The Correlation of Quantitative Ultrasound Measures and Supraspinatus Tendon Quality: A Pilot Study

Gerald A Ferrer[1], R. Matthew Miller[1], Masahito Yoshida[2], James H. Wang[1,2], Volker Musahl[1,2], Richard E. Debski[1,2]*

[1]Department of Bioengineering, Swanson School of Engineering, University of Pittsburgh, Pittsburgh, Pennsylvania, USA, [2]Department of Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Abstract

Background: The objective of this study was to determine the feasibility of assessing tendon quality as quantified by histology through changes in quantitative ultrasound measures. **Methods:** Eight cadaveric shoulders (four with a small supraspinatus tendon tear) were examined using conventional B-mode ultrasound in the transverse plane by internally rotating and hyperextending the humerus. Quantitative ultrasound measures (skewness, kurtosis, variance, and echogenicity) were calculated based on the grayscale distribution of the ultrasound image taken of the supraspinatus tendon near the insertion site. The specimens were then dissected to the supraspinatus tendon where tendon biopsies were taken near the insertion site, mid-substance, and myotendinous junction. Through histology, tendon quality was evaluated based on collagen fiber organization, fatty infiltration, nuclei shape, and cellularity. Correlations between quantitative ultrasound measures and histological grades of tendon quality were determined through Pearson or Spearman's rho correlations. **Results:** A total of three significant correlations between quantitative ultrasound measures and histological parameters of tendon quality were found. Significant correlations between kurtosis and cellularity at the insertion site ($r = 0.724$) ($P < 0.05$) as well as variance and fatty infiltration at the myotendinous junction ($\rho = -0.843$) ($P < 0.05$) and for the whole tendon ($\rho = -0.826$) ($P < 0.05$) were found. **Conclusion:** The results show the potential for quantitative ultrasound measures to assess factors of tendon quality that can only be determined through histology. With further development of the methodology that utilizes quantitative ultrasound measures, clinicians might be able to evaluate the tendon quality noninvasively in future.

Keywords: Grayscale, histology, quantitative ultrasound, supraspinatus tendon

Introduction

Rotator cuff tears are an important clinical issue with a prevalence rate reported to be 20%–30% in the general population.[1] In the United States alone, rotator cuff tears account for over 4.5 million physician visits each year, resulting in treatment costs over $3 billion annually.[2] Treatment of rotator cuff tears by physical therapy accounts for about 25% of individuals,[3] and surgical repair has been reported to fail up 69% of the time.[4] Tear propagation from physical therapy and re-tears occurring at the tendon–suture interface following a surgical repair are common occurrences, possibly due to poor tissue quality.[4,5] As a result, a need exists to reduce the failure rate of treatment by allowing clinicians to assess tendon quality quickly and accurately to predict the effectiveness of the treatment utilized.

Ultrasound is a noninvasive, cost-efficient, accurate, and reliable imaging modality that is becoming more widely used by clinicians to diagnose, evaluate, and treat rotator cuff tears.[6-11] However, clinicians use ultrasound as a subjective tool to assess whether a rotator cuff tear is present and the overall quality of the tendon. The primary quantitative measurements made with ultrasound are measurements of tissue geometry such as tear size, tendon width, thickness, and cross-sectional area.[12-14] Recently, quantitative ultrasound measures have been shown to be a reliable technique for determining tendon quality by analyzing the grayscale distribution of an ultrasound image.[15-21] In these studies, an

Address for correspondence: Prof. Richard E. Debski, 408 Center for Bioengineering, 300 Technology Drive, Pittsburgh, Pennsylvania 15219, USA. E-mail: genesis1@pitt.edu

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interpretation of features that describe the ultrasound image echotexture (echogenicity, variance, skewness, and kurtosis) was explained. Increased tendinopathy was associated with increased measures of skewness and kurtosis as well as decreased measures of variance and echogenicity, with a healthy tendon expected to exhibit highly aligned collagen fibers creating a striped pattern of alternating dark and light bands.[16,17] While quantitative ultrasound measures have been established to quantify overall tendon quality,[15,17] specific factors that can affect these measures have yet to be established.

Histology has been used extensively to determine specific factors that influence the quality of the tendon.[22-26] These studies have shown poor tendon quality being associated with changes in collagen fiber organization, nuclei shape, cellularity, and fatty infiltration. Understanding tendon quality is important since poor tendon quality relates to decreased mechanical properties of the tendon.[22] Reduced mechanical properties due to pathologic changes may be one reason for high failure rates of surgical repairs.

