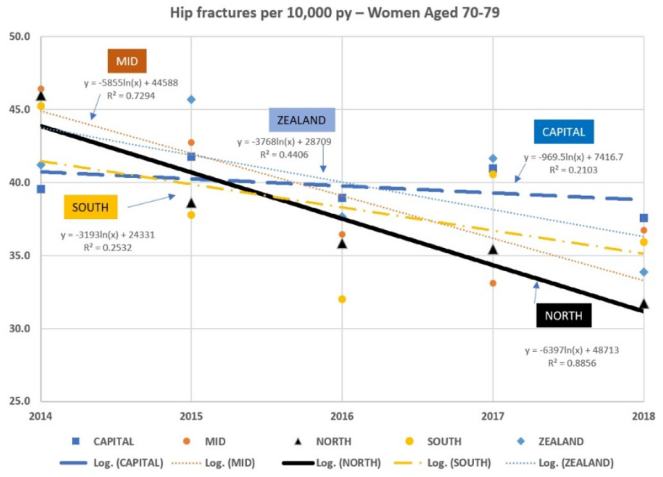
	Age 70-79 Hip fractures per 10,000 p.y.		Age 80+ Hip fractures per 10,000 p.y.	
Women	**2014**	**2018**	**2014**	**2018**
Capital reg.	39.6	37.6	155.1	140.5
Mid reg.	46.4	36.7	191.4	151.8
South reg.	45.2	35.9	179.1	169.0
Zealand reg.	41.2	33.9	200.8	157.0
North reg.	**46.0**	**31.7**	**188.9**	**139.3**
σ	*2.8*	*2.1*	*15.6*	*11.0*
Men				
Capital reg.	24.4	23.6	76.4	72.5
Mid reg.	28.6	30.3	99.9	89.5
South reg.	30.0	26.6	100.4	97.7
Zealand reg.	27.0	21.9	97.5	80.6
North reg.	**24.9**	**26.1**	**98.6**	**74.9**
σ	*2.1*	*2.8*	*9.1*	*9.4*



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Concurrent Oral Poster Presentations 1: Basic / Translational: Bone Diseases

P007

Involvement of Irisin in age-related osteoporosis: positive correlation with BMD in older adult patients and inhibitory effect on the senescent marker p21 in osteoblasts

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Background/Introduction: We previously showed that treatment with the myokine Irisin ameliorates disuse-induced osteoporosis and muscular atrophy in mice. In humans, we and other authors showed a positive correlation between irisin and bone mineral density (BMD) in children and young adult athletes.

Purpose: Few studies have been conducted so far to investigate the links between circulating Irisin, its precursor, FNDC5, in skeletal muscle, and bone status in the same population of human subjects. Therefore, we evaluated possible correlations of Irisin serum levels with BMD and key parameters obtained from bone and muscle biopsies in a cohort of older adult patients. To address whether Irisin may be effective in delaying the cellular aging process, we also investigated its possible senolytic action on osteoblast cell cultures *in vitro*.

Methods: Sixty-two patients (age 68.71± 12.31) undergoing total hip or knee replacement were recruited and divided into osteopenic/osteoporotic patients and healthy subjects. Serum Irisin concentration, BMD, and in bone and muscle biopsies mRNAs of Osteocalcin (*Ocn*), Irisin precursor (*Fndc5*), and *p21* were measured.

Results: Irisin serum levels negatively correlated with age (r=-0.515; p=0.000018) and positively correlated with femoral (r=0.619; p=0.001) and vertebral (r=0.201; p=0.0001) BMD. Irisin was also positively associated with *Fndc5* mRNA in muscle biopsies (r=0.248; p=0.016), as well as with *Ocn* mRNA in bone biopsies (r=0.708; p=0.006). Of note, we found lower irisin levels (p=0.0011) in patients with osteopenia/osteoporosis (OP) compared to healthy controls. By analyzing the senescence marker *p21*, we found a significant increase in its expression in the bone biopsies of OP patients compared to controls. Additionally, *in vitro* data on murine osteoblasts showed that irisin downregulates *p21* mRNA levels (3-fold, p=0.03) compared to untreated cells.

Conclusion(s): Overall, these results indicate that, given the emerging role of irisin as an osteoanabolic agent, it could also represent a possible senolytic agent to delay age-related osteoporosis.

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P010

Repair of a critical size defect in osteoporotic mice

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Background/Introduction: To prevent loss of bone mass and deterioration of microarchitecture in osteoporosis, bisophosphonates (BP) are the therapy of choice. Patients treated with BP, however, may still suffer from fractures and large bone defects. Biomaterials, such as CaP ceramics, may be used to fill critical-size defects, eventually in combination with osteogenic growth factors (bone morphogenetic protein-2; BMP2). L51P, an engineered BMP2 variant, which binds BMP antagonists, may cause an increase of the biological efficacy of BMP2.

Purpose: It is hypothesized, that prolonged BP therapy interferes with biomaterial turnover. To test this, the turnover of ßTCP implant ceramics was studied in a long bone critical-size defect in ovariectomized (*OVX*) mice treated with BP.

Methods: Eight weeks after *OVX*, treatment with BP commenced. Five weeks later, a femoral defect (3.5 mm) was generated and stabilized, using an internal osteosynthesis system. βTCP cylinders loaded with 0.25µg or 2.5µg BMP2, 2.5µg L51P, 0.25µg BMP2/2.5µg L51P and control implants were fitted into the defects. Femora were collected 6 and 12 weeks post-implantation.

