Background: A persistent atrophy of muscle fibers and an accumulation of fat, collectively referred to as fatty degeneration, commonly occur in patients with chronic rotator cuff tears. The etiology of fatty degeneration and function of the residual rotator cuff musculature have not been well characterized in humans. We hypothesized that muscles from patients with chronic rotator cuff tears have reduced muscle fiber force production, disordered myofibrils, and an accumulation of fat vacuoles.

Methods: The contractility of muscle fibers from biopsy specimens of supraspinatus muscles of 13 patients with chronic full-thickness posterosuperior rotator cuff tears was measured and compared with data from healthy vastus lateralis muscle fibers. Correlations between muscle fiber contractility, American Shoulder and Elbow Surgeons (ASES) scores, and tear size were analyzed. Histology and electron microscopy were also performed.

Results: Torn supraspinatus muscles had a 30% reduction in maximum isometric force production and a 29% reduction in normalized force compared with controls. Normalized supraspinatus fiber force positively correlated with ASES score and negatively correlated with tear size. Disordered sarcomeres were noted, along with an accumulation of lipid-laden macrophages in the extracellular matrix surrounding supraspinatus muscle fibers.

Conclusions: Patients with chronic supraspinatus tears have significant reductions in muscle fiber force production. Force production also correlates with ASES scores and tear size. The structural and functional muscle dysfunction of the residual muscle fibers is independent of the additional area taken up by fibrotic...
Rotator cuff tears are among the most debilitating and frequent upper extremity injuries, with more than 250,000 surgical repairs performed annually in the United States. Although there have been important improvements in surgical repair and rehabilitation techniques, many patients continue to have symptoms after repair, and re-tear rates for surgical repair of full-thickness tears remain high. A set of common pathologic changes often occurs in patients with chronically torn rotator cuff muscles, including muscle fiber atrophy, fibrosis, and accumulation of fat within and around muscle fibers. These changes are commonly referred to as fatty degeneration. The severity of fatty degeneration is positively correlated with poor functional outcomes, and despite successful surgical repair of the tear of the torn rotator cuff, fatty degeneration often does not improve after repair and for some patients continues to worsen over time.

Given the anatomic and biomechanical complexity of the shoulder girdle, it can be challenging to specifically isolate the rotator cuff during strength testing in the clinical setting, making it difficult to measure rotator cuff function by conventional biomechanical testing methods. Whole muscle force measurements have been performed in rotator cuff muscles from sheep and rats, but these techniques are invasive and can be difficult to perform in a fashion that allows the animal to recover. Whereas whole muscle force measurements can be informative, force measurements from single muscle fibers obtained from small tissue biopsy specimens provide a wealth of information about the function of muscle as a whole and are predictive of strength measurements performed at the whole muscle level. Using a rat model of full-thickness rotator cuff tears, we measured force production of individual muscle fibers and demonstrated a 40% reduction in maximum isometric force (F₀) and an 18% reduction in specific force (sF₀, defined as F₀ normalized to the muscle fiber cross-sectional area [CSA]) in torn muscles compared with controls. This reduction in F₀ and sF₀ at the level of individual muscle cells indicates that chronic rotator cuff tears disrupted the abundance or function of myofibrils, which are the fundamental contractile structures of muscle cells. Further, whereas fat accumulation was previously noted to occur in animal models of chronic rotator cuff tears, we identified a pool of macrophages that accumulate around fatty plaques present in injured rotator cuff muscles. Although these studies provided insight into potential mechanisms that result in muscle weakness after rotator cuff tears, the pathophysiologic mechanism of fatty infiltration and the impact of chronic tears on the ability of the rotator cuff muscle fibers to generate force in humans have not been defined.

The primary objective of this study was to measure the contractile and morphologic properties of muscle fibers from patients with chronic rotator cuff tears. A secondary objective was to determine if there were correlations between muscle fiber contractility and American Shoulder and Elbow Surgeons (ASES) survey instrument scores or the size of the tear. We hypothesized that patients with rotator cuff tears would have reduced muscle fiber force production compared with healthy muscle fibers and that force production would be positively correlated with ASES scores and negatively correlated with tear size.