By providing a quantitative assessment of specific factors of tendon quality through conventional B-mode ultrasound images, clinicians could adapt their surgical technique accordingly to minimize the failure rate. Therefore, the objective of this study was to determine the feasibility of assessing tendon quality as quantified by histology through changes in quantitative ultrasound measures.

Methods

Eight fresh-frozen cadaveric shoulders (2 male, 6 female, 63 ± 12 years) were used for this study, in which the specimens were acquired with approval from the University of Pittsburgh Committee for Oversight of Research and Clinical Training Involving Decedents. As part of the screening process for all shoulder specimens acquired in our laboratory, ultrasound scans are performed to detect rotator cuff tears. Four of the cadaveric shoulders that were obtained over time had a small tear in the supraspinatus tendon (<1 cm in the anterior–posterior direction) confirmed after dissection, and the tear size was measured with digital calipers. All specimens were stored at −20°C and allowed to thaw overnight at room temperature before use.

Ultrasound imaging and analysis

All ultrasound images were obtained prior to dissection by single orthopedic surgeon with over 15 years of expertise in musculoskeletal ultrasound for the assessment of rotator cuff tears. Ultrasound images were taken with the specimen in a position of hyperextension and internal rotation of the humerus to expose as much of the supraspinatus tendon as possible under the acromion (i.e. simulated Crass position).[27-30]

A transverse view of the widest part of the supraspinatus tendon near its insertion to the humerus for the purposes of minimizing the effects of anisotropy on tendon appearance for the quantitative ultrasound measure analysis.[16] Furthermore, this approach represents the clinical approach to viewing the supraspinatus tendon in the transverse view. Images were obtained using an ultrasound machine (LOGIQ S8, General Electric Healthcare, Chicago, IL, United States) equipped with a 61 mm footprint linear transducer (ML6-15 Transducer, General Electric Healthcare, Chicago, IL, United States) (850 × 649 pixels) [Figure 1]. To minimize variability between the images obtained between specimens, only one ultrasound examiner obtained the ultrasound images, the long head of the biceps tendon was used as a consistent reference point between specimens, and the relevant parameters of the ultrasound system did not change across all examinations (frame rate = 18 fps, frequency = 18 MHz, gain = 65 dB, AO% =100). Furthermore, the ultrasound examiner was blinded to the results of the quantitative ultrasound measures, as described below, to minimize any bias when obtaining the ultrasound image.

Another examiner was responsible for evaluating the quantitative ultrasound measures for the image from each specimen. A single examiner was utilized to evaluate the quantitative ultrasound measures, as preliminary tests and prior studies have established low interrater reliability.[16] A 1 cm wide region of interest located 1 cm posterior from the posterior margin of the long head of the biceps tendon was manually selected using ImageJ (National Institutes of Health, Bethesda, Maryland, United States) to capture the middle third of the supraspinatus tendon. From the selected region of interest, quantitative ultrasound measures (skewness, kurtosis, variance,

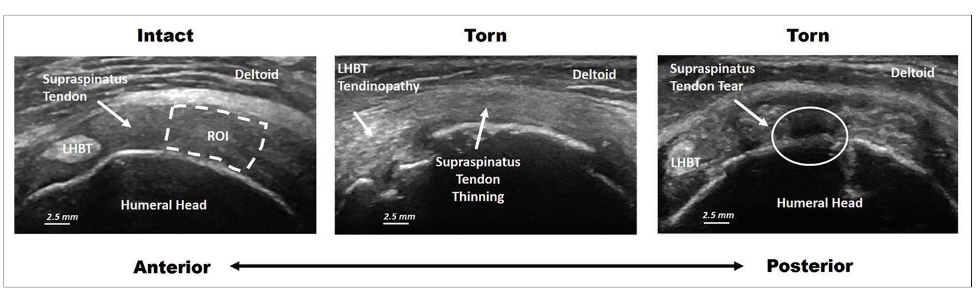

Figure 1: Transverse view of an intact and two torn supraspinatus tendons. A 1 cm wide region of interest was measured 1 cm away from the posterior margin of the long head of the biceps tendon. Quantitative ultrasound measures were made based on the grayscale distribution within the region of interest. The middle image demonstrates signs of long head of the biceps tendon tendinopathy and supraspinatus tendon thinning, suggestive of a tear. For the far-right image, the dark area within the circle indicates the location of the tear. Damage to the surface of the humeral head can be observed for both the middle and far-right image

and echogenicity) were calculated using MATLAB (Mathworks, Natick, MA, United States) based on a histogram that describes the grayscale distribution or echotexture as a measure of tendon quality.[16] The histogram contains information about each pixel in the ultrasound image where each pixel in the ultrasound images represents a grayscale value ranging from 0 (black) to 255 (white). All histograms were verified to be normally distributed and the full 0–255 range was used for the analysis. Skewness, kurtosis, and variance were determined through first-order statistics and represent the asymmetry, pointiness, and spread of the grayscale histogram. Echogenicity was determined as the mean grayscale value of the region of interest.

