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The effects of chronic unloading and gap formation on tendon-to-bone healing in a rat model of massive rotator cuff tears

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Abstract

The objective of this study was to understand the effect of pre-repair rotator cuff chronicity on post-repair healing outcomes using a chronic and acute multi-tendon rat rotator cuff injury model. Full-thickness dual tendon injuries (supra- and infraspinatus) were created unilaterally in adult male Sprague Dawley rats, and left chronically detached for 8 or 16 weeks. After chronic detachment, tears were repaired and acute dual tendon injuries were created and immediately repaired on contralateral shoulders. Tissue level outcomes for bone, tendon, and muscle were assessed 4 or 8 weeks after repair using histology, microcomputed tomography, biomechanical testing, and biochemical assays. Substantial gap formation was seen in 35% of acute repairs and 44% of chronic repairs. Gap formation negatively correlated with mechanical and structural outcomes for both healing time points regardless of injury duration. Bone and histomorphometry, as well as biomechanics, were similar between acute and chronic injury and repair regardless of chronicity and duration of healing. This study was the first to implement a multi-tendon rotator cuff injury with surgical repair following both chronic and acute injuries. Massive tear in a rodent model resulted in gap formation regardless of injury duration which had detrimental effects on repair outcomes.

Keywords

shoulder; repair; chronicity; healing; rotator cuff

INTRODUCTION

Rotator cuff disease is a degenerative condition that can lead to significant shoulder pain, muscle atrophy, and tendon rupture, severely limiting upper extremity function. Surgical treatment of rotator cuff defects to restore function and treat pain is one of the most common orthopaedic procedures in the United States, with over 250,000 repairs performed each year¹. However, rotator cuff tendon healing is unpredictable, with short and mid-term failure rates ranging from 30–94%^{2,3}. Many factors have been associated with impaired rotator cuff

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tendon healing following repair, including increased age⁴, tear size², tendon and bone degenerative changes⁵, and gap formation between the repaired tendon and its bony insertion shortly after surgical repair⁶. Recently, we and others have shown that magnitude and duration of tendon injury prior to repair can strongly influence rotator cuff health^{7,8}, musculotendinous stiffness at time of repair⁹, and bone quality¹⁰. These factors result in an increased likelihood of developing a recurrent tendon defect after repair^{2,6}. However, little is understood about the mechanisms leading to poor healing outcomes following rotator cuff repair¹¹, and potential mediators of failed rotator cuff repair healing are difficult to control and assess in the clinical setting.

Chronic rotator cuff tears are associated with tendon retraction¹², fatty accumulation⁷, and muscle atrophy¹³. Prolonged detachment of rotator cuff tendons has been shown to lead to increased muscle stiffness¹⁴ and repair tension⁹, which has detrimental effects on post-repair healing outcomes. Additionally, chronic rotator cuff injury has been associated with reduced bone quality at the greater tuberosity¹⁵, with increased bone loss associated with impaired post-repair healing outcomes¹⁰.

Clinically, efforts have been made to improve surgical techniques in order to improve tendon healing. Double-row repair, for example, has shown promise in improving healing rates following rotator cuff repair¹⁶, especially in larger tears. However, degenerative changes to the muscles such as atrophy and fatty accumulation are irreversible at a certain point, regardless of the repair technique. The role of chronicity in large rotator cuff injuries is of particular interest as chronic tears have been linked to an increased likelihood of repair failure^{2,7}. The current study was performed to determine the effect of pre-repair rotator cuff chronicity on post-repair healing outcomes in the setting of a massive tear using an established model for rotator cuff repair. We hypothesized that: (1) increased chronicity of tendon injury prior to repair would be associated with impaired healing outcomes compared to acute injury prior to repair, and (2) repair site continuity, as measured by gap formation, would correlate with healing outcomes as measured by impaired repair site continuity, decreased bone quality, reduced tendon-to-bone mechanical outcomes, and altered muscle biochemistry.

