Development of Fatty Atrophy After Neurologic and Rotator Cuff Injuries in an Animal Model of Rotator Cuff Pathology

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Background: Detachment of a tendon from its osseous insertion, as can be the case with severe rotator cuff injuries, leads to atrophy of and increased fat in the corresponding muscle. We sought to validate a rotator cuff injury model in the rabbit and to test the hypothesis that tenotomy of a rotator cuff tendon would consistently create muscle atrophy and fatty degeneration analogous to the changes that occur after injury to a nerve innervating the same muscle.

Methods: New Zealand white rabbits were divided into three groups: (1) partial rotator cuff tear without retraction of the muscle, (2) complete rotator cuff tear with retraction of the muscle, and (3) nerve transection of the subscapular nerve. Animals were killed at two or six weeks after injury, and the muscles were analyzed for weight, cross-sectional area, myosin fiber-type composition, and fat content. In addition, the subscapular nerve was harvested at two weeks and evaluated for neuronal injury.

Results: At six weeks after injury, the rabbit muscles in the complete tenotomy and nerve transection groups had significant decreases in wet mass and increases in fat content relative to the control groups. Fat accumulation had a similar spatial pattern at six weeks in both the nerve transection and complete tenotomy groups. Such changes were not seen in the partial tenotomy group. No change was found in muscle myosin fiber-type composition. At two weeks after injury, subscapular nerves in the complete tenotomy group showed gross evidence of neuronal injury.

Conclusions: This study establishes the rabbit subscapularis muscle as a valid model to study the muscular changes associated with rotator cuff tears. Our data suggest that the muscular changes associated with complete tenotomy are comparable with those seen with denervation of the muscle and suggest that chronic rotator cuff tears may induce a neurologic injury.

Clinical Relevance: Chronic rotator cuff injuries are associated with neuronal injury of the affected muscle. As such, neuronal injury may explain the histopathologic changes that have been observed following chronic rotator cuff tears.

Detachment of a tendon from its osseous insertion and the subsequent retraction of the myotendinous unit lead to characteristic changes in the muscle. Immediately, the tensile forces exerted on the muscle are unloaded and the length of the muscle decreases. This shortening translates to a decreased sarcomere length, myofiber disorganization, and, consequently, a compensatory decrease in sarcomere number (both longitudinally and radially)^{1,2}. Such changes decrease the maximum tension and overall strength of the muscle. Disuse of the unloaded muscle results in a significant loss of muscle mass (approximately 60%) with a decrease in muscle fiber cross-

sectional area and an increase in fat content³⁻⁵. Furthermore, chronically detached muscles become stiffer and shorter than normal tendon-muscle units, and the passive load required to reduce and repair the tendon becomes excessive^{6,7}.

Denervation of a muscle is also known to reproducibly create architectural changes such as a decrease in cross-sectional area and fatty atrophy in a muscle group⁸. The loss of propagation of action potential through the neuromuscular junction prevents contraction of the muscle fibers and leads to disuse atrophy. As early as two weeks after denervation in rats, muscle mass decreased by as much as 54%⁹.

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Chronic rotator cuff tears may manifest with the clinical attributes of atrophy, increased connective tissue formation, and fatty infiltration^{1,10,11}. The techniques used to measure the amount of muscle atrophy and fat content in the human shoulder have traditionally been limited to semiquantitative imaging modalities, such as computed tomography and magnetic resonance imaging^{11,12}. Early changes often may include edema and hematoma formation localized in the injured muscles. After weeks, there can be a relative increase in both fat and extracellular water content. Chronic muscle tears may result in progressive fatty infiltration and loss of fluid, with minimal fibrosis. Although such changes are seen, relatively little is known about the pathogenesis of these changes.

To better understand the pathogenesis of this biologic phenomenon, an animal model that can be used to accurately and quantitatively evaluate the evolution of rotator cuff injuries has been developed^{13,14}. The rabbit subscapularis muscle has been shown to have similar anatomical and biomechanical properties to those of the human supraspinatus muscle (Fig. 1).

