sham. Articular cartilage lesion severity scores (mean and maximum) were similar in WT and Socs2-/- mice with either DMM, or with ageing. Micro-CT analysis revealed decreases in SCB thickness (WT: 0.15mm±0.003; KO: 0.11mm±0.003; P<0.001), epiphysial trabecular number, and thickness in the medial compartment of Socs2-/-, in comparison to WT mice (P<0.001). DMM had no effect on the SCB thickness in comparison to sham in either genotype.

**Conclusions:** These data suggest that enhanced GH signalling through SOCS2 deletion accelerates growth plate fusion, however this has no effect on osteoarthritis vulnerability in this model.

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**P175**
**Stabilization of UHRF1 in synovial fibroblasts is a novel therapeutic strategy for rheumatoid arthritis**

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**Abstracts**

**Introduction:** Rheumatoid arthritis (RA) is a systemic autoimmune disease. RA is characterized by chronic synovial inflammation with aberrant epigenetic alterations. Epigenetic regulation has been suggested as therapeutic strategy, however, epigenetic regulatory mechanisms underlying RA pathogenesis remain largely unknown. Here, we showed that Ubiquitin-like protein, was administrated

**Methods and Results:** We found that murine arthritis tissue and human RA tissue, particularly synovial fibroblasts (SF), exhibit remarkable up-regulation of Uhrf1 mRNA. SF-specific Uhrf1 conditional knockout (cKO) mice showed more severe articular phenotypes associated with hyperplastic synovium and apoptosis resistance of SF than littermate control. To reveal Uhrf1 function, RNA-seq and MBD-seq were performed using SF obtained from control and cKO mice. Integrative genome-wide analyses of the transcriptome and methylome showed that expression of several cytokines including Ccl20, Tnfsf11 and Csf3 were up-regulated in Uhrf1-deficient SF accompanied with reduced DNA methylation signatures. In RA patients, Da528, Th17 accumulation and apoptosis resistance were negatively correlated with Uhrf1 expression in synovium. Besides, DNA methyltransferases (DNMT1, DNMT3A and DNMT3B) mRNA levels were comparable between OA and RA tissue and not correlated with disease activity. Finally, we assessed whether Uhrf1 stabilization contribute to improvement for arthritis pathogenesis. Ryuvidine, which was identified as a candidate chemical compound to stabilize Uhrf1 protein, was administrated

in arthritis model mice. As the results, arthritis pathogenesis was ameliorated by the treatment with Ryuvidine. Also, development of organoid derived from RA-SF was suppressed by Ryuvidine. (P-value<0.05 in all cases)

**Conclusion:** Our results demonstrated that UHRF1 expressed in SF can contribute to suppress multiple pathogenic events associated with RA such as Th17 recruitment, SF apoptosis, suggesting that targeting UHRF1 could represent a novel therapeutic strategy for RA.

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**P176**
**More Bone with Less Minerals: The Effects of Dietary Phosphorus on the Zebrafish Skeleton**

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**Abstracts**

**Background/Introduction:** Dietary phosphorus (P) is essential for mineralisation of the calcium-phosphate based vertebrate skeleton. P-deficiency can cause growth retardation, osteomalacia and bone deformities, both in teleosts and in mammals. Conversely, excess P supply triggers soft tissue calcification and bone hypermineralisation.

**Purpose:** Teleosts obtain calcium from the water through the gills, but they depend on dietary P intake. In Atlantic salmon, dietary P-deficiency causes a complete stop of bone mineralisation but bone matrix formation is unaffected. This study tested the effects of dietary P-deficiency and oversupply in zebrafish.

**Methods:** Three experimental diets containing 0.5% (low P, LP), 1.0% (control) and 1.5% (high P, HP) P were used to treat juvenile wild-type zebrafish for two months (ethical approval No.260/2020-PR). Alizarin red, X-rays, synchrotron X-ray tomographic microscopy, nanodentistology, histology and transmission electron microscopy were used to analyse bone morphology, mineralisation and mechanical properties.

**Results:** LP zebrafish display hypomineralised bones compared to controls (P<0.05), albeit without deformities. The diet LP increases the production of non-mineralised bone matrix. Osteoblasts have enlarged endoplasmic reticulum cisternae, indicative for increased collagen synthesis. The HP diet promotes growth (13.5±3.6 mm vs. 12.2±3.1 mm, P<0.05) but also a higher frequency of vertebral centra fusions (28% vs. 5%, p=0.006).

**Conclusion:** Low dietary P content stimulates the formation of non-mineralised bone without inducing malformations in zebrafish. In contrast, a high dietary P content promotes mineralisation, increases bone stiffness and leads to vertebral body fusions. This new zebrafish model shows that bone formation and mineralisation can be uncoupled. It is also a useful tool to understand the
mechanisms underlying osteomalacia and abnormal mineralisation related to variations in P metabolism.

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### P177

**Kinetics of bone loss in the murine hindlimb unloading model at two temperatures: standard 22°C vs. thermoneutral 28°C**

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The aim of this study was to investigate the effect of thermal stress on bone loss.

