obesity-linked OA treatment. To directly test this hypothesis, we evaluated the impact of KD in a murine model combining obesity and osteoarthritis (12127-2017110911058255v2).

Obese mice on a high fat diet (HFD) received knee medial meniscus destabilization at week 16 to induce OA, then were fed one of three diets ad libitum: HFD; KD; Control Diet (CD). 8 weeks later animals were killed and organs collected.

BHB levels increased tenfold in KD group only (0.1 mM for all groups before diet switch, 1.6 mM upon switch to KD; $p < 0{,}001$). Glycemia remained high in HFD (10.2 mM), but decreased in CD (8.2 mM) and even more in KD (6 mM)($p < 0{,}001$). HFD mice continued to gain weight, while CD and KD lost weight. The Kondziela test showed no variation in muscle strength over the 8-week treatment in CD and KD and lower performance in HFD group. MMP13 expression in cartilage of CD was lower than in HFD and even lower in KD (twofold, p = 0.005).

Our results validated the efficacy of KD to induce a raise in BHB levels and weight loss in obese OA mice, without a negative impact on muscle function. We can now further appraise its effects on osteoarthritis in this model.

Keywords: Osteoarthritis, obesity, ketogenic diet, BHB, epigenetics

P191

Development of a novel biologic agent for treating RA

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Rheumatoid Arthritis (RA) is a chronic inflammatory autoimmune disease characterized by serious synovitis accompanying cartilage and bone erosion. Chimeric protein of the extracellular domain of CTLA-4 with IgG1 Fc region (CTLA-4Ig) is a unique biologic clinically used as abatacept. Although CTLA-4Ig shows efficacy in RA patients showing poor responses to a neutralizing antibody for an inflammatory cytokine, the proportion of clinical remission achievement is not yet high. Prevention of joint bone destruction is also not enough. Here, we constructed a chimeric protein construct of CTLA-4Ig fused with anti-RANKL scFv at the C-terminal (CTLA-4Ig $\sim$ scFv) to improve the pharmacological effect of CTLA-4Ig. Anti-RANKL scFv was obtained by screening a phage library displaying randomized scFv. In vitro assay using ELISA confirmed that the binding affinity of CTLA-4Ig $\sim$ scFv to CD86 extracellular domain is about 5 nM, which is comparable with that of CTLA-4Ig. The binding affinity of CTLA-4Ig $\sim$ scFv to RANKL extracellular domain was about 1 nM. Then, we compared the curative effect of CTLA-4Ig $\sim$ scFv with that of CTLA-4Ig using collagen-induced arthritis (CIA) mouse model. Mice with all the paws starting to swell at day 9 after the second immunization were divided randomly to 3 treatment groups; CTLA-4Ig (n = 18), CTLA-4Ig $\sim$ scFv (n = 19) and vehicle (n = 18). Each mouse was administered with CTLA-4Ig 200 μg/day, CTLA-4Ig $\sim$ scFv 200 μg/day or vehicle from day 10 to day 16. At day 17, mice were sacrificed and frozen thin-sections of hindleg knee joints were prepared. Histological assessments of cartilage and bone destruction were performed. CTLA-4Ig $\sim$ scFv significantly reduced ($p < 0.01$) mature osteoclasts formation compared to the vehicle group, while the effect of CTLA-4Ig was not significant. As for the cartilage erosion, tendency of reduction in CTLA-4Ig $\sim$ scFv treated group was observed but the difference was not significant. Collectively, introduction of anti-RANKL scFv to CTLA-4Ig improved the curative effect in CIA mouse model.

Keywords: RA, biologics, RANKL

P195

Metatarsal open fracture model in rats for in vivo investigation of secondary bone healing

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Objectives: The open and close fracture model frequently utilized for laboratory investigation are associated with varying degree of complications ranging from high degree of fracture comminution to severe associated soft tissue injury which interfere with fracture healing. This study aimed at developing an improved quality and reproducibility of an experimental open fracture model in rat metatarsal with minimal complications.

Methods: Standard open mid shaft transverse metatarsal fracture was produced with bone cutting forceps in 28 rats. The study was approved by the Institutional Animal Care and Used Committee of

Universiti Putra Malaysia (R028/2015). Six weeks old rats, weight range between 190 and 220 g were used. They were housed and feed according standard guideline of IACUC. Aanaesthesia was achieved using intramuscular injections of ketamine and xylaxin at respective dose of 70 and 7 mg kg^{-1}.Tramadol HCl was administered subcutaneously at 4 mg kg^{-1}e for analgesia. The open fracture was created at the left metatarsal in all the rats. The fracture complications, pattern of fracture produced, fracture consolidation, histological and radiographic healing of the fracture were assessed and evaluated.

Results: The fracture produced in the mid metatarsal shaft of all rats was 100% transverse, 73% located at mid shaft, no fracture comminution was observed. Mild fracture angulations were recorded. Minimal soft tissue injury was recorded immediate post surgery but, but no infection and delayed union observed. Varying degree of weight bearing lameness was also recorded but seize at day sixth onwards post operative. Callus index observed was peaked at week 2 and 3 but declined at week 7^{th} during consolidation period. The fracture line disappeared completely at week seven postoperative. There was positive correlation between the histological and radiographic healing scores.

Conclusions: The metatarsal open fracture model could be considered to be a suitable model for in vivo study of secondary fracture healing.

P196

Activation of pro-apoptotic caspases in zebrafish skeletogenesis

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Caspases belong to a family of highly conserved aspartate-specific cysteine proteases best known for their critical roles in mediating apoptosis and inflammatory responses. As transducers and executioners of programmed cell death, caspases are especially important during development, for the elimination of unwanted cells contributes to tissue morphogenesis. More recently, caspases also emerged as mediators of non-apoptotic signalling associated with several cellular and physiological functions. This includes skeletal development.

The most common species to investigate novel functions of caspases in vivo is the mouse. Here we focused on another model organism, the zebrafish (*Danio rerio*), attractive for skeletal research because of accessible and transparent developmental stages. As the information about caspases in zebrafish is scarce with focus on pre-osteogenic embryonic stages, we performed a basic screening of pro-apoptotic caspase activation during skeletal development.

Presence of cells positive for individual caspases was evaluated by immunohistochemistry. So far, we detected executioner caspases (caspase-3, − 7) during early embryonic development in the cartilage of the pharyngeal arches (caspase-7) and in the neurocranial cartilages (caspase-3). The initiator caspase-9 was found in the developing cartilage of the pharyngeal arches and in the brain cells. More recent data from this ongoing study and correlations of active caspases with apoptotic cells detected by TUNEL assay will be presented at the meeting.

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P197

Deletion of *SREBF1*, a functional bone-muscle pleiotropic gene, alters arachidonic acid-related lipid signaling in zebrafish

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Recent bivariate analysis for bone mineral density and lean mass identified several genomic loci as having an effect on both traits. A signal on 17p11.2 included Sterol Regulatory Element-Binding Factor 1 (*SREBF1*). *SREBF1* codes for SREBP-1 protein, a transcription factor also known as an adipocyte differentiation factor; its role in lipid homeostasis could be essential for understanding its pleiotropic functions on both muscle and bone.

We established a zebrafish *SREBF1* knockout (KO) model by CRISPR-Cas9 technology (R23 fs allele).

We used dorsal muscles of adult KO for *Lipidomics Profiling* performed by liquid chromatography-tandem mass spectrometry using targeted method for profiling and quantification of Lipid Mediators (LMs). We profiled 48 LMs derived from various essential polyunsaturated fatty acids (PUFA) to determine potential targets regulated by *SREBF1*. Ratio of ω-6 PUFA derivatives (arachidonic acid, AA) to ω-3 PUFA derivatives (eicosapentaenoic acid, EPA) in fish was 1:4.93 in males and 1:5.69 in females. After adjusting for sex and weight, we found that the levels of 11,12-epoxyeicosatrienoic acid (11,12-EET) were linearly associated with the number of R23 fs alleles.

We also compared gene expression between KO and WT zebrafish by genome-wide RNA-seqGenes were considered to be differentially expressed if the false discovery rate (FDR) was less than 0.05 and there was at least twofold difference in expression.. Significantly enriched pathways included *Fatty acid elongation, Linoleic acid metabolism, Arachidonic acid metabolism, Adipocytokine signaling*, and *DNA replication*.

In conclusion, KO of *SREBF1* affects lipid-signaling mediators rather specifically. EETs, metabolites of arachidonic acid through cytochrome P450/epoxygenase, play an important role in insulin-mediated augmentation of microvascular blood flow in skeletal muscle. EETs attenuate inflammatory signaling, while enhance adipocytokine signaling, implying that their modulation could have major therapeutic potential in the treatment of musculoskeletal disease. These findings could lead to new understanding of *SREBF1* biology for musculoskeletal homeostasis.

P198

Characterising the interplay between inflammation and skeletal remodelling during fracture repair in zebrafish

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Fracture risk increases with age and with reduced bone density. Increased fracture risk is observed in the metabolic bone disease osteoporosis which affects more than 27 million people in the EU. Immune cells play a key role in maintaining skeletal homeostasis throughout life. However, the role of inflammation in regulating the

response to skeletal injury and subsequent remodelling is less well understood.

Zebrafish are an excellent model organism for studying skeletal disease as they are genetically amenable, most disease pathways are highly conserved, and they offer excellent imaging possibilities. Zebrafish caudal fins contain up to 400 bony segments; fractures can be introduced into these segments and the processes surrounding skeletal remodelling dynamically imaged.

Using Alizarin red staining to visualise bone, we observed the presence of bone calluses resulting from spontaneous fractures in the fin bones of aged (> 2 years), but not young (< 1.5 year) wild type (wt) zebrafish. To understand fracture accumulation in older fish we induced fractures in wt fish crossed into transgenic reporters for macrophages, neutrophils, TNFalpha, Wnt signalling and skeletal reporters for osteoblasts and osteoclasts. We observe recruitment of immune cells by 2 h post injury, peaking at 2 days post-injury (d.p.i.) for neutrophils, and at 7 d.p.i for macrophages. Inflammation peaks from 1 to 2 d.p.i., where TNFalpha colocalises with both macrophages and bone. Skeletal remodelling is slower, with new bone formation observed (Calcein labelling) by 7 d.p.i.

We have also identified a mutant with reduced bone density, which has significantly increased susceptibility to spontaneous fracture. Preliminary analyses of bone remodelling suggests that this mutant accumulates bone calluses similar to those seen in aged wt fish as early as 6 months old. Using computational modelling and induced fracture experiments in zebrafish, we are uncovering the biological and biomechanical factors underpinning fracture susceptibility.

Keywords: Zebrafish, bone-density, osteoporosis, fracture, inflammation

P199

The curious case of bone pathology in Osx1-GFP::Cre mice

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Osx1-GFP::Cre, Tet-Off mice are commonly used for osteoblast-specific knockout or overexpression of different genes despite bone abnormalities reported in these animals. To manipulate gene expression at early stages in osteoblast differentiation in a G610C mouse model of osteogenesis imperfecta, we crossed heterozygous G610C ($G610C^{+/-}$) with hemizygous Osx1-GFP::Cre ($Osx1\text{-}GFP::Cre^{+/-}$) animals. Consistent with previous reports, we observed mild bone pathology (mostly undermineralization of the skull) in $G610C^{-/-}$; $Osx1\text{-}GFP::Cre^{+/-}$ pups, which was noticeable only during the first several weeks after birth. In contrast, all $G610C^{+/-}$; $Osx1\text{-}GFP::Cre^{+/-}$ pups were stillborn and had extreme scapula, clavicle and rib deformities, severe undermineralization of the skull, yet no pronounced long bone abnormalities in limbs. In animals treated with doxycycline until weaning to prevent Osx1-GFP::Cre expression, the subsequent activation of the transgene had positive rather than negative effects on bone, e.g., significantly increasing the amount of trabecular bone in distal femur of both $G610C^{+/-}$ and $G610C^{-/-}$ mice ($P < 0.001$). We argue that the lethal skeletal deformities in untreated mice resulted from a combination of cell stress caused by very high Osx1-GFP::Cre expression in differentiating embryonic osteoblasts with the cell stress caused by the G610C mutation. Apparently, lower Osx1-GFP::Cre expression in doxycycline-treated G610C mice after weaning did not produce cell stress and therefore did not affect bone pathology associated with the G610C mutation. The origin of the positive effects of the transgene on bone after weaning is presently unclear.

Keywords: Osteogenesis imperfecta, osterix-Cre, G610C mouse, Osx1-GFP::Cre mouse

P200

Mice lacking Plastin-3 display a specific defect of cortical bone acquisition

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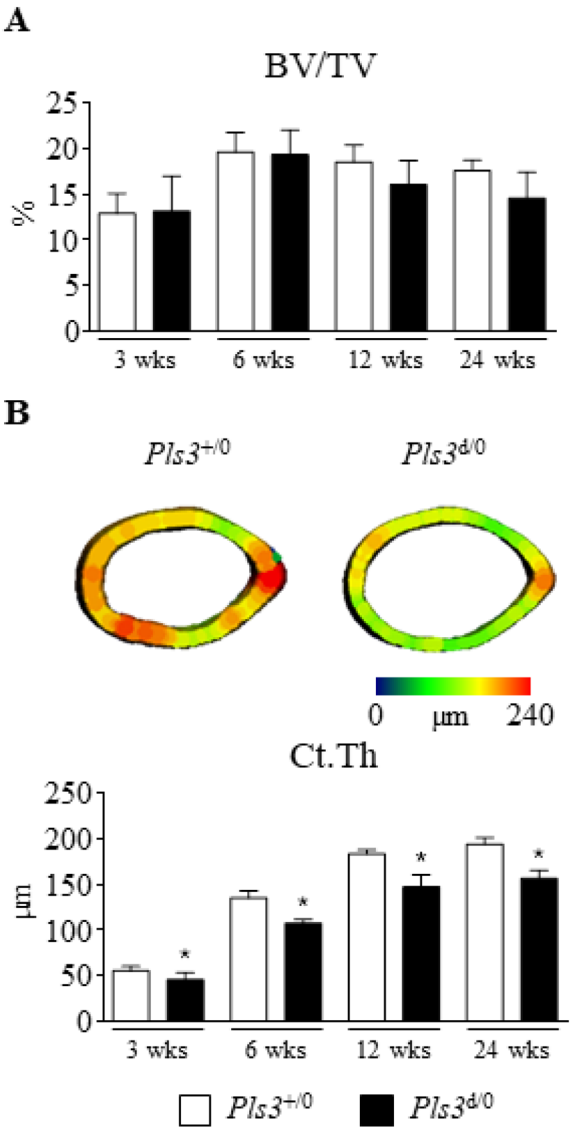
Although inactivating mutations of *PLS3*, encoding the actin-bundling protein Plastin-3, have been identified to cause X-linked osteoporosis, the cellular and molecular influence of Pls3 on bone remodeling is poorly defined. Moreover, although a previous study has demonstrated moderate osteopenia in 12 week-old *Pls3*-deficient mice based on μCT scanning, there is no reported analysis of such a model on the basis of undecalcified histology and bone-specific histomorphometry. To fill this knowledge gap we applied a deep phenotyping approach and studied *Pls3*-deficient mice at different ages. Surprisingly, we did not detect significant differences between wildtype and *Pls3*-deficient littermates with respect to trabecular bone mass (Fig.A). Remarkably however, the cortical thickness in the femur, was significantly reduced in *Pls3*-deficient mice in all age groups (Fig.B). We additionally studied the ex vivo behavior of *Pls3*-deficient primary osteoblasts, which displayed moderately impaired mineralization capacity and subtle changes of cell morphology. Of note, the expression of *Sfrp4* was significantly reduced in *Pls3*-deficient cultures (0.57 ± 0.08 r.e.), a potentially relevant finding, since Sfrp4 inactivation, in mice and humans, specifically causes cortical thinning. We finally investigated, if *Pls3*-deficiency would impair the osteoanabolic influence of parathyroid hormone (PTH). For that purpose we applied daily injections of PTH into wildtype and *Pls3*-deficient mice and found a similar response regardless of the genotype. Taken together, our data reveal that Pls3-deficiency in mice only recapitulates the cortical bone phenotype of individuals with X-linked osteoporosis by negatively affecting the early stage of cortical bone acquisition.

Keywords: Bone remodeling, Cortical bone, Plastin-3, X-linked osteoporosis



[Figure]

P201

Development of CRISPR/Cas9-based dual-fluorescent reporter mouse models to study in vivo single cell mechanomics of osteoblasts and osteoclasts

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Objectives: Refined in vivo models are needed to better understand mechano-molecular mechanisms crucial for bone adaptation and regeneration. Here, we employ CRISPR/Cas9 genome editing technology (plasmid, guideRNA, Cas9) to generate dual-fluorescent reporter mice for studying in vivo single cell mechanomics of osteoblasts and osteoclasts.
Methods: To label osteoblast (Integrin binding sialoprotein—*Ibsp*) and osteoclast-specific targets (tartrate-resistant acid phosphatase type 5—*Acp-5*; Calcitonin receptor—*Calcr*) with fluorescent proteins (eGFP, mCherry), we used CRISPR/Cas9 genome editing: Donor templates were designed (Fig. 1A) and inserted into puC57 vectors. GuideRNAs were selected based on specificity, efficiency, off-target predictions and distance from insertion site. After in vitro digestions, CRISPR/Cas9 reagents (guideRNA combinations: *Ibsp* + *Acp-5*; *Ibsp* + *Calcr*) were injected into C57BL/6 J zygotes and transferred to Swiss Webster recipient mice (approved by veterinary authorities).
Results: In vitro digestions of target-specific PCR fragments showed functioning of guideRNAs and Cas9 protein (Fig. 1B). After in vivo application, 12 and 13 pups were born for the co-injected guideRNA combinations (*Ibsp* + *Acp*; *Ibsp* + *Calcr*), respectively. PCR genotyping (Fig. 1C) identified 7 potential founders. For all targets, complete reporter integration at the intended locus was seen and confirmed by sequencing. So far, the animals do not show phenotypic constraints and are currently back-crossed to C57BL/6 J mice with ongoing further characterization (mRNA/protein expression, off-target assessment).
Conclusions: The dual-fluorescent reporter mouse lines will allow to rapidly identify osteoblasts and osteoclasts in samples from in vivo experiments (tail loading, femur defect loading) for spatially resolved single cell mechanomics during bone adaptation and regeneration.

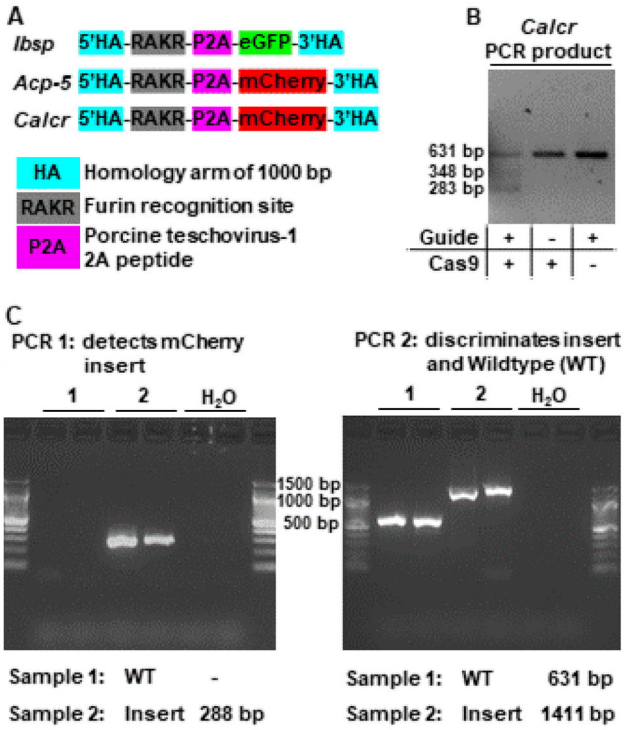

Figure 1. Fluorescence-labeling of osteoblasts (*Ibsp*) and osteoclasts (*Acp-5*, *Calcr*) using CRISPR/Cas9 genome editing. **A:** Target-specific donor plasmids. **B:** *In vitro* digestion of *Calcr* PCR products. **C:** *Calcr* genotyping PCRs.

[Development of CRISPR/Cas9-based dual-fluorescent reporter mouse models for bone cells.]

P203

Methods of diagnosis and treatment in a patient with calcific supraspinal tendinitis case report

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Objectives: The objective of the study is to bring to the attention of a 45-year-old patient presenting in the Clinical Rehabilitation Hospital for pain and significant limitation of left shoulder mobility in order to establish a diagnosis and appropriate recovery treatment.
Materials and methods: A 45-year-old patient is presented in April 2016 in our service after approximately 6 weeks of pain, without any connection to physical effort or trauma, combined with a significant reduction in active and passive mobility. In the joint evaluation active flexion 70°, passive flexion 90°; active abduction 10°, passive abduction 50° (with more pain on recovery); internal and external rotation practically impossible.
Radiographic changes of scapular-humeral periarthritis were described in the radiological examination of the left shoulder.

The ultrasound examination of the shoulder reveals the incomplete supraspinal tendon with a hypoecogenic area that changes the outline of the tendon and a syndrome of the subacromo-subdeloid conflict. Under the ultrasound guidance, the tendon is punctured and aspirated a white-yellow creamy, cretaceous, folowed by Diprophos and Xiline administration.

Morphopathological examination and electron microscopy have identified hydroxyapatite crystals.
Results: Two weeks after the ultrasound-guided puncture, where the patient followed physiokinetotherapy and anti-inflammatory drug therapy, pain and shoulder mobility improved significantly ($\alpha < 0.05$); active flexion without pain 90°, 180° with pain; active abduction 110°, passive abduction 140°.
Conclusions: The patient´s progression was favorable with pain relief and improved shoulder amplitude after a complex and appropriate treatment, ultrasound examination facilitating diagnosis and treatment.
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Keywords: Calcific tendinitis, ultrasound, physical activity.

P204

Sarcopenia feature selection and risk prediction using machine learning

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Purpose: To verify the usefulness of machine learning for selecting risk factors and developing predictive models for patients with sarcopenia.

Materials and methods: We collected medical records from Korean postmenopausal women based on Korea National Health and Nutrition Examination Surveys. Training data set was used to construct models based on popular machine learning algorithms such as support vector machines (SVM), random forests, and logistic regression (LR) based on simple surveys.
Results: A total of 4020 patients aged 65 years and over were enrolled in this study, including 1698 (42.2%) males and 2322 (57.8%) females. The 10 most important risk factors in men were BMI, RBC, BUN, vitamin D, ferritin, fiber intake (g/day), primary diastolic blood pressure, WBC, fat intake (g/day), age, GPT, niacin intake (mg/day), protein intake (g/day), fasting blood sugar, and water intake (g/day). The 10 most important risk factors in women were BMI, water intake (g/day), WBC, RBC, iron intake (mg/day), BUN, HDL, protein intake (g/day), fiber consumption (g/day), vitamin C intake (mg/day), PTH, niacin intake (mg/day), carotene intake (μg/day), potassium intake (mg/day), calcium intake (mg/day), sodium intake (mg/day), retinol intake (μg/day), and age. After ROC analysis, the AUC for each machine learning model was not significantly different for both genders.
Conclusions: The most cost-effective method in clinical practice is to make feature selection with random forest model and expert knowledge and to make disease prediction by verifying several machine learning models. However, validation of the prediction model developed through future studies is needed.
Keywords: Machine learning, feature selection, risk prediction, sarcopenia

P205

Running-exercise conditioning improves the healing of non-critical size bone defects in male Wistar rats

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This study aims to investigate the most osteogenic exercise-conditioning program for bone-defect repair in Wistar rats. We hypothesized that high-intensity-interval running will improve the healing of non-critical size bone defects.

Thirty male Wistar rats were divided in three groups (n = 10): Sedentary Control (SC), Continuous running (CR, 45 min at moderate speed) and Intermittent running (IR, 5 min at moderate speed, followed by 2 min of intensive running and 1 min of passive recovery). Training lasted for 8 weeks (5 days/week, 45 min/day).

