High-Speed Treadmill Running Does Not Induce a Tendinopathic Phenotype in Rat Achilles Tendon

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Introduction

Achilles tendon pathology comprises an increasing and consequential clinical burden,[1,2] but robust and reproducible preclinical animal models of Achilles tendinopathy are lacking. Overuse is a common etiology of tendon pathology, and exercise-induced overuse has been considered a promising mechanism for creating a clinically relevant tendinopathy model. In rat Achilles tendon, treadmill running at moderate speed (17-20 m/min) results in variable structural and functional outcomes,[3-7] failing to induce a consistent tendinopathy phenotype. Effects of running at higher speeds (> 25 m/min) on Achilles tendon structure and function have not been thoroughly investigated, though early results have shown potential for a tendinopathic phenotype.[7,8] Therefore, the objective of this study was to rigorously assess the structural and biomechanical impacts of high-speed treadmill running on rat Achilles tendon. We hypothesized that 16 weeks of high-speed treadmill running would induce an overuse tendinopathy phenotype characterized by matrix disorganization, rounded cell morphology, and reduced tensile mechanical properties.

Methods

Sprague-Dawley rats (~400 g) were randomized into two groups: cage activity (n = 12) and running (n = 9). The running group underwent a 3-week acclimation protocol followed by 16 weeks of high-speed treadmill running (27 m/min, 10° incline, 1 hour/day, 5 days/week); mild electrical shock was used at the back of the treadmill to encourage running. After 16 weeks, Achilles tendons were harvested bilaterally for histological and mechanical assessment. For histology, ankles were prepared for paraffin histology with standard techniques,[9] sectioned sagittally (7 mm thickness), stained serially with DRAQ5TM (abcam, Waltham, MA, USA) then 0.1 % toluidine blue, and imaged (10X magnification). Midsubstance regions (~1.3 × 0.65 mm) from two sections per tendon were analyzed (CellProfilerTM[10]) for cell count and nuclear shape. Tendons designated for mechanical testing were first μCT imaged (10 μm resolution, μCT35, Scanco Medical, Brüttisellen, Switzerland) to identify heterotopic ossification (HO). Images were segmented and HO volume was quantified with Amira 6.7 (Thermo Fisher Scientific, Waltham, MA). After scanning, tendons were prepared[9] and tested with a viscoelastic testing protocol (preconditioning; stress relaxation at 9% strain; sinusoidal frequency sweeps at 0.1, 1, 5, and 10 Hz) followed by a quasi-static ramp (0.3% strain/s) to failure with image capture for optical strain measurement. Digital image correlation software (Vic2D, Correlated Solutions, Irmo, SC) was used to determine strain distributions along the length of the tendon at the transition point, mid-linear region (2 × transition strain), and

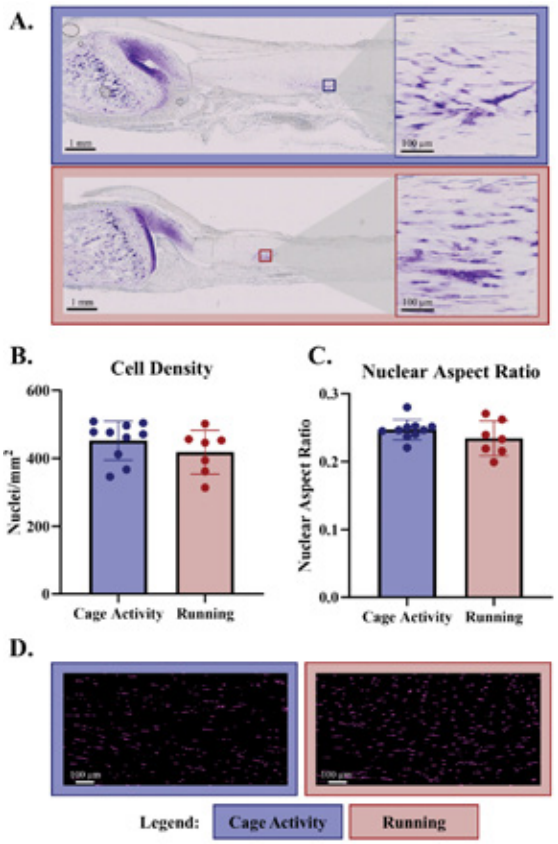

Figure 1. Both cage activity (blue) and running (red) tendons contain discrete regions of disorganization with rounded cells, indicative of HO **(A)**; running did not impact cell density **(B)** or nuclear aspect ratio **(C)** in the midsubstance (representative images shown in **D)**.