Materials and methods

Subjects

All subjects provided informed, written consent before participation in this study. Subjects who were 18 years of age or older, who had a full-thickness supraspinatus tear as diagnosed by ultrasound or magnetic resonance imaging, had a history of shoulder pain for at least 5 years, and consented to undergo arthroscopic rotator cuff examination were eligible for participation in the study. Patients who were undergoing revision rotator cuff tear, had previous shoulder or upper extremity surgery, or had a history of a myopathy or metabolic or rheumatologic disorder were excluded from participation in the study. Patients also completed the ASES survey instrument to measure shoulder-specific function 1 week before surgery. The ASES activities of daily living (ADL) subscale for the involved shoulder as well as the composite score that includes the ADL of the uninvolved shoulder and a visual analog pain scale were used for analysis.

Diagnostic imaging

The diagnosis of a full-thickness supraspinatus tear was assessed from ultrasound or magnetic resonance imaging studies before surgery. The gap distance between the free tendon end and the anatomic footprint on the humeral head was measured from scans in coronal and sagittal planes. A single board-certified and fellowship-trained musculoskeletal radiologist read the imaging studies and calculated the gap distances with Imagecast PACS 3.6 software (IDX Systems, Burlington, VT, USA).
**Surgical repair and muscle biopsy**

A single board-certified and fellowship-trained orthopedic surgeon performed surgical repairs and muscle biopsies. All patients had a posterosuperior rotator cuff tear, and the torn supraspinatus tendon was fully mobilized and repaired to the anatomic footprint by a transosseous-equivalent, double-row technique. After repair, biopsy specimens were taken from the distal quarter of the supraspinatus with an arthroscopic duckbill basket punch instrument (Arthrex, Naples, FL, USA). The biopsied tissue was immediately trimmed, prepared, and processed for muscle fiber contractility measurements or imaging studies.

**Muscle fiber contractility**

The contractility of muscle fibers was measured by previously described techniques and solutions. Briefly, bundles of muscle fibers approximately 5 mm in length and 0.5 mm in diameter were dissected from supraspinatus muscle biopsy specimens and exposed to skinnning solution for 30 minutes. Bundles were then transferred to storage solution for 16 hours at 4°C and then stored at −80°C until use. On the day of an experiment, bundles of muscle fibers were thawed on ice, and single fibers were pulled from bundles with fine mirror-finished forceps. Fibers were then placed in a chamber containing relaxing solution and secured at one end to a force transducer (Model 403A; Aurora Scientific, Aurora, ON, USA) and at the other end to a servomotor (Model 322C, Aurora Scientific). Two ties of 10-0 monofilament nylon suture were used to secure each end of the fiber to the force transducer and servomotor. With a laser diffraction measurement system, the length of the fiber (L₀) was adjusted to obtain a sarcomere length of 2.7 μm, which is the optimum length for human fibers based on the thin filament length measurements of Walker. The average CSA of the fiber was calculated assuming an elliptical fiber cross section, with diameters obtained at 5 different positions along the fiber from high-magnification images of the top and the side views. Maximum isometric force (F₀) of the fiber was elicited by immersing the fiber in a high calcium-containing activation solution. Specific force (sF₀) was calculated by dividing F₀ by CSA. Up to 20 fast fibers were tested from each subject with a supraspinatus tear, and those fibers that did not demonstrate tearing or slipping were used for analysis. The average value from each subject was used in statistical analyses. Single-fiber contractility measurements in the current study were compared with the values obtained from healthy vastus lateralis biopsy specimens from the 65- to 80-year-old pooled male and female pre-training cohorts (N = 27 subjects) that were previously reported.

