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Biaxial Tensile Testing and Constitutive Modeling of Human Supraspinatus Tendon

The heterogeneous composition and mechanical properties of the supraspinatus tendon offer an opportunity for studying the structure-function relationships of fibrous musculoskeletal connective tissues. Previous uniaxial testing has demonstrated a correlation between the collagen fiber angle distribution and tendon mechanics in response to tensile loading both parallel and transverse to the tendon longitudinal axis. However, the planar mechanics of the supraspinatus tendon may be more appropriately characterized through biaxial tensile testing, which avoids the limitation of nonphysiologic traction-free boundary conditions present during uniaxial testing. Combined with a structural constitutive model, biaxial testing can help identify the specific structural mechanisms underlying the tendon's two-dimensional mechanical behavior. Therefore, the objective of this study was to evaluate the contribution of collagen fiber organization to the planar tensile mechanics of the human supraspinatus tendon by fitting biaxial tensile data with a structural constitutive model that incorporates a sample-specific angular distribution of nonlinear fibers. Regional samples were tested under several biaxial boundary conditions while simultaneously measuring the collagen fiber orientations via polarized light imaging. The histograms of fiber angles were fit with a von Mises probability distribution and input into a hyperelastic constitutive model incorporating the contributions of the uncrimped fibers. Samples with a wide fiber angle distribution produced greater transverse stresses than more highly aligned samples. The structural model fit the longitudinal stresses well (median $R^2 \geq 0.96$) and was validated by successfully predicting the stress response to a mechanical protocol not used for parameter estimation. The transverse stresses were fit less well with greater errors observed for less aligned samples. Sensitivity analyses and relatively affine fiber kinematics suggest that these errors are not due to inaccuracies in measuring the collagen fiber organization. More likely, additional strain energy terms representing fiber-fiber interactions are necessary to provide a closer approximation of the transverse stresses. Nevertheless, this approach demonstrated that the longitudinal tensile mechanics of the supraspinatus tendon are primarily dependent on the moduli, crimp, and angular distribution of its collagen fibers. These results add to the existing knowledge of structure-function relationships in fibrous musculoskeletal tissue, which is valuable for understanding the etiology of degenerative disease, developing effective tissue engineering design strategies, and predicting outcomes of tissue repair. [DOI: 10.1115/1.4005852]

Keywords: tendon mechanics, structure-function, collagen fiber alignment, biaxial tension, constitutive model

1 Introduction

The supraspinatus tendon is part of the myotendinous complex that makes up the rotator cuff and helps provide glenohumeral joint stability and abduction [1]. Like most tendons, it is composed primarily of water, collagen, and small leucine-rich proteoglycans [2,3]. However, unlike most tendons, the supraspinatus has heterogeneous compositional and mechanical properties. Rather than simply running parallel to the tendon's longitudinal axis, collagen fibers follow wide angular distributions that vary across the tendon length and thickness [4–6]. The longitudinal tensile modulus, tensile failure stress, and compressive stiffness

also exhibit regional variations [6–9], suggesting a strong relationship between the local structure and function of the supraspinatus tendon. Such complexity likely reflects the multiaxial mechanical environment of the supraspinatus tendon resulting from its position over the humeral head, contact with the acromion, anatomical confluence with the infraspinatus tendon, and the wide range of shoulder motion [10–16]. Investigating the structure-function relationships of the supraspinatus tendon may further explain the significance of its atypical material properties and offer insight into the mechanical behavior of fibrous musculoskeletal connective tissues in general.

Previous studies have used uniaxial tensile loading applied both parallel and transverse to the tendon axis to investigate the relationship between collagen fiber organization and regional supraspinatus tendon mechanics [8,17]. Specifically, highly aligned regions of the supraspinatus were shown to have significantly greater longitudinal tensile moduli than regions with a wider fiber angle distribution. Regions with less fiber alignment exhibited much higher moduli transverse to the tendon axis, with

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some regions displaying near planar isotropic behavior [17]. Nevertheless, results from uniaxial testing may not appropriately characterize the planar mechanics of tissues that experience multi-axial loads in vivo, such as the supraspinatus tendon. For example, not constraining the unloaded edges of the annulus fibrosus of the intervertebral disc during uniaxial tension produces lower apparent mechanical properties compared to using more physiologic boundary conditions [18,19]. Biaxial tensile testing obviates these concerns by providing control of the two-dimensional tissue strain and has been used to evaluate the planar mechanics of a wide variety of biological tissues [19–25].

