Muscle Fibers are Injured at the Time of Acute and Chronic Rotator Cuff Repair

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Abstract

Background Rotator cuff tears are a common source of shoulder pain and disability. Even after surgical repair, some patients continue to have reduced function and progression of fatty degeneration. Because patients with chronic cuff tears often experience muscle shortening, it is possible that repairing the tendon to its anatomic footprint induces a stretch-induced muscle injury that could contribute to failures of the repair and perhaps ongoing pain. **Questions/purposes** We hypothesized that, compared with acutely torn and repaired muscles, the stretch that is required to repair a chronically torn cuff would result in

more muscle fiber damage. Specifically, we asked: (1) Is there muscle fiber damage that occurs from repair of an acutely torn rotator cuff and does it vary by location in the muscle; and (2) is the damage greater in the case of repair of a chronic injury?

Methods We used an open surgical approach to create a full-thickness rotator cuff tear in rats, and measured changes in muscle mass, length, and the number of fibers containing the membrane impermeable Evans Blue Dye after acute (1 day) or chronic (28 days) cuff tear or repair in rats. Differences between groups were tested using a one-way ANOVA followed by Tukey's post hoc sorting.

Results Chronic tears resulted in 24% to 35% decreases in mass and a 20% decrease in length. The repair of acutely and chronically torn muscles resulted in damage to 90% of fibers in the distal portion of the muscle. In the proximal portion, no differences between the acutely torn and repaired groups and controls were observed, whereas repairing the chronically torn group resulted in injury to almost 70% of fibers.

Conclusions In a rat model, marked injury to muscle fibers is induced when the tendons of torn rotator cuffs are repaired to their anatomic footprint.

Clinical Relevance In this animal model, we found that repair of chronically torn cuff muscles results in extensive injury throughout the muscle. Based on these findings, we posit that inducing a widespread injury at the time of surgical repair of chronically torn rotator cuff muscles may contribute to the problems of failed repairs or continued progression of fatty degeneration that is observed in some patients that undergo rotator cuff repair. Therapeutic interventions to protect muscle fiber membranes potentially could enhance outcomes for patients undergoing rotator cuff repair. To evaluate this, future studies that evaluate the use of membrane sealing compounds or drugs that

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upregulate endogenous membrane-sealing proteins are warranted.

Introduction

Rotator cuff tears are among the most common and incapacitating upper extremity injuries with more than 250,000 surgical repairs performed each year in the United States [4]. Although notable improvements have been made in surgical repair and rehabilitation techniques, many patients continue to have symptoms after repair and the frequency of repeat tears after surgical repair of full-thickness tears remains unacceptably high [3, 7]. A set of pathologic changes often occurs in torn rotator cuff muscles, including atrophy of muscle fibers, an accumulation of lipid in and around muscle fibers, and fibrosis [11]. These changes are commonly referred to as “fatty degeneration,” and despite successful surgical repair of the tear, fatty degeneration frequently does not improve after repair and correlates with poor functional outcomes [9]. The cellular and molecular etiology of fatty degeneration is not fully understood; gaining greater insight into the physiologic processes that regulate muscle fiber regeneration may improve the treatment of patients with chronic rotator cuff tears.

The rotator cuff muscles of patients with chronic tears are markedly shorter than those of patients with intact cuff muscles [26]. Sudden and severe lengthening of the muscle fiber can damage the plasma membrane (sarcolemma), leading to a sustained influx of calcium ions in the fiber [5, 25]. A persistent elevation in calcium can lead to muscle spasm and activation of calcium-dependent proteases known as calpains, which degrade contractile proteins and reduce muscle force production [10, 23]. Calpain activation is also important for induction of adipogenesis [22]. Because the surgical repair of chronically torn rotator cuff muscles involves a rapidly induced and persistent lengthening of the muscle, the plasma membranes of rotator cuff muscles may be damaged during repair, and this injury may contribute to the persistent pain and progression of fatty degeneration that sometimes occur in patients after surgical repair of large or massive tears.