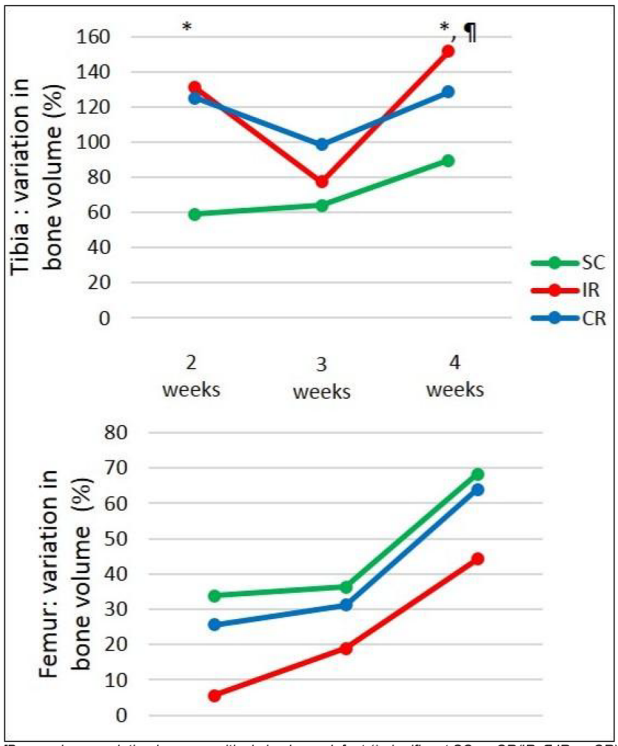
Then, a surgical procedure was performed (bone defect in the right tibia and femur). No exercise was performed during convalescence. The healing-tissue microarchitectural parameters were assessed once a week for 4 weeks by microCT, and serum alkaline phosphatase (ALP) by an enzymatic assay at 4 weeks. Experimental protocols were approved by the local Animal Ethical Committee.

Comparisons were made using a Kruskal–Wallis test, then a Mann-Witney test when applicable, with significance set at $p < 0.05$.

The percentage of bone-volume variation in the tibia was significantly higher in CE and IE at 2 and 4 weeks, compared to SC, and in IE compared to CE at 4 weeks (Fig 1). In CE and IE, this percentage was higher in the tibia than in the femur at 2 and 4 weeks. ALP dosages were similar in all groups.

Continuous and Intermittent exercise-conditioning improves the healing of non-critical size bone defects in Wistar rats. The tibia may be a better model than the femur to study bone repair.

High-intensity-interval exercise, bone repair, rat



[Bone volume variation in a non-critical size bone defect (significant SC vs CR/IR; ¶ IR vs CR)]*

P206

Modulation for extracellular matrix synthesis using follistatin gene synthesis in scar tissue fibroblasts from patients with flexor tendon adhesions

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Introduction: Tendon injuries of the hand require surgical repair to function properly. Excess scarring and adhesions to adjacent tissues after tendon repair is one of the common complications and compromise the natural gliding mechanics of the flexor tendons. Follistatin (FST), a single-chain glycosylated protein, binds and neutralizes TGF-b superfamily, and decreases inflammation and fibrosis. In this study, we hypothesized that overexpression of FST using a gene therapy approach will modulate excessive extracellular matrix (ECM) synthesis and alpha smooth muscle actin (a-SMA) expression in scar tissue fibroblasts from patients with severe scar adhesions after flexor tendon repair.
Methods: To test the anti-fibrotic effect of adenovirus-follistatin construct (Ad-FST), the cells from patients who underwent tenolysis surgery were utilized. After serum starvation, scar tissue fibroblasts were exposed to Ad-FST and cultured for 24 h. The mRNA expression levels of collagens and MMPs were analyzed. Various ECM proteins were estimated using Western blotting.
Results: Scar tissue fibroblastic cells with Ad-FST demonstrated a 30% and 20% reduction in collagen III and MMP-1 mRNA expressions respectively, a 70% increase in level of MMP-3 gene expression.($p < 0.05$) In addition, scar tissue fibroblastic cells with Ad-FST showed a 70% increase in TIMP 4 and phosphorylation of ERK protein expression. ($p < 0.05$) Also, scar tissue fibroblastic cells with Ad-FST demonstrated a 20% inhibition of fibronectin, PAI-1, a-SMA and phosphorylation of P38. ($p < 0.05$)
Discussion: In the current study, FST involves in the process of ECM remodeling by reducing the expression of collagen III and increasing the expression of MMP-3 and TIMP 1 in the scar tissue fibroblastic cells. Hence FST seems to affect collagen synthesis at the transcriptional and translational level and collagen degradation via MMPs and TIMPs in scar tissue fibroblasts from patients with tendon adhesions after tendon injuries.
Keywords: Follistatin, fibroblast, collagen III, MMPs, TIMPs

P209

Effect of a complex exercise programme on postural balance, endurance and falls in women with establilshed osteoporosis

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Based on evidences the successful decrease of the risk of fragility fractures in advanced osteoporosis can be accomplished if the antiosteoporotic medication has been combined with complex exercise programme.

Aim of our randomised, controlled study was to investigate the efficacy of a complex exercise programme, including traditional physical exercise´s elements, sensomotor and aerobic training with different progressivity levels among women with established osteoporosis. In terms of postural balance assessments, both performance-based and quantitative static and dynamic postural balance tests. Regarding the assessment of aerobic capacity, bicycle ergometric, and spirometric examinations were carried out.

Based on our study it appears that the intervention group which completed a sensomotoric balance training programme performed significantly better in keeping balance, confirmed by performance-based, static and dynamic posturometric tests.

There was no significant difference in the number of falls at the end of the one-year balance training programme, but there were half as many falls among participants of the intervention group as in the control group.

Significance of our study is that both performance-based and computer-based methods were used to evaluate the effect of a complex exercise programme on postural balance and staying power in women with advanced osteoporosis. It is also a novel that traditional strengthening exercises were combined with elements of proprioceptive posture training.

Results provided evidence that balance exercise programme combined with aerobic elements influenced simultaneously the muscular, respiratory, cardiovascular systems and resulted in improved aerobic endurance. This resulted in a more precise performance of the exercises, reduced completion time and improved participants´ mobility. rendering clinical significance for this study.

Our results confirm that exercise programmes, which aim to address reduced proprioception, to strengthen sensomotor function and aerobic capacity, in addition to strengthening muscles and endurance, are effective in reducing the number of falls owing to balance instability for established osteoporotic women.

P210

Physiotherapy of patients with bone tumor or metastatic bone disease

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Physiotherapy of cancer patients is one of the most controversial issues. Until now, physiotherapy was not suggested or only in strictly limited accessibility for those patients who had malignant disease. The majority of articles have reported beneficial effects on cancer patients and only few articles mentioned that certain electrotherapy treatment could be harmful.

Physiotherapy exercises are safe in case of bone metastasis or bone tumors, but it should be adjusted to the type and stadium of the tumor and the anticancer therapy.

During the physiotherapy special attention need to be paid to avoid falls and fractures.

Pathologic fractures frequently cause immobility. In these cases in order to keep the normal range of motion of the joints light exercises are recommended. Care should be taken to avoid loading the affected area in order to prevent trauma.

In bone metastases percutaneous electrical stimulation therapy has been reported to have a beneficial effect on opioid resistant pain. No side effect or tumor propagation was observed in any of the studies.

In multiple myeloma it is the activity of the disease that primarily determines the form of exercises. In plateau phase the best result is expected with intensive home made exercises. In contrast, for patients with advanced disease milder intensity, short termed exercises repeated frequently are suggested.

After bone marrow transplantation mobilization exercises are needed as soon as possible, but at the beginning intensity of the movements should be low.

Of course, each patient requires an individual assessment, however, if we exclude the possibility of tumor recurrence and metastasis, most of physiotherapy procedures can be used safely. In our presentation we would like to give an overview of the efficacy and safety of physiotherapy that can be applied in patients with bone-related tumors, based on the latest publications.

Keywords: Bone tumor, metastases, cancer, physiotherapy

P211

Effectiveness of occupational therapy in the management of femoral fractures in extended care units for elderly patients

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Objectives: From January to December 2018 103 elderly patients with femoral fractures have accessed this service. They have been assessed through a multifaceted (orthopedic-geriatric-rehabilitative) approach using MMSE, BADL, IADL, Barthel Index. Prior to fracture Barthel Index showed an average score ± 70/100.

Results: In 63 patients standing position recovery started 3 days after prosthesis due to femoral medial fracture. They were dismissed after a 15/25-day hospitalization. 34 elderly subjects recovering from osteosynthesis regained the sitting position in 2–3 days, load tests were made between 7 and 14 days and they left the unit 30/45 days after admittance. At discharge 21 subjects were moved to the Extended Care Unit where they followed an Occupational Therapy (OT) programme to recover autonomy: 1) teach how to shift while in bed; 2) teach how to leave the bed using the healthy side of one's body; 3) prescribe aids to improve ambulation; 4) teach how to take steps; 5) perform lower limbs mobilization through specific exercises in a joined team with the physiotherapist. The group including patients following the programme was then compared to that formed by 18 subjects not included in the same programme. A 2-month OT programme showed improvement of motor skills detected through scales scores (BADL 3.5/6 > 4.3/6—IADL 2.6/8 > 5.3/8 − Barthel Index 50/100 > 90/100) while control group scores were worse (BADL 3.4/6 > 4.0/6 − IADL 2.8/8 > 4–8/8 − Barthel Index 50/100 > 80/100).

Conclusions: Effectiveness of a OP programme in patients recovering from femoral fractures and osteosynthesis was evaluated. Comparing the BADL–IADL–Barthel Index scores at admittance and at discharge from the extended care unit, the aim of the integrated rehabilitative OT programme was to reestablish the functional condition prior to fracture.

Keywords: Occupational Therapy Femoral Fracture Elderly Patients

P213

Relationship between body composition analysis and fracture risk in middle-aged and elderly women living in Northern China

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Objectives: Adipose tissue is closely related to bone metabolism, and the relationship between obesity and bone health remains unclear. The purpose of this study was to investigate the relationship to fracture risk through body composition analysis.

Methods: We included 249 middle-aged men and women living in the community of Hohhot, including 46 males and 203 females, aged 40–90 years. Body composition and bone mineral density were measured using a dual-energy X-ray absorptiometry (DXA), and fracture risk was further analyzed by FRAX tool.

Results: The mean age was 62 years old, femoral neck bone mineral density was 0.62 g/cm^2 for females and 0.72 g/cm^2 for males. The risk of major fractures in the next 10 years was 6.8 for females, 3.2 for males, and hip fracture for the next 10 years was 2.7 for female and 1.3 for male. The percentage of female body fat was 36%, and the percentage of male body fat was 25%. The amount of lean tissue in males was higher than that in females, and the percentage of fat was lower than that in females. The fracture of the main part of the female was related to the age and the percentage of body fat, and was negatively correlated with the bone density of the femoral neck, the amount of lean tissue and the amount of lean tissue in the limbs, and the lean tissue index. Hip fracture was positively correlated with age, and was negatively correlated with femoral neck bone density, body lean tissue mass, and lean tissue mass in the extremities.

Conclusions: This results suggest that body composition contributes to the analysis of fracture risk, analyzes the characteristics of body composition analysis indicators, and investigates relevant factors for disease prevention and treatment.

Keywords: Postmenopausal women; Osteoporosis; bone densitometry; body composition; Northern China

P214

Relation between serum albumin and sarcopenia in osteoporotic hip fracture patients

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Introduction: The close relationship between muscle and bone had been called muscle-bone interactions which were appreciated as biomechanical stimuli, stress and strain forces applied to the skeleton influence on the change of both bone and muscle.

The muscle acts as a storage pool for serum albumin. Studies investigating the association between serum albumin and muscle measures are controversial and have reported inconsistent results, which might be explained using different muscle measures, study designs, demographic characteristics, and/or different adjustment models

The aim of the present study was to investigate the relationship between factors related to osteoporosis, sarcopenia, and albumin.

Methods: From November 2015 to August 2016, medical records and radiographs of patients who suffered from femoral neck fracture and intertrochanteric fracture were retrospectively reviewed. Muscle mass was measured using whole-body DXA. To adjust the muscle mass with height, appendicular lean mass (ALM)/height2 was used. Muscle strength was measured using Jamar dynamometer. Serum albumin was measured within 6 h from arrival at the emergency department. Regression analysis was used to analyze the effect of albumin-adjusted with vitamin D, the C-reactive protein (CRP) on grip strength, muscle mass, and sarcopenia.

Results: In hip fracture patients, serum albumin was positively associated with (ALM)/height2, grip strength, and diagnosis of sarcopenia ($p = 0.048$, $p = 0.045$, $p = 0.018$, respectively). However, the significance was weakened as Vitamin D and CRP were adjusted in the relation of serum albumin and grip strength (n.s.). The only positive association between serum albumin and diagnosis of sarcopenia has remained significant after adjustment of Vitamin D and CRP ($p = 0.026$).

Conclusions: Serum albumin was positively associated with the diagnostic criteria of sarcopenia according to the Asian Working Group in the hip fracture patients.

P215

Sarcopenia defined by bioelectrical impedance and handgrip strength analysis in elderly patients with new stroke or transitory ischemic attack

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Objectives: The aim of the study was to assess body composition, muscle strength, and prevalence of pre-sarcopenia and sarcopenia in patients suffering from new stroke of transitory ischemic attack (TIA).

Methods: The study group consisted of 36 patients (21 women and 15 men). The average age of patients was 75.1 ± 8.9 years. 6 patients (17%) were diagnosed with TIA. We performed bioelectrical impedance analysis (BMI) for the evaluation of body composition. Skeletal muscle mass (SMM) was calculated by the following equation: SMM (kg) = 0.566·Fat free mass. Skeletal muscle mass index (SMMI) was calculated as SMM (kg)/height (m)2. Muscle strength was measured using a Jamar hand dynamometer.

Results: The cutoff values for SMMI and grip strength were according to the European Working Group on Sarcopenia in Older People (EWGSOP) consensus. According to the study, the mean BMI was 26.8 ± 5.3. The weight mean was 85.7 ± 22.0 kg in men, 65.8 ± 13.7 kg in women. The mean fat mass value was 20 ± SD 9.9 kg, fat free mass mean was 53.5 ± 11.4 kg. Handgrip strength in men was 31.3 ± 14.6 kg, in women 15.8 ± 7.0 kg. According to the EWGSOP consensus 9 out of 21 women (43%) and three of 15 men (20%) had values lower than the cutoff. Mean SMM in men was 35.8 ± 4.7 kg, in women 26.3 ± 4.3 kg. SMMI in men was 11.79 ± 1.01 kg/m2 and 10.44 ± 1.49 kg/m2 in women. In our study no one met EWGSOP criteria for sarcopenia diagnosis, but three patients were diagnosed with presarcopenia. 12 patients demonstrated low handgrip strength. No one demonstrated presence of sarcopenic obesity according to NHANES III criteria.

Conclusions: Elderly patients with new stroke or TIA assessed with BMI and handgrip strength did not demonstrate high prevalence of sarcopenia in our study. High prevalence of low handgrip strength was not associated with low SMM or low SMMI.

Keywords: Sarcopenia, stroke, TIA, elderly, handgrip

P216

Influence of muscle strength and balance on quality of life in postmenopausal osteoporosis women

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Objective: The purpose of this study was to evaluate muscle strength and balance in postmenopausal osteoporosis women and to correlate them with quality of life.

Methods: 48 postmenopausal women (60.3 ± 6.7 years) diagnosed with osteoporosis were included in this cross-sectional study. The weight, height, menopausal duration were registered. Osteoporosis was confirmed by bone mineral density measurement, performed at the lumbar spine (L_2-L_4) and at the femoral neck, by the dual-energy X-ray absorbtiometry method. Knee extensor strength was measured by the isokinetical method at the angular velocity of 120°/s, using a Gymnex Iso 2 Dynamometer. Balance was evaluated by Timed Up and Go test (TUG), Chair Raising test (CRT), Berg Balance Scale (BBS). Quality of life was assessed by Qualeffo-41 questionnaire (Romanian version). Analyses of the relationships between strength and balance parameters and quality of life scores were done.

Results: TUG score negatively correlated with knee extensor strength ($r = -0.354$, $p < 0.05$) and positively correlated with Qualeffo-41 ($r = 0.582$, $p < 0.05$). Negative correlation was found between the CRT score and the knee extensor strengths ($r = -0.322$, $p < 0.05$), while positive correlation was found between the CRT score and the Qualeffo-41 Jobs around the house subscale ($r = 0.396$, $p < 0.05$). BBS score positively correlated with knee extensor strength ($r = -0.517$, $p < 0.05$) and negatively correlated with Qualeffo-41 ($r = -0.543$, $p < 0.05$). Qualleffo-41 also negatively correlated with knee extensor strength ($r = -0.318$, $p < 0.05$).

Conclusions: Muscle strength and balance significantly correlated with quality of life in postmenopausal osteoporosis women. These results suggest the importance of physical exercise in this category of patients, where rehabilitation programs should emphasize corrective balance and muscle strengthening in order to improve quality of life.
Keywords: Muscle strength, balance, quality of life, osteoporosis

P217

Importance of sonoelastography in prediction of Achilles tendon rupture

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Objectives: The concept of assessing the mechanical properties of the Achilles tendon after reconstruction using ultrasound shear wave elastography (SWE) has been recently introduced into the clinical practice. It was demonstrated that about 6% of patients with ruptured Achilles tendon experience the rupture of contralateral tendon in the future. Our main objective was to estimate the risk for rupture of contralateral tendon in patients who underwent surgical reconstruction of ruptured Achilles. Additionally, we proposed a novel questionnaire for assessment of healthy individuals and patients after Achilles tendon surgical reconstruction.
Methods and Materials: Twenty-four patients who underwent surgical repair of the ruptured Achilles tendon and twelve aged matched healthy controls were examined with ultrasound SWE, and elastograms were analyzed by using proposed new software. Functional outcomes were assessed with American Orthopedic Foot and Ankle Society (AOFAS) scoring system and subjective rating system which we introduced and validated.
Results: The stiffness of injured tendon was markedly decreased (by 42%, $P < 0.01$) compared to contralateral tendon of the same patient. Both AOFAS score and our novel subjective assessment scale positively correlate with ultrasound SWE values in ruptured Achilles tendons. The stiffness of contralateral Achilles tendons in patients was 23% lower than among healthy individuals ($P < 0.05$).
Conclusions: Irrespective of the lack of difference in the subjective feeling assessed by AOFAS and proposed novel subjective questionnaire, the contralateral tendon in the patients with reconstructed Achilles tendon had significantly lower stiffness than healthy individuals. Therefore, contralateral tendons in patients who suffered from rupture are more prone to future ruptures.
Keywords: Achilles tendon, shear wave elastography, stiffness

P218

Association between body composition, osteoporosis, and diabetes in Korean elderly subjects (KNHANES 2008–2010)

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Objectives: It is well known that diabetes, osteoporosis, fragility fracture and sarcopenia are to increase with age. The objective of the study was to investigate the association of osteoporosis and sarcopenia with diabetic patients aged 65 years or older.

Materials and methods: We used the database from the 2008 to 2010 the Korea National Health and Nutrition Examination Surveys (KNHANES). A total of 2533 subjects aged 65 years or older were selected (1170 men and 1363 women). Bone mineral density and body composition measurement through dual energy X-ray absorptiometry (DXA) were performed in all subjects.
Results: Study subjects with men($n = 1170$) were aged mean 71.6 ± 4.9 years old and women ($n = 1363$) were aged mean 71.8 ± 5.1 years old. The prevalence of DM was 20.8% in men and 21.3% in women. BMI in diabetic group was higher than in non-diabetic group, irrespective of gender. There was no difference of age between diabetic and non-diabetic group. The prevalence of osteoporosis was lower in diabetic group compared with non-diabetic group in men (8.2% vs. 12.6%, $p = 0.009$) as well as in women (48.3% vs. 58.5%, $p = 0.003$). The prevalence of sarcopenia was higher in subjects with DM than in non-DM subjects (28.8% vs. 15.6%, $P < 0.001$ in men, 25.9% vs. 17.2%, $P = 0.001$ in women). Diabetic group have higher prevalence of sarcopenic-obesity in men (14.4% vs. 8.1%, $p < 0.001$) as well as in women (18.3% vs. 11.3%)
Conclusions: Elderly diabetic subjects shows lower prevalence of osteoporosis, but have higher prevalence of sarcopenia and sarcopenic-obesity, irrespective gender.
Keywords: Osteoporosis, sarcopenia, sarcopenic obesity, diabetes Mellitus

P221

Radiofrequency echographic multi spectrometry for diagnosis of osteoporosis in senile women

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Objectives: Senile osteoporosis is defined as low bone mineral density (BMD) and increased bone breakage in people over 70 years. Furthermore, vitamin D deficiency is common among senile people. Up to today Dual-energy X-ray absorptiometry (DXA) was the only one method for assessment of BMD of axial skeleton. Recent published studies introduced novel approach—Radiofrequency echographic multi spectrometry (REMS) for BMD assessment of the both-lumbar spine and femoral neck. The aim of this study was to assess the incidence of osteoporosis in senile women based on BMD values measured with REMS technology.
Materials and methods: 28 senile Caucasian white women aged 75.6 ± 4.5 years were included in the study. REMS technology was used to measure bone mineral density (BMD) of lumbar spine and femoral neck. T-score greater than -2.5 and lower than -1.0 standard deviations below the mean for normal young white women was defined as osteopenia. T-score lower than -2.5 standard deviations was classified as osteoporosis. Normal range of serum vitamin D was attributed to range between 30 and 50 ng/ml.
Results: According to BMD of lumbar spine we found 78.6% incidence of osteoporosis and 17.9% incidence of osteopenia. Only 3.5% of the senile women had normal BMD of the lumbar spine. After assessing the femoral neck BMD, we could diagnose 53.6% women with osteoporosis and 39.3% women with osteopenia. 7.1% women were with normal femoral neck BMD. The mean serum vitamin D level was 28.42 ± 18.78 ng/ml (range 16.3–48.7), which was below the normal range.
Conclusions: In the current study we could establish high incidence of osteoporosis among senile women through REMS technology and low mean serum level of vitamin D. Furthermore, senile women

tended to develop more often spinal osteoporosis than osteoporosis at femoral neck.
Keywords: Osteoporosis, senile women, REMS

P222

Orthopedic articles on surgery for osteoporotic fractures mentioned little about the treatment of osteoporosis

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Purpose: To report the proportion of postoperative medical managements for osteoporosis among the orthopedic articles about surgical treatment for osteoporotic fractures
Materials and methods: The 'Korean Orthopaedic Association' databases were queried with the terms 'osteoporosis OR osteoporotic AND fracture'. Search criteria for inclusion in this study included studies in Korean, published since January, 2006 until December, 2015. The studies about
1) conservative treatment for osteoporotic fracture,
2) arthroplasty after osteoporotic fracture,
3) osteoporosis medication,
4) imaging tools as dual-energy X-ray absorptiometry,
5) review articles or case reports, and
6) epidemiology were excluded and total 35 articles were included in the analysis.
Results: The categorization according to the fracture site identified in the total 35 articles included 21 spine, 5 hip joint, 5 upper arm, 3 wrist joint, and 1 ankle joint. 32 articles (91.4%) described the detailed or upper limit of T-score as diagnosis of osteoporosis and no detailed description of the T-score was found in the remaining 3 articles. Total 9 articles (25.7%) introduced that the authors had done the medical treatment of osteoporosis during the follow-up periods. Among these articles, only 4 articles described the detailed contents about the period of treatment and the drug used in the treatment.
Conclusions: The proportion of articles describing the treatment of osteoporosis after surgery is only 25.7%, compared with the number of articles (91.4%) describing the T-score. In the article on surgical treatment of osteoporotic fractures, explanation of postoperative osteoporosis treatment is further required.
Keywords: Fracture, osteoporosis, orthopedic, article

Author	Year	Treatment initiation or duration	Prescribed pharmacologic drug	Remarks
Park	2006	At discharge ~	Alendronate	
Kim	2007	Postoperative 3 months ~	Alendronate or risedronate	
Ahn	2009	–	Bisphosphonate	Evaluate compliance rate at postoperative 6,12 months
Yim	2012	For more 1 year	Risedronate or alendronate or ibandronate	Evaulate compliance rate between groups

[Description about osteoporosis medication]

P223

Diagnostic strategy for elderly patients with isolated greater trochanter fractures on plain radiographs

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Isolated greater trochanter (GT) fractures are relatively rare and few studies have assessed the appropriate diagnostic and therapeutic strategies for these fractures. When initial plain radiographs show an isolated GT fracture, underestimation of occult intertrochanteric extension may result in displacement of a previously non-displaced fracture. This study examined the clinical results and value of different diagnostic strategies in elderly patients with isolated GT fractures on plain radiographs. Between January 2010 and January 2015, 30 patients with initial plain radiographs showing isolated GT fractures were examined using MRI, bone scanning and/or CT for suspected occult intertrochanteric extension. We assessed the sensitivity, specificity, and positive and negative predictive value of each test. In addition, we noted the location of the fracture or soft-tissue injury on MRI in addition to treatment results. All 30 patients had osteoporosis and fractures caused by minor trauma. MRI revealed isolated GT fractures in nine patients and occult intertrochanteric fractures in 21 patients. Using the MRI-based diagnosis as a reference, the results showed that plain radiographs, bone scans, and CT scans can be used for supplementary examination but they are not appropriate as confirmatory tests for these fractures. However, in patients with both isolated GT fractures seen on plain radiographs and increased uptake in only the GT area on bone scans, MRI revealed isolated GT fractures. The fractures were treated surgically in 20 patients and conservatively in 10 patients with satisfactory clinical results. We confirmed that MRI-based examination is useful in all symptomatic elderly patients whose plain radiographic findings reveal isolated GT fractures. However, we suggest that there is a need to establish a diagnostic strategy through increased understanding of the available diagnostic methods. We believe that surgical treatment should be considered in patients with occult intertrochanteric fractures that are detected on MRI.
Keywords: Trochanter fracture, Occult, Radiographs

P224

Correlation of bone mineral densities and body composition between GE-Lunar prodigy and Osteosys primus

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Purpose: The aim of this study was to investigate the correlation between BMD and muscle mass measured by Osteosys primus® and GE Lunar Prodigy®. In addition, we calculate the conversion formula between two devices.
Methods: The subjects were men and women aged 20 s years. Study subjects with a body mass index (BMI) greater than or equal to 17 kg/m2 and less than or equal to 35 kg/m2 were negative in the pregnancy test at the time of screening, and participants who voluntarily participated in the study and agreed to the written consent. The study exclusion criteria were those with scoliosis, osteoarthritis, rigid vertebral osteomalacia, or other clinical vertebral deformities, previous experience with adverse events after previous DXA or radiography, with mental illnesses such as severe depression, pregnant or lactating women, artificial pacemakers, or implanted cardiac pacemakers. All participants were scanned twice on both Osteosys Primus (OsteoSys, Seoul, Korea) and GE-Lunar Prodigy (Madison, WI, USA) DXA systems using each manufacturer's standard scan and positioning protocols.