While constitutive models are necessary to describe the mechanical behavior of a given material, choosing the appropriate model is important for meaningful interpretation of experimental data. For example, natural variability of tissue structure in biological samples can impair accurate calculations of material properties using pure phenomenological models [26,27]. In contrast, structural models directly incorporate tissue composition and organization, thereby accounting for inter-specimen structural differences. Furthermore, these models enable identification of the individual mechanisms underlying tissue mechanical behavior and allow for quantification of their relative importance. Specifically, Lanir [28,29] developed a model that integrates the strain energy of individual fibers oriented over a given angular distribution to determine the global tissue mechanical response. Application of this model to biaxial tensile data enables the evaluation of the relationship between the collagen fiber orientation and planar tissue mechanics of the supraspinatus tendon under near physiologic loading conditions.

The objective of this study was to evaluate the contribution of collagen fiber organization to the planar tensile mechanics of the human supraspinatus tendon by fitting biaxial tensile data with a structural constitutive model that incorporates a sample-specific angular distribution of nonlinear fibers. We hypothesized that the measured levels of both longitudinal and transverse stresses will be correlated with the angular distribution of collagen fibers and that the model will successfully capture the relationship between mechanical anisotropy and fiber organization.

2 Methods

2.1 Sample Preparation. A total of forty-eight samples were harvested from eight human supraspinatus tendons (age 69 ± 6 years). Twenty-seven samples were used during preliminary testing and twenty-one samples were used for the data collection presented here. Donors with a prior history of shoulder injury were excluded from the study, as were tendons with visible tears. Similar to the protocol of Lake et al. [8], $10 \times 10 \text{ mm}^2$ samples were cut from three regions of each tendon (anterior, posterior, and medial) with their edges parallel and perpendicular to the tendon longitudinal axis (Fig. 1(a)). They were then frozen and sharply bisected at their midplane thickness, yielding bursal- and joint-sided samples for each region. The twenty-one samples were equally divided among the anterior, posterior, and medial regions. For each region, roughly half of the samples came from either the bursal ($n=11$) or joint ($n=10$) surfaces. A freezing stage microtome (SM2400, Leica Microsystems, Germany) was used to trim the samples to a thickness of $400 \mu\text{m}$. After thawing the samples in phosphate buffered saline (PBS) for approximately 15 min at room temperature, their thickness was measured using a noncontact laser device.

To provide attachment points for mechanical testing, cloth sandpaper tabs were glued (Loctite 454, Henkel, Germany) to each edge of the sample, with $\sim 1 \text{ mm}$ overlap between the tab and the tissue (Fig. 1(b)). The width of each tab was measured with a digital caliper, and the average value was multiplied by the sample thickness to determine the cross-sectional area for stress calculations. A 0.5 mm diameter brass marker (Small Parts, Miramar, FL) was attached to each sandpaper tab to provide for optical strain control of the grips. The sample surface was speckle-coated

Fig. 1 (a) Schematic of regions where supraspinatus tendon samples were harvested. (b) Image of speckle-coated sample in reference configuration with markers (arrow) attached to sandpaper tabs via transparent plastic (dashed line). (c) Biaxial testing device with the crossed linear polarizers above and below the sample loaded in the center of the PBS bath.

with Verhoeff's stain to enable post-test tissue strain analysis via digital image correlation. Note that while grip-to-grip strains were used to provide consistent sample boundary conditions during testing, all data analysis and model fitting were performed on the optically-measured tissue strains.

2.2 Biaxial Testing. Following preparation, the sample was equilibrated in PBS for 1 h before testing. During this time, 2-0 silk suture was secured to the free end of each sandpaper tab by metal hooks. The sutures were then looped around the pulleys of a custom biaxial testing device [19] (Fig. 1(c)). Four independent motors applied loads to the sample both parallel (longitudinal direction) and perpendicular (transverse direction) to the tendon axis. A high-resolution digital camera fitted with a telecentric lens (NT63-730, Edmund Optics, Barrington, NJ) tracked the Green-Lagrange strains of the brass markers attached to the tabs. All tests were performed via grip-to-grip strain control at a constant rate of 0.002 s^{-1} , with the sample immersed in a bath of room temperature PBS. In addition, crossed linear polarizers were placed above and below the sample in order to measure its collagen fiber organization [8]. The polarizers were continuously rotated at 0.4 rev/s and images were acquired every 15 deg from 0 deg to 180 deg for post-test fiber alignment analysis. The longitudinal direction of the sample was defined as 0 deg .

