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Tensile properties and fiber alignment of human supraspinatus tendon in the transverse direction demonstrate inhomogeneity, nonlinearity and regional isotropy

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Abstract

A recent study (Lake et al. 2009) reported the properties of human supraspinatus tendon (SST) tested along the predominant fiber direction. The SST was found to have a relatively disperse distribution of collagen fibers, which may represent an adaptation to multiaxial loads imposed by the complex loading environment of the rotator cuff. However, the multiaxial mechanical properties of human SST remain unknown. The objective of this study, therefore, was to evaluate the mechanical properties, fiber alignment, change in alignment with applied load, and structure-function relationships of SST in transverse testing. Samples from six SST locations were tested in uniaxial tension with samples oriented transverse to the tendon long-axis. Polarized light imaging was used to quantify collagen fiber alignment and change in alignment under applied load. The mechanical properties of samples taken near the tendon-bone insertion were much greater on the bursal surface compared to the joint surface (e.g., bursal moduli 15–30 times greater than joint; $p < 0.001$). In fact, the transverse moduli values of the bursal samples were very similar to values obtained from samples tested along the tendon long-axis (Lake et al., In press, JOR). This key and unexpected finding suggests planar mechanical isotropy for bursal surface samples near the insertion, which may be due to complex *in vivo* loading. Organizationally, fiber distributions became less aligned along the tendon long-axis in the toe-region of the stress-strain response. Alignment changes occurred to a slightly lesser degree in the linear-region, suggesting that movement of collagen fibers may play a role in mechanical nonlinearity. Transverse mechanical properties were significantly correlated with fiber alignment (e.g., for linear-region modulus $r_s = 0.74$, $p < 0.0001$), demonstrating strong structure-function relationships. These results greatly enhance current understanding of the properties of human SST and provide clinicians and scientists with vital information in attempting to treat or replace this complex tissue.

Keywords

transverse properties; supraspinatus tendon; collagen fiber alignment; structure-function; polarized light imaging

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Introduction

Collagen fibers are the primary structural component of tendon and are generally organized along the tendon's long-axis. Since most tendons are loaded primarily along this predominant fiber direction, several studies have evaluated the "longitudinal" uniaxial tensile properties of various human tendons (e.g. (Butler et al. 1984; Carlson et al. 1993; Itoi et al. 1995; Lewis and Shaw 1997; McGough et al. 1996; Nakajima et al. 1994)). Some tendons may be subjected to loads that are more complex than solely tensile forces. However, there is little data available regarding more complex mechanical characteristics of tendon. One study found that the transverse (perpendicular to long-axis) modulus of sheep flexor tendon is approximately two orders of magnitude less than the longitudinal modulus (Lynch et al. 2003), which is similar to what was found for human medial collateral ligament (Quapp and Weiss 1998). These small transverse values are not unexpected, since these tissues consist of a very highly aligned collagen fiber network. It is not known if transverse mechanical properties are greater for tendons that have a more complex structural organization or are subjected to a more complex physiologic loading environment.

One tendon that functions in a particularly complex loading environment is the human supraspinatus tendon (SST), one of the four tendons that comprise the rotator cuff of the shoulder. Degeneration and tears of the SST are common and are often treated surgically. However, limited success in treatment strategies illustrates the need to better understand the properties of this tissue. Recent work quantified the degree of collagen fiber alignment in different regions of the tendon and demonstrated a highly inhomogeneous tissue, with areas near the tendon-bone insertion exhibiting a relatively low degree of fiber alignment (Lake et al. 2009). This study also reported location-dependent longitudinal mechanical properties that significantly correlated with degree of fiber alignment, demonstrating strong structure-function relationships. The unique properties of SST near the tendon-bone insertion may result from multiaxial loading *in vivo*. In addition to the large range of motion of the shoulder joint, the SST is constrained on either side by other soft tissues and must repeatedly pass underneath the coracoacromial arch. This functional environment may subject the SST to compression, shear and/or off-axis tensile (e.g., transverse) forces. However, the multiaxial mechanical properties of SST have not been described. In particular, the tensile properties in the transverse direction, which together with the properties in the longitudinal direction provide information regarding the level of planar anisotropy, have not been evaluated nor has the relationship between the inhomogeneous fiber organization and these mechanical properties been reported.

