Diet-Induced Obesity Deteriorates Cancellous Bone Structure Despite Increased Blood Perfusion

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INTRODUCTION: Over half of adults and an increasing number of children and adolescents in developed countries are overweight or obese[1]. Overweight individuals often have larger bones thought to protect them from fracture[2], but other factors like microstructure and material properties also contribute to bone strength[3] and are negatively impacted in murine obesity models[4-6]. Since bone is highly vascularized and will not develop or repair properly without sufficient blood flow[7], and obesity is associated with vascular degenerating pathologies[8] (e.g., type 2 diabetes mellitus, coronary heart disease, stroke), we hypothesize that microstructural degeneration is associated with altered vascular supply to bone tissue. Since exercise is both angio- and osteogenic, we further hypothesize that moderate aerobic exercise will modulate the effects of obesity on osteovasculature.

METHODS: With approval of the IACUC at North Carolina State University, sixteen 5-week-old C56Bl/6J male mice (Jackson Labs) were fed a high fat diet (HFD, 60% kcal fat) or a matched control fat diet (CFD, 10% kcal fat) for 18 weeks. After 10 weeks of diet (15 wk of age), HFD and CFD mice were divided into exercise (EX) or sedentary (SED) groups (n=4 each). For 8 weeks, exercise mice ran on a treadmill (8 m/min, 37 min, 5 days/wk, 5° incline), and sedentary mice were placed on a stationary treadmill. Body mass and serum glucose concentration were recorded weekly, and glucose tolerance tests were performed 13 and 18 weeks after diet initiation. After 18 weeks of diet, blood perfusion in the tibiae was measured *in vivo* using laser Doppler flowmetry (LDF)[9]. After LDF, mice were euthanized, blood samples were drawn, and femora and tibiae were dissected. Serum concentrations of BMP2 and VEGF were determined with ELISA. Left femora were scanned with micro-computed tomography (μCT) and reconstructed at a 10-μm voxel size. Density and microarchitecture were analyzed in the mid-diaphysis and distal metaphysis[10]. Right femora were tested in three-point bending to determine structural and estimated material properties. The effects of diet and exercise were examined using two-way ANOVA with Tukey post-hoc tests using α=0.05 (R).

RESULTS: HFD mice tended to have greater body mass than CFD mice throughout the study ($p<0.023$ for 15-18 wk of diet), resulting in a 32.7% heavier phenotype after 18 weeks of diet (32.4±1.7g CFD, 43.0±5.2g HFD). HFD tended to have negative effects on fasting glucose concentrations ($p<0.011$ for 17, 18 wk of diet). While exercise did not significantly affect body mass or fasting glucose concentrations, it did improve glucose tolerance in HFD mice after 14 weeks of diet, but the effect was abolished after 18 weeks of diet (Fig. 1A). Both HFD and treadmill exercise increased tibial blood perfusion (Fig. 1B). BMP2 was higher in HFD EX vs. CFD EX mice ($p=0.034$), and VEGF serum concentrations were higher in HFD SED vs. HFD EX mice ($p=0.015$) (Fig. 2).

Exercise had no effect on bone microstructure or mechanical properties. Compared with CFD, HFD negatively impacted cancellous bone volume fraction ($p=0.0069$), trabecular number ($p=0.0006$), and trabecular separation ($p<0.0001$) in the distal femoral metaphysis (Fig. 3). Degree of anisotropy tended to be lower in HFD mice (-8.6% vs. CFD, $p = 0.097$), suggesting preferential loss of longitudinally aligned trabeculae. Mid-diaphyseal μCT measurements were not significantly different between exercise or diet groups. In three-point bending, HFD femora had significantly lower yield load (-21.5% vs. CFD, $p=0.013$) and ultimate load (-13.9% vs. CFD, $p=0.048$).

DISCUSSION: Diet-induced obesity in mice caused structural deterioration of important regions of the femora that moderate treadmill exercise could not prevent. Exercise caused increased blood perfusion in the tibia but did not induce changes in the various bone measures. Since HFD mice exhibited symptoms of metabolic disease (glucose intolerance), we expected diabetes-related vascular damage and decreased blood perfusion within the tibia. However, we observed that tibial blood perfusion was actually increased in the HFD group, which may be due to increased adiposity within the bone marrow space. Adipose is an endocrine organ that stimulates chronic inflammation which is pro-angiogenic and could be responsible for increased vascular supply in the tibia[11,12]. Further histology will be performed to quantify the blood vessel numbers in the tibia.

SIGNIFICANCE: We have directly measured increased blood flow *in vivo* within the hindlimb bones due to both diet-induced obesity and exercise.

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ACKNOWLEDGMENTS: This work was supported by NIH K12HD073945 (JHC). We thank Dr. T. Bateman and E. Livingston for μCT support.

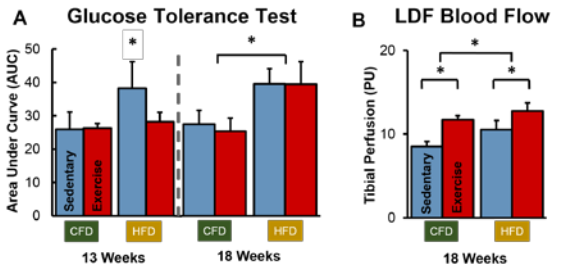

Figure 1: **A)** Exercise for 4 wks conserved glucose tolerance with HFD to CFD levels (13 wks diet), but the benefit was lost by 18 wks diet. **B)** Tibial blood flow from LDF was increased with both HFD and exercise. *p<0.05.

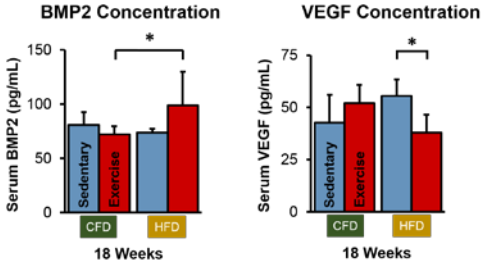

Figure 2: The combination of high fat diet and exercise significantly increased BMP2 and decreased VEGF serum concentrations. *p<0.05.

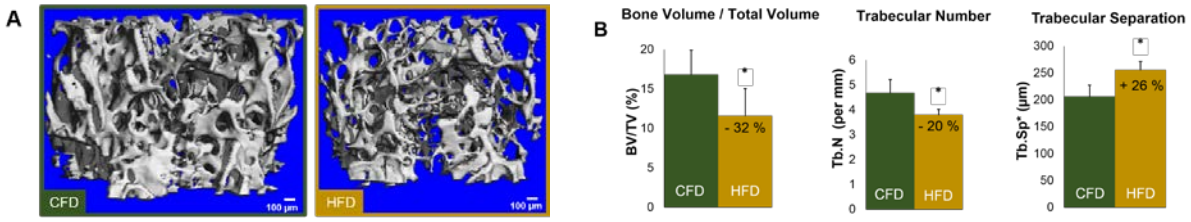

Figure 3: **A)** Reconstructed μCT scans of the distal femoral metaphysis show deteriorated microarchitecture with HFD marked by **B)** decreased bone volume fraction and trabecular number and increased trabecular separation. *p<0.05 for HFD vs. CFD.