Results: Compared with the GE-Lunar Prodigy, the mean BMDs of Osteosys Primus were underestimated to 7.3% in L1, 2% in L2, 2.4% in L3, 1% in L4, 2.5% in L1-4, 5.8% in femur neck, 4.9% in femur trochanter, 4% in femur shaft, and 1.9% in total femur, respectively. Compared with the GE-Lunar Prodigy, the mean lean mass of Osteosys Primus were underestimated to 3.7% (179.4 g) in arms, and 7.1% (1104.72 g) in legs, respectively.
Conclusions: There were a very high correlation of BMD and muscle mass between Osteosys Primus and GE lunar prodigy. In addition, measurement values of Osteosys Primus were consistently lowered by 1–7.1% compared to measurement values of GE Lunar prodigy.
Keywords: Osteosys Primus, GE lunar prodigy, DXA, fat, lean.

P225

Incidence of osteopenia and osteoporosis among postmenopausal women through radiofrequency echographic multi spectrometry

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Recent published studies introduced an innovative echographic approach, defined as Radiofrequency Echographic Multi Spectrometry (REMS), which is applicable on both sites—femoral neck and lumbar spine. Furthermore, significant correlations between BMD values of lumbar spine and femoral neck measured by REMS and Dual-energy X-ray absorptiometry (DXA) has been demonstrated in these studies. Our aim was to assess the incidence of osteopenia and osteoporosis among postmenopausal women through REMS technology.

In this study, a total of 100 postmenopausal Caucasian white women aged 65 ± 10 years underwent echographic scan using REMS technology. The study was carried out in two Bulgarian cities by certified operator. T-scores of lumbar spine and femoral neck have been assessed. T-score greater than -2.5 and lower than -1.0 standard deviations below the mean for normal young white women was defined as osteopenia. T-score lower than -2.5 standard deviations was classified as osteoporosis.

49% (49/100) women have been diagnosed with osteopenia according to T-score values of lumbar spine. The incidence of osteopenia of the femoral neck was higher—52% (52/100) compared to those of the lumbar spine. Osteoporosis of lumbar spine was found in 39% of the postmenopausal women (39/100). 29% (29/100) women had osteoporosis of the femoral neck and this incidence was lower than the incidence of osteoporosis of the lumbar spine. 12% of the women (12/100) had normal bone mineral density (BMD) of lumbar spine and 19% (19/100) had normal BMD of femoral neck.

Using REMS approach we could identify higher incidence of osteoporosis of lumbar spine compared to those of femoral neck among postmenopausal women. Conversely, osteopenia was more likely to be present on femoral neck. REMS technology is promising alternative for assessment of osteoporosis.
Keywords: Osteoporosis, postmenopausal women, REMS

P226

Neglected bodily senses in women living with vertebral fractures: a focus group study

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Background: Older women are at risk of osteoporosis and associated fragility fracture. Vertebral fractures are an extremely important sequalae of osteoporosis as they are associated with reduced quality of life and one of the highest risks of future fracture. Despite this, the majority of vertebral fractures are not detected in clinical care. This study aimed to characterise women's descriptions of their bodily senses relating to vertebral fractures, to inform development of a screening tool for use in clinical care to identify women who require further investigation with spinal radiographs.
Methods: Four qualitative focus groups (n = 19) were conducted with women diagnosed with vertebral fracture. Groups were purposively sampled to include a range of ages, some participants with one fracture and some with more than one. All participants were aware they had a vertebral fracture. Data were analysed thematically and Eccleston's 'Ten Neglected Bodily Senses' was used as a theoretical framework to guide analysis.
Results: Experiences of vertebral fractures could be characterised using seven of Eccleston's neglected senses: pain, movement, fatigue, balance, pressure, appetite and breathing. Pain was the dominant sensation, and all participants explained how pain built with activity, reaching a "peak". Most participants had become physically shorter making some feel "squashed" which put pressure on other body parts and participants expressed a desire to "stretch" and lengthen their bodies. Related to this, some described appetite loss or sense of restricted breathing. Participants also experienced a sense of being "pulled" forwards, which impacted on balance and exacerbated fear of falling.
Conclusions: This work contributes to an understanding of the characteristics of vertebral fractures. Novel descriptions identified by this study not present in the literature, but recognised by clinicians, include a crescendo of pain during activities, and a sensation of being pulled forward.
Keywords: Vertebral fractures, Pain, Symptoms, Qualitative research

P227

Quality assurance of bone densitometry: 3 years experiences of a nation-wide follow-up in Hungary by using ESP Phantom

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Accuracy and precision errors in bone densitometry have silent but major impact to the clinical management of osteoporosis. Internal errors develop slowly, so they will be recognized after a significant time. We have developed a standard protocol for the earliest detection of instrumental shifts, preventing false results in the patients' management.

In 2015 and 2017, the European Spine Phantom (ESP) was scanned 11 times, without reposition, by the same technologist. Lunar and Hologic densitometres were involved, representing > 75% of all devices in Hungary. Group-specific average and standard deviation was separately calculated for both two groups of machines. Devices found to be out of $\pm$ 1 SD and out of $\pm$ 2 SD have been detected. All participatinf densitometry laboratories took their results and in case of malposition an urgent service was initiated, followed by controll measurement of ESP.

For Lunar, 43.5% of the devices were found out of 1 SD range in 2015, and none out of 2 SD range. In 2017 only 25% ($p = 0.23$), respectively, included 7.1% out of 2 SD range as well. The distinct improvement can be partly explained by changing the old densitometers to new ones in one third of devices. For Hologic, 27.7% of the scanners were found out of 1 SD range (included 5.5% out of 2 SD), while two years later 35.2% ($p = 0.72$) out of 1 SD but none out of 2 SD. One quarter of the old devices were renewed in the studied period.

Our project contributed to advancing quality of densitometry in Hungary. In spite of servicing the current alterations, the error in the groups seems again being produced, pointing at need of a continous quality control project. However, higher renewal rate can promise long-term improvement in quality of bone densitometry in the country.

Densitometry—Quality

P228

Can forearm bone mineral density predict late reduction loss of distal radius fracture treated by cast immobilization?

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Introduction: Osteoporosis is known as one of risk factors for late reduction loss in distal radius fracture. Bone mineral density (BMD) of lumbar or hip is generally used as a standard measure for osteoporosis, however, this would not reflect the local bone density. The purpose of this study was to investigate whether forearm BMD is associate with late reduction loss in patients with distal radius fracture.

Methods: From march, 2016 to June, 2018, total 108 patients with distal radius fracture were retrospectively reviewed. Patients were divided into two groups according to the presence of reduction loss evaluated from radiograph taken at least 6 weeks after injury. Reduction loss was regarded as present if radial inclination less than 10′, volar tilt more than 20′, less than − 10′ or ulnar variance more than 3 mm. Lumbar BMD as well as forearm BMD (total and metaphysis) were measured and compared between two groups.

Results: Reduction loss was observed in 44 patients (40%). Forearm BMD had moderate correlation with lumbar BMD and age. The total and metaphyseal BMD of forearm was less in patients with reduction loss compared to patients without reduction loss; however it is not statistically significant (—2.9 vs. − 2.5 for total [$p = 0.141$], − 2.0 vs. − 2.4 for metaphysic [$p = 0.104$]). Lumbar BMD is similar between two groups.

Conclusions: Forearm BMD alone was not an independent prognostic factor. However, it could have more value on predicting late reduction loss than lumbar BMD. Through future study we expect forearm BMD can be used as one of prognostic factors combined with other known factors.

P229

Study of cortical and trabecular compartments through 3d-shaper in lung transplanted patients

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Introduction: Bone architecture could be altered by glucocorticoids(GC) in lung transplanted patients(LTP).Volumetric bone mineral density(BMDv) would help to study bone architecture.

Objectives: Evaluate the change in BMD, BMD cortical superficial, BMDv trabecular and BMDv integral measured before and after LT.

Methods: LTP evaluated by rheumatology were included.Prior to LT, demographic characteristics, diagnosis of lung disease and risc factors(RF) of low bone mass(BM) were collected.Patients were grouped into 3 groups according to the type of lung disease:Chronic Obstructive Pulmonary Disease(COPD), Intersticial Lung Diseases(ILD) and other pathologies.DXA(GE-LUNAR) and 3D-SHAPER software(v2.7, Galgo Medical) were made before and 6 months after LT to evaluated BM and BMDv.

Results: 47 LTP were included, mean age of 56.6 ± 8.7 years.ILD group had the higher proportion of patients with low calcium intake and high doses of GC.The proportion of smokers was higher in COPD.Before LT OP prevalence in all LTP was 25.5% and in COPD was 63.6% ($p < 0.05$)0.18 patients started osteoactive treatment (COPD was the most treated group).OP prevalence post LT was 23.7% (n = 38).BMD and 3D-SHAPER values and their variation are shown in Table 1.

Conclusions: Prevalence of some RFs was different between lung disease groups.Before LT COPD presented worse BM, was the most treated group and had the lowest proportion of patients with GC at high doses.They experienced a significant improvement in BMD and volumetric measurements with respect to other groups who loss values.Trabecular BMDv was the most altered measure of 3D-SHAPER, the greater decrease was in LPT with high doses of GC and the lower in those with osteoactive treatment.

[Table 1. BMD and 3D-SHAPER values.]

P230

Transcultural adaptation and psychometric properties of the Korean version of the quality of life questionnaire of the European Foundation for Osteoporosis (QUALEFFO-41)

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Purpose: The aim of this study was to translate the Quality of Life Questionnaire of the European Foundation for Osteoporosis (QUALEFFO-41) for Korean patients and then validate the Korean version of QUALEFFO-41.

Methods: Translation and transcultural adaptation of the QUALEFFO-41 was conducted according to the international recommendations. Ninety-seven patients (mean age, 73.6 years) with osteoporosis were participated in validating the Korean version of QUALEFFO-41. To test reliability, internal consistency was evaluated using Cronbach's alpha coefficient. To test validity, convergent validity was assessed using correlation with the SF-12 and EQ-5D and discriminant validity was assessed using ROC curve analysis.

Results: The English version of QUALEFFO-41 was translated and adapted to Korean without notable discrepancies The Korean QUALEFFO-41 had good reliability with Cronbach's alpha ranging from 0.733 to 0.942. QUALEFFO-41 had good correlations to SF-12 and EQ-5D. Compared to subjects without history of fracture, those with history of fracture showed significantly worse score according to

QUALEFFO-41, but not according to SF-12 or EQ-5D. ROC curve analysis revealed that physical function domain of QUALEFFO-41 had significant ability to discriminate between subjects with and without history of fracture, while SF-12 or EQ-5D did not.
Conclusions: The Korean version of the QUALEFFO-41 demonstrated relevant internal consistency, convergent validity, and discriminant validity, which can be recommended to evaluate quality of life in Korean.
Keywords: quality of life, osteoporosis, translation, Korean

P231

Discrimination of hip fracture type for men with spatial differences in the distribution of bone

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Introduction: Little is known about the spatial distribution differences in volumetric bone mineral density and cortical bone structure at the proximal femur between femoral neck fractures and trochanteric fractures. Fracture types play a distinct role in predictors, but few studies have subdivided fracture into types. Further, dual-energy X-ray (DXA)-based methods or quantitative computed tomography (QCT) measures or combined assessments have shown limited ability in discrimination of hip fracture types.
Methods: In this case–control study, a total of 67 men with fragility hip fractures, 33 with femoral neck (FN) fractures (mean ± SD age: 77.4 ± 9.5 years) and 34 with trochanteric (TR) fractures (76.9 ± 9.5 years), and 115 control subjects (71.0 ± 6.3 years) were included for the comparisons. By using cortical bone mapping(CBM) based on QCT data, we accurately assess the spatial distribution of cortical and trabecular bone related to hip fracture type. Differences in the spatial distributions of cortical bone mineral density (CBMD), cortical bone thickness(CTh), cortical mass surface density (CM), and endocortical trabecular bone mineral density (ECTD) were investigated using surface-based statistical parametric mapping (SPM). We compared these spatial distributions between controls and both types of fracture, and between the two types of fracture.
Results: Using SPM, we showed that all spatial assessments were significantly different in fracture cases versus cohort in some specific regions, although CBMD, CTh and CM were not different in regions appropriate to fracture type. We also found spatially heterogeneous endocortical trabecular bone mineral density differences between control subjects and subjects with hip fracture that varied by fracture type.SPM results of direct comparisons of two fracture types indicated specific superior regions of femoral neck related cortical thickness differences between FN and TR cases.
Keywords: Femoral neck fracture, trochanter fracture, SPM, QCT

P232

Radiofrequency echographic multi spectrometry (REMS) and trabecular bone score (TBS) are significantly associated with fragility fracture in postmenopausal women

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The diagnosis of osteoporosis is based on bone mineral density (BMD) assessed by dual-energy X-ray absorptiometry scans. The limitation of BMD is that considers only the density of the bone and fails in measuring bone microarchitecture. The Trabecular Bone Score (TBS) is a texture parameter related to bone microarchitecture that may provide skeletal information. Recently, an innovative echographic approach for osteoporosis diagnosis, defined as Radiofrequency Echographic Multi Spectrometry (REMS), has been introduced and clinically validated. This study aimed to evaluate BMD and TBS evaluated by DXA and BMD assessed by REMS technique (REMS-BMD) in women with or without fractures.

In 199 postmenopausal women (age 61.5 ± 13.3 years) BMD was assessed at lumbar spine (BMD-LS), at femoral neck (BMD-FN) and at total femur (BMD-TF) by using a DXA device (Hologic, Discovery); TBS was calculated using TBS iNsight software. In all women, an echographic scan of the same anatomical sites was performed with the REMS approach (Echolight S.p.a.).

Both the TBS and the BMD values were lower in women with vertebral fractures respect to women without vertebral fractures ($p < 0.01$). Also REMS-BMD was lower in women with vertebral fractures respect to women without vertebral fractures ($p < 0.01$). Moreover, densitometric values provided by the two techniques showed an high degree of Pearson's correlation at every site (BMD-LS $r = 0.28$, $p < 0.001$; BMD-FN $r = 0.26$, $p < 0.001$ and BMD-FT $r = 0.29$, $p < 0.001$). Both BMD-LS and REMS BMD-LS showed a significant ($p < 0.001$) positive correlation with TBS. Both REMS BMD and TBS are significantly associated with fragility fracture in postmenopausal osteoporosis women ($b = -1.209$; $p < 0.001$ and $b = -0.678$; $p < 0.05$ respectively).

In conclusion, both TBS and REMS BMD seem to be better associated with the presence of fragility fracture with respect to BMD-LS in postmenopausal women.

P233

Utility of Trabecular Bone Score (TBS) for fracture risk assessment in glucocorticoid-induced osteoporosis

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Glucocorticoid-induced osteoporosis (GIOP) is a common form of secondary osteoporosis (OP). Fractures in GIOP frequently occur with higher bone mineral density (BMD) values than expected. The Trabecular Bone Score (TBS) provides information about bone microarchitecture and fracture risk independently of BMD. Therefore, TBS measurement could be useful for identifying patients with high fracture risk associated with glucocorticoid (GC) treatment.
Objectives: To analyse the utility of TBS for fracture risk assessment in GC-treated patients compared to BMD assessment, the gold-standard diagnostic test.
Methods: 127 patients on GC treatment (≥ 5 mg/day) were included (mean age 62 ± 18 years, 63% women) in this cross-sectional study. The medical history, anthropometric data, bone metabolism parameters, lumbar and femoral BMD (DXA) (considering OP:

T-score ≤ -2.5), TBS (considering degraded microarchitecture [DMA]: < 1.230) and dorsolumbar X-ray (to assess vertebral fractures [VF]) were evaluated. BMD and TBS sensitivity, specificity, and positive and negative predictive values (PPV, NPV) were evaluated to determine the diagnostic accuracy of the two methods.
Results: Most of the patients were receiving GC treatment for vasculitis or polymyalgia rheumatica during 47.7 ± 69 months at a mean daily dose of 14.5 mg. 17% had VF, 28% any type of fragility fracture (VF + non-VF), 29% OP and 71% DMA. In patients with VF, low TBS was more frequent than densitometric OP (76%, p = 0.03 *vs.*38%, p = n.s). Patients with any fragility fracture showed similar results (69%, p = 0.02 *vs.*36%, p = n.s). The diagnostic accuracy of TBS was greater than BMD on evaluating VF, with a sensitivity, specificity, PPV and NPV of 0.76, 0.53, 0.25 and 0.92 for TBS, and 0.38, 0.72, 0.22, and 0.85 for BMD, respectively. Specificity increased to 0.89 for VF and 0.9 for any fragility fracture on combining both assessments (OP + DMA).
Conclusions: TBS has greater discriminative power than BMD measurement and could be useful as a complementary tool for fracture risk assessment in GIOP.
Keywords: Glucocorticoid-induced osteoporosis; fracture risk; Trabecular Bone Score;

P234

Association between muscle force and power with different pQCT and DXA-derived parameters in middle-aged adults

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Jumping mechanography (JM) and peripheral Quantitative Computed Tomography (pQCT) have recently gained interest as powerful techniques for the assessment of muscle function and bone geometry. These techniques constitute useful tools for the investigation of the muscle-bone relationship. The aim of this study was to evaluate the association between JM-derived muscle force and power with different pQCT- and DXA-derived parameters. Data was obtained from 568 participants (mean age: 49.8 ± 4.99) from a population-based cohort with available data for cross-sectional analysis. Maximum muscle force and power were assessed using single 2-legged jump. pQCT scans were performed at 4% and 66% of the tibial length, measuring bone geometry parameters such as cortical and trabecular bone density, area and circumference. Next, dual-energy X-ray absorptiometry (DXA) was performed and areal bone mineral density (aBMD), lean mass and fat mass were derived. All variables were standardized prior linear regression analyses with standardized β-coefficients presented; corrected for age, sex and weight. Our results showed strong positive association between maximum force and trabecular density at 4% (β: 0.174; 95% CI: 0.033 to 0.315), cortical area (β: 0.192; 95% CI: 0.088 to 0.295) and thickness (β: 0.077; 95% CI:0 0.047 to 0.306) at 66%, but not with cortical density at 66% (β: 0.006; 95% CI: − 0.127 to 0.139), endosteal circumference (β: − 0.022; 95% CI: − 0.159 to 0.115) and periosteal circumference (β:0 0.037; 95% CI: − 0.085 to 0.158). Maximum power followed the same trend and was also associated with periosteal circumference (β: 0.153; 95% CI: 0.023 to 0.282). Finally, muscle force and power were also associated with all DXA-derived bone and body composition parameters. In conclusion, maximum force and power are associated with specific pQCT bone parameters and DXA outcomes. Therefore, they may serve as important clinical markers for musculoskeletal decline in older adults.

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Difference in bone Loss in patients with erosive and non-erosive hand osteoarthritis: a two-year longitudinal study

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Background: Hand osteoarthritis (OA) and its more severe subset erosive hand OA are common causes of pain and morbidity.
Objectives: To compare the change of bone mineral density (BMD) between patients with erosive and non-erosive hand OA in a two-year longitudinal study.
Methods: Consecutive patients with symptomatic HOA fulfilling ACR criteria were included in this study. Erosive hand OA was defined by at least one erosive interphalangeal joint. All patients underwent DEXA examination of lumbar spine, total femur and femur neck was performed at the baseline and after two years.
Results: Altogether, 144 patients (15 male) with symptomatic nodal HOA were included in this study and followed between April 2012 and January 2018. Out of these patients, 82 had erosive disease after two years. The disease duration ($p < 0.01$) was significantly higher in patients with erosive compared with non-erosive disease at baseline. Osteoporosis (T-score < -2.5 SD) was diagnosed in 12.5% (9/72) of patients with erosive hand OA and in 8.06% (5/57) of patients with non-erosive hand OA. BMD was significantly lowered in patients with erosive compared with non-erosive disease at baseline.T-scores were also significantly lowered in patients with erosive compared with non-erosive disease.

Furthermore, we found significant decrease in BMD in patients with erosive compared with non-erosive disease over two years (lumbar spine: − 3.30% vs. 1.06%, $p < 0.05$, total femur (− 1.58% vs. 0.82%, $p < 0.05$) and femur neck (− 3.2% vs. 0.02%, $p < 0.05$). The decrease in T- score of lumbar spine (− 3.66% vs. 15.52%, $p < 0.01$) and total femur (− 4.29% vs. 7.68%, $p < 0.05$) was also significantly higher in erosive compared with non-erosive hand OA.
Conclusions: These results suggest that patients with erosive hand OA are at higher risk for the development of general bone loss.

P240

Controlling hypoxia-inducible factor-2a is critical to maintaining bone homeostasis in mice

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Pathological bone loss is caused by an imbalance between bone formation and resorption. The bone microenvironments are hypoxic, and hypoxia-inducible factor (HIF) is known to play notable roles in bone remodeling. However, the relevant functions of HIF-2a are not well understood. Here, we have shown that HIF-2a deficiency in mice

enhances bone mass through effects on differentiation of osteoblasts and osteoclasts. In vitro analyses revealed that HIF-2a inhibits osteoblast differentiation by targeting *Twist2* and stimulates RANKL-induced osteoclastogenesis via regulation of *Traf6*. Additionally, HIF-2a appears to contribute to the crosstalk between osteoblasts and osteoclasts by directly targeting RANKL in osteoprogenitor cells. Experiments performed with osteoblast- and osteoclast-specific conditional knockout mice supported a role of HIF-2a in this crosstalk. HIF-2a deficiency alleviated ovariectomy- and aging-induced bone loss in mice and specific inhibition of HIF-2a with ZINC04179524 significantly blocked RANKL-mediated osteoclastogenesis. Collectively, our results suggest that HIF-2a functions as a catabolic regulator in bone remodeling critical for the maintenance of bone homeostasis.