MATERIALS AND METHODS

Study design

Adult male Sprague-Dawley rats (N=58) were used for this study following institutional Animal Studies Committee approval. Animals (N=52) were anesthetized with isoflurane carried by oxygen gas and subjected to unilateral, full thickness rotator cuff injuries of the supraspinatus (SS) and infraspinatus (IS) tendons without repair as described in the supplemental material on the right shoulder as well as sham injuries to the contralateral limb at time zero (t_0) (Figure 1). Rotator cuff tendon injuries in the right shoulders were left unrepaired for either 8 or 16 weeks, at which time SS and IS tendon injuries were surgically repaired, as described in the supplemental material, and the contralateral (sham) limb was subjected to acute SS and IS tendon injuries with immediate surgical repair. Following repair, a period of healing of either 4 or 8 weeks was allowed prior to euthanasia via carbon dioxide asphyxiation and thoracotomy (Figure 1). Thus, animals belonged to one of four experimental groups: all animals had either an eight or sixteen week chronic injury, and were sacrificed at four or eight weeks after repair (N=12–14 per group). All animals had an acute injury and repair on the contralateral side at the time of chronic repair. Previous studies established that 8 weeks of SS and IS tenotomy led to slight rotator cuff degeneration and 16 weeks of SS and IS tenotomy led to moderate rotator cuff degeneration⁸. An additional group of age-matched, uninjured animals (N=6) was used as controls.

Gross evaluation and microcomputed tomography

At the time of dissection postmortem, repair continuity was investigated by an orthopaedic surgeon (LC). The amount of gap formation between the SS tendon and anatomic bony insertion was measured using digital calipers (Absolute Digimatic CD-4" CSX, Mitutoyo America Corporation, Aurora, IL, USA). Specimens were carefully dissected to maintain scar and tendon continuity but with complete removal of skeletal muscle. Specimens allocated for histology (N=3 for each group) were immediately fixed in 4% paraformaldehyde. For bone morphometry, humeri of specimens designated for histology as well as for biomechanical testing (described below) were scanned in air using micro-computed tomography (microCT; Scanco μ CT40, Switzerland) with 20 μ m resolution at 45kV/177 μ A and 300ms integration time. Analysis of bone morphometry was performed using a volume of interest spanning approximately 2.5mm of the epiphysis of the humeral head from the proximal end, superior to the growth plate that included the insertional landmarks of the SS and IS tendons and excluded the cortical bone. Bone volume fraction (BV/TV), bone mineral density (BMD), trabecular architecture (thickness (Tb.Th.), number (Tb.N.), and separation (Tb.S.)) were measured using the manufacturer's software (Scanco, Switzerland). In addition to bone morphometry, scar volume was measured as all soft, non-mineralized tissue in a 4mm region near the tendon-to-bone attachment of the SS by thresholding against air. Following scanning, specimens were prepared for histologic assessment as described in the supplemental material or prepared for biomechanical testing.

