SHORT COMMUNICATIONS 1

Bone microarchitecture and strength in long-standing type 1 diabetes

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Background: Type 1 diabetes (T1DM) is associated with an increased fracture risk, specifically at non-vertebral sites. The influence of glycaemic control and microvascular disease on skeletal health in long-standing T1DM remains largely unknown. We aimed to assess areal (aBMD) and volumetric bone mineral density (vBMD), bone microarchitecture, bone turnover and estimated bone strength in patients with long-standing T1DM.
Methods: We recruited 59 patients with T1DM (disease duration 37.7±9.0 yrs.; age 59.9±9.9 yrs.; BMI 25.5±3.7 kg/m^2; 5-year median HbA1c 7.1% [IQR 6.82-7.40]) and 77 non-diabetic controls. Dual-energy X-ray absorptiometry, high-resolution peripheral quantitative computed tomography at the ultradistal radius and tibia and biochemical markers of bone turnover were assessed. Group comparisons were performed after adjustment for age, gender and BMI.
Results: Patients with T1DM had lower aBMD at the hip (p<0.001), distal radius (p=0.01), lumbar spine (p=0.04) and femoral neck (p=0.05) as compared to controls. CTX, a marker of bone resorption, was significantly lower in T1DM (p=0.005). At the distal radius there were no significant differences in vBMD and bone microarchitecture between both groups. In contrast, patients with T1DM had lower cortical thickness (estimate -0.14 [-0.24, -0.05], p<0.01) and lower cortical vBMD (-28.66 [-54.38, -2.93], p=0.03) at the ultradistal tibia. Bone strength and bone stiffness at the tibia were significantly reduced in T1DM compared to controls. Both the altered cortical microarchitecture and decreased bone strength and stiffness were dependent on the presence of diabetic peripheral neuropathy
Conclusion: In addition to a reduced aBMD and decreased bone resorption long-standing, well-controlled T1DM is associated with a cortical bone deficit at the ultradistal tibia with reduced bone strength and stiffness. Diabetic neuropathy was found to be a determinant of cortical bone structure and bone strength at the tibia potentially contributing to the increased non-vertebral fracture risk.

Associations of calcium intake and calcium from various sources with blood lipids in a population of older women and men with high calcium intake

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Background: Promoting calcium intake is a cornerstone for osteoporosis management. Some individuals limit dairy product consumption (a major calcium source), due to their high content in saturated fats and their perceived negative impact on lipid profiles. This study explored the associations of total calcium and calcium from various sources with blood lipids in community-dwelling elderly.
Methods: We used data from 717 elderly (80% women, mean age 71±2 years) from the Geneva Retirees Cohort. Dietary calcium was assessed at several timepoints using a validated food frequency questionnaire and calcium supplement use was recorded. Blood lipids were treated as categorical variables to distinguish those with normal and abnormal levels.
Results: Increasing total calcium intake was associated with lower risks for high total cholesterol (P=0.038) and triglycerides (P=0.007), and low HDL-cholesterol (P=0.010). Dairy calcium (P=0.031), especially, calcium from milk (P=0.044) and milk-based desserts (P=0.039) i.e., low-fat (P=0.022) and non-fermented (P=0.005) dairies were associated with a lower risk for high total cholesterol. Greater calcium intakes from total dairies (P=0.020), milk (P=0.020) and non-fermented dairies (P=0.027) were associated with a lower risk for hypertriglyceridemia. No

association was observed between calcium from non-dairy sources, cheese or high-fat dairies and blood lipids. Calcium though supplements was inversely associated with hypertriglyceridemia (P=0.022) and low HDL-cholesterol (P=0.001), but not after adjustments.
Conclusions: Our results suggest that higher calcium intakes from diet or supplements are not adversely associated with blood lipids in elderly, whilst total, and particularly low-fat, dairies are valuable calcium sources potentially related to favorable lipid profiles.

Effects of transdermal testosterone and/or monthly vitamin D on fall risk in pre-frail hypogonadal men age 65 and older: a double blind 2x2 factorial design randomized placebo-controlled trial.

Bischoff-Ferrari HA, Gaengler S, Muenzer T, Dawson-Hughes B, Lang W, Theiler R, Egli A, Freystätter G.