**Introduction:** Hindlimb unloading (HLU) is an established rodent model to study the effects of microgravity and mechanical discharge on the skeleton. We suspended mice for different times to investigate the kinetics of bone loss. Suspended mice were isolated and with limited possibility to behaviourally respond to temperature, are more prone to the cold stress that impacts bone biology at standard housing temperatures. To further explore such non-mechanical confounding factor in our system, we conducted experiments at standard and thermoneutral temperatures.

**Methods:** 16-week-old C57BL/6j male mice were acclimatized for 4 weeks at 22°C or 28°C, then submitted to HLU or kept unsuspended in pair-fed control cages (CONT) for 5, 14 and 21 days (ethical approval 201611231457342-V6).

**Results:** Analysis by in vivo microCT shows that femur trabecular BV/TV decreases in HLU at 5d and 14d compared to respective CONT (P=0.002 at 22°C, p=0.01 at 28°C; p=0.02 at 22°C, p=0.0001 at 28°C) and the loss continues at 21d (P=0.0001 at 22°C, p=0.0001 at 28°C). At mid-diaphysis, ex vivo analysis shows a decrease in cortical thickness in HLU compared to respective CONT at 21d (P=0.0001 at 22°C, p=0.0001 at 28°C) and only at 22°C at 14d (P=0.05).

Trabecular bone percentage loss of CONT is higher at 22°C than 28°C (P=0.0194 at 14d). Cortical thickness of CONT is lower compared to that at 28°C (P=0.0001 at 14d).

Osteoclast differentiation and activity gene expression changes following HLU, in particular early and at 22°C (MCSF, RANKL, TRAP, Ctsk), broadly consistent with histomorphometry results. Expression of apoptosis, senescence and autophagy genes is not affected by HLU at any temperature.

**Conclusions:** Bone loss continues to occur at 21d of suspension. Housing temperature has complex effects on bone, influencing response to unloading, but without preventing bone loss. At thermoneutrality, HLU-induced bone resorption is clearly observed, but not accompanied by an increase in osteoclast activators RANKL and MCSF.

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### P178

**Lugol as a chemical enabler of enhanced imaging for bone-muscle tissues in fish model**

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Flawless functioning of the musculoskeletal system is essential for healthy life and successful aging. Bone and muscle are the most abundant tissues in human body, while their genetic and metabolic failures can result in aging-related diseases like osteoporosis and sarcopenia. Zebrafish has highly similar musculoskeletal system with humans, which promote its rapid utilization for bone-muscle research. The knockout (KO) of srebf1 fish demonstrated a decreased BMD and altered lipids in adult zebrafish muscle. Therefore, we utilized srebf1 KO zebrafish as a model for better understanding of osteoporosis and sarcopenia.

To explore the consequences of a loss of slow muscle fibers’ mass (by fat infiltration) on muscle health and the effect of Lugol on the vertebral BMD using srebf1+/− model.

Adult male and female fish, knockout for srebf1 gene and srebf1+/− (WT) siblings were either overfed for 45 days or provided a regular diet (control), with 13-15 fish per group. Lugol contrasting solution was applied to formaldehyde-fixed fish to stain soft tissue. BMD and soft tissue attenuation were measured using micro-CT SKYSCAN 1172 (Bruker), lipids were quantified by Oil Red O staining in the same fish.

Both srebf1+/− and srebf1−/− overfed fish gained body weight and BMI significantly (P=0.015 and P=0.022) compared to WT and KO controls. We found no differences between the genotypes or diet groups in the Lugol-stained vertebral BMD. Concomitantly, overfeeding of srebf1−/− fish significantly (P<0.0087, P<0.000001) reduced fast and slow muscle fibers mass compared to srebf1−/− control siblings, while slow muscle fibers demonstrated significant increase (P=0.0001) in lipids in comparison to fast muscle fibers.

This study confirmed that Lugol staining did not alter BMD of the adult zebrafish. Lugol staining for micro-CT scanning provides a unique framework to study simultaneously bone and muscle/fat tissues in zebrafish, for capturing manifestations of osteoporosis and sarcopenia.

**Keywords:** Zebrafish; Lugol staining; Lipids; Bone-muscle diseases

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### P179

**Differences between the effects of anti-catabolic zoledronate and anabolic teriparatide treatments on bone in adult ovariec-tomized rats**


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*Background/Introduction:* Human osteoporosis is a systemic skeletal disease characterized by low bone mass and increased bone fragility. Effective osteoporosis therapies are available including anti-catabolic and anabolic agents. These treatments have been associated with adverse effects on skeleton including atypical fractures and osteosarcoma. New therapies are needed with improved efficacy/safety ratio.

**Purpose:** We compared the effects of anti-catabolic zoledronate and anabolic teriparatide treatments on bone in adult ovariec-tomized (OVX) rats used as the major small animal model of postmenopausal osteoporosis in nonclinical efficacy studies.

**Methods:** Female Sprague-Dawley rats were ovariec-tomized or Sham-operated at 6 months of age. OVX rats were treated subcutaneously with zoledronate at 20 μg/kg, teriparatide at 40 μg/kg/d or with vehicle, and Sham-operated rats with vehicle for 2 months. Treatment effects