Biochemistry

For biochemical assays (N=9), bilateral SS and IS muscles from a single experimental time point (acute and chronic 16wk injury and repair with 8wk healing duration) were harvested upon sacrifice halved along the length of the muscle, with one half frozen in liquid nitrogen and stored at -80°C until use in biochemical assays and the other half stored in glycerinated storage solution at 4°C until mechanical testing. Small ($\sim 2\text{mm}$ thick) samples of frozen muscle were homogenized in appropriate solutions for titin, collagen, and myosin heavy chain (MHC) distribution assays, respectively. For titin molecular weight assays, frozen muscle samples were pulverized and placed in sample buffer (8M urea, 2M thiourea, 30% glycerol, 0.05M Tris (pH 6.8), 75mM DTT, 3% SDS, 0.05% bromophenol blue)¹, vortexed thoroughly, and subsequently centrifuged. The molecular mass of titin in muscle samples was determined using sodium dodecyl sulfate-vertical agarose gel electrophoresis (SDS-VAGE)¹, as previously described². Human cadaver soleus (3.72 MDa) and rat cardiac muscle (2.99 MDa) were used as titin standards. Gels were run at 4°C for 4hr at a constant current of 15mA. A representative titin gel is shown in Figure S-2. For collagen assays, frozen muscle samples were hydrolyzed in 6N HCl, baked overnight at 110°C , and collagen content was determined using a colorimetric analysis for hydroxyproline following addition of chloramine T and pDAB³. Hydroxyproline content was converted to μg of collagen per mg of muscle ($\mu\text{g}/\text{mg}$) using a constant (7.46). For MHC distribution assays, frozen muscle samples were homogenized (250mM sucrose, 100mM KCl, 20mM Trizma base, 5mM EDTA, pH 6.8) with protease inhibitors on ice, then centrifuged. Supernatant were removed and pellets were resuspended in 200 μL of washing solution (175mM KCl, 20mM Trizma base, 2mM EDTA, 0.5% Triton X-100, pH 6.8). Samples were centrifuged again, supernatant removed, and pellets resuspended in 300 μL resuspension buffer (150mM KCl, 20mM Trizma base, pH 7.0). Protein was quantified using standard bovine serum albumin protein assays (ThermoScientific, Rockford, IL, USA) and MHC distribution of samples was determined using SDS-PAGE gel electrophoresis⁴ (Figure S-2).

Biomechanics

For muscle biomechanical testing (N = 9), muscle halves were tested within 2 weeks of harvest. Passive single-fiber and fiber-bundle muscle mechanics were tested as follows

using previously established methods⁵. Muscle fiber and fiber bundles were removed from storage solution and placed in relaxing solution⁵ where single fiber segments or fiber bundles (approximately 20 fibers) were carefully dissected and mounted in a chamber in a custom apparatus at room temperature. Fibers/bundles were secured using silk suture on one end to a force transducer (Model 405A, sensitivity 10V/g, Aurora Scientific, Ontario, Canada) and on the other end to a rigid titanium wire attached to a rotational bearing (Newport MT-RS; Irvine, CA). Sarcomere length was measured using transillumination with 7mW He-Ne laser⁶. Fiber diameters were measured in three locations along the fiber and averaged. Cross-sectional area was computed from this measurement assuming cylindrical muscle fiber geometry. After measurement of diameter and length, samples were stretched in 250 μm increments and force-displacement data were collected after 3 minutes of stress relaxation by measuring sarcomere length and tension. Both fibers and fiber bundles were tested in duplicate. Tension and sarcomere length data were converted to stress and strain, respectively, and fitted with a quadratic equation between 2–4 μm . Tangent moduli of these curves were calculated by taking the derivative of the quadratic equation and evaluation at a sarcomere length of 3.2 μm . Muscle fiber and fiber bundles were tested in duplicate. Tension and sarcomere length data were converted to stress and strain, respectively, and fitted with a quadratic equation between 2–4 μm . Tangent moduli of these curves were calculated by taking the derivative of the quadratic equation and evaluation at a sarcomere length of 3.2 μm .

For tendon-to-bone biomechanical testing (N = 10 in each group), cross sectional area of the SS and IS tendons were measured near the attachment sites using microCT. Growth plates were then secured using surgical wire and humeri were potted in poly-methyl-methacrylate. Repair-site sutures were cut and carefully removed to ensure no contribution of the suture during tension-to-failure tests. Specimens were tested in a phosphate buffered saline bath at 37 °C. Tests were performed on a uniaxial mechanical testing frame (Instron 5866, Norwood, MA) with an in-line 500 N load cell, custom fixtures and a thin-film top grip (Imada, Northbrook, IL). Tests were performed for tensile loading of the IS first with 5 cycles of preconditioning to 5 % strain at 0.1 %/sec, followed by stress relaxation (100 %/sec ramp, 5 % strain hold for 300 sec), a period of rest (300 sec), and extension to 3 N at a rate of 0.2 %/sec. Specimens were then reoriented for tensile loading of the SS with similar preloading and stress relaxation tests; however, for SS tendons, tendons were extended to failure at a rate of 0.2 %/sec. Strain was measured globally as grip-to-grip displacement relative to the initial gauge length for each tendon. Stiffness and Young's modulus were calculated by measuring the slope of the linear region of force-length and stress-strain curves, respectively, for both IS and SS tendons. Failure mechanisms were assessed for SS and maximum load, maximum stress, and toughness were calculated for the SS tendon.

