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Rat supraspinatus muscle atrophy after tendon detachment

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Abstract

Rotator cuff tears are one of the most common tendon disorders found in the healthy population. Tendon tears not only affect the biomechanical properties of the tendon, but can also lead to debilitation of the muscles attached to the damaged tendons. The changes that occur in the muscle after tendon detachment are not well understood. A rat rotator cuff model was utilized to determine the time course of changes that occur in the supraspinatus muscle after tendon detachment. It was hypothesized that the lack of load on the supraspinatus muscle would cause a significant decrease in muscle mass and a conversion of muscle fiber properties toward those of fast fiber types. Tendons were detached at the insertion on the humerus without repair. Muscle mass, morphology and fiber properties were measured at one, two, four, eight, and 16 weeks after detachment. Tendon detachment resulted in a rapid loss of muscle mass, an increase in the proportion of fast muscle fibers, and an increase in the fibrotic content of the muscle bed, concomitant with the appearance of adhesions of the tendon to surrounding surfaces. At 16 weeks post-detachment, muscle mass and the fiber properties in the deep muscle layers returned to normal levels. However, the fiber shifts observed in the superficial layers persisted throughout the experiment. These results suggest that load returned to the muscle via adhesions to surrounding surfaces, which may be sufficient to reverse changes in muscle mass.

Keywords: Supraspinatus muscle; Chronic; Atrophy; Tendon; Shoulder

Introduction

Rotator cuff tears of the shoulder represent a common and challenging orthopaedic problem [23]. While many of these tears are asymptomatic, detachment of the tendon can not only lead to pain, but can also lead to atrophy of the associated muscle, accumulation of fat in the areas normally occupied by muscle, and a subsequent loss in shoulder stability and function [13,15,16]. The reason for these changes may be a combination of lack of muscle activity due to the pain of movement, and a lack of force transmission from the muscle to the bone and the potential shortened state of the muscle fibers due to tendon detachment. Subacromial decompression can be performed to remove pain and tendon repair can be performed to restore a strong connection between the bone, tendon, and muscle. However, the delay between the time of injury and surgical repair is thought to inhibit the healing process, resulting in weak, fatty muscles even after repair. Therefore, a more complete understanding of the muscle changes that occur with time from injury to repair is needed to improve the surgical outcome of chronic rotator cuff tears [3,13].

Significant progress toward understanding the changes in the rotator cuff muscle following tendon

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injury has been made utilizing a variety of approaches such as non-invasive CT and MRI measurements in patients [11,30]. In addition to these approaches, several animal models (e.g., rabbit and sheep) have been developed to study the changes in the muscle following supraspinatus tendon detachment [1,4,10,19]. In the rabbit, muscle atrophy increased and muscle function decreased with time after tendon detachment and fatty infiltration of the muscle occurred as early as 4 weeks after tendon detachment [1]. Similar results were found in more comprehensive studies using the same animal model [8–10]. More recently, the effect of a delayed repair on the fatty infiltration of the muscle after tendon repair has been studied. It was found that the supraspinatus muscle did not recover after tendon repair [19] and interestingly, fatty infiltration increased following repair. This suggests that although tendons are intact and load is restored, complete muscle repair is not established.

While all models have specific advantages and disadvantages, the anatomy and function of the rat rotator cuff most closely resembles that of the human cuff (other than certain non-human primates). Specifically, the rat has an enclosed arch created by the acromion, coracoid, and clavicle, through which the supraspinatus tendon passes repetitively during shoulder motion [25]. Since the role of the acromion and the enclosed arch is believed to be critical and is at a minimum controversial in the pathogenesis of rotator cuff injuries, an animal model that includes this feature has certain advantages for rotator cuff injury and repair studies. This model has been used previously to address a variety of rotator cuff tendon injury and repair problems [26,28]. The objective of this study was to determine the time course of changes to the supraspinatus muscle in an established rat model of rotator cuff tendon detachment injury [25] to understand the nature of muscle atrophy following tendon detachment and to determine if the potential changes in rat muscle recapitulate those found in muscles from human patients. We hypothesize that tendon detachment would lead to a significant decrease in supraspinatus muscle mass, conversions in fiber properties toward those of fast fiber types, and an increase in the proportion of connective tissue within the muscle bed.

