The role of repair tension on tendon to bone healing in an animal model of chronic rotator cuff tears

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Abstract

Rotator cuff tendon tears are one of the most common shoulder injuries. Although surgical repair is typically beneficial, re-tearing of the tendons frequently occurs. It is generally accepted that healing is worse for chronic tears than acute tears, but the reasons for this are unknown. One potential cause may be the large tensions that are sometimes required to repair chronically torn tendons back to bone (i.e., repair tension). Therefore, the objective of this study was to utilize an animal model of chronic rotator cuff repairs to investigate the role of increased repair tension on tendon to bone healing. We hypothesized that an increase in repair tension would be related to detrimental changes to the healing insertion site. To test this hypothesis, the supraspinatus tendon of rats was surgically detached and then repaired immediately or after a delay of 2, 4, or 16 weeks. The repair tension was measured using a tensiometer and the mechanical properties, collagen organization, and protein expression of the healing insertion site were evaluated 4 and/or 16 weeks following repair. We found that the repair tension increased with time following detachment, and was related to a decrease in the failure properties and viscoelastic peak stress and an increase in cross-sectional area and stiffness of the insertion site. Therefore, repair tension should be minimized in the clinical setting. Future studies will include additional animal model studies involving the relationship between tension and muscle properties and a clinical study investigating the role of repair tension on repair failure.

Keywords: Supraspinatus tendon; Rotator cuff; Shoulder; Chronic; Tension; Animal model

1. Introduction

There are more than 3 million visits to physicians each year in the United States concerning shoulder-related injuries (American Academy of Orthopaedic Surgeons, 1999–2003). One of the most common injuries are tears to the rotator cuff tendons of the shoulder, which are often associated with pain, instability, and decreased shoulder function (Cofield, 1985). When conservative treatments are not successful in managing this condition, surgery is often performed to repair the torn tendons back to bone. While this is typically beneficial, re-tearing of the tendons following repair frequently occurs partly because of inadequate tendon to bone healing (Harryman et al., 1991).

It is generally accepted that healing is worse for chronic tears than for acute tears, but the reasons for this are unclear (Warner and Gerber, 1998). Chronic tears are characterized, in part, by a retracted and stiff musculotendinous unit, and large tensions are sometimes required to repair the tendon back to bone (Hersche and Gerber, 1998). Since mechanical forces are thought to play a large role in the maintenance, remodeling, and healing of the tendon to bone insertion site (Woo et al., 1982), one potential cause for poor healing may be the large repair tensions. Evidence for this comes from a clinical study that has shown that an increase in repair tension has a negative effect on surgical outcome (Davidson and Rivenburgh, 2000).
However, this study did not specifically address the effect of tension on tendon to bone healing. Therefore, the objective of the current study was to use a previously published rat model of chronic rotator cuff repair (Gimbel et al., 2004a,c; Thomopoulos et al., 2002) to investigate the role of increased tension on tendon to bone healing of the rotator cuff.

Although there are certainly many other factors, aside from tension, that play an important role in healing of chronic tears (Iannotti, 1998), we chose to focus on tension since it can be utilized in clinical decision making. It can be directly measured during surgery and altered post-operatively by abducting the shoulder. Animal models are ideal for investigating the role of tension on tendon to bone healing since a consistent injury and repair scenario can be employed and the mechanical properties of the tendon to bone insertion site can be measured (Soslowsky et al., 1996). For the current study, the supraspinatus tendon of the rat was surgically detached and a delayed repair was performed to mimic the increased repair tension of chronic rotator cuff tears in humans. Repair tension was measured during surgery using a tensiometer and correlated to the mechanical properties of the healing insertion site. Collagen organization and protein expression at the insertion were also measured to evaluate potential causes for alterations in the mechanical properties. We hypothesized that an increase in tension would be related to detrimental changes in the mechanical properties, collagen organization, and protein expression of the healing insertion site.

