Exercise Protocol Induces Muscle, Tendon, and Bone Adaptations in the Rat Shoulder

[1]Sarah I. Rooney, MSE
[1]Emanuele Loro, PhD
[1,2]Joseph J. Sarver, PhD
[1,3]Cathryn D. Peltz, PhD
[1]Michael W. Hast, PhD
[1]Wei-Ju Tseng, MSE
[1]X. Sherry Liu, PhD
[1]Tejvir S. Khurana, PhD
[1]Louis J. Soslowsky, PhD

[1]University of Pennsylvania, Philadelphia, PA, USA

[2]Drexel University, Philadelphia, PA, USA

[3]Henry Ford Hospital, Detroit, MI, USA.

Introduction

Rotator cuff tendinopathy, which primarily affects the supraspinatus tendon, is a common clinical condition. While late-stage tendinopathy has been fairly well characterized, earlier stages of tendon degeneration have not, partially due to the lack of an appropriate comparison model system. It is unknown how tendinopathy progresses from an early, treatable stage to a chronic, irreversible stage. A rat model of supraspinatus overuse[1] has suggested mechanisms governing tendon degeneration; however, delineating which changes are pathologic or simply physiologic adaptions to increased loading remains a question. The development of a non-injurious exercise model to which overuse can be compared is critical to the advancement of tendinopathy research; this model has not been created previously. Therefore, the objective of this study was to develop and characterize a rat exercise model that induces systemic and local shoulder adaptations. We hypothesized that a mild treadmill training protocol would produce adaptations consistent with exercise in the supraspinatus tendon and muscle and the humerus.

Methods

Adult, male Sprague-Dawley rats were divided into exercise (EX; n=8) and control cage activity (CA; n=8) groups (IACUC approved). EX rats ran on a flat treadmill at 10 m/min, 1 h/day, 5 days/wk, for 12 weeks[2] while CA rats maintained normal cage activity. Upon completion, rats were sacrificed, weighed, and stored at -20°C.

Tissue Harvest

Rats were thawed, and the right supraspinatus (supra) muscle and tendon were dissected and weighed, and muscle cross-sectional area (CSA) was measured with a custom laser device.[3] Tendon at the insertion site was isolated from the muscle for *o*-Hydroxy-proline (OHP) assay. The superficial and deep regions of the supra muscle[4] were collected separately for protein analysis. The heart and retroperitoneal and epididymal fat pads were dissected and weighed.

Tendon Mechanics

Rats were thawed, and the left supra tendon was dissected and prepared for tensile mechanical testing with preconditioning, stress-relaxation, and ramp to failure.[5]

Tendon Collagen

OHP, a measure of collagen content, was determined and normalized to tendon wet weight.[6,7]

Muscle Protein

A Western blot was performed to quantify mitochondrial proteins. Total protein from the supra muscle superficial region was probed for oxidative phosphorylation complexes I-V using Total OXPHOS antibody cocktail (MitoSciences). Bands were visualized by chemiluminescence, imaged, and analyzed with commercial software. Mitochondrial proteins were normalized to α-tubulin, and the relative quantity of target protein in the EX group compared to CA was calculated.

Bone μCT

μCT (Scanco VivaCT40) was used to determine trabecular and cortical bone structure. For trabecular bone, a 2mm region just distal to the growth plate was scanned (15μm isotropic voxels); for cortical bone, a 1.5 mm region at 60% of the humerus length was scanned (35μm isotropic voxels). Bone was segmented from marrow using a global thresholding technique and then subjected to standard microstructural analysis.

Bone 3-Point Bending

A custom fixture attached to an Instron created a 3-point bend in the humeral shaft until fracture.[8]

Statistics

Comparisons between EX and CA were made with 1-tailed t-tests for significance ($p \leq 0.05$) and trends ($p \leq 0.1$).

Results

EX rats had reduced body (−7%) and fat pad (retroperitoneal: -45%, epididymal: −39%) mass. An 8% increase in supra muscle mass was measured, and no change was detected in heart mass (Figure 1). Supra muscle CSA significantly increased 10% with exercise (Table 1).