To determine whether the plasma membrane of muscle fibers from chronically torn rotator cuff muscles is damaged during repair, we used a well-established rat model of full-thickness rotator cuff tears [12, 18, 24]. Evans Blue Dye (EBD) is a water-soluble, membrane-impermeable dye that accumulates in the cytosol of muscle fibers that have sustained an injury to their plasma membrane [14]. We hypothesized that, compared with acutely torn and repaired rotator cuff muscles, the substantial acute stretch that is required to repair a chronically torn and shortened rotator

cuff would result in more muscle fiber damage as measured by an increase in EBD positive (EBD+) muscle fibers. We asked: (1) Is there muscle fiber damage that occurs from repair of an acutely torn rotator cuff and does it vary by location in the muscle; and (2) is the damage greater in the case of repair of a chronic injury?

Materials and Methods

This study was approved by the University of Michigan Institutional Animal Care and Use Committee. Thirty male Sprague-Dawley retired breeder rats were placed in six groups: (1) nonoperated controls; (2) sham surgery; (3) acute tear no repair; (4) acute tear and repair; (5) chronic tear no repair; and (6) chronic tear and repair (Fig. 1). The surgical procedure was described in previous studies [12, 13, 17]. In brief, rats were anesthetized with 2% isoflurane and the skin above the shoulder was shaved and scrubbed with chlorhexidine/isopropanol. A deltoid-splitting transacromial approach was used to expose the supraspinatus tendon, but the muscle belly was not observed or manipulated. For the acute groups, the supraspinatus tendon was sharply detached from its footprint on the humerus and immediately repaired to the same footprint, and the surgical site was closed as described subsequently. For the chronic groups, the tendon was detached in a similar fashion and completely encased in sterile nonpyrogenic surgical tubing (Pharmed[®] BPT; Saint-Gobain, Akron, OH, USA) that was secured to the tendon using a modified Mason-Allen stitch. This approach prevented the tendon from forming adhesions to the surrounding connective tissue and allowed the muscle to freely retract. The deltoid muscle and skin were closed and the animals were allowed to recover for 28 days. To repair the torn tendon, a modified Mason-Allen stitch using 5-0 two-arm Ethibond suture (Ethicon, Somerville, NJ, USA) was placed in the tendon stump. The tuberosity was lightly decorticated until bleeding was noted, and soft tissue and fibrocartilage was débrided from the insertion site. Crossed bone tunnels were created at the anterior and posterior portions of the insertion site using 0.7-mm K-wire and the tendon was affixed to its original anatomic footprint. The sham group received a skin and deltoid-splitting incision; care was taken not to handle or traumatize the supraspinatus muscle or tendon. In all surgical procedures, the deltoid was closed using 4-0 chromic gut and the skin was closed with a subcutaneous running suture of 4-0 Vicryl[®] (Ethicon) and GLUture (Abbott Laboratories, Abbott Park, IL, USA). Subcutaneous buprenorphine (0.05 mg/kg) was administered for analgesia during postoperative recovery. Ad libitum weightbearing and cage activity were allowed, and rats were monitored for signs of distress or infection.

Fig. 1A–C (A) The timeline of events (in days) for the six experimental groups in the study is shown. (B) The location of sections of the muscle used for histologic analysis are shown. (C) A representative histologic image shows fibers containing EBD. Blue = EBD; red = WGA lectin, which is used to label the extracellular matrix (ECM); green = DAPI, which is used to label nuclei.

Twenty-four hours before tissue harvest, each rat received an intraperitoneal injection of 100 mg of EBD dissolved in 10 mL of phosphate-buffered saline (Sigma Aldrich, St Louis, MO, USA) per 1 kg rat mass [14]. At harvest, rats were anesthetized with sodium pentobarbital (50 mg/kg), the supraspinatus muscles were removed, the length was determined using digital calipers, and the mass was recorded. Animals were humanely euthanized by an overdose of sodium pentobarbital followed by induction of bilateral pneumothorax. Owing to technical problems, three muscles in the chronic tear and repair group, and one

muscle from each other group, were lost at the time of harvest.