Background: Low testosterone blood levels have been associated with an increased risk of falling in older men, however evidence from randomized controlled trials is lacking. Also, combined benefits with vitamin D supplementation are unknown.
Methods: To test whether transdermal testosterone at a dose of 75 mg per day and/or 24'000 IU Vitamin D once per month reduce the fall risk in community dwelling men age 65 and older with low total testosterone levels (<11.30 nmol/l) and fulfilling at least 1 criteria of Fried-based frailty criteria. The primary outcomes were number of persons who fell and the rate of falls, assessed prospectively at two follow-up clinical visits (6 and 12 months) and four follow-up phone calls (2, 4, 8, and 10 months). Analyses adjusted for age, fall history, person-time and the baseline measures of BMI, 25-(OH)D, total testosterone level, and short physical performance test battery score (SPPB). As there were no significant interactions between treatments, main effects are presented.
Results: 553 of 1126 men met the pre-screening inclusion criteria, and of those only 91 men met the blood level targets to be enrolled in the trial (mean age: 72.2± 5.9 years, baseline mean total testosterone blood levels: 10.8 ±3.0 mmol/L, mean 25(OH)D concentration: 26.8±7.6 ng/ml (20.9% below 20 ng/ml)). Over 12 months, 38 participants had 74 falls. The odds of falling was not significantly influenced by testosterone versus no testosterone (OR = 0.62 (0.23, 1.68)), but participants who received monthly vitamin D versus no monthly vitamin D had a 2.6-fold increased odds of falling (OR = 2.63 (1.03, 6.70)). The rate of falls was neither influenced by testosterone (IRR = 0.69 (0.37, 1.30), nor by vitamin D (IRR = 1.8 (0.94, 3.42)), significantly. Only men treated with testosterone and achieving the highest quartile of total testosterone levels at follow-up had a reduced rate of falls (IRR = 0.15 (0.02, 0.94)).
Conclusion: Transdermal testosterone did not reduce the odds or the rate of falling significantly, although a benefit among those achieving the highest testosterone blood levels cannot be excluded. Conversely, monthly vitamin D increased the odds of falling independent of testosterone supplementation.

SHORT COMMUNICATIONS 2

Sarcopenia prevalence and incidence in Swiss postmenopausal women: The OsteoLaus Cohort.

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Background: Sarcopenia is a progressive generalized loss of skeletal muscle strength and mass, leading to muscle fragility and susceptibility to falls, fracture and death in the aging population.
Aim: To assess the prevalence and incidence of sarcopenia as based on different definitions in a Swiss population.
Methods: Postmenopausal women from the OsteoLaus cohort underwent body composition (Lunar Dual-energy X-Ray Absorptiometry) and handgrip strength (HGS) (Jamar Dynamometer) measures during the third and fourth visits (mean follow-up 3.2years). Sarcopenia was defined based on HGS and appendicular lean mass (ALM)/height2 (EWGSOP-ALMI-2019/2009), or HGS and ALM (EWGSOP-ALM-2019, FNIH-ALM-2017,) or HGS and ALM/BMI (FNIH-BMI-2017/2014) or ALM/height2 (IWG), or HGS (EWGSOP-HGS-2019).
Results: 817 women participated in both visits (mean±SD: age 68.91±6.67 years, BMI 26.08±4.75 Kg/height2, HGS 24.69±5.87 Kg, ALM 17.01±2.52 Kg). Nine (1.1%), 22(2.7%), 25(3.6%), 39(4.8%), 55(6.7%), 7(0.9%), 115(14.1%) and

53(6.5%) individuals were sarcopenic as based on the EWGSOP-ALMI-2019, EWGSOP-ALMI-2009, EWGSOP-ALM-2019, FNIH-ALM-2017, FNIH-BMI-2017, FNIH-BMI-2014, IWG and EWGSOP-HGS-2019 definitions, respectively. Incident rate of sarcopenia per 100 person-years were 0.4, 0.8, 1.3, 1.5, 2.3, 0.8, 1.5 and 4.0 as based on the same definitions, respectively. Using Bonferroni correction, prevalent major osteoporotic and hip fracture were only associated with the EWGSOP-HGS-2019 definition.
Conclusions: Up to 10-fold variations were seen in both prevalence and incidence rates of sarcopenia as diagnosed by the various definitions in Swiss postmenopausal women. Only muscle strength derived from HGS was associated with fractures. The lack of a robust definition of sarcopenia with clinical significance is a barrier to its management.

Development of a personalized fall rate prediction model: the GERICO cohort analysis

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Background: Fragility hip fractures in elderly people negatively impact their quality of life, resulting in increased morbidity and mortality. Fractures are primarily caused by falls in individuals with decreased bone strength who are unable to cushion their falls. Accordingly, the number of falls has a direct relationship to fracture risk. The purpose of this analysis was the development of a statistical model to predict future fall rate using personalized risk predictors.
Methods: In the prospective cohort GERICO, several fall risk factor variables were collected in community-dwelling older adults at two timepoints (T1, T2). The fall number experienced during 12 months prior to the examination were recorded. Rate ratios (RR) for the fall number at T2 were computed for age, sex, reported fall number at T1, physical performance tests, comorbidity and medication number with negative binomial regression models.
Results: The analysis included 605 participants (male: 123, female: 482). The fall number at T1 was the strongest risk factor with a RR of 2.60 (95 % confidence interval (CI) 1.55 – 4.39) for 3 falls, RR of 2.64 (95 % CI 1.06 – 6.56) for 4 falls and RR of 10.22 (95 % CI 6.27 – 16.66) for 5 and more falls. All other predictors showed no clear association to the number of reported falls.
Conclusion: In the GERICO cohort, history of falls as predictor information for a personalized fall rate estimate is a crucial information. Specifically, individuals having experienced three and more falls are expected to experience multiple falls again.