Preliminary testing demonstrated that there was considerable inter-sample variability in the stress and strain values defining the linear-region of the stress-strain curve. Therefore, it was not possible to test each sample to a single maximum stress or strain level. In order to consistently test all samples to the linear-region without causing failure, a custom algorithm was developed that tracked the real-time slope of the stress-strain response according to the following protocol. First, the sample was equibiaxially pre-stained from an unloaded position to 0.02 strain. The sample was then longitudinally stretched while holding the transverse direction fixed. The custom algorithm monitored the slope of the longitudinal stress-strain response by fitting a line in real-time through

Fig. 2 Schematic of the custom algorithm used to monitor the slope of the longitudinal stress-strain curve in real-time by fitting the most recent 15 s of data at each time point ($t_1, t_2, t_3, \dots$). Once the slope dropped by 0.5% from the maximum value (at t_4), the test was stopped (t) and the final recorded stress was used as the limit for the subsequent mechanical testing. Note spacing between time points is exaggerated for clarity.

the stress versus nominal grip-to-grip strain data obtained over the last 15 s (0.03 strain). This calculation was updated every 0.2 s (e.g., at $t_1, t_2, t_3, \dots$, in Fig. 2). When the real-time slope of the stress-strain response fell by more than 0.5% below the maximum computed value (e.g., at t_4), the linear-region was considered to have been reached and the test was stopped. The longitudinal stress at this final time point (t^*) was used as the linear-region stress limit for subsequent mechanical testing.

Each sample was tested as follows. After the 1 h equilibration in PBS, the custom algorithm was run to find an initial guess of the linear-region stress limit used for preconditioning only. The sample was preconditioned by prestraining to 0.02 equibiaxial strain and repetitively loading the longitudinal direction up to the initial stress limit for five cycles while holding the transverse direction fixed. Immediately following the preconditioning, the algorithm was run a second time to determine the final stress limit for the data collection protocols. The sample was then fully unloaded and allowed to recover for one hour in the PBS bath, after which the position of the optical strain markers was recorded. This position was used as the reference configuration and defined the zero-strain state for all tests. Polarized light images were also captured to define the zero-strain fiber alignment.

A set of four protocols were used for data collection. Each consisted of equibiaxially prestraining the sample to 0.02 strain and then loading the longitudinal direction to the final linear-region stress limit for five cycles with the following strain ratios ($E_{11}:E_{22}$, where E_{11} is the longitudinal strain and E_{22} is the transverse strain) – 1:0, unloaded, 1:0, 4:1. For the unloaded test, the transverse direction was allowed to freely contract. These boundary conditions were chosen to cover a large range of strain environments without damaging the sample. The results from the two 1:0 tests were compared to confirm that they were repeatable and no damage had occurred. Images for fiber alignment analysis were captured every 0.01 strain in the longitudinal direction. Data and images from only the final loading curve for the unloaded, repeat 1:0, and 4:1 tests were used for experimental data analysis and model fitting.

2.3 Experimental Data Analysis. Two-dimensional displacement maps of the sample surface were calculated via digital image correlation (Vic2D 2009, Correlated Solutions, Columbia, SC) of images collected during each loading protocol. From these displacement maps, a single deformation gradient tensor $\mathbf{F}$ was obtained for each image by finding the least-squares solution to the following over-constrained system of equations

$$\begin{bmatrix} x_{1,1} & \dots & x_{1,i} & \dots & x_{1,n} \\ x_{2,1} & \dots & x_{2,i} & \dots & x_{2,n} \end{bmatrix} = \begin{bmatrix} F_{11} & F_{12} & A_1 \\ F_{21} & F_{22} & A_2 \end{bmatrix} \begin{bmatrix} X_{1,1} & \dots & X_{1,i} & \dots & X_{1,n} \\ X_{2,1} & \dots & X_{2,i} & \dots & X_{2,n} \\ 1 & 1 & 1 & 1 & 1 \end{bmatrix} \quad (1)$$

where $x_{1,i}$ and $x_{2,i}$ are the longitudinal and transverse positions, respectively, of each correlated point in the deformed configuration, $X_{1,i}$ and $X_{2,i}$ are the positions of the same points in the reference configuration, $\{F_{11}, F_{12}, F_{21}, F_{22}\}$ are the components of $\mathbf{F}$, and $\{A_1, A_2\}$ are the components of a translation vector. Points located within strain concentrations near the grips were excluded from the above calculation. For each image, the tensor $\mathbf{F}$ obtained from Eq. (1) was then used to calculate the tissue stretch in the longitudinal and transverse directions.