The muscle mass of acutely injured rats was different from that of the chronic groups, but no differences were observed between acute groups (Fig. 2A). Compared with controls, rats that had a chronic supraspinatus tear but did not undergo repair had a 35% (364.2 mg/556.4 mg) decrease in muscle mass compared with controls (Fig. 2A), whereas rats that had a chronic tear and also underwent repair had a 24% (425.4 mg/556.4 mg) decrease in wet mass compared with controls (Fig. 2A). Both groups of rats that underwent a chronic tear experienced an approximate 20% (18.6 mm/25.8 mm for chronic tear and no repair; 18.9 mm/25.8 mm for chronic tear and repair) decrease in supraspinatus length at the time of harvest when compared with the muscle length of the control, sham, and acute groups (Fig. 2B).

After measuring mass and length, the muscles were divided into distal and proximal segments, placed in Tissue-Tek® (Sakura, Torrance, CA, USA), frozen in isopentane cooled with liquid nitrogen, and stored at -80° C. Ten-micron sections of tissue from the midproximal and middistal regions were used for analysis (Fig. 1B). Sections were fixed with 4% paraformaldehyde and incubated with wheat germ agglutinin (WGA) lectin AF488 (Invitrogen, Carlsbad, CA, USA) to mark the extracellular matrix and 4',6-diamidino-2-phenylindole, dihydrochloride (DAPI) to identify nuclei. EBD is a fluorescent molecule and was identified using a far red fluorescent filter set. Sections were observed using a Zeiss Axioplan 2 microscope equipped with an AxioCam camera (Carl Zeiss Microscopy, Jena, Germany). Three random fields per section were taken using the $\times 10$ objective, and the number of total and EBD+ fibers was quantified using ImageJ (National Institutes of Health, Bethesda, MD, USA) (Fig. 1C).

Based on the work of Kostrominova et al. [16] and preliminary studies in our laboratory, to detect a 30% difference between groups with a power of 0.80 required seven muscles per group, and we added an additional three to account for unexpected losses or technical problems (Table 1). A one-way ANOVA ($\alpha = 0.05$) and Tukey's post hoc sorting were used to evaluate the differences between groups. Prism 6.0 software (GraphPad, La Jolla, CA, USA) was used for statistical analyses.

Results

In the sham and acute tear and repair groups, there were no significant differences observed in the number of EBD+ fibers in the midproximal region, but a significant difference was observed in the middistal region (Fig. 3A).

Fig. 2A–B Supraspinatus (A) muscle mass and (B) muscle length of the six experimental groups are shown. Values are mean $\pm$ SD. There were nine muscles from each group except the chronic tear and repair group, in which there were seven. The differences between groups were tested using a one-way ANOVA ($\alpha = 0.05$) followed by Tukey's post hoc sorting. The ANOVA values for muscle mass are

$F = 20.20$, $p < 0.0001$, and $R^2 = 0.70$; and for muscle length, $F = 61.48$, $p < 0.0001$, and $R^2 = 0.87$. The post hoc sorting labels are: a = different ($p < 0.05$) from control; b = different ($p < 0.05$) from sham; c = different ($p < 0.05$) from acute tear no repair; d = different ($p < 0.05$) from acute tear and repair; and e = different ($p < 0.05$) from chronic tear no repair.

Table 1. Data for muscle mass, muscle length, and percentage of Evans Blue Dye-positive (EBD+) muscle fibers

Variable	Control	Sham	Acute tear no repair	Acute tear and repair	Chronic tear no repair	Chronic tear and repair
Number	9	9	9	9	9	7
Mass (mg)						
Mean	556.4	560.2	593.1	649.8	364.2 ^{a,b,c,d}	425.4 ^{a,b,c,d}
Lower 95% CI	493.1	530.4	526.8	582.5	309.5	376.2
Upper 95% CI	619.6	590	659.3	717.1	418.9	474.7
Length (mm)						
Mean	25.8	26.2	25.1	25.9	18.6 ^{a,b,c,d}	18.9 ^{a,b,c,d}
Lower 95% CI	25.3	25.8	23.9	24.1	18.0	17.8
Upper 95% CI	26.3	26.6	26.4	27.6	19.3	20.1
Middistal segments						
Mean	2.6	26.5 ^a	88.2 ^{a,b}	87.8 ^{a,b}	2.7 ^{b,c,d}	88.3 ^{a,b,e}
Lower 95% CI	0.1	5.4	73.6	77.3	0.8	79.4
Upper 95% CI	5.2	47.6	102.9	98.4	4.6	97.3
Midproximal segments						
Mean	3.1	10.4	7.4	25.1	2.7	68.8 ^{a,b,c,d,e}
Lower 95% CI	0.6	-1.9	-0.9	-4.8	1.4	45.0
Upper 95% CI	5.6	22.7	15.6	54.9	4.0	92.6