Effect of Dalcroze Eurhythmics Exercise on Physical Function and Muscle in Sarcopenic Older Adults: The SARCARE Randomized Controlled Trial

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Background: Most therapeutic interventions for sarcopenia currently under development focus on increasing muscle mass. Given the growing evidence pointing towards the key role of the central nervous system in sarcopenia, it can be hypothesized that specific interventions influencing this system may also exert a significant effect on physical function in sarcopenic elders. Dalcroze Eurhythmics (DE) exercise has previously been shown to increase physical performances and reduce falls in older adults at high risk for falls [1], but also to modify central nervous system activity. The SARCARE trial was mainly designed to determine i) the effectiveness of a DE exercise intervention in improving physical function and reducing falls among sarcopenic older adults, and ii) whether exercise-related benefits are associated with changes at central level. Here we report the effects on physical function and muscle.

Methods: The SARCARE study (ISRCTN39600964) is a single-centre, single-blind, two-arm, randomized controlled trial, in which community-dwelling sarcopenic adults aged ≥65 years were randomized (1:1) to i) a DE exercise group (2x/week 1-hour supervised group-based) or ii) a CONtrol group (no exercise), and assessed at baseline, 6- and 12-month. Both groups received also regular educational lectures on relevant topics for older adults. The primary outcome was change in Short Physical Performance Battery (SPPB) score at 12 months. Secondary outcomes included, among others, changes in muscle strength and mass (assessed by DXA), falls, cognition, and quality of life.
Results: Among 196 randomized participants (mean age, 75.2 years; 89% women), 171 (87%) completed the study. The adherence rate to the DE exercise intervention was 81%. At 12 months, physical function improved in the DE group compared to CON group (group*time interaction for SPPB score: 1.48, 95%CI 0.98 to 1.98; p<0.001). Significant improvements were also found in the DE group on muscle strength (5x chair stand time and handgrip strength, p<0.01 for both) compared to CON group, while no effect was found on muscle mass (appendicular lean mass/height^2: -0.01, 95%CI -0.09 to 0.09, p=0.95).
Conclusions: A 12-month DE exercise program was effective in improving physical function and muscle strength in sarcopenic older adults, without change in muscle mass. Further analysis should help to clarify the mechanisms underlying the muscular benefits of DE training.
References: [1] Trombetti et al. Arch Intern Med 2011
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SBMS

Micro tensile properties of Osteogenesis Imperfecta ECM are not inferior to healthy control

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Osteogenesis Imperfecta (OI) is a genetic, collagen-related bone disease that increases bone fragility. A recent micropillar compression study revealed that OI bone extracellular matrix (ECM) is not inferior to healthy and increases with mineralization[1]. Thus, we hypothesize that the collagen defect alters the properties in tension.
In three transiliac biopsies (healthy n=1, OI type I n=1, and OI type III n=1), 23 micro tensile (healthy n=8, OI type I n=7, and OI type III n=8) were fabricated (gauge size 10x5x2µm^3) and loaded under tension until failure[2]. Ultimate strength and loading modulus were extracted from the resulting stress-strain curve. Then, fracture surfaces were graded according to the mineralized collagen fibers alignment into axial, mixed, and transversal fracture surface types (FST). Additionally, the biopsies were scanned with µCT, and the tissue mineral density (TMD) of the cortices was extracted. OI biopsies revealed 100 mgHA/cm^3 higher TMB than healthy biopsy. Interestingly, loading modulus and ultimate strength were not inferior in OI than healthy ECM. Moreover, loading modulus and ultimate strength depend on the FST (axial> mixed> transversal). Loading modulus (R^2= 0.556, p= 2.65e-05) and the ultimate strength (R^2=0.46, p= 2.2e-04) can be significantly predicted using FST and TMD as factor.
This study is limited by its small sample size and dry testing condition. Nevertheless, our study indicates that the brittleness of OI bone may be caused at a higher level (e.g., cortical thickness or porosity).

