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Biceps tenotomy in the presence of a supraspinatus tear alters the adjacent intact tendons and glenoid cartilage

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Introduction

The shoulder is the most mobile joint in the body and thus relies on dynamic restraint from the rotator cuff, which includes muscles and tendon from the supraspinatus, infraspinatus, upper and lower subscapularis, and teres minor (Fig. 1)(Anderson et al., 2012). Injuries involving the rotator cuff are common, and can lead to pain and joint dysfunction. These injuries are often sports-related and believed to be due to overuse, indicated by increases in cellularity, changes in cell morphology, decreased collagen fiber alignment, and decreased tendon mechanics (Soslowsky et al., 2000). These overuse injuries are common in athletes and workers who repeatedly perform overhead movements, such as swimming or throwing (Andrews et al., 1985; Mazoué and Andrews, 2006; Namdari et al., 2011). It is not uncommon for these groups of patients to return to their sport or activity shortly after treatment. If a rotator cuff tear is present, this can cause force imbalances, which have negative implications for the surrounding tissues if left untreated (Peltz et al., 2009; Perry et al., 2009; Reuther et al., 2013b). While past studies have determined the mechanical mechanisms by which rotator cuff tears lead to further joint damage (Reuther et al., 2014, 2013a, 2013b; Thomas et al., 2014), the implications of associated surgical procedures, such as full detachment of the long head of the biceps (LHB) via tenotomy, have yet to be fully examined.

The role of the LHB tendon as a shoulder stabilizer is debated; some believe it provides minimal stability (Levy et al., 2001; Yamaguchi et al., 1997) while others believe it assists the supraspinatus tendon in acting as a humeral head depressor (Alexander et al., 2013; Kido et al., 2000, 1998). Clinically, it has previously been reported that the LHB will develop pain and structural damage in the presence of a rotator cuff tear (Biz et al., 2012; Boileau et al., 2004; Mazzocca et al., 2013). Studies using a rat rotator cuff model have demonstrated structural damage in the LHB in the presence of an isolated supraspinatus-only (SO) tear (Peltz et al., 2011, 2010, 2009; Reuther et al., 2013b). Surgeons commonly recommend arthroscopic tenotomy in response to chronic symptoms in the LHB associated with rotator

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Conflicts of Interest

The authors have no conflicts of interest to report.

cuff tears. While LHB tenotomy in the presence of a rotator cuff tear has been shown to reduce pain and improve function (Kim et al., 2012; Szabo et al., 2008; Zhang et al., 2013), the effects of this surgery on the surrounding joint structures, including the intact rotator cuff and glenohumeral cartilage, are relatively unknown.

Past studies using our rotator cuff rat model have shown that different variants of rotator cuff tendon injury and overuse results in varying amounts of damage to the glenohumeral joint (Peltz et al., 2011, 2010, 2009, Reuther et al., 2014, 2013a, 2013b, 2012; Thomas et al., 2014). For example, concurrent detachment of the supraspinatus and infraspinatus (SI) tendons resulted in decreased glenohumeral cartilage thickness after 4 weeks of cage activity (Reuther et al., 2012). When overuse was applied to this same system, it resulted in decreased elastic modulus and thinning of the glenohumeral cartilage and alterations in shoulder function when compared to uninjured (Reuther et al., 2013a). This concurrent SI tear also showed decreased properties in the surrounding joint when compared to an SO tear in the presence of overuse (Reuther et al., 2014). Additionally, an LHB tenotomy in the presence of an SI rotator cuff tear resulted in improvements in subscapularis tendon properties, but minimal changes in shoulder function (Thomas et al., 2014). However, the effect of an LHB tenotomy, in the presence of an SO tear and overuse activity, on shoulder function, intact rotator cuff properties, and cartilage properties is currently unknown.

Therefore, the purpose of this study was to investigate the effect of full detachment of the LHB via tenotomy in the presence of an SO rotator cuff tear followed by overuse activity on ambulatory function and mechanical and histologic properties of the remaining rotator cuff tendons and glenohumeral cartilage. We hypothesized that an LHB tenotomy in the presence of an SO tear, followed by overuse, would result in negative changes in the surrounding tissues demonstrated by a decrease in joint function and mechanics and increased cellular activity in the intact tendons and glenoid cartilage.