Although rabbit models have been shown to be a reliable and reproducible model for the study of rotator cuff pathology, previous animal models have failed to effectively and consistently reproduce this phenomenon^{15,16}. Limitations to the previous models include size of the animals, gait patterns, and variation in gross anatomy as compared with the anatomy of humans¹³. We made use of a rabbit model to validate the rabbit subscapularis muscle as a reproducible model for rotator cuff injury, examine the pathologic changes related to fat infiltration, and test our hypothesis that rotator cuff tenotomy creates an injury that is analogous to that seen with denervation of the muscle.

Materials and Methods

Creation of the Animal Model

Twenty-four adult male New Zealand white rabbits (Oregon Health and Science University, Portland, Oregon), weighing between 3 and 4 kg, were randomized into three groups (eight rabbits in each group): (1) partial rotator cuff tear without retraction of the muscle, (2) complete rotator cuff tear with

Fig. 1

Anatomy of rabbit and human in the sagittal view. Computer-generated (left) and gross-anatomy (right) images of the glenoid and scapula of a rabbit (A and B) and a human (C and D), illustrating the similarity of the rabbit subscapularis tendon (passing under the lesser tuberosity) to the human supraspinatus tendon.

retraction of the muscle, and (3) transection of the subscapular nerve. The left subscapularis tendon, muscle, and associated subscapular nerve from each rabbit served as the experimental rotator cuff unit, while the contralateral right shoulder served as a sham surgical control.

Surgeries were performed as previously described¹⁴. The rabbits were fully anesthetized with ketamine and xylazine and given prophylactic penicillin prior to all procedures. A skin incision was made just inferior to the clavicle, and the deltopectoral interval was split and retracted to gain access to the shoulder. The coracobrachialis muscle was observed as covering the subscapularis tendon attachment and was incised and not repaired (Fig. 2). The subscapularis tendon was visualized, and a right-angle clamp was inserted to expose the entire tendon at its insertion on the lesser tuberosity of the humerus. In the partial rotator cuff tenotomy group, the superior half of the subscapularis tendon was sharply transected with a number-11 scalpel blade at its insertion to the humerus. The complete transection was carried out in a similar fashion but with complete incision of the tendon. The tendon was clamped to prevent retraction of the myotendinous unit until a 5-mm Penrose Drain (C.R. Bard, Covington, Georgia) could be sutured to the tendon to prevent it from scarring down. The clamp was then released, and the muscle immediately retracted approximately 3 mm from its humeral insertion site. This degree of retraction is approximately one-fourth the size of the average rabbit humeral head size, which is approximately 13 mm. In the nerve transection group, the same surgical approach was used to expose the tendon and the nerve innervating the subscapularis muscle. Electrical stimulation (Surgical Nerve Locator/Stimulator; Allegiance Healthcare, McGaw Park, Illinois) was used to identify the subscapular nerve at the point at which it pierced the muscle fascia, and the nerve was then sharply transected with a number-11 scalpel blade. Through the same approach, a sham surgical procedure was performed on the contralateral (right) shoulder of each animal to visualize the rotator cuff without inducing any injury to the tendon. All surgical wounds were closed at two levels: with use of 3-0 Vicryl (polyglactin) sutures (Ethicon-Johnson and Johnson, Somerville, New Jersey) to reapproximate the muscle, and with use of 2-0 Nylon (Ethicon-Johnson and

Johnson) interrupted sutures to close the skin. The rabbits were given intramuscular buprenorphine immediately after recovery from anesthesia, the day after surgery, and as needed for pain. All animals were allowed normal cage activity after surgery. The experimental protocol described in this study was approved by the Animal Care and Use Committee at our institution and is in accordance with National Institutes of Health and United States Department of Agriculture guidelines for the use and care of laboratory animals.

Specimen Harvest

The twenty-four rabbits were randomly killed at either two or six weeks from the time of model creation. At each time point, twelve rabbits (four animals per group) were anesthetized and given a lethal dose of sodium pentobarbital. The subscapularis muscle, tendon, neuromuscular junction, and subscapular nerve were harvested bilaterally from each animal. Specimens were immediately wrapped in aluminum foil, placed on dry ice, and stored at -80°C in a freezer until analyzed. The complete tenotomy group was evaluated for the presence of tendon reattachment, which had not occurred in any animal. Additionally, the subscapular nerve transection was confirmed in all animals by direct visualization in that group.