Investigating the origins of bone fragility in diabetes using multiscale experiments

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Men and women with type 2 diabetes have a 2 to 3-fold increased fracture risk. With the global epidemic of diabetes, touching one in 11 adults worldwide, understanding the mechanisms underlying diabetic bone fragility is an urgency. Contrary to standard bone fragility diseases such as osteoporosis, type 2 diabetes is not associated with a low bone mass but it is clearly caused by changes in bone quality (i.e., material properties, structure). Using a rat model of polygenic Type 2 Diabetes Mellitus (T2DM) combined with multi-scale experiments from the nanoscale to the

macroscale (including synchrotron small-angle X-ray scattering and micro-tomography experiments), we demonstrate that diabetes significantly reduces whole-bone strength and toughness for a given bone mass, and we quantify the roles of T2DM-induced deficits in material properties versus bone structure in long bones, vertebrae and discs. Deficits in material properties in diabetic rat bones were associated with increased non-enzymatic crosslinking (i.e., advanced glycation end-products) and impaired collagen fibril deformation. These findings provide insight into factors that increase bone fragility for a given bone mass in T2DM. Type 2 diabetes is not only associated with less biomechanically efficient bone structure, but diabetes also reduces tissue ductility by limiting collagen fibril deformation at the nanoscale, which ultimately reduces the maximum load capacity and energy dissipation of the bone.

Association of bone microstructure and short-term radiographic outcomes in unicompartmental knee arthroplasty

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Background: Unicompartmental knee arthroplasty (UKA) is common for patients with only medial or only lateral severe osteoarthritis (OA) of the knee. Loosening of the tibial component is the major complications. The role of bone microstructure in UKA loosening was investigated in this study.

Methods: Patients registered for UKA between March and August 2021 were recruited. Leg angles and cement distribution were evaluated from pre- and intra-operative radiographs. Subchondral tibial condyles collected during the surgery and scanned with microCT as described earlier [1]. Bone volume fraction (BV/TV), trabecular thickness (Tb.th) and bone mineral density (BMD) were evaluated in three ROIs (A, B and C) defined by the implant geometry (fig. 1 & 2). At 6 weeks, bone-implant contact was categorized in each ROI as good/radiolucent. Associations between radiolucent lines (RLLs), microstructure and cement distribution were evaluated with generalized linear modeling, adjusted for demographics and leg alignment.

Results: Twenty-one patients were recruited (21 knees, 11 females, 65±10 years old, 17 Medial, 21 SIGMA DePuy Synthes with a uni fixed-bearing tibial tray. BV/TV ranged from 31±8 % in ROI A, 41±10 % in B and 50±17 % in C. Tb.th and BMD followed similar trends. Cement depth was significantly ($p<0.001$) inversely proportional to BV/TV. At 6 weeks, radiolucent gaps were visible in 4 knees. Age ($p = 0.004$), BV/TV ($p = 0.045$) and BMD ($p = 0.049$) showed a significant association with RLLs presence.

Conclusions: Should these preliminary results be confirmed by the currently running study (target N=230), bone microstructure may be an additional biomarker for predicting risks of UKA implant loosening.

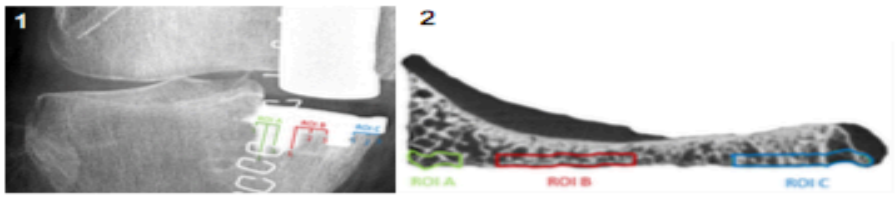

Fig. 1 – Definitions of ROIs in intra-operative radiographs were used for evaluating cement distribution. (2) Spatially equivalent ROIs were generated in the microCT scans for bone microstructure analysis.

Towards a bone-on-a-chip with physiological trabecular structure by combining biomimetic coating and high-resolution 3D printing

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Background: Bone is a highly organized, mineralized tissue assembled from nano to macro scales, characterized by hierarchical networks. However, current 3D in vitro bone models have limitations in mimicking the bone composition and architecture.
Hypothesis: In this work, we aim at developing an in vitro bone model including a 3D printed, high-resolution trabecular microstructure.
Methods: A 3D printed bone-like trabecular structure was obtained via DLP printing of an acrylated resin. Subsequently, a biomimetic coating was introduced for improving bone-like properties (simulated body fluid 10X). Calcium Phosphate (CaP) deposition efficiency was investigated by calcium quantification, alizarin red staining and SEM/EDX surface analysis. We verified cell adhesion and proliferation through live/dead staining and Alamar blue assay together with the differentiation of bone marrow-derived mesenchymal stem cells (BMSCs) and monocytes into osteoblasts (OBs) and osteoclast (OCs) respectively. Finally, the trabecular bone was integrated in a bone-on-a-chip device and colonized with bone cells and endothelial cells (EC) for generating a vascular network.
Results: Successful CaP coating was confirmed by SEM/EDX and alizarin red staining on the coated samples surface, as compared to non-coated control. CaP coating promoted cell attachment and proliferation within 7 days. We additionally obtained a successful differentiation of monocytes and BMSCs into OCs and OBs, respectively. The bone-on-a-chip device allowed the direct seeding of bone cells on top of the trabeculae and a homogeneous distribution of EC-laden fibrin gel within the trabeculae.
Conclusions: We successfully fabricated a multicellular bone-on-a-chip device, exploiting SBF surface coating of a 3D printed trabecular bone-like structure.