Keywords: Bone homeostasis, osteoblast, osteoclast, osteoporosis

P241

Association between RUNX2 gene polymorphism and osteopenia among Asian elderly: a population-based case–control study

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Background: Osteopenia is generally prevalent in the elderly population. Patients with osteopenia have higher risk of fracture and seriously affects their quality of life. The proportion of the elderly over 65 years old in Taiwan will reach 14.6% in 2019. Therefore, it is foreseeable that the number of patients with osteopenia will increase dramatically in next decades. The cause of osteopenia is relatively complicated. In addition to environmental risk factors, genetic factors also play an important role. Recent GWAS studies have found that bone density is associated with many genes. Runt-related transcription factor 2 (RUNX2) gene is a key transcription factor of osteoblast differentiation and apoptosis, which make this gene a biologically plausible risk factor for osteopenia. This study aimed to investigate association between RUNX2 gene polymorphism and osteopenia among Asian elderly population.

Materials and methods: We performed a case–control study and recruited 418 participants who received health examination at Health Management Center of Tri-Service General Hospital from March 2015 to August 2017. Demographic data were obtained by structured questionnaire, and bone mass density was measured by dual-energy x-ray absorptiometry (DEXA). Subjects with T-score lower than − 1 was classified as osteopenia case group, t-score higher than -1 was classified as healthy control group. All data analyses were done by using R software version 3.4.2.

Results: After adjusted for age, sex, and BMI, subjects with CT genotype shows insignificantly 14% higher risk of having osteopenia (OR = 1.14, 95% CI = 0.64–2.00). Subjects with TT genotype shows insignificantly 15% lower risk of having osteopenia (OR = 0.85, 95% CI = 0.06–11.90).

Conclusions: Our study failed to detect significant association between RUNX2 rs11755164 gene polymorphism and osteopenia among Asian elderly in present study.

Keywords: Gene polymorphism, Bone mineral density, Osteopenia, RUNX2

P242

Analysis of treatment, diagnostic measures and complications of osteoporosis in patients with new low-energy fractures

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Objectives: We assessed osteoporosis risk factors, the level of osteoporosis treatment and detection coverage in patients with low-energy fractures. Also we evaluated patients' comorbidity.

Materials and methods: The study group included 135 patients (105 women) with new low-energy. The average age was 66.5 ± 16.9 years.

Results: According to the interview, 38 patients (28.1%) had at least one low-energy fracture in the past. Only five patients (13.2%) with previous low-energy fractures were properly examined. 12 patients (8.9%) had results of osteodensitometry; eight of them had osteoporosis and three patients—osteopenia. 3 patients received treatment with bisphosphonates (alendronic and zoledronic acids) and 1 patient with denosumab. Five patients received vitamin D and calcium supplementation during a period of 1 to 8 years. Four patients didn't receive any treatment after the diagnosis of osteoporosis.

24 (17.8%) patients were current smokers. 31 (23.0%) patients had smoked in the past. The cumulative duration of smoking was 21.2 ± 17.4 years.

12 patients (8.9%) consumed alcohol beverages once in a week, three patients consume alcoholic drinks 3-7 times a week. The others consumed alcohol episodically.

9 patients (6.7%) received glucocorticoids for a long period of time. None of them was consulted about the risk of osteoporosis.

43 patients (31.9%) had spontaneous falls in the the past year. The average number was 2.9 ± 2.6.

The majority of the patients demonstrated comorbidity. 54 (40.0%) had ischemic heart disease, 81 patients (60.0%)—hypertensive disease. 36 (26.7%) patients had congestive heart failure. 8 patients (5.9%) were diagnosed with chronic kidney disease. 26 patients (19.3%) had a diagnosis of cerebrovascular insufficiency.

Conclusions: Patients with low-energy fractures were not diagnosed properly and did not receive appropriate therapy before the fracture. Patients demonstrated significant comorbidity that complicated their further therapy and rehabilitation.

Keywords: Osteoporosis, epidemiology, comorbidity, risk factors

P243

Association between VDR rs2228570 gene polymorphism and osteopenia among Asian elderly: a population-based case–control study

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Background: Osteopenia is generally prevalent in the elderly population. Patients with osteopenia have higher risk of fracture and seriously affects their quality of life. The proportion of the elderly over 65 years old in Taiwan will reach 14.6% in 2019. Therefore, it is foreseeable that the number of patients with osteopenia will increase dramatically in next decades. The cause of osteopenia is relatively complicated. In addition to environmental risk factors, genetic factors also play an important role. Recent GWAS studies have found that

bone density is associated with many genes. Vitamin D Receptor (VDR) gene polymorphism is reported to influence calcium absorption, which make this gene a biologically plausible risk factor for osteopenia. This study aimed to investigating association between VDR rs2228570 gene polymorphism and osteopenia among Asian population.

Materials and methods: We performed a case–control study and recruited 418 participants who received health examination at Health Management Center of Tri-Service General Hospital from March 2015 to August 2017. Demographic data were obtained by structured questionnaire, and bone mass density was measured by dual-energy x-ray absorptiometry (DEXA). Subjects with T-score lower than -1 was classified as osteopenia case group, t-score higher than -1 was classified as healthy control group. All data analyses were done by using R software version 3.4.2.

Results: After adjusted for age, sex, and BMI, subjects with AA genotype shows insignificantly 21% higher risk of having osteopenia (OR = 1.21, 95% CI = 0.69–2.13). Subjects with A allele shows insignificantly 10% higher risk of having osteopenia (OR = 1.10, 95% CI = 0.84-1.44).

Conclusions: Our study failed to detect significant association between VDR rs2228570 gene polymorphism and osteopenia among Asian elderly in present study.

Keywords: Gene polymorphism, Bone mineral density, Osteopenia, VDR rs2228570

P244

Primary hyperparathyroidism surgery. Our experience

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Introduction: We analyze our surgical experience of the primary hyperparathyroidism.

Methods: A retrospective study was conducted on 60 patients operated of primary hyperparathyroidism (PHPT) from 2005 to 2017. We collected epidemiological and clinical data, biochemical parameters, surgical characteristics included the measurement of intraoperative parathormone (ioPTH) and osteoporosis data.

Results: Sixty operated patients aged 55, 5 ± 15, 3 years were studied. The main surgical criterium was the hypercalcemia, but 15% of the patients had normocalcemic hyperparathyroidism. Patients who achieved healing after surgery were significantly younger. Patients with normocalcemic hyperparathyroidism had lower PTH in all measurements, higher levels of vitamin D and creatinine. We compared the clinical characteristics in patients with osteoporosis with respect to those who didn´t have it. People with osteoporosis had more peripheral fractures. We found significant results in terms of the age of the patients, higher in those with osteoporosis (63 ± 11.6 years vs 49 ± 15.6; $p < 0.000$). The preoperative calciuria was lower in patients with OP (220.4 mg/24 h ± 107.6 vs 446.7 ± 214.7; $p < 0.000$) and ioPTH at 15 min was significantly higher in patients with smaller bone masses (82.9 pg/mL ± 58.0 vs 45.3 ± 39.0 $p < 0.013$). Patients with PPH had a worse densitometric value in the lumbar spine before surgery (BMD in Femoral Neck preoperative -1.7 ± 0, 9 vs BMD in Lumbar Spine -2,5 ± 1,1). There was improvement in both the femoral neck (BMD -2. 1 ± 1, 1) and the lumbar spine (-2,1 ± 1,1) after surgery although it was not significant.

Conclusions: Primary hyperparathyroidism occurs with an increasingly broad spectrum. As it is demonstrated in the literature, the densitometric results after the PHPT surgery of our series, improve and especially at lumbar spine.

The measurement of ioPTH is a useful tool in the surgical treatment and it provides a prognostic value in the follow-up of these patients.

P245

Influence of bone health and vitamin D status in postmenopausal osteoporosis subjects in northern China

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Objective: Vitamin D deficiency/insufficiency is common worldwide, as well as in Inner Mongolia. The objective of this study was to investigate the association between Vitamin D deficiency with bone health in Hohhot city for Latitude 40.8°N from April to June.

Methods: A total of 173 Han and Mongolian subjects from Hohhot, aged 30-83 years, mean 60 years old, were randomly selected in subjects who were tested at the Second Affiliated Hospital of Inner Mongolia Medical University in April, May and June 2016. The serum 25-hydroxyvitamin D (25OHD) was measured by Roche Cobas e602 automatic electrochemiluminescence instrument. Vitamin D, Bone Mineral Density, 10 years probability of fracture risk were measured. The data were statistically analyzed using SAS UE edition.

Results: A total of 173 subjects were analyzed. 152 were female and 21 were male. The mean age was 63 years old. The 25OHD level was 17.8 ng/mL for male and 18.1 ng/mL for female. 62% of subjects were was VD deficiency ((< 20 ng/mL) and 28% was VD insufficient (25OHD 20 $\sim$ 30 ng/mL). The serum 25-hydroxyvitamin D level in each group was lower than the normal value. Among them, the age of 25-hydroxyvitamin D was significantly lower in the elderly women. In the middle-aged group level ($P < 0.05$). Vitamin D levels in older women are mainly distributed in moderate to severe deficiencies.

Conclusions: The incidence of vitamin D deficiency is high among women living in Hohhot, regardless of age, especially in middle-aged and elderly women who are at high risk of vitamin D deficiency. Effective measures should be taken to prevent vitamin D deficiency and reduce the health effects of vitamin D deficiency.

Keywords: Vitamin D; Hohhot City; Northern China

P246

Prevalence of modifiable and non modifiable risk factors of osteoporosis in health care workers of < 40 years at tertiary health center of remote India

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Introduction: Osteoporosis is a global problem occurring in every geographic area and affecting 150 million men and women worldwide. Osteoporosis is defined as a reduction of bone mass (or density) or the presence of a fragility fracture. Aim of the study is to gain knowledge of prevalence and identifying risk factors among health care workers and in turn increasing the awareness, education, prevention, and treatment of osteoporosis.

Materials and methods: This study is based on International Osteoporosis Foundation (one minute risk test) questionnaire performed on health care workers over the age of 18 years at a tertiary health center in a remote region of India. A total of 470 health care

professionals were included in this study of which 180 were women and 290 men

Results: Non-modifiable risk factors i.e. family history was positive in 36.55% male and 48.3% females. H/O Rheumatoid arthritis(M-7.58%, F-11.1%), Corticosteroid use(M-6.89% F-8.33%), H/O Type 1 DM, overactive thyroid, increased PTH(M-8.62% F-5%), Nutritional disorders(M-8.62% F-8.33%), Recurrent falls (m-1.37% f-1.11%). Modifiable risk factors such as Alcohol intake (> 2 units/day, M-33.10% F-13.8%), Smoking (M-46.55% F-21.11%), Sun light exposure(< 10 min, M-6.55% F-40.55%), Physical activity (< 30 min/day, M- 14.44% F-42.22%), Allergies to dairy products with no calcium supplementation(M-13.44% F-23.10%) were calculated.

Conclusions: In spite of an awareness of osteoporosis and its risk factors among health professionals, high prevalence of modifiable and non modifiable risk factors was found. Since more than 50% of general population in this part of world is illiterate, a much higher prevalence is expected in then. There is a need to educate this high risk population at the earliest to prevent this disease by implementing national or international health strategies to tackle this increasing global health problem.

P247

Risk of hip and subtrochanteric or femoral shaft fractures after bisphosphonate use in Korea women: an analysis using Korean national sample cohort

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Concern about bisphosphonate-associated subtrochanteric and femoral shaft(ST/FS) fractureshas been extremely raised in Asia. However, its real risk is still debatable, because there is no study to estimate risk and benefit of bisphosphonatefrom Asian population. Our objective was to evaluate the risk of typical hip fractures and ST/FS fracturesamong bisphosphonate users using nationwide database in Korea.

This retrospective cohort study used National Health Insurance Service-National Sample Cohort.We evaluated occurrence of the ST/FS and femoral neck and intertrochanteric (FN/IT) fracturesafter the index date among female bisphosphonate new users. Incidence rate of ST/FS and FN/IT fractures were compared between long-term users (≥ 1 year) and short-term users (< 1 year).

Among 46,420 bisphosphonate users, we identified14,689 long-term users and 21,840 short-term users. During the study period, 61 long-term users and 36 short-term users had ST/FS fractures, while 204 long-term users and 511 short-term users had FN/IT fractures. The incidence rate of ST/FS fractures was 67.1/100,000 person-years (95% CI, 50.3 to 83.9) in the long-term users and 31.2/100,000 person-years (95% CI, 21.0 to 41.4) in the short-term users. The risk of ST/FS fractures was significantly higher in long-term users than in short-term users (adjusted hazard ratio = 2.345, 95% CI 1.541 to 3.569)

The incidence rate of FN/IT fractures was 225.5/1,000 person-years (95% CI, 194.6 to 256.5) in the long-term users and 448.6/1000 person-years (95% CI, 409.7 to 487.5) in the short-term users. The risk of FN/IT fractures was significantly lower in long-term users than in short-term users (adjusted HR = 0.578, 95% CI, 0.490 to 0.682)

Our study showed that bisphosphonate had benefits of hip fracture prevention and suggested that the raised concerns about bisphosphonate-associated ST/FS fractures in East Asia may be overestimated than the reality, although the use of bisphosphonate might increase the risk of ST/FS fractures.

Keywords: Atypical femoral fracture, bisphosphonate, national cohort

P248

Serum iron indices are associated with bone mineral density in premenopausal women

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Background: Osteoporosis is characterized by a decrease in bone mineral density (BMD) and increased risk for fragility fractures. Notably, the serum iron level may interact with the bone health status. This study investigated correlations of BMD with serum iron and hemoglobin levels and total iron binding capacity (TIBC).

Methods: We performed a retrospective analysis of the medical records of premenopausal women in South Korea. The study protocol was approved by the Kyungpook National University Chilgok Hospital Institutional Review Board. BMD and Z scores of BMD were verified using dual-energy X-ray absorption. Participants were stratified into quartiles for analyses of the associations of serum iron, TIBC, and hemoglobin levels with BMD.

Results: A simple linear regression analysis revealed associations of iron [β: − 0.001; standard error (SE), 0.001; $p < 0.001$], hemoglobin levels (β: 0.015; SE, 0.003; $p < 0.001$), and TIBC (β: 0.001; SE, 0.001; $p < 0.001$) with changes in BMD, and this pattern was also observed in a multiple linear regression analysis. Multivariate logistic regression analysis of iron and TIBC for low BMD revealed odds ratios of 1.005 ($p < 0.001$) and 0.995 ($p < 0.001$), respectively.

Conclusions: This study demonstrated clear relationships of changes in BMD with serum iron levels and TIBC and thus confirms the usefulness of these markers in the clinical evaluation of iron storage and BMD in younger women.

Keywords: Bone, Osteopenia, Trace mineral, Pre-menopause, Women

P249

Osteoporotic fractures in the elderly: is hyponatremia player or bystander?

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Objectives: To evaluate the role of hyponatremia in fragility fractures and mortality in elderly.

Methods: 284 hospitalized geriatric patients without secondary causes of hyponatremia or osteoporosis were recruited. Age, sex,

comorbidities, drugs, previous fragility fractures were recorded. Blood levels of sodium, potassium, phosphate, calcium were measured. Cognitive function, nutrition, muscular strength, balance were evaluated by standard tests. Normo- and hypo-natremic patients were compared for the analysed variables. The patients´ mortality rate was recorded with a follow-up after hospital discharge. The Ethics Committee of our Hospital approved the study.

Results: Hyponatremic patients were more malnourished (BMI, MNA score), without significant differences in cognitive performance (MMSE), in risk of falls (Tinetti scale) nor in muscle strength. Interestingly, hyponatremic patients reported higher prevalence of fragility fractures

Variable	Normonatremic (234) Median (IQR)	Hyponatremic (50) Median (IQR)	p
MMSE	24.8 (22.2–26.7)	24.6 (22.2–26.3)	0.503
MNA	21.5 (18.5–24.5)	20.0 (17.0–22.5)	0.005
BMI (Kg/m^2)	25.3 (22.7–29.0)	22.6 (20.9–24.6)	0.000
Force to the dynamometer (Kgp)	20.0 (14.0–28.0)	18.0 (12.0–24.0)	0.099
Tinetti score	17.0 (12.0–23.0)	18.0 (12.0–24.0)	0.556
Previous fragility fractures (%)	22 (44.0%)	59 (25.2%)	0.008

[Hypo/normo-natremic patients variables]

Survival analysis showed that hyponatremia at baseline was associated with higher mortality rate (p = 0.005), Hazard Ratio (HR) crude = 1.80 (95% CI 1.19–2.71), HR adjusted for co-morbidities, sex, age = 1.75 (1.16–2.65).

Conclusions: Our result shows that hyponatremic patients are generally more affected by malnutrition and highlights the roles of hyponatremia as worse prognosis indicator and risk for fragility fractures, regardless to other variables as age, sex, co-morbidities. Serum sodium is an easily available and affordable biochemical measurement in clinical practice; hence, the assessment of hyponatremia could be used as an index of worse outcome in old patients, supplementing the patient assessment clinical scales.

Keywords: Hyponatremia, fracture risk, fragility fracture, mortality

P250

Management of osteoporosis in patients living with HIV—a systematic review and meta-analysis

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Objectives: Osteoporosis may be a major comorbidity in patients living with HIV (PLHIV). The aim of this systematic review and meta-analysis is to assess the evidence on fracture risk in PLHIV, bone mineral density (BMD) in PLHIV compared to controls, longitudinal changes in BMD in PLHIV and effect of antiosteoporotic treatment in PLHIV.

Methods: A systematic literature search was conducted using The databases Medline at Pubmed and Embase were explored using the search terms in free-text: "HIV" and "fracture", "fracture", "HIV" and "bone turnover", and "HIV" and"bone mineral density". Data was extracted by reviewer one and two. Eligibility criteria follow the aim of the study and include randomized controlled trials and observational studies. Meta-analysis was performed using random effects model assessing fracture risk, BMD compared to controls, and changes in BMD.

Results: 2397 papers were identified of these 142 were included in the systematic review and of these 84 were included in the meta-analysis. The risk of a fragility fracture (RR = 1.51, 95% CI: 1.41;1.63) and hip fracture (RR = 4.05, 95% CI: 2.99;5.49) were increased. PLHIV have lower BMD at the hip (z-score − 0.31, 95% CI: − 0.46; − 0.27) and lumbar spine (z-score − 0.36, 95% CI: − 0.39; − 0.15) compared to controls. At initiation of antiretroviral treatment (ART), treatment naive PLHIV had a BMD decline of − 2.68% (95% CI: − 3.11; − 2.25) at the lumbar spine within one year, whereas treatment experienced PLHIV had no BMD change wihtin one year.

Conclusions: PLHIV are more susceptible to fractures and experience a rapid decline in BMD at ART initiation, however the fracture risk does not seem to be fully explained by a reduced BMD. Early initiation of anti-osteoporotic therapy may be of importance in PLHIV.

Keywords: HIV, osteoporosis, meta-analysis, fracture risk

P251

Gastrectomy is associated with increased risk of osteoporotic fracture in community-dwelling elderly men 20 years or more after the surgery: Formen Cohort Study

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Purpose: Many studies have reported that patients with history of gastrectomy have lower areal bone mineral density (aBMD) and higher fracture risk than those without. However, population-based studies on this topic are scarce. In addition, little work has been done on bone metabolic status of patients with gastrectomy in long-term time frame. This study aimed to clarify the association of gastrectomy with aBMD, bone metabolism markers and fracture risk in community-dwelling elderly Japanese men.

Methods: A total of 1992 men aged ≥ 65 years completed baseline measurements including aBMD at the spine and hip, serum levels of intact parathyroid hormone (PTH), intact osteocalcin (OC), tartrate-resistant acid phosphatase isoenzyme 5b (TRACP5b) and undercarboxylated OC (ucOC), and an interview regarding past disease history including gastrectomy. Osteoporotic fractures (OPFs) that occurred during the 5-year follow-up period were determined through structured interviews.

Results: After excluding participants with type 1 diabetes mellitus and those with missing values, 1985 men including 132 men with history of gastrectomy were analyzed. Men with gastrectomy had significantly higher PTH, TRACP5b and ucOC levels and lower aBMD than those without, and significantly higher risk of OPF after adjusting for covariates including aBMD (hazard ratio (HR): 2.55, 95% confidence interval (CI): 1.17, 5.55). Risk of OPF was not significantly different by cause of gastrectomy (peptic ulcer or cancer), while the increase in risk was more evident in participants who survived 20 years or more after gastrectomy (HR: 3.52, 95% CI: 1.45, 8.54).

Conclusions: History of gastrectomy was associated with elevated bone resorption, decreased aBMD and increased fracture risk in

community-dwelling elderly men. This increase in fracture risk was more prominent long after gastrectomy. Gastrectomized men should be observed long term for the management of osteoporosis and fracture risk.

Keywords: Gastrectomy, gastric cancer, gastric ulcer, osteoporotic fracture, population-based cohort study

P252

Hematopoietic autophagy deterioration links to osteoporosis

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Objectives: Disorders of hematopoiesis affect the skeletal system. We examine the correlation between hematopoietic system autophagy and bone homeostasis.

Findings: In clinical, we found positive correlation between red blood cell count and femur neck bone mineral density (BMD) in 4964 healthy samples, pearson = − 0.197, P < 0.01. Femur-derived bone marrow of 30 patients was obtained from young normal BMD (BMD > − 1.0, age < 40 years) or aged osteoporosis (BMD < − 2.5, age > 60 years) during total hip replacement surgery. Human hematopoietic stem progenitor cells (CD34 + CD45 +) LC3 protein was inhibited in aged osteoporosis patients (43.62 ± 11.92% compared to 21.67 ± 3.57%) associated with descending autophagy gene expression, with Atg7, Atg5, Atg12, LC3b, Lam2a, P62 involved (P < 0.05).

Deletion of Atg7 gene in hematopoietic system mice (Atg7f/f; Vav-iCre) were established, which led to autophagy dysfunction in hematopoietic system. Atg7 null in hematopoietic system caused low BMD (0.046 ± 0.018 compared to 0.172 ± 0.026 g/cm^3, p < 0.05), low bone formation rate (1.06 ± 0.12 compared to 1.58 ± 0.11 um/d, P < 0.05) and weak bone biomechanical strength properties (Load 9.12 ± 0.98 compared to 14.98 ± 0.87 N, P < 0.05). Scanning electron microscope, H&E and Masson staining depicted trabecular microstructure destruction. However, there is no size difference in skeleton Alcian blue and Alizarin red S staining. Immunofluorescence of cortical bone revealed abnormal osteocyte size and number, osteocyte DNA damaged and increased ROS in Atg7f/f; Vav-iCre mice, accompanied by loss of type H vessels. Bone homeostasis related gene SP7, RUNX2, BMP2, BMP6, CTSK, TRAP5 (P < 0.05) were inhibited in Atg7f/f; Vav-iCre mice. Integrative proteomics functional enrichment showed Atg7f/f; Vav-iCre mice bone tissue skeletal systme morphogenesis and development were down-regulated, with collagen1 inhibition by KEGG analysis, confirming by collagen1 immunohistochemistry staining.

Conclusions: Deterioration of autophagy in hematopoietic system inhibited osteogenesis in clinical and animal model. This work suggests that hematopoietic autophagy have potential to maintain bone homeostasis, especially collagen1 in extracelluar matrix, as well as type H vessels, possibly contributing to osteogenesis.

P253

Seasonally dependent decrease of the number of fractures in Poland in the years 2010–2015

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Introduction: Falls are the main risk factors of extravertebral fractures. The study aimed to examine trends in fracture incidence in the last years and their correlation with temperature and number of snowy days.

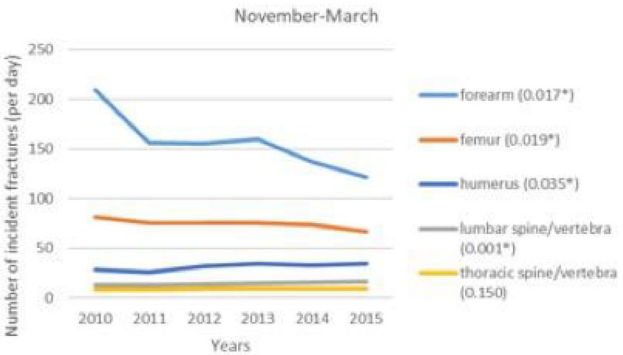
Methods: A database of the National Health Fund, covering almost all fractures in Poland in the years between 2010 and 2015, was analyzed. Patients older than 50y were included.

