Cholesterol Reduction Limits Obesity-Induced Bone Loss via *Phlpp2*

Ismael Y. Karkache[1], Elizabeth K. Vu[1], Samuel W. Mitchell[1], Mitchell Shimak[1], Grant Kim[1], Barbara Azevedo Machado[1], Emily Chavez[1], Kim C. Mansky[1], Elizabeth W. Bradley
[1]University of Minnesota, Minneapolis, MN
Karka010@umn.edu

Disclosures: Ismael Y. Karkache (N), Elizabeth K. Vu (N), Samuel W. Mitchell (N), Mitchell Shimak (N), Grant Kim (N), Barbara Azevedo Machado (N), Emily Chavez (N), Kim C. Mansky (N), Elizabeth W. Bradley (5-Medtronic, 8-BMC Musculoskeletal Disorders, Connective Tissue Research)

INTRODUCTION: Diet-induced obesity increases the incidence of negative health outcomes such as diabetes and hypercholesterolemia, but the effects of obesity on bone mass are complex. Additionally, hypercholesterolemia is common in postmenopausal individuals, a group with an increased prevalence of osteoporosis. While high LDL levels associate with diminished bone mass independent of obesity, the contribution of hypercholesterolemia to the effects of diet-induced obesity and potential cellular and molecular mechanisms remain poorly defined. Substantial data supports that high cellular cholesterol levels positively impact osteoclast differentiation. Activating mutations of Phlpp2 strongly correlate with high LDL levels in humans, suggesting that Phlpp2 may mediate increased osteoclastogenesis associated with hypercholesterolemia. In this study, we sought to determine if hypercholesterolemia mediates detrimental effects of diet-induced obesity on bone mass via Phlpp2.

METHODS: We generated female C57Bl/6J mice deficient in *Phlpp2* in the myeloid lineage (Phlpp2 cKO_{LysM}) by crossing mice with floxed *Phlpp2* alleles (*Phlpp2* fl/fl) with those harboring a LysM-Cre allele. Cre-negative, sex-matched littermates were used as controls. Starting from weaning age (4 weeks) mice were fed either a normal chow (NC, n=7-10) or high fat diet (HFD, n=8-10). A group of HFD-fed mice were also administered the cholesterol-lowering agent simvastatin (HFD+S, n=7-8). Mice within groups were aged to skeletal maturity (12 weeks) and micro-CT analyses at the distal and mid-shaft femur was performed. We also sectioned and TRAP/FAST Green stained tibiae to look at bone surface osteoclasts and bone microarchitecture. We employed three-way ANOVA and post-hoc singular comparisons.

RESULTS: Our preliminary work showed that deletion of *Phlpp2* increased cortical thickness in female mice only. It is for this reason that we used female mice for this study. All results were normalized to the weights of the mice. Micro-CT analyses revealed of *Phlpp2* cKO_{LysM} mice demonstrated enhanced cortical thickness (Ct. Th) (10±0.5%) as well as increased cortical bone volume per total volume (BV/TV) (4%±1.9%). We next determined if *Phlpp2* ablation protected against HFD-induced bone loss. Micro-CT analyses demonstrated that HFD-fed control littermates exhibited reduced femoral mid-shaft cortical bone (-33±7.4% Ct.BV/TV, -30±7.1% Ct.Th). In contrast, HFD-fed mice showed improvements in bone mass (+13±5.5% Ct.BV/TV, +16±4.8% Ct.Th) when administered simvastatin. Interestingly, while *Phlpp2* cKO_{LysM} HFD mice also exhibited reduced cortical bone at a similar scale to the control (-29.6±6.1% Ct.BV/TV, -26.7±5.5% Ct.Th) when adjusted for weight, we did not see any significant improvement to these parameters when *Phlpp2* cKO_{LysM} HFD mice were administered simvastatin (-0.003±10.2% Ct.BV/TV, +3.5±9.5% Ct.Th) (Figure 1). In our histomorphometry, we saw a substantial increase BV/TV in both the HFD and HFD + S groups in our control mice (+116±4.7%, +127.1±3.6%) but only in the HFD + S group for our *Phlpp2* cKO_{LysM} mice (+178.8±6.69%). We also observed significant reductions in Ct.Th and for both HFD (-55.3±7%) and HFD + S (-47.7±5.8%), albeit only in the knockout mice. Osteoclasts per Bone Surface were reduced solely by diet in the control mice (-58.3±16.1%) and by diet and treatment in the *Phlpp2* cKO_{LysM} HFD mice (-55.3±6.5%, -47.7±7.1%) (Figure 2).