Two-dimensional maps of fiber alignment were also generated [8]. The pixels in the central region of the sample were grouped into a grid of 20×20 regions. For each deformed image, the grid coordinates were transformed by the deformation gradient tensor $\mathbf{F}$ to ensure that each grid region represented the same area of tissue throughout the test. The fluctuation of the average pixel intensity within each region as a function of the polarizer angle was fit by a sinusoidal curve with 90 deg periodicity. The principal fiber direction within each grid region was assigned to the angle resulting in minimum transmitted light intensity. Ambiguity in this assignment was resolved by assuming that during the unloaded test the fiber direction would rotate towards the loading direction [8]. The fiber angles over the entire sample were binned to produce an empirical histogram of the fiber distribution and the spread of this distribution at zero-strain was quantified by the circular variance, where lower values indicate higher alignment.

Affine fiber kinematics were evaluated by comparing the change in the empirical histogram at the end of the 4:1 test with that predicted by the deformation gradient tensor under an affine assumption. The degree of affine kinematics was quantified by the range and offset (where larger values indicate less affine behavior). The range and offset represent differences in the spread and central location, respectively, of the empirical and predicted histograms of fiber angles [30].

To compare with previous uniaxial data, the longitudinal linear-region modulus of the unloaded test was determined by a bilinear least-squares fit of the longitudinal stress-stretch curve. The linear-region modulus of the unloaded test and the ratio of the maximum transverse to maximum longitudinal stress (σ_{ratio}) of the 4:1 test were correlated with circular variance to evaluate experimental structure-function relationships.

2.4 Model Formulation. The structural model was based on a formulation by Sacks [29] of a general constitutive model developed by Lanir [28]. Briefly, the tendon samples were idealized as a fiber-reinforced hyperelastic material composed of a planar angular distribution of fibers embedded in a compliant ground substance (i.e., extra-fibrillar matrix). It was assumed that the extra-fibrillar matrix does not contribute to the mechanical response of the tissue given previous testing of highly aligned tendons that found the tensile moduli in the transverse direction orders-of-magnitude lower than the moduli measured along the dominant fiber direction [31]. The fibers themselves were assumed to be crimped in the reference configuration with a stochastic distribution of uncrimping lengths. A γ -probability distribution was chosen to represent the fiber uncrimping stretch (i.e., point at which the fiber is fully uncrimped)

$$G(\lambda_c - 1) = (\lambda_c - 1)^{\alpha-1} \frac{e^{-(\lambda_c-1)/\beta}}{\beta^\alpha \Gamma(\alpha)} \quad (2)$$

where λ_c is the fiber uncrimping stretch, and α and β are positive constants that determine the shape of the distribution. Given this

distribution, the mean uncrimping stretch ($\bar{\lambda}_c$) and the standard deviation of uncrimping (λ_c^{SD}) are, respectively

$$\bar{\lambda}_c = \alpha\beta + 1, \quad \lambda_c^{SD} = \sqrt{\alpha\beta^2} \quad (3)$$

The fibers were assumed to begin bearing load only after they are fully straightened, at which point they produce a linear stress response

$$S_f = K\varepsilon_f$$

where

$$\varepsilon_f = \frac{1}{2} \left(\frac{\lambda^2}{\lambda_c^2} - 1 \right), \quad \lambda^2 = \mathbf{N}^T \mathbf{F}^T \mathbf{F} \mathbf{N} \quad (4)$$

In these equations, S_f is the 2nd Piola-Kirchhoff stress of the fiber, K is the fiber modulus, λ is the fiber stretch, ε_f is the Green-Lagrange strain of the fiber referenced to the uncrimping length of the fiber (i.e., $\varepsilon_f = 0$ when $\lambda = \lambda_c$), and $\mathbf{N}$ is the unit vector describing the fiber direction. Therefore, the stress produced by a population of fibers at a given orientation $\mathbf{N}$ and stretch λ is

$$S_f = \frac{K}{2} \int_1^\lambda G(\lambda_c - 1) \left(\frac{\lambda^2}{\lambda_c^2} - 1 \right) d\lambda_c \quad (5)$$

