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Short communication

The influence of testing angle on the biomechanical properties of the rat supraspinatus tendon

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ABSTRACT

Rotator cuff tears are a common shoulder pathology. The rat supraspinatus tendon model is commonly employed for preclinical assessment of rotator cuff pathology or regeneration. However, there is a lack of a standardized biomechanical testing protocol; previous studies have tested the tendon at abduction angles ranging from $-15°$ to $90°$. This study aimed to assess the effect of abduction/testing angle on the biomechanical properties of the rat supraspinatus tendon. Fourty-eight shoulders ($n=12$/group) from healthy Sprague-Dawley rats were randomized to 4 testing angle groups: 0° (corresponding to 90° abduction), 30°, 60°, and 90° (0° abduction). Biomechanical testing of the supraspinatus was performed, consisting of stress-relaxation and load-to-failure. Mechanical properties were calculated, and nonlinear tensile modeling was performed via the Quasilinear Viscoelastic (QLV) and Structurally Based Elastic (SBE) models. Results indicate that testing angle significantly affects supraspinatus tendon biomechanics. Stiffness and modulus significantly decreased with increasing testing angle (stiffness: 20.93 ± 5.8 N/mm at 0° vs. 6.12 ± 1.0 N/mm at 90°, $P < .001$; modulus: 59.51 ± 34.0 MPa at 0° vs. 22.37 ± 7.4 MPa at 90°, $P = .002$). Testing angle correlated significantly to ultimate strain, yield strain, and all coefficients of the SBE and QLV models, implying differences in collagen fiber crimp patterns and viscoelastic behavior as a function of testing angle. These results suggest that differences in testing methodology, in particular testing angle, significantly affect the measured mechanical properties of the supraspinatus tendon. Future studies may consider utilizing testing angles of 0°–30°, at which tendon stiffness is maximized, and full standardization of rat rotator cuff testing protocols is necessary.

1. Introduction

Rotator cuff (RC) tears are one of the most common shoulder injuries in the United States (Aurora et al., 2007; Galatz et al., 2006; Gimbel et al., 2007b; Mannava et al., 2011). While surgical repair is a successful treatment option to alleviate pain and restore function, failure rates of RC repair remain unacceptably high, ranging from ~15% to up to -90% depending on retraction and tear size (Aurora et al., 2007; Hein et al., 2015; Levy et al., 2013; Peltz et al., 2010a). The most common site of rotator cuff tear is the supraspinatus tendon (Matava et al., 2005; Ubthoff and Loehr, 1987), and a substantial portion of clinical and preclinical research is focused on the injury and repair of this tendon (Boileau et al., 2005; Carpenter et al., 1998; Galatz et al., 2006; Liem et al., 2007; Longo et al., 2008; Peltz et al., 2009, 2010a). The supraspinatus tendon inserts in a fan-shaped enthesis onto the proximal humerus (Thomopoulos et al., 2006). Collagen fibers are predominantly oriented perpendicular to the tidemark at the tendon-to-bone insertion, deviate outwards with the natural "splay" of the fan-shaped enthesis (Thomopoulos et al., 2006), and run parallel down the length of the tendon (Thomopoulos et al., 2006, 2003b). Fiber orientation and crimp frequency are known to be heterogenous, and previous studies have demonstrated changes in supraspinatus fiber alignment and crimp frequency in response to tensile loading (Lake et al., 2009; Miller et al., 2012a, 2012b). These phenomena have been suggested as the bases for the nonlinear structural response of the tendon in tension (Lake et al., 2009; Miller et al., 2012a, 2012b).

The rat supraspinatus model has been extensively utilized as a model for RC pathology, repair, and regeneration (Carpenter et al., 1998; Cohen et al., 2006; Galatz et al., 2006; Gimbel et al., 2007b;

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Levy et al., 2013; Mannava et al., 2011; Mikolyzk et al., 2009; Peltz et al., 2010a; Ross et al., 2015), and results from biomechanical testing of the intact and repaired rotator cuff tendon are often used to draw conclusions regarding the overall integrity of the RC and/or success of a regenerative approach. However, there is a lack of a standardized testing protocol to assess rat RC biomechanics. Previously-published testing methodologies differ in many aspects, including strain rate to failure, endpoint strain and duration of stress-relaxation, as well as preload magnitude and duration. Consequently, reports of the biomechanical properties of the supraspinatus tendon vary widely in literature (Carpenter et al., 1998; Gimbel et al., 2004, 2007a; Ross et al., 2015; Thomopoulos et al., 2003a). Previous studies have evaluated the rat supraspinatus tendon at a range of abduction angles, including −15°, 0°, 20°, 50° and 90° (Carpenter et al., 1998; Galatz et al., 2006; Levy et al., 2013; Peltz et al., 2010a; Thomopoulos et al., 2003a), as well as at unspecified angles (Cohen et al., 2006; Gimbel et al., 2004, 2007a; Mannava et al., 2011; Thomopoulos et al., 2003b). The supraspinatus tendon and enthesis may exhibit differential biomechanical properties at varying angles of abduction due to differences in fiber orientation and crimping frequency, but, to date, no data exists assessing this potential effect.

The aim of this study was to evaluate the effect of abduction testing angle on the biomechanical properties of the rat supraspinatus tendon. We hypothesized that biomechanical parameters of the tendon will vary significantly as a function of testing angle.

