# Bone and Mineral Metabolism

## BONE, FROM BENCH TO BEDSIDE

### *PTH Protects Osteocytes From Oxidative Stress and Cellular Senescence*

*Yuhei Uda, MS, Roberto Santos, MS, Alejandro Kochen, BS, Carly Newell, N/A, Tim Y. Huang, BS, Paola Divieti Pajevic, MD, PhD.*
Boston University School of Dental Medicine, Boston, MA, USA.

Age-induced osteoporosis is characterized by a progressive decline in bone formation and increase in bone resorption with uncoupled activities of osteoblasts and osteoclasts. Parathyroid hormone (PTH) is used in the clinic to treat osteoporosis due to its anabolic actions on bone via binding to the PTH receptor (PPR). The receptor is highly expressed in cells of the osteoblastic lineage, including osteocytes. Osteocytes are the most abundant cells in bone and serve as a key regulator of bone remodeling. Despite the significant role of PPR signaling in skeletal homeostasis, its function in osteocytes during aging remains unclear. We have gathered preliminary data demonstrating that mice lacking PPR predominantly in osteocytes (Dmp1-$PPR^{KO}$) have marked age-induced bone loss due to increased bone resorption and suppressed bone formation. These mice, with aging, develop characteristics of skeletal senescence: a decrease in osteoprogenitors and an increase in bone marrow adiposity and $p16^{Ink4a}$/*Cdkn2a* expression in bone. Since senescence of cells in the bone microenvironment has been reported as a cause of age-induced bone loss, we hypothesized that PPR signaling protects osteocytes from senescence. To test this hypothesis, we generated osteocytes (Ocy454-12H), in which the PPR expression was ablated using CRISPR/Cas9 technique. Ocy454-12H-$PPR^{KO}$ and Ocy454-12H-$PPR^{Ctrl}$ cells were treated with PTH followed by an exposure to hydrogen peroxide ($H_2O_2$). High levels of intracellular reactive oxygen species (ROS), including $H_2O_2$, promote protein and DNA oxidation, resulting in cell death and senescence. PTH treatment significantly suppressed the increase in $H_2O_2$-induced cell death, measured by resazurin-based assays, in $PPR^{Ctrl}$ but not in $PPR^{KO}$ cells. We analyzed intracellular ROS levels using a fluorescent probe and found that PTH treatment significantly suppressed the increase in ROS upon $H_2O_2$ exposure, suggesting an antioxidant function of PTH in osteocytes. To further investigate if PTH prevents osteocytes from oxidative stress-induced senescence, we examined senescence-associated β-galactosidase (SA β-gal) activity in cells that were treated with PTH followed by an exposure to low doses of $H_2O_2$. Compared to untreated and $PPR^{KO}$ groups, treatment with PTH significantly decreased the number of SA β-gal positive cells, demonstrating that PPR signaling protects osteocytes, and possibly other osteoblastic cells, from $H_2O_2$-induced cellular senescence. PTH treatment reduced mRNA expression of p21/*Cdkn1a*. Taken together these results demonstrate that PPR signaling is important to protect osteocytes from cellular senescence.

# Bone and Mineral Metabolism

## BONE, FROM BENCH TO BEDSIDE

### *Sarcopenic Obesity Indices Are Major Determinants of Bone Strength in Older Adults With Obesity*

*Giulia Gregori, MSc[1], Arjun Paudyal, MSc[1], Yann Barnouin, PhD[2], Alessandra Celli, MSc[3], Reina Condevillamar Villareal, MD[1], Clifford Qualls, PhD[3], Dennis Tan Villareal, MD[4].*
[1]Baylor College of Medicine/Michael E DeBakey VA Medical Center, Houston, TX, USA, Houston, TX, USA, [2]BAYLOR COLLEGE OF MEDICINE, Houston, TX, USA, [3]Baylor College of Medicine, Houston, TX, USA, [4]Baylor College of Medicine/Michael E DeBakey VA Medical Center, Houston, TX, USA.