Assuming that the angular distribution of fibers can be described by a von Mises probability density function $[R(\theta)]$, the stress response of all the fiber populations, and hence, of the entire tissue sample is

$$\mathbf{P} = \frac{K}{2} \int_{-\pi/2}^{\pi/2} R(\theta) \int_1^\lambda G(\lambda_c - 1) \left(\frac{\lambda^2}{\lambda_c^2} - 1 \right) \mathbf{F}[\mathbf{N} \otimes \mathbf{N}] d\lambda_c d\theta, \quad (6)$$

where $\mathbf{P}$ is the 1st Piola-Kirchhoff stress tensor ($\mathbf{P} = \mathbf{F}\mathbf{S}$), and θ is the angle of the fiber population described by the unit vector $\mathbf{N}$.

2.5 Parameter Estimation and Model Validation. For each sample, a semicircular von Mises distribution was fit to the zero-strain empirical histogram of the fiber angles (Fig. 3). The three model parameters K , α , and β were estimated by simultaneously fitting Eq. (6) to the experimental stresses measured during the final cycle of both the unloaded and 4:1 protocols. Specifically, a trust-region-reflective least-squares algorithm (Matlab, MathWorks, Natick, MA) minimized the error between the model and

Fig. 3 Representative fit of the empirical histogram of fiber angles with a von Mises probability density function

experimental stress tensors (P_{11} = longitudinal stress, P_{22} = transverse stress, and $P_{12} = P_{21} = 0$) corresponding to the deformation gradient tensors ($\mathbf{F}$) calculated from each image. In order to assess its predictive ability, the model was validated by fitting the experimental stresses of the 1:0 test using the model parameters determined from the fit of the other two loading protocols.

The quality of the model fits was quantified by the Nash-Sutcliffe efficiency (R^2), computed as

$$R^2 = 1 - \frac{\sum (y_j - f_j)^2}{\sum (y_j - \bar{y})^2} \quad (7)$$

where y_j is the experimental stress at the j th datapoint, f_j is the model stress at the same datapoint, and $\bar{y}$ is the mean experimental stress. This metric is analogous to the coefficient of determination in that it represents the amount of variation in the experimental data explained by the model [32]. However, this definition of R^2 ranges from $-\infty$ to 1, where 1 indicates a perfect fit and values < 0 indicate that the model performs worse than a horizontal line located at the mean experimental value [33]. The relative importance of the errors between the model and experimental transverse stresses was evaluated by normalizing the maximum transverse error by the maximum longitudinal stress ($\hat{\sigma}_{\text{error}}$).

2.6 Sensitivity Analysis. A sensitivity analysis was performed to determine the model sensitivity to the measured fiber angle distribution. The effects of perturbing the two von Mises distribution parameters (i.e., the mean fiber angle and circular variance) were evaluated by calculating the sum of squares error (SSE) between the model and experimental stresses for all protocols using the estimated model parameters

$$\text{SSE} = \sum_{i=1}^6 \sum_j (y_{ij} - f_{ij})^2 \quad (8)$$

where i refers to each of the six stresses measured for each sample (3 protocols $\times$ 2 directions = 6 stresses). The sum of squares error was calculated over the full range of the mean fiber angle (180 deg) and the circular variance (0 – 0.5). These values were normalized to the SSE produced by the experimentally measured von Mises parameters and graphed as a contour plot. The model sensitivity to the mean fiber angle was quantified by calculating the range in which this parameter could be perturbed while keeping the circular variance fixed and producing a normalized SSE of less than two. The same procedure was used to calculate the perturbation range for the circular variance but with the mean fiber angle held fixed.

2.7 Statistics. Correlations of circular variance with the longitudinal linear-region modulus of the unloaded test and with σ_{ratio} of the 4:1 test were evaluated. Due to the departure from normality of several parameter values, as determined by a D'Agostino's K^2 test, all correlations were evaluated using the nonparametric Spearman rank correlation coefficient (r_s). Nonparametric correlations with circular variance were also performed for the affine kinematics metrics, $\hat{\sigma}_{\text{error}}$, fiber modulus, $\bar{\lambda}_c$, λ_c^{SD} , and the perturbation ranges of the mean fiber angle and circular variance. Statistical significance was set at $p < 0.05$ and all data are presented as median values with interquartile ranges.