Results: there was a significant decrease of the yearly incidence of forearm (from 71,907 to 54,169) and femur (from 27,291 to 25,002) fractures that was dependent mainly on the decrease during winter months (Figure 1). The differences in the incidence of humerus and vertebral fractures were not seasonally dependent. The number of fractures was related to the weather. Table 1 shows Pearson's correlation coefficients between weather variables (based on meteorological station in Warsaw) and number of fractures (for period November-March).

Conclusions: Even in a short-time observation period (2010–2015) a climate-dependent decrease of the incidence of forearm and femur fractures can be observed

Table 1. Pearson's correlation coefficients between weather variables and number of fractures.]

	Forearm	Femur	Humerus	Lumbar spine	Thoracic spine
Mean temperature in December	−0.813*	− 0.838*	0.389	0.599	0.158
Mean temperature in January	−0.888*	− 0.870*	0.311	0.495	0.096
Mean temperature in February	−0.357	− 0.429	0.512	0.678	0.401
Number of days with snow (per year)	0.823*	0.698	−0.176	−0.544	−0.131
Mean annual temperature	−0.930*	−0.949*	0.554	0.829*	0.332



[Number of fractures in November-March]

P254

Secular trend of lumbar and femur bone mineral density measurements in women above 50 over 10 years

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Purpose: A question have been raised since previous studies reported a contradictory finding that age-adjusted bone mineral density (BMD) in postmenopausal women decreases in developed countries while incidence of osteoporotic fracture decreases or remains stable. We investigated secular trend in age-adjusted vertebral, and femoral bone mineral density measurements over last 10 years in postmenopausal women as well as changes in factors related to BMD.
Methods: We analyzed lumbar, femur neck and total femur BMD of 5605 women above 50, measured by DXA in years of 2008–2009, 2012–2013 and 2016–2017.
Results: Bone mineral content, BMD, T-score at lumbar spine and proximal femur in postmenopausal women declined significantly as time passed by. (vertebral BMD 1.08 ± 0.17 for 2008–2009, 1.06 ± 0.17 for 2012–2013, 1.06 ± 0.17 for 2016–2017 P for trend < 0.001, femoral neck BMD 0.87 ± 0.13 for 2008–2009, 0.85 ± 0.12 for 2012–2013, 0.84 ± 0.12 for 2016–2017 P for trend < 0.001, Total femur BMD 0.94 ± 0.13 for 2008–2009, 0.91 ± 0.13 for 2012–2013, 0.90 ± 0.12 for 2016–2017 P for trend < 0.001)
Decreasing trend in lumbar and femoral BMD in women aged 50–59 was prominent compared to that in women aged over 70. After adjustment with age, BMI, renal function, liver function, history of diabetes, calcium and vitamin D supplementation, alcohol intake, smoking, and physical activity, the secular trend of BMD remained significant. Temporal changes of these BMD related factors were not in accordance with the BMD change, except for exercise. (P for trend < 0.001)
Conclusions: BMD in postmenopausal women has declined significantly over last 10 years. This finding would suggest a menace to rebound the incidence of osteoporotic fracture in future.

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Risk factors associated with the development of fractures in glucocorticoid treated patients. The role of hypogonadism

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Glucocorticoid-induced osteoporosis(GIOP) is a common form of secondary osteoporosis(OP). Fractures in GIOP frequently occur with higher bone mineral density(BMD) than expected and typically at treatment initiation, complicating the identification of patients at risk for fracture.
Objectives: Identify risk factors associated with fragility fracture development in GC-treated patients.
Methods: 127 patients(aged 62 ± 18 years, 63% women, 46% postmenopausal) on GC treatment(≥ 5 mg/day, > 3 months) were included. Clinical data collected included: risk factors for OP and fractures, dose and GC-treatment duration, previous fractures and disease activity, anthropometric data, bone metabolism parameters(including gonadal axis study), BMD analysis(DXA; OP: T-score ≤ -2.5), TBS(degraded microarchitecture [DMA]: $<$ 1.230), dorsolumbar X-ray (assessing vertebral fractures[VF]) and FRAX risk(GC-adjusted).
Results: Most patients received GC treatment for vasculitis or polymyalgia rheumatica during 47.7 ± 69 months(mean daily dose: 14.5 mg). 17% had VF, 28% had fragility fracture(VF + non-VF), 29% OP and 71% DMA. Patients with VF received more GC-boluses(57.1% vs. 29.5%, $p = 0.03$), were older(68 ± 13 vs. 60 ± 19 years, $p = 0.02$), postmenopausal (100% vs. 67%, $p = 0.015$) and/or men with testosterone < 250 ng/dL(57% vs. 11%, $p = 0.017$) and had lower TBS values(1.100 vs. 1.220, $p < 0.001$) and higher FRAX risk(17 vs. 9, $p = 0.003$); patients with fragility fractures showed higher accumulated GC-doses(6.1 ± 13 vs. 8 ± 18 g, $p = 0.046$). On multivariate analysis, hypogonadism(OR 14.3; IC95% 2.2- > 100, $p = 0.01$) and having received GC-boluses(OR 3.40; IC95% 1–11.8, $p = 0.01$) were the principal factors associated with VF. Hypogonadism(OR 7.1; IC95% 1.5–38.7, $p = 0.01$) and having a FRAX > 20(OR 6.97; IC95% 1.3–51.7, $p = 0.02$) were factors related to the presence of fragility fractures. Men with testosterone < 250 ng/dL had higher BMI(29.4 vs. 26.3, $p = 0.005$) and disease activity(ESR 23 vs. 12, $p = 0.005$) and lower TBS(1.050 vs. 1.210, $p < 0.001$); age, daily and cumulated GC doses were similar to subjects with normal testosterone levels.
Conclusions: Hypogonadism is a major risk factor for developing fractures in GC-treated men and women, whereas receiving GC-boluses is related to VF, indicating the importance of evaluating the gonadal axis in these patients.
Keywords: Glucocorticoid-induced osteoporosis; vertebral fracture; hypogonadism.

P257

Inflammatory bowel disease: a nationwide study of hip fracture and mortality risk

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Bone loss is commonly observed in patients with inflammatory bowel diseases (IBD) at all ages. Osteoporotic bone can have devastating consequences, particularly in elderly patients. Hip fractures are associated with excess mortality in the general population. For IBD patients, there are only limited data available. Therefore, we aimed to assess hip fracture risk and all-cause mortality risk among IBD patients.

In a nationwide nested case–control study, 56,821 hip fracture cases aged ≥ 50 years and 113,718 age-, sex- and region-matched non-hip fracture controls were analyzed. A history of IBD was assessed from data of all Austrian social health insurance funds between 2012 and 2016. Cox regression analysis was used to calculate mortality risk and logistic regression for fracture risk.

We identified 531 patients with IBD (25.0% men, mean age 81.2 years, SD 9.7). The prevalence of Crohn's disease (CD) and ulcerative colitis (UC) was higher among hip fracture cases compared

to controls (211 CD and 299 UC vs. 67 CD and 145 UC per 100 000, respectively). Analysis adjusted for anti-osteoporotic and glucocorticoid treatment showed that IBD patients had increased risk of hip fracture (OR 2.37, 95%CI 2.00–2.81), while patients with CD revealed a higher hip fracture risk compared to the UC patients (OR 3.10, 95%CI 2.33–4.14 and OR 2.02, 95%CI 1.63–2.51, respectively). The overall mortality risk after hip fracture was higher among IBD patients, particularly for women (adjusted HR 1.33, 95%CI 1.07–1.64). The risk was greater for CD (HR 1.61, 95%CI 1.18–2.19, $p = 0.003$) and not significant for UC (HR 1.15, 95%CI 0.86–1.55, $p = 0.34$).

In this nationwide study, IBD patients of advanced age had a more than twofold increased risk for sustaining a hip fracture and an increased risk of death.

This study emphasizes, with recent data, that priority should be given to improve bone health in elderly IBD patients.

P258

Anterolateral femoral bowing and loss of thigh muscle are associated with occurrence of atypical femoral fracture: effect of failed tension band mechanism in mid-thigh

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The purpose of this study was to characterize anterolateral bowing of the femur using X-rays and muscular atrophy in the mid-thigh using computed tomography (CT) in patients with AFFs. We then compared the results with those of an intertrochanteric fracture to understand whether these measures act as causative factors of AFFs. From January 2009 to December 2015, 37 patients with complete AFF and 12 patients with incomplete AFF were enrolled in this study. Lateral femoral bowing, anterior femoral bowing, crosssectional area (CSA), and attenuation coefficient of thigh muscles in the AFF group are measured and compare with those in the intertrochanteric fracture group. Lateral and anterior femoral bowing in the AFF group were significantly higher than those in the intertrochanteric fracture group. The level of fracture was found to be significantly associated with lateral and anterior femoral bowing ($r = 0.569$, $r^2 = 0.324$, $p < 0.001$; $r = -0.530$, $r^2 = 0.281$, $p < 0.001$, respectively). Total CSA and CSA of anterior and medial compartments were significantly lower in the AFF group ($p < 0.05$). The attenuation coefficient of the total thigh muscle and all three compartments in the AFF group were significantly lower than those in the intertrochanteric fracture group ($p < 0.05$). This study demonstrated that anterolateral femoral bowing was highly associated with the occurrence of AFF. Atrophy of thigh muscles was found in patients with AFF. We suggest that anterolateral femoral bowing and loss of thigh muscles can be the causative factors of the occurrence of AFF.

Keywords: Atypical femoral fracture, Femoral bowing, Thigh muscle

P266

Influence of drug-based osteoporosis therapy on physical performance parameters and pain

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Background: Osteoporosis is a multifactorial medical condition which is increasing in prevalence. It is considered to be a primary cause of fractures, morbidity and chronic pain. Training to improve muscles and coordination can be helpful to support the sufferer to manage everyday tasks, sooth pain and to prevent accidents. However, the effect of a targeted drug based therapy on these parameters remains uncertain.

Methods: During an eighteen month prospective study twenty five osteoporosis patients aged 48 to 78 (65.0 ± 8.3) received a therapy using osteoporosis specific drugs. The treatment results analysed (pre/post) both physical capabilities and pain.

Gathered data were analysed using SPSS (SPSS Inc., Chicago, USA). First the data were descriptively evaluated. The quantitative characteristics were outlined by use of mean, standard deviation (sd) and number of available data sets as interval mean $\pm$ SD. The qualitative evaluation utilised absolute and percentage frequency.

Depending on the results of the Shapiro-Wilke test on standard distribution, we used the corresponding t-test to evaluate the variation of each parameter throughout the measurements, or we used the Wilcoxon Signed Rank Test. For testing qualitative characteristics and analysing the categorical frequencies we applied the Chi^2-test. One this was complete, we calculated the effect size r. All p-values are the result of a double-sided statistical tests and in general is $p \leq 0.05$ considered significant.

Results: No changes became apparent for trunc strength, mobility and coordination ($p > 0.05$). Patients showed significant loss in body height ($p < 0.001$), grip strength in the right hand decreased significantly ($p = 0.006$) and the pain niveau dropped significantly ($p = 0.001$). Changing factors for a therapeutic success were a Teriparatide intake ($p = 0.043$), physical activity ($p = 0.021$) and tendentially the BMI ($p = 0.073$).

Conclusions: Pain is lowered by osteoporosis medication, but for physical performance indicators there are no or even declining effects.

Keywords: Grip strength, mobility, osteoporosis, pain

P267

Clinical effectiveness and safety of Denosumab in ESRF patients on dialysis: a systematic review

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Introduction: Adult patients with end stage kidney disease (ESRD) on dialysis are at high risk of fractures due to chronic kidney disease mineral bone disorder (MBD) and osteoporosis. Most of the osteoporosis drug treatments are not licenced for use in dialysis patients. Denosumab (DN) is a human monoclonal antibody licenced for the treatment of osteoporosis. There is scarcity of literature about the clinical effectiveness and safety of DN in end stage renal disease on dialysis.

Aim: To systematically review the available scientific literature about the clinical effectiveness and safety of DN in dialysis population.

Methods: Clinical question was designed using patient, intervention, comparison & outcome (PICO) framework. A systematic literature search was conducted using Cochrane, Embase, Pubmed, CINAHL and Medline databases for publications related to the use of DN in adult dialysis population. References list and grey literature using Google scholar were searched for relevant publications. Search was limited to publications in English language and adult population.

Results: One hundred and sixty seven titles and abstracts were screened by two authors independently. Nine studies fulfilled the study inclusion and exclusion criteria. There was dearth of literature

evaluating the clinical effectiveness of DN. The literature about safety was also limited but it consistently highlighted the risk of progressive and time dependant hypocalcaemia after administration of DN in dialysis patients. It was severe and symptomatic in few cases.
Conclusions: DN could be used to treat osteoporosis in dialysis population who are at high risk of fractures however they should be closely monitored for adverse events especially hypocalcaemia. It could be serious however with adequate monitoring and Calcium/vitamin D supplementation it could be managed. Adequately powered, randomized controlled studies with longer follow up are required to assess the effectiveness of DN in dialysis population.

P268

Treatment of osteoporotic hip fracture in asian elderly patients (osteosynthesis versus endoprosthesis)

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Background: The purpose of this study was to compare the clinical results between osteosynthesis and endoprosthesis for femoral neck fractures in asian elderly patients, and to analysis the factors that may affect the failure of osteosynthesis.
Methods: A retrospective review of 382 hips over 65-year old with femoral neck fracture was done. Within non-displaced fracture group, 81 cases (56.6%) underwent internal fixation (IF) and with 62 cases (43.3%) having bipolar hemiarthroplasty (BPHA). As for displaced fracture group, 60 cases (25.1%) underwent internal fixation (IF) with 179 cases (74.8%) having BPHA. Average follow-up period for the patients was 36.8 months. Analysis was conducted on complications depending on fracture types and osteoporosis, and clinical evaluation was done on gait capability by using Koval walking ability.
Results: As for Koval score for clinical assessment, in non-displaced group, IF group showed change of 1.3 from 1.5 before operation to 2.8 during the last follow-up, while BPHA group recorded change of 1.07 from 1.54 to 2.61 during final follow-up. Though BPHA group showed lower reduction in gait decrease, it was not statistically significant difference ($p = 0.093$). As for complications, BPHA group showed statistically significant lower percentage of complications compared to IF group, ($p = 0.017$) but re-operation rate was no significant differences ($p = 0.17$).
Conclusions: Endoprosthetic replacement could be a primary option for displaced femoral neck fracture in elderly asian patients. The choice of surgical treatment methods of non-displaced fracture in elderly asian patients should be determined carefully considering the age and the presence of osteoporosis.
Keywords: Osteoprosis, hip, femur neck fracture, osteosynthesis, endoprosthesis

P270

The influence of nacre nutritional supplementation on bone preservation in aged mice

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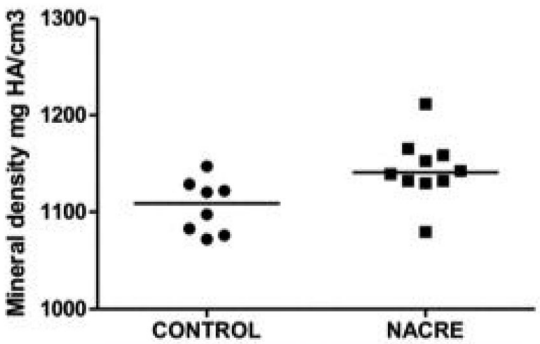
Nacre, or mother-of-pearl, is a calcium carbonate composite produced by bivalves such as pearl oysters, as an internal shell coating. Nacre powder and extracted molecules can prevent bone loss in an ovariectomy-induced osteoporosis mouse model (Kim et al., Biomaterials, 33:7489, 2012). In this study we have investigated the effect of nacre powder supplementation in aged mice.

Nacre powder was administrated orally to 22 months old female mice (25 mg/kg/day), 28 days (n = 10), when water was given to control (n = 8). The microarchitecture of trabecular and midshaft cortical bone was assessed by micro-CT. Biochemical markers CTX, osteocalcin and P1NP were measured in serum. Gene expression of bone formation and resorption markers was investigated in the femoral cortex and the bone marrow.

We demonstrated that nacre powder treatment of aged mice led to significantly higher femoral trabecular mineral density (figure 1) and trabecular thickness. After 1 month bone cellular activity appeared to be reduced, as suggested by lower serum CTX, osteocalcin and P1NP. No significant variation was observed for Runx2, osteopontin, osteocalcin, Rank, Rankl, Cathepsin K, Osteoprotegerin, BMP-2, BMP-4 and TRAcP gene expression. Nacre powder was well tolerated and induced neither variation of weight nor side effects in other organs assessed by anapathology.

Thus, nacre supplementation showed positive effect on bone quantity in 22 months old mice. Further investigations will complete this study with longer administration. In addition of being a source of calcium, nacre contains bone bioactive compounds currently under study in our lab.
Keywords: Nacre, Osteoporosis, Ageing, Bone remodelling



Mineral density	Mann-Whitney test
	p=0,0056**

[Figure 1]

P271

Effect of a synthetic osteoconductive bone graft substitute with zeta potential control (geneX-ds) in the treatment of intertrochanteric fracture

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Introduction: The aims of the present study were to identify the clinical effect of application of geneX-ds in elderly patients with intertrochanteric fracture treated using PFNA.
Materials and Methods: From March 2014 to October 2017, 235 patients (65 men and 168 women) were enrolled in this study. All patients received surgical treatment using PFNA. We compared the preoperative details and surgical outcomes, including radiologic outcome (postoperative reduction, tip apex distance, sliding distance of helical blade, union, and union time) and clinical outcomes (Harris Hip Score and the walking ability at the last follow-up) between the geneX-ds group and no geneX-ds group. each groups. Pearson's Chi square test or Fisher's exact test for categorical variables, and the independent Student's test or Mann–Whitney U-test for continuous variables were used for the analysis, where appropriate.
Results: In patients with unstable fracture who achieved anatomical or extramedullary type of reduction, the average sliding distance at 1, 3, 12 months were 4.9 mm, 7.5mmm and 8.1 mm in geneX-ds group and 7.5, 10.8, and 12.1 mm in no geneX-ds group, respectively. There were significant differences in the sliding distance at 1, 3, 12 months between these two groups.
Conclusions: The use of synthetic osteoconductive bone graft substitute with zeta potential control may have positive effect on controlled sliding of helical blade and the fracture healing of intertrochanteric fracture.
Keywords: Intertrochanteric fracture, Zeta potential control, Bone graft substitute, Proximal femoral nail antirotation, Osteoporosis

P272

Efficacy and safety of vertebroplasty and kyphoplasty in osteoporotic vertebral compression fracture with posterior cortical bone injury

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Introduction: Vertebral compression fracture with posterior cortical bone injury has increased the risk of leakage of bone cement and has been considered as a relative contraindication of vertebroplasty and Kyphoplasty. The purpose of this study was to evaluate the clinical effect of vertebroplasty and kyphosplasty in osteoporotic compression fractures and evaluate the cement leakage in patients with posterior cortical bone injuries using CT.
Materials and methods: We retrospectively analyzed the patients who underwent vertebroplasty or kyphoplasty for OVCF between July 2011 and October 2016. The patients were divided into two groups according to the presence of posterior cortical bone injuries. Clinical evaluation was performed prior to the operation, at immediate postoperatively and 1-year follow-ups. Radiography were taken preoperatively, immediate postoperatively, and 1-year follow-ups. Anterior and posterior vertebral height was measured in lateral radiographs. Postoperative radiographs and computed tomography were also reviewed for leakage and canal enchroachment.
Results: Total of 647 vertebroplasty or kyphoplasty were performed. Among 647 vertebral bodies, 291 were compression fractures and 356 were burst fractures. The overall leakage rate was 33.14% in the compression fracture and 31.95% in the burst fracture. Compression fracture group and stable burst fracture group showed no difference in total leakage rate regardless of the procedure. Both vertebroplasty and Kyphoplasty in the vertebral body with osteoporotic burst fracture showed improvement in anterior vertebral height ratio and kyhotic angle after the operation, and Kyphoplasty showed better result than vertebroplasty. The degree of canal enchroachment was improved both vertebroplasty and kyphoplasty group in burst fractures.
Conclusions: Vertebroplasty and kyphoplasty in burst fractures showed no clinically important difference in cement leakage rate compared with compression fracture and showed clinical and radiologic improvement. Vertebroplasty and kyphoplasty are good treatment options with safety and efficacy in osteoporotic burst fractures.
Keywords: Osteoporotic fracture, vertebroplasty, kyphoplasty, cement leakage

P273

Is really full length inramedullary nail necessary for treatment for atypical subtrochanteric femur fracture?

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Purpose: The purpose of this retrospective study was to evaluate whether length of nail would influence on the radiologic and hemodynamic outcomes in the treatment of atypical insufficiency subtrochanteric fractures.
Materials and Methods: Seventy-two consecutive fractures with atypical insufficiency subtrochanteric fractures who had undergone intramedullary fixation using proximal femoral anti-rotation nail (PFNA, diameter, 10, 11, 12 mm; length, 200,240, 300, 340, 380 mm, Synthes, Oberdorf, Switzerland) between March 2010 and March 2016 were followed-up for over 12 months. Patients were classified partial nail used group (200, 240 mm) and full length nail used group (300, 340, 380 mm). For radiological assessment, time to union, change of neck-shaft angle, and leg length discrepancy (LLD) were measured. For hemodynamic parameter evaluation, operation time and total blood loss until postoperative 24 h were investigated.
Results: Radiologically, there were no significant difference between partial length nail group and Full length nail group in union period, change of neck-shaft angle and leg length discrepancy (LLD) (In order, $p = 0.429$, $p = 0.273$, $p = 0.359$). Clinically, There were no significant difference total blood loss but difference in operation time (In order, $p = 0.249$, $p = 0.034$). There were three operation related complications. One lateral cortical thinning around nail tip occurred in short nail used group. Four intraoperative iatrogenic fractures on ipsilateral side occurring nail insertion were occurred in long nail used group. There was one ipsilateral secondary fracture after operation in partial nail length groups.
Conclusion: Full nail length PFNA for atypical insufficiency subtrochanter fracture was not more efficient except ipsilateral femoral secondary fracture and has disadvantage in respect of long operation time.

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The clinical utility of TRACP-5b to monitor intravenous zoledronate and denosumab treatment of osteoporosis

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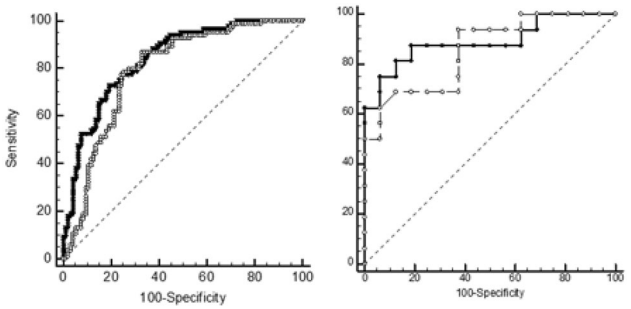
Background: Bone turnover markers can be used to monitor response to osteoporosis treatment. Tartrate resistance acid phosphatase (TRACP-5b) is a bone resorption marker that reflects osteoclast number and has minimal biological variation. We commonly use the bone formation marker PINP in our clinical practice to identify treatment response.
Objectives: To compare the diagnostic accuracy of TRACP-5b to PINP to assess the response of patients to zoledronate and denosumab therapy in a clinical setting.
Methods: 97 patients (74 female and 23 males, mean age 70) receiving 5 mg annual intravenous zoledronate for osteoporosis were recruited after at least one infusion. 97 patients receiving no treatment were recruited as group-matched controls. 16 patients (15 females and 1 male, mean age 76) receiving at least their second dose of 6 monthly 60 mg subcutaneous denosumab and 16 matched controls were recruited. TRACP-5b (ELISA, Nittobo) and PINP (automated immunoassay, iSYS-IDS) were measured in serum in the non-fasting state between 0800 and 1700.
Results: ROC curve analysis was used to compare TRACP-5b and PINP Figure. The AUC for TRACP-5b and PINP were 0.79 and 0.84, respectively in the zoledronate group. The difference between the AUCs were 0.048, p = 0.233. The AUC for TRACP-5b and PINP were 0.85 and 0.89, respectively in the denosumab group. The difference between the AUCs was 0.037, p = 0.545.
Conclusions: TRACP5b and PINP have similar diagnostic accuracy in identifying patients receiving parenteral anti-resorptive treatments for osteoporosis
Keywords: Osteoporosis, Bone turnover markers, Treatment



[ROC: PINP (black circles) and TRACP-5b (white cricles). Zoledronic acid (left) denosumab (right)]

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Outcome of osteoporosis evaluation, treatment and follow-up in patients referred to a specialised outpatient clinic compared to patients in care of general practitioners

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Aim: Effect of patient care by osteoporosis-specialists versus general-practitioner on compliance and adherence to treatment for patient eligible for per oral bisphosphonates (po-BP)
Methods: Register study. Data extracted from the DXA-scanners in the period 2005–2013.
Inclusion criteria: T-score $\leq$ -2,5 in spine or hip, treatment naïve, eligible for po-BP.