Statistics

For morphometry results, a two-factor analysis of variance (ANOVA) was used to determine the effect of tendon injury duration (acute, chronic 8 week, chronic 16 week) and healing duration (4 week, 8 week) on BV/TV, BMD, Tb.Th., Tb.N., and Tb.S. Post-hoc analyses using Fisher's partial least squared differences were used to determine significant interactions between tendon injury duration and healing duration, and paired t-tests were used for intra-animal comparisons between acute/repair and chronic injury/repair outcomes. For biochemical and passive mechanical results, comparisons were made using paired t-tests to identify differences between injury duration (acute or chronic 16 week) following 8 weeks of healing on collagen content, titin molecular weight, single-fiber moduli and bundle moduli. A one-way ANOVA (injury duration) with repeated measures of % MHC (Type I, IIa, IIx, and IIb) was used to determine distribution of MHCs for SS and IS muscle following injury duration (acute or chronic 16 week) and 8 weeks of healing. For tendon-to-

bone biomechanical results, a two-factor ANOVA was used to determine the influence of injury and healing durations with a covariate of gap length. Bartlett Chi-square tests were used to compare between categorical variables (durations of injury/healing) and length of gap formation. Pearson correlations were used to determine the relationship between gap length and structural and mechanical outcomes. Post-hoc analyses of correlations between gap formation and biomechanical and morphometric outcomes was performed using Bonferroni probabilities. Significance was set to an alpha level of $p < 0.05$. All statistical analyses were performed using Systat 11.

RESULTS

At time of repair, chronic tendon injuries were more difficult to repair than acute injuries. Specifically, increased scar formation near the bony footprint and retracted tendons in the chronic injury were always present in the chronic injury shoulder, whereas intact tendons did not demonstrate any retraction, as these tendons were not previously detached. Marginal scar formation in the acutely injured shoulder was limited to the acromion, with scar formation not found near the bony footprints of the SS and IS at time of acute injury and repair. Post-repair healing with substantial tendon retraction, as measured by $>1\text{mm}$ of gap formation between the SS tendon suture and the native SS insertion site, was seen in 15 out of 43 (35%) acute repairs and 19 out of 43 (44%) chronic repairs. A representative repair with minimal gap formation is illustrated in Figure 2A, indicated by close proximity of the tendon suture to the bone and congruent insertion morphology. Healing with continuity but with substantial gap formation is illustrated in Figure 2B, highlighted by increased gap length from the tendon suture to the bone along with disorganized insertion morphology and increased scar formation. There were no significant correlations between injury or healing duration and length of gap formation (Bartlett Chi-square probability = 0.527). Gap formation was not correlated with either acute or chronic tears, nor did it correlate with duration of healing. However, scar volume was correlated with gap length (Figure 2C).