Differences between groups were tested using a one-way ANOVA ($\alpha = 0.05$) followed by Tukey's post hoc sorting; ^a different ($p < 0.05$) from control; ^b different ($p < 0.05$) from sham; ^c different ($p < 0.05$) from acute tear no repair; ^d different ($p < 0.05$) from acute tear and repair; ^e different ($p < 0.05$) from chronic tear no repair.

For the sham group, 27% of the fibers were EBD+, and the remaining groups had almost 90% EBD+ fibers (Fig. 3B).

For chronic tears that were not repaired, no differences in EBD+ fibers in the midproximal or middistal regions

were observed. However, in the chronic tear and repair group, the midproximal region had nearly 70% EBD+ fibers, and this value was significantly different from all other groups (Fig. 3A). At the middistal region, 88% of the

Fig. 3A–B The percentages of EBD-positive (EBD+) fibers in the (A) midproximal and (B) middistal regions of the supraspinatus muscles are shown. Values are mean $\pm$ SD. There were nine muscles from each group except the chronic tear and repair group in which there were seven. The differences between groups were tested using a one-way ANOVA ($\alpha = 0.05$) followed by Tukey's post hoc sorting. The ANOVA values for EBD+ fibers in the middistal region are:

$F = 70.03$, $p < 0.0001$, and $R^2 = 0.88$. The ANOVA values for EBD+ fibers in the midproximal region are: $F = 11.98$, $p < 0.0001$, and $R^2 = 0.56$. The post hoc sorting labels are: a = different ($p < 0.05$) from control; b = different ($p < 0.05$) from sham; c = different ($p < 0.05$) from acute tear no repair; d = different ($p < 0.05$) from acute tear and repair; and e = different ($p < 0.05$) from chronic tear no repair.

fibers were EBD+, similar to the values from the acute tear groups (Fig. 3B).

Discussion

Chronic rotator cuff tears are a frequent and debilitating injury, and for many patients, symptoms will progress with time despite undergoing surgical repair. Because the cuff muscles of patients with chronic tears are substantially shorter than those of patients with intact cuff muscles [26], sudden lengthening can damage the plasma membrane of the fiber [5], and slow and progressive lengthening of chronically torn rotator cuff muscles can reverse fatty degeneration [8], the purpose of this study was to determine whether repairing chronically torn cuff tendons would induce an iatrogenic injury to the muscle. The results from the study indicate that acutely and chronically torn rotator cuff muscle fibers are damaged owing to surgical repair, although the chronically torn group shows much greater damage throughout the muscle.

There are several limitations to this study. Although the rat is a widely accepted animal model for the study of cuff tears, rats do not have the severity of fatty degeneration develop seen in humans [11, 12]. We did not directly measure muscle contractility, but EBD is widely used to detect muscle plasma membrane damage and the amount of EBD+ fibers correlates well with declines in whole muscle

force production [14, 16]. EBD was analyzed 24 hours past injection; accumulation was not evaluated at other times. Owing to the presence of EBD in muscles, we did not directly measure gene expression or biochemical markers of atrophy or inflammation, because the presence of this dye makes many biochemical measures difficult. However, there already is a large body of work that has evaluated these markers at similar times [12, 13, 18, 24]. We also did not directly measure tendon retraction, although we did take care to ensure the tendon was secured in silicone and at the time of harvest verified the tendon encased in silicone was free of lateral adhesions to surrounding connective tissue. We used an open surgical model while most rotator cuff repairs in patients are performed with a minimally invasive or arthroscopic approach, and the indirect inflammation from an arthroscopic approach may be less than what would occur in open surgical repair.