**Histology**

Histology was performed as previously described. Biopsied tissue was rinsed free of blood with saline and was snap frozen in Tissue-Tek (Sakura, Torrance, CA, USA) with isopentane cooled in liquid nitrogen and stored at −80°C until use. Muscles were sectioned at a thickness of 10 μm and prepared for immunohistochemistry. To identify lipid deposition and macrophages, sections were fixed in 4% paraformaldehyde, permeabilized in 0.1% Triton X-100 in phosphate-buffered saline, blocked in 5% goat serum, and incubated with biotinylated antibodies against the macrophage-specific antigen CD68 (AbCam, Cambridge, MA, USA) as well as BODIPY FL to identify lipid (Invitrogen, Grand Island, NY, USA), DAPI (Sigma-Aldrich, St Louis, MO, USA) to identify nuclei, and wheat germ agglutinin conjugated to Alexa Fluor 555 (WGA-AF555; Invitrogen) to identify extracellular matrix. Streptavidin conjugated to Alexa Fluor 647 (Invitrogen) was used to detect CD68 antibodies. Sections were visualized with an Axioplan 2 microscope (Zeiss, Thornwood, NY, USA) equipped with AxioCam cameras (Zeiss). Sections were taken with use of the 10× objective to visualize lipid and extracellular matrix, and as the macrophage signal is appreciable only under high-power magnification, the 40× objective was used to identify macrophages, extracellular matrix, and lipid signal.

**Transmission electron microscopy**

Biopsied tissue used for electron microscopy was rinsed free of blood with saline and fixed immediately with 2.5% glutaraldehyde solution in 0.1 M Sorensen buffer (Electron Microscopy Sciences, Hatfield, PA, USA). Tissue was then post-fixed with 1% osmium tetroxide in 0.1 M Sorensen buffer (Electron Microscopy Sciences), dehydrated through a graded ethanol series, and then embedded in an epoxy resin (Sigma-Aldrich). One-micron sections were cut on an ultramicrotome and stained with 1% toluidine blue for evaluation by light microscopy. Ultrathin sections were obtained by cutting specimens longitudinally with a diamond knife ultramicrotome and post-staining with uranyl acetate and lead citrate. Sections were visualized with a CM-100 transmission electron microscope equipped with a high-resolution digital camera (Philips, Mahwah, NJ, USA).

**Statistics**

Values in the text are presented as mean ± standard deviation. Tukey box plots are used to present data in Figure 1. Differences between size and contractility values of torn supraspinatus muscles in the current study were compared with values from the vastus lateralis muscles of healthy control 60- to 85-year-old subjects by t tests (α = .05). Linear regression analysis was also used to determine the correlation between muscle fiber contractility and other measured parameters. Prism 6.0 software (GraphPad Software, La Jolla, CA, USA) was used to conduct statistical analyses.

**Results**

The age of patients in this study was 53 ± 7 years (range, 45-70 years), with 8 men and 5 women. Patients had an ASES ADL score of 13 ± 4.8 (range, 5-19 of a possible 30) for the involved shoulder and a composite ASES score of 46 ± 18 (range, 13-75 of a possible 100). The biopsy specimens of supraspinatus muscles of patients in this study showed the typical fat accumulation as has been described previously (Fig. 1, A), with the presence of canonical adipocytes as well as numerous large lipid droplets present in and around muscle fibers. By transmission electron microscopy, highly disordered sarcomeres with a loss of uniform force-transmitting Z discs and abnormally shaped...
mitochondria were also present throughout multiple biopsy specimens (Fig. 1, D). Areas of fibrosis and large vesicles containing lipids, mitochondria, and sarcomeric proteins were also observed (Fig. 1, E). An accumulation of multinuclear complexes of macrophages (Fig. 1, B, C, and F) was also frequently noted.