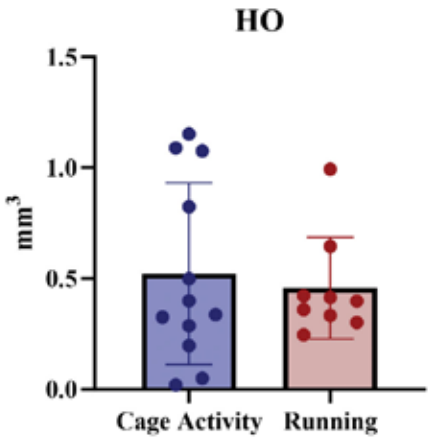

Figure 2. Tendons from both activity groups demonstrated HO by μCT. Running did not influence HO volume.

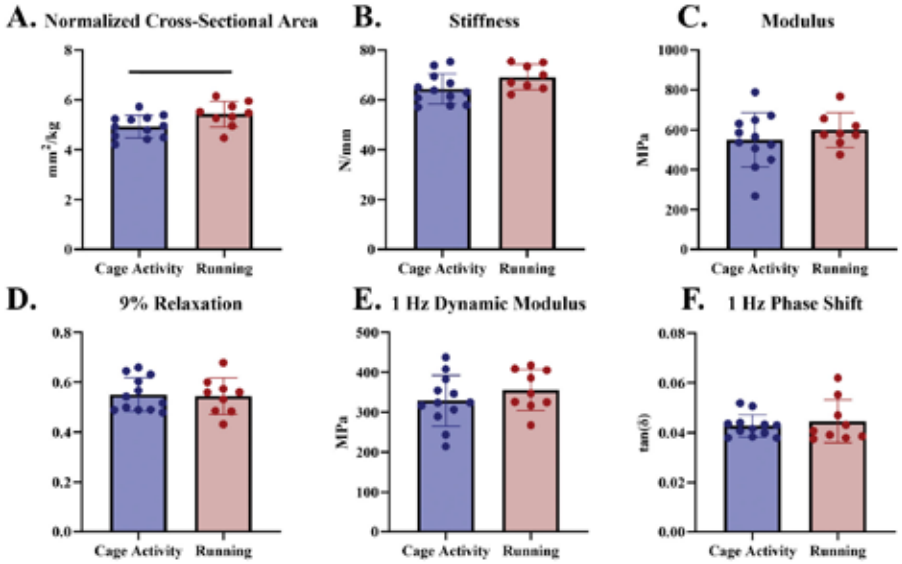

Figure 3. Running increased Achilles tendon CSA normalized to body weight **(A)**; neither stiffness **(B)** nor optical modulus **(C)** were influenced by treadmill running. Similarly, percent relaxation **(D)**; dynamic modulus **(E)**; and phase shift **(F)** were unaffected by treadmill running (data shown for 1 Hz, consistent across frequencies).

failure. T-tests were used to compare histological and mechanical properties between cage activity and running groups, and 2-way repeated measures ANOVAs with Šídák's multiple comparison tests were used to assess differences in regional strain and modulus between activity groups. Significance was set at $p < 0.05$.

Results

All histology samples demonstrated varying amounts of discrete pockets of matrix disorganization, increased staining intensity, and rounded cell morphology, demonstrating an HO phenotype (Figure 1A). In regions of interest, chosen to exclude regions of suspected HO, cell density and nuclear shape were unaffected by treadmill running (Figure 1B-D). In contralateral limbs, we consistently detected the presence of HO on µCT, though HO volume (Figure 2) and mineral density (data not shown) were unaffected by activity level. While running was associated with a decrease in cross-sectional area (CSA, $p = 0.04$), when normalized to body weight, runners demonstrated increased normalized CSA ($p = 0.02$). Despite this, no differences were detected between groups in viscoelastic(relaxation at 9% strain, dynamic modulus, phase shift) or elastic (stiffness, modulus) mechanical properties (Figure 3A-F). Local strain and modulus varied along the tendon length as expected ($p < 0.05$) but were unaffected by activity group (data not shown).

Discussion

Contrary to our hypothesis, 16 weeks of high-speed treadmill running did not induce an overuse tendinopathy phenotype. While rat Achilles tendon is a well-established model for investigations of HO,[11] previous studies of impacts of treadmill running on rat Achilles tendon have not considered potential impacts of HO on tendon structure and biomechanics. We speculate that the high incidence of HO may impact the consistency of both histological and mechanical findings from previous rat Achilles tendon tendinopathy models. Future methods for inducing Achilles tendinopathy should consider alternative approaches to achieve a reproducible phenotype.

Acknowledgements

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