2. Methods

2.1. Study design

A total of 95 rats were used in this study. Ten of the rats were not operated upon and served as an uninjured control. For the remaining rats, a tear was surgically created bilaterally by sharply detaching the supraspinatus tendon from its insertion site on the humerus using a scalpel as described in a previous publication (Gimbel et al., 2004c). The tear was then either immediately repaired (n = 40) or repaired after a delay of 2 (n = 15), 4 (n = 15), or 16 (n = 15) weeks using suture and a transosseous bone tunnel as described previously (Thomopoulos et al., 2002). For 15 of the 40 animals in the immediate repair group, the suture was cut after the repair was performed and the tendon was allowed to freely retract allowing scar tissue to form between the tendon end and insertion site under no-tension. Therefore, there were six study groups; an uninjured control group, a no-tension group, a 0 week immediate repair group, a 2 week delayed repair group, a 4 week delayed repair group, and a 16 week delayed repair group.

Prior to repair, the tendon was mobilized, the end of the tendon was identified by a suture knot placed at the time of detachment and grasped with double-armed 5-0 prolene suture. The scar tissue between the tendon end and insertion site was removed using a scalpel and bur. If repair tension measurements were performed for the shoulder, the peak tension required to reappose the tendon to its insertion site (i.e., repair tension) was measured using a custom tensiometer (Gimbel et al., 2004a). Following tension testing, the repair was completed by passing the suture through a transverse drill hole in the humerus and tying the tendon back to bone. Rats were allowed cage activity until the time of sacrifice at 4 or 16 weeks after repair. Mechanical, organizational, and immunohistochemical analyses were performed on shoulders at the insertion site 4 and/or 16 weeks after repair. This study was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Pennsylvania.

2.2. Mechanical analysis

Mechanical testing of the supraspinatus tendon to bone insertion site was performed as described in a previous publication (Thomopoulos et al., 2003a,b) 4 weeks following repair for at least one shoulder from 10 animals in all groups, except for the 0 week immediate repair group, where at least one shoulder from 25 animals were tested. Prior to testing, the humerus and supraspinatus tendon were isolated, Verhoff stain lines were applied, and the tendon area (width multiplied by thickness) was measured at the insertion (Soslowsky et al., 1994). The tendon–bone unit was then immersed in a 39 °C PBS bath, attached to an Instron materials testing machine (Model# 5543), preloaded to 0.1 N, preconditioned for 10 cycles from 0.1 to 0.5 N (1.875%/s rate, 8 mm gage length), and held for 300 s. A stress relaxation experiment was then performed (5% strain, 31.25%/s rate, 600 s relaxation period) followed by a constant strain rate ramp experiment (0.1875%/s rate) until failure. Local strain at the insertion strain was measured using an optical method (Derwin et al., 1994).

The peak load, equilibrium load, load ratio (peak/ equilibrium), and peak and equilibrium stress were calculated from the relaxation experiment. The data was also directly fit to Fung’s Quasilinear Viscoelastic (QLV) model (Fung, 1972) to extract additional elastic (A and B) and viscous (C, τ1, and τ2) parameters as described in previous publications (Gimbel et al., 2004b; Thomopoulos et al., 2003a,b). From the ramp to failure experiment, the stiffness and modulus were determined from the near-linear region and failure load and stress (i.e., ultimate load and stress) were determined if failure occurred at the insertion. Mechanical testing data was not analyzed in a small number of animals due to errors.
in dissection. If both shoulders were tested, the average for both shoulders was taken to be the resulting value for that animal. The repair tension values and mechanical properties were averaged for each group and compared using an ANOVA followed by a Fisher’s post hoc test for all properties except failure load and stress. Additionally, each animal was considered individually and the properties, including failure load and stress, were correlated with tension using a linear regression. Significance was defined as $p<0.05$.

2.3. Histology and collagen organization analysis

Histological and organizational analysis was performed at both 4 and 16 weeks following repair. Using standard histologic methods, 5µm sections of the bone–tendon–muscle unit from one shoulder of five animals in each group were cut and stained with hematoxylin and eosin (H&E). Specimens were then qualitatively evaluated along the tendon to bone insertion site for tissue structure and cell number. The angular deviation (AD) of the collagen, a measure of the spread in the fiber orientations, was also quantitatively measured using a polarized light microscopy method described in previous publications (Gimbel et al., 2004c; Thomopoulos et al., 2003a). A larger AD represents a more disorganized tissue. The AD was statistically compared ($p<0.05$) between groups at each time point using an ANOVA followed by a Fisher’s post hoc test.

