# Treadmill Running Does Not Induce Mechanical Changes in the Rat Subscapularis Tendon

Chet S. Friday[1], Courtney A. Nuss[1], Jeremy D. Eekhoff[1], Louis J. Soslowsky[1], Andrew F. Kuntz[1,2]
[1]University of Pennsylvania, Philadelphia, PA, [2]Corporal Michael J. Crescenz VA Medical Center, Philadelphia, PA
Chet.Friday@Pennmedicine.upenn.edu

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**INTRODUCTION:** Rotator cuff tendinopathy, often caused by overuse,[1,2] results in functional deficits and pain,[3] presenting a substantial clinical problem that impacts people of all ages and levels of physical activity.[4] Previous studies in rat models have shown that exercise-induced overuse results in tendinopathy in the supraspinatus tendon.[5] However, the impact of increased activity on the remainder of the rotator cuff is unknown. Although previously believed to be an infrequent source of clinical pathology, the subscapularis is now recognized as a common cause of rotator cuff tendinopathy.[6] Therefore, the objective of this study was to evaluate the impact of treadmill running on inducing tendinopathy in the subscapularis tendon in the rat model. We hypothesized that high levels of treadmill running would lead to tendinopathy and result in decreased mechanical properties in the upper and lower bands of the subscapularis.

**METHODS:** Treadmill Protocol: Forty-five 16-week-old male Sprague-Dawley rats were subjected to one of three levels of exercise by treadmill running – cage activity (CA), moderate-speed running (MSR, 17 m/min at 10° decline)[5], or high-speed running (HSR, 22 m/min at 10° decline). Animals subjected to running protocols underwent an acclimation period of two weeks in the MSR group and three weeks in the HSR group, followed by four weeks of treadmill running (1 hour per day, 5 days per week). Animals were sacrificed after completion of the treadmill protocol for mechanical testing. Sample Preparation: Subscapularis and supraspinatus tendons were harvested with the left humerus and fine dissected free of non-tendon soft tissue. Subscapularis tendons were separated into upper and lower bands by cutting through the clear delineation point, in order to test the two distinct bands of the subscapularis individually (Fig 1). Tendon cross-sectional areas were measured using a laser-based device[7] and humeri were potted in polymethyl-methacrylate for testing. Mechanical Testing and Analysis: Tendons were secured in custom fixtures at a gauge length of 8mm from the insertion. Testing was performed on an Instron 5542 test frame (Instron, Norwood, MA), and consisted of preconditioning (30 cycles between 0.125% and 0.375% strain at 0.25 Hz), stress relaxation (6% strain for 10 minutes), and quasi-static ramp-to-failure (0.1 mm/s). Tendons were then analyzed for elastic properties (stiffness, modulus), viscoelastic properties (percent relaxation), and failure properties (maximum force, maximum stress) using custom MATLAB scripts. Samples that failed at the grip were excluded from failure property analysis. Data was analyzed using one-way ANOVA followed by a Tukey's post-hoc test ($\alpha = 0.05$).

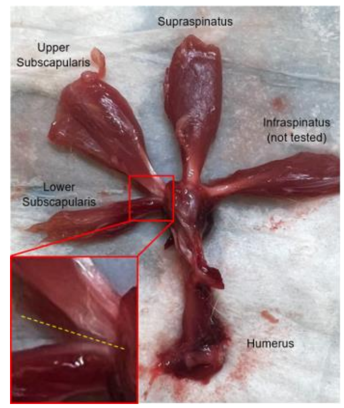
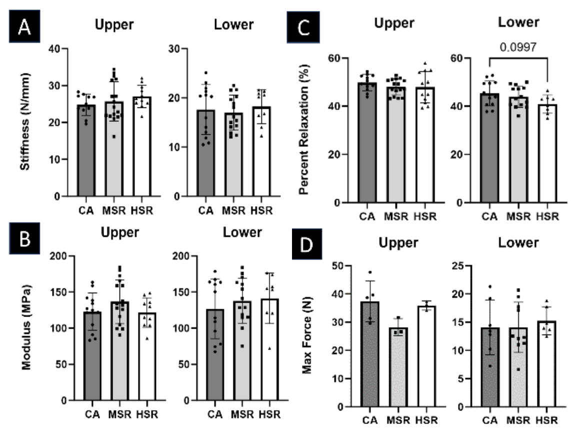



Fig. 1: Photograph of dissected humerus with rotator cuff tendons attached, with delineation point between upper and lower bands of the subscapularis highlighted in yellow.

**RESULTS:** Upper and lower bands of the subscapularis tendon showed no differences in stiffness (Fig. 2A), grip modulus (Fig. 2B), or percent relaxation (Fig. 2C) between activity levels. Additionally, upper and lower bands showed no differences in maximum force (Fig. 2D) or maximum stress (Fig. 2E) between activity levels. Similarly, supraspinatus tendons showed no differences in any mechanical properties between activity levels (*data not shown*).

**DISCUSSION:** This study demonstrated that the downhill treadmill running protocols utilized did not induce mechanical changes consistent with overuse in the subscapularis tendon. Although there were no mechanical changes observed, it is possible that treadmill running may have induced biological and/or histopathological changes that have not been evaluated to date. Prior studies using the MSR model focused on the supraspinatus tendon.[5,8,9] The lack of observed mechanical changes in the subscapularis indicates that the model may not be suitable for this tendon, perhaps due to the unique organization, composition, local loading environment, or relative location of the acromion to the supraspinatus when compared to the subscapularis. Interestingly, this study did not find mechanical changes in the supraspinatus tendon, adding to the mixed results of the treadmill model,[5,8,9] which may be affected by protocol variables such as treadmill inclination, as well as duration and time of day of running. Taken together, the results of this study in the subscapularis and supraspinatus indicate the described protocol does not induce mechanical changes consistent with tendinopathy that may lead to rotator cuff tears. While recent clinical studies have highlighted the high and previously unrecognized incidence of subscapularis tears,[6] the relationship of subscapularis tears to preexisting tendon damage, potentially due to overuse, remains unknown and further studies are needed.



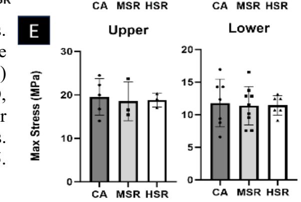




Fig. 2: Mechanical testing results. No significant differences were found between groups for (2A, 2B) elastic, (2C) viscoelastic, or (2D, 2E) failure properties of upper or lower bands of the subscapularis. 2A-2C: n between 8-16, p > .05. 2D-2E: n between 3- 10, p > .05.

**CLINICAL SIGNFICANCE:** Increased physical activity did not result in tendinopathy in the subscapularis, suggesting the possibility that exercise-induce overuse does not lead to subscapularis tendon damage and tears in the absence of other contributing factors.

**REFERENCES:** [1] Gomoll+ Arthritis Rheumatol, 2004. [2] van der Windt+ Ann Rheum Dis, 1995. [3] Millar+ Nat Rev Dis Primers, 2021. [4] Hopkins+ Asia Pac J Sports Med Arthrosc Rehabil Technol, 2016. [5] Soslowsky+, JSES, 2000. [6] Bennett+, Arthroscopy, 2001. [7] Favata PhD Thesis, 2006. [8] Parks+ J Orthop Res, 2017. [9] Attia+ J Orthop Res, 2011.

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