Data on compliance/adherence was extracted from Danish medicine database.

All patients had DXA-scans evaluated by a specialist in osteoporosis; initiation of treatment and patient care differed between the study populations.

GP population (GPP): treatment initiated and patient cared by a GP.

Specialist Population (SP): treatment initiated and patient cared by an osteoporosis specialist.
Results: 11,201 DXA-scans was performed and of these 3670 met the inclusion criteria (GPP 2181/SP 1514). The GPP consisted of significantly more men, the population was older, had a shorter education, lower income jobs and more comorbidities (Charlson Comorbidity Index). No difference in baseline T-score or presence of major osteoporotic fracture (MOP).
More patients in the GPP was adherent to po-BP after 12 mo. ($p < 0.0001$), but significantly more patients in SP started other osteoporosis treatment within the first 12 mo. ($p < 0.0001$). No difference was found in overall adherence to treatment of osteoporosis.

12 mo.	SP	GPP
Adherence to po-BP	52.2%	65.5%
Other osteoporosis treatment	13.3%	3.5%
No treatment	34.5%	31.0%

[Adherence after 12 months]

Conclusions: Patients in the care of a specialist were more likely to receive other osteoporosis-treatment than po-BP but the overall compliance and adherence to treatment was not different in the two populations after 12 mo.
More knowledge on side-effects and treatment alternatives may be the explanation and may impact adherence to treatment > 12 mo. as well as future fracture risk.
Keywords: Bisphosphonate, compliance, adherence

P276

Denosumab in patients with chronic renal failure

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Objectives: The aim of this report is to study the efficacy and tolerability of denosumab in patients with chronic renal failure (CRF).
Materials and methods: Patients with CRF who received a single 60 mg subcutaneous dose of denosumab every 6 months for an minimum period of 24 months were included. Data collected included information about the following: CRF stage, fracture history, relevant medications, adverse events, serum creatinine, calcium, phosphorus, magnesium levels and ECG before treatment and every week within the first 8 weeks of treatment and every 3 months after that, vitamin D and Parathormone (PTH) levels prior to dosing, bone mineral density before and every year after treatment.
Results: 48 patients (44 female, 4 male; 11 patients with CRF stage 1, 12 patients—CRF-2, 11 patients—CRF- 3, 10 patients—CRF-4, 4 patients—CRF-5) were identified. Mean duration of treatment was 2.8 years, average eGFR—44 ml/min/1.73 m^2, mean patient age—61 years (range 42–86), mean BMI—26 (range 18–37). The average

calcium prior to dosing was 2.41 mmol/l (2.11 to 2.58) falling to 2,01 mmol/l (1.84–2.29) after dosing. Average PTH level prior to dosing was 148 ng/l (38–345) and after was 743 ng/l (154–1642). Hypocalcemia was detected in 18 of the patients studied. 6 patients developed severe hypocalcemia (calcium less than 1.94 mmol/l) and 3 of them developed seizure and prolonged QTc.

Conclusions: Denosumab is a very effective treatment of osteoporosis.. Denosumab cause a small, but not clinically significant reduction in serum calcium in most patients with CRF. Patients with severe renal dysfunction may have an increased risk of developing hypocalcemia. In patients with preexisting hypocalcemia, serum calcium and vitamin D should be monitored more frequenty throughout treatment duration.

Keywords: osteoporosis, treatment, chronic renal failure, hypocalcemia, denosumab.

P277

Regional positive effects of acarbose on bone properties in female mice

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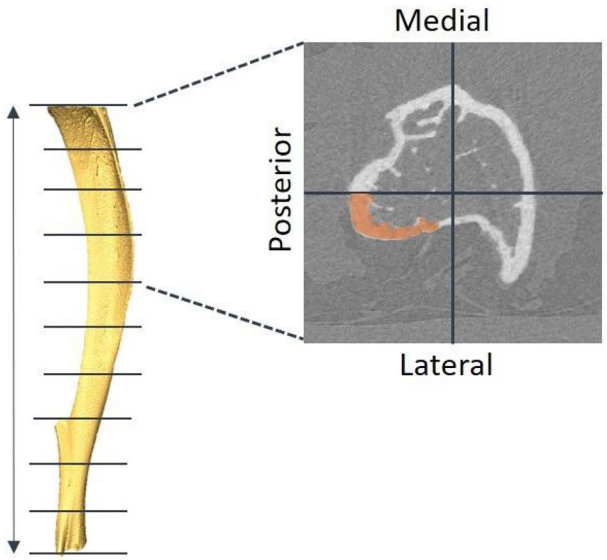
Acarbose, used for treating diabetes, has recently been shown to target the molecular mechanisms of ageing by increasing lifespan in mice. It is unknown if this molecule can slow down bone loss with age. To test this hypothesis, UM-HET3 mice were treated with acarbose. Approved by the University of Michigan's Institutional Animal Care and Use Committee. At 12 (n = 17) or 22-months (n = 21), mice were culled with their untreated controls (n = 23 and n = 29, respectively), their right tibiae imaged by micro-computed tomography (voxel size: 10.4 μm) and bone properties assessed using: standard morphometric analyses to characterise the proximal trabecular and diaphyseal cortical bone; and a novel method involving a detailed spatial analysis of the densitometric properties of the entire tibia, divided into 40 individual regions to assess heterogeneous effects of acarbose on bone volume fraction (BV/TV) and tissue mineral density (TMD). No significant effects were observed using standard morphometric methodology. Spatial analysis showed negligible effects in male mice. In female 12-month mice, positive acarbose effects were found in BV/TV, in the proximal, lateral and posterior regions of the bone (7–29%, $p < 0.05$). In 22-month female mice, there were positive effects on TMD in the same regions (2–6%, $p < 0.05$). This study provides evidence that acarbose has positive effects on bone structure in female mice, in specific regions of the tibia. These effects were only detected by the detailed spatial analysis of the densitometric properties of the tibia.



[Effects of acarbose on BV/TV and TMD in the lateral and posterior regions of the tibia]

P278

IGF-I and Pamidronate affects differently mandible than other skeletal sites

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Bone loss induced by ovariectomy (OVX) affects less the mandible than other skeletal sites. The mandible is subjected to cyclical, heavy and abrupt forces during mastication preventing part of bone loss after ovariectomy. Whether this modifies its response to osteotropic agents is not known.

We investigated the effect of IGF-I (anabolic) and pamidronate (APD, anti-catabolic) on BMD (DXA) and trabecular/cortical micro-architecture (micro-CT) of mandibular alveolar bone in OVX rats. Forty-four 4-month-old female Sprague–Dawley rats underwent trans-abdominal OVX or sham operation (SHAM). After 9 weeks, OVX animals were randomly allocated into 4 groups. Two of them received IGF-I by osmotic mini-pumps implanted subcutaneously. The two other OVX groups and the SHAM received the vehicle alone. Then, one group which received IGF-I and one OVX control group received subcutaneous injections of pamidronate (APD). The SHAM and the other OVX groups received the vehicle. Values were obtained at the end of the study, significances of differences were evaluated by ANOVA.

As compared to OVX group, IGF- and APD-treatment increased BMD in OVX animals (+6% $p < 0.05$ and +7% $p < 0.01$), while OVX did not influence significantly mandibular BMD. Trabecular micro-architecture was not influenced by IGF. However, APD increased BV/TV (+21% $p < 0.05$), and trabecular thickness (+24% $p < 0.01$) and number (+8% $p < 0.05$). OVX affected trabecular number and spacing (− 8% $p < 0.05$ and +12% $p < 0.05$). Only IGF-I increased cortical thickness (+20% $p < 0.05$), which may explain the concurrent BMD increase.

Mandibular trabecular bone responds favorably after OVX to APD but not to IGF-I, while cortical bone seems to respond favorably only to the latter. These results confirm that the mandible responds differently than other skeletal sites to osteotropic agents, possibly due to functional (mechanical loading and stimulation of bone formation),

morphological (high trabecular thickness), and embryological differences (membranous ossification).

P279

Zoledronate, but not solely Calcium and Vitamin D, can prevent the accelerated periprosthetic bone loss after total hip arthroplasty in patients with low systematic BMD

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Purpose: The aim of the study was to investigate the influence of preoperative systematic BMD on periprosthetic bone remodeling after total hip arthroplasty (THA) and the efficiency of zoledronate (ZOL) treatment on postoperative periprosthetic bone preservation.
Methods: This multicenter, prospective cohort study was conducted in 4 centers between Jan 2015 and May 2018. Patients were assigned to Normal BMD, Osteopenia and Osteoporosis + ZOL group according to T value of hip and spine measured by DEXA pre-operation. Patients with osteopenia received daily oral calcium (600 mg/d) and vitamin D (0.5ug/d), while patients in Osteoporosis + ZOL group received additional ZOL (5 mg/year). Periprosthetic BMD in seven Gruen zones were measured within 7 days, 3 months, 12 months post-operation and annually thereafter.
Results: 81 patients completed the 1st year follow-up and the mean follow-up time was 1.3 year. There were significant decreases of total Gruen zone (− 4.55%, $P < 0.05$) and Gruen zone 1(− 10.38%, $P < 0.01$) in patients with osteopenia during the 1st postoperative year, while no significant difference was found in the Normal BMD and Osteoporosis + ZOL group. Patients with osteopenia showed a greater bone loss in Gruen zone 1 (− 10.38% vs. − 3.08%) than patients with normal systemic BMD at 12 months after THA, without reaching to significant level. Patients in Osteoporosis + ZOL group showed increase of BMD in Gruen zone 1 (+16.01%), which had significant difference when compared with Normal BMD ($P < 0.05$) and Osteopenia group ($P < 0.001$). Multiple linear regression analysis illustrated that low preoperative systemic BMD was predictive of bone loss in Gruen zone 1 at 12 months after THA ($R^2 = 0.40$,$P < 0.05$).
Conclusions: Low systematic BMD is the risk factor of postoperative periprosthetic bone loss. Zoledronate, but not solely Calcium and Vitamin D, can prevent the accelerated postoperative periprosthetic bone loss in patients with low systematic BMD.
Keywords: total hip arthroplasty, periprosthetic bone remodeling, BMD, zoledronate

P280

Longitudinal effects of PTH(1–34) and mechanical loading on trabecular and cortical bone morphometry in the ovariectomized mouse

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Objectives: To determine longitudinal effects of parathyroid-hormone (PTH(1–34)) treatment alone and in combination with mechanical loading on bone morphometry in ovariectomized mice.
Methods: With ethical approval, 24 C57BL/6 mice were ovariectomized at 14-weeks old and randomised into four groups (n = 6/group): [PTH] PTH(1–34); [ML] mechanical loading; [PTH + ML] PTH(1–34) and loading; [OVX] untreated. PTH(1–34) injections were administered 5 days/week (100 μg/kg/day) during weeks 18–22. The right tibia was loaded non-invasively at weeks 19 and 21 (12 N, 40 cycles/day, 3 days/week). The right tibia was in vivo microCT-scanned (10 μm^3/voxel) every two weeks from week 14–24. Bone morphometric properties were computed in tibia trabecular metaphysis: bone volume fraction (Tb.BV/TV); in cortical midshaft: total area (Tt.Ar), thickness (Ct.Th). Between-group differences were evaluated (ANCOVA, adjusted for baseline and Bonferroni post hoc tests). Statistical significance, $p < 0.05$.
Results: In ML and PTH + ML, Tb.BV/TV was significantly higher than both PTH and OVX at week 22 (percent difference: +38 to 60%, $p < 0.001$) and was higher in ML than PTH + ML (+16%, $p = 0.001$). Tb.BV/TV did not increase in PTH. At weeks 20–24, Tt.Ar was higher in all treatments than OVX (+6 to +22%, $p = 0.001$–0.037), and both Tt.Ar and Ct.Th were higher in PTH + ML than PTH (weeks 22–24: +6 to +16%, $p = 0.002$–0.005).
Conclusions: PTH had less bone anabolic effects on OVX mice compared to ML. Combined treatment may have additional benefit to cortical bone, whereas PTH limits the osteogenic benefit of loading on trabecular morphometry. Thus, concurrent treatment may not be beneficial in the treatment of oestrogen-deficient bone loss in this mouse model.

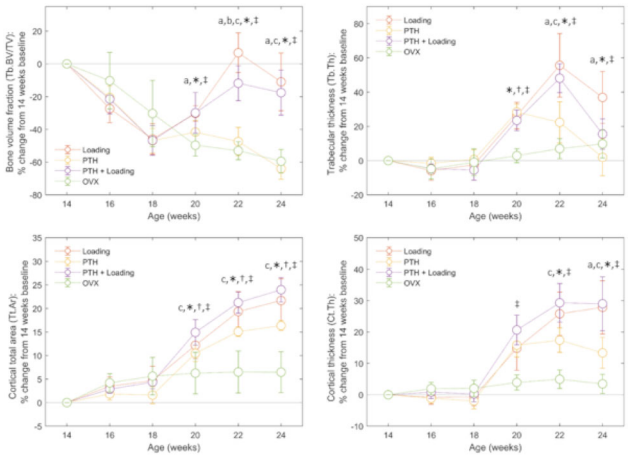

Figure 1 Mean percent change from baseline in bone morphometry. Statistical significance p<0.05 (Bonferroni-correction): [a]ML vs PTH, [b]ML vs PTH+ML, [c]PTH vs PTH+ML, [*]ML vs OVX; [†]PTH vs OVX, [‡]ML+PTH vs OVX

P281

Hip fracture risk and antiresorptive treatment in patients with type 2 diabetes: a nationwide population based case control study

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Background and aims: There is very little evidence regarding the efficacy of antiosteoporotic treatment in diabetic patients. The aim of this study was to assess the risk of hip fracture (HF) among diabetic patients with type 2 diabetes (DM-2) and to assess the fracture

efficacy of bisphosphonates (BPs) and denosumab (DMAB) among these patients.
Methods: In this nationwide case–control study we analysed data on 56,830 subjects (aged $\geq$ 50) who sustained a HF between 2012 and 2016 with follow-up until 2017, and an age-, sex-, and regionally matched control population without HF (113,724 subjects).We identified DM-2 patients based on a prescription of anti-diabetic medications (ATC code A10B: blood glucose lowering drugs, excluding insulin). Logistic regression was used to explore associations.
Results: There were 15,310 (9.3%) users of anti-diabetic medications. The mean age of diabetic patients was 82 $\pm$ 8.7 years with 10,896 (71.2%) of women. Analysis adjusted for age, sex and all medications prescribed before sustaining a HF showed, that diabetic patients were at higher risk of sustaining a HF (OR 1.12, 95%CI 1.08–1.16, P < 0.001) compared to non-diabetic patients.
Before sustaining a HF, 186 (1.2%) of DM-2 patients were treated with DMAB, 126 (0.8%) with zoledronic acid, 617 (4.0%) with ibandronic acid, 772 (5.0%) with alendronic acid and 275 (1.8%) with risedronic acid. DM-2 patients treated with DMAB had lower risk of sustaining HF whereas patients treated with BPs had slightly higher risk compared to patients without treatment. Although, after full adjustment for all confounders results were non-significant (DMAB OR 0.73, 95%CI 0.49–1.10, p = 0.13; risedronic acid OR 1.17, 95%CI 0.91–1.17, p = 0.22; alendronic acid OR 1.09, 95%CI 0.94–1.28, p = 0.26, ibandronic acid OR 1.11, 95%CI 0.94–1.33, p = 0.23, zoledronic acid OR 1.24, 95%CI 0.85–1.82, p = 0.27).
Conclusions: Antiresorptive treatment seems not to prevent HF among elderly patients with DM-2.
Keywords: Hip fracture, Diabetes mellitus-II, bisphosphonates, denosumab

P286

Microstructural analyses of historical bone samples presenting with tuberculosis

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Objectives: Tuberculosis is among the leading causes of death from infectious diseases and affects many organ systems including the skeleton. Our aim was to investigate the microstructure of bone samples diagnosed with tuberculosis from the pre-antibiotic era.
Methods: We studied 20 vertebral bodies, 20 femora and 20 tibiae with tuberculosis from the 19th and early 20th century. Moreover, 10 femora and 10 tibiae from body donors and 10 unaffected vertebrae were investigated for comparisons. The microstructure of the bones was assessed by micro computed tomography (Viscom X 8060 II). Semi-quantitative analyses of the trabecular and cortical bone compartments were performed.
Results: In the vertebrae, the femora and the tibiae with tuberculosis, a decrease of trabecular thickness, an increase of trabecular number and trabecular defects were seen. The diseased femora and tibiae were affected by cortical porosity. Cortical thickness was increased in the affected femora but decreased in the tibiae. Ankylosis was observed in 55% of the femora, but only in 25% of the tibiae with tuberculosis.
Conclusions: Skeletal tuberculosis uniformly affects the trabecular compartment. In contrast, different disease manifestations of the cortices of femora and tibiae were observed.
Keywords: Tuberculosis, Microstructure, Trabecular defect, μCT, Diseases

P287

Novel ELISA for the detection of intact FGF23

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Objectives: Fibroblast growth factor 23 (FGF23) is a bone-derived hormone, suppressing renal phosphate reabsorption and vitamin D synthesis, and stimulating calcium reabsorption in distal tubules of the kidney. The bioactive intact FGF23 contains 251 amino acids and is glycosylated and phosphorylated. Its activity is mediated by binding to FGFR/Klotho receptor complex at the target cell surface. FGF23 is cleaved between Arg179 and Ser180 to an inactive N- and C-terminal fragment. Increased serum concentrations of intact FGF23 are a hallmark of renal phosphate-wasting diseases such as ADHR, X-linked hypophosphatemia (XLH), tumor-induced osteomalacia, or autosomal recessive hypophosphatemic rikets.
Methods: Here, we show the development, characterization and validation of a new intact FGF23 ELISA. Epitopes of both monoclonal antibodies were analyzed by overlapping linear peptides spotted to a microarray and binding kinetics were determined with biolayer interferometry. The assay was validated according to standard quality guidelines regarding its specificity, precision, robustness, accuracy and linearity. Assay performance as well as sample measurements of apparently healthy and diseased human subjects were compared with other commercially available assays.
Results: The structural epitope of the coating antibody is in the N-terminal part of FGF23, whereas the labelled detection antibody detects a linear epitope at the C-terminal fragment. Both antibodies bind with high affinity. The immunoassay generates highly specific signals for human intact FGF23. Accuracy, parallelism, as well as intra- and inter-assay precision are within the standard of acceptance with 80–120% and < 10% CV respectively. The correlation of apparently healthy and diseased samples compared with existing assays on the market are quite good ($R^2 > 0.95$).
Conclusions: This well-characterized intact FGF23 ELISA can be used for the measurement of human serum and plasma samples and may support further FGF23 research in the field of bone and mineral diseases.
Keywords: Intact FGF23, fibroblast growth factor, sandwich ELISA, biomarker

P288

Difference of alveolar bone loss in ligature-induced periodontitis model according to mouse strain

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Periodontitis is a common dental disease characterized by inflammation-induced progressive damage to the tooth-supporting structures including the alveolar bone. The ligature-induced periodontitis model using mice has been used in research related to periodontitis. However, there is no standard about strain and age of mice in this animal model. In this study, to promoting the standard of ligature-induced periodontitis model we examined the difference of alveolar bone loss (to CEJ from alveolar bone) after tooth ligation according to mouse strain.

Five week old C57BL/6, BALB/C and ICR mice were used (non-Ligature group : CTL-group, Ligature group: Li-group). The left maxillary second molar was ligated using 6–0 silk. Two weeks later, the alveolar bone loss of the CTL-group and the Li-group was observed using CT images.

The alveolar bone loss in Li-groups of C57BL/6, BALB/C and ICR mice was significantly more than those CTL-groups. In Li-groups, alveolar bone loss of C57BL/6 mice was the most compared to other mouse strains. However, there was no statistically significant difference between other Li-groups because the deviation of alveolar bone loss was large in C57BL/6 mice Li-group. In ICR mice Li-group the deviation of alveolar bone loss was small. In conclusion, significant difference of alveolar bone loss between control and experimental group is observed in all mouse strains. Because the deviation of alveolar bone loss in Li-group of ICR mice is smaller than other mouse strains, the ligature-induced periodontitis model using ICR mouse may provide the useful experimental results.

Keywords: Periodontitis, Tooth ligation, Alveolar bone loss, Mouse strain

P289

Degenerative medial meniscus retains some protective effect against osteoarthritis-induced subchondral bone changes

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Introduction: Cartilage, menisci and subchondral bone are a functional unit. In a previous study, we found a significant protective effect of the meniscus on cartilage and subchondral bone structure in normal knees, we extended the investigation to osteoarthritic (OA) knee specimens.

Design: In a sample of 46 knee specimens (26 females), 11 (6 females) were considered osteoarthritic according to the Kellgren-Lawrence classification. Meniscal grading was performed. Micro-CT images were used to analyze cartilage thickness, subchondral plate thickness (SBP_Th) and the micro-architecture of subchondral bone such as BV/TV(%), trabecular thickness Tb.Th (mm), spacing Tb.Sp (mm), and number Tb.N (1/mm), Structure Model Index and degree of anisotropy. Bone micro-architecture analysis was performed at different depths (1–5 mm, 6–10 mm and 11–15 mm) in two different locations of the medial tibial plateau: one peripheral covered by the meniscus and one central not covered by the meniscus.

Meniscal grade (grade 3 or 4) was significantly higher for OA than normal knees (grade 1 or 2). with $P < 0.001$. Cart_Th was systematically lower with OA but not always significantly. SBP_Th did not differ between OA and normal knees. Knee areas without meniscal coverage and those with destroyed meniscus showed bone sclerosis, defined as increased bone volume to total volume (BV/TV), increased Tb.N, thick trabeculae with reduced spacing and a more plate-like architecture. The protective effect of meniscal coverage was observed in the 5–6 mm of the subchondral bone closest to the joint. As compared with normal knees, OA knees showed significantly increased bone sclerosis ($P = 0.05$–0.001) at the peripheral location; only BV/TV ($P = 0.03$) and trabecular number ($P = 0.02$) differed between OA and control knees at the central location not covered by meniscus.

Conclusions: A degenerative meniscus still retains some of its protective effects on subchondral bone.

P290

Male spontaneously hypertensive rats develop poor bone mass and strength

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Hypertension and osteoporosis are among major healthcare problems worldwide. Interestingly, clinical studies have shown that hypertensive patients exhibit bone loss, especially in elderly people. Men are considered to be at higher risk with earlier development and progression of high blood pressure. Thus, it is possible that hypertension in men may lead to low bone mass and poor bone quality. However, the previous findings on skeletal changes in male hypertensive patients were controversial. This discrepancy in skeletal phenotypes might be influenced by genetic background, nutrition, lifestyle and other confounding factors. Herein, we addressed skeletal changes in male spontaneously hypertensive rat (SHR), a genetic rat model of essential hypertension together with age-matched normotensive controls. This study has been approved by the institutional ethics committee. Male SHR at 12 weeks of age were selected with significant increases in systolic and diastolic blood pressures for 6–8 weeks (i.e., the onset of high blood pressure in this model is at 4–6 weeks). Volumetric bone mineral density (vBMD) and the physical properties of long bone were examined using micro-computed tomography and three-point bending test, respectively. We found that trabecular and cortical vBMD as well as cortical thickness of femur were significantly decreased in male SHR ($p < 0.05$) compared to normotensive rats. In addition, bone geometry parameters (periosteal and endosteal perimeter) were markedly decreased in these hypertensive male rats ($p < 0.01$). Furthermore, these rats also exhibited the impairment of bone strength as indicated by decrease in maximum load (i.e., ultimate force resulting in fracture, $p < 0.001$). Our findings suggested that development of high blood pressure in young adult male SHR resulted in poor bone phenotypes both in quantity and quality. Understanding the mechanism in which high blood pressure leads to bone phenotype alteration may represent new option for prevention of bone loss and fracture in hypertensive patients.