We next determined if the structural changes observed by histology and electron microscopy would affect muscle fiber force production. As most of the patients seeking rotator cuff repair had a long history of reduced shoulder function and atrophy, and many had bilateral symptoms, it was not possible to use another shoulder girdle muscle or the contralateral side as a control. We therefore compared the contractile and morphologic values of fibers from the current study with a previously published study from our laboratory that measured similar parameters from vastus lateralis fibers from otherwise healthy 60- to 85-year-old male and female subjects. As shown in Figure 2, CSA of the fibers from the healthy controls in the previous study (VL, Fig. 2, A; \( P = .871 \)) was similar to that of the fibers from patients with supraspinatus tears in the current study (SSP), but there was a 30% reduction in maximum isometric force (\( F_o \), Fig. 2, B; \( P < .002 \)) and a 29% reduction in specific force (\( sF_o \), Fig. 2, C; \( P < .001 \)) in patients with chronic supraspinatus tears. A video demonstrating force measurement from a healthy vastus lateralis muscle fiber and from a chronically torn supraspinatus muscle is shown in Supplemental Video 1.

Finally, we evaluated whether different clinical parameters correlate with supraspinatus muscle fiber morphology and contractility. ASES ADL scores did not correlate with tear size, but ASES total score did negatively correlate with tear size (Fig. 3, A and B). The size of the tear did not correlate with muscle fiber CSA or \( F_o \) (Fig. 4, A and B), but a negative correlation was observed between tear size and \( sF_o \) (Fig. 4, C). No correlations were observed between ASES ADL scores of the affected shoulder and fiber CSA or \( F_o \) (Fig. 5, A and B), but a positive correlation was observed between ASES ADL scores of the affected shoulder and \( sF_o \) (Fig. 5, C). Similar results were observed for the composite ASES scores (Fig. 5, D-F). We also did not observe significant correlations between age and ASES...
ADL score ($R^2 < 0.01; P = .92$), ASES total score ($R^2 = .02; P = .66$), CSA ($R^2 = 0.02; P = .68$), $F_o$ ($R^2 = 0.04; P = .45$) and $sF_o$ ($R^2 = 0.03; P = .55$). As no differences with regard to age were observed, these data are not presented in graphical format.

**Discussion**

Understanding the pathophysiologic changes that occur in the muscles of patients with chronic rotator cuff tears is of critical importance to improving outcomes for these patients. This was the first study, to our knowledge, that measured the contractility and characterized the ultrastructure of muscles at the single-cell level from patients with chronic rotator cuff tears and correlated these changes to outcomes scores and imaging studies. We identified a striking deficit in specific force production of the residual muscle fibers and also observed structural abnormalities in the architecture of muscle contractile proteins and abnormalities in the shape of mitochondria surrounding lipid droplets. Further, we have identified a population of lipid-laden macrophages present in the extracellular matrix of patients with torn rotator cuff muscles that may offer insights into the pathophysiologic process and therapeutic modulation of fatty infiltration of the rotator cuff musculature in patients with chronic tears. Finally, normalized force production was observed to have a negative correlation with tear size and a positive correlation with ASES scores. Combined, these results identify chronic structural and mechanical changes in torn rotator cuff muscles that may explain the poor functional capacity of the muscles after repair and provide biologic support for the utility of ASES scores and gap size in predicting muscle function in patients with rotator cuff tears.

Myofibrils, which are composed of repeating segments of sarcomeres, are the fundamental organelles within muscle fibers that generate force. The forces generated within sarcomeres are transmitted laterally to the extracellular matrix surrounding muscle fibers by $Z$ discs. An increase in the number of myofibrils in parallel will increase the total force production of muscle, whereas a decrease will lead to reduced total muscle force production. Exercise or inactivity can change the size and total force-generating capacity of muscle fibers, but the change in the number of myofibrils in healthy muscles is proportional to the change in size of the fiber. The ratio of muscle fiber CSA to maximum isometric force production ($F_o$) is specific force ($sF_o$), and decreases in $sF_o$ typically suggest a deficit in the production of healthy, properly aligned sarcomeres. $sF_o$ is therefore an important functional biomarker of the health of a muscle fiber, and decreases in $sF_o$ can indicate bona fide pathologic changes in the muscle tissue. In addition, whereas CSA and $F_o$ can change between different muscles in the body, $sF_o$ is a parameter that is thought to be consistent across healthy fibers.
containing the same myosin isoforms from different muscles in the body and is not anticipated to change on the basis of the anatomic location of the muscles. Previous studies have identified no differences in sFo between fibers from upper and lower extremity muscles in humans or any aging-associated changes in healthy rotator cuff muscles in adult and old rats. The sFo of type II fibers from lower extremity muscles are therefore expected to be similar to the sFo values of type II fibers from muscles of the upper extremity.