Effect of strain on naïve human MSC differentiation, an in vitro bioreactor study.

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Background: In bone healing, mechanical stimulation is known to enhance callus formation and secondary bone healing, therein interfragmentary strain was proven a key factor [1, 2]. Optimal magnitude of the deformation, its frequency, the optimal loading starting point and its duration are still unknown. At the cellular level, the influence of those parameters on the process of hypertrophic cartilage formation and remodeling are still not fully understood.
Hypothesis: Strain applied to naïve Bone Marrow derived Mesenchymal Stem Cells (BM-MSC) in vitro induces their differentiation towards hypertrophic chondrocytes-like phenotype.
Methods: Cellularised 8% Gelma hydrogel were subjected to 0, 10 and 30% of 5sec strain every 2hours, 24h a day for 14 days using a multiwell bioreactor (StrainBot, RISystem) and in presence of Chondro-permissive medium (CP) (DMEM high Glucose, 1% NEAA, 10 µM ITS, 50 µg/mL ascorbic acid, and 100 mM Dex), CP-plus medium (CP+, 2ng/mL TGFβ1-containing CP), or chondrogenic medium (C+, 10ng/mL TGFβ1-containing CP). Cell viability, gene expression and histology staining at day 0 and 14. (n=3 donors*) were analyzed.
Results: DNA quantification and live/dead staining revealed homogenous cell viability, density and distribution over time and in all tested conditions. No significant regulation of RUNX2, ALP, SOX or RUNX2/Sox9 ratio were observed according to the percentage of strain in CP nor CP+ medium. Yet, in CP medium Coll1, Coll 2, ACAN, COMP, MMP13 and Col10 were upregulated in response to 10% strain compared to 0% or 30%. In CP+ medium, the percentage of

strain applied showed less (Col1, COMP and Col10) or inverted (Col2, ACAN, MMP13) regulation effect. Histology showed larger and rounder cells in CP medium and 10% strain.
Conclusion: Naïve MSCs tend to develop a hypertrophy like phenotype in response to 10% strain. 10% strain induces stronger cellular response compared to 0% or 30%. In our model, Strain and TGFβ1 do not act in synergy but seem to define different cells differentiation routes.

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*# KEK-ZH-NR: 2010–0444/0
Funded by the AO Foundation.StrainBot sponsored by RISystemAG & PERRENS 101 GmbH.

Shorter resting period between cycles induces stronger MSC response to mechanical stimuli.

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Fracture healing outcomes depend on their mechanical environment [1]. In vivo studies demonstrated that mechanical stimulation enhances the process of fracture healing, where interfragmentary strain is key factor [2, 3]. Cyclic compressive displacement was shown beneficial for callus formation and further bone bridging in vivo, but the optimal loading protocol and its effect at the cellular level are not fully defined.
Using a multi-well bioreactor (StrainBot, RISystem) we aim to study the effect of resting periods between deformation cycles on human bone marrow-derived mesenchymal stem cells (BM-MSC)* in presence of chondro-permissive medium. Cells (n=3 donors) embedded in 8% gelatin methacryol gels (GelMa) experienced two axial deformation protocols for 14 days: P1: 10% strain 5 sec – 2 hours pause; P2: 10% strain 5 sec – 10 sec pause. Samples subjected to low (5 sec-2 hours) and high (5 sec-10 sec) deformation frequency for 14 days continuously (time equivalent, te) were analysed by qPCR, histology, glycosaminoglycan (GAG) and DNA quantification. Likewise, samples experiencing an equivalent number of cycles (cycle equivalent, ce, 168 cycles), either with high or low frequency were analysed.

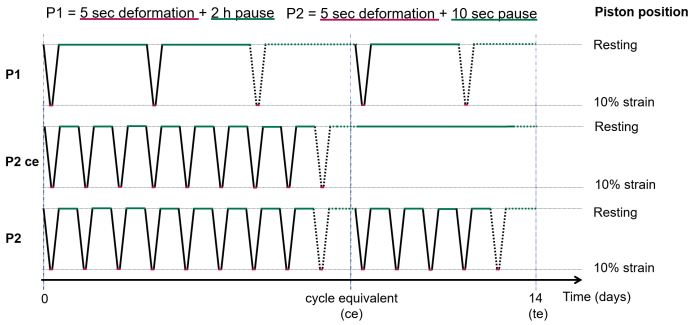

Figure 1: Schematic overview of loading study design

Our results showed that strain alone enables BM-MSCs differentiation as shown by upregulation of MMP13, Col10 and COMP genes when compared to no strain after 14 days. A shorter pause showed stronger cellular response regarding those genes. The cycle-equivalent group (P2ce) showed an increased chondrogenic gene expression. Though DNA quantification at day 14 showed a slightly decreased in P2te and P2ce compared to Day 0. Further, GAG/DNA is lower in P2te and P2ce compared to P1 at Day 14. Histology did not show a clear trend of the morphology of the cells in mechanically loaded groups. If those preliminary findings are promising, interindividual variations were large, more donors will need to be tested to confirm these results.