2.4. Protein expression analysis

Immunohistochemical analysis was performed at 4 and 16 weeks following repair. Sections from one shoulder of five animals in each group were immunostained with antibodies for multiple collagens (types I, II, III, and XII) and proteoglycans (aggrecan, decorin, and biglycan) using 3,3’-diaminobenzidine (i.e., DAB) as described in a previous publication (Yokota et al., 2005). A specimen from each of the groups and a negative control was represented in each staining session, but each post-repair time point (i.e., 4 and 16 weeks) was done separately. The sections were then viewed under a light microscope and a digital image was taken at the insertion. Staining intensity, excluding the background, was measured using a custom Matlab program and each group was given a grade of undetectable or minimal (−), low (+), moderate (++), or high (+++). The grading scale was determined separately for each protein. This method was validated by comparing the grades obtained using this method to the average grade of three independent investigators who evaluated the same slides in a blinded manner.

3. Results

3.1. Repair tension and mechanical results

Repair tension increased with time from injury to repair as shown in Fig. 1. The geometric and mechanical properties were significantly different between repair groups and tension was linearly related to these changes. The average cross-sectional area and stiffness generally increased as the tension increased (Fig. 2a and b) and were significantly correlated with tension (Table 1). The modulus was not different between repair groups (Fig. 2c). Failure during mechanical testing occurred at the tendon to bone insertion site for 56%, 38%, 45%, 25%, and 70% of the specimens in the no-tension, 0, 2, 4, and 16 week repair groups. Failure load (Fig. 3a, Table 1) and stress (Fig. 3b, Table 1) were negatively and significantly correlated with tension. These correlations were significant despite the low $r^2$ values, which ranged from 0.22 to 0.56 (Table 1).

There were only a few significant differences in the viscoelastic properties and they were not necessarily linearly related to tension. The QLV parameter C was significantly decreased for the 2 week repair group relative to the no-tension and 16 week repair groups (Fig. 4a), and the load ratio showed a similar trend. The average peak stress generally decreased as tension increased (Fig. 4b) and was significantly correlated with tension (Table 1).

Lastly, the geometrical and mechanical properties of the repair groups were significantly different from the uninjured control group. The area of the injury groups was significantly larger and the modulus and the stiffness were significantly lower than the uninjured control (Fig. 2). Furthermore (e.g., Fig. 4), the uninjured specimens reached a significantly higher peak load and stress than the injury groups and maintained this with a
significantly higher equilibrium load and stress. Lastly, the elastic QLV parameters $A$ and $B$ were significantly larger than the injury groups and the uninjured specimens exhibited less relaxation at a faster rate than the injury groups as demonstrated by a significant decrease in $C$, load ratio, and $\tau_1$.

### 3.2. Histology and collagen organization results

The uninjured insertion site had parallel collagen fibers inserting uniformly into bone and a fibrocartilaginous transition zone was observed. The healing tissue 4 weeks following repair showed a large increase in cellularity, poor integration of the collagen fibers into the bone, and a significant increase in angular deviation of the collagen relative to the uninjured control (Table 2). At 16 weeks, the tissue showed signs of remodeling with a decrease in cellularity, improved integration of the collagen fibers into the bone, and a decrease in the angular deviation of the collagen relative to 4 weeks (Table 2).

### 3.3. Protein expression results

Differences in protein expression were detected between the repair groups, over time, and relative to the uninjured control (Table 3). There was a general increase in the expression for matrix proteins relative to the uninjured control 4 weeks following repair for all repair groups except the no-tension group. The expression for the no-tension and uninjured group was generally low, whereas the expression for the other groups was generally moderate. Four weeks after repair, expression for decorin and biglycan were either low or moderate and mainly contrasting, although the expression for these two proteins was not the same between injury groups. Protein expression generally diminished at 16 weeks following repair except for decorin and biglycan, which generally remained elevated relative to control.
The current study utilized a supraspinatus tendon detachment and delayed repair rat model to mimic the repair of chronic rotator cuff tears in humans. We measured the tension required to re-approse the tendon to its insertion site at the time of repair and the mechanical properties, collagen organization, and protein expression of the healing insertion site following repair to determine whether repair tension is related to healing. We found that repair tension significantly increased early after detachment and progressively increased with additional time. Moreover, the relationship between tension and healing was more complicated than initially expected. We found that tension was related to the greatest extent with a decrease in the failure stress. This was accompanied by a decrease in the failure load and viscoelastic peak stress and an increase in area and stiffness.

