CHARACTERIZATION OF SUPRASPINATUS MUSCLE AFTER TENDON DETACHMENT: A MORPHOLOGICAL AND HISTOLOGICAL STUDY IN A RAT MODEL

*University of Pennsylvania, Philadelphia, PA

Introduction: Rotator cuff injuries to the shoulder are common and debilitating conditions, often requiring physical therapy to strengthen musculature or surgery to reattach the load bearing musculotendinous unit. While tendon repair can often be performed surgically, information on deleterious changes that may be present in the muscle are not well understood. Several animal models have been developed to alter the muscle loading environment including immobilization, tenotomy, and hindlimb suspension [1]. However, very few studies addressing changes in rotator cuff muscles have been performed [2-4]. Therefore, the objective of this study is to determine the time course of changes to the supraspinatus muscle in an established rat model of rotator cuff tendon detachment injury [5]. We hypothesize that the lack of load on the supraspinatus muscle will lead to a significant decrease in muscle mass and conversions in fiber properties toward those of fast fiber types.

Materials and Methods: Eleven Sprague-Dawley rats were used in this study (IACUC approved). Eight rats were operated upon bilaterally to fully detach the supraspinatus tendon at its bony insertion site. The tendon was allowed to freely retract creating a gap approximately 4mm from its insertion. Rats were sacrificed at one and four weeks post-injury (n=4 each condition). The remaining three uninjured rats served as controls.

At sacrifice, the supraspinatus tendon and muscle were exposed and removed for histological analysis. The wet weight of each muscle was measured, after which the muscles were surrounded in embedding medium (Tissue-Tek) and rapidly frozen in liquid nitrogen cooled isopentane. Cryosections were stained with H&E and trichrome for morphological analysis and to assess collagen infiltration. Immunohistochemistry was used to determine myosin heavy chain composition [6] and fiber size distribution. Microscopy was performed on a Leica DMR microscope (Leica Microsystems). Image acquisition and analysis was carried out using a MicroMax digital camera system (Princeton Instruments, Inc.) and imaging software (OpenLab, Improvision).

Between group differences were compared using an ANOVA followed by Fisher’s post hoc test. Statistical significance was at p<0.05.

Results: Muscles exhibited a statistically significant loss in mass by one week after tendon detachment. There was a 13% decrease in muscle mass at both 1 and 4 weeks after the injury compared to uninjured controls (Table I).

To assess the source of muscle mass loss, muscle cryosections were subjected to immunohistochemistry with anti-laminin to outline the muscle fibers. Representative images are shown in Figure 1. Image analysis revealed a significant decrease in fiber size. To illustrate the change in fiber size, the number of fibers per high-powered field was calculated for each muscle cross section in six different fields. As the size of the fibers decreased, the number of fibers per high-powered field significantly increased (Figure 2).

Muscle fiber type has been shown to shift toward faster fiber types when there is a lack of muscle activity. In order to address whether a fiber type shift occurred in this animal model, muscle cryosections were immunostained with antibodies which recognize the myosin heavy chain isosforms (Table I). There was a statistically significant decrease in MHC I and MHC IIA fiber types following detachment and a slight increase in MHC IIB, although this result was not significant.

With prolonged periods of disuse, muscle can exhibit an increase in fibrotic tissue. Cryosections were stained with trichrome to assess whether this occurred. Collagen content was not markedly changed, even after four weeks post injury (data not shown).

Discussion: This study characterized the modifications to the rat supraspinatus muscle associated with tenotomy. To our knowledge, this is the first examination of the rat supraspinatus muscle in control and unloaded conditions. We hypothesized that the detached tendon would cause a decrease in muscle mass due to the lack of load on the muscle. Our observations support this hypothesis in that the supraspinatus muscle undergoes a rapid and statistically significant decrease in muscle mass and fiber size. We also hypothesized that there would be a shift toward fast fiber types upon unloading. This shift was partially present as a significant decrease was quantitated in both MHC I fibers and in MHC IIA fibers over time. However, no change existed in MHC IIB fibers with time. This finding suggests that nervous activity, which is maintained in this model, may partially modulate changes in fiber properties in the absence of muscle load, but that nervous activity is insufficient to maintain muscle mass. Further fiber type shifts might occur later after decreases in muscle mass. Increased durations of tendon detachment might reveal these fiber type conversions, in addition to other modifications, such as fibrotic infiltration, which was not observed by 4 weeks. The rapid decline in muscle mass after tenotomy shows that the muscle is a very sensitive indicator of the state of tendon attachment. This finding suggests that noninvasive methods which can monitor muscle size could serve as an important method of analysis for tendon detachment and repair. Future studies will investigate the effects of longer periods of tendon detachment on muscle properties, as well as the mechanisms through which these changes occur.


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Figure 1: Muscle fiber size decreases by one week after tenotomy. Immunohistochemistry of muscle cross-sections with anti-laminin revealed that the distribution of fiber size diminished with unloading. Scale bar, 100 µm.

Figure 2: Density of fibers increases after tenotomy. The number of fibers per high-powered field (HPF) indicates that the size muscle fibers decreases after one week of tendon detachment. p<0.05 compared to control.