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sarahilk@seas.upenn.edu

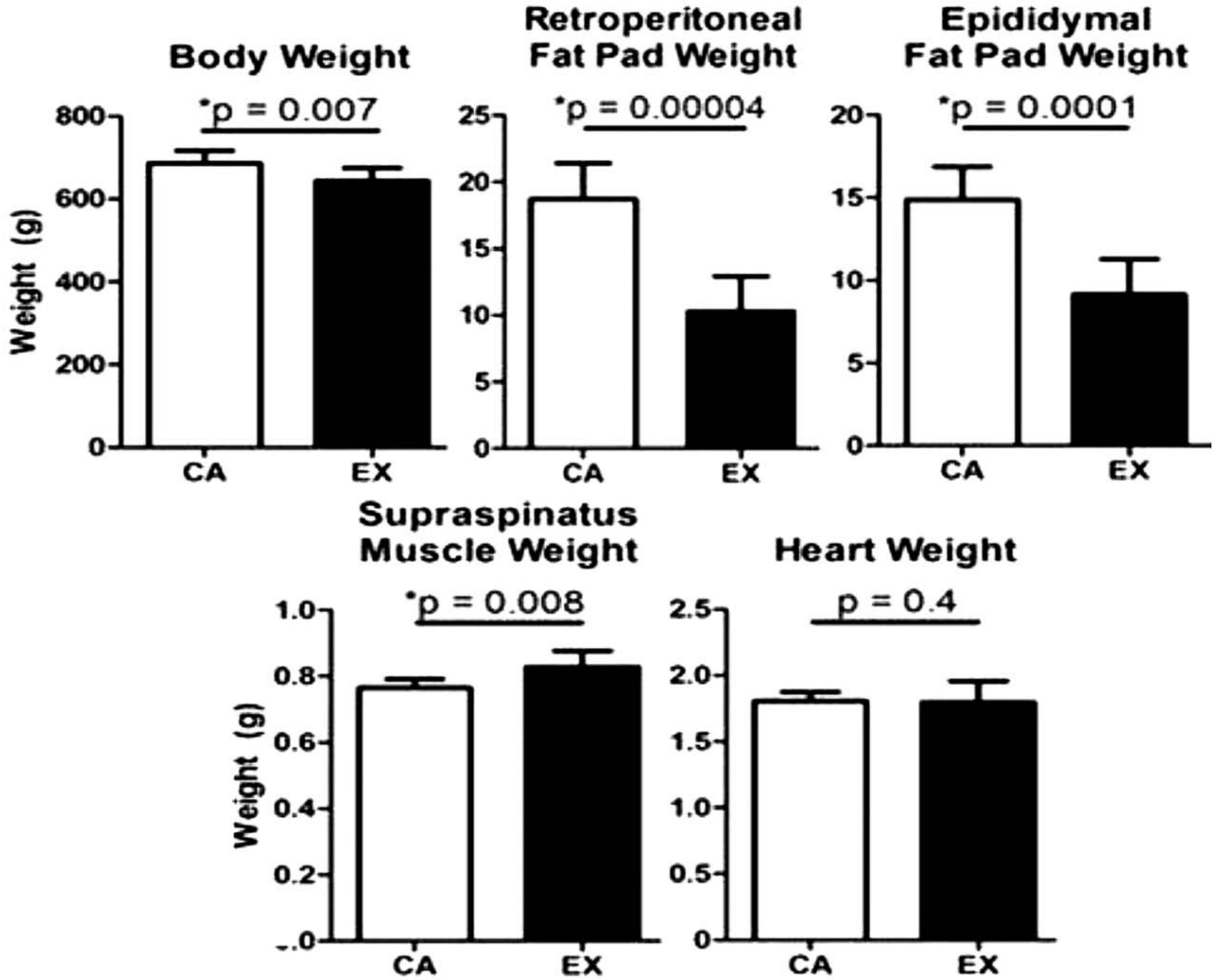

Figure 1. Consistent with adaptations to exercise, EX rats had decreased body and fat pad weight, increased supra muscle weight and no change in heart weight (Mean ± StDev).