Histology

Following ultrasound imaging and analysis, all shoulders were dissected, isolating the supraspinatus tendon for the procurement of tissue biopsies for histology. Tissue biopsy samples (~2 mm × 4 mm) were taken near the insertion site, mid-substance, and myotendinous junction for each supraspinatus tendon, ensuring that the longer edge of the rectangle is parallel to the long axis of the tendon. After each piece of tissue was excised, the sample was placed in a histology cassette with the distal edge of the tissue sample at the beveled end of the cassette. The samples were then fixed in 10% buffered formalin for a minimum of 72 h and were oriented such that the posterior side is the first side to be sectioned. Following fixation and paraffin embedding, each sample was sectioned at a 5-µm thickness and was cut along the long axis of the tendon such that three slices were obtained throughout the anterior–posterior width of each tissue biopsy. Each slice was stained with hematoxylin and eosin (H and E) to visualize tendon morphology. Light microscopy was used to image each slice with a ×20 objective lens across the full tendon thickness where images were saved for the analysis at a later time. Since the tendon consists of layers that are not tendon proper (capsule and bursa), a set percentage of the articular–bursal thickness was removed to ensure the analysis only included the tendon.[24]

All histological images were graded for tendon quality using four parameters in a blinded fashion by three independent observers using a semi-quantitative scale. The four parameters chosen to evaluate tendon quality were as follows: collagen fiber organization, nuclei shape, hypercellularity, and fatty infiltration.[23-26] The semi-quantitative grading scale was adapted from a previous study to create a 4-point scale of tendon quality, with a higher score indicating poor tendon quality and a larger area of the tendon being affected (0 = no change, 1 = slight localized change <25% of tendon area, 2 = multifocal change 25%–50% of tendon area, and 3 = diffuse or global change >50% of tendon area).[22] Poor collagen fiber organization was evaluated as less aligned collagen fibers [Figure 2d = Poor collagen fiber organization; b = Highly aligned collagen fibers]. Nuclei shape indicative of poor tendon quality was evaluated as the increased presence of rounded and less elongated nuclei (Black arrow in Figure 2a and c). Hypercellularity was identified as an increase in the number of cells to form a cluster [Figure 2c]. Fatty infiltration was determined as the presence of white globules [Arrowhead in Figure 2e and f].

The average grade between the three observers for each image was used to represent the tendon quality for each image. For each biopsy sample, the average grade of the three slices was