Histological Analysis of Skeletal Muscle

Wet Mass of Muscle

The subscapularis muscle was dissected en bloc from its origin along the subscapular fossa of the scapula and the distal tendinous attachment. The wet mass of the subscapularis muscle was measured with use of a digital scale immediately after dissection (Denver Instruments, Arvada, Colorado).

Quantification of Muscle-Fiber

Cross-Sectional Area

To determine muscle-fiber cross-sectional area, a transverse section of the subscapularis muscle was harvested from the mid-portion of the muscle, mounted on cork with use of Tissue Tek (Miles Laboratories, Elkhart, Indiana), and frozen with use of isopentane cooled by liquid nitrogen. Cross-sections (20 μm thick) were cut with use of a cryostat, mounted on poly-L-lysine-coated glass slides (Fisher Scientific, Pittsburgh, Penn-

Fig. 2

Gross-anatomy photographs of the rabbit scapula, showing the subscapularis fossa (A), the glenoid (B), and the muscle anatomy (C). The images demonstrate the osseous tunnel of the subscapularis tendon.

sylvania), and stained with hematoxylin and eosin. Sections were visualized at 200× magnification (Leica Microsystems, Wetzlar, Germany) and images were recorded with use of a digital camera (DP71; Olympus, Center Valley, Pennsylvania). The cross-sectional areas of fifty random muscle fibers per muscle were subsequently measured with use of ImageJ v1.32 image-analysis software (National Institutes of Health, Bethesda, Maryland). Muscle samples were analyzed in a random order, and the investigator was blinded as to which muscle was being analyzed.

Morphometric Quantification of Intramuscular

Fat Content

To estimate the percentage of fat content of the subscapularis muscle, the entire muscle was cut in cross section and stained with oil red O (Fisher Scientific), a lysochrome diazo dye (fat-soluble). Cross sections were from the distal (i.e., close to the tendon), the middle, or the proximal (i.e., the point of attachment to the fossa) aspect of the entire subscapularis muscle. The muscles were sectioned as described above, mounted on slides, and allowed to dry for twenty-four hours at -70°C . Sections were then rehydrated in double-distilled water for one minute and propylene glycol for ten minutes. The sections were then stained with oil red O at room temperature and mildly rocked for two hours. The oil red O staining solution was prepared by mixing 0.7 mg of oil red O stain in 100 mL of propylene glycol at 100°C . Sections were then washed in 85% propylene glycol for five minutes, followed by three washes in double-distilled water. Counterstaining was done with use of hematoxylin for two minutes, followed again by three washes in water. Slides were allowed to dry at room temperature and were then coverslipped with glycerol. The slides were then recorded as images as described above. Digital images were made at specific step lengths in both the x and y plane ($100\ \mu\text{m}$), throughout the entire cross section of the muscle. This allowed unbiased, objective, and systematic sampling of the entire muscle cross section. Each digital image was analyzed. The area of fat (staining red) and the total subscapularis muscle fiber area were measured with use of the ImageJ software. The ratio of fat area relative to total area was used to calculate the percentage of intramuscular fat for each sample.

Expression of Whole Muscle Myosin Heavy Chain Isoforms Myofibrillar Protein Extraction

Purified myofibril preparations were extracted with use of techniques described previously^{17,18}. Briefly, this process included homogenization of a 150-mg cross section of the subscapularis muscle cut from the midsubstance in a solution (solution A; pH 6.8) containing 250 mM sucrose, 100 mM KCl, 20 mM tris(hydroxymethyl)aminomethane (Tris), and 5 mM ethylenediaminetetraacetic acid (EDTA). The homogenate was centrifuged at 1000g for ten minutes at 4°C . The resulting pellet was resuspended in a solution (solution B; pH 7.0) containing 175 mM KCl and 20 mM Tris and centrifuged as described above. The resulting pellet was again suspended in solution B and adjusted to a protein concentration of 6 mg/mL

Fig. 3

En bloc wet mass of the subscapularis muscle on the normal and injured sides at two weeks (A) and six weeks (B) after injury. At six weeks, there is a significant decrease in wet mass in the complete tenotomy (Comp) and nerve transection groups. The partial tenotomy group showed no significant change in wet mass at either two or six weeks. I-bars indicate standard error of the mean.

with use of the biuret technique. Myofibrils were then stored at 1 mg/mL and at -20°C in a solution containing 50% glycerol, 50 mM $\text{Na}_4\text{P}_2\text{O}_7$, 2.5 mM ethylene glycol-bis(β -aminoethyl ether)-N,N,N',N'-tetraacetic acid, and 1 mM 2-mercaptoethanol (pH 8.8).