Therefore, the objective of this study was to evaluate the mechanical properties, fiber alignment and structure-function relationships of human supraspinatus tendon in transverse testing. In addition, polarized light imaging was used to quantify, for the first time, changes in collagen fiber alignment in tendon subjected to transverse loading. The hypotheses were that: 1) similar to the previous longitudinal loading response (Lake et al. 2009), initial fiber alignment and changes in alignment during transverse loading will be location-dependent, 2) transverse mechanical properties will be nonlinear and inhomogeneous, with the largest values in posterior samples due to a more disperse fiber distribution, and 3) structure-function relationships will be demonstrated by significant correlations between mechanical and organizational properties.

Methods

Sample preparation

Eleven supraspinatus tendons (SST) were harvested from fresh frozen human cadaveric shoulders (average age 53.2 ± 12.1 years; range 35–67 years old) with no reported history of injury to the shoulder or rotator cuff. Tendons with tears were excluded. The SST was dissected

free from all surrounding tissue and rectangular full-thickness samples (~20×5mm) were cut transverse to the tendon long-axis from three distinct tendon locations (anterior, posterior, medial; Figure 1).

Samples were frozen to a microtome stage (Leica, Wetzlar, Germany) using embedding medium. After leveling off the tendon surface, approximately 200µm of tissue was removed prior to cutting an approximately 400µm-thick tissue slice representing the bursal (superior) tendon surface. The sample was then removed from the microtome, turned over, and refrozen to the stage. Once again the tendon surface was leveled off and approximately 200µm of tissue was removed. An approximately 400µm-thick slice was then cut representing the joint (inferior) surface (order of cutting bursal vs. joint samples was randomized). Thus each shoulder yielded six location-specific test samples (Figure 1): anterior-bursal (AB), anterior-joint (AJ), posterior-bursal (PB), posterior-joint (PJ), medial-bursal (MB), and medial-joint (MJ). A rectangular sample shape was maintained (rather than dog-bone shaped) in order to provide a larger total area for collagen fiber orientation analysis and to minimize cutting of fibers. Four to eight 0.5-mm diameter brass beads were glued to the tendon surface in a grid pattern for strain measurement. Cross-sectional area was measured using a laser device (Favata 2006) and length was measured using digital calipers. After being secured in custom clamps, the median gauge length for all samples was 13.9mm and the median aspect ratio was 3.1.

Mechanical testing

Samples were placed in a PBS bath and loaded in a tensile testing machine (Instron, Norwood, MA) integrated with a custom-built polarized light imaging system to quantify fiber alignment during testing (Lake et al. 2009). This system consists of a linear backlight (Dolan-Jenner, Boxborough, MA), two linear polarizing sheets (Edmund Optics, Barrington, NJ) oriented perpendicular to one another on either side of the test sample, a stepper motor (Lin Engineering, Santa Clara, CA) for polarizer rotation, and a high resolution digital camera (Imaging Solutions Group, Fairport, NY) for image acquisition. Tendon samples were ramped to failure from a slack position to ensure that the toe-region was obtained. A constant, quasi-static strain rate of 0.1%/sec was used, which is in the range of rates used in previous transverse tendon testing (Lynch et al. 2003). Based on preliminary studies, a tare stress of 0.0075 MPa was used to establish the zero stress-strain state. Images were acquired every 5 seconds for optical strain analysis and sets of 15 images were acquired every 10 seconds as the polarizers rotated through a 135° range for analysis of collagen fiber alignment.

Data analysis

A custom Matlab program (Matlab, Natick, MA) was used to optically track the surface beads and calculate two-dimensional Lagrangian strain using tensor algebra. Stress was calculated as force divided by initial cross-sectional area. Using the least squares method, a bilinear curvefit was applied to the stress-strain data in order to quantify the modulus in the toe- and linear-regions, as well as the stress and strain values corresponding to the transition point of the bilinear fit (Figure 2). Poisson's ratio was calculated from the 2D strain data as the negative of the ratio of strain perpendicular to the loading direction divided by the strain in the loading direction (Lynch et al. 2003).