2. Methods

2.1. Specimen preparation and biomechanical testing

Under an Institutional Animal Care and Use Committee (IACUC)-approved protocol for an unrelated study, healthy, adult, female Sprague-Dawley rats aged 14 weeks were euthanized via CO_2 asphyxiation. The humerus and attached supraspinatus tendon were dissected and prepared for mechanical testing. Cross-sectional area (CSA) was determined via a laser-based system, as previously described (Ross et al., 2015; Thomopoulos et al., 2003a) (see Supplemental information). Specimens were randomized into four test groups ($n=12$ per group) based on testing angle: 0°, 30°, 60°, and 90°, which represent a range of abduction angles (i.e. 0° testing angle is 90° of abduction). Specimens were mounted at their respective testing angles (Fig. 1) in an environmental chamber containing normal saline (.1 M, pH 7.4, 39.1 ± 1 °C) on a materials testing system (Insight 5, MTS, Eden Prairie, Minnesota, USA). Stress-relaxation testing was performed for 300 s at .1 N, followed by load-to-failure testing at a rate of .3% s^{-1}, as previously described (Peltz et al., 2009, 2010b). Full details of mechanical testing methodology are available in the supplemental methods. Peak stress, equilibrium stress, relaxation rate and percent relaxation were calculated from stress relaxation testing. Stiffness, elastic modulus, yield stress, yield strain, ultimate stress, and ultimate strain were calculated from failure testing.

2.2. Quasi-linear viscoelasticity model

Viscoelastic behavior of the tendons during stress-relaxation was modeled using the quasi-linear viscoelasticity (QLV) model, a widely used nonlinear model of tendon and ligament behavior (Fung, 1972; Gimbel et al., 2004; Kwan et al., 1993; Nigul and Nigul, 1987; Peltz et al., 2010b; Woo et al., 1981; Woo, 1982), as described in the Supplemental information. QLV elastic constants A, B, and viscous constants C, τ_1, and τ_2 were derived from stress-relaxation data using curve-fitting with a custom-built MATLAB program (v2013a, The Mathworks, Nattick, MA).

2.3. Structurally based elastic model

The structurally based elastic (SBE) model (Peltz et al., 2010b; Sverdlik and Lanir, 2002) was used to derive fiber slack length (μ) and fiber slack length standard deviation (σ) to model collagen fiber recruitment during load-to-failure testing, as described in the Supplemental information. SBE constants were derived from load-to-failure testing using curve-fitting with a custom MATLAB program.

2.4. Statistical analysis

The normality and equal variance assumptions were confirmed using the Shapiro–Wilk test and Levene's test, respectively. 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 testing angle and mechanical properties. Significance was determined at $P<.05$.

3. Results

3.1. Biomechanics

All specimens successfully underwent mechanical testing to yield $n=12$/group for all comparisons. Complete tabulated results of all measured variables can be found in Supplemental information. There were no significant differences in tendon CSA between specimens from varying testing angles. In results from stress relaxation testing, no significant differences in peak stress or relaxation rate were observed between testing angles. Equilibrium stress was significantly higher at 30° compared to 90°. Percent (%) relaxation was significantly higher at 90° compared to 0° and 30° (Fig. 2C). Testing angle correlated significantly to % relaxation ($\rho=.480$, $P=.001$).

In load-to-failure testing, tendons tested at 90° exhibited significantly lower stiffness compared to all other testing angles, and tendons tested at 60° also exhibited lower stiffness compared to 0° and 30° (Fig. 2A). Modulus was also significantly lower in 90° tendons compared to 0° and 30° (Fig. 2B). Tendons tested at 90° exhibited significant increase in ultimate strain compared to 0° and 30° and a significant increase in yield strain compared to those tested at 30°. Ultimate stress and yield stress did not vary significantly between groups. Testing angle correlated significantly to

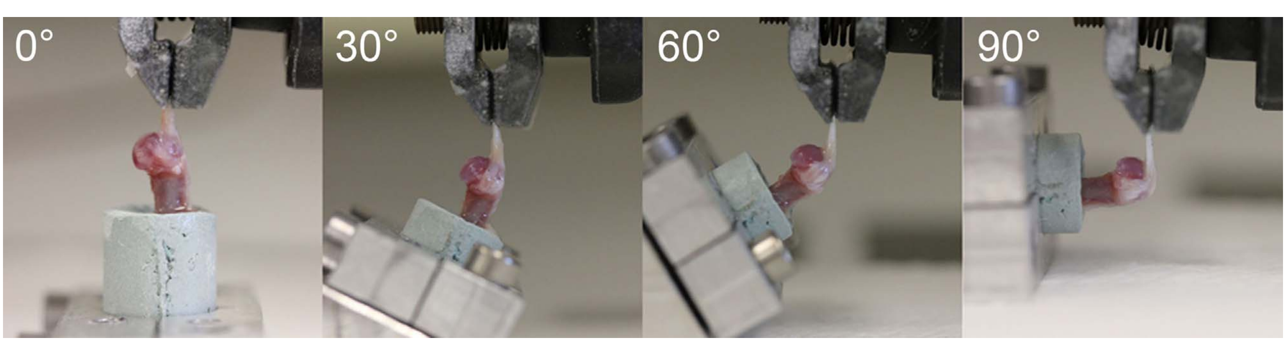

Fig. 1. Testing angles of 0°, 30°, 60°, and 90°, depicted without the attached humeral head compression plate or environmental test chamber used during final testing. Specimens were potted in polyester resin and mounted in a custom testing fixture. Fixed angle blocks were used to achieve the 4 testing angles.

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