Electrophoretic Separation of Myosin Heavy**Chain Isoforms**

Myosin heavy chain protein isoforms were separated by techniques previously developed and tested¹⁹. The separating gel solution contained 8% acrylamide, 0.16% bis-acrylamide, 30% glycerol, 0.4% sodium dodecyl sulfate (SDS), 0.2 M Tris (pH 8.8), and 0.1 M glycine. This solution was degassed for approximately fifteen minutes. Polymerization was then initiated by adding N,N,N',N'-tetramethylethylenediamine (TEMED; 0.05% final concentration) and ammonium persulfate (0.1% final concentration) to the separating gel solution. After the separating gel had been poured, it was layered with ethyl alcohol and allowed to polymerize for approximately thirty minutes. Once the separating gel was polymerized, the stacking gel was poured. The composition of the stacking gel was 4% acrylamide, 0.08% bis-acrylamide, 30% glycerol, 70 mM Tris (pH 6.7), 4 mM EDTA, and 0.4% SDS. This solution was also degassed for fifteen minutes before addition of TEMED (0.05% final concentration) and ammonium persulfate (0.1% final concentration). The composition of the running buffer was 0.1 M Tris, 0.15 M glycine, and 0.1% SDS. Myofibril samples were denatured with use of a sample buffer solution containing 5% β -mercaptoethanol, 100 mM Tris-base, 5% glycerol, 4% SDS, and bromophenol blue. Approximately 1 μ g of protein was loaded into each well. Electrophoresis was performed with use of an SG-200 vertical-slab gel system (CBS Scientific, Del Mar, California). Gels were run by using a constant voltage of 275 V for approximately twenty-four hours. This method separated the fast Type IIA, fast Type IIX, fast Type IIB, and slow Type I myosin heavy chain isoforms (order of migration). Myosin heavy chain protein isoform bands were stained with Coomassie Blue G-250. The myosin heavy chain protein isoform bands were scanned and quantified with use of a Molecular Dynamics Personal Densitometer (Molecular Dynamics, Sunnyvale, California).

Nerve Histology

The right subscapular nerve was harvested at two weeks following complete tenotomy of the subscapularis tendon (in four specimens) and also following transection of the nerve (in four specimens). The two-week time point was chosen because, at that time point, the nerve remains identifiable with consistent evidence of Wallerian degeneration in any nerve transection model and can serve as a positive control. In both cases, the contralateral subscapular nerve served as a control. Following harvest, specimens were fixed in 4% paraformaldehyde and frozen in paraffin blocks. The frozen specimens were then divided into 5- μ m sections, stained with 0.6% Sudan black B fat stain, and visualized with use of a light microscope (IX71; Olympus).

Statistical Analysis

All values were expressed as the mean and the standard error of the mean. Comparisons of each specimen's mean were performed with use of the unpaired Student t test. A p value of <0.05 was considered significant.

Source of Funding

NIH funding was received by Dr. Gupta; however, that funding was not directly related to this project.

Results**Animal Surgery**

All twenty-four animals survived the surgical procedure, and there were no instances of infection or other complications. Several animals chewed their sutures and required reclosure of the surgical wounds.

Histological Analysis of Skeletal Muscle**Wet Mass of Muscle**

At two weeks, the wet mass of the muscles in the nerve transection group decreased from 6.09 ± 0.61 g to 4.00 ± 0.25 g,

Fig. 4

Muscle cross-sectional area for the subscapularis muscle on the normal and injured sides at two weeks (A) and six weeks (B) after injury. There was a significant decrease in cross-sectional area in the nerve transection and complete tenotomy (Comp) groups at both two and six weeks after injury. I-bars indicate standard error of the mean.

which was a change of 34.3% compared with that of the control muscles ($p < 0.001$), whereas the wet mass of the partial tenotomy and complete tenotomy groups showed no significant change (Fig. 3, A). By six weeks, the muscles in the complete tenotomy and nerve transection groups had a significant decrease in wet mass from 6.08 ± 0.11 g to 5.46 ± 0.28 g ($p < 0.05$) and 6.10 ± 0.29 g to 5.13 ± 0.18 g ($p < 0.02$), respectively, as compared with control muscles (Fig. 3, B). These data show that the atrophic process in the nerve transection model precedes that of the complete tenotomy injury. At six weeks, however, there was comparable loss of muscle mass between the two groups.

