Rat Supraspinatus Tendon Responds Acutely and Chronically to Exercise

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Introduction

Tendons respond to exercise by adapting their extracellular matrix (ECM). Prior studies found increased[1,2] or unchanged[3,4] tendon stiffness with chronic exercise; however, these changes have not been characterized over time, and it is unknown how acute responses following a single exercise bout lead to adaptations with repeated bouts. Previously, we showed that acute and chronic gene responses to exercise are distinct, and matrix metalloproteinase (MMP) expression is altered.[5] MMPs facilitate ECM turnover and are increased with tendinopathy. Whether MMPs are a physiologic response to load or pathologic in tendon degeneration is unknown. Therefore, the objective of this study was to identify acute responses and chronic adaptations of supraspinatus tendon to non-injurious exercise. We hypothesized that chronic exercise increases tendon mechanical properties, and MMP activity increases acutely.

Methods

One hundred fifty six male, Sprague-Dawley rats (400-450g, IACUC approved) were divided into acute or chronic exercise (EX) or cage activity (CA) groups.[5,6] Animals in acute groups were euthanized 3, 12, 24, 48, or 72 hours upon completion of a single bout of exercise (10 m/min, 1 hr) on a flat treadmill (EX3h, EX12, EX24, EX48, EX72). A control, treadmill-trained group (CA-T) did not undergo a single bout of exercise. A second control group maintained normal cage activity for the duration (CA24) and was used to measure MMP activity. Animals in chronic EX groups walked on a flat treadmill (10 m/min, 1 hr/day, 5 days/wk) for 3 days, 1 wk, 2 wks, or 8 wks (EX3d, EX1, EX2, EX8). Rats were euthanized 72 hr after their final exercise session to avoid potentially confounding acute effects of exercise. Two control groups maintained normal CA for an early (CA2) or later (CA8) time point. Tendon histology and MMP activity were measured for all groups. Tendon mechanics were determined in the EX24, CA-T, EX2, CA2, EX8, and CA8 groups. *Mechanics:* Stiffness was calculated as the slope of the linear region of the load-displacement curve during a ramp to failure at 0.3%/s. Modulus was calculated from optical strain and stress measurements. Dynamic modulus and tangent of the phase angle between the stress and strain were calculated from a frequency sweep (0.1, 1, 2, 10 Hz) of 10 sine cycles at 8% strain with 0.125% strain amplitude. Percent relaxation was calculated from a 300s stress-relaxation test at 8% strain. *Histology:* 7μm sections of paraffin-embedded bone-tendon-muscle units were stained with H&E and imaged with polarized light (chronic groups only) to determine the circular standard deviation of the collagen alignment. Cell density and shape were quantified with commercial software (Bioquant). *MMP Activity:* A SensoLyte 520 generic MMP assay kit (Anaspec) was used to determine MMP activity, following the manufacturer's protocol. Dilutions of purified human MMP-13 were used to create a standard curve. *Statistics:* To determine the effects of exercise on tendon mechanical properties, t-tests were used to compare EX and CA groups separately for each time point. Significance was set at $p \leq 0.05$ and trends at $p \leq 0.1$. To determine the effects of exercise on tendon histological properties (cell density, cell shape, organization for chronic), 1-way ANOVAs were performed for the acute groups (EX3h, EX12, EX24, EX48, EX72, CA-T) and the early chronic groups (EX3d, EX1, EX2, CA2). If the ANOVA was significant ($p \leq 0.05$) or a trend ($p \leq 0.1$), then pairwise comparisons were performed with Fisher's tests. A t-test was used to compare EX8-CA8 for tendon histology and MMP activity. A 1-way ANOVA was used to compare MMP activity in the early chronic groups. To determine the acute effects of exercise on MMP activity, after confirming no differences in MMP activity between them, CA-T and CA24 control groups were pooled to form a single control group (CA-P). Then, a 1-way ANOVA was performed for the EX3h, EX12, EX24, EX48, EX72, and CA-P groups with Fisher's tests for pairwise comparisons. All data is presented as mean + standard deviation.

Results

A single bout of exercise resulted in mild trends toward reduced tendon mechanical properties, but 2 or 8 weeks of chronic exercise led to increased tendon mechanics (Figure 1). Dynamic modulus increased after 8 weeks of exercise, and tan(δ) decreased after 2 weeks of exercise (not shown). Cell density was not affected acutely or chronically (not shown).