3 Results

3.1 Experimental. The samples were cut to a median thickness of 408 (1st and 3rd quartiles: 346, 475) μm and a median cross-sectional area of 2.7 (2.4, 3.0) mm^2 . Using the real-time curve-fitting algorithm, the longitudinal linear-region stress limit was 1.31 (1.05, 2.49) MPa, which corresponded to a linear-region

Fig. 4 Significant correlations were observed between circular variance (where a low variance represents more highly aligned fibers) for (a) the longitudinal linear-region modulus from the unloaded boundary condition test, (b) the ratio of maximum transverse to maximum longitudinal stress (σ_{ratio}) from the 4:1 test, and (c) the range (where higher values indicate less affine behavior)

stretch of 1.073 (1.055, 1.103). As expected, the longitudinal linear-region modulus of the unloaded boundary condition test was negatively correlated with zero-strain circular variance ($r_s = -0.61$, $p < 0.01$; see Fig. 4(a)). Additionally, the σ_{ratio} was positively correlated with the circular variance ($r_s = 0.71$, $p < 0.001$; see Fig. 4(b)), indicating that the less aligned samples produced a greater transverse stress response. The values for the affine kinematics metrics were relatively low (i.e., behavior was generally affine), with range values of 4.7 deg (3.0 deg, 10.6 deg) and offset absolute values of 2.8 deg (1.3 deg, 3.8 deg). Only the

range was positively correlated with the circular variance ($r_s = 0.76$, $p < 0.0001$; see Fig. 4(c)).

3.2 Parameter Estimation and Model Validation. Excellent fits of the structural model to the longitudinal direction stresses (P_{11}) were observed (Fig. 5), with median R^2 values of 0.97 for parameter estimation (Table 1). Model validation using the 1:0 test was equally successful, with median R^2 values of 0.96. For the transverse direction (P_{22}), the fits produced median R^2 values

Fig. 5 An example of the fits of the structural model to the experimental data for the (a),(b) 4:1 test, (c),(d) unloaded test, and (e),(f) 1:0 test. Note that the 1:0 test was not used to determine the model parameters and, therefore, represents a validation of the model.

Table 1 Experimental and Model Results

	Quartiles		
	Median	1st	3rd
Experimental results			
Longitudinal modulus (MPa)	69.2	41.7	95.1
Circular variance	0.120	0.026	0.219
Model parameters			
Fiber modulus (K; MPa)	98.7	71.6	183.5
Mean uncrimping stretch ($\bar{\lambda}_c$)	1.065	1.035	1.079
S.D. of uncrimping (λ_c^{SD})	0.012	0.007	0.027
α	14.9	9.9	43.8
$\beta (\times 10^{-3})$	3.3	1.1	8.9
Parameter estimation			
Avg. longitudinal R^2	0.97	0.94	0.99
4:1 transverse R^2	0.50	−0.17	0.76
Validation			
Longitudinal R^2	0.96	0.93	0.98
Transverse R^2	0.53	−20.5	0.76

of 0.50 for estimation and 0.53 for validation, with negative R^2 values obtained for one-third of the samples (Fig. 6(a)). When the magnitudes of the errors for the transverse stress were normalized by the maximum measured longitudinal stress ($\hat{\sigma}_{error}$), the transverse stress errors were relatively small for the more aligned samples (low circular variance), with a positive correlation between the $\hat{\sigma}_{error}$ and the circular variance ($r_s = 0.66$, $p = 0.001$; see Fig. 6(b)).

The median value for the fiber modulus (K) was 98.7 (1st and 3rd quartiles: 71.6, 183.5) MPa, for $\bar{\lambda}_c$ was 1.065 (1.035, 1.079), and for λ_c^{SD} was 0.012 (0.007, 0.027). No correlation was found between the fiber modulus and circular variance, while moderate positive correlations were observed for $\bar{\lambda}_c$ ($r_s = 0.45$, $p < 0.05$) and λ_c^{SD} ($r_s = 0.46$, $p < 0.04$; Fig. 7). Finally, the von Mises distribution fit the histograms of the empirical fiber angles well with R^2 values of 0.91 (0.84, 0.95).