Methods

46 adult male Sprague-Dawley rats (400–450 g) were used in this study. To acclimate the animals to their treadmill training environment, they were subjected to a 2-week training period, followed by 4 weeks of overuse activity (downhill (10°) treadmill running at 17 m/min for 1h/day, 5 days/week) (Soslowsky et al., 2000) to generate a clinically relevant tendinopathic condition in the supraspinatus tendon. Next, the animals were randomized into two groups: unilateral supraspinatus-only (SO) tendon detachment or detachment of the supraspinatus and LHB tendons (SB), as previously described (Peltz et al., 2009; Thomopoulos et al., 2002). This study was approved by the Institutional Animal Care and Use Committee. After surgery, animals were allowed 1 week of cage activity before gradually returning to the overuse training over 2 weeks. After treadmill training, all animals underwent an additional 5 weeks of overuse activity (Reuther et al., 2014, 2013a, 2013b; Thomas et al., 2014). The treadmill training protocol, including overuse, was constant across both groups. Sacrifice of both groups occurred 8 weeks after surgical detachment of the tendons. During dissection, animals were checked to confirm that the tendons detached at the time of surgery remained detached during training. The supraspinatus tendon always fills in with some scar tissue, which is normal for animal studies. This tissue is dramatically

mechanically inferior to native tendon and it is unlikely that load bearing capacity is achieved. The LHB tendon is known to be a highly tensioned tendon (Narvani et al., 2013) and retracted into the bicipital groove after surgery. For histology, tissues were immediately fixed in formalin. The remaining animals were stored intact at -20°C before mechanical testing. All methods utilized in the following sub-sections have been reported previously as referenced.

Quantitative Ambulatory Assessment

Forelimb gait and ground reaction forces were quantified using an instrumented walkway (Sarver et al., 2010). These parameters have been shown to be decreased in the presence of an injury (Sarver et al., 2010), and assist with the clinical translation of tissue-level animal studies where invasive methods of testing are not an option. Data was collected 1 day prior to tendon detachment to obtain baseline values, then collected at 3, 7, 14, 28, 42 and 56 days post-surgery. Ground reaction force data, including medial/lateral, braking, propulsion, and vertical forces were collected for each walk via force/torque cells that were mounted to two 70 mm long acrylic force plates. Cameras beneath the walkway were able to collect both ventral and sagittal (left and right) views of the animal due to mirrors placed along the sides of the walkway (Fig 2). Paw print analysis allowed for measurement of several temporal and spatial parameters, including stride length, step width and speed. At each time, at least two walks were recorded per animal, as well as body weight. For each animal, parameters were averaged across walks and normalized to body weight for each day.

Tendon Mechanical Testing

The animals were thawed, and the scapula and humerus were dissected with the infraspinatus and subscapularis tendons intact. Tendon testing was performed as previously described (Peltz et al., 2009; Thomas et al., 2012). Briefly, stain lines for local optical strain measurement (at insertion and midsubstance) were placed on the upper and lower bands of the subscapularis and infraspinatus tendons. Cross-sectional area was measured using a custom laser device (Favata, 2006). The scapula and humerus were embedded in a holding fixture using PMMA, gripped with cyanoacrylate annealed sand paper in custom grips, and immersed in PBS at 37°C . Tensile testing was performed as follows: preload to 0.08 N, preconditioning (10 cycles of 0.1–0.5 N at a rate of 1% strain/s), stress relaxation to 5% strain at a rate of 5% strain/s for 600 seconds, and ramp to failure at 0.3% strain/s. Stress was calculated as force divided by initial area, and 2D Lagrangian strain was determined from stain line displacements that were measured from images using custom texture tracking software (Perry et al., 2009).

Cartilage Mechanical Testing

The glenoid was prepared for cartilage mechanical testing by sharply detaching the LHB at its insertion on the superior rim of the glenoid in the SO group. The glenoid was then preserved by wrapping in soft tissue and freezing (-20°C).