Table 1. EX rats showed adaptations consistent with exercise in supraspinatus muscle and tendon and trabecular (Tb) and cortical (Ct) humerus bone. (mean ± StDev, * = significant, + = trend).

Tissue	Measurment	CA	EX	p-value
Muscle	CSA (mm^2)	34.3±3.9	37.7±2.6	*0.04
Tendon	CSA mm^2)	1.97±0.42	1.97±0.28	0.5
	Modulus (MPa)	100±42	112±38	0.3
	% Relaxation	61±9	68±5	*0.04
	Collager/Wet Weight (%)	25±5	28±1	−0.07
Bone	Tb Conn. D ($1/mm^3$)	60±12	70±12	−0.07
	Tb N (1/mm)	3.1±0.7	3.5±0.4	−0.10
	Tb Sp (mm)	0.34±0.09	0.28±0.04	−0.08
	Tb DA	1.79±0.07	1.86±0.07	*0.03
	Ct BMD ($mgHA/cm^3$)	1116±12	1126±10	*0.05
	Ct TMD ($mgHA/cm^3$)	1212±18	1227±7	*0.02

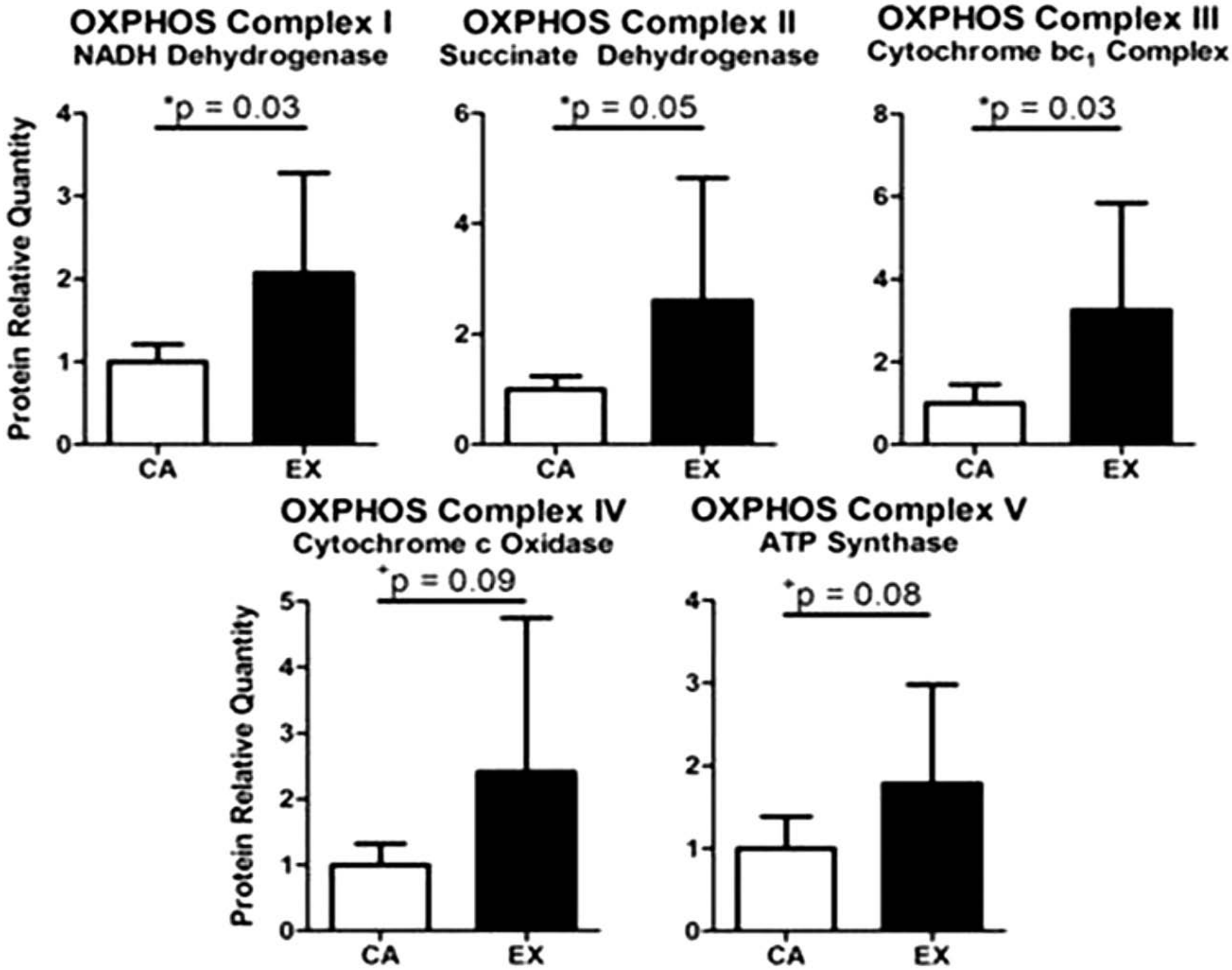

Figure 2. Consistent with adaptations to aerobic exercise, EX rats had increased expression of oxidative phosphorylation proteins. Here reported as relative quantity of expression in EX compared to CA rats after normalization with Tubulin (Mean ± StDev).

Supra tendon CSA and elastic modulus were not different between groups (Table 1). Percent relaxation significantly increased in the EX group (Table 1). Collagen content of the supra tendon normalized to wet weight trended toward an increase observed in the EX group (Table 1). Supra muscle had increased oxidative phosphorylation proteins (Figure 2).

Trabecular bone in the EX group demonstrated trends toward increased trabecular number (Tb.N) and connectivity (Conn.D) and decreased spacing (Tb.Sp) as well as significantly greater degree of anisotropy (DA, Table 1). No differences were found in trabecular bone volume, structure model index, thickness, bone mineral density (BMD), or tissue mineral density (TMD, not shown). Cortical bone in the EX group had significantly increased BMD and TMD (Table 1) with no changes in volume or cortical thickness (data not shown). No differences were found for second moment of area, max load, max displacement, modulus, flexural rigidity, or max stress (data not shown).

Discussion