Although repair tension was not the only factor that changed with delayed repair, we believe that tension is, in part, responsible for changes in healing since our findings are consistent with some previous investigations. Remodeling of the patellar tendon in response to elevated stress has been investigated by surgically removing the lateral portions of the tissue (Hayashi, 1996; Yamamoto et al., 1999). The authors found that elevating the stress by 33% and 100% resulted in a decrease in the tensile strength for some tendons and an increase in the cross-sectional area partially restoring the failure load. In a previous study using the same model as the current study (Thomopoulos et al., 2003b), post-operative exercise, which most likely increases the

### Table 2
Collagen angular deviation of the healing insertion site 4 and 16 weeks following repair

<table>
<thead>
<tr>
<th></th>
<th>Angular Deviation (°)</th>
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<tbody>
<tr>
<td></td>
<td>4 weeks after repair</td>
</tr>
<tr>
<td>No-tension</td>
<td>42 ± 7*</td>
</tr>
<tr>
<td>Immediate repair</td>
<td>44 ± 7*</td>
</tr>
<tr>
<td>2 week delayed repair</td>
<td>37 ± 9</td>
</tr>
<tr>
<td>4 week delayed repair</td>
<td>43 ± 11*</td>
</tr>
<tr>
<td>16 week delayed repair</td>
<td>45 ± 12*</td>
</tr>
<tr>
<td>Uninjured</td>
<td>27 ± 5</td>
</tr>
</tbody>
</table>

The data is presented as mean ± standard deviation. The (*) designates a significant difference versus the uninjured control.

### 4. Discussion

The current study utilized a supraspinatus tendon detachment and delayed repair rat model to mimic the repair of chronic rotator cuff tears in humans. We measured the tension required to re-approse the tendon to its insertion site at the time of repair and the mechanical properties, collagen organization, and protein expression of the healing insertion site following repair to determine whether repair tension is related to healing. We found that repair tension significantly increased early after detachment and progressively increased with additional time. Moreover, the relationship between tension and healing was more complicated than initially expected. We found that tension was related to the greatest extent with a decrease in the failure stress. This was accompanied by a decrease in the failure load and viscoelastic peak stress and an increase in area and stiffness.

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tension, was found to result in an increase in the area of the healing insertion site. Lastly, the ultimate load of the healing flexor tendon in the dog was slightly, but not significantly, decreased by delaying repair for 21 days (Gelberman et al., 1991). This may partially support the decrease in failure load observed in the current study. Together with the current study, it appears that an increase in tension can have a detrimental effect on the failure properties of healing tissues and stimulate the production of scar tissue. The underlying mechanism responsible for the changes in the current study may be that increased tension may damage the insertion site, preventing proper integration of the tendon into bone, while at the same time stimulating cellular activity, increasing the amount of poor quality scar tissue produced.

Tension did not appear to be related to the collagen organization of the insertion site since we found no differences in the angular deviation of the collagen 4 weeks following repair. This finding is consistent with the similar modulus between groups, but somewhat surprising since an increase in tension might be expected to align the collagen fibers in the direction of loading. However, loading at the insertion is most likely multi-directional and larger tensions may produce larger loads in more than one direction or other aspects of organization may have changed that are not reflected in the angular deviation measurement. Lastly, organization improved toward normal from 4 to 16 weeks following repair, but still did not appear to be related to tension since it remained similar between groups.