For cartilage thickness measurements (Reuther et al., 2012), each scapula was thawed and immersed in PBS containing protease inhibitors (cOmplete ULTRA tablets, Sigma Aldrich, St. Louis, MO) at room temperature. Specimens were scanned at 0.25-mm increments using

a 55 MHz ultrasound probe (VisualSonics, Inc., Toronto, Ontario, Canada) in plane with the scapula. Captured B-mode images of each scan were segmented by selecting the cartilage and bony surfaces of the glenoid. The three-dimensional positions of these surfaces were reconstructed and used to determine cartilage thickness maps. Each map was divided into six regions (center (C), posterior-superior (PS), posterior-inferior (PI), anterior-superior (AS), anterior-inferior (AI), and superior (S)) and a mean thickness was computed for each region. After ultrasound scanning, specimens were wrapped in soft tissue and frozen (-20°C) until mechanical testing.

For cartilage mechanical testing (Reuther et al., 2012), each scapula was thawed and immersed in PBS containing a protease inhibitor cocktail at room temperature. Utilizing a 0.5-mm-diameter, nonporous spherical indenter, cartilage indentation testing was performed. Briefly, a preload (0.005 N) was followed by eight stepwise stress relaxation tests (8- μm ramp at 2 $\mu\text{m}/\text{second}$ followed by a 300-second hold). The scapula was repositioned for each localized region such that the indenter tip was perpendicular to the cartilage surface in each region. Cartilage thickness for indentation testing was determined by identifying the indentation location on each thickness map. Equilibrium elastic modulus was calculated, as described (Hayes et al., 1972), at 20% indentation and assuming Poisson's ratio ($\nu = 0.30$).

Histology

For histology, rotator cuff samples were left intact as bone-tendon-muscle units. For the glenoid cartilage, the glenoid was detached from the rest of the scapula at the glenoid neck. All samples were processed, longitudinal sections (7 μm) were collected, and tendon samples were stained with hematoxylin and eosin to assess cellularity and cell morphology, which, when increased, indicate higher levels of tissue remodeling. Cartilage samples were stained with safranin O, fast green and iron hematoxylin to visualize cartilage, mineralized tissue, and cellular nuclei, respectively. This gives insight into both the structure of the glenohumeral cartilage and cellular activity levels to elucidate the mechanisms behind the changes that are seen in joint-level function and cartilage-specific mechanics. Hematoxylin and eosin-stained tendon sections were imaged at the insertion site and midsubstance of each tendon at 200x magnification and were graded on a scale from one to three for cellularity and cell shape (a value of three corresponded to the largest cell number and most rounded cell shape, respectively) (Carpenter et al., 1998). Cartilage sections were imaged at 200x magnification in five regions (C, PS, PI, AS and AI) corresponding to the indentation locations and graded using a modified Mankin Score (Salo et al., 2002), with a higher score corresponding with more osteoarthritic characteristics. All histology scoring (for tendon and cartilage) was performed by three blinded investigators, and the mode of these three values was taken as the score for each specimen (Dourte et al., 2010).

Statistics

Mean and variation from preliminary studies were used to determine that 10 animals would be necessary to achieve a power of 80% at $p < 0.05$ for ambulatory assessment and tendon and cartilage mechanics. Previous studies have determined 5 animals contains sufficient power for histology. For ambulation data, to determine the effect of group and time, significance was assessed using a two-way ANOVA with repeated measures on time. If a

significant interaction effect was observed, follow-up paired *t*-tests were conducted to determine where the significant interactions occurred. Due to the nature of measuring rat ambulation, multiple imputations (~15% of data) were conducted on the ambulation data to allow for a repeated measures analysis. For tendon mechanics, significance was assessed using one-tail *t*-tests. For cartilage thickness and modulus, a one-tail Welch's unequal variances *t*-test was used for analysis. For tendon and cartilage histology, median grades were compared between groups within each region using a Mann-Whitney test. For all comparisons, significance was set at $p < 0.05$.

Results

The ambulatory, mechanical and histologic results for the SO group has been published in a previous study (Reuther et al., 2013b).

Ambulatory Data

The detachment of the LHB in the presence of a rotator cuff injury did not show any significant changes for ground reaction forces, temporal or spatial measures concerning ambulatory activity.