Muscle-Fiber Cross-Sectional Area Quantification

The muscle-fiber cross-sectional area decreased at two weeks from $5180 \pm 226 \mu\text{m}^2$ to $3954 \pm 187 \mu\text{m}^2$ (23.7%, $p < 0.03$) in the complete tenotomy group and from $5331 \pm 181 \mu\text{m}^2$ to $3964 \pm 186 \mu\text{m}^2$ (25.6%, $p < 0.02$) in the nerve transection group when compared with that of the control muscles (Fig. 4, A). At six weeks, the cross-sectional area in the muscles that underwent complete tenotomy decreased from $5626 \pm 144 \mu\text{m}^2$ to $4557 \pm 127 \mu\text{m}^2$ (19.0%, $p < 0.04$), and, in the muscles that underwent nerve transection, the cross-sectional area decreased from $5641 \pm 193 \mu\text{m}^2$ to $3857 \pm 198 \mu\text{m}^2$ (31.6%, $p < 0.03$) (Fig. 4, B). The degree of cross-sectional area reduction was more dramatic in the nerve transection group but appeared to occur along a similar time line. Animals with partial-thickness tenotomies did not show a significant change in cross-sectional area as compared with the change in area on the control side at either two or six weeks.

Morphometric Quantification of Intramuscular Fat Content

At six weeks, the fat content of the subscapularis muscle was significantly greater in both the complete tenotomy and nerve transection groups than in the control groups at the proximal, middle, and distal aspects of the muscle (Figs. 5 and 6). There was no significant change in fatty infiltration of muscles in all three groups at two weeks or in the partial tenotomy group at six weeks except for the middle aspect of the muscle (Fig. 6). In the complete tenotomy group at six weeks, the percent of fat content was $11.86\% \pm 2.85\%$ ($p < 0.02$), $7.44\% \pm 1.88\%$ ($p < 0.02$), and $3.65\% \pm 0.96\%$ ($p < 0.04$) in the distal, middle, and proximal aspects of the muscle, respectively. In the nerve transection group, a similar pattern was noted: $11.35\% \pm 3.04\%$ ($p < 0.05$), $5.9\% \pm 0.91\%$ ($p < 0.02$), and $4.72\% \pm 1.05\%$ ($p < 0.05$) in the distal, middle, and proximal aspects, respectively. At the six-week time point, the fat content of both the nerve transection and complete tenotomy groups was similar. These findings suggest that the process of fatty degeneration is slower than the process of muscle atrophy. The two models of muscle injury progressed at the same rate over time with respect to fatty muscle content. Additionally, the similar pattern of muscle atrophy and fat infiltration in the complete tenotomy group compared with the pattern in the denervated group suggests that perhaps there may be a neuronal injury component to complete rotator cuff tears.

Whole Muscle Myosin Heavy Chain Isoform Expression

The myosin heavy chain isoform phenotype was not affected by the extent of rotator cuff tear or innervation state.

Fig. 5

Photomicrographs of cross sections of the subscapularis muscle stained with oil red O and counterstained with hematoxylin in the control group (A, two weeks; D, six weeks), complete tenotomy group (B, two weeks; E, six weeks), and nerve transection group (C, two weeks; F, six weeks). The pattern of fatty infiltration is similar at six weeks in the complete tenotomy (E) and nerve transection (F) groups (original magnification, $\times 200$).

Fig. 6

Fatty infiltration of distal (Dist), middle (Mid), and proximal (Prox) aspects of the muscle in all three groups at two weeks (left side of graph) and six weeks (right side of graph) after injury. At six weeks, the amount of fatty infiltration was significant in the nerve transection group and the complete tenotomy group. In addition, the pattern of infiltration was similar. I-bars indicate standard error of the mean.