Microcomputed tomography was used to evaluate the effects of tendon detachment and repair on the bony architecture of the humeral head. Bone morphometry was similar between acute injury and repair with 8 weeks of healing and chronic injury and repair with 8 weeks of healing (Figure 3A). Injury and repair had a substantial effect on bone morphometric outcomes compared to uninjured, age-matched controls. All injury groups (acute and chronic) at both healing time points demonstrated significantly reduced trabecular BV/TV (Figure 3B) and trabecular BMD (Table 1) compared to uninjured, age-matched control animals. Bone morphometric outcomes did not differ in the presence or absence of gap formation for all injury and repair groups ($R^2 = 0.13$ for BV/TV, $R^2 = 0.06$ for BMD, $R^2 < 0.03$ for TbTh, TbN, and TbTh). Nearly all injury/repair groups (with the exception of acute injury-and-repair with 4 weeks healing) demonstrated significantly reduced Tb.N. compared to uninjured control animals (Figure 3C). Chronically injured and repaired humeri demonstrated trends for decreased Tb.N. (Figure 3C), increased Tb.S. (Table 1), and increased Tb.Th. (Table 1) compared to the contralateral, acutely injured and repaired limb, although these differences were not always statistically significant. There was a significant difference in Tb.S. between acute injury and repair and chronic 16 weeks injury and repair. Additionally, there was a significant effect of healing time, with an increase in Tb.S. and a decrease in Tb.N. between 4 weeks and 8 weeks of healing. Osteoclast surface area, measured using TRAP-positive staining, relative to total trabecular bone surface area (% osteoclast surface area), demonstrated increased coverage for all experimental groups and healing durations compared to age-matched uninjured controls (Figure S-1). There were no marked differences in tissue histopathology between acute and chronic injury groups for either 4 or 8 week healing time points (Table S-1).

There was no significant difference in titin molecular weight for the IS muscle (S-Table 2). No significant differences were seen in collagen content between acute and chronic (16 week) injury following 8 weeks of healing for either SS or IS muscles (S-Table 2). No significant differences were observed in passive mechanical outcomes at the fiber level between acute and chronic injury duration with 8 weeks of healing for either SS or IS muscles (Figure 4A). While significant differences were demonstrated between SS and IS muscles for MHC ratios of Type II fibers (Figure 4C), no significant differences were observed in MHC ratios between acute and chronic (16 week) injured groups following 8 weeks of healing for either SS or IS muscles (Figure 4C).

When examining the biomechanical adaptations of rotator cuff muscles following injury and repair, there was a significant increase in titin molecular weight when comparing acute and chronic (16 week) injury with 8 week of healing for the SS muscle ($p = 0.03$; Table S-2). Representative stress-strain plots of SS and IS muscles from acute injury & repair and chronic (16wk) injury & repair at both the fiber-level and bundle-level muscle mechanical outcomes are illustrated in Figure S-3. A significant increase in passive mechanical stiffness was observed following chronic (16 week) injury and repair, compared to acute injury and repair, at the bundle level for the IS, but not the SS, muscle following 8 week of healing (Figure 4B). For SS tendons, longer healing duration led to increased ultimate load (Figure 5A). However, there was no significant effect of injury group for most structural and material properties. Toughness was significantly reduced in the chronic (8 week) injury duration group compared to the contralateral acute injury group after 4 weeks of healing (Table 2). Additionally, chronic (8 week) injury duration led to a significant decrease in stiffness and Young's modulus for IS tendons following 4 week healing duration compared to their contralateral, acute injury and repair limbs (Table 2). Mechanical outcomes were negatively correlated with gap length for both healing durations. Specifically, gap length was the strongest predictor of SS mechanical properties, particularly maximum load following 4 weeks of healing (Figure 5B and C), as well as Young's modulus, maximum stress, and toughness (data not shown).

DISCUSSION

The influence of large rotator cuff tears on shoulder function and surgical reparability is a pressing clinical concern. A clinically-translatable animal model that can control for tendon tear magnitude, duration of detachment, and duration of healing may allow for the identification of targets for treatment, therapy, and rehabilitation following repair of the injury. In the current study, we investigated outcomes for the repair of large rotator cuff tears in a rodent model in the setting of acute and chronic tendon injuries. Previous reports demonstrated that the size of the rotator cuff tear correlates positively with fatty accumulation, atrophy¹⁶ and stiffness⁹ of the muscle, likely contributing to poor healing outcomes post repair². We have demonstrated in previous work that increased duration of rotator cuff injury via SS and IS tenotomy in the rat model can lead to gross degenerative changes in the skeletal muscle physiology⁷. Specifically, dual tenotomy of the SS and IS sustained for 8 or 16 weeks led to increased fatty accumulation⁸ in the SS and IS muscles. These parameters have been associated with clinical degeneration, and may hinder reparability, healing, and musculoskeletal health of the rotator cuff².