In the current study, we tested the contractility of permeabilized fibers from patients with chronic supraspinatus (SSP) tears and compared these values with those obtained from the uninjured, untrained vastus lateralis (VL) muscles of healthy 65- to 80-year-old men and women. Whereas the size of the fibers was similar between the control VL and injured SSP muscles, SSP muscles had a reduction in Fo and a subsequent decrease in sFo. These findings are consistent with reductions in sFo that were observed in rat models of rotator cuff tears. There was malalignment in the orientation of Z discs, which likely disrupts the proper transmission of force generated in the myofilaments to the cytoskeleton of the fiber. Although there was an accumulation of lipid and fibrotic extracellular matrix, as optimal force production requires precise alignment of the contractile proteins, we speculate that the sarcomeric disorder observed in the electron micrographs contributed to the reduction in sFo observed in the fibers from injured SSP muscles. There was also a negative correlation between sFo and objective and subjective clinical measurements. sFo was negatively correlated with the gap size of the tendon tear, and this is consistent with the clinical finding that patients with greater gap sizes tend to have worse clinical outcomes. sFo was also positively correlated with subjective, patient-completed ASES surveys, suggesting that there is a connection between patient-perceived shoulder

Figure 4  Correlations between single-fiber contractility parameters and the size of the gap between the free tendon end and the anatomic attachment of torn supraspinatus (SSP) muscles. (A) Cross-sectional area (CSA), (B) maximum isometric force (Fo), and (C) specific force (sFo, Fo normalized to cross-sectional area) of fibers as a function of SSP tear gap size.

Figure 5  Correlations between ASES scores and single-fiber contractility parameters of torn supraspinatus (SSP) muscles. ASES affected shoulder ADL score as a function of (A) cross-sectional area, (B) maximum isometric force (Fo), and (C) specific force (sFo, Fo normalized to cross-sectional area) of torn SSP fibers. ASES composite score as a function of (D) cross-sectional area, (E) maximum isometric force (Fo), and (F) specific force (sFo, Fo normalized to cross-sectional area) of torn SSP fibers.
function and the ability of SSP muscles to generate force. The reduction in sFo in the current study is consistent with the findings of Gerber et al., who identified a negative correlation between whole muscle force production and force normalized to anatomic CSA in patients undergoing rotator cuff repair. However, Gerber postulated that the reduction in normalized force production was due to fat and connective tissue taking up area in muscle cross sections. Whereour results are in general agreement, the findings of the current study suggest that the decrease in sFo occurs at the level of the muscle fiber independent of the additional area taken up by fibrotic tissue and fat in a muscle cross section. In addition, although the prevalence of rotator cuff tears increases with age, we did not observe correlations between age and muscle fiber contractility, ASES scores, or tear size. Overall, our results indicate that patients with chronic rotator cuff tears have highly disordered sarcomeres that prevent proper force generation and that parameters used for clinical decision making, such as gap size and patient-perceived function, correlate well with rotator cuff sFo values.

The ultrastructure of the rotator cuff musculature in patients with chronic rotator cuff tears as reported in the current study is of paramount importance to understanding of the pathophysiologic mechanism of fatty infiltration and atrophy. The deposition of fat in and around muscle fibers is commonly observed after chronic rotator cuff tear. The ontogeny of fat accumulation and the specific cells and signaling pathways that drive this process in rotator cuff tears remain largely unknown. Atherosclerosis is a well-studied condition that is marked by an accumulation of lipid and local tissue inflammation and shares some similar features with chronic rotator cuff tears. In atherosclerosis, a population of fatty macrophages, often referred to as foam cells, take up high levels of lipid and promote local tissue inflammation. We previously identified a population of lipid-laden macrophages in rat models of chronic rotator cuff tears. In the current study, we also report the presence of lipid-laden macrophages, as identified by the presence of lipid droplets and staining for the pan-macrophage marker CD68. These lipid-laden macrophages in torn SSP muscles also share similar morphologic features with foam cells visualized with transmission electron microscopy. As foam cells promote local tissue inflammation in blood vessels, current interventions that prevent or decrease foam cell accumulation are being studied as potential therapeutic modalities in the treatment of atherosclerosis. Although it is not known whether the lipid-laden macrophages present in torn rotator cuff muscles play a role in tissue inflammation or muscle function, given the important role that these cells have in atherosclerosis, this is an area of research that deserves further study.

