Rat plantarflexor tendon biomechanics and composition are minimally affected by 12 weeks of high-volume loading

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INTRODUCTION: Tendinopathy is a debilitating condition that affects 2.35 per 1000 individuals aged 21-60 years[1] and risk factors include increases in activity, limited recovery time, and repetitive movements.[2] Repetitively loaded tendons, such as the Achilles tendon, are particularly susceptible to tendinopathy and account for 10% of all running-related injuries.[3] Treadmill running[4] is a common strategy to attempt to study Achilles tendon overuse in rodents, yet a chronic Achilles tendon loading protocol in these small animal models is challenging to achieve because Achilles tendon stresses during running in humans far exceed those during running in rodents.[5,6] Thus, the biomechanical and compositional changes to the rat Achilles tendon following chronic loading are unknown. The purpose of this study was to determine the structural and functional response of the rat plantarflexor tendons (Achilles and plantaris tendons) to long-term repetitive ankle loading using a novel small animal dynamometer. We hypothesized that increasing tendon loading would cause degenerative changes to the Achilles and plantaris tendons, leading to a higher cross-sectional area (CSA) and inferior mechanical properties.

METHODS: Loading protocol: We divided twenty-three 12-week male Sprague Dawley rats into one control and three experimental groups (IACUC approved). We used male rats in this study because men are particularly at risk of developing Achilles tendinopathy and eventual rupture.[7] The experimental group was anesthetized and mounted on a custom small animal dynamometer (Fig. 1A).[8] Hind limbs were affixed in a splint with the knee in full extension and the resting ankle angle at 90°. The ankle was driven through 90 Nmm of torque for 5000 cycles at 2.5 Hz, where 90 Nmm is estimated to be 2-3x greater than torques generated during uphill rodent running.[9] The experimental groups received this high-volume tendon loading bilaterally, and this protocol was repeated five times per week for 4, 8, or 12 weeks. Cage activity controls were euthanized at 4 weeks, and experimental animals were euthanized the morning after the last loading bout at 4, 8, or 12 weeks. Biomechanics (n=5-6/group): We measured tendon CSA using a custom laser device and applied Verhoeff stain lines at the tendon midsubstance for optical strain analysis. Achilles tendons were secured by fixing the hindpaw in polymethylmethacrylate at 120° and clamping the proximal tendon between sandpaper grips. Plantaris tendons were clamped between sandpaper grips on each side. All tendons were tested in a 37° C 1X PBS bath. Our testing protocol consisted of a 0.5 or 1 N preload, 10 cycles of preconditioning at 0.5% strain, stress relaxations at 3%, and 6% grip strain followed by frequency sweeps at 0.1, 1, 5, and 10 Hz, and a ramp-to-failure at 0.1%/s. We measured elastic (stiffness, modulus, local modulus at the tendon midsubstance) and viscoelastic (dynamic modulus, phase shift (tan δ), and percent relaxation) properties. Biochemistry (n=5-6/group): Achilles and plantaris tendons were digested in Proteinase K overnight at 55°C prior to quantifying glycosaminoglycan (GAG) content using the 1,9-dimethylmethylene blue assay and CS as standard.[10] Statistics: Data was assessed for normality using the Shapiro-Wilk test. Comparisons across control and experimental groups were conducted using one-way ANOVAs with Bonferonni post-hoc tests. Significance was set at $\alpha=0.05$.

RESULTS: Achilles and plantaris tendon composition was unaffected by loading. Specifically, there were no changes to CSA, water content, or GAG content following loading treatment (Fig. 1B-C, top row). There were no hypothesized adaptations of structural or material properties to long term loading (Fig. 1B-C, middle row). While half of 12-week Achilles tendons failed at the tendon midsubstance (rather than the normal mode of failure at the insertion), stiffness, max strain, and modulus ($p=0.051$ for plantaris, $p=0.66$ for Achilles) were unaffected by loading treatment. We did observe an increase in max force following treatment, but only in the plantaris tendon ($p=0.042$ for plantaris, $p=0.27$ for Achilles). Finally, we observed minimal adaptations of Achilles or plantaris tendon viscoelasticity (Fig. 1B-C, bottom row), including no changes to dynamic modulus or tan δ and a decrease in stress relaxation in the plantaris tendon ($p=0.029$ for plantaris, $p=0.099$ for Achilles) following loading treatment.