Findings from the current study, however, suggest that the impact of tear size, especially as it relates to the potential for post-repair gap formation, may dominate the healing outcomes, regardless of tear chronicity. We demonstrated that gaps often form between the tendon and bone after repair of large rotator cuff injuries in a rodent model. Gap formation correlated with impaired tendon structural and mechanical outcomes regardless of duration of initial tendon injury. This finding emphasizes the importance of initial fixation strength on repair

site integrity and healing, particularly following massive rotator cuff tears. Clinically, repair of large or massive tears often lead to high retear rates², regardless of duration of tendon injury. Improved surgical techniques and modifications have been suggested to encourage better healing outcomes post-repair, including the implementation of double-row repair¹⁷ and augmentation of repairs with scaffolds¹⁸. The terminology “failure with continuity” has been used to describe retraction of the tendon following repair without a recurrent defect⁶. It is unclear from the current study precisely what the timing and influence of gap formation had on functional adaptations to the rotator cuff complex. However, our data suggest that gap formation is the strongest predictor of repair success, overwhelming any effect from tear chronicity.

Other models of tendon repair, such as in the flexor tendon, have demonstrated a critical role for repair site congruency following surgical intervention^{19,20}. In the canine model of flexor tendon repair, a critical length of gap formation (>3mm) has been shown to lead to reduced strength and stiffness, independent of healing duration¹⁹. Several factors have been suggested to improve flexor tendon repair site congruency, such as post-repair mobilization²¹, muscle loading²², repair site tension²³, and gliding resistance²⁴, particularly at early healing time points. Of particular interest is the role of repair site loading, and its influence on repair site integrity and mobilization. While early controlled motion prevents adhesion formation following flexor tendon repair, positioning of the joint during rehabilitation can influence the magnitude of load observed at the repair site, and thus can influence the integrity of repair²⁵.

Although not addressed in the present study, the loading across the rotator cuff repair site may play a critical role in the formation of gap. It is speculated that tension of rotator cuff tendons at time of repair, as well as orientation and accuracy of positioning of the repaired tendons back to their bony footprints on the humeral head, played a role in gap formation following injury. In support of this premise, previous work has demonstrated that reduced loading during the post-operative period, in the form of cast immobilization, is beneficial to tendon-to-bone healing in the rat model²⁶. In the present study, we did not implement cast immobilization nor did we encourage exercise and increased mobility following rotator cuff repair. Immobilization was not feasible due to the bilateral study design that was used (i.e., both shoulders would need to be immobilized). The lack of post-repair immobilization is a limitation of this study, as gap formation may have occurred shortly after repair and continued to elongate throughout the healing duration.

The only time point that demonstrated differences between chronic and acute injury was the earliest time point (8 week chronic injury) with the shortest duration of healing (4 week), of which SS tendon-to-bone toughness and IS stiffness were significantly impaired in the chronic injury and repair shoulder compared to the contralateral acute injury and repair shoulder. While the SS tendon-to-bone attachment was robustly characterized in terms of mechanical and structural outcomes for the present study, it would be worthwhile to assess the mechanical outcomes of the IS in future studies. Previous work implementing rotator cuff injury and repair in a rodent model have focused primarily on the SS tendon-to-bone attachment^{9,10}; however, little emphasis has been placed on the injury and repair characteristics of the IS and the combinatorial role of dual-tendon (SS and IS) injury on healing. While the SS tendon is the most commonly observed rotator cuff tendon to present a tear²⁷, multi-tendon injuries can have greater clinical implications, such as increased risk of retear after repair² and decreased shoulder function²⁸. In the current study, we focused on determining the failure characteristics of the previously well-described SS tendon-to-bone attachment instead of the IS attachment. Due to overlapping SS and IS footprints of the repaired entheses, only one tendon could be tested to failure (i.e., the mechanics of the second tendon would be compromised during failure testing of the first tendon). Future work

will investigate healing of the IS in the multi-tendon rotator cuff injury model in order to better understand the reparative process following rotator cuff repair.