Fiber alignment was calculated throughout testing as described previously (Lake et al. 2009). Briefly, images of the tendon surface were divided into rectangular areas. Pixel intensities were summed by area, per image and plotted vs. corresponding angle of polarizer rotation. A sine wave was fit to the intensity-angle data and used to determine the angle corresponding to minimum pixel intensity, which represents the average direction of the area's collagen fiber alignment. Alignment maps and histograms were created by compiling values from all areas (Figure 3). Circular variance (VAR), a measure of the distribution of collagen fiber alignment

with respect to the tendon long-axis, was calculated at zero strain, transition strain (intersection of the toe- and linear-regions) and linear-region strain (2X transition strain). The zero and transition strain VAR values were compared to evaluate the change of fiber alignment in the toe-region, while the transition and linear strain VAR values were compared to evaluate the change of alignment in the linear-region.

Statistical analysis

Shapiro-Wilk tests indicated non-normally distributed data, so non-parametric statistical tests were utilized for all data analysis. Significance was set at $p < 0.05$, trends at $0.05 < p < 0.1$, and Bonferroni corrections were made for number of comparisons. Mechanical parameter values were compared for tendon location using Kruskal-Wallis tests followed by Mann-Whitney post-hoc tests. To evaluate the mechanical anisotropy of each SST tendon location, Mann-Whitney tests were used to compare transverse toe- and linear-region moduli to corresponding longitudinal moduli from our previous study (Lake et al. 2009). Changes in alignment were evaluated using Friedman tests to compare VAR_{zero} to VAR_{transition} (toe-region alignment change) and VAR_{transition} to VAR_{linear} (linear-region alignment change), followed by Wilcoxon signed-rank post-hoc tests. Spearman rank correlation coefficients were calculated to evaluate correlations between VAR_{zero} values and each of the mechanical properties. Due to the non-normal data distribution, all data is presented as median values and interquartile range.

Results

Results demonstrate highly inhomogeneous and nonlinear (note scale of y-axes) moduli values (Figure 4). For the anterior and posterior locations, both toe- and linear-region moduli values were much larger in the bursal samples than in the joint (e.g., AB moduli are approximately 30X greater than AJ). Moduli from the medial samples were very small; MB values were significantly less than both AB and PB values and MJ moduli were significantly less than PJ. For transition strain, the medial samples were significantly greater than both anterior and posterior tendon locations (Table 1). For transition stress and Poisson's ratio (Table 1), the values from the medial samples were significantly smaller than both anterior and posterior samples. In addition, both transition stress and Poisson's ratio exhibited significant differences through the tendon thickness (bursal > joint) in the anterior and posterior (but not medial) samples.

Circular variance values (VAR) were seen to increase under transverse load (Figure 5), corresponding to decreasing collagen fiber alignment along the tendon long-axis (e.g., Figure 3). All tendon locations demonstrated at least a trend towards alignment change in the toe-region (evaluated by comparing VAR_{zero} and VAR_{transition} values within each tendon location). In general, significant changes in fiber alignment continued in the linear-region (evaluated by comparing VAR_{transition} and VAR_{linear} values within each location), although the effect appears to be slightly less pronounced. As hypothesized, the initial fiber alignment prior to testing (VAR_{zero}) was inhomogeneous (Figure 6a). Specifically, MB samples were significantly more organized than either AB or PB, while PJ was also significantly more aligned than PB.

Initial fiber alignment was found to correlate significantly with all mechanical parameters calculated in this study. A plot of linear-region modulus vs. VAR_{zero} ($r_s = 0.74$, $p < 0.0001$) on a log scale demonstrates an exponential relationship between initial alignment and transverse tensile modulus (Figure 6b). Toe-region modulus ($r_s = 0.73$, $p < 0.0001$) and transition stress ($r_s = 0.73$, $p < 0.0001$) exhibit a similar positive nonlinear dependence on fiber alignment, while Poisson's ratio is also significantly correlated ($r_s = 0.67$, $p < 0.0001$). Finally, transition strain exhibits a negative dependence ($r_s = -0.49$, $p = 0.0001$) on VAR_{zero}. The strong correlation of

mechanical and organizational parameters demonstrates a strong structure-function relationship in SST.