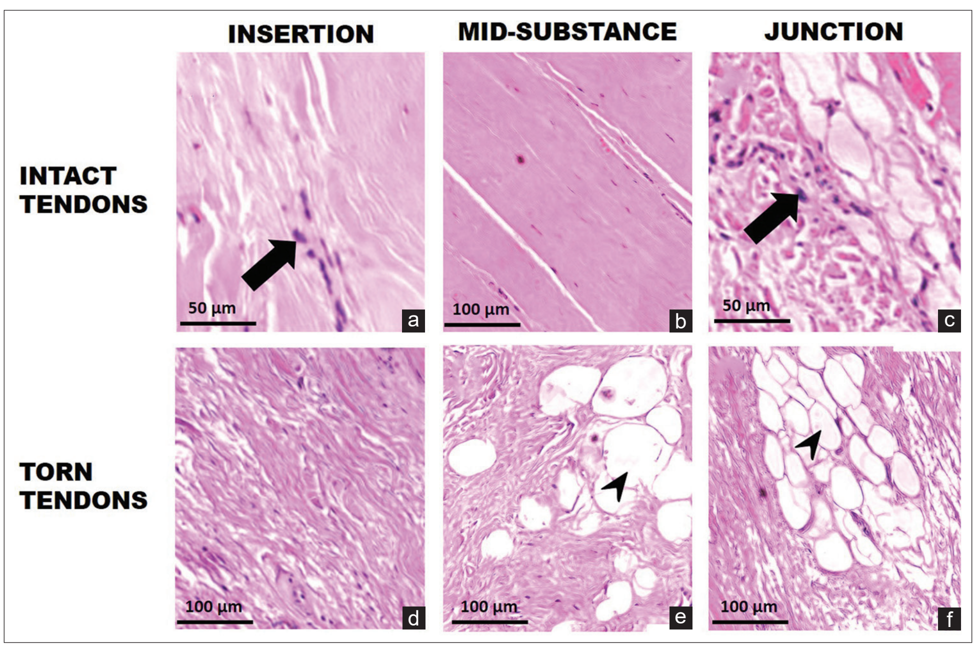

Figure 2: H and E-stained histological images. The top row shows histological images from an intact tendon (a-c), while the bottom row shows histological images from a tendon with a tear (d-f). Each column indicates the location where the histological image was obtained from (insertion site = a and d, mid-substance = b and e or myotendinous junction = c and f). Both intact and torn tendons exhibited changes in tendon quality at all location sites in terms of fatty infiltration (black arrowhead), more rounded nuclei shape (black arrow), increased cellularity and disorganized collagen fibers

used to represent tendon quality for that location site. The summation of the grades for all four parameters represented the overall tendon quality. Tendon quality of the "entire tendon" was determined as the average grade between all three locations (insertion, mid-substance, and myotendinous junction).

Statistics

A Kolmogrov–Smirnov test was performed to determine if the data were normally distributed. When appropriate, nonparametric tests were performed to account for nonnormally distributed data. The intraclass correlation coefficient (ICC) determined the inter-rater reliability of grading the histology images. A two-way mixed ANOVA with a *post hoc* Bonferroni test evaluated histological grades of tendon quality based on the location site (insertion, mid-substance, and junction) and tendon state (intact and torn). Independent samples *t*-tests or Mann–Whitney U-tests determined the effect of tendon state (intact and torn) on quantitative ultrasound measures and histological grades of tendon quality. The correlation between quantitative ultrasound measures and histological grades of tendon quality was determined through Pearson or Spearman's rho correlation calculations. Significance was set at $P < 0.05$.

Results

The four shoulders with a tear had an anterior–posterior tear width of 14.8 ± 11.8 mm and a medial–lateral tear width of 11.0 ± 7.0 mm when measured with the shoulder positioned at 0° of abduction. Histological grading between the three examiners showed good inter-rater reliability for overall tendon quality (ICC = 0.89) as well as for each individual parameter of collagen fiber organization, cellularity, nuclei shape, and fatty infiltration (ICC = 0.76, 0.77, 0.81, and 0.94, respectively). Qualitatively, most specimens with a tear exhibited damage to the long head of the biceps tendon and the articular surface of the humeral head [Figure 1].

Quantitative ultrasound measures showed a wide range of values for all tendons, with no statistically significant difference for any of the quantitative ultrasound measures between the intact and torn tendons (skewness: $P = 0.114$; kurtosis: $P = 0.234$; variance: $P = 0.114$; and echogenicity: $P = 0.718$) [Table 1]. Specifically, the quantitative ultrasound measures of variance and echogenicity tended to be higher for the intact tendons (903 ± 551 and 93 ± 16, respectively) versus the torn tendons (383 ± 83 and 85 ± 19, respectively). The measures of skewness and kurtosis tended to be lower for the intact tendons (0.1 ± 0.1 and-0.1 ± 0.5, respectively) compared to the torn tendons (0.6 ± 0.5 and 1.6 ± 1.5, respectively). In addition, the intact tendons showed more variability than torn tendons for each quantitative ultrasound measure.

From the histological analysis, collagen fiber disorganization, rounded nuclei shape, hypercellularity, and fatty infiltration were present throughout the tendon [Table 2]. Various degrees of tendon quality were exhibited among the location sites [Figure 2]. Regardless of the presence of a tear, significantly more fatty infiltration at the myotendinous junction was observed compared to the insertion and mid-substance [Table 2, $P < 0.001$]. Furthermore, tendons with a tear had twice as much fatty infiltration than intact tendons [Table 2, $P < 0.05$]. No other histological parameters of tendon quality showed significant differences between location sites or tendon state.