3.3 Sensitivity Analysis. A sensitivity analysis was performed to determine the effect of the measured mean fiber angle and circular variance parameters on the model fit performance. For the majority of samples, the isometric lines of the normalized SSE produced a kidney-shaped contour (Figs. 8(a) and 8(b)). The model fits using the experimentally measured fiber parameters for ten of these samples were located near the center of the contours (Fig. 8(a)), whereas for eight samples, the fits were at an offset (Fig. 8(b)). The contour plots of the remaining three samples had an altogether different shape (Fig. 8(c)). Across all samples, the

mean fiber angle could be perturbed over a median range of 14.3 deg (1st and 3rd quartiles: 5.9 deg, 27.6 deg) before the SSE doubled. These values were positively correlated with circular variance ($r_s = 0.53$, $p = 0.01$) and represent 7.9% (3.3%, 15.3%) of the full 180 deg parameter scale. The perturbation range for the circular variance parameter had a median of 0.092 (0.065, 0.169), which represents 18.4% (13.0%, 33.8%) of the full scale, and was also positively correlated with the experimentally measured circular variance ($r_s = 0.75$, $p < 0.0001$).

4 Discussion

This study evaluated the contribution of collagen fiber alignment to the planar tensile mechanics of the supraspinatus tendon. In agreement with our hypothesis, there was a clear correlation between fiber organization and the transverse and longitudinal stresses. First, samples with a higher degree of fiber alignment exhibited a greater longitudinal linear-region modulus than less aligned samples (Fig. 4(a)), similar to results from previous uniaxial testing [8]. Second, the ratio of transverse to longitudinal stresses (σ_{ratio}) was greater for samples with a wider distribution of fiber angles, with the transverse stress of the least aligned sample reaching 37% of the longitudinal stress in response to a 4:1 (long:trans) strain ratio (Fig. 4(b)). These results further support the importance of collagen fiber organization to tissue mechanics and demonstrate that regions of the supraspinatus tendon are capable of supporting large stresses applied perpendicular to the longitudinal axis of the tendon. The particular regions exhibiting this capacity were not specifically identified since this study was not powered to conduct regional comparisons of the results. Instead, the focus was to obtain samples with a range of fiber alignments in order to effectively use the structural model to evaluate the general relationship between fiber alignment and planar mechanics.

The structural model was successful in fitting the longitudinal stresses and in describing the structure-function relationship between fiber alignment and longitudinal mechanics. The R^2 values obtained for the fits of the longitudinal stresses were near unity for all mechanical protocols, including the 1:0 test used for model validation, which demonstrates the predictive success of the model. In addition, the lack of correlation between the fiber modulus parameter and the circular variance suggests that the model isolated the individual contributions of the fiber moduli and their angular variation to the overall tissue properties. The success of this model, which only incorporates fibers with a given angular distribution, strongly suggests that the longitudinal mechanics of the supraspinatus tendon primarily depend on the moduli, crimp, and angular distribution of the collagen fibers.

The model fits of the transverse stresses, in contrast to the longitudinal direction, were relatively poor for about half of the samples. It should be noted that for highly aligned samples, low

Fig. 6 (a) For one-third of the samples, the model fits of the transverse stresses from the 4:1 test produced negative R^2 values. (b) Normalizing the model errors for the transverse stress by the maximum longitudinal stress ($\hat{\sigma}_{error}$) demonstrates that the errors for the more highly aligned samples are relatively inconsequential in comparison to samples with a wider distribution of fibers.

Fig. 7 (a) Fiber modulus was not correlated with circular variance, while positive correlations were observed for both (b) the mean uncrimping stretch (λ_c), and (c) the standard deviation of uncrimping (λ_c^{SD})

Fig. 8 Representative sensitivity plots for the normalized SSE, given changes in the mean fiber angle and circular variance. Isometric lines indicate fold increases in the SSE compared to the model fits using the experimentally measured fiber angle parameters ($\bullet$). The majority of plots were kidney-shaped, with the fits of 10/21 samples located (a) near the center of the contours, and those of 8/21 samples (b) at an offset. The plots for three samples had (c) a different shape.

transverse R^2 values are slightly misleading. For these samples, errors on the order of 10 kPa produce very low, and sometimes negative, R^2 values since such highly aligned tendons produce nearly zero transverse stress. A more informative analysis is to normalize the maximum error in the transverse direction between the model and experimental results by the maximum longitudinal stress. This accounts for the importance of the errors in the transverse direction in context of the two-dimensional mechanics of the sample. Plotting this value ($\hat{\sigma}_{error}$) versus the fiber circular variance indicates that the transverse stress errors for highly aligned samples are, indeed, relatively inconsequential in comparison to samples with a greater circular variance (Fig. 6(b)).

