Influence Of Abduction Testing Angle On Biomechanics Properties Of The Rat Supraspinatus Tendon

Ryan Pomajzl, MD, Tristan Maerz, MS, Michael D. Kurdziel, MS, Michael Newton, BS, Abigail Davidson, Kevin Baker, PhD, Kyle Anderson, MD.
Beaumont Health System, Royal Oak, MI, USA.

Disclosures:

Introduction: Clinically, failure rates of healing after rotator cuff (RTC) tear and subsequent repair remains unacceptably high ranging from 16% for non-retracted tears in young subjects to 94% in massive RTC tears. It is estimated that RTC tears account for 4.5 million physician visits per year with over 250,000 surgeries performed annually in the U.S. Surgical advancements and treatments are a continuous topic of investigation. The rat rotator cuff model has been used extensively over the past 10-15 years as a model for rotator cuff disease, repair, and regeneration. Biomechanical testing of the intact and repaired tendon is widespread in literature, and biomechanics are often used to make conclusions regarding healing. There is a lack of a standardized biomechanical testing protocol to assess rat rotator cuff biomechanics, and studies have evaluated the supraspinatus at -15°, 0°, 15°, and 90° of abduction. As the supraspinatus tendon inserts in a fan-shaped enthesis, it is unclear how these changes in methodology impact biomechanical parameters and their subsequent implications in tendon-to-bone healing. Our study aimed to evaluate the extrinsic and intrinsic biomechanical parameters of the rat supraspinatus tendon in varying degrees of abduction angle during testing.

Methods: Shoulders from 30 mature, healthy female Sprague-Dawley rats were harvested en bloc and frozen at -20°C. Before testing, shoulders were thawed in cool saline and dissected to isolate the humerus and attached supraspinatus tendon. The tendon was meticulously dissected from its muscle belly. The humerus was potted in a polyester resin in a neutral position. Cross-sectional area (CSA) of each tendon was calculated using a laser-based system and averaged from a series of three measurements. Specimens were randomized into four test groups (n=6 per group) based on testing abduction angle: 0°, 30°, 60°, and 90° (Figure 1) and mounted to a materials testing frame (MTS Insight 5, Eden Prairie, MN, USA) in an environmental saline bath (pH 7.4) maintained at 39.1°C. A humeral head bracing plate was added to eliminate tensile failures of the humeral epiphysis, a common failure mode in unsecured humeri. The proximal end of the tendon was held in a clamp lined with delicate task wipes. Gage length was measured prior to testing, varying between 2.5-3.5mm, and all strain rates were adjusted accordingly. Specimens were then subjected to stress relaxation and ultimate failure testing. Briefly, specimens were preloaded to 0.1N, preconditioned for 10 cycles from 0.1 to 0.3 N at a rate of 0.5Hz, and then held at 0.1N for 300s. Specimens were then immediately elongated to a strain of 5% at a rate of 50%/s followed by a 1200s relaxation period. The specimens were allowed to fully recover prior to ultimate failure testing. For failure testing, specimens were preloaded to 0.1N, preconditioned for 10 cycles from 0.1N to 0.3N, and then loaded at a rate of 0.3%/s until failure was noted. Load and displacement data were collected via MTS software and analyzed with Matlab (v2013a, MathWorks, Natick, MA, USA). Peak stress, equilibrium stress, relaxation rate and percent relaxation were calculated from stress relaxation testing. Stiffness, elastic modulus, ultimate stress, ultimate strain, yield stress, and yield strain were calculated from failure testing. Parameters were compared between groups using one-way analysis of variance (ANOVA) with a modified Bonferroni post-hoc comparison with adjustment for type I error. Spearman rank order correlation was used to analyze correlations between abduction angle and mechanical properties to analyze trends with increasing abduction angle.

Results: There were no differences in CSA between investigational groups. The elastic modulus (Figure 2, top) was significantly greater at 30° (68.8 ± 24.3 MPa) of abduction compared to all testing angles of 0° (37.9 ± 15.4 MPa; P=0.017), 60° (30.2 ± 3.8 MPa; P=0.002), and 90° (22.0 ± 9.0 MPa; P<0.001). Similarly, tendon stiffness (Figure 2, bottom) was significantly greater at 0° of abduction (17.1 ± 4.0 N/mm) compared to both 60° (11.8 ± 1.8 N/mm; P=0.03) and 90° (6.3 ± 0.8 N/mm; P<.001). 30° of abduction (18.1 ± 3.7 N/mm) compared to 60° (P=.008) and 90° (P<.001), and 60° compared to 90° (P=0.023). Yield extension at 30° and 60° were significantly lower compared to 90° of abduction (0.34 ± 0.07 mm to 0.70 ± 0.32 mm, P=0.14 and 0.38 ± 0.05 mm to 0.70 ± 0.32 mm, P=.038, respectively). Notably, tendons at 90° of abduction had greater % relaxation during the stress relaxation experiment (63.1 ± 6.2%) compared to 0° (38.7 ± 16.1%, P=0.001), 30° (36.5 ± 3.5%, P<0.001), and 60° (47.2 ± 6.15, P=.014). Interestingly, there were no significant differences in ultimate stress between testing groups. There were numerous significant correlations between abduction angle and the following biomechanical parameters: yield load (r=−0.533, P=0.007, Fig 3A), % relaxation (r=−0.716, P<.001, Fig 3B), relaxation rate (r=−0.495, P=0.014, Fig 3C), stiffness (r=−0.808, P < .001), and modulus (r=−0.495, P=0.012).

Discussion: The rat supraspinatus tendon enthesis is a fan-shaped structure able to accommodate a large range of motion in
abduction-adduction. Though most studies in the literature do not share a standardized biomechanical testing protocol, this study demonstrates that the abduction angle at which the rat supraspinatus tendon is biomechanically tested has a profound impact on numerous biomechanical parameters. Data from our study indicates that the rat supraspinatus tendon is tightest in 30° of abduction with significantly greater stiffness and elastic modulus compared to higher angles of abduction. Numerous biomechanical parameters had strong correlations to increasing abduction angle. For example, our data shows that the yield load of the rat supraspinatus tendon decreases with increasing abduction angle. Furthermore, stiffness and modulus demonstrated a strong negative correlation with increasing abduction angle. This suggests that tendon fiber orientation and stiffness inherently vary across the range of motion spectrum. Future histologic analysis of collagen orientation in addition to QLV material constant derivation will further elucidate the effect of tendon orientation on tested and derived biomechanical properties.

**Significance:** The rat rotator cuff is an established and popular rotator cuff model. Most studies in the literature do not share a standardized biomechanical testing protocol, and data from this study indicates that the testing abduction angle has a significant effect on stress relaxation and failure biomechanical parameters.

**Acknowledgments:**

![Figure 1](image)

**Figure 1.** Rat supraspinatus tendon attached to a soft tissue clamp at 0°, 30°, 60°, and 90° of abduction prior to stress relaxation and ultimate failure testing.

**References:**
Figure 2. Rat supraspinatus tendon elastic modulus (MPa) (top) and stiffness (N/mm) (bottom) at 4 abduction angles after undergoing biomechanical testing. Overall, tendons tested at lower abduction angles were significantly stiffer than tendons tested at 60° and 90°. Tendons at 30° had a significantly greater compressive modulus than all other test groups.
Figure 3. Rat supraspinatus yield load (N) (A), % relaxation (B), and relaxation rate (1/min) (C) had