**Background:** The increasing number of older adults with obesity is a growing public health problem because of increased risk of fractures especially at the ankle and upper leg despite normal or high bone mineral density. Among the contributory factors for fracture risk in this population may be aging- and obesity- associated physical frailty and impaired bone quality. However, how the adverse changes in physical function and body composition in this aging and obese population contribute to bone quality as assessed by finite element analyses (FEA) of bone strength has not been determined. **Methods:** One-hundred sixty-nine older (age ≥ 65 yrs.) adults with obesity (BMI ≥ 30 $kg/m^2$) were recruited to participate in lifestyle intervention trials at our Medical Center. All underwent baseline measurements of bone strength (failure load [N] and stiffness [$N.mm^{-1}$]) as estimated using FEA from high-resolution peripheral quantitative tomography (HR-pQCT) of the distal radius and tibia. In addition, body composition (appendicular lean mass/BMI [$ALM_{BMI}$], fat mass/$height^2$ [FMI]) was assessed by dual-energy x-ray absorptiometry (DXA) and physical function by the modified physical performance test (PPT), knee extension strength (isokinetic dynamometry), hand grip strength, and 4-meter gait speed. **Results:** Bivariate analyses showed that $ALM_{BMI}$ (r=.57 to .58), FMI (r=-.16 to -.17), gait speed (r=.20 to .21), grip strength (.56 to .57), and knee extension strength (r=.40 to .42) correlated with stiffness and failure load at the distal radius (all P<0.05). In addition, $ALM_{BMI}$ (r=.65 to .67), FMI (r=-.22 to .23), gait speed (r+.18 to .19), grip strength (r=.58 to .59), and knee extension strength (r=.44 to .45) correlated with stiffness and failure load at the distal tibia (all P<0.05). Controlling for age and sex, multiple regression analyses revealed that $ALM_{BMI}$ (β=.34 to .35) and grip strength (β=.28 to .29) were the independent predictors of stiffness and failure load at the distal radius, explaining 45% to 46% of the variance in stiffness and failure load (P<0.001). On the other hand, multiple regression analyses revealed that $ALM_{BMI}$ (β=.45 to .52), grip strength (β=.27 to .28), and FMI (β=.17 to .18) were the independent predictors of stiffness and failure load at the distal tibia, explaining 74% to 75% of the variance in stiffness and failure load (P<0.001). **Conclusions:** These findings suggest the importance of preserving muscle mass while reducing fat mass and improving physical function to maintain bone quality and decrease the risk of fractures when older adults with obesity undergo lifestyle intervention.

# Bone and Mineral Metabolism

## BONE, FROM BENCH TO BEDSIDE

### *Sustained Morphine Delivery Suppresses Bone Formation and Alters Metabolic and Circulating miRNA Profiles in Mice*

*Adriana Lelis Carvalho, PhD[1], Daniel J. Brooks, MS[2], Deborah Barlow, BS[3], Karen Houseknecht, PhD[4], Mary Bouxsein, PhD[2], Jane B. Lian, PhD[5], Tamara King, PhD[3], Nicholas H. Farina, PhD[6], Katherine J. Motyl, PhD[1].*
[1]Maine Medical Center Research Institute, Scarborough, ME, USA, [2]Beth Israel Deaconess Medical Center, Boston, MA, USA, [3]University of New England, Biddeford, ME, USA, [4]University of New England, Portland, ME, USA, [5]University of Vermont College of Medicine, Burlington, VT, USA, [6]University of Vermont, Burlington, VT, USA.

Opioid use is associated with a three to four-fold increase in fracture risk, which is higher in males compared to females. Although one component of that risk may be falls, there is also evidence of altered bone remodeling. However, the mechanisms of these effects are not entirely clear. Our aim was to develop a mouse model of opioid-induced bone loss and study the impact on bone turnover and potential miRNA-mediated regulatory mechanisms. We evaluated the effects of sustained morphine treatment on the skeleton and metabolism of male and female C57BL/6J mice by treating with vehicle (0.9% saline) (n=9–11) or morphine (18 mg/kg) (n=11) using subcutaneous osmotic minipumps for 25 days. Morphine did not influence body weight or food intake, but did reduce fat mass in both sexes. All mice treated with morphine had higher resting energy expenditure and respiratory quotient, indicating a shift toward carbohydrate metabolism. After 25 days of treatment, microComputed Tomography (μCT) analyses indicated that morphine-treated male mice lost 15% of trabecular bone volume fraction (Tb.BV/TV) and 14% of Tb. bone mineral density (BMD) ($p<0.05$) in the distal femur compared to vehicle, but there were no changes in cortical bone. Females did not lose bone, suggesting differences may be hormone-related. Despite these sex differences, males and females had similar levels of morphine exposure, measured by LC-MS/MS. Histomorphometric analyses demonstrated that in males, morphine reduced bone formation rate ($p<0.05$) compared to vehicle, but did not impact osteoclast parameters. Consistent with this, morphine reduced bone formation marker gene expression in the tibia of males (including *Bglap* and *Dmp1*). Circulating miRNA profiles were interrogated in serum collected at day 12 from vehicle- and morphine-treated male and female mice. While very few changes were present in females, there were 14 differentially expressed miRNAs in males treated with morphine that reached a threshold of ≥ 2-fold change and $p<0.01$. After target pathway analysis (DIANA miR Path V.3 using experimentally validated miRNA/mRNA targets (Tarbase V.7)), we found that the four upregulated miRNAs (miR-484, -223-3p, -328-3p, and -3107-3p) were associated with 13 enriched KEGG pathways ($p<0.05$), including fatty acid metabolism pathways ($p<0.001$). Fatty acid metabolism has recently been linked to osteoblast function, and suppression of such pathways is consistent with the finding of increased respiratory quotient. In summary, morphine leads to trabecular bone loss due to reduced bone formation in males. miRNA findings indicate this may be due to altered metabolic control of mineralization. Further investigation into hormonal and metabolic dependency of morphine-induced bone formation changes could lead to clinical mitigation strategies for preventing the adverse effects of opioids on bone health.