P294

Hypophosphatasia, bilateral hip fractures and seizures

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Case report: A 19-year-old man was sitting playing a computer game and lost consciousness. On regaining consciousness he was confused, had bilateral hip painandwas unable to weight bear. He was admitted to hospital.

His medical history included hypophosphatasia diagnosed when he was 6 months, two ventriculoperitoneal shunts inserted and prior bilateral femur shaft fractures following jumping from a wall that were repaired with intramedullary nails. He also had easily chipped teeth. He was taking Tramadol and Etoricoxib. He did not drink alcohol nor use recreational drugs. He excessively played computer games.

Clinical examination was normal apart from bruises on the tongue.

Full blood count, kidney, liver and thyroid functions, calcium and phosphate were normal. Alkaline phosphatase was < 20 IU/L(N 40-120).X-ray showed bilateral hip fracture.EEG showed slow background and anterior dominant sharp transients.

He underwent bilateral intramedullary fixation.

Discussion: The patient presented with bilateral hip fracture, which is extremely rare. He had rachitic bones, due to hypophosphatasia, with previous lowtrauma fractures. The previously inserted intramedullary nails may have altered the biomecheanical forces and the impact of the trauma, increasing the stress on the neck of femur and increasing the risk of hip fracture.

The patient had risk factors for epileptic activity. Infantile and childhood hypophosphatasia may be associated with pyridoxine-responsive seizures. Video games can evoke seizures. Also seizurescan be a complication of ventriculoperitoneal shunt surgery or malfunction of the shunt.

Although it was the first episode, He was treated as having an epileptic seizure, based on the multiple risk factors and the EEG findings, especially that the consequences of having other seizures might adversely affect the bone healing postoperatively.

Hypophosphatasia is extremely rare and prior to enzyme replacement therapy, survival to adulthood with childhood-onset severe hypophosphatasia was uncommon.Enzyme replacement therapy in this patient is likely to reduce his risk of future fractures.

P295

Endocrine osteoporosis: about 35 cases

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Endocrine osteoporosis is a rare complication to endocrinopathies. It can accompany or even reveal endocrine disease. Its severity is mainly related to fracture risk and the high risk of morbidity and mortality. The purpose of our work is to determine the osteodensitometric profile and the risk of fracture in patients followed for endocrinopathy.

Materials and methods: Descriptive cross-sectional study of 35 patients followed for osteoporosis secondary to endocrinopathy during the period from 2014 to 2018.

Results: The average age of patients was 56.05 years old. The sex ratio (F/H) was 0.19. The average BMI was 26.4 kg/m^2. Postmenopausal women accounted for 48% of the study population.15 patients were followed for hyperthyroidism (48%), 10 for hyperparathyroidism (32%), 8 for hypercorticism (25%) and 2 for hypogonadism (6%). The duration of evolution of endocrinopathy at the time of diagnosis of osteoporosis was 6 years. BMD was more common in the spine with diagnosed osteoporosis in 48%. The mean T score was − 2.98 at the lumbar spine with an average BMD of 0.765. Osteoporosis in the femoral neck was found in 44% of cases. The mean T score was − 2.28 with a mean femoral BMD of 0.669. In the forearm, osteoporosis was found in 34% of patients. The mean T score was − 2.37 with an average BMD of 0.532. An average T score less than − 3 in the forearm was noted in 80% of patients followed for hyperparathyroidism.11 patients (35%) had a fracture of which 63% were at the vertebral level.

Conclusions: A well conducted etiological assessment, in search of an endocrine pathology is indicated in front of any osteoporosis.

Keywords: Endocrinopathy, bone, DXA, osteoporosis, fracture

P296

Reversible hypophosphatemic osteomalacia in a patient in dialysis therapy

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Osteomalacia is a bone disease rarely reported in patients with chronic kidney disease in western countries. The main cause remains vitamin D deficiency and is reversible with repletion of high doses of calcidiol. We report the case of a patient with reversible osteomalacia.

A 51 year-old man was referred for pain in the left hip. His medical history included a nephropathy requiring hemodialysis and bilateral hip replacements. Serum levels showed normal calcium (2.2 mmol/l), low phosphate (0.48 mmol/L), high PTH (437 ng/mL, X4) and normal 25OH-vitamin D (61 nmol/L). BMD was: 1.094 g/cm^2, T-score 0). A bone biopsy revealed severe osteomalacia and bone marrow fibrosis. Vitamin D and calcium supplementation were optimized, targeting higher levels of 25(OH)-vitamin D.

An extrusion of the distal screw of the hip prosthesis appeared few months later. The second bone biopsy revealed the persistence of osteomalacia and mild secondary hyperparathyroidism. Biological disorders were unchanged despite optimized vitamin D supplementation. Oral phosphorus supplementation was introduced (1600 mg/day), resulting in the normalization of the serum phosphate levels within few days (0.54 to 1.25 mmol/l). The BMD increased significantly within five months at the lumbar spine (1.079 to 1.222 g/cm2, T-score 0.2 to 1.2 SD). Histological regression of osteomalacia was marked (osteoid volume/bone volume (53% to 25%), along with bone alkaline phosphatase levels (43 to 20 µg/l). No other bone event occurred to date.

This observation with 3 consecutives biopsies shows that hypophosphatemia remains a cause of osteomalacia in CKD patient and is reversible with phosphate supplementation. This highlights that bone and mineral disorders in CKD could be monitored with serial BMD measurements.

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Correlation of serum 25-hydroxyvitamine D level with serum LDL, calcium level in Korean women

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Introduction: Sarcopenia is a syndrome of reduced muscle mass, strength and function. Sarcopenia is a risk factor of falling, disability, and mortality and handgrip strength is a valid predictor of muscle strength. Therefore we investigated to determine the correlation between serum vitamin D (25-OHD) level and lipid and electrolyte level among Korean people.
Materials and methods: A total of 589 persons, 340 men (mean 37.1 ± 7.3 years, 19–60 years) and 249 women (mean age 35.4 ± 8.3 years, 18–61 years), who had taken serum 25 (OH) vit D level from February to October 2018 to 2014 at Ewha Womans University Hospital Health Promotion Center were included in this study. Demographic data including age, height, weight, BMI, waist and hip circumference and serum 25-hydroxyvitamin D level was collected. Serum level of lipid profile (Total cholesterol, LDL, HDL, TG), electrolyte (calcium, magnesium, phosphorus) were also collected. The analysis was done using Pearson´s correlation and Fisher test. P value < 0.05 was considered significant.
Results: Mean serum 25-OHD level of men and women were 21.1 ± 8.2 (3–70) ng/mL and 20.4 ± 8.4 (4.4–63.87)ng/mL, respectively. Statistical analysis revealed that Vitamin D had positive correlation with serum lipid profile, especially LDL cholesterol level and serum electrolyte level, especially calcium level were significantly correlated with serum vitamin D (25-OHD) level in Korean women.
Conclusions: Serum 25-OHD level was correlated with serum lipid and electrolyte level. Considering the previous data on serum vitD level among Korean people, nowadays the importance of vitamin D intake was emphasized on mass media and this may contribute to the increase of mean serum vitD level.

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Osteoporosis and hearing loss in older koreans: findings from the Korea National Health and Nutrition Examination Survey (KNHANES) 2009–2011

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Objectives: To determine the relationship between age-related hearing impairment and osteoporosis by investigating the relationship between hearing loss and cortical bone density evaluated from femur neck bone mineral density (BMD).
Methods: We used data from the Korea National Health and Nutrition Examination Survey (KNHANES) to examine the associations between osteoporosis and ARHI from 2009 to 2011. Total number of participants was 4861 including 2273 men and 2588 women aged 50 years or older. Osteoporosis was defined as a BMD 2.5 standard deviations (SDs) below according to the World Health Organization (WHO) diagnostic classification. ARHI was defined as the pure-tone averages (PTA) of test frequencies 0.5, 1, 2, and 4 kHz at a threshold of 40 dB or higher on the more impaired hearing side.
Results: Total femur T-score ($p < 0.001$), L-spine T-score ($p < 0.001$) and, femur neck T-score ($p < 0.001$) were significantly lower in the osteoporosis group compared to the normal group. Thresholds of PTA were significantly different in normal compared to osteopenia, and osteoporosis groups. In addition, there were significantly higher PTA thresholds in the osteoporosis group compared to other groups ($p < 0.001$). After adjusting for all covariates, the odds ratio for hearing loss was significantly increased by 1.7-fold with reduced femur neck BMD ($p < 0.01$). However, L-spine BMD was not statistically associated with hearing loss. ($p = 0.22$)
Conclusions: Our results suggest that osteoporosis is significantly associated with a risk of hearing loss. In addition, L-spine BMD was not correlated with hearing loss, only femur neck BMD was significantly correlated.
Keywords: Osteoporosis; Femur neck; Age-related hearing impairment; Pure tone audiometry.

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Effect of Roux-en-Y gastric bypass on bone marrow adipose tissue and bone mineral density in non-diabetic postmenopausal women

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Introduction: Bariatric surgery effectively reduces body weight and improves metabolic health, but is also associated with increased fracture risk. Bone marrow adipose tissue (BMAT) could be a possible mediator of the increased fracture risk following bariatric surgery, since high BMAT is also associated with increased fracture risk in anorexia nervosa, a disease characterized by low body weight. The aim of this study is to determine the effect of bariatric surgery induced weight loss on BMAT and bone mineral density (BMD) in postmenopausal women.
Methods: The study was approved by the local medical ethics committee. We included 17 postmenopausal, non-diabetic obese women, scheduled for laparoscopic Roux-en-Y gastric bypass surgery (RYGB). We determined bone marrow fat signal fraction (BMAT) of L3-L5, measured quantitative chemical shift imaging and volumetric BMD (vBMD) of L3–4, measured by QCT, before surgery and 3 and 12 months after surgery. Data were analyzed by linear mixed model.
Results: BMAT was negatively associated with vBMD at baseline ($R^2 = 0.41$ $p = 0.005$). Body weight decreased after surgery from 106 ± 15 [baseline] to 91 ± 13 [3 months] and 74 ± 10 kg [12 months, $p < 0.001$]. BMAT decreased after surgery from 52 ± 8% [baseline] to 50 ± 8% [3 months] and 46 ± 7%

[12 months, $p < 0.001$]. vBMD decreased after surgery from 104 ± 27 [baseline] to 95 ± 21 [3 months, $p = 0.001$] and 98 ± 26 mg/cm^3 [12 months, $p = 0.080$]. Calcium and vitamin D did not change after surgery.

Conclusions: We show a decrease in BMAT 12 months after RYGB and a decrease in vBMD 3 months after RYGB. Our results are inconsistent with the current literature, as previous research shows no change in BMAT following RYGB in non-diabetic women. These differences might be explained by the small number of postmenopausal women in these studies and could indicate that BMAT and bone metabolism following RYGB might be regulated differently in pre- and postmenopausal women.

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Cardiovascular autonomic neuropathy as a cause of impaired physical functioning in subjects with chronic hypoparathyroidism

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Background: Hypoparathyroid(hypoPT) patients treated with calcium and vitamin D supplementation show an impairment of Quality of life(QoL), an increase of fatigue and higher risk of mortality. Cardiovascular autonomic neuropathy(CAN) is characterized by an impairment of the cardiovascular autonomic system and it is associated with increased mortality and fatigability. We have demonstrated that hypoPT patients show an increased risk of CAN. No studies have investigated the association between CAN and QoL in hypoPT.

Aim: We tested the hypothesis that CAN would be associated with impaired QOL measures in subjects with chronic post-surgical hypoparathyroidism.

Design: We enrolled 48 subjects with hypoPT treated with calcium and calcitriol, and 38 healthy subjects who underwent thyroidectomy. Subjects completed the RAND36-Item Short Form(SF-36) Health Survey, evaluating physical(PCS) and mental(MCS) health. QOL was evaluated by Fatigue score(FG). CAN was assessed by cardiovascular autonomic reflex tests(CARTs). Participants were considered to have "early CAN"(EC) if they had one abnormal result in the CARTs and "definite CAN"(DC) with two or more abnormal results.

Results: Compared to no HypoPT group, participants with HypoPT had a lower PCS(313.9, SD 77.4 vs 276.6, SD 89.9, $P = 0.042$) and a lower FG(42.3, SD 7.5 vs 38.6, SD 7.7, $P = 0.031$. In the hypoPT group, compared with patients without CAN, only participants with DC had a statistically significant lower FG ($-$ 9.3 points, $P = 0.004$). These results were confirmed after adjustment for age, calcium concentration, TSH and calcium supplementation, with β: $-$ 5.57($P = 0.060$) for EC and $-$ 9.91($P = 0.002$) for DC. The corresponding figures for PCS were $-$ 57.41 for EC($P = 0.126$) and $-$ 85.26 for DC($P = 0.026$). The corresponding figures for total SF-36 were $-$ 89.68($P = 0.207$) for EC and $-$ 151.55($P = 0.039$) for DC. No statistically significant differences were found for MCS.

Conclusions: Definite CAN may represent a cause of impaired QoL in patients with chronic post-surgical hypoparathyroidism. The presence of cardiovascular autonomic neuropathy could explain the fatigue, a common complaint in patient with hypoparathyroidism.

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Abdominal aortic calcification and cardiovascular events: a systematic review and meta-analysis of observational studies

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Abdominal aortic calcification (AAC) is commonly seen on images taken for vertebral fracture assessment. The clinical importance of AAC remains poorly understood. We therefore undertook a systematic review and meta-analyses of studies reporting AAC and incident cardiovascular (CV) events or all-cause mortality. We identified 52 studies (46 cohorts, 36,092 participants) published before March 2018, from the Medline and Embase databases. Summary risk ratios (RR) were estimated from the 46 cohorts using random-effects models comparing lowest reported AAC group (referent) to all other AAC groups combined (any-more advanced AAC). Due to clinical heterogeneity, the studies from chronic kidney disease (CKD) patients and the general population were meta-analyzed separately. We identified moderate quality evidence based on GRADE ratings from studies of the general population, that people with any-more advanced AAC had higher risk of CV events; RR, 1.83 (95%CI, 1.40 to 2.39), $P < 0.001$, fatal CV events; RR, 1.85 (95%CI, 1.44 to 2.39), $P < 0.001$ and all-cause mortality RR, 2.11 (95%CI, 1.61 to 2.76), $P < 0.001$. Increasing severity of AAC was associated with increased risk for all outcomes (all $P < 0.001$). In CKD patients, there was moderate-high quality evidence that patients with any-more advanced AAC had higher risk of CV events; RR, 3.47 (95%CI, 2.21 to 5.45), $P < 0.001$, fatal CV events; RR, 3.69 (95%CI, 2.32 to 5.85), $P < 0.001$ and all-cause mortality; RR, 2.30 (95%CI, 1.86 to 2.85), $P < 0.001$. There were insufficient studies from other clinical populations to meta-analyze. In conclusion, when AAC, or more advanced AAC are seen on lateral spine images, these individuals are at a substantially higher risk of future CV events with poorer prognosis. This is particularly evident in CKD patients and individuals from the general population with more advanced AAC. Capturing and providing this information may help clinicians manage patients' cardiovascular disease risk.

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Identification of an ATP6V1B1 variant in two related patients with distal renal tubular acidosis

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Background: Distal renal tubular acidosis (dRTA) with hearing impairment is an inherited disease caused by mutations in the ATP6V1B1 or ATP6V0A4 genes encoding ATPase subunits of a proton pump. Clinical manifestations for dRTA include metabolic acidosis, hypokalemia, alkaline urine, nephrocalcinosis and progressive hearing impairment.
Objectives: To identify the genetic cause of two patients with dRTA in a Sudanese cohort.
Methods: Two genetically related Sudanese children presented with metabolic acidosis, hypokalemia, nephrocalcinosis, and rickets in a clinic in Khartoum, Sudan. One child was found having sensorineural deafness, which occurs in one third of patients with inherited dRTA. The genetically related parents of both children did not have any symptoms. Following whole exome sequencing on whole-blood derived DNA and quality control steps, Spotfire software (http://spotfire.tibco.com) was used to screen for potential variants.
Results: The variant shared by both patients, located in the gene ATP6V1B1, was identified as a nonsynonymous single nucleotide variation (rs114234874; G/A) in exon 6 with an allele frequency of 0.02–0.03 in the total and African population (Exac browser, http://exac.broadinstitute.org/). Various other selection criteria were used including autosomal inheritance pattern, high conservation (CADD score 18.8) and damage score (varied from tolerant to damaging depending on the tool used).
Conclusions: The variant found in the two children has never been reported before in patients with dRTA. Despite not being identified as a mutation (MAF > 0.01), the potential pathogenic nature of the variant may be responsible for dRTA in the two patients but requires further study in a larger population and supported by fundamental studies.

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Longitudinal follow up of bone density in children with inflammatory bowel diseases

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Objectives: Children with inflammatory bowel disease (IBD) are prone to low bone mineral density (BMD). We examined longitudinal changes in BMD, and identified predictors of improvement.
Methods: A retrospective longitudinal study of two BMD measurements at the lumbar spine (L1-L4) and total body less head (TBLH) by dual-energy X-ray absorptiometry (DXA) of pediatric patients with IBD. The change in BMD (ΔL1–4 z-score) was examined for correlation with age, anthropometric measurements, disease activity, and treatment.
Results: Forty-one patients (age at diagnosis 12.1 ± 3.5 years, 18 males) were included. At the first DXA scan, mean L1–4 z-score (-1.64 ± 1.02) and TBLH z-score (-1.62 ± 1.03) were lower than expected ($p < 0.01$). L1–4 z-score at first scan correlated positively with height-SDS ($R = 0.44$, $p < 0.01$) and weight-SDS ($R = 0.50$, $p < 0.01$); and at second scan with weight SDS ($R = 0.34$, $p = 0.04$); and negatively with inductions per year ($R = -0.46$, $p < 0.01$) and glucocorticoid courses ($R = -0.30$, $p = 0.05$). There was a trend towards improvement in L1–4 z-scores (-1.45 ± 0.83 vs. -1.64 ± 1.02, $p = 0.12$) and TBLH z-scores (-1.28 ± 0.88 vs. -1.62 ± 1.03, $p = 0.08$). ΔL1–4 z-scores correlated positively with Δweight-SDS, Δheight-SDS, and ΔBMI-SDS, and with age at second scan ($R = 0.55$, $p < 0.01$; $R = 0.42$, $p < 0.01$; $R = 0.42$, $p = 0.01$; $R = 0.35$, $p = 0.02$, respectively); and negatively with the L1–4 z-score and TBLH z-score at the first scan ($R = -0.63$, $p < 0.01$; $R = -0.63$, $p < 0.01$, respectively). Stepwise linear regression analysis identified L1–4 z-score at first scan and Δweight-SDS as independent predictors of ΔL1–4 z-scores.
Conclusions: BMD correlated with anthropometric measurements and glucocorticoid treatment. Improvement in BMD was more pronounced in patients whose weight improved or with low BMD at the first scan.

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Intact mouse model to determine bioavailability of phosphorus from infant formulas

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Mineral bioavailability from food is influenced by multiple factors. To evaluate phosphorus (Pi) bioaccessibility from products under different digestive conditions from amino-acid based formulations, we previously used an in vitrodigestion setup.

Here we developed a mouse model to evaluate Pi bioavailability using parameters of Pi homeostasis as readouts. 46-day old males ($n = 45$) were fed an egg-white based low Pi diet (LPD) containing 0.6%Ca, 0.02%Pi ad libitum for two weeks. At 60 days of age, 15 mice continued LPD, 15 mice were switched to egg-white based control diet (COD, 0.6%Ca, 0.3%Pi), and 15 mice were switched to an amino acid-based diet (AAD, 0.6%Ca, 0.4%Pi) and administered the proton pump inhibitor pantoprazole (40 mg/Kg/d). Diets were matched for caloric content. Spot urines were collected at 60, 64, 67, 70 days of age, and at day 74 mice were sacrificed for urine and terminal blood collections.

At baseline, Pi/creatinine was not significantly different among the three groups. Urine Pi/creatinine peaked at day 70 in AAD and COD fed mice, with little change in LPD fed mice. At day 74, AAD and COD comparably restored plasma Pi and urine Pi/creatinine (table). Mice gained less weight on AAD and LPD when compared to COD diet. Pantoprazole expectedly raised stomach pH in mice.

Our findings suggest that Pi bioaccessibility measured in our in vitromodels translates well into Pi bioavailability in mice.The amino acid-based formula tested could restore parameters of phosphate homeostasis even with neutralized stomach pH.

	LPD	COD	AAD
Urine-Pi/Urine-creatinine (mg/mg)	0.059 ± 0.01	1.27 ± 0.3, p = ns vs. LPD	2.55 ± 0.3, p < 0.0001 vs. LPD, p = 0.04 vs. COD
Plasma-Pi (mg/dl)	4.7 ± 0.7	10.8 ± 0.7, p < 0.0001 vs. LPD	9.3 ± 0.6, p < 0.0001 vs. LPD, p = 0.3 vs. COD
Weight (g)	22.4 ± 0.19	24.5 ± 0.3, p = 0.0003 vs. LPD	23.1 ± 0.5, p = ns vs. LPD, p = 0.01 vs. COD
Stomach pH	5.4 ± 0.2	4.9 ± 0.15, p = ns vs. LPD	6.6 ± 0.2, p < 0.0001 vs. LPD, p < 0.0001 vs. COD

Results of 74-day old mice after 14 days on three different diets

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Bone and gaucher disease

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Gaucher disease (MG) is the most common lysosomal storage disease in children. Osteoskeletal manifestations are frequent, associated with disability and poor quality of life. Recombinant glucocerebrosidase has been recognized as a gold standard in adult and pediatric patients. The main objective of our study was to measure bone mineral density (BMD) in children with this disease under this enzyme replacement therapy (ERT).

Observation 1: O. A., aged 12, followed for a type 1 MG since the age of 5, put under TES for 1 month. It presents diffuse bone pain associated with a splenomegaly of 16 cm. The densitometric evaluation found a lumbar spine Z-score at − 1.0, − 0.9 at the femoral neck, − 0.7 at the total hip level.

Observation 2: M. B., aged 10, followed for a type 1 MG since the age of 2 years, under TES for 6 years. It is asymptomatic with an isolated 17 cm SPM. His densitometric found a lumbar spine Z-score at − 1.4, 2.0 at the femoral neck and 1.7 at the total hip.

Observation 3: A. M., 7 years old, followed for a MG since the age of 4 years. At the densitometric, a lumbar spine Z-score was at − 0.6, at − 0.1 at the femoral neck, − 0.0 at the total hip level.

Discussion and conclusions: It is an inherited autosomal recessive disease caused by a deficiency of the lysosomal enzyme beta-glucocerebrosidase. It is classified into three phenotypes, the most common type 1 (94%). Glucocerebroside accumulation in lysosomes of the monocyte-macrophage is responsible for common clinical features. Bone manifestations are common and indicate an aggressive form. In our patients, there was one case of osteopenia. It is therefore important to recognize the disease at an early stage, in order to initiate appropriate therapy and prevent progression and irreversible complications.

Keywords: Bone, DXA, Gauche disease, Osteoporosis, Glucocerebrosidase

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Metabolic phenotyping in the rare bone disease alkaptonuria: LC-QTOF-MS analysis of a new targeted *HGD*$^{-/-}$ mouse model identifies previously unreported urinary metabolite alterations

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Alkaptonuria (AKU) is a rare 'inborn error' of tyrosine metabolism caused by lack of the enzyme homogentisate 1,2-dioxygenase. The primary metabolic consequence of AKU is increased circulating homogentisic acid (HGA), which accumulates throughout the body over many years, particularly in cartilage of load-bearing joints. Deposited HGA causes a striking pigmentation in tissue, a process termed 'ochronosis'; AKU is also known as 'black bone disease'. Ochronosis inevitably causes severe early-onset osteoarthropathy. This study employed a metabolomics approach to investigate for the first time the wider metabolic consequences of *HGD*-deficiency in a new targeted *HGD-/-* mouse model which recapitulates the human AKU phenotype.

Mouse urine was collected from 15 *HGD*$^{-/-}$ (mean age(± SD) 13(0.3) weeks) and 14 *HGD*$^{+/-}$ (mean age 11(0.3) weeks) male BALB/c mice. Samples were analysed by LC-QTOF-MS (Agilent). HPLC was performed by reversed-phase gradient-elution over 1–12 min. The QTOF mass spectrometer was operated in negative followed by positive polarity, mass range 50–1700. Data were mined using two complementary approaches; first using an accurate-mass/retention-time database developed in-house from 619 metabolite standards and second using an accurate-mass database of metabolites related to wider phenylalanine, tryptophan and tyrosine metabolism.