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[2] R. Hente, S.M. Perren, Acta Chir Orthop Traumatol Cech 85(6) (2018) 385-391.[3] S.M. Perren, J. Cordey, Springer, Berlin, Heidelberg, NY, 1980, pp. 63-77
*(KEK-ZH-NR: 2010–0444/0)
Funded by the AO Foundation.StrainBot sponsored by RISystemAG & PERRENS 101 GmbH.

Identification of the core regulatory networks regulating subchondral bone and marrow adipose tissue remodeling in human knee osteoarthritis

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Background: Subchondral bone and marrow adipose tissue (BMAT) contained therein undergo elevated turnover and remodeling in osteoarthritis (OA). However, our understanding of gene regulatory networks in individual BMAT-resident cell populations remains limited.
Hypothesis Core regulatory networks in OA BMAT may be inferred from bulk tissue transcriptomics using computational cell deconvolution.
Methods: We conducted comparative transcriptomics using three independent studies comprising subchondral bone and BMAT from non-OA controls (n=20), non-sclerotic (n=45) and sclerotic (n=59) OA samples. Cell deconvolution (adipocyte/pre-adipocyte/fibroblast/endothelial) of differentially expressed genes (DEGs) was performed using single-cell atlases of human adipose tissue. Cell type-specific DEGs were functionally annotated and core regulatory networks inferred by transcription factor (TF) binding site enrichment.
Results: 534 upregulated and 363 downregulated DEGs were shared between ≥2 datasets. Upregulated DEGs were expressed in fibroblasts (38%), pre-adipocytes (27%) and endothelial cells (22%). Dowregulated DEGs were predominantly expressed by endothelial cells (31%) and adipocytes (25%). We inferred TF networks driving upregulated DEGs in pre-adipocytes (*PRRX1/SNAI2/TWIST1*) and fibroblasts (*SP7/SMAD4/RUNX2/DLX5*) and loss of *PPARG/SOX17/SNAI1*-driven gene expression in adipocytes and endothelial cells. Pre-adipocyte DEGs were enriched for collagen fibril organization and ossification. Fibroblast DEGs associated with biomineral tissue development and negative regulation of angiogenesis. Downregulated DEGs were enriched for triglyceride catabolism (adipocytes) and regulation of vascular permeability (endothelial).
Conclusion: These data provide detailed insight into cellular and molecular mechanisms underpinning subchondral bone and BMAT remodeling in OA. An expansion of pre-adipocyte populations along altered function of BMAT adipocytes might represent a previously unrecognized regulatory mechanism and novel treatment target in OA subchondral bone.

Inositol Phosphatase SHIP1 – a Regulator of Osteoclast Lineage Cell Development and Activity

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Introduction: Src-homology (SH) 2 domain-containing inositol-5-phosphatase 1 (SHIP1) is a negative regulator of PI3K/Akt pathway that is expressed in hematopoietic cells. Osteoclast (OC) development depends on two essential pathways activated by receptor activator of RANKL and CSF-1. Both pathways involve PI3K and may therefore be regulated by SHIP1. SHIP1-deficient mice ($SHIP^{styx/styx}$) are characterized by low bone density and this study aimed to investigate cellular mechanisms leading to this phenotype.
Methods: MicroCT analysis of vertebrae and femora was performed to evaluate bone structure. To study OC development, progenitor cells (OPC) from $SHIP^{styx/styx}$ mice and control mice were cultured with RANKL and CSF-1. Osteoclastogenesis was assessed using cell viability assay and by determining TRAP activity. OC capacity to dissolve amorphous calcium phosphate (CaP) was determined.
Results: BV/TV of vertebrae and femora of $SHIP^{styx/styx}$ mice was decreased compared to wt animals (40% and 35%, respectively). Trabecular number in vertebrae from $SHIP^{styx/styx}$ mice was increased by 26%, while thickness was decreased by 30%. In femora from $SHIP^{styx/styx}$, trabecular thickness was reduced by 25%, whereas trabecular number remained unchanged. In vitro, $SHIP^{styx/styx}$ OPC showed a 1.5-fold increased proliferation compared to controls, yet the

number of OPC-derived OC was reduced by 40%. The capacity of SHIP$^{styx/styx}$ OC to dissolve CaP was decreased by 60% compared to controls.
Conclusion: Our data indicates a central role for SHIP1 in OC development and activity. The low bone mass phenotype in SHIP$^{styx/styx}$ mice, however, may be caused by reduced bone formation or by systemic inflammatory condition characteristic of SHIP1-deficient mice.