Tendon Properties

The lower subscapularis tendon did not show any changes in the area of the insertion or midsubstance between groups. However, the upper subscapularis tendon showed an increase in insertion area ($p = 0.05$) in the presence of LHB tenotomy (Fig. 3). Increases in midsubstance areas were also found in the infraspinatus ($p = 0.003$) and upper subscapularis ($p = 0.04$) tendons (Fig. 4). An increase in tendon midsubstance elastic modulus was also seen in the upper subscapularis ($p = 0.04$) tendon (Fig. 5). LHB tenotomy did not reveal statistically significant changes in elastic modulus in the insertion of the infraspinatus, lower subscapularis or upper subscapularis tendons. Histology showed a significantly increased score for cell shape ($p = 0.05$) in the midsubstance of the infraspinatus tendon, signifying that the cells present are more metabolically active (Table 1). The results showed no statistical changes in the lower subscapularis or upper subscapularis tendons in cellularity or cell shape in any region.

Cartilage Properties

The LHB tenotomy group showed a significant decrease ($p = 0.006$) in glenohumeral cartilage thickness in the AS region and a significant increase ($p = 0.02$) in the S region when compared to the intact LHB group (Fig. 6). The LHB tenotomy group also showed significantly greater equilibrium elastic modulus in the C ($p = 0.02$) and AS ($p = 0.006$) regions (Fig. 7). Histology also showed significant increases in modified Mankin score (Table 2) in the LHB tenotomy group in the C ($p = 0.03$), AS ($p = 0.007$) and PS ($p = 0.006$) regions of the glenohumeral cartilage (Fig. 8).

Discussion

Decreased joint function and pain are commonly associated with the LHB after experiencing a rotator cuff tear. To relieve these symptoms, a tenotomy is commonly performed on the LHB in conjunction with supraspinatus repair. However, the effect that this procedure has on the surrounding joint structure and function is unknown. Therefore, we hypothesized that LHB tenotomy in the presence of an SO tear, followed by overuse, would result in changes in the surrounding tissues demonstrated by a decrease in joint function and mechanics and increased cellular activity in the intact tendons and glenoid cartilage.

This study does have several limitations. Despite the similarities in bony anatomy, the rat rotator cuff model is not capable of perfectly replicating the human conditions seen clinically due to the different loading patterns seen in quadrupeds. However, the rat shoulder does produce large amounts of glenohumeral forward flexion, which increases the risk for subacromial impingement in the rotator cuff and LHB tendons. This allows the rat rotator cuff model to replicate repetitive overhead activity as seen in humans (Soslowky et al., 1996). We also acknowledge the limitations in the surgical procedure. This study performed an acute surgical detachment of the supraspinatus and LHB tendons. Rotator cuff tears usually develop gradually through chronic, degenerative conditions in the tendon. While this is not equivalent to the human condition, the animals did receive an overuse protocol for 4 weeks prior to tenotomy, which has been shown to create a tendinopathic condition of the supraspinatus tendon (Soslowky et al., 2000). The LHB tenotomy was also performed at the time of the acute detachment of the supraspinatus tendon, rather than what is done clinically where the LHB tenotomy occurs months or years after injury. Despite these limitations, our study design allows for a well-controlled comparison to investigate the role of the LHB tendon in the development of joint damage. Our results demonstrate that tenotomy of the LHB tendon in the presence of an SO tendon tear results in altered mechanics of the infraspinatus and upper subscapularis tendons and decreased histologic and mechanical properties in the glenohumeral cartilage. LHB tenotomy did not reveal significant changes in joint function.

We demonstrated that the LHB tenotomy did not result in any functional changes. This is consistent with past studies in this animal model (Thomas et al., 2014) that showed no changes in joint function when the LHB tenotomy was performed in the presence of a rotator cuff tear involving multiple tendons. However, this is inconsistent with clinical studies, which found improved joint function when a LHB tenotomy was performed in the presence of a rotator cuff tear in humans (Kim et al., 2012; Walch et al., 2005; Zhang et al., 2013). The improvements in joint function that are associated with LHB tenotomy could be due to decreases in pain, and not necessarily improvement in overall joint health (Kim et al., 2012; Zhang et al., 2013).