DISCUSSION: Our study further highlights the impact obesity has on bone. We likewise demonstrate that detrimental effects of obesity-induced bone loss are mediated in part by hypercholesterolemia. Moreover, we show that cholesterol-mediated effects of diet-induced bone loss are *Phlpp2*-dependent. The apparent enhancement of trabecular bone seen in our histomorphometry suggests two-fold impact of obesity that differs by bone compartment and may not wholly be dependent on cholesterol status. The number of bone surface osteoclasts in our HFD control mice was rescued by treatment with simvastatin, but not in our *Phlpp2* cKO_{LysM} HFD mice, suggesting this too may be *Phlpp2* dependent.

SIGNIFICANCE: Our work is significant both scientifically, because it sheds new light on the role of *Phlpp2* in bone biology. It is clinically significant because it highlights a potential avenue to treat bone loss in hypercholesterolemic women.

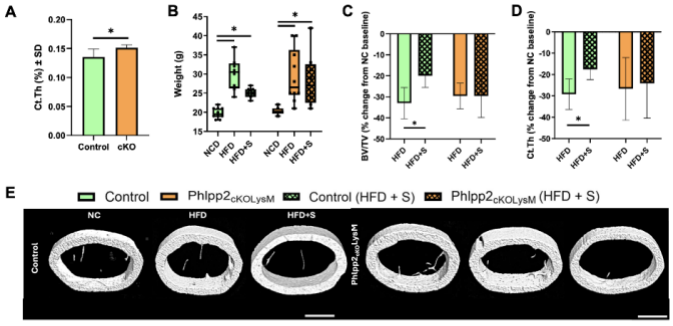

Figure 1: Cortical thickness is diminished by diet-induced obesity but alleviated by cholesterol reduction. Asterisks indicate statistical significance (*p <0.05). **A)** Average cortical thickness (Ct.Th) of midshaft femoral cortex in normal chow (NC) fed control and cKO female mice. **B)** Weights of control and cKO female mice fed either NC or HFD, and a subset of HFD mice administered simvastatin, taken directly prior to sacrifice at 12 weeks of age. **C,D)** Average Ct.BV/TV (C) and Ct.Th (D) of HFD diet groups of female mice, reported as a percent change from the average in the NC baseline group, respective to genotype. **E)** 3D reconstructions of the midshaft femoral cortex. Scale bar is 500 microns.

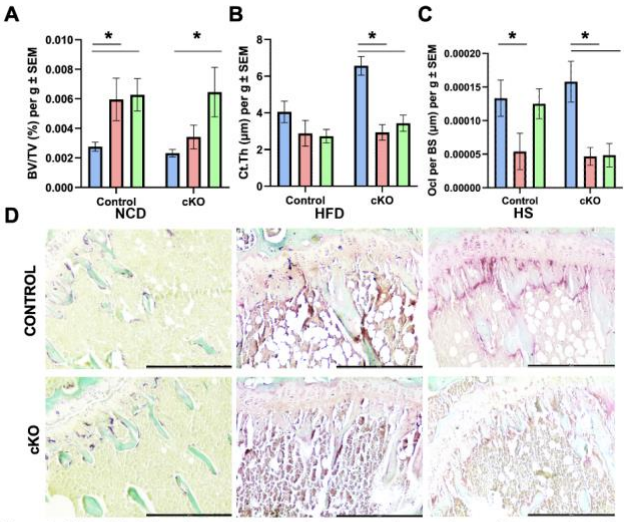

Figure 2: Phlpp2 deletion alters diet induced changes to bone microarchitecture. Asterisks indicate statistical significance (*p <0.05). We obtained 7-micron-thick sections from the tibiae of our control and Phlpp2 cKOLysM mice proximal to the growth plate. We TRAP/FAST Green stained these sections to visualize trabecular bone and bone-lining TRAP positive cells (e.g., osteoclasts). **A-C)** Average A) Bone Volume per Total Volume, B) Cortical Thickness, and C) Osteoclasts per Bone Surface across the entire span of the growth plate. **D)** Representative images of our stained tissue sections. We measured cortical bone approximately 1mm distal to the growth plate. Scale bar is 100 microns.