Repair of a critical-size defect in estrogen-deficient mice treated with bisphosphonates

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Background: Bisphosphonates (BP) prevent bone loss in post-menopausal osteoporosis. Since the inhibition of bone resorption by BP will cause decreased bone formation, extended treatment may impair bone healing.
Hypothesis: In this study, it is hypothesized, that BP therapy interferes with biomaterial turnover. To test this, β-tri-calcium phosphate (βTCP) ceramics coated with Bone Morphogenetic Protein-2 (BMP2) and L51P, a BMP2 analogue inactivating BMP-antagonists, were inserted into critical-size bone defects in estrogen-deficient mice under BP therapy.
Methods: Eight weeks after ovariectomy (OVX) or sham operation of NMRI mice, bone mass was assessed and BP therapy started. After five weeks, a femoral critical-size defect was generated, filled with βTCP cylinders loaded with 0.25µg or 2.5µg BMP2, 2.5µg L51P, 0.25µg BMP2/2.5µg L51P or empty controls and rigidly fixed. Femora were collected 12 weeks post-op.
Results: Micro-computed-tomography (MicroCT) revealed low bone formation in defects fitted with control implants or ceramics loaded with 2.5µg L51P and 0.25µg BMP2 in sham and OVX mice +/- BP. In contrast, cylinders loaded with 0.25µg BMP2/2.5µg L51P and 2.5µg BMP2 induced high bone formation and biomaterial turnover in sham and OVX animals, especially under BP therapy. The relative expression levels of transcripts encoding collagen I were 150 times higher than those for collagen II and 5000 times higher than for collagen X.
Conclusion: MicroCT results demonstrated the potential of L51P to increase the osteogenic efficiency of BMP2 in healing of ceramic fitted critical-size bone defects. The relative expression of different collagens suggests intramembranous bone formation in the rigidly fixed defects

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Imatinib treatment reduces bone lesion progression in a murine model of fibrous dysplasia

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Fibrous Dysplasia of bone (FD) is a rare genetic debilitating disease caused by gain-of-function mutations of the GNAS gene, encoding Gsα protein. FD bone lesions, characterized by marrow fibrosis, abnormal bone trabeculae, thin cortices and defective bone mineralization, are the consequences of impaired osteogenic differentiation and aberrant proliferation of mesenchymal stem cells, and elevated osteoclastogenesis. Platelet-derived growth factor B (PDGFB) and PDGF receptor type β (PDGFRβ) are overexpressed in human FD bone lesions. To determine whether PDGF/PDGFR signaling contributes to FD pathogenesis, 2-month-old GnasR201C-expressing mice were fed a control diet or a diet containing the PDGFR inhibitor Imatinib (0.5 mg/kg/day) for 4 months. Their bone phenotypes were analyzed by microCT and gene expression analyses. GnasR201C mice exhibited increases in Pdgfb and Pdgfrb expressions in femurs in comparison to wildtype littermates. GnasR201C mice showed increased cortical bone volume with coexisting sclerostic and lytic areas at femoral midshaft, augmented total volume and trabecular bone volume at proximal femur, and decreased volumetric bone mineral density (vBMD). Imatinib treatment did not affect trabecular bone abnormalities in GnasR201C mice, but partially prevented increases in cortical bone volume at femoral midshaft and total volume at proximal femur, and decreases in vBMD. Finally, Imatinib treatment reduced expressions of FD markers, such as Rankl, osteocalcin, fibronectin and Timp1, in femurs of GnasR201C mice. In conclusion, our results

show that Imatinib treatment reduces progression of bone lesions in a murine model of FD, and suggest that PDGFR signaling could be involved in the pathogenesis of this skeletal disease.

SG-BOND (RARE BONE DISEASES)

Late diagnosis of a rhizomelic punctuate chondrodysplasia type 1 due to a *PEX7* gene mutation in a 27 year-old woman

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A 27 year-old woman with intellectual disability, polyepiphyseal dysplasia, and "calcific tendinopathy" of the left shoulder was referred to the rare bone disease clinic for a diagnostic workup. She had bilateral joint replacement for "hip dysplasia" as a teenager, and was known for bilateral cataracts and hypoacusia. Her height was 1.45 m. Her nose was bulbous and her dentition was disorganised. She had brachydactyly, bilateral elbow flexum, lumbar hyperlordosis and slight dorsal cyphosis. The left ankle had no motion range, and that of the right ankle was limited.
Radiographs at age 27 years showed epiphyseal dysplasia of both shoulders and elbows, and fusion of the anterior aspect of the C3 to C6 vertebrae. A CT-scan including the left forearm and the ankles had shown ossification of the radio-ulnar interosseous membrane and bilateral tibiofibular ankyloses with bony bridges anterior to the distal tibio-fibular syndesmoses.
A retrospective reanalysis of childhood radiographs revealed stippled calcifications ("puncta") around several joints. Next-generation sequencing analyses revealed a truncating variant, c.45_52dup, p.His18Argfs*35, in the *PEX7* gene at the homozygous state, confirming the diagnosis rhizomelic punctuate chondrodysplasia (rCDP) type 1. Usually a severe disorder diagnosed in the newborn, some rare rCDP type 1 patients may present beyond infancy. The NGS sequencing technique may give surprising results and allow to identify "childhood onset" disorders in adults. When evaluating adults with constitutional bone diseases, inspection of childhood radiographs may allow to observe diagnostic signs (like the "puncta" in this case) that are no longer visible in the adult radiographs.