Nerve Morphometry

After transection, the subscapular nerve underwent Wallerian degeneration with gross evidence of axonal disintegration and myelin debris formation. After complete tenotomy of the rabbit subscapular tendon, this nerve also underwent neuronal degeneration with evidence of myelin degeneration and axonal changes.

Discussion

Our results demonstrate that the complete, chronically detached subscapularis muscle in this rabbit model changes significantly, with a decrease in muscle mass, a decreased cross-sectional area, and an increase in fat content. The muscle fiber type was not changed as compared with that of the control groups. In addition, we showed that the increase in fat exhibited a spatial pattern, with higher fat accumulation distally (closer to the tendon attachment as compared with proximally). Denervated muscle showed a similar fat accumulation pattern, both temporally and spatially. Furthermore, the nerve specimens from the rabbits that underwent complete tenotomy revealed evidence of neuronal injury, as did the nerve specimens from animals in which the nerve was transected.

Rotator cuff pathology is associated with progressive and likely irreversible degenerative changes of the rotator cuff muscles. The amount of atrophy and fatty degeneration influences several clinical parameters, such as strength and loss of function, and may affect the results of rotator cuff repair^{2,10,20-22}. Despite the importance of these factors, there are few studies that address the quantitative changes present in the muscle following rotator cuff injury, and there is limited understanding of the mechanisms behind these processes^{22,23}. The creation

of a nonhuman model that accurately reproduces the clinical condition is therefore an important step in developing a mechanistic approach to understanding the pathogenesis and repair of these types of tendon tears.

Previous animal models of rotator cuff injury have been associated with mixed outcomes with regard to the development of fatty atrophy and overall cuff pathology^{2,12,15,16,24-27}. At both eight and sixteen weeks after tendon detachment, no significant difference was found in the proportion of fat in the rat supraspinatus muscle¹⁵. Gerber et al.²⁶ and Coleman et al.²⁷ reported significant fat infiltration of the detached infraspinatus muscle in sheep models but showed differing outcomes with regard to reversal of the fatty degeneration process. Studies in the rabbit supraspinatus muscle have shown fatty degeneration beginning as early as four weeks, with a peak at six weeks and with slow reversal by twelve weeks²⁴. In studies in which the effects of early and delayed reattachment of torn rotator cuff tendons in rabbits were evaluated, it was found that the reattachment of the tendon at either time point reversed the atrophy or fat accumulation^{12,16}.

We found a significant increase in fat content in chronically detached (tenotomy) muscles at six weeks after injury, with minimal fat accumulation at two weeks after injury. The fat accumulation exhibited a distinct spatial variation. Distal to the origin of the subscapularis muscle, there was a higher accumulation of fat than there was proximally, demonstrating a nonuniform pattern. Another study of chronically detached rabbit supraspinatus muscles showed an increase in fat content from 1.3% in normal muscle to 5.4% in the detached muscle at twenty-four weeks after injury, although minimal changes were

noted at twelve weeks after injury¹². Additionally, in a chronic-injury infraspinatus model in sheep, fat accumulation was noted to be 6.2% at eighteen weeks after injury²⁷. Although the data regarding the increase of fat in these other models are similar to our data, a primary limitation of those studies is that the samples were taken from one location in the studied muscle, commonly the midportion. Furthermore, the present magnetic resonance imaging method of evaluating fatty atrophy in the supraspinatus muscle involves a single image obtained just medial to the scapular spine and may not be sufficient to assess this important biologic parameter. The experimental data have shown a spatial pattern of fat accumulation in the affected muscle, with a higher percentage of fat accumulation in the distal aspects and a milder accumulation proximally; therefore, an analysis of a limited region of the muscle may inaccurately measure the amount of accumulation within the supraspinatus.

The data from this study also support the concept that the amount of infiltration of fat in the subscapularis muscle is directly related to the extent of tendon injury, which is consistent with findings in humans. In computed tomography studies performed in humans, the closer the tendon stump is to the glenoid fossa, the higher is the percentage of fat accumulation^{23,28}. Furthermore, more severe fatty degeneration is noted with larger tears that extend into the infraspinatus from the supraspinatus. In this study, partial tenotomy showed a minimal decrease in mass and minimal fat accumulation as compared with what was seen in control muscles. Complete tenotomy with retraction of the muscle resulted in extensive fat accumulation and a decrease in weight. However, as this is only a gross comparison between complete and partial tenotomy, additional studies in which the amount of retraction is varied following a complete tenotomy are needed to determine if our model is entirely consistent with the findings that have been observed in humans.

The use of the term *fatty degeneration* has been called into question by investigators who have sought to understand the spatial location and source of the fatty tissue. Several investigators have studied the distribution of fat following rotator cuff injury in animal models, and these investigators have found significant amounts of extramuscular and intramuscular fat accumulation^{10,12,16,26,29}. Gerber et al. used electron microscopy to document that the process is not fatty degeneration but rather a fatty infiltration, with the remaining muscle fibers atrophied but still capable of recovery²⁶. In that study, the histology showed both extrafascicular and interfascicular fat accumulation, with the greatest amount of fat being found between the fascicles in all groups. Whether the increased fat content is a result of proliferation of existing adipocytes, the invasion of extramuscular adipocytes, or a differentiation of pluripotent cells toward mature adipocytes is an important area that warrants further investigation.

Neuronal injury in rotator cuff tears has been suggested, yet little validation has been offered³⁰. Cadaver studies have shown a significant increase in tension of the suprascapular nerve at the notch with associated tendon retraction³⁰. Additionally, electromyographic studies of the suprascapular nerve have shown slowing of conduction and the F-wave action po-

tential in complete rotator cuff tears, suggesting an injury to the neuromuscular axis³¹. Furthermore, neural cell adhesion molecule (NCAM), a marker expressed by denervated muscle cells, was shown to be elevated as early as one week following tenotomy of the rabbit extensor digitorum longus muscle. It was proposed in that study that the increase in NCAM may be secondary to an acute neuronal injury¹. In that study, histological evaluation of the subscapular nerve following complete tenotomy showed both axonal damage and myelin breakdown. Thus, findings of atrophy, cross-sectional area loss, and increased fat in the muscles of animals with a tenotomy may in part be explained by neuronal injury and are consistent with the hypothesis that a rotator cuff tear, and the associated myotendinous retraction, may induce an injury to the primary motor nerve unit.

This neural injury may contribute to the irreversibility of the muscle injury and delayed healing after primary rotator cuff repair. Degenerative changes in tenotomy models are less severe when there is interruption of either the afferent pathways or sensory outflow^{32,33}. When the nerve to a tenotomized muscle is stimulated, there is an increase in the amplitude of the monosynaptic reflex^{1,34,35}. It is possible that neural injury induced by retraction of the myotendinous unit has an important role in the degenerative changes observed in the muscle fibers following tenotomy. In the human, the suprascapular nerve is relatively tethered in the suprascapular notch. Similarly, the rabbit subscapular nerve is tethered to the adjacent osseous architecture and thus it is possible that a neuronal injury, in the setting of retraction of the myotendinous unit, occurs by a similar mechanism in both humans and rabbits^{13,14}.

This study employed a technique of whole-muscle myosin heavy chain electrophoresis separation to evaluate changes in muscle fiber type¹⁹. Our finding of no significant change in muscle fiber type in any of the models at either time point is not the first report of this finding in the literature^{1,36,37}. A study of the effect of tenotomy compared with denervation on the expression of myosin heavy chains in a rat model found only a transient shift between various isoforms without long-term changes³⁶. However, our findings are inconsistent with those of a recent study involving tenotomy of the rat supraspinatus muscle, which showed an increase in the proportion of fast muscle fibers¹⁵. These inconsistencies in the literature might reflect differences in species.

In conclusion, we show evidence of a reproducible nerve injury following complete tenotomy of the rabbit subscapularis myotendinous unit, suggesting that the biologic changes noted in chronic rotator cuff injuries may be explained by neuronal injury. As chronically torn rotator cuffs in human studies have shown limited functional outcomes over the long term³⁸⁻⁴⁰, such changes might explain the irreversible nature of muscle weakness and atrophy in chronic rotator cuff injuries with muscle retraction³⁰. Medial retraction of a massively torn supraspinatus is believed to place tension on the suprascapular nerve at the scapular notch. Furthermore, there are several reports of decompression or mobilization of the suprascapular nerve during rotator cuff repairs to improve functional outcome and decrease pain. Further work is still required to more fully

define the relationship between neuronal injury and the associated biologic changes within the muscle following tenotomy. ■

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