Comparing profiles of *HGD*$^{-/-}$ *and HGD*$^{+/-}$ mice (Benjamini-Hochberg-adjusted $p < 0.05$) revealed a number of metabolite alterations previously unreported in AKU. Fourteen metabolites were increased in *HGD*$^{-/-}$, and eight were decreased. Together, these changes indicate alteration to metabolites of tyrosine degradation other than HGA, and several other associated pathways including tryptophan, catecholamine and citrate and potentially ochronotic pigment formation.

Although AKU is a single gene defect in tyrosine metabolism, these data show that there are unpredicted consequences on other metabolic pathways. The data support a host of targeted follow-up studies to further understand AKU metabolism and moreover, support our technique as an invaluable phenotyping strategy for biomarker discovery in other rare diseases.

Keywords: Alkaptonuria, metabolomics, metabolic-phenotyping

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TNAP inhibition in periodontal ligament cells during osteogenic differentiation leads to differential expression of mineralization and inflammation associated genes

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Hypophosphatasia (HPP) is a rare hereditary disease manifesting symptomatically, including bone and dental mineralization defects, and caused by malfunctions of tissue-nonspecific alkaline phosphatase (TNAP) due to mutations within the *ALPL* gene. Its leading dental symptom is premature loss of deciduous teeth due to failing periodontium. Furthermore, HPP patients may suffer from impaired dental mineralization and have an increased risk for the development of periodontitis. Nevertheless, the molecular mechanisms behind those changes remain largely unknown.

We isolated dental stem cells from periodontal ligament (PDLSCs) and dental pulp (DPSCs) of human third molars and confirmed their mesenchymal stem cell character by surface marker analysis via flow cytometry and their differentiation capacity into adipocytes and osteoblasts via qPCR, Alizarin Red- and Oil Red O-staining. Additionally, RNAseq and subsequent qPCR analyses were performed to unravel the influence of TNAP inhibitor levamisole during osteogenic differentiation in PDLSCs.

Flow cytometry revealed positive expression of CD44, CD90, CD105, CD73, and CD146, whereas expression of hematopoietic markers CD11b, CD14, CD19, CD31, and CD45 was detected in less than 2% of all analyzed samples. Differentiation assays showed a low ability of PDLSC and DPSC populations for adipogenic differentiation, whereas osteogenic differentiation capacity was more prominent. Levamisole treatment completely abolished matrix formation during osteogenic differentiation. RNAseq with PDLSCs revealed differential expression of dentin matrix acidic phosphoprotein 1 (DMP1), which was downregulated in levamisole treated cells (adj p 0.017), and revealed the upregulation of purinergic P_2X_7 receptor upon levamisole treatment (adj p 0.0002).

While DMP1 is known to have anti-inflammatory properties and is promoting mineralization, pro-inflammatory stimulation has been described for the P_2X_7 receptor. Therefore, differential expression of those genes during osteogenic differentiation under the influence of levamisole may help to gain better insights into the molecular mechanisms contributing to the dental symptoms of HPP patients.

TNAP: hypophosphatasia, dental, inflammation, mineralization

P319

Biophysical analysis of novel disease-causing mutation of CLCN7 gene

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CLC-7 is an intracellular chloride-proton antiporter member of the CLC protein family. In complex with its accessory protein, Ostm-1 localizes to the lysosomes where it is important for protein degradation and to the ruffled border of osteoclasts, where it thought to play a critical role in the acidification of the resorption lacuna and in the osteoclast-mediated bone resorption.

ClC-7 KO mice phenotype revealed severe osteopetrosis, retinal degeneration and neurodegeneration associated with lysosomal storage. Consistently several mutations in the human CLCN7 gene were identified in patients with osteopetrosis, a disease characterized by dense and fragile bones associated to highly heterogeneous clinical symptoms.

We investigated the effects of nine undescribed CLC-7 mutations identified in patients diagnosed with osteopetrosis with a different level of severity.

We observed by confocal microscopy that the co-localization of CLC-7 and Ostm1 to lysosomes is preserved for both CLC-7 mutants. Moreover to determine the effects of CLC-7 mutations on the transporter activity we analyzed the patch-clamp recording exploiting a plasma-membrane-targeted CLC-7 that overcomes the limit of the lysosomal localization of the protein.

Preliminary results reveal that 4 CLC-7 mutants lose their functionality, while the other five did not abolish completely chloride currents, but showed a behavior similar to the WT or alterations in the kinetic of activation or in the total currents recorded.

We also studied whether the Cl^-/H^+ coupling is affected by the mutations using an optical assay that employs the E_2GFP/DsRed Cl_2/pH sensor fused to the C-terminus of CLC-7. We found that coupled Chloride/proton transport is conserved.

P320

Gain-of-function of TMEM16E/ANO5 scrambling activity causes gnathodiaphyseal dysplasia (GDD), an autosomal-dominant generalized skeletal syndrome

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Gnathodiaphyseal dysplasia (GDD, OMIM: 166260) is an autosomal-dominant generalized skeletal syndrome characterized by fibro-osseous lesions of the jawbones and associated with long and tubular bone dysplasia and fragility (first described by Akasaka et al. 1969; Riminucci et al. 2001). The skeletal disease is associated to different mutations in the human *TMEM16E* (*ANO5* or *GDD1*) gene, encoding for a membrane protein highly expressed in bone tissue, such as calvaria, femur and mandibule. TMEM16E belongs to a small family of membrane proteins, named TMEM16 or anoctamins, which function either as ion transporters or as scramblases, facilitating the movement of phospholipids, among which also phosphatidylserine (PtdSer), between the leaflets of the membrane bilayer.

TMEM16E is mainly expressed in intracellular membranes, and its physiological function and role in the pathophysiology of the disease are currently unclear.

We have recently shown that human TMEM16E overexpressed in mammalian cell lines displays partial plasma membrane localization and gives rise to phospholipid scrambling as well as non-selective ionic currents in presence of high intracellular Ca^{2+} (Di Zanni et al. 2018). Nine GDD mutations have been identified leading to amino acid exchanges at six positions, mostly localized in the first and in the second extracellular loop of TMEM16E. We used patch-clamp experiments and annexin-V binding assays to study the effect of the GDD-causing mutation pThr513Ile, located in the second extracellular loop, and showed phospholipid scrambling activity and large time-dependent ion currents even at low cytosolic Ca^{2+} concentrations. Additionally, we studied the mutations involving highly conserved cysteine residues localized in the first extracellular loop. These mutations, despite causing reduced plasma membrane targeting, showed a gain-of-function phenotype for scramblase activity, similar to the mutation p.Thr513Ile.

Our data provide evidence for a gain-of-function phenotype related to GDD mutations in TMEM16E.

The first three authors equally contributed.

P328

Radiological index in the flat vertebra

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Introduction and Purpose: Spine is a mechanical structure, which disposes their vertebral bodies in harmony with their stature. Defects in type II collagen gene are described in which, flat vertebra can be found, but the current vertebral indexes, don´t measure a relation between a person height and his vertebra.

Materials and methods: Patients attending physician since 1994, both sexes, 20–55 years old, in whom Type II collagen disease or vertebral dysplasia was suspected, were selected for the study. A control group was created from patients that didn´t fulfill the last inclusion criteria. Eighth dorsal flat vertebra in a lateral chest radiograph were assessed. Figure 1.

Finally, a descriptive study was carried out and a comparative study of average of vertebral index of the eight dorsal vertebra (VIDV8) results was applied: VIDV8 = 10 x LVD8/(HVD8 x stature).

Results: 174 subjects were analyzed, 84/90(study group/control one), both homogeneous and without statistically significant differences in sex, age and height. The VIDV8 value, did not show any significant difference compared to the previous variables, except for patient cohort, with an average value of 10.1 $Meters^{-1}$ in control group, and 12.5 $Meters^{-1}$ in pathological one ($p < 0.001$). To a value of 11,108 $Meters^{-1}$ the sensitivity is 90.5% and specificity 92.2%.

Conclusions: The VIDV8 is stable for the variables sex, age, height, and weight. To an outcome of 11,108 $Meters^{-1}$, it discriminates both groups with sensitivity of 90.5% and specificity of 92.2%, in order to avoid the ambiguity of the explorer.

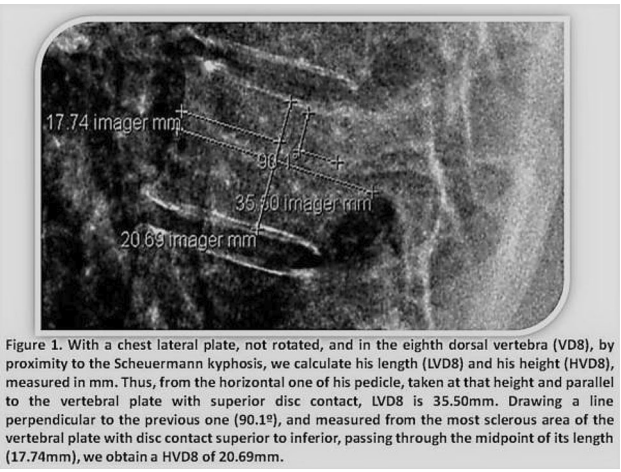

Figure 1. With a chest lateral plate, not rotated, and in the eighth dorsal vertebra (VD8), by proximity to the Scheuermann kyphosis, we calculate his length (LVD8) and his height (HVD8), measured in mm. Thus, from the horizontal one of his pedicle, taken at that height and parallel to the vertebral plate with superior disc contact, LVD8 is 35.50mm. Drawing a line perpendicular to the previous one (90.1º), and measured from the most sclerous area of the vertebral plate with disc contact superior to inferior, passing through the midpoint of its length (17.74mm), we obtain a HVD8 of 20.69mm.

P329

Bone Islands

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Osteopoikilosis is a rare autosomal dominant sclerosing disease. In this case-report, a 17-year-old boy presented multiple radio-opaque ovoid or circular lesions (enostoses) in the epiphyses and metaphyses of the long bones and pelvis. The subsequent molecular analysis of the LEM domain-containing protein 3 gene has identified the presence of a heterozygous germline mutation c.1754dup. The identical genetic mutation and radiological pattern has been observed in the father of the patient.

Keywords: Osteopoikilosis, sclerosing bone dysplasia, LEMD3 gene, enostoses

P330

Lymphangiomatosis: a rare entity presented in two patients

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Lymphangiomas are benign, slow-growing tumors composed of lymphatic vessels. Lymphangiomatosis is a rare disease developing in multiple body systems typically seen in isolation without clinical consequences. Solid organs, soft tissues and bones are the most common sites of involvement. Radiography, CT, MR and biopsy can be used for diagnosis. Radiography findings of the bone show well-defined osteolytic lesions. Biopsy of involved bone structures is mostly not effective for diagnosis.

We present two cases treated in our hospital. A 59-year old man with acute abdomen issues was operated in 2011. Laparotomy revealed mesenteric lymphangioma cystica. In 2016 a CT exam showed cystic lesions in spleen, mesentery and retroperitoneum. His neck pain began in 2017. The MRI studies of the spine demonstrated lytic lesions in the cervical, thoracic and lumbar vertebrae, which was consistent with his diagnosis of lymphangiomatosis. The bone biopsy didn't show identifiable lining of the cyst. Seven years follow-up with mediastinal, abdominal and vertebral CT and MRI did not show complications or increase in the size of the mass in our patient.

A 49-year old woman had pain and swelling above the left pelvis in the soft tissue at the age of two. She had three operations: in 1972, 1976 and 1992. Biopsy showed the lymphocytes and dilated lymphatic channel with cyst formation, compatible with cystic lymphangioma. In 2005, she had low back pain. MRI showed recidivist lymphangiomatosis in the same place near the left pelvis. In 14 years of follow-up, she had no complication.

In imaging, our patients showed signs of lymphangiomatosis involving mediastinum, retroperitoneum, mesentery, soft tissue and bones. Routine laboratory studies were normal. The patients have received no treatment. The CT and MR images of the bone should be enough for definitive diagnoses without the need for excisional biopsy.

Keywords: Lymphangiomatosis, bone, MRI, biopsy

P331

Identification of a homozygous p.Arg101His mutation in MMP2 in a 15-year-old boy with Winchester-Torg syndrome

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Winchester-Torg syndrome (WTS) is a very rare progressive skeletal dysplasia inherited in an autosomal recessive manner and classified as an osteolysis syndrome. Patients with WTS can show a wide spectrum of symptoms including peripheral osteolysis, osteoporosis, interphalangeal joint erosions, subcutaneous fibrocollagenous nodules and facial dysmorphism. So far, only a few loss-of-function

mutations in two genes encoding related matrix metalloproteinases (MMP), namely *MMP2* and *MMP14*, have been identified in patients with WTS. MMP2 is involved in the degradation of extracellular matrix. MMP14 is a member of the membrane-type MMP (MT-MMP) subfamily which can interact with MMP2 and can cleave its prodomain. This interaction is essential for the function of MMP2.

We report a 15-year-old Palestinian boy from consanguineous parents who was referred because of progressive joint contractures and muscle weakness. Physical examination revealed a dystrophic appearance, generalised muscle weakness, and multiple joint contractures. Tendon reflexes were preserved. Cognition was normal. There was no facial dysmorphism. On radiographs extensive osteolysis in the wrists was seen, as well as severe generalised osteoporosis. Using homozygosity mapping, a homozygous region on chromosome 16q12.2, which includes the *MMP2* gene, was identified. Mutation screening of the coding regions of *MMP2* using Sanger sequencing, demonstrated the presence of a homozygous missense mutation c.302G > A; p.Arg101His (NM_004530.5). The p.Arg101His mutation has already been reported in a consanguineous Saudi Arabian family. It is located in the prodomain of MMP2 which is highly conserved between members of the MMP gene family. Functional assays have shown that the p.Arg101His mutation is abolishing the enzymatic activity of MMP2.

In conclusion, the diagnosis of WTS should be considered in patients presenting with progressive contractures, pain and swelling starting in hands and feet, certainly when there is no evidence for an inflammatory joint disease. Radiographs should be performed and thoroughly evaluated for signs of progressive osteolysis.

P332

A new ALPL gene mutation case report

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Mutations within the tissue non-specific alkaline phosphatase gene [*ALPL*] are the molecular basis of hypophosphatasia [HPP], a rare disease characterized by reduced serum levels of alkaline phosphatase [ALP], defective bone and teeth mineralization, with a very variable phenotype presentation.

Genetic analysis of the *ALPL* gene was requested for a 59-year-old female, coming to our attention in order to confirm a clinical diagnosis of HPP. She referred frequent dental problems (caries) throughout her life, and discovery of low serum levels of ALP since about age 20. She was not in menopause, even if she had menstrual irregularities. Dual energy-X rays Absorption [DXA] analysis, performed by Hologic Discovery Horizon A, revealed T-scores of − 3 at lumbar spine, − 2.3 at total hip and − 3 at femoral neck respectively.

The *ALPL* gene (NM_000478.4) sequencing was obtained by MySeq Illumina technology in our laboratory, and identified the c.327C > A (p.Asp109Glu) variant, in heterozygous form, in the coding sequence of exon 5, not yet reported in the *ALPL* database (http://www.sesep.uvsq.fr/03_hypo_mutations.php).

Since both her 89-year-old mother and her 64-year-old brother had always showed low serum levels of ALP (< 35U/L), recurring dental problems (mainly caries), and bone problems (mother: multiple vertebral and hip fractures and diagnosis of osteoporosis; brother: DXA T-scores − 2.6 at lumbar spine, − 1 at total hip and − 1.6 at femoral neck), we decided to extend the genetic analysis to these relatives, and identified the same genetic variant in both.

These data highlight the importance of genetic analysis in mild HPP phenotypes in order to confirm the clinical diagnosis and establish a more suitable and beneficial therapeutic approach in these patients.

Keywords: Hypophosphatasia, adults, alkaline phosphatase, fractures, teeth

P333

1-Alpha-hydroxylase-deficiency—lost in transition

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Introduction: Active Vitamin D (1,25(OH)2VD) stems from hydroxylation of 25 OH vitamin D (25OHD) by 1-alpha-hydroxylase.

Loss of function of 1-alpha-hydroxylase causes vitamin D-dependent rickets type 1A, VDDR1A (OMIM #264700). This ultra-rare disease presents with hypocalcemia and rickets early in life. Treatment with calcitriol and monitoring of therapy is needed lifelong. At initial presentation the diagnosis may be suspected based on the typical laboratory constellation.

Case Reports: We present clinical, biochemical and treatment data of 5 patients with VDDR1A of various ages (5; 13; 16; 41 and 43 years). Diagnostic work up and treatment in the now 13 year old boy was uncomplicated. In contrast, the now 16 year old boy was misdiagnosed as PHP1b and treated accordingly. Not before his baby brother presented with typical signs of VDDR1A was the correct diagnosis established and treatment adjusted. The 41 year old women with VDDR1A transitioned from pediatric care to adult care in 1995. Subsequently, diagnoses and therapies changed repeatedly along with relocations and change of physicians. Development of renal insufficiency following a longterm calcium- and calcitriol supplementation resulted in re-assessment and re-diagnosis of VDDR1A at age 39. Her older brother (43) was lost in transition aswell but untreated.

Conclusions: VDDR1A may be suspected in the presence of characteristic clinical and laboratory findings at initial presentation, but diagnostic workup can be challenging in patients who receive calcium-relevant medication. A structured transition of care program including patient empowerment for adolescents is crucial to avoid mistreatment in later life.

Keywords: 1-alpha-hydroxylase-deficiency, Transition, Rickets, VDDR1, rare disease

P334

A novel LRP4 mutation in a middle-aged woman with widely distributed osteosclerosis

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Diffused osteosclerosis is an unusual radiographic finding with variant causes. A 40-year-old premenopausal woman came with a 7-year history of back pain, which firstly appeared at the lumber site and progressed to bilateral chest region. She had no remarkable anamnesis nor medication history and had not fractured. Radiographic results revealed severe diffuse osteosclerosis of vertebral bodies and calvarium, with no syndactyly. Dual-energy X-ray absorptiometry (DXA) BMD Z-scores suggested that she had dense bone diffusely

affecting her axial skeleton (L1-L4, Z-score +10.9; L2-L4, Z-score +10.6). Serum calcium, phosphorus levels and parathyroid hormone (PTH) were consistently normal, T-25-hydroxyvitamin D (T25OHD) 17.2 ng/ml. However, bone turnover markers were within normal range except for beta-CTX which measured 2.5-fold above the reference limits. Those seemed unmatched with the radiographic findings. Notably, we found a novel LRP4 mutation, leading to the substitution of glutamine at position 1601 in cytosine (c.1601G > C;p.R534T) by next generation sequencing and confirmed it by Sanger sequencing. Reporter assays demonstrated that this mutation led to impaired sclerostin inhibition of Wnt signaling, which is in line with the previous reported LRP4 mutations. In case to better understand the interactions between LRP4 mutation and sclerostin, we evaluated the circulating sclerostin level of serum and found a moderate increase that might be due to the failure of sclerostin binding to the mutated LRP4 protein. For diagnosis, skeletal fluorosis or aberrant phosphate homeostasis did not explain her osteosclerosis. Previous reports on LRP4 mutations affecting bone account for sclerosteosis or Cenani-Lenz syndactyly syndrome, characterized by bone dysplasia and limb abnormality. Considering ours is found in the heterozygous state, the haploinsufficiency or a dominant negative effect may explain the mild phenotype of our patient. Further localization studies will be helpful. Or if not, it will be a new cause for widely distributed osteosclerosis.

Keywords: LRP4, osteosclerosis, sclerostin, bone turnover markers, DXA

P335

Fracture prevalence and BMD in Danish adults with hypophosphatasia (HPP)

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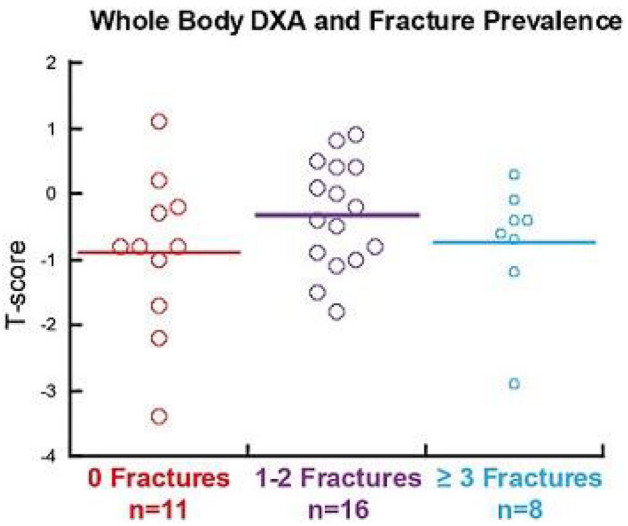
Introduction: Hypophosphatasia (HPP), a rare inborn disease caused by mutation (s) in *ALPL*, associates with reduced bone mineralization, due to impaired activity of alkaline phosphatase. Clinical findings are highly variable and often involve fractures. However, little is known about the bone quality and its association to fracture risk in HPP. The objective was to asses BMD and the fracture prevalence in adult HPP.
Methods: A nationwide observational study, including adults with genetic verified HPP, classified as compound heterozygote (CHZ; n = 5) and heterozygote (HZ; n = 30). DXA-scans (Hologic, Inc., USA) were performed to evaluate T-scores of the whole body, forearm, hip and the lumbar spine (L1-L4). Fracture prevalence was obtained from a questionnaire and the clinical journal.
Results: DXA scans revealed a higher mean T-score (± SD) of L1-L4 and the total hip in CHZ-HPP [2.06 (± 0.8); 0.94 (± 0.9)], compared with HZ-carriers [− 0.3 (± 1.5); − 0.1 (± 1.1)]. In comparison, means (± SD) of the forearm T-scores were lower and differed less between these two groups [CHZ:− 0.92 (± 0.7); HZ: − 0.8 (± 1.2)]. Further, low T-scores (whole body) were not associated with a higher number of fractures (Figure 1). Among all participants, 14% were wrongly diagnosed with osteoporosis.



[Figure 1: T-scores with means (whole body) and number of fractures in adults with HPP (CHZ and HZ).]

Conclusions: The majority of HPP patients show BMD within normal range, and osteoporosis is not common among Danish HPP patients. A DXA measurement may not be sufficient to asses fracture risk in HPP patients. Therefore, other investigations are required to evaluate the bone quality in adult HPP.

Keywords: Hypophosphatasia, *ALPL*, BMD, Osteoporosis, Fracture

P336

Novel imaging approaches to the quantification of musculoskeletal alterations in X-linked hypophosphatemic rickets (XLH)

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Background: X-linked hypophosphatemia (XLH) is a rare genetic disorder of phosphate metabolism caused by mutations in the PHEX gene. This pilot study aims to apply novel imaging techniques to asses the musculoskeletal phenotype of XLH patients by bidirectional axial transmission (BDAT) ultrasound, magnetic resonance spectroscopy (MRS) and high resolution peripheral quantitative computed tomography (HR-pQCT).
Methods: BDAT bone ultrasound of the radius and tibia was performed in eight XLH patients aged between 4.2 and 20.8 years and compared to thirthy healthy controls aged between 5.8 and 22.8 years. Nine participants opted to participate in additional HR-pQCT scanning and/or MRS.
Results: Bone ultrasound was feasible in patients and controls as young as 4 years of age. The velocity of the first arriving signal (V_{FAS}) in BDAT ultrasound was significantly lower in XLH patients compared to healthy controls: In the radius, mean V_{FAS} of XLH patients and controls was 3553 ± 196 and 3873 ± 143 m/s, respectively (− 8.3%; $p < 0.001$). In the tibia, it was 3531 ± 156 and 3757 ± 119 m/s, respectively (− 6.0%; $p = 0.019$). HR-pQCT

showed a higher trabecular thickness in XLH patients (+16.7%; p = 0.021). By MRS, we found a reduction of intramyocellular lipids in the soleus muscle in XLH patients (− 35.4%; p = 0.038).

Conclusions: BDAT bone ultrasound revealed significant differences in cortical bone quality of young XLH patients as compared to controls. Regular monitoring of XLH patients by a radiation-free technology such as BDAT might provide valuable information on bone quality and contribute to the optimization of treatment. Further studies are needed to establish this affordable and time efficient method in the XLH patient cohort.