National healthcare pathway for patients with Hereditary Multiple Exostoses

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Hereditary multiple exostoses (HME) is a rare autosomal dominant genetic disorder characterised by osteochondromas (exostoses) arising near the growth plate; it is caused by loss of function mutations in the *EXT1* and *EXT2* genes.
Osteochondromas are benign tumours mainly located on the knees, shoulders, wrists, ribs and vertebrae. They may cause pain, bone deformities, local neurovascular compression and spinal compression. In 1 to 5% of cases, their thin cartilage cap may degenerate into chondrosarcoma. Patients with HME therefore require a close follow-up to detect and prevent complications from childhood through adulthood.A clear diagnostic and therapeutic clinical pathway is

crucial to improve patient care and facilitate management by healthcare professionals. In terms of imaging, a fine balance has to be found between accuracy of information, irradiation and cost-effectiveness.
At Lausanne University Hospital, we follow 23 children and 20 adults with HME. An expert panel including a rheumatologist, a geneticist and both a paediatric and adult orthopaedic surgeons, has been formed and aims to delineate a national care pathway for patients with HME. Our mission is to identify the best possible management plan from the scientific literature, from our own experience and from an analysis of practises in other national and international hospitals specialized in constitutional bone diseases.
The aim is to create a coherent, effective and easy to understand care pathway for patients with HME in Switzerland.

Molecular characterization of a newly reported MBTPS2 variant in a fetus affected with severe Osteogenesis Imperfecta

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Objectives: We described the first X-linked recessive form of Osteogenesis imperfecta caused by MBTPS2 missense mutations in patients presenting with moderate to severe phenotypes. Missense mutations in MBTPS2 laying in proximity to those found in OI, cause the dermatological condition IFAP/ KFSD without skeletal abnormalities. RNA-sequencing based transcriptomics analysis of MBTPS2-OI fibroblasts showed alterations in the expression of genes involved in lipid metabolism, cartilage and bone development (1). Furthermore, we showed a reduction in collagen deposition in the ECM by immunofluorescence analysis (1). A male proband terminated at gestational week 21/40 for severe skeletal dysplasia presented with shortening of all long bones and bowing of the femurs and tibiae. Post-delivery examination, radiology and autopsy confirmed the clinical diagnosis of OI. WES based brittle bone dysplasia panel identified a previously unreported c.516A>C; p.Glu172Asp variant in MBTPS2 classified as variant of uncertain significance. Functional studies have been undertaken to infer pathogenicity of this variant.
Methods: Total RNA was extracted from cultured fibroblasts of the aborted MBTPS2-OI fetus, an aborted COL1A2(Gly553Asp)-OI fetus and two previously characterized MBTPS2(Leu505Phe)-OI and IFAP/KFSD patients. Gene expression changes of FADS1, DHCR24, CHST3, VEGFA, ADAMTS12, DKK1 and COL1A1 showing differential expression in MBTPS2-OI were measured by RT-qPCR. Immunofluorescent staining was performed to investigate extracellular matrix protein deposition by fibroblasts in culture. Furthermore, lipids extracted from cultured fibroblasts were measured by GC-MS/MS.
Results: In the fibroblasts of the MBTPS2-OI aborted fetus we observed: i) changes in the expression of the selected genes involved in lipid metabolism (FADS1 and DHCR24), coupled with changes in the relative ratios of cellular fatty acids, as well as genes involved in skeletal/cartilage/bone development (CHST3, VEGFA, ADAMTS12, COL1A1 and DKK1); ii) a reduction in collagen deposition in the ECM by immunofluorescent staining. These alterations were similar to those documented in MBTPS2-OI patients (1).
Conclusions: Together, these findings support pathogenicity of the newly identified MBTPS2 p.Glu172Asp variant as an OI causing variant, without overlap at the molecular level with IFAP/KFSD caused by MBTPS2 variants. This study shows the value of implementing data sets identified in a multi omics study to the molecular characterization of a genetic variant of unknown significance.

1 Lim PJ, Marfurt S, et al. Front Genet. 2021;12:662751. Epub 2021/06/08. doi: 10.3389/fgene.2021.662751.