Disuse Causes Bone Loss via Different Mechanisms in Male and Female Mice

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INTRODUCTION: Disuse causes extensive bone and muscle loss, increasing the risk of developing osteopenia, sarcopenia, and bone fractures. Our lab has shown that genetic variation influences the magnitude of bone and skeletal muscle loss from disuse in inbred mice[1,2]. Inbred mice have limited genetic diversity. To overcome this limitation, we used mice of the diversity outbred (DO) mouse population. DO mice were developed by cross-breeding 8 highly genetically diverse inbred founder strains (C57Bl/6J, A/J, 129S1/SvImJ, NOD/ShiLtJ, NZO/HlLtJ, CAST/EiJ, PWK/PhJ and WSB/EiJ)[3]. We hypothesized that genetic variation would affect the magnitude of bone loss from disuse via single limb immobilization in genetically diverse, DO mice.

METHODS: All procedures were done with the approval of the VCU IACUC. Thirty female and thirty male 16-week-old DO mice had the right hind limb immobilized in a cast for 3 weeks, and then the mice were sacrificed. Femurs were scanned by in vivo micro-CT at baseline on day 1 and 3 weeks later on day 22. Femur mechanical properties were measured by 3-point bending to failure. RNA was collected from marrow-flushed tibias, and RNA sequencing was performed on the left and right tibia of every mouse.

RESULTS: Mice had significantly greater cortical area/total area in the right femur than the left femur at baseline. Immobilization of the right limb resulted in significant decreases in femur cortical area/total area and epiphyseal trabecular bone volume (Fig 1). Cortical TMD was increased in the immobilized femur of female mice, but not in male mice. Femur strength was lower in the immobilized limb in female mice, but not male mice. Female mice had 586 significantly upregulated differentially expressed genes (DEGs) in the immobilized limb and 138 downregulated DEGs. Male mice had 547 upregulated DEGs and 130 downregulated DEGs. The most significant upregulated Gene Ontology (GO) in female mice was Multinuclear Osteoclast Differentiation (Table 1). This GO was not significantly upregulated in males. There were similar significantly upregulated GOs in female and male mice relating to extracellular matrix organization. However, there were drastic differences in which GOs were significantly downregulated, suggesting sex differences in which mechanisms regulate bone loss from disuse.

DISCUSSION: This is the first study to investigate how disuse affects skeletal properties and transcriptomic changes using genetically unique subjects. We found that, despite genetic differences, disuse causes cortical and trabecular bone loss in every mouse. Genetics influenced the magnitude of bone loss. Female and male mice had similar magnitudes of bone loss, but there were greater changes in TMD and bone strength in the females. Transcriptomic analysis revealed female mice had different downregulated gene ontologies indicating sex differences in the response to disuse.

SIGNIFICANCE: Genetic variation, limb and sex influence bone and muscle mass, underscoring the importance of evaluating interventions in both sexes, both limbs and a genetically diverse population. Disuse causes bone loss throughout a genetically diverse population, but there are sex differences in which mechanisms are involved.

REFERENCES: 1. Friedman et al., Bone Reports 2021. 2. Maroni et al., JMNI 2021. 3. Svenson et al., Genetics 2012.

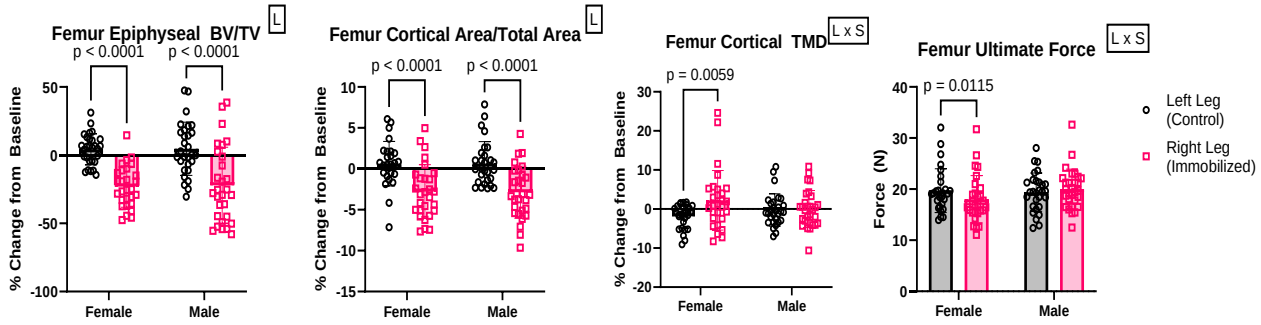

Figure 1. Femur epiphyseal BV/TV, mid-diaphyseal cortical area fraction, tissue mineral density, and ultimate force (mean ± SD) after three weeks of single limb immobilization of the right leg in 16-week old diversity outbred mice. Immobilization decreased bone mass in male and female mice and decreased bone strength in female mice. L - significant main effect of limb; L x S - significant limb x sex interaction (2-Way RM ANOVA).

Table 1. The most significantly upregulated and downregulated Gene Ontologies in immobilized limbs, compared to control limbs.

	Upregulated			Downregulated	
Rank	Gene Ontology - Female	Gene Ontology - Male	Rank	Gene Ontology - Female	Gene Ontology - Male
1	Multinuclear Osteoclast Differentiation	Collagen Fibril Organization	1	Myofibril Assembly	Macropinocytosis
2	Collagen Fibril Organization	Extracellular Matrix Organization	2	Sarcomere Organization	Hemostasis
3	Extracellular Matrix Organization	Extracellular Structure Organization	3	Striated Muscle Contraction	Pinocytosis
4	Extracellular Structure Organization	Regulation of Basement Membrane Organization	4	Striated Muscle Hypertrophy	Regulation of Superoxide Anion Generation
5	Proteoglycan Catabolic Process	External Encapsulating Structure Organization	5	Muscle Contraction	Regulation of Leukocyte Degranulation