Previous studies have evaluated gross changes in morphologic features of muscle fibers in other models of chronic tendon tears [1, 2]. As soon as 1 day after a full-thickness tendon tear, the plasma membrane of muscles has a crinkled appearance with the presence of focal lesions. Additionally, rapid degradation of sarcomeres, which are the active force-generating structures in muscles, often is observed [1, 2]. Both of these processes continue to worsen during the first few weeks after a chronic tear, at which point the membranes are finally reorganized and repaired [2]. In the current study, a chronic tear resulted in a major

decrease in the mass and length of supraspinatus muscles. Nearly all of the fibers in the middistal region of the two acute tear groups and the chronic tear and repair group contained EBD 1 day after surgery. However, the middistal region of the chronic tear no repair group showed only a few EBD+ fibers and was not different from the controls. Although we anticipated that the chronic tear and repair would lead to a lot of damage, the amount of injury in the acute tear groups was surprising. These results suggest that acute changes to the length of a muscle can result in a lot of damage to muscle fiber plasma membranes and are consistent with findings from previous studies that evaluated muscle damage and acute changes in muscle length [1, 2].

Although care was taken not to damage the sham-operated muscles during surgery, more than 1/4 of the muscle fibers in this group were EBD+, suggesting that changes in muscle length alone are not entirely responsible for the membrane damage observed in this study. Although the length of the sham muscles did not change, there was injury to the deltoid muscle and overlying skin and connective tissue because of the surgical incision. The inflammation that occurs after muscle and connective injury can induce the expression of proinflammatory cytokines and activate proteolytic enzymes that can disrupt stable muscle fiber membranes [6, 27]. The observed damage in the acute tear groups and chronic tear and repair groups therefore likely comes about because of mechanical damage to the fibers and indirect activation of proinflammatory signaling molecules and proteolytic enzymes.

A gradient of damage was seen moving from distal to proximal. In rats, the length of individual fibers is approximately 40% of the whole muscle length [19], so changes in fibers located in the distal portion of the muscle may not reflect changes in fibers located in the proximal portion. Although the middistal portion showed widespread damage in the acute tear and chronic tear and repair groups, the midproximal portion was protected from injury for most experimental conditions. However, nearly 70% of fibers in the chronic tear and repair group were EBD+. Because no differences in EBD+ fibers were observed in the acute tear groups or the chronic tear no repair group, and the proximal portion of the muscle is not exposed during surgeries, the widespread injury seen in the midproximal portion of the chronic tear and repair group likely directly occurs because of the sudden 20% lengthening of the chronically shortened muscles. The degree of shortening and lengthening we observed is similar to what is observed clinically. In humans, the length of an intact supraspinatus muscle is approximately 11 cm [19], and because many patients have chronic tears that are 2 to 3 cm in length [20], surgical repair of a torn rotator cuff may result in sudden 20% or greater changes in length that may induce extensive injury throughout the muscle.

Although improvements have been made in rotator cuff repair and rehabilitation techniques, for many patients with chronic cuff tears, fatty degeneration frequently does not improve after repair and can continue to worsen with time [9]. The results from our study suggest that repairing either acutely or chronically torn rotator cuff tears causes damage to muscle fibers, but the degree of the injury in chronically torn rotator cuff muscles is much more extensive. For patients with chronic tears, this injury likely further deteriorates a muscle that already is weakened from a period of fatty degeneration and may explain why some patients have worsening of fatty degeneration after surgical repair. Developing a way to protect muscle fiber membranes from stretch-induced injury potentially could prevent some of the difficulties of failed repairs or continued progression of fatty degeneration that is observed at times in patients who undergo rotator cuff repair. Membrane sealing molecules like poloxamer 188 [21] or pharmaceutical compounds that upregulate endogenous membrane sealing proteins like dysferlin [15] could help to protect muscles from lengthening injury after rotator cuff repair, and warrant further study in preclinical models of rotator cuff tear.

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