

Murine Model of Cardiometabolic HFpEF Displays Decreased Bone Density and Worsened Bone Morphology

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INTRODUCTION: Heart-failure with preserved ejection fraction (HFpEF) accounts for fifty percent of heart failure cases, and its prevalence has grown under the umbrella of increased metabolic dysfunction, obesity, and hypertension. Metabolic dysfunction, obesity, and hypertension also have negative implications for osteoporosis and fracture incidence, yet little research has evaluated bone health in the context of HFpEF. Here we evaluate bone density and morphology in a murine model of HFpEF, with or without exercise intervention. We hypothesized HFpEF mice would have lower bone density and poorer bone morphology compared to control mice. Further, we expected the increased loading from voluntary wheel running to provide protection against the expected detriments to bone-related outcomes in HFpEF mice.

METHODS: Twenty-five nine-week-old C57BL/6N mice were randomized to chow, high-fat (60%) diet (HFD), or HFpEF, induced by HFD plus L-arginine methyl ester (L-name at 0.5g/L added to cage water bottle), for 10-weeks. The combination of metabolic stress (induced by 60% HFD) and hypertension (induced by L-name, nitric oxide inhibitor) readily induces HFpEF in male mice, hence the sex dependent cohort. Four weeks into dietary interventions, mice were single housed with the dietary cohorts randomly split into sedentary and exercise (voluntary wheel running) groups. Mice were euthanized at 19-weeks old and hind-limb long bones were harvested, cleaned of soft-tissue, fixed in 10% neutral buffered formalin for 48-hours, and stored in phosphate buffered saline at 4°C until μ CT scanning. Right-side tibias were scanned on a Quantum GX II μ CT with the following parameters: 18mm FOV, 50kV, 160 μ A, high-resolution, 58min. 3D volumes were reconstructed at 10 μ m voxel size, and each tibia required 3 scans to capture the full tibia. Tibias were stitched and analyzed in Dragonfly World 2025.1 using the bone analysis wizard using specific regions of interest (ROI) for trabecular (4mm section of proximal diaphysis) and cortical (2mm section of diaphysis above tibia-fibular junction) outcomes. Bone density was assessed by averaging Hounsfield unit (HU) values within bone labeled voxels for the entire tibia. Statistical analysis was performed in GraphPad prism using two-way ANOVA to determine main effects (diet, exercise, interaction) followed by Tukey's post-hoc testing for group differences, $\alpha=0.05$. Animal work was approved by Pennington Biomedical's IACUC.

RESULTS SECTION: Our mouse model of HFpEF, induced with 60%HFD and L-name, showed significant bone detriments compared to mice on both chow and 60% high fat diets. Outcomes for trabecular morphology showed significant decreases in trabecular bone volume (BV/TV) and poorer structural model index (transition from plate-like trabecular structure to rod-like) in HFpEF mice compared to controls, a significant difference not seen with HFD alone. Cortical outcomes were similar, with HFpEF mice displaying significantly reduced cortical area and decreased endosteal and periosteal perimeters compared to both HFD and control mice. Bone density, assessed based on average HU values within bone label voxels for the entire tibia, demonstrated a clear and significant decrease in bone density for HFpEF mice compared to both chow and HFD mice, where HFD mice displayed lower density compared to controls and higher density compared to HFpEF mice. With respect to 6-weeks of voluntary wheel running, results suggest a modest negative effect on bone. No significant effects of exercise were noted within diet groups (chow, HFD, HFpEF), but main effects for exercise showed a significant negative effect on bone density.

DISCUSSION: Here we show the combination of HFD and L-name, well characterized to induce HFpEF in mice, has negative implications for bone structure and density. These results were found with a relatively short intervention time for bone-related outcomes (10-weeks). Further, and contrary to our original hypothesis, 6-weeks of voluntary wheel running resulted in decreased bone density. While our results provide novel evidence for HFpEF as a negative effector of bone, in addition to HFD alone, they are limited by the model of HFpEF used here (HFD+L-name). Nitric oxide signaling is implicated in bone anabolic signaling and the use of L-name, a nitric oxide synthase inhibitor, limits the interpretation of our results. Future work could utilize other models of HFpEF, such as the ZSF1 obese rat. We are also limited by the relatively short intervention time of 10-weeks. In the context of bone changes as an effect of diet, interventions are more commonly conducted for >12-weeks. A longer intervention could have revealed a clear delineation between chow, HFD, and HFpEF groups as well as a more robust assessment of what role voluntary aerobic exercise may play in managing HFpEF when bone is a concern.

SIGNIFICANCE/CLINICAL RELEVANCE: (1-2 sentences): Metabolic dysfunction, hypertension, and obesity are a growing healthcare problem worldwide, downstream co-morbidities included. Here we've provided early evidence for HFpEF as a predictor of bone dysfunction and show aerobic exercise alone may harm bone in the context of managing HFpEF.