Discussion

As hypothesized, the mechanical data for human SST tested transverse to the predominant fiber direction were highly inhomogeneous and nonlinear. Contrary to our hypothesis, however, the posterior samples did not demonstrate the strongest mechanical behavior. Significant differences were not found between anterior and posterior samples, but rather between bursal and joint samples, which is different from longitudinal mechanical tests (Lake et al. 2009). A comparison of longitudinal and transverse properties demonstrates significant mechanical anisotropy (Figure 7), where longitudinal moduli are significantly larger than transverse for medial samples ($\sim 10^3$ X greater), as well as for AJ and PJ samples (~ 10 X greater). Unexpectedly, however, there were no differences between the longitudinal and transverse moduli values for AB and PB, suggesting planar mechanical isotropy for these locations. While the highly anisotropic results for most of the SST locations (AJ, PJ, MB and MJ) are similar to what has been reported for multiaxial testing of flexor tendon (Lynch et al. 2003) and medial collateral ligament (Quapp and Weiss 1998), the planar isotropic mechanical results (AB and PB locations) contrast dramatically with these previous tendon/ligament studies. This highlights the unique characteristics of the SST, particularly the bursal-side tissue near the insertion (AB/PB).

Collagen fiber alignment and changes in alignment under transverse load were found to be location-dependent, as hypothesized. Samples became less aligned along the tendon long-axis when tested in transverse tension (Figure 8). Consistent changes of alignment in the toe-region supports the notion that fiber movement is partly responsible for the nonlinear mechanical response of tendon (Guerin and Elliott 2005). However, continued alignment changes in the linear-region suggest that other factors must also contribute to mechanical nonlinearity. The uncrimping of collagen under load has been implicated in causing stress-strain nonlinearity in tendon (Hansen et al. 2002; Morgan et al. 2006), while transverse nonlinearity may also be due to interactions between the fibers and extrafibrillar matrix. Unfortunately, the imaging approach used in this study does not allow for concurrent evaluation of collagen crimp or fiber-matrix interactions.

Statistically significant correlations were observed between mechanical and organizational properties. Specifically, samples that have a more disperse initial fiber distribution demonstrate a stiffer mechanical response in transverse loading. The effect of fiber alignment on moduli values is particularly pronounced, as indicated by the exponential relationship between these properties. As a result of this exponential relationship, changes in initial fiber alignment ($\Delta \text{VAR}_{\text{zero}}$) have little effect on mechanical properties when there is a high degree of overall alignment (at small VAR_{zero} values); however, even small $\Delta \text{VAR}_{\text{zero}}$ will result in a large change in mechanical properties when overall alignment is relatively low (at large VAR_{zero} values). Also interesting is the negative correlation of VAR_{zero} with transition strain. In particular, highly aligned samples experience significantly more strain before reaching the transition from toe- to linear-region. In the extreme case, this translates to a large difference in median values of 6% vs. 26% transition strain for AB and MB, respectively (Table 1). Taken together, these significant correlations indicate a strong relationship between the structure and function of human SST.

This study provides the first quantitative description of changes in fiber alignment in tendon under transverse load, as well as descriptions of the mechanical behavior and collagen fiber alignment of human SST. These properties may be due to the complex loading environment of the rotator cuff. The anterior and posterior samples near the tendon-bone insertion may

experience multiaxial and/or combined loads which could lead to decreased fiber alignment relative to the medial samples, which likely experience primarily uniaxial tensile forces. In addition, the large increase in transverse properties for the anterior/posterior bursal samples may be an adaptation to loading on the bursal surface by the coracoacromial arch (such as compression or shear forces due to acromial impingement). This explanation is supported by the data from the medial samples, which are located further away from both the tendon-bone insertion and the acromion and subsequently exhibit no bursal-joint differences.

This study is not without limitations. First, the measurable range of the polarized light imaging system utilized in this study is 90° rather than the full 180° range of possible values. By making the assumption that fibers will always change orientation towards the direction of loading, we were able to make corrections in order to consider the full 180° range of possible angle values. Second, the transverse data from the current study have been compared to previously reported longitudinal data from the same six tendon locations. While the samples are taken from the same general tendon location, by nature the orientation of the transverse/longitudinal samples are different. Therefore, the tissues contained within these orthogonal samples are slightly different. Third, due to the natural anatomical variability of cadaver tissues there was a range in geometrical size of tendon samples. In a few cases, smaller tendons led to the use of samples that were smaller than the desired size ($20 \times 5 \text{ mm}$). In addition, due to a limited amount of tissue available for testing, the aspect ratio of the samples was lower than desired. Considering the significant differences in this dataset, we believe that these limitations regarding sample size had little effect on the results. Finally, it is possible that collagen fibers run out of the (length-width) plane, in which case these fibers could be cut during our microtome sample sectioning technique. We have not noticed evidence for such fibers during dissections and sample preparation, therefore we believe that this effect, while possible, would be minimal and would not significantly affect the results of this study.