The LHB tenotomy group showed an increase in the insertion area of the upper subscapularis, increase in the midsubstance area of the infraspinatus and upper subscapularis, and an increase in the upper subscapularis elastic modulus. The infraspinatus tendon had a rounded cell shape, which is often seen in injured tendons (Soslowky et al., 2000). The changes seen in the infraspinatus and upper subscapularis tendons could be

caused by an interruption in the anterior-posterior force balance, which has been shown to be important to joint health and function (Reuther et al., 2014; Thomas et al., 2014). The anterior-posterior force balance is disrupted as a result of the LHB tenotomy, which has been shown to stabilize the humeral head and prevent translation in both the anterior and superior directions (Alexander et al., 2013).

The LHB tenotomy group showed increased thickness in the S region of the glenohumeral cartilage and a decrease in thickness in the AS region. The equilibrium elastic modulus showed increases in both the C and AS regions in the presence of the LHB tenotomy. Additionally, this group also showed an increased modified Mankin score, which represents an increase in arthritic properties, for cartilage histology in the C, AS, and PS regions of the cartilage. Past studies have shown that glenohumeral cartilage properties are decreased in the presence of rotator cuff tears (Reuther et al., 2013a, 2013b, 2012). These results show that LHB tenotomy exacerbates the negative effects associated with overuse activity after an SO tear (Reuther et al., 2013b, 2012). The altered mechanics of the glenohumeral cartilage, combined with the increased modified Mankin score, suggests that LHB tenotomy in the presence of an SO rotator cuff tear alters the loading in the superior half of the glenohumeral cartilage which could be due to increased humeral head translation in the absence of the LHB tendon (Alexander et al., 2013).

Clinically, this study shows the importance of the LHB tendon in the presence of a rotator cuff tear for the health of the remaining, intact tendons and glenohumeral cartilage health, which agrees with previous studies (Reuther et al., 2013a, 2013b, 2012). Cartilage damage and humeral head instability after LHB tenotomy has also been shown to be of concern among physicians, especially for young patients and athletes (Eakin et al., 1998; Elser et al., 2011). Our results indicate that the properties of the surrounding tendons and glenoid cartilage are altered in the presence of the LHB tenotomy, perhaps due to decreased joint stability (Alexander et al., 2013; Reuther et al., 2014; Thomas et al., 2014). Future studies are needed for further clinical investigation concerning LHB tenotomy in the presence of a rotator cuff tear in humans to find if the short-term pain-relief of the LHB tenotomy results in increased joint damage long-term. Future studies should also explore changes in the rotator cuff muscles as a result of injury and overuse to see a more comprehensive view of the tissues involved in this model.

In conclusion, our study showed that LHB tenotomy in the presence of an SO rotator cuff tear in a rat model had no effects on joint function. However, adjacent tendons showed altered mechanical properties and substantial effects were seen in the glenohumeral cartilage for both mechanical and histologic properties. This study provides evidence that LHB tenotomy in the presence of an SO tear negatively effects the surrounding joint structure. While an established in vivo animal model that mimics the human condition was used, randomized control clinical trials are needed to further examine the long-term consequences of LHB tenotomy on the glenohumeral joint.

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Figure 1.

Anterior and posterior views of the rotator cuff. The four muscles and tendons that make up the rotator cuff and provide dynamic support are the supraspinatus (SS), infraspinatus (IS), subscapularis, and teres minor (TM). The LHB tendon (arrowhead) also provides support to the humeral head by preventing anterior translation. Reprinted from Sports Medicine Clinics, Vol 31, Anderson MW, Brennan C, Mittal A, Imaging Evaluation of the Rotator Cuff, 605–631, Copyright 2012, with permission from Elsevier.

Figure 2.

Image of the instrumented walkway used to collect ambulatory data. The walkway is constructed from acrylic polymer that is 15 mm thick, 100 mm wide and 600 mm long. Two force/torque cells were mounted to two 70 mm long, 15 mm thick acrylic force plates, placed 150 mm from walkway entrance. Mirrors were placed along the sides of the walkway to provide multiple viewpoints for analysis.