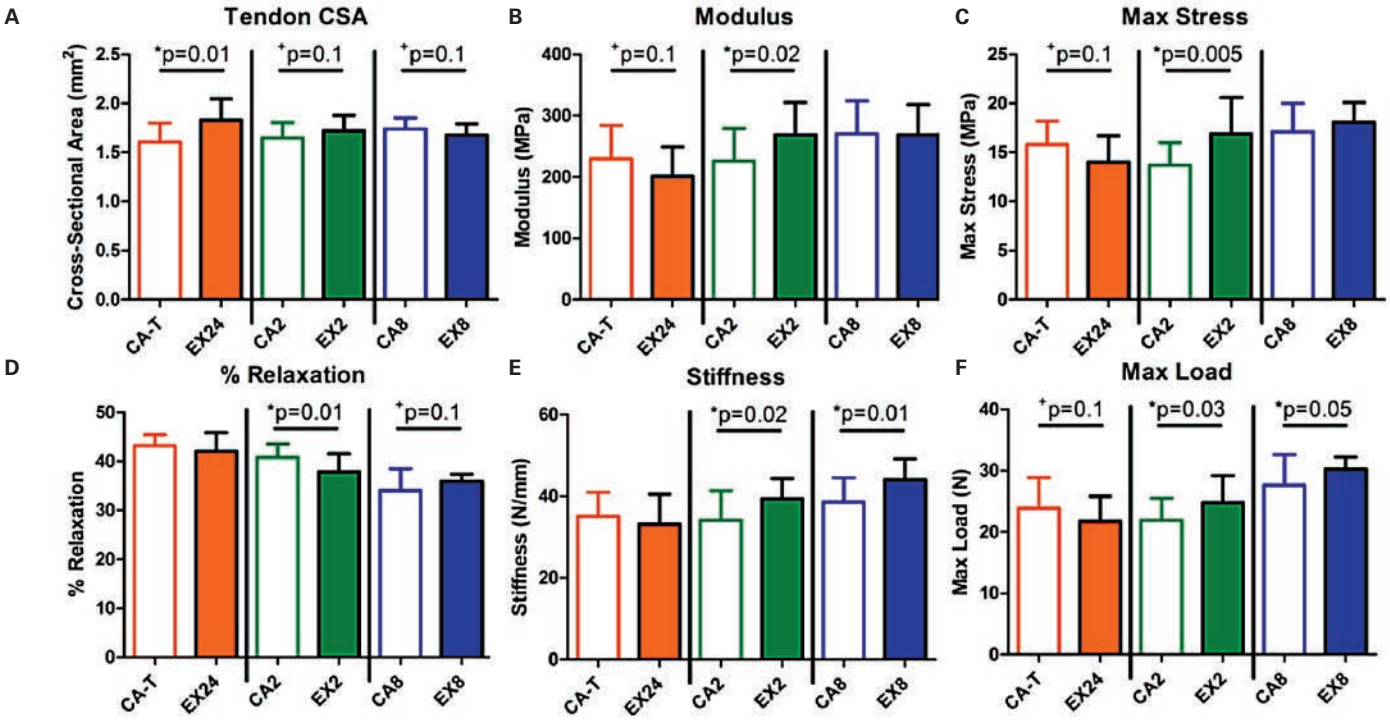

Figure 1. Tendon Mechanics. A single bout of EX followed by 24 hr of rest led to increased tendon cross-sectional area (A) and trends toward decreased modulus (B), max stress (C), and max load (F), suggesting mildly inferior properties. Chronic EX resulted in beneficial adaptations after 2 weeks and 8 weeks. n = 9-12 acute, 12-17 at 2 wks, 11-13 at 8 wks.

Cells became rounder with chronic exercise and 48 hours after a single bout of exercise (not shown). All tendons were highly organized, and chronic exercise did not affect collagen organization (not shown). MMP activity decreased 12, 24, and 48 hours after a single bout of exercise and returned to baseline by 72 hours (Figure 2A). MMP activity decreased after 8 weeks of chronic exercise (Figure 2B).