Results: *OVX* led within eight weeks to a significant decrease in femoral total bone density in comparison to *sham* animals (p value 0.0001). In addition, analysis of uteri dry weight verified a shrinkage of uteri in *OVX* animals at the study endpoint (p value 0.0067). *OVX* mice under BP therapy, which received βTCP implants loaded with 0.25µg BMP2, 0.25µg BMP2/2.5µg L51P and 2.5µg BMP2, showed a strong induction of bone growth. In comparison, *OVX* animals without BP

medication showed formation of calcified tissue in the groups with 0.25μg BMP2/2.5μg L51P and 2.5μg BMP2 implants only.

Conclusion(s): The results indicate synergistic effects of BMP2 and L51P on bone healing. Moreover, BP caused a reduction in implant turnover. Therefore, efficiency of healing of biomaterial-filled bone defects might be impaired in patients treated with BP due to blocked implant removal.

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P159

Effect of hormone replacement therapy on bone formation quality and mineralization regulation mechanisms in early postmenopausal women

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Background/Introduction: Post-menopausal osteoporosis is characterized by a negative imbalance between bone formation and bone resorption resulting in a net bone loss, increasing the risk of fracture. One of the earliest interventions to protect against this was hormonal replacement therapy (HRT).

Purpose: Bone strength depends on both the amount and quality of bone, the latter including compositional / material and structural properties. Bone compositional / material properties are greatly dependent on both patient-, and tissue-age.

Methods: Raman spectroscopy is an analytical tool ideally suited for the determination of bone compositional / material properties as a function of tissue age as it is capable of analyzing areas ~1 x 1 μm^2 in tetracycline labeled bone forming areas. Using such analysis of humeri from an ovariectomized primate animal model, we have previously shown that loss of estrogen results in alteration in the mineralization regulation mechanisms by osteoid organic matrix attributes at actively forming bone surfaces.

Results: In the present work we used Raman microspectroscopic analysis to analyze paired iliac crest biopsies obtained from 10 postmenopausal women at baseline and after 2 years treatment with HRT, to investigate the effects of this treatment on bone material / compositional properties at precisely defined micro-areas and tissue ages. Specifically, we analyzed osteoid, three tissue ages at forming cortical and trabecular surfaces (based on the presence of double fluorescent labels), and interstitial bone. The following parameters were measured: mineral / matrix ratio, mineral maturity / crystallinity, and tissue water, glycosaminoglycan, and pyridinoline content.

Conclusion(s): The results indicated significant correlations between osteoid proteoglycans, sulfated proteoglycans, pyridinoline, and earliest mineralized tissue mineral content, suggesting that in addition to changes in bone turnover rates, HRT affects the osteoid composition, as well as fibrillogenesis and mineralization regulation mechanisms.

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P104

Lrp5 mutant and crispant zebrafish faithfully model human osteoporosis, establishing the zebrafish as a platform for CRISPR-based functional screening of osteoporosis candidate genes

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Background/Introduction: Genome-wide association studies (GWAS) have improved our understanding of the genetic architecture of common, complex diseases such as osteoporosis. Nevertheless, to attribute functional skeletal contributions of candidate genes to osteoporosis-related traits there is a need for efficient and cost-effective *in vivo* functional testing.

Purpose: This can be achieved through CRISPR-based reverse genetic screens, where phenotyping is traditionally performed in stable germline KO mutants. However, recently it was shown that first-generation (F0) mosaic mutant zebrafish (so-called crispants) recapitulate the phenotype of germline KOs.

Methods: To deliver proof-of-concept for functional validation of osteoporosis candidate genes through crispant screening, we compared a crispant to a stable KO zebrafish model for the *lrp5* gene. In humans, recessive loss-of-function mutations in *LRP5*, a co-receptor in the Wnt signaling pathway, cause Osteoporosis-pseudoglioma syndrome. In addition, several GWAS studies identified *LRP5* as a major risk locus for osteoporosis-related phenotypes.

Results: In this study, we showed that early stage *lrp5* KO larvae display decreased notochord mineralization ($P<0.0001$) and malformations of the head cartilage. Quantitative microCT and mass-spectrometry element analysis of the adult skeleton revealed decreased vertebral bone volume ($P<0.005$) and bone mineralization ($P<0.001$), hallmark features of osteoporosis. Furthermore, regenerating fin tissue displayed reduced Wnt signaling activity in *lrp5* KO adults. Additionally, *lrp5* crispants were generated by micro-injecting one-cell stage embryos with CRISPR RNP complexes containing Cas9 and a two-part gRNA (tracrRNA:crRNA duplex). Next-generation sequencing analysis of adult crispant tissue revealed a mean out-of-frame mutation rate of 76%, resulting in strongly reduced levels of Lrp5 protein. These crispants generally showed a milder, but nonetheless highly comparable skeletal phenotype and a similarly reduced Wnt pathway response compared to *lrp5* KO mutants.

Conclusion(s): In conclusion, we show through faithful modeling of LRP5-related primary osteoporosis, that crispant screening in zebrafish is a promising approach for rapid functional screening of osteoporosis candidate genes.

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P179

Early life stress does not affect bone mass in male mice but induces an osteopenic phenotype in female mice

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