# Bone and Mineral Metabolism

## BONE, FROM BENCH TO BEDSIDE

### *Therapeutic Potentials of Dimeric $^{Cys25}$PTH(1–34) Peptide for Osteoporosis and Fracture Healing of the Bones- Buy One, Get One Free*

*Doori Park, PhD[1], Yoshiaki Kitaura, DDS, PhD[2], Thomas Dean, MS[3], Shinsuke Ohba, DDS, PhD[2], Thomas James Gardella, PhD[4], Ung-il Chung, MD, PhD[2], Soo Young Lee, PhD[5], Sihoon Lee, MD, PhD[6].*
[1]Ewha Womans University, Seoul, Korea, Republic of, [2]The University of Tokyo Graduate School of Engineering, Tokyo, Japan, [3]Massachusetts General Hospital, Boston, MA, USA, [4]MASSACHUSETTS GENERAL Hospital, Boston, MA, USA, [5]Ewha Womans University, Seoull, Korea, Republic of, [6]Gachon University College of Medicine, Incheon, Korea, Republic of.

**Objective:** The extraordinarily high bone densities identified in the hypoparathyroidism patients originating from PTH R25C mutation suggested the possibility that this modified protein has unique biologic effects and contributes to the gain of bone volume. Interestingly, western blot of cell lysates stably transfected with PTH R25C construct revealed that $^{Cys25}$PTH(1–84) formed a dimer, presumably due to disulfide bonding of the cysteine residues. This study aims to study the characteristics of $^{Cys25}$PTH(1–34) dimeric peptide (Dimer) both *in vitro* and *in vivo* for potential therapeutic application of Dimer as a novel anabolic agent. **Methods:** Identity of the chemically synthesized Dimer was confirmed by its molecular weight and purity using MS and HPLC, respectively. Basic characteristics, for example, ability to bind to PTHR and cAMP production were investigated using a variety of cells. In addition, the ligand-receptor internalization was investigated using TMR tagged Dimer. *In vivo* characteristics, such as calcemic and phosphatemic responses, pharmacokinetics and pharmacodynamics, were assessed in CD1 mice. The osteoanabolic effects of Dimer were assessed using a fracture-healing model, a calvarial-injection model and an OVX mouse model. **Results:** *In vivo* study showed that Dimer has similar calcemic and phosphatemic responses to PTH(1–34; WT). In cell assays, Dimer showed a similar cAMP production but slightly lower binding affinity compared to WT. Dimer-receptor complex was internalized into the cells. Surprisingly, Dimer showed a potent anabolic effect in the fracture-healing model in mice measured as the callus volume fraction by microCT. We also observed a comparable anabolic effect of Dimer in calvarial-injection model and OVX model. **Conclusions:** Dimeric $^{Cys25}$PTH(1–84) peptide might play a substantial role in the high bone mass in hypoparathyroidism patients, originating from the PTH R25C mutation. This may be translated into the development of potential therapeutic modality for the treatment of osteoporosis and fracture healing using Dimer.

# Bone and Mineral Metabolism

## FRACTURE PREVENTION AND TREATMENT

### *Efficacy of Low Dose Denosumab in Maintaining Bone Mineral Density in Postmenopausal Women With Osteoporosis Switching From 60mg to 30mg 6 Monthly: A Real World, Prospective Observational Study*