Table 1: Quantitative ultrasound measures and histological grades for significant correlations

Specimen	Kurtosis	Variance	Skewness	Echogenicity	Cellularity (insertion)	Fatty infiltration (junction)	Fatty infiltration (entire tendon)
1 (intact)	0.2	376	0.1	92	0.5	1.0	0.3
2 (intact)	−0.4	1343	0.1	88	1.0	0.4	0.1
3 (intact)	−0.8	1414	−0.1	114	0.3	0.2	0.1
4 (intact)	0.4	481	0.2	78	0.5	0.2	0.2
5 (torn)	0.2	275	0.1	112	0.6	2.1	1.2
6 (torn)	1.5	404	0.8	75	0.9	1.7	0.7
7 (torn)	1.0	376	0.2	69	0.5	1.4	0.6
8 (torn)	3.6	476	1.3	84	1.3	0.4	0.4

Table 2: Tendon quality: Grades of histological parameters (mean±standard deviation)

	Fiber organization	Nuclei shape	Increased cellularity	Fatty infiltration	Sum
Insertion	2.5±0.3	2.5±0.03	0.6±0.3	0.2±0.2	5.9±0.7
Mid-substance	2.4±0.4	2.4±0.4	0.6±0.2	0.3±0.3	5.6±0.9
Junction	2.4±0.3	2.4±0.3	0.8±0.4	0.9±0.6	6.4±1.1
P	0.349	0.168	0.616	<0.001	0.051
Intact	2.5±0.2	2.5±0.3	0.6±0.2	0.3±0.3	5.9±0.7
Torn	2.3±0.4	2.4±0.4	0.8±0.3	0.6±0.6	6.1±1.2
P	0.328	0.546	0.163	0.033	0.476

The histological parameters of tendon quality at each individual location site showed poor tendon quality for tendons with a tear. At the mid-substance, the overall tendon quality was graded to be 19% higher in torn tendons (6.4 ± 0.6) than intact tendons (5.4 ± 1.3) ($P < 0.05$). In addition, torn tendons had more fatty infiltration than intact tendons at the mid-substance, 0.5 ± 0.4 and 0.1 ± 0.1, respectively ($P < 0.05$). No significant difference was found between the intact and torn tendons for collagen fiber organization, cellularity, cell shape, or fatty infiltration at the insertion site or myotendinous junction.

A total of three significant correlations between quantitative ultrasound measures and histological parameters were found [Table 1]. A significant correlation was found at the insertion site between kurtosis and increased cellularity ($r = 0.724$, $P < 0.05$) [Figure 3]. No correlation between quantitative ultrasound measures and histological parameters was found at the mid-substance. At the myotendinous junction, a significant inverse correlation between variance and fatty infiltration existed ($\rho = -0.843$, $P < 0.01$) [Figure 4]. When considering the entire tendon, a significant inverse correlation between variance and fatty infiltration was also found ($\rho = -0.826$, $P < 0.05$) [Figure 4].

Discussion

The findings from this study show the potential for using quantitative ultrasound measures to analyze the grayscale echotexture of an ultrasound image and assess changes in tendon quality as quantified through histology. Certain changes in tendon quality could be detected because of their effect on the echotexture of an ultrasound image (e.g., fatty infiltration will make the ultrasound image having hyperechoic regions). While previous studies have shown quantitative ultrasound measures correlate to clinical pathologies,[15,17,21] no direct evidence has been provided correlating quantitative ultrasound measurements to tendon morphology.

The quantitative ultrasound measures showed no statistically significant differences between the intact and torn tendons, but the trends observed were similar to previous studies in terms of healthier tendons exhibiting higher measures of variance and echogenicity as well as lower measures of kurtosis and skewness.[15,17] Comparisons of the raw values of each quantitative ultrasound measure to previous studies are not possible due to differences in ultrasound examiners and ultrasound settings (e.g., frequency and gain). Nonetheless, this study reinforces the notion that with further development and analyses, quantitative ultrasound measures could be useful to assess tendon quality as long as a repeatable protocol is used to obtain repeatable images and inter-examiner variability is eliminated.[15-17,20]

The strong positive correlation between cellularity and kurtosis indicates that a sharp peak in the grayscale histogram of the ultrasound image relates to hypercellular regions found in the tendon. The other correlations were between variance and fatty infiltration. Variance measures the spread of the grayscale values, while fatty infiltration indicates the amount of fatty infiltration. The correlations indicate that increased kurtosis and decreased variance are associated with increased supraspinatus tendon degeneration, consistent with prior studies that found increased kurtosis and decreased variance correlated to poor supraspinatus and biceps tendon health.[17] The correlations found in this study suggest that quantitative ultrasound measures could be used to assess specific factors that affect tendon quality which can only be otherwise assessed through histology or magnetic resonance imaging. The ability for clinicians to evaluate the changes in tendon quality is important since material properties of the tendon may be reduced as a result, making rotator cuff tear initiation and propagation more likely.[26,31]