After 12 weeks of treadmill training, rats showed systemic (decreased body and fat pad mass) and local shoulder (tendon, muscle, bone) changes consistent with exercise. Heart mass did not change, indicating that this protocol does not tax the cardiovascular system. No changes were seen in tendon elastic properties, but stress-relaxation significantly increased. Other studies on tendon mechanics after training show mixed results but often find no differences in modulus or CSA.[9] This study suggests that tendons may have greater viscoelastic than elastic adaptations to training, which could be due to increased fluid retention. EX tendons had decreased dry-to-wet weight ratio (not shown), consistent with increased percent relaxation. Unlike the established supra overuse model, tendons did not show decreased mechanics, indicating that this training is non-injurious to the tendon.[1] Supra muscle showed hypertrophy (increased weight and cross-sectional area), indicative of a response to loading, and increased expression of oxidative phosphorylation proteins, indicative of endurance training. Humerus trabecular bone had increased anisotropic orientation, consistent with load-induced bone remodeling. Cortical bone showed increased bone and tissue mineral density, with no change in volume, suggesting that changes in bone mass are due to increased tissue mineralization. Other studies have also shown no changes in humerus mechanics following moderate treadmill training.[10] Although the adaptations to exercise found in this study are mild, they are consistent and present across multiple tissues using multiple assays. This study is limited by the quality of tissue available, sample size, and single time point investigated. In conclusion, this is the first non-injurious rat shoulder exercise model, which can be compared to the previously established overuse model[1] to differentiate between beneficial adaptation and maladaptation in response to loading.

Significance

This study establishes the first rat exercise protocol that induces adaptations in the shoulder, and future research can use this as a comparison model to study how the supraspinatus tendon adapts to loading and undergoes degeneration with overuse.

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