Materials and methods

Surgical injury model

A total of 24 Sprague–Dawley rats were used in this study. Twenty rats were operated upon bilaterally under general anesthesia to fully detach the supraspinatus tendon at its bony insertion site utilizing a previously described procedure [2,12,27]. Briefly, the supraspinatus was exposed and visualized as it passed under the bony arch created by the acromion, coracoid, and clavicle to its insertion on the greater tuberosity of the proximal humerus. The supraspinatus tendon was separated from adjacent tissues and detached sharply at its insertion on the greater tuberosity using a scalpel blade. The attachments of synergist muscles remained intact during the procedure. A 5-0 proline suture with long tails was attached to the tendon stump to enable identification of the tendon end at sacrifice. The musculotendinous unit was allowed to freely retract without attempt at repair creating a gap approximately 4 mm from its insertion. The overlying deltoid muscle and skin was then closed and the rats were allowed unrestricted cage activity. Rats were sacrificed at one, two, four, eight, and 16 weeks post-detachment ($n = 4$ each timepoint). The remaining four rats did not undergo tendon detachment, and served as uninjured controls for this study. All animal experiments were approved by the University of Pennsylvania's Institutional Animal Care and Use Committee.

Histological assessment of collagen and fat infiltration

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, Torrance, CA) and rapidly frozen in liquid nitrogen cooled isopentane. Muscles were stored at −80 °C for subsequent analysis. Frozen cross-sections (10 μm thick) were taken from the proximal, mid-belly and distal portions of the supraspinatus muscle. Masson's trichrome was utilized to assess the presence of collagen infiltration and fibrosis. Oil red O staining was utilized to assess the presence of fat infiltration. Microscopy was performed on a Leitz DMR microscope (Leica Microsystems, Bannockburn, IL). Image acquisition and analysis was carried out using a MicroMAX digital camera system (Princeton Instruments, Inc., Trenton, NJ) and imaging software (OpenLab, Improvision, UK). Six high powered fields were analyzed per muscle cross-section to determine the proportion of collagen or fat infiltration in the muscles. For collagen content, high powered fields were acquired away from the central tendon, which could potentially obscure differences. These images were then quantitatively analyzed to determine the area of fibrotic tissue per high powered field. The analysis was performed by applying a consistent threshold to each image and calculating the area of fibrotic tissue using the above imaging software (OpenLab, Improvision, UK).

Immunohistochemistry

Fiber size analysis. Immunostaining with polyclonal Laminin antibody (Neomarkers, Fremont, CA) was utilized to measure muscle fiber size distribution. Muscle cryosections (10 μm thick) were incubated in 5% bovine serum albumin (BSA) in PBS for 20 min, and then incubated in the same solution plus anti-laminin (1:200) overnight at 4 °C. After several washes in PBS, the sections were incubated in Rhodamine-conjugated anti-rabbit (1:200) (Jackson Immunoresearch Laboratories, Inc., West Grove, PA) in 5%BSA for 1 h at room temperature. After several more washes in PBS, sections were mounted in aqueous mounting medium containing DAPI (Vector Laboratories, Burlingame, CA) to counterstain the nuclei. Stained sections were visualized on an epifluorescent microscope, as described above. For each muscle cross-section, six high power fields (HPF) were acquired for analysis. The number of fibers per HPF was counted to determine if there is a significant change in fiber size. For example, if the fibers decrease in size, then the number per HPF will increase.

Fiber type composition. Immunohistochemistry was also used to determine myosin heavy chain composition [24]. Primary antibody dilutions were as follows: Type I myosin (BA-F8), 1:50; Type IIa myosin (SC-71), 1:10; Type IIb myosin (BF-F3), 1:3; Embryonic myosin (BF-45), 1:50. FITC-conjugated, goat anti-mouse IgM antibodies and donkey anti-mouse IgG (H + L) (Jackson Immunoresearch Laboratories, Inc., West Grove, PA) were used as secondary antibodies. Microscopy was performed on an epifluorescent microscope, as described above. Preliminary experiments revealed an asymmetric fiber type distribution across the supraspinatus muscle. Therefore, the fiber type compositions in the superficial and deep layers were analyzed independently, with images of five high powered fields acquired for each region.

Statistical analysis

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