This paper has several limitations. We did not have access to healthy, age-matched control SSP muscles, as this would require performing arthroscopic biopsies of a large cohort of subjects, and instead used the VL as a control muscle. The results are also biased toward patients with rotator cuff tears who chose to undergo surgery. Whereas the sFo production of healthy VL and SSP muscles is anticipated to be similar, it is possible that the sFo for healthy SSP muscles is different from that of VL muscles. Although we correlated muscle fiber contractility by tear size and ASES scores, we could not perform correlations based on Goutallier score. Not all patients had magnetic resonance imaging scans; some had only ultrasound performed to verify the clinical diagnosis of a rotator cuff tear and did not have reliable images to characterize the severity of fatty infiltration. As many patients with chronic, full-thickness tears do not know the specific time when their injury occurred, we do not know whether the time since suffering a tear has an impact on SSP muscle contractility. We also took biopsy specimens at the time of surgical repair, but we did not perform repeated biopsies to determine if repair is able to improve the contractile properties of torn SSP muscles. These limitations notwithstanding, the study provided important insight into the cellular and molecular pathophysiology of chronically torn rotator cuff muscles and provided biologic corroboration for commonly used clinical assessment parameters and tools that are used to predict rotator cuff function.

Despite improvements in surgical repair and rehabilitation techniques, rotator cuff tears remain among the most debilitating and frequent upper extremity injuries. Current treatment options are limited and are mostly aimed at addressing symptoms and not treating the underlying pathologic process. For many patients who undergo successful repair of their chronically torn rotator cuff, fatty degeneration continues to progress over time. The repair of chronically torn rotator cuff muscles subjects the chronically shortened muscle to a substantial and rapid length change that may be injurious to the muscle. Given the changes in myofibril architecture observed in the current study, inducing such a rapid length change at the time of surgical repair likely generates a second injury that can be difficult for the muscle to recover from. Using a sheep model, Gerber et al demonstrated that a slow, progressive lengthening of chronically torn rotator cuff muscles could reduce fatty degeneration and restore normal muscle architecture. This approach probably allows the muscle to slowly remodel and to adapt to new tension being placed on it. Therapeutic interventions that target sarcomere and membrane stability may therefore be beneficial in enhancing the regeneration of chronically torn rotator cuff muscles and improve overall patient outcomes.

Conclusions

Patients with chronic rotator cuff tears often have a persistent atrophy of muscle fibers and an accumulation of fatty and fibrotic tissue, collectively referred to as
fatty degeneration. Although several animal studies have provided insight into potential mechanisms that result in muscle weakness after rotator cuff tears, the pathophysiologic mechanism of fatty infiltration and the impact of chronic tears on the ability of the rotator cuff muscle fibers to generate force in humans had not been defined. The current study demonstrated that patients with chronic rotator cuff tears have significant reductions in muscle fiber force production, disrupted myofibril architecture, and accumulation of fatty macrophages in the muscle extracellular matrix. We also observed that normalized force production had a negative correlation with tear size and a positive correlation with ASES scores. This work helps explain the cellular and molecular basis for the reduced upper extremity strength and functional biologic validation for the use of ASES scores and tear size measurements in predicting shoulder function, and will, it is hoped, contribute to the development of future therapies to improve functional outcomes and enhance rotator cuff regeneration.

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**Supplementary data**

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