All tendons, regardless of their tendon state (i.e. torn or intact), exhibited similar tendon quality. No statistical differences were found between the intact and torn group for the quantitative ultrasound measures. In addition, most histological parameters of degeneration (collagen fiber organization, nuclei shape, and cellularity) showed no significant differences between the intact and torn tendons. Only fatty infiltration and overall tendon quality exhibited differences between the intact and torn tendons, which were similar to findings from the previous histological studies.[24,32]

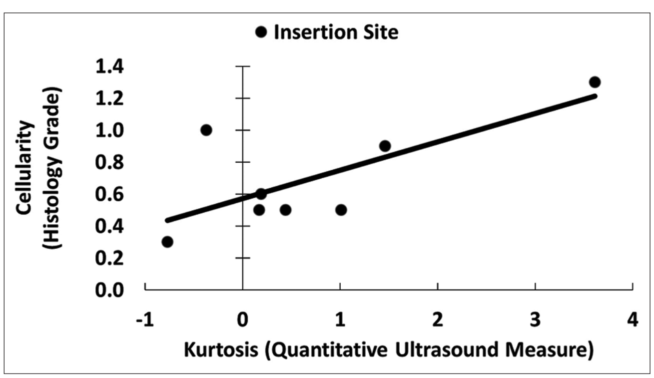

Figure 3: Strong positive correlation between kurtosis and cellularity at the insertion site ($r = 0.724$, $P < 0.05$)

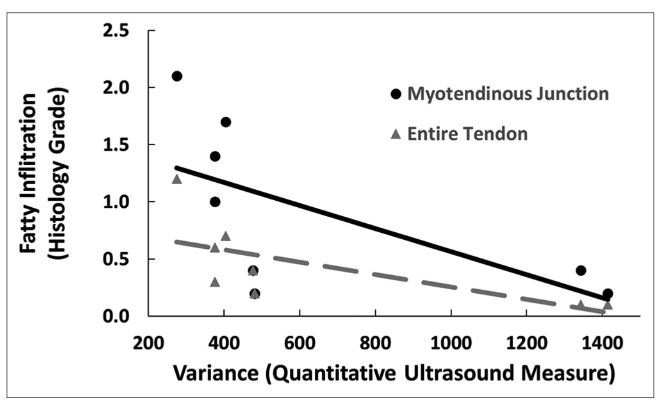

Figure 4: Strong inverse correlation between variance and fatty infiltration at both the myotendinous junction and the entire tendon ($\rho = -0.843$ and $\rho = -0.826$, respectively) ($P < 0.05$). Plotted data represents raw data rather than "ranked" data used for Spearman's rho calculation

A limitation of this study is that only the transverse view of the supraspinatus tendon was imaged by ultrasound, corresponding most closely to the insertion site of the tendon. The analysis of the supraspinatus tendon near the myotendinous junction using the transverse view is not possible due to interference from the acromion. This imaging protocol was previously verified to obtain repeatable images of the supraspinatus tendon for quantitative ultrasound measure analyses while maximizing the amount of the tendon that can be imaged.[16] The quantitative ultrasound measures are sensitive to the conventional B-mode image acquired. Care must be taken to thoroughly verify the repeatability of the ultrasound images acquired to account for the anisotropy of the supraspinatus tendon and the influence of the surrounding bones. Therefore, while a long-axis view of the supraspinatus tendon may have provided more information about multiple tendon locations, an established repeatable protocol does not yet exist.

Based on the findings in this study, further development of an imaging protocol for a long-axis supraspinatus tendon view may possibly reveal even more correlations to changes in tendon quality. In addition, only a small subset of factors that affect tendon quality could be investigated since only H and E staining was used. However, the use of additional staining techniques is not likely to change the results found for the subset of factors that altered tendon quality investigated in this study. Future studies will utilize additional staining techniques to understand different factors that may affect tendon quality (e.g. collagen content, fat, and glycosaminoglycans present in the extracellular matrix). To differentiate quantitative ultrasound measurements between intact and torn tendons, *post hoc* power analysis showed that 71 additional samples in each group were needed.

Conclusion

From this study, the foundation for a new methodology of quantitative ultrasound measures has been established for clinicians to use ultrasonography as a diagnostic tool to noninvasively and