DISCUSSION: Contrary to our hypotheses, we observed small changes to plantaris tendon biomechanics, no changes to Achilles tendon biomechanics, and no changes to composition in either tendon following 4, 8, or 12 weeks of high-volume ankle loading. These results are consistent with prior studies examining Achilles tendon loading using an inclined treadmill, where even 16 weeks of daily running bouts resulted in no changes to Achilles tendon structure-function.[11] A recent study found that plantaris tendon biomechanics adapt before eventually degenerating into a tendinopathy-like phenotype in a synergist ablation model,[12] suggesting that the tendon response to overloading may be dose-dependent. However, a tendinopathy phenotype in the rat Achilles tendon may be more challenging to create without an added comorbidity, such as advanced age or diabetes.[13] Future work will examine these models of tendon degeneration and the effects of our loading protocol on the calcaneus and triceps surae of the rats used in this study.

SIGNIFICANCE: This study investigated the biomechanical properties and composition of the Achilles and plantaris tendons following repeated loading at ankle torques greater than those during running. These studies can elucidate adaptations to long-term loading during tendinopathy pathogenesis.

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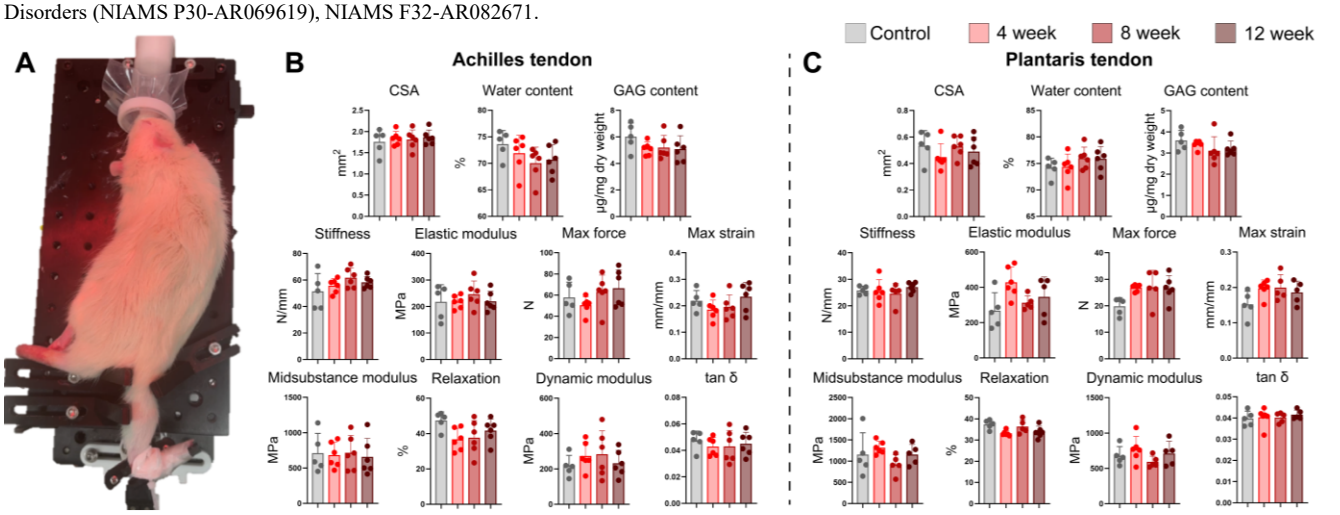

FIGURE 1: (A) Custom passive dorsiflexion system. (B) Achilles and (C) plantaris tendons were minimally affected by 4, 8, and 12 weeks of high-volume loading treatment, with minimal or negligible changes to composition, structural, material, and viscoelastic properties (1 Hz, 6% strain shown).