Disuse Differentially Affects Achilles Tendon Composition in Male and Female DO Mice

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INTRODUCTION: Hierarchical collagen fibers are the primary source of strength and function in tendons and ligaments.[1,2] It is well established that mechanical cues are critical to the development and maintenance of these collagen fibers, and disuse or reductions in mechanical loading significantly reduces tendon mechanics; however the exact mechanism is largely unknown.[1-4] There is a need to better understand cellular response to unloading in tendons to limit the deleterious effects of inactivity, prevent degeneration with long-term space travel, and develop better rehabilitation protocols post injury.[2,3,5] While the effects of disuse have been well studied in muscles and bones, the effects of unloading in tendons has had considerably less research.[5] Further, most studies evaluating tendon response to disuse involve the use of inbred mice or rats, which lack the genetic variability seen in humans and limit the potential impact of these studies on human tissues. Diversity outbred (DO) mice are developed by cross-breeding 8 genetically diverse inbred founder strains of mice—C57Bl/6J, A/J, 129S1/SvImJ, NOD/ShiLtJ, NZO/HlLtJ, CAST/EiJ, PWK/PhJ and WSB/EiJ—resulting in a mouse population with high levels of genetic variation, which more closely match the response of humans to tendon disuse.[4] The objective of this study was to investigate the response of Achilles tendons to disuse via single limb immobilization in genetically variable DO mice to better understand tenocyte response to unloading in males and females.

METHODS: All procedures were approved by the VCU IACUC. Thirty female and thirty male 16-week-old DO mice had their right hind limbs immobilized in a cast for 3 weeks as part of a larger study evaluating the effects of disuse on bone and muscle adaptation. After 3 weeks, the mice were sacrificed and the Achilles tendons from both the cast and contralateral, uncast hind limbs were removed. Tendons were randomly divided between analysis techniques, with N=9 fixed for organization analysis, N=10 frozen for mechanical analysis, and N=11 stored for compositional analysis from each sex. Cast and uncast tendons were analyzed for organization using confocal reflectance microscopy as previously described.[6] Tendon mechanical properties were assessed with tendons immersed in a PBS bath and exposed to a stress relaxation test at 5% strain held for 10 minutes followed by a ramp to failure at 0.5% strain/s to determine elastic properties. Tendons for compositional analysis were weighed wet, lyophilized, and weighed dry to determine percent water, and the DNA, glycosaminoglycan, and collagen content were determined via Picogreen, DMMB, and hydroxyproline assays, respectively. Tendon composition was normalized to wet weight and is reported as the ratio of composition in the cast tendon over the uncast tendon. Statistical analysis was performed using one-way and paired two-way ANOVA with Tukey's post-hoc test ($p<0.05$).

RESULTS: When viewed under confocal microscopy, there were no strong metamorphic differences between the Achilles tendons, regardless of mouse sex or cast condition (Fig 1A). However, preliminary mechanical tests demonstrated a general trend of decreased elastic modulus and ultimate tensile strength (UTS) in unloaded/casted tendons compared to contralateral controls in male mice. Interestingly, females do not appear to have a decrease in tendon mechanics in cast tendons compared to contralateral controls (Fig 1B). Mirroring differences in mechanics, males and females had differences in composition with disuse. Female mice had a general trend of increasing DNA and GAG accumulation in cast tendons, while males had a significant decrease in collagen concentration in the cast tendon compared to contralateral control tendons (Fig 1C). Neither male nor female tendons had differences in percent water between cast and uncast tendons (Fig 1C).

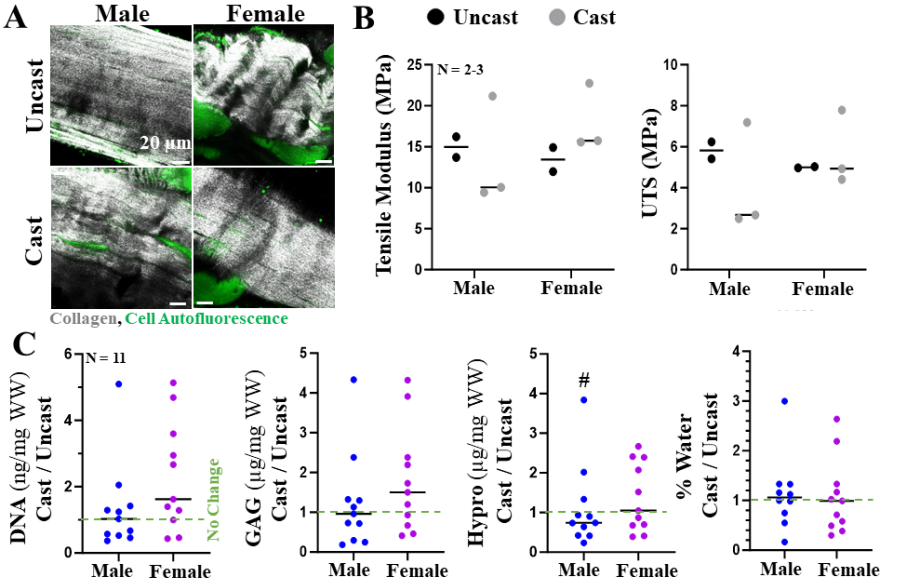

Figure 1: A) confocal reflectance of collagen organization, B) Preliminary tensile modulus & ultimate tensile strength (UTS), and C) DNA, GAG, collagen, and % water of cast compared to uncast tendons in DO mice. #Significance to uncast tendons ($p<0.05$).

DISCUSSION: While the Achilles tendons of male and female DO mice don't appear to have changes in collagen organization in response to 3 weeks of disuse (Fig 1A), they demonstrate shifts in mechanics based on sex and cast condition (Fig 1B). Specifically, cast tendons from male mice appear to have decreased mechanics compared to both female cast tendons and uncast tendons from both sexes (Fig 1B). The reduction in tendon mechanics without macroscopic changes in organization or percent water largely correlates with previously studies performed in other inbred strain male animal models,[2] however the differential response in females is more surprising. These differences between male and female tendons could be due differential regulation of composition in response to disuse. Male mice had significantly reduced collagen content in cast tendons, while females tended to have increased DNA and GAG concentrations in cast tendons, with no change in collagen concentration (Fig 1C). Overall, this suggests that 3 weeks of disuse in the Achilles tendons of genetically variable DO mice leads to differential disruptions of tendon composition based on the sex of the mouse, which produces differential changes in mechanics of disused tendons. Mechanical tests are on-going to increase N and better evaluate viscoelastic and toe-region mechanics. Further, on-going work is evaluating gene expression to confirm differences in composition, as well as evaluating LOX expression to determine whether disuse effects collagen crosslinking, previously speculated to be a major contributor to decreased mechanics in tendons due to disuse.[2,4]

SIGNIFICANCE: This study is one of the first to assess the effects of disuse on the Achilles tendon of genetically variable mice and to evaluate the effect of disuse in males and females. The results of this study so far suggest tendons from males and females respond differentially to disuse. A better understanding of how tendons from males and females differentially respond to disuse will help to limit the deleterious effects of inactivity which occur with prolonged bedrest, prevent degeneration with long-term space travel, and will help to develop better rehabilitation protocols post injury.[2,3,5]

REFERENCES: 1. Galloway+*J Bone Surg Am* 2013; 2. Magnusoon+*J of Physiology* 2018; 3. Roffino+*Life Sci Space Res* 2021; 4. Almeida-Silveira+*Eur J Appl Physiol* 200; 5. Liphart+*NPJ Microgravity* 2023; 6. Puetzer+*Biomat 2021*