There are several important clinical implications of this study. As mentioned, the bursal-joint differences observed in the anterior and posterior, but not medial, samples suggests that complex loading may play a significant role in altering properties near the insertion. Overall, the data shows that the human SST is a very complex tendon with unique properties. It is important for clinicians and scientists to understand and appreciate the complexity of these multiaxial properties and the nature of structure-function relationships in order to properly diagnose, treat, prevent or repair SST injury. For example, while several synthetic and biologic grafts have been used to augment or replace torn SST, these approaches have generally been unsuccessful. This may be because these graft materials have not matched the complex mechanical properties of native SST (such as nonlinearity and inhomogeneity). In addition, clinicians should consider multiaxial properties when repairing or replacing the SST. Specifically, a repair strategy needs to not only reconstitute the tensile properties along the tendon long-axis, but also reestablish mechanical integrity in other loading directions (e.g., transverse) so that the repair can properly function in the physiologic environment. Overall, the limited success of treatment strategies to date illustrates the difficulty in treating a complex tendon whose native properties are poorly understood. This study greatly enhances the characterization of native SST properties and, in addition, these results provide necessary guidelines for the development of synthetic or biologic tendon replacements.

In conclusion, this study provided insight into the transverse mechanical properties of tendon and described the effect of transverse loading on collagen fiber alignment. In addition, this study has illustrated the inhomogeneous mechanical and organizational properties, as well as strong structure-function relationships, in human SST. The most unexpected finding of this work is the apparent planar mechanical isotropy of bursal samples near the tendon-bone insertion.

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Figure 1.
Harvest location of six human supraspinatus tendon samples (schematic represents a tendon taken from a right shoulder)

Figure 2.
Example bilinear curvefit of stress-strain tensile data; arrows indicate the zero, transition and linear strain values

Figure 3. Sample alignment maps and histograms at zero, transition and linear strain levels demonstrate decreasing fiber alignment with increased strain (VAR values = 0.07, 0.13, and 0.23, respectively)

Figure 4. Modulus in the toe- (left) and linear-region (right) depict inhomogeneous and nonlinear (note scale of y-axes) transverse tensile properties of human supraspinatus tendon (median $\pm$ interquartile range, *=significant)

Figure 5.

Circular variance values (VAR) show inhomogeneous fiber distributions, decreasing alignment (increasing VAR) in the toe-region (zero to transition strain) of all tendon locations and in the linear-region (transition to linear strain) of all locations except PB (median $\pm$ interquartile range; *=significant, #=trend)

Figure 6.

(A) VAR values at zero strain are significantly different by tendon location demonstrating organizational inhomogeneity (median $\pm$ interquartile range; *=significant); (B) VAR_{zero} values correlate significantly with mechanical parameters such as linear-region modulus (r_s =Spearman rank correlation coefficient)

Figure 7.

Linear-region moduli values for human supraspinatus tendon tested in both transverse and longitudinal tension; the values within the AB and PB locations are not different, demonstrating mechanical isotropy in these locations (*=significant)

Figure 8.

Schematic of fiber realignment during transverse tensile test; fiber A reorients in the direction of loading, while fiber B remains parallel to the tendon long-axis

Table 1

Median and interquartile ranges for transition strain, transition stress and Poisson's ratio calculated from transverse tensile tests of human supraspinatus tendon (matching letters indicate statistically significant differences, $p < 0.05$)

	Transition strain			Transition stress (MPa)			Poisson's ratio					
	25%	Median	75%	25%	Median	75%	25%	Median	75%			
AB	0.04	0.06	0.07	a	0.15	0.41	0.49	d,f	1.67	2.04	2.73	i,k
AJ	0.05	0.11	0.26		0.02	0.03	0.06	d	0.46	0.70	1.03	i
PB	0.07	0.09	0.10	b	0.15	0.25	0.45	e,g	1.02	1.39	2.16	j,l
PJ	0.08	0.09	0.11	c	0.02	0.04	0.10	e,h	0.72	0.86	0.98	j,m
MB	0.16	0.26	0.36	a,b	0.00	0.01	0.02	f,g	0.14	0.28	0.40	k,l
MJ	0.13	0.19	0.30	c	0.01	0.02	0.03	h	0.12	0.30	0.70	m