There were several limitations to the current study. First, the surgical repair employed a single-suture method to repair both SS and IS tendons to their native bony footprints. Previous work by Gimbel, et al, has demonstrated that chronic detachment of the SS tendon can lead to increased repair-site tension, which may in turn contribute to decreased mechanical properties⁹. Although not directly explored, it is possible that multi-tendon injuries to the rodent rotator cuff led to increased repair-site tension in the present study, leading to increased gap formation compared to previous studies of single rotator cuff tendon injuries. Second, we only assessed structural and mechanical outcomes at post-healing time points, and did not characterize repair gap formation, bone morphometry, or tendon organization at the time of repair. Chronic tendon injuries likely had detrimental effects on the epiphyseal bone, such as increased osteoclast activity and resorption, decreased bone volume, and impaired trabecular organization. While immobilization and whole-joint unloading has been previously shown to play a role in bone remodeling in other load-bearing joints²⁹ and tendon-to-bone attachments²², little work has been done to investigate the direct role of rotator cuff tendon unloading on bone morphometric adaptations near the bony footprints of the rotator cuff tendons¹⁰. However, recent clinical findings suggest the importance of characterizing bone quality, as it likely has implications on the success of arthroscopic rotator cuff repair³⁰. Therefore, future work will investigate the role of chronic tendon detachment with varying magnitudes of injury severity on bony adaptations without surgical repair. Lastly, this study did not quantitatively assess the presence of gaps at time of repair, but qualitative assessment at time of repair suggested potential difficulties in repair of the chronic side, but not the acute side, due to increased scarring and tendon retraction. However, differences between acute and chronic sides following 4 or 8 weeks of repair were not identified, which may suggest a critical role of repair and a potential masking of degenerative changes due to repair site failure via gap formation.

Massive rotator cuff tears are prone to failure after surgical repair, presumably due to poor healing. We hypothesized that tear chronicity would have a greater impact on repair healing than tear size, however, we found that tear size influenced gap formation and that this factor had a substantial impact on repair characteristics. Clinically, this supports maximizing the mechanical strength of the repair in order to minimize tendon pull off. While chronic changes in the muscles proximal to repair may render tears irreparable, tears will likely heal better when tendon remains well approximated to its bony insertion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Schematic of study design. Large, chronic rotator cuff injuries (Chronic injury) were induced via tenotomy of SS and IS on the right shoulders at time zero (t_0 , thin arrow), with Sham injuries in the left shoulders. After a period of 8 or 16 weeks of chronic injury (gray bars), rotator cuff injuries in the right shoulder were repaired (Repair, bold arrow) and an acute injury to the SS and IS in the left shoulder was induced and immediately repaired (Acute injury and repair; bold arrow). A 4 or 8 weeks period of healing post-repair was assigned prior to sacrifice (white bars), denoted by "X", and shoulders were processed for post-mortem assays.

Figure 2. Representative histological sections stained with Masson's trichrome of a post-repair SS tendon-to-bone attachment with (A) minimal gap formation and (B) substantial gap formation. (C) Gap length significantly correlated with scar volume at the tendon-bone attachment site. [Yellow arrowheads indicate location of suture holes].

Figure 3.

(A, B) Representative, intra-animal microCT reconstructions of the humeral head of acute injury-and-repair with 8wks of healing and 16week chronic injury-and-repair with 8wks of healing demonstrate slight morphological differences between sides. (C) BV/TV was significantly reduced following injury-and-repair, regardless of injury duration prior to repair, following both 4wks of healing and 8wks of healing compared to the uninjured control group. (D) Tb.N. also decreased following injury-and-repair. Tb.N. demonstrated a healing-duration and injury-duration trend. [* $p < 0.05$ compared to uninjured control.]

Figure 4.