For less aligned samples, the discrepancy between the model and experimental transverse stresses is not insignificant. One possible reason for this could be inaccuracies in representing the fiber angles with a von Mises distribution. However, using the directly measured fiber angle data in the model rather than the von Mises distributions did not substantially improve the fits (data not shown). Alternatively, simplifications in the model may account for the poorer fits. For example, contributions from the extra-fibrillar matrix were not included in the model. Adding a strain energy term for the matrix would likely not improve the model fits given the extremely weak response of highly aligned supraspinatus tendon samples when loaded transverse to the fiber direction [17]. More likely, terms that represent fiber-fiber interactions, such as collagen fiber entanglements, may be more useful. Non-continuum approaches (such as fiber network models [34]) may also be helpful by relaxing the requirements of a spatially homogeneous fiber distribution, homogeneous tissue deformations, and affine fiber kinematics.

An important and novel attribute of this study was the custom algorithm that ensured each sample was tested to a comparable point within the linear-region of its stress response. Although this algorithm introduced additional complexity to the mechanical protocol, the alternative of using a single maximum strain or stress level was not feasible, as is evident from the substantial variation of the values that define the linear-region. The algorithm indicated when the linear-region had been reached through real-time measurements of the tangent modulus of the stress-strain curve. This technique successfully mitigated the effects of large inter-sample variability and allowed general conclusions to be drawn concerning the experimental and modeling results.

The model parameters obtained from this study are comparable with measurements from the literature. The estimated collagen fiber moduli fall within the measured range of 49 MPa to 3 GPa obtained from previous experimental studies [35-40]. It is difficult to make a more precise comparison since these values vary substantially with tissue type, fiber diameter, and testing method. In addition, it is unclear what physical scale with which the fiber moduli from this study should be associated. The fiber uncrimping stretch, on the contrary, is larger than that previously experimentally measured. Testing of rat tail tendons has found that crimp wave patterns are fully removed by about 4% elongation [41,42]. In the current study, a fiber elongation of 7.2% was necessary to fully uncrimp the collagen fibers, based on the 95th-percentile of the uncrimping distribution generated by the median uncrimping parameters. This difference may be due to preconditioning the samples, leading to a recoverable increase in uncrimping strains [43]. More likely, however, preloading may explain the difference in uncrimping, since the current study used an unloaded zero-

strain condition, whereas the previous experiments used a pre-loaded state, which reduces the size of the toe-region [20]. Finally, it is unknown how the characteristics of collagen fiber crimping (i.e., frequency, period, etc.) in the human supraspinatus tendon compare with those found in rat tail tendons.

A limitation of this study is that transmitted polarized light gives a through-thickness average of collagen orientation rather than identifying individual fibers. Nevertheless, the relatively low values for the affine kinematics metrics indicate that orientation changes in the measured angles were consistent with the tissue-level deformations, suggesting that the measured angles are representative of true collagen structures. In addition, sensitivity analyses of the model fits demonstrated that, for the majority of samples, there was a kidney-shaped region where the fits were relatively insensitive to errors in the mean fiber angle and circular variance. This pattern is similar to that found in a previous application of this model [29]. Overall, the model was more sensitive to changes in the mean fiber angle than in the circular variance. A doubling of the SSE was produced by perturbing the mean fiber angle by 7.9% of the full scale, while a perturbation of 18.4% was required for the circular variance. The sizes of both perturbation ranges increased with the measured circular variance. These results suggest that while the model is moderately insensitive to small changes in the fiber angle distribution, large errors in fiber angle measurements may produce inaccurate model predictions, especially for more highly aligned samples.

In conclusion, this study demonstrates that the longitudinal mechanics of the supraspinatus tendon is primarily dependent on the moduli, crimp, and angular distribution of its collagen fibers. A structural model incorporating these characteristics successfully fit and predicted the longitudinal stresses produced in response to a variety of multiaxial loads similar to those likely presented in vivo. However, the level of transverse stress could not be adequately accounted for by the model, which may need additional terms to represent possible interactions between tissue components. This study helps isolate the specific mechanisms responsible for the tissue-level planar mechanics of the supraspinatus tendon. Such information is critical for understanding the etiology of degenerative tendon tears, developing effective tissue engineering design strategies, and predicting outcomes of tissue repair.

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