Discussion

Rat supraspinatus tendons demonstrated acute responses and chronic adaptations to exercise. The mild trends toward decreased mechanics following a single exercise bout may initiate and foster chronic adaptations. Although these differences were minor acutely, they were sufficient to result in significant beneficial adaptations chronically. Generic MMP

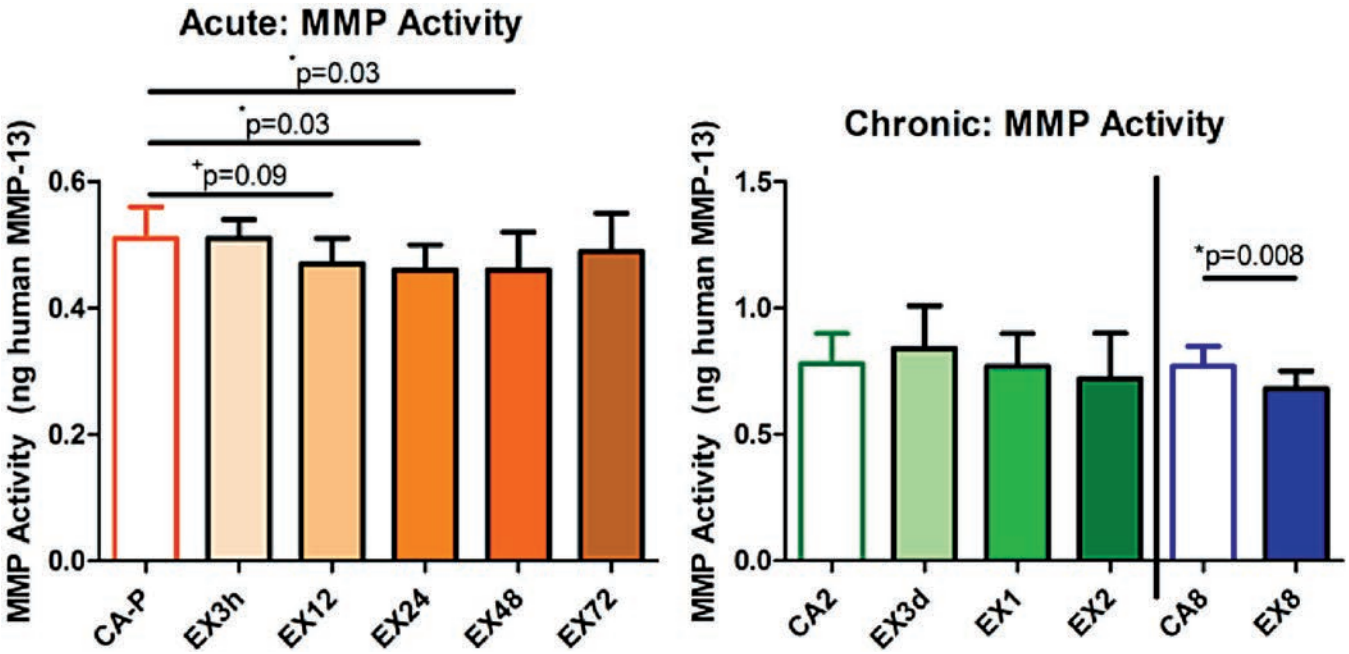

Figure 2. MMP Activity. MMP activity decreased after a single bout of EX (A) and 8 weeks of EX (B). n = 6-8 acute EX, n = 14 CA-P, n = 8-12 chronic.

activity decreased with exercise. Although some studies have found increases in MMPs following exercise, others have found decreases.[7] Decreased MMP activity may indicate an anabolic instead of catabolic response and contrasts the response seen with injury. This study investigated a single, previously validated exercise protocol, and it is unknown how results would change with increased intensity. Additionally, tendon mechanics were measured at a single acute time point, and it is unknown how exercise immediately alters these properties or when they return to baseline. Taken together, results suggest that mild, acute decreases in MMP activity and tendon mechanics following a single exercise bout lead to enhanced tendon mechanical adaptations with repeated exercise bouts. This study provides a foundation for future work to distinguish beneficial from detrimental responses to exercise to develop new strategies to prevent and treat overuse injuries.

Significance

This study helps define the acute and chronic temporal response of supraspinatus tendon to load in an *in vivo* model. Results provide a framework for future studies to develop efficient exercise protocols that minimize risk of overuse injury.

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