

Investigating the Effects of Injury and Exercise in Modulating Achilles Tendon Fluid Clearance

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INTRODUCTION: Achilles tendinopathy is a chronic and degenerative disease with long symptom duration and high socioeconomic cost.^{1,2} Tendinopathy pathogenesis remains incompletely understood, stalling therapeutic efforts. Interstitial fluid content is increased in tendinopathy, but little is known about the role of fluid transport in the disease process or as a potential therapeutic target.³ We've shown in previous work that we can quantitatively evaluate fluid clearance rates in healthy rat Achilles tendons using the injection of near-infrared (NIR) tracers, demonstrating that venous clearance rates are significantly faster than lymphatic clearance rates.⁴ We have shown that rehabilitative treadmill running can improve fluid clearance in both healthy and diseased rat knee joints in the context of osteoarthritis.^{5,6} Similar rehabilitative protocols, such as the Alfredson protocol, are the clinical gold standard for Achilles tendinopathy treatment. In this study, we had two objectives: 1) Characterize the effect of injury on Achilles tendon venous and lymphatic clearance rates, and 2) Investigate how a moderate treadmill running exercise protocol affects fluid clearance in healthy rat Achilles tendons. *We hypothesized that injury would initially increase (2 week timepoint) but eventually decrease (8 week timepoints) clearance rates, and that clearance rates would be increased after a single bout of running exercise.*

METHODS: All procedures were conducted in accordance with IACUC protocol. Skeletally mature female Sprague-Dawley rats were used for all experiments. We used female rats to avoid potential behavioral confounds from visual or olfactory interactions between sexes. **Fluid Clearance:** Fluid clearance rates were measured through imaging of near-infrared fluorescent tracers. The venous clearing (680RD, Li-Cor) and lymphatic clearing (800CW-20kDa PEG) tracers were co-injected intratendinously and imaged over time using an IVIS Spectrum (Revvity). The fluorescence in the tendon region of interest was measured and quantified in LivingImage (Revvity). Values were normalized to the timepoint immediately after injection and fit from the maximum values with exponential decay curves. From these data, initial clearance was calculated as the difference between the 0- and 1-hour images (*Exercise* only), and overall clearance was calculated as the time constant of decay of the exponential fit. **Injury:** Rats (N = 20) received a surgically induced unilateral Achilles tendon full thickness transection that was immediately repaired with suture. Animals were divided into three groups, with fluid clearance rates measured at 2 (N = 10), 4 (N = 5), and 8 (N = 5) weeks after injury. A group of naïve control rats (N = 5) was also included in this study. **Exercise:** Healthy rats (N = 4) all underwent two experimental protocols. Fluid clearance rates were measured at baseline (Sedentary) and after two weeks of pretraining on a custom modified NordicTrack treadmill (Exercise). There were 2 weeks of washout between the two protocols. For the exercise experiment, the animals completed a single bout of treadmill running at 15 meters/minute for 30 minutes between the 0- and 1-hour images after injection. Data were analyzed for each limb individually in GraphPad (Prism) with an alpha level of 0.05 and are presented as mean ± standard deviation.

RESULTS SECTION: **Injury:** Both lymphatic and venous overall clearance rates were significantly altered after injury compared to naïve controls and contralateral limbs. Injured tendons had faster venous clearance rates at 4 and 8 weeks compared to contralateral limbs, while contralateral tendons at 8 weeks had slower venous clearance than controls. For lymphatic clearance rates, injured tendons had faster clearance than controls and contralateral limbs at 2 weeks, and slower clearance than controls and contralateral limbs at 8 weeks. **Exercise:** Exercised healthy rat Achilles tendons did not have significantly different overall (p = 0.883) or initial (p = 0.431) venous clearance rates when compared to sedentary baseline measures (Fig. 2). Exercise also did not significantly change overall (p = 0.158) or initial (p = 0.491) lymphatic clearance rates when compared to the sedentary data (Fig. 3).

DISCUSSION: As we hypothesized, lymphatic clearance rates initially increased after injury but eventually decreased to a rate slower than healthy tendons by the 8 week timepoint. Venous clearance rates did not match this hypothesis, as we saw significantly faster clearance in injured limbs through the 8 week timepoint. This decreased lymphatic clearance seen 8 weeks after tendon injury could factor into the increased interstitial fluid content seen clinically in tendon disease. Contrary to our exercise hypothesis, we saw no significant difference in either lymphatic or venous tracer clearance rates after a single bout of moderate running exercise. This exercise protocol was potentially not at a high enough intensity to increase clearance in healthy rats, but further work should be done to understand the effect of different exercise protocols after injury. Changing ankle kinematics through graded running or utilizing periodic running bouts could change the loading intensity or loading rate of the tendon and therefore have a larger effect on fluid related outcomes.

SIGNIFICANCE/CLINICAL RELEVANCE: The role of fluid clearance pathways in tendon pathology is not fully understood. Poor fluid content management may lead to unresolved inflammation. This study examines potential methods for improving tendon fluid clearance.

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IMAGES AND TABLES:

