Exploring the Biologics of Rotator Cuff Injury and Advancing Repair

Student: Bryce Macek

Supervisors: Dr. Jeff Leiter, Dr. Peter MacDonald

Department of Orthopedic Surgery

Rotator cuff tears are a common problem associated with muscle atrophy and fatty infiltration. These changes may be progressive and even irreversible despite successful repair. A deeper understanding of the cellular processes contributing to these degenerative changes is needed to predict outcomes. The objectives of the present study are to: 1) characterize rotator cuff tears through clinical exam and MRI, 2) compare muscle atrophy at the cellular level via muscle biopsy of torn supraspinatus and deltoid, 3) determine if cuff tear size is related to clinical variables. Ten patients with clinical and MRI evidence of a rotator cuff tear were biopsied from supraspinatus and deltoid muscles during arthroscopy. Samples were stained with hematoxylin and eosin to determine fiber diameter. Fiber diameter of the deltoid muscle was greater than the supraspinatus ($p<.001$). Distribution of fiber diameter of the supraspinatus and deltoid muscles did not follow a normal distribution, which may indicate muscle atrophy. The deltoid muscle of seven patients did follow a normal distribution compared to three supraspinatus muscles. The results of this study suggest that the deltoid muscle is a viable option to use as a control in microscopic studies of rotator cuff muscles. When combined with the other phases of this research project the results of this study have the potential to provide insight into the mechanisms responsible for muscle atrophy and fatty infiltration in shoulder injury. This information can be used to guide new treatments and increase the effectiveness of current interventions.

Gratefully acknowledge the support entirely or in part by the following sponsors; CIHR, Dr. H.T. Thorlakson Foundation, MMSF, MHRC, Associate Dean (Research), Faculty of Medicine, St. Boniface Research Foundation, Health Sciences Centre Research Foundation, and Pan Am Clinic Foundation.

Introduction

Rotator cuff tears are a major cause of disability within Manitoba and throughout the industrialized world and often affect individuals between 35 and 70 years of age. Rotator cuff tears are associated with muscle atrophy and fatty infiltration, which may be caused by disuse, denervation or intrinsic changes within. These changes are progressive and may be irreparable despite successful surgical tendon reattachment[1].

Anatomy

The human shoulder comprises two joints. The acromioclavicular joint, formed by the articulation of the distal clavicle with the acromion, lies just above the glenohumeral joint, which is formed by the humeral head and glenoid fossa of the scapula. Several muscles attach and insert around the joint. Superficially, the pectoralis, deltoid, latissimus dorsi, teres major and biceps brachii cross the shoulder. Of particular importance in stabilization and control of the glenohumeral joint are the four, deep rotator cuff muscles; supraspinatus, infraspinatus, teres minor and subscapularis. The supraspinatus muscle inserts most superiorly at the greater tubercle of the humerus and abducts the arm for the initial fifteen degrees. The infraspinatus and teres minor muscles, two external rotators, insert inferiorly and posteriorly. Subscapularis, which internally rotates the humerus, inserts at the lesser tubercle and its anterior approach, combined with the superior and inferior insertion of the aforementioned muscles forms a tight 'cuff' around the humeral head. It is interesting to note that the suprascapular nerve, which innervates supraspinatus and infraspinatus, passes beneath the transverse scapular ligament, courses laterally along the floor of the supraspinous fossa of the scapula and around the spinoglenoid notch. These sites become potential points of nerve compression[2] that may lead to, or be a result of, tear of the supraspinatus muscle. The nerve gives two motor branches to the supraspinatus, the first of which is larger and occurs at or near the transverse scapular ligament. In addition to its motor supply, the suprascapular nerve also has two articular branches that relay sensory information from around the glenohumeral joint and surrounding strucutres[3].

Spectrum of disease

Injury to the rotator cuff is thought to occur along a continuum. Rotator cuff tendonopathy, a non-inflammatory degenerative tendon condition commonly referred to as shoulder impingement, is an early form of disease. Considered an overuse injury, it may be associated with repetitive overhead activity and ageing. Patients commonly present with pain or weakness with shoulder abduction. Injury progression can lead to actual tearing of the rotator cuff tendons from their insertion on the humerus. Tears can be full thickness or partial; partial tears are often described as being either bursal or articular referring to which side of the tendon is torn.

Prevalence of rotator cuff tears

While reports of rotator cuff tear prevalence vary, these injuries are a common problem. A recent population based study[4] found a prevalence of 21%. One cadaveric study demonstrated partial thickness tears in 28% of subjects as well as full thickness tears in 30%[5]. Using 456 cadaveric shoulders, Lehman et al[6] observed a full tear in 17%, whereas Reilly et al[7], in a large review comprising 2553 shoulders, report 12% having a full tear. Ultrasound has also been used to assess incidence. Tempelhof[8] reported on 411 asymptomatic patients of which 23% (96) had full thickness tears. Furthermore, he found that while 13% of patients in their fifties had tears, 31% of those in their seventies had a tear. Perhaps not surprisingly, 51% of participants who were eighty or older had a tear. Indeed, incidence is related to age. Milgrom[9] showed, using 90 asymptomatic patients, an increase in prevalence of tears between those in their forties and those fifty of older. MRI studies have also shown increased prevalence with age for both full and partial tears[10].

Mechanism of injury

Tears of the rotator cuff can result from traumatic injury where the tendon is forcefully pulled from its insertion; more commonly, the rotator cuff degenerates over time. While incompletely understood, the pathogenesis of chronic tears can be thought of in terms of intrinsic or extrinsic mechanisms. Intrinsic mechanisms refer to inherent properties of the tendon itself that predispose to injury. Studies have identified an area of hypovascularity in the supraspinatus tendon near its insertion , which, among tears, was associated with more extensive degeneration compared to other areas[11]. Others[12,13] have observed histological changes within torn rotator cuff tendons including disorganized collagen architecture and chondroid metaplasia. The structures around the tendon, particularly the acromion and the coraco-acromial ligament, may cause mechanical damage from above to the tendon as it travels below. While Neer[14] postulated compression of the tendon underneath the coraco-acromial arch, Bigliani[15] described patients as having either a flat, curved or hooked acromion.

Clinical assessment of rotator cuff pathology

Patient history typically includes stiffness, pain or loss of function. There may or may not be history of a traumatic event such as a fall on an outstretched hand. Pain may be worse at night leading to sleep loss. Several physical examination procedures can raise suspicion of rotator cuff problems. Inspection may reveal atrophy of the supraspinatus or infraspinatus muscles. Upper extremity weakness of external rotation, internal rotation or abduction may be present[16]. Perhaps not surprisingly, full thickness and larger tears have been associated with increased stiffness and greater functional deficits[16–18]. However, another study determined that pain or range of motion findings could not distinguish between full and partial thickness tears[19]. Furthermore, as mentioned above, cuff tears can be completely asymptomatic[10]. These findings indicate that clinical exam does not provide much detail regarding tears. Indeed, even special tests and signs such as a painful arc of abduction, Neer's, Hawkin's and Jobe's tests do not distinguish different pathologies on the spectrum of shoulder impingement and in light of their variable accuracy, findings should be considered cautiously[20,21]. While direct visualization at surgery is the gold standard for diagnosing a cuff tear, imaging studies provide an accurate and non-invasive work up for shoulder impingement. Ultrasound[22] and MRI[23,24] are both accurate and comparable[25] in detecting full thickness tears. However, ultrasound is much less reliable at detecting partial thickness tears and requires skillful technique and interpretation[22,26]. MRI accuracy at detecting rotator cuff tears, as well as its ability to distinguish and recognize several pathologies of the shoulder make it a valuable tool for assessing shoulder pain and loss of function[24].

Natural history and clinical decision making

With clinical suspicion of rotator cuff pathology and tear evidence on imaging, the orthopedic surgeon and patient must decide on some course of action. Factors concerning the patient most certainly include relief of pain and restoration of function. The surgeon must be able to inform the patient of potential benefits and risks with attempted cuff repair. Due to the obvious costs and risks associated with surgery, it is logical to first assess the merits of conservative, non-operative management. Maman et al[27] reported on 59 cuff tears at a mean follow up of 20 months. They found that more than 50% of full thickness tears increased in size compared to only 8% of partial thickness tears. For those older than 60 years, 54% had progressed while only 17% of patients 60 years or younger had progression. Another study[28] examined conservatively managed massive cuff tears (full thickness tears involving two or more tendons) at a mean 48-month follow up. Tear sizes increased and 50% of tears considered reparable at diagnosis were deemed irreparable at follow up. Conversely, Fucentese et al[29] found no significant change in mean tear size of isolated supraspinatus ruptures at minimum 2 years follow up. Furthermore, they noted that active range of motion for flexion, abduction and external rotation did not differ from diagnosis to follow up. While the clinical course of conservatively managed cuff tears may not be fully understood, tear size, tendon involvement, and age appear to have some influence. Numerous studies have looked at the risks and benefits of rotator cuff repair[30–33]. Unfortunately, massive tears have the possibility of re-tearing. Re-tear rates as high as 34%[31] and 44%[30] have been reported. Interestingly, despite structural failure of repair, clinical improvements in pain, range of motion, and functional status were still evident in patients with re-tears[30,31]. However strength may not recover fully[30]. Repair of isolated supraspinatus tears, although smaller in size, still have the potential for re-rupture[34]. Patient age with respect to outcome has conflicting findings. Voigt et al[34] suggest that patients older than 60 years are less likely to heal whereas Djah et al[33] reported improved outcomes in a study of patients older than 65 years.

Other factors relating directly to the muscle must be considered in order to further understand rotator cuff pathology and the potential for full restoration of structure and function. Following tendon tear, the muscle belly retracts medially[1]. Muscle atrophy ensues. Atrophy has also been shown to persist despite successful tendon reattachment[35]. This could explain why strength is often not regained. Another muscular change following tendon tear is fatty infiltration of the muscle[1,36]. Both muscle atrophy and fatty infiltration are related to severity of tear size[37,38] and are associated with poorer outcomes[35] after repair. Studies[39,40] have attempted to quantify these degenerate changes through CT and MRI. Imaging is readily available to the clinician and is useful in predicting outcomes based on tear size, and severity of atrophy and fat infiltration. However, analysis of pathological muscle tissue in patients with rotator cuff tears could provide us with a deeper understanding of the mechanisms associated with the degenerative change seen on MRI.

Advancing Repair

By understanding the cellular processes occurring in pathological muscle, and correlating these with clinical indices, better prediction of outcomes may become possible. Additionally, pharmacologic treatment options may become available which could augment surgical repair by attenuating and/or reversing atrophy and fatty infiltration. In other words, a more complete appreciation of the cellular details and mechanisms surrounding atrophy and fatty infiltration are necessary. Assessing muscle biopsies is an obvious approach. However, few studies[37,41–43] have involved biopsy of the human rotator cuff. Several abnormalities indicating pathological change can be assessed[44]. Hematoxylin and Eosin staining can reveal changes in muscle fiber size and shape as well as changes in nuclear location. We know variability of fiber size can characterize a pathological state. More advanced staining techniques can provide further information about fiber type patterns, cellular reactions, and protein expression. Other studies have turned to analysis of gene expression. Schmutz et al[37] found an upregulation of several enzymes involved in proteolytic pathways.

To our knowledge, there are very few studies regarding the human rotator cuff where all biopsies are obtained arthroscopically. Since the majority of rotator cuff surgeries are now being performed with this minimally invasive technique, we felt it necessary to establish a technique that allowed for the retrieval of muscle biopsies. The purpose of this study was to first characterize rotator cuff (supraspinatus) tears through clinical examination and MRI. Secondly, we wanted to compare muscle atrophy at the cellular level via muscle biopsy of the supraspinatus and deltoid muscles. Finally, we wanted to record the following variables that may have an association with findings of the overall study which extends beyond this research: age, height, weight, BMI, time from injury to surgery, smoking status, muscle atrophy and fatty infiltration.

Methods and Materials

Prior to the initiation of any study activities, approval was obtained from the local research ethics board and regional health authority research review board. Potential participants for the study were screened from the surgical wait list of participating surgeons and contacted by research staff to determine if the patient was willing to participate. After explaining the study in detail, potential participants were asked to complete an informed consent form. Eleven patients (n=11) agreed to participate in the study. All individuals had clinical evidence (history, physical exam and MRI findings mentioned above) suggesting a rotator cuff tear and had failed conservative management.

Muscle samples for cell culture

Culture media (Basal growth media (BGM): sterile DMEM+20% serum replacement+antibiotic+antimycotic, pH 7.4) was prepared in Dr. Anderson's lab, University of Manitoba. Prior to surgery, 240uL of BrdU was added to 12mL of BGM. This was then divided equally into separate 12mL tubes. One tube received 30uL of ISDN and would serve as media for the treatment group. The other tube was used as a control. Both the treatment tube and the control tube were divided equally. Thus four tubes for the cell culture experiment were taken to the operating room. Two contained ISDN and two did not.

Muscle samples for sections

One hour prior to surgery, two tubes containing 10mL 4% paraformaldehyde (PFA) were prepared for biopsy and transported to the operating theater.

Muscle pieces for western blotting

One hour prior to surgery, two RNA-later eppendorf tubes (1.5mL) were labeled, prepared for biopsy and transported to the operating theater.

Biopsy procedure

Surgery was performed at the Pan Am Clinic. During surgery, three biopsies were obtained from both the torn supraspinatus muscle and from the anterior deltoid (six total) using an arthroscopic basket punch. The deltoid was chosen as the control muscle since contralateral, asymptomatic rotator cuff tears are common[10]. Other rotator cuff biopsy studies[41,43] have also taken this approach. Specimens were inspected to ensure muscle tissue was present and any extra-muscular fat and connective tissue was removed in the operating room with a scalpel. For each muscle, the three specimens were divided in pieces and placed in corresponding conical tubes: 1) PFA 2) BGM+BrdU+ISDN, 3) BGM+BrdU, 3) and 4) RNA-later. Please note that tubes #2, 3 and 4 were obtained for phases of this project that are beyond the scope of the research study described here within. The four tubes containing BGM were kept at room temperature and the two PFA and two RNA-later tubes were kept on ice. Specimens were immediately transported to Dr. Anderson's lab at the University of Manitoba.

Cryostat sectioning

Once at the lab, samples that were placed in PFA were stored for 24 hours in four degrees Celsius. The tissue was then placed in cryoprotectant at four degrees and allowed to sink. Specimens were then frozen in isopentane and sectioned with a cryostat (International Cryostat MODEL CTI). Seven-µm sections were placed onto silanated slides.

Hematoxylin and Eosin Staining

The slides were stained with hematoxylin and eosin (H&E), to allow visualization of individual muscle fibers by light microscopy for measurement.

Measurement of fiber diameter

Measurements of fiber diameter, to characterize fiber size as per our objective, were carried out. Muscle sections were viewed at 10x magnification. Several pictures were taken of each section to ensure that at least 50 fibers could be measured from each specimen. ImageJ software was used to measure individual fiber diameter. During the measurement process, the pictures were blinded for patient and muscle (supraspinatus vs. deltoid). Due to the variability in appearance between the different sections, special attention was made to a uniform approach. Additionally, fibers that were not oriented approximately longitudinally were excluded.

MRI assessment

MRI scans took place at the clinic and were performed using a 1.5T Siemens MAGNETOM scanner (Siemens, Erlangen, Germany). As a quantitative measure of atrophy, cross sectional area of the supraspinatus muscle belly and of the supraspinatus fossa are calculated at the scapular Y-view[45]. Standardization is achieved by expressing muscle area relative to fossa area as the occupation ratio[45,46]. The tangent sign[46] is a qualitative indicator of atrophy. In the Y-view a line is drawn connecting the coracoid to the scapular spine. A positive sign is observed when muscle belly is completely below the line. In order to assess fatty infiltration, the Goutallier[39] CT scan classification system recently adapted for MRI[40] will be applied to the supraspinatus and infraspinatus. The scale runs from 0-4 describing intramuscular fat. A grade of 0 suggests no intramuscular fat. A score of 1 implies small streaks of fat but no significant accumulation. Grade 2 corresponds to significant fat but not as much as muscle. Grade 3 describes an amount of fat equal to that of muscle. Grade 4 is seen where there is more fat than muscle. MRI measurements were made first by finding the Y-view as described above. The image was exported and the previously described measurements were made with the use of ImageJ software.

Statistical analysis

The mean (SD) diameter of muscle fibers from the supraspinatus and deltoid muscles were determined for both the sample population as well as each individual participant. A student's T-test was used to determine the difference in muscle fiber diameter of supraspinatus muscle fibers compared to deltoid fibers grouped from all study participants. Paired T-tests were utilized to determine if there was a difference in muscle fiber diameter between supraspinatus and deltoid muscles from the same participant. Shapiro-Wilk tests were executed to test for normality of the distribution of the diameter of muscle fibers within study participants and between fibers collected from all study participants. The significance level was set at $p<.05$.

Results

Patient demographic data

As mentioned previously, 11 patients had completed an informed consent form and were enrolled in the study. There was no rotator cuff tear found at arthroscopy for one patient. No biopsy was taken and they were excluded from analysis. Ten patients (7 male, 3 female) were included in the analysis. Patient demographic information can be found in Table 1. Average age was 58.5 years (49-65). Three patients smoked or had a history of smoking. Six patients related their shoulder symptoms to an identifiable injury. Average time between onset of symptoms and surgery was 95 weeks. However, removal of an extreme outlier gives an average of 48 weeks (n=9).

Patient MRI findings

Routine MRI data for all 10 patients is displayed in Table 2. Nine of 10 patients had a full thickness supraspinatus tear. Two patients had tear extension to the infraspinatus tendon.

Patient MRI measurements

Table 3 displays measurements as previously described.

Arthroscopic findings

Arthroscopy confirmed a full thickness rotator cuff tear in nine patients and a partial thickness tear in one patient. Other diagnoses made at surgery included one labral tear, one partial subscapularis tear and three Type-2 SLAP lesions. Rotator cuff repair was performed in nine patients (all full-thickness tears). Biceps tenotomy (n=7), acromioplasty (n=10), bursectomy (n=4) and labral debridement (n=1) were also performed.

Muscle biopsy results

The mean deltoid muscle fiber diameter is greater than the diameter of fibers from the supraspinatus muscle when data from all participants is pooled together (Table 4). Within individuals, nine of the ten participants had a greater deltoid muscle fiber diameter compared to supraspinatus fibers ($p<.001$). The fiber diameter of the deltoid muscle in participant #9 was less than ($p<.001$) the fiber size of the supraspinatus muscle.

Distribution analysis of fibers from the deltoid and supraspinatus muscles indicated that data from neither muscle followed a normal distribution ($p<.001$, Figure 4). The distribution of muscle fibers from the supraspinatus muscle of seven out of ten participants also did not follow a normal distribution. Conversely, seven out of ten participants demonstrated that the fiber diameter of the deltoid muscle did demonstrate normality.

Discussion

The results of this study confirmed the difference in fiber diameter between the deltoid and supraspinatus muscles. This was evident not only in an analysis of the entire sample but within each individual patient, with the exception of one. Further, the normality of the distribution of fiber diameter was rejected when data for the entire sample was analyzed. Conversely, within participants the distribution of fiber area was normal in seven of ten deltoid muscles. The normality of the distribution of fiber area of the supraspinatus muscle was rejected in seven of ten patients and only normal in three. Since the normality of the distribution of muscle fibers has been used as a criteria to establish a normal (healthy) muscle, our preliminary results suggest that deltoid muscle may be a viable option as a control muscle when studying the rotator cuff.

Other studies have also compared human supraspinatus and deltoid in the setting of rotator cuff tears[41,43]. Irlenbusch[41] attempted to characterize change in fiber size between the supraspinatus and deltoid across a spectrum of rotator cuff disease states. In contrast to our study, samples were obtained via an open surgical technique. In addition to hematoxylin and eosin staining, the group also performed a Gomori trichrome stain as well as the nicotinamide adenine dinucleotide dehydrogenase reaction to distinguish between fast and slow muscle fibers. Fiber cross sectional area was used as the morphological marker of fiber size whereas the present study uses fiber diameter. A total of 37 samples were analyzed. Twenty-six of these samples were from rotator cuff tears. The present study examined 10 samples, all from rotator cuff tears. Interestingly, while our population consisted of almost entirely full-thickness tears (n=9), Irlenbusch reported 16 partial-thickness tears compared to 10 full thickness tears. A closer examination between studies with respect to patients with full thickness supraspinatus tears reveals some noteworthy comparisons. Among complete supraspinatus tears, the average patient age in their study was 54 (n=10). The average age of patients with a complete supraspinatus tear in our study is 58 (n=9). 80% of patients in their study were male, while 78% are male in ours. Perhaps the most interesting comparison, regarding atrophy and muscle itself, comes from that of duration of injury before the operation. They reported an average time of 36 weeks. Excluding one patient who reported 10 years of shoulder pain, for our group, the average time between injury/onset of symptoms to surgery is 43 weeks. This represents a difference of seven weeks. Atrophy of torn rotator cuff muscles is progressive[35]. It would make an interesting comparison between our two studies based on this difference. However, the fact that different indexes of size (cross sectional area vs. diameter) were used might make it difficult to compare.

Taking a functional approach to muscle fibers, Einarsson et al[43] also compared supraspinatus tissue from torn muscle to ipsilateral deltoid. Seven patients with a mean age of 66 years (range: 51-78) were biopsied with a vacuum needle biopsy. They also reported an average "tear age", defined as time from onset of symptoms to time of surgery, of 14 months (56 weeks). Compared to the present study, the difference is 13 weeks. While the main focus of their study was on mechanical testing of fibers, a comparison of fiber diameter between supraspinatus and deltoid showed no significant difference.

Steinbacher et al[42] examined supraspinatus biopsies of 18 patients with an average age of 62.5 years. They compared three different groups. Group 1 (n=6) consisted of patients with supraspinatus tears of 3-5cm. Group 2 (n=6) contained patients with tears greater than 5cm. Group 3 (n=6) was a control group of patients with intact rotator cuffs who underwent subacromial decompression for impingement. In contrast the present study, only contains one patient with a tear larger than 3 cm. This team used digital planimetry with light microscopy and quantitative stereology on electron microscopy to examine cellular and subcellular changes of muscle. They reported increased amount of intracellular lipid within Group 2 compared to Group 1. As discussed, fatty infiltration occurs in addition to atrophy. Research must focus on the underlying mechanisms of both processes. Also, Group 2 showed a decrease in the relative number of myofibrils.

Another biopsy approach, undertaken by Schmutz et al[37], has focused on gene expression of various transcription factors and proteins involved in the proteolytic cascade. While they did not identify a predominating pathway, many factors were elevated in large tears.

Of course the present study is not free of limitations. The arthroscopic basket punch technique used in obtaining the samples prevented the orientation of muscle fibers during sectioning. This resulted in the vast majority of fibers being oriented in the longitudinal direction as opposed to the cross sectional view. Unfortunately, this absence of cross sectional architecture prevents identification of many pathological changes[44]. However, measurements of fiber diameter, to characterize fiber size as per our objective, could be carried out. In addition to longitudinally oriented fibers, others were noted to pierce the plane of section at various angles. There was a high degree of variability of fiber number between specimens.

References

1. Gerber C, Meyer DC, Schneeberger AG, Hoppeler H, von Rechenberg B. Effect of tendon release and delayed repair on the structure of the muscles of the rotator cuff: an experimental study in sheep. *J Bone Joint Surg Am*. 2004;86-A(9):1973–1982.
2. Albritton MJ, Graham RD, Richards RS 2nd, Basamania CJ. An anatomic study of the effects on the suprascapular nerve due to retraction of the supraspinatus muscle after a rotator cuff tear. *J Shoulder Elbow Surg*. 2003;12(5):497–500.
3. Aszmann OC, Dellon AL, Birely BT, McFarland EG. Innervation of the human shoulder joint and its implications for surgery. *Clin. Orthop. Relat. Res.* 1996;(330):202–207.
4. Yamamoto A, Takagishi K, Osawa T, et al. Prevalence and risk factors of a rotator cuff tear in the general population. *Journal of Shoulder and Elbow Surgery*. 2010;19(1):116–120.
5. Jerosch J, Müller T, Castro WH. The incidence of rotator cuff rupture. An anatomic study. *Acta Orthop Belg*. 1991;57(2):124–129.
6. Lehman C, Cuomo F, Kummer FJ, Zuckerman JD. The incidence of full thickness rotator cuff tears in a large cadaveric population. *Bull Hosp Jt Dis*. 1995;54(1):30–31.
7. Reilly P, Macleod I, Macfarlane R, Windley J, Emery R. Dead men and radiologists don't lie: a review of cadaveric and radiological studies of rotator cuff tear prevalence. *Annals of The Royal College of Surgeons of England*. 2006;88(2):116–121.
8. Tempelhof S, Rupp S, Seil R. Age-related prevalence of rotator cuff tears in asymptomatic shoulders. *J Shoulder Elbow Surg*. 1999;8(4):296–299.
9. Milgrom C, Schaffler M, Gilbert S, van Holsbeeck M. Rotator-cuff changes in asymptomatic adults. The effect of age, hand dominance and gender. *J Bone Joint Surg Br*. 1995;77(2):296–298.
10. Sher JS, Uribe JW, Posada A, Murphy BJ, Zlatkin MB. Abnormal findings on magnetic resonance images of asymptomatic shoulders. *J Bone Joint Surg Am*. 1995;77(1):10–15.
11. Rathbun JB, Macnab I. The microvascular pattern of the rotator cuff. *J Bone Joint Surg Br*. 1970;52(3):540–553.
12. Fukuda H, Hamada K, Yamanaka K. Pathology and pathogenesis of bursal-side rotator cuff tears viewed from en bloc histologic sections. *Clin. Orthop. Relat. Res.* 1990;(254):75–80.
13. Longo UG, Berton A, Khan WS, Maffulli N, Denaro V. Histopathology of rotator cuff tears. *Sports Med Arthrosc*. 2011;19(3):227–236.
14. Neer CS 2nd. Anterior acromioplasty for the chronic impingement syndrome in the shoulder: a preliminary report. *J Bone Joint Surg Am*. 1972;54(1):41–50.
15. Bigliani LU, Ticker JB, Flatow EL, Soslowsky LJ, Mow VC. The relationship of acromial architecture to rotator cuff disease. *Clin Sports Med*. 1991;10(4):823–838.
16. McCabe RA, Nicholas SJ, Montgomery KD, Finneran JJ, McHugh MP. The effect of rotator cuff tear size on shoulder strength and range of motion. *J Orthop Sports Phys Ther*. 2005;35(3):130–135.

17. Mall NA, Kim HM, Keener JD, et al. Symptomatic progression of asymptomatic rotator cuff tears: a prospective study of clinical and sonographic variables. *J Bone Joint Surg Am*. 2010;92(16):2623–2633.
18. Seo S-S, Choi J-S, An K-C, Kim J-H, Kim S-B. The factors affecting stiffness occurring with rotator cuff tear. *J Shoulder Elbow Surg*. 2012;21(3):304–309.
19. Brownlow HC, Smith C, Corner T, Neen D, Pennington R. Pain and stiffness in partial-thickness rotator cuff tears. *Am J. Orthop.* 2009;38(7):338–340.
20. Park HB, Yokota A, Gill HS, El Rassi G, McFarland EG. Diagnostic accuracy of clinical tests for the different degrees of subacromial impingement syndrome. *J Bone Joint Surg Am*. 2005;87(7):1446–1455.
21. Kelly SM, Brittle N, Allen GM. The value of physical tests for subacromial impingement syndrome: a study of diagnostic accuracy. *Clin Rehabil*. 2010;24(2):149–158.
22. Teefey SA, Hasan SA, Middleton WD, et al. Ultrasonography of the rotator cuff. A comparison of ultrasonographic and arthroscopic findings in one hundred consecutive cases. *J Bone Joint Surg Am*. 2000;82(4):498–504.
23. Kneeland JB, Middleton WD, Carrera GF, et al. MR imaging of the shoulder: diagnosis of rotator cuff tears. *AJR Am J Roentgenol*. 1987;149(2):333–337.
24. Iannotti JP, Zlatkin MB, Esterhai JL, et al. Magnetic resonance imaging of the shoulder. Sensitivity, specificity, and predictive value. *J Bone Joint Surg Am*. 1991;73(1):17–29.
25. Naqvi GA, Jadaan M, Harrington P. Accuracy of ultrasonography and magnetic resonance imaging for detection of full thickness rotator cuff tears. *Int J Shoulder Surg*. 2009;3(4):94–97.
26. Ok J-H, Kim Y-S, Kim J-M, Yoo T-W. Learning curve of office-based ultrasonography for rotator cuff tendons tears. *Knee surgery, sports traumatology, arthroscopy: official journal of the ESSKA*. 2012. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22735978. Accessed July 25, 2012.
27. Maman E, Harris C, White L, et al. Outcome of nonoperative treatment of symptomatic rotator cuff tears monitored by magnetic resonance imaging. *J Bone Joint Surg Am*. 2009;91(8):1898–1906.
28. Zingg PO, Jost B, Sukthankar A, et al. Clinical and structural outcomes of nonoperative management of massive rotator cuff tears. *J Bone Joint Surg Am*. 2007;89(9):1928–1934.
29. Fucentese SF, von Roll AL, Pfirrmann CWA, Gerber C, Jost B. Evolution of nonoperatively treated symptomatic isolated full-thickness supraspinatus tears. *J Bone Joint Surg Am*. 2012;94(9):801–808.
30. Bartl C, Kouloumentas P, Holzapfel K, et al. Long-term outcome and structural integrity following open repair of massive rotator cuff tears. *Int J Shoulder Surg*. 2012;6(1):1–8.
31. Gerber C, Fuchs B, Hodler J. The results of repair of massive tears of the rotator cuff. *J Bone Joint Surg Am*. 2000;82(4):505–515.
32. Peters KS, McCallum S, Briggs L, Murrell GAC. A comparison of outcomes after arthroscopic repair of partial versus small or medium-sized full-thickness rotator cuff tears. *J Bone Joint Surg Am*. 2012;94(12):1078–1085.
33. Djahangiri A, Cozzolino A, Zanetti M, et al. Outcome of single-tendon rotator cuff repair in patients aged older than 65 years. *Journal of shoulder and elbow surgery / American Shoulder and Elbow Surgeons ... [et al.]*. 2012. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22743069. Accessed July 25, 2012.

34. Voigt C, Bosse C, Vosshenrich R, Schulz AP, Lill H. Arthroscopic supraspinatus tendon repair with suture-bridging technique: functional outcome and magnetic resonance imaging. *Am J Sports Med*. 2010;38(5):983–991.
35. Gladstone JN, Bishop JY, Lo IKY, Flatow EL. Fatty infiltration and atrophy of the rotator cuff do not improve after rotator cuff repair and correlate with poor functional outcome. *Am J Sports Med*. 2007;35(5):719–728.
36. Kim HM, Dahiya N, Teefey SA, et al. Relationship of tear size and location to fatty degeneration of the rotator cuff. *J Bone Joint Surg Am*. 2010;92(4):829–839.
37. Schmutz S, Fuchs T, Regenfelder F, et al. Expression of atrophy mRNA relates to tendon tear size in supraspinatus muscle. *Clin. Orthop. Relat. Res.* 2009;467(2):457–464.
38. Barry JJ, Lansdown DA, Cheung S, Feeley BT, Ma CB. The relationship between tear severity, fatty infiltration, and muscle atrophy in the supraspinatus. *Journal of shoulder and elbow surgery / American Shoulder and Elbow Surgeons ... [et al.]*. 2012. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22541866. Accessed June 22, 2012.
39. Goutallier D, Postel JM, Bernageau J, Lavau L, Voisin MC. Fatty muscle degeneration in cuff ruptures. Pre- and postoperative evaluation by CT scan. *Clin. Orthop. Relat. Res.* 1994;(304):78–83.
40. Fuchs B, Weishaupt D, Zanetti M, Hodler J, Gerber C. Fatty degeneration of the muscles of the rotator cuff: assessment by computed tomography versus magnetic resonance imaging. *J Shoulder Elbow Surg*. 1999;8(6):599–605.
41. Irlenbusch U, Gansen H-K. Muscle biopsy investigations on neuromuscular insufficiency of the rotator cuff: a contribution to the functional impingement of the shoulder joint. *J Shoulder Elbow Surg*. 2003;12(5):422–426.
42. Steinbacher P, Tauber M, Kogler S, et al. Effects of rotator cuff ruptures on the cellular and intracellular composition of the human supraspinatus muscle. *Tissue Cell*. 2010;42(1):37–41.
43. Einarsson F, Runesson E, Karlsson J, Fridén J. Muscle biopsies from the supraspinatus in retracted rotator cuff tears respond normally to passive mechanical testing: a pilot study. *Knee Surg Sports Traumatol Arthrosc*. 2011;19(3):503–507.
44. Dubowitz V, Sewry C. *Muscle Biopsy A Practical Approach*. 3rd ed. Philadephia: Saunders Elsevier; 2007.
45. Thomazeau H, Rolland Y, Lucas C, Duval JM, Langlais F. Atrophy of the supraspinatus belly. Assessment by MRI in 55 patients with rotator cuff pathology. *Acta Orthop Scand*. 1996;67(3):264–268.
46. Zanetti M, Gerber C, Hodler J. Quantitative assessment of the muscles of the rotator cuff with magnetic resonance imaging. *Invest Radiol*. 1998;33(3):163–170.

Table 1. Patient Demographics

F=female, M=male, Ht.=height, Wt.=weight, BMI=body mass index

Participant ID	Age	Sex	Ht.(m)	Wt. (kg)	BMI	Smoking	Onset of symptoms to surgery (weeks)
001	65	F	1.51	80.0	35.1	Yes	73
002	49	M	1.83	107.5	32.1	No	33
003	57	M	1.80	86.2	26.5	No	23
004	59	M	1.85	106.5	31.0	No	56
005	62	M	1.77	102.0	32.6	No	57
006	62	M	1.79	98.3	30.5	Yes	520
007	56	M	1.80	125.0	38.4	Yes	69
008	65	M	1.76	80.3	25.9	No	61
009	58	F	1.62	55.7	21.1	No	38
010	52	F	1.60	62.6	24.4	No	17

Table 2. Patient MRI Data

Participant ID	Tear size (cm)	Thickness	Tendons Torn
001	N/A	Partial	Supraspinatus
002	1.0	Full	Supraspinatus
003	2.6x4.2	Full	Supraspinatus with infraspinatus extension
004	1.8x2	Full	Supraspinatus
005	1.5	Full	Supraspinatus with infraspinatus extension
006	3.8	Full	Supraspinatus
007	2.0	Full	Supraspinatus
008	3.2	Full	Supraspinatus
009	2.0	Full	Supraspinatus
010	2.0	Full	Supraspinatus

Table 3. Patient MRI Measurements

Participant ID	Tangent Sign	Occupancy Ratio	Goutallier Score (0-4)
001	Negative	0.85	1
002	Negative	0.85	1
003	Negative	0.71	1
004	Negative	0.83	1
005	Negative	0.86	2
006	Negative	0.31	3
007	N/A	N/A	N/A
008	Negative	0.61	0
009	Negative	0.80	1
010	Negative	0.96	1

Table 4. **Fiber diameter of supraspinatus and deltoid muscle fibers compared within and between individual study participants.**

Deltoid (Delt) muscle fibers have a greater fiber diameter (p<.05) than fibers from the supraspinatus (SS) muscle when the means (SD) of all muscle fibers from each study participant are combined and analyzed with a student's T-test. The Shapiro-Wilk test was performed to determine the normality of the distribution of both the supraspinatus and deltoid muscle fibers. The distribution of muscle fibers from the supraspinatus and deltoid muscles of all study participants did not follow a normal distribution (p<.001 and p<.001, respectively). The significance level was set at p<.05.

Participant ID	SS µm (SD)	n	Normality	Delt µm (SD)	n	Normality	Mean diff Delt - SS	p-value
All	18.6 (8.0)	818	<.001	23.4 (8.8)	615	<.001	5.5	<.001
001	13.8 (2.9)	56	.030	28.9 (9.3)	56	.877	15.2	<.001
002	16.9 (4.2)	56	.251	25.7 (12.9)	56	<.001	8.8	<.001
003	19.1 (7.6)	96	<.001					
004	19.7 (4.1)	74	.041	23.1 (7.6)	74	<.001	3.4	<.001
005	18.2 (6.3)	73	.021	25.7 (8.2)	74	<.001	7.6	<.001
006	11.0 (2.5)	20	.953	15.4 (2.5)	20	.276	4.4	<.001
007	23.7 (7.8)	78	.042	27.8 (8.3)	78	.052	4.0	<.001
008	18.7 (4.6)	76	.312	21.5 (8.1)	76	.091	2.8	<.001
009	21.7 (7.0)	80	.017	17.4 (3.8)	80	.235	-4.3	<.001
010	9.5 (1.7)	101	.004	18.6 (4.7)	101	.294	9.2	<.001

Figure 1. Distribution of the diameter of fibers from the supraspinatus and deltoid muscles of all study participants.

Muscle fibers from the supraspinatus and deltoid muscles of all participants did not follow a normal distribution as indicated by the Shapiro-Wilk test ($p<.001$ and $p<.001$, respectively). Since a normal distribution indicates the muscle is normal, it is difficult to determine if both muscle groups were pathological at the time of surgery.