Figure 3.

Tendon insertion area for the adjacent intact tendons (infraspinatus, upper subscapularis, lower subscapularis) for SO and SB groups. The group that received the biceps tenotomy had an increased insertion area in the upper subscapularis tendon ($p = 0.05$) when compared to the group without the biceps tenotomy. Data are shown as mean $\pm$ SD.

Figure 4.

Tendon midsubstance area for the adjacent intact tendons for SO and SB groups. The group that received the biceps tenotomy had an increased midsubstance area in the infraspinatus tendon ($p = 0.003$) and upper subscapularis tendon ($p = 0.04$) when compared to the group without the biceps tenotomy. Data are shown as mean $\pm$ SD.

Figure 5.

Tendon midsubstance modulus for the adjacent intact tendons for SO and SB groups. The group that received the biceps tenotomy showed an increase midsubstance modulus in the upper subscapularis tendon ($p = 0.04$) when compared to the group without the biceps tenotomy. Data are shown as mean $\pm$ SD.

Glenoid Cartilage Thickness

Figure 6.

Glenoid cartilage thickness at 6 regions (center (C), anterior-superior (AS), anterior-inferior (AI), posterior-superior (PS), posterior-inferior (PI), and superior (S)). The SB group had significantly lower thickness in the AS region ($p = 0.006$) and significantly higher thickness in the S region ($p = 0.02$) when compared to the SO group. Data are shown as mean $\pm$ SD.

Figure 7.

Glenoid cartilage modulus at 6 regions (center (C), anterior-superior (AS), anterior-inferior (AI), posterior-superior (PS), posterior-inferior (PI), and superior (S)). The SB group had significantly higher modulus in the C ($p = 0.02$) and AS ($p = 0.006$) regions when compared to the SO group. Data are shown as mean $\pm$ SD.

Figure 8.

Representative images of the glenoid cartilage are displayed (stain, safranin O, fast green and iron hematoxylin; original magnification, x200). Modified Mankin score was significantly increased in (A) the group with the biceps tenotomy compared to (B) the group without the biceps tenotomy.

Tendon histology was analyzed for differences in cell shape and cell density of the infraspinatus tendon. A significantly more rounded cell shape ($p = 0.05$) was found in the midsubstance of the infraspinatus tendon in the biceps tenotomy group when compared to the group without the biceps tenotomy. Data are shown as mean $\pm$ SD.

Table 1

Tendon	Location	Cell Shape		p-value	Cell Density		p-value
		SO	SB		SO	SB	
Infraspinatus	Ins	2.4 $\pm$ 0.9	2.6 $\pm$ 0.5	0.5	2.2 $\pm$ 0.8	2.0 $\pm$ 0.7	0.4
	Mid	1.00 $\pm$ 0.0	2.20 $\pm$ 0.8	0.05*	2.00 $\pm$ 1.0	1.60 $\pm$ 0.9	0.3

Table 2

The differences in modified Mankin score were analyzed in 5 different regions of the glenoid cartilage (center (C), anterior-superior (AS), anterior-inferior (AI), posterior-superior (PS), posterior-inferior (PI), and superior (S)). A significantly increased modified Mankin score was shown for the SB group in the C ($p = 0.03$), AS ($p = 0.007$) and PS ($p = 0.006$) regions when compared to SO. Values are expressed as median and interquartile range (Q1–Q3). SO = unilateral detachment of the supraspinatus; SB = unilateral detachment of the supraspinatus and long head of the biceps.

Region	Group	Modified Mankin Score	p-value
Center	SO	2.67 (2–4.33)	0.03*
	SB	6.00 (5.42–6.25)	
Anterior-Superior	SO	4.33 (4.33–4.33)	0.007*
	SB	6.67 (6.00–7.00)	
Anterior-Inferior	SO	4.67 (4.00–5.00)	0.1
	SB	5.33 (5.33–6.67)	
Posterior-Superior	SO	3.00 (2.67–4.00)	0.006*
	SB	5.00 (4.67–5.00)	
Posterior-Inferior	SO	4.67 (3.33–5.67)	0.2
	SB	5.33 (5.33–6.00)	