(A) There were no differences between groups for muscle fiber tangent modulus. (B) There was a significant increase in bundle tangent modulus following chronic (16wk) injury and repair compared to acute injury-and-repair for the IS muscle. (C) No differences were observed between myosin heavy chain (MHC) fiber distribution when comparing acute injury-and-repair and chronic (16wk) injury-and-repair for SS and IS muscles. [Bar indicates significant difference, paired t-test ($p < 0.05$)].

Figure 5.

(A) The maximum load of SS tendon-to-bone repairs increased from 4wks to 8wks of healing duration, but was not influenced by chronic injury duration. (B) Maximum load was negatively correlated with gap length after 4wks of healing, and (C) this correlation was weaker after 8wks of healing. A significantly higher maximum load was observed for 8wks of healing compared to 4wks of healing.

Table 1

Bone morphometry parameters (BMD, Tb.Th., and Tb.S.) for uninjured controls as well as acute and chronic rotator cuff injuries with repair after 4wk and 8wk of healing.

Healing duration	Group	BMD ($\mu\text{g HA/mm}^3$)	Tb.Th. (mm)	Tb.S. (mm)
--	Control	444.5 $\pm$ 37.1	0.11 $\pm$ 0.01	0.35 $\pm$ 0.02
4wk healing	Acute	366.2 $\pm$ 43.0 *	0.10 $\pm$ 0.02	0.38 $\pm$ 0.06
	Chronic (8wk)	361.2 $\pm$ 51.8 *	0.10 $\pm$ 0.01	0.41 $\pm$ 0.09
	Chronic (16wk)	365.7 $\pm$ 32.9 *	0.11 $\pm$ 0.02	0.40 $\pm$ 0.05 *
8wk healing	Acute	362.5 $\pm$ 35.1 *	0.11 $\pm$ 0.01	0.43 $\pm$ 0.05 *
	Chronic (8wk)	360.3 $\pm$ 68.6 *	0.11 $\pm$ 0.01	0.46 $\pm$ 0.13 *
	Chronic (16wk)	309.7 $\pm$ 70.1 *	0.11 $\pm$ 0.01	0.56 $\pm$ 0.16 *

* significantly different than Control.

Tendon-to-bone biomechanics results for acute and chronic rotator cuff injuries with repair after 4wk and 8wk of healing.

Table 2

Tendon	Healing duration	Group	Stiffness (N/mm)	E (MPa)	σ_{max} (MPa)	Toughness (MPa)
SS	4wk healing	Acute	11.7 ± 9.37	7.6 ± 7.46	1.47 ± 0.84	0.29 ± 0.15
		Chronic (8wk)	9.6 ± 5.56	8.9 ± 8.15	1.36 ± 0.58	0.20 ± 0.06*
		Chronic (16wk)	9.2 ± 5.84	8.7 ± 3.82	1.54 ± 0.90	0.24 ± 0.17
	8wk healing	Acute	16.3 ± 7.20	14.2 ± 11.5	1.73 ± 0.93	0.29 ± 0.13
		Chronic (8wk)	11.0 ± 10.23	6.5 ± 4.93	1.66 ± 1.06	0.43 ± 0.18
		Chronic (16wk)	18.3 ± 7.74	19.3 ± 14.4	2.16 ± 1.11	0.21 ± 0.16
IS	4wk healing	Acute	7.2 ± 2.08	7.2 ± 5.23	---	---
		Chronic (8wk)	5.4 ± 1.90*	3.8 ± 1.34*	---	---
		Chronic (16wk)	6.8 ± 3.64	4.8 ± 3.33	---	---
	8wk healing	Acute	8.1 ± 2.92	7.4 ± 4.81	---	---
		Chronic (8wk)	6.8 ± 3.64	5.5 ± 2.88	---	---
		Chronic (16wk)	11.9 ± 7.74	9.3 ± 4.33	---	---

E = elastic modulus; σ_{max} = maximum stress.

* significantly different than acute injury and repair group for the same healing duration. Failure properties were not obtained for the IS tendons.