Tension did, however, appear to be related to protein expression at the insertion. We expected that an increase in tension would result in a general decrease in protein expression. This was based on a previous study which found that a lower post-operative activity level generally had a greater mRNA expression (Thomopoulos et al., 2003b). Contrary to our expectation, we found that protein expression generally increased with tension, since the group with the lowest tension (i.e., the no-tension group) generally had the lowest protein expression. Although unexpected, this is supported by the smaller cross-sectional area for the no-tension group. Interestingly, this general trend was true for both aggrecan and collagen type II, which typically indicate a more well-established insertion (Thomopoulos et al., 2003a,b), even though the failure properties of the insertion were decreased with increased tension. However, increased tension may have resulted in larger shearing and/or compressive forces at the insertion (Vogel and Koob, 1989) which may have stimulated the expression of these proteins while at the same time damaging the structure of the insertion site. Lastly, protein expression generally diminished with time, with the exception of decorin and biglycan. These two proteins are thought to play contrasting roles in collagen fibrillogenesis and be important for the remodeling and maturation of scar tissue (Plaas et al., 2000). The continually elevated expression of these proteins suggests that they may serve an important role in the long-term maturation of the scar tissue.

The findings of the current study may provide insight into the reason for the high re-tear rate of repaired rotator cuff tendons in the clinical setting and how to possibly prevent these failures. Since we found that an increase in tension was indeed related to a decrease in failure properties, tendon to bone failure may be more likely for higher tensions in the clinical setting. This may partially explain the decrease in function found in patients with larger repair tensions (Davidson and Rivenburgh, 2000). Early repair or abducting the arm postoperatively may reduce the tension and prevent the likelihood of tendon to bone failure.

### Table 3
Protein expression at the tendon to bone insertion site 4 and 16 weeks following repair

<table>
<thead>
<tr>
<th></th>
<th>Col I</th>
<th>Col II</th>
<th>Col III</th>
<th>Col XII</th>
<th>Aggrecan</th>
<th>Biglycan</th>
<th>Decorin</th>
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<tbody>
<tr>
<td>4 weeks after repair</td>
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<tr>
<td>No-tension</td>
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<td>+</td>
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<tr>
<td>Immediate repair</td>
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<td>++</td>
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<td>++</td>
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<tr>
<td>2 week delayed repair</td>
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<tr>
<td>4 week delayed repair</td>
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<td>16 week delayed repair</td>
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<tr>
<td>16 weeks after repair</td>
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<td>No-tension</td>
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<td>Immediate repair</td>
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<td>2 week delayed repair</td>
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<td>4 week delayed repair</td>
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<td>16 week delayed repair</td>
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<tr>
<td>Uninjured</td>
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Expression was separately graded for each protein as undetectable or minimal (−), low (+), moderate (++), and high (+++).
There are some limitations of this study that should be considered. First, multiple factors other than tension are changing with time from injury to repair. Although we found that tension is indeed significantly related to healing, it is unlikely that tension is entirely responsible for these changes since other factors are likely to play an important role as well. This may explain the relatively low $r^2$ values that we obtained. Second, we did not measure how the passive tension changes for each group following repair. While tension may eventually become equivalent between groups, it appears that the changes in initial tension were sufficient to affect healing. Third, this is an animal model study and delayed repair may not be the same as chronic repair in humans. This is demonstrated by the large increase in cross-sectional area following delayed repair in the rat, which may not occur in humans. Future studies will investigate additional methods to model chronic repair in humans. Lastly, we did not evaluate failure of the repair since the scar tissue which is produced in response to the repair, as is typical in these types of surgical experimental models, made it difficult to determine whether failure occurred 4 or 16 week following surgery. We do not believe that failure of the repair is a significant problem, however, since previous studies in this animal model have optimized the surgical technique to prevent failure.

In summary, the repair tension rapidly increased following injury and was related to a decrease in failure properties and viscoelastic peak stress and an increase in tissue area and stiffness. The underlying mechanism responsible for these changes is unknown, but an increase in tension may cause damage while stimulating the production of scar tissue. The detrimental changes to the failure properties suggest that repair tension should be minimized, possibly by abducting the shoulder, to reduce the chance of failure in the clinical setting. Future studies will include a clinical study evaluating the relationship between repair tension, shoulder abduction, and repair outcome. Future studies will also include additional animal model studies involving the relationship between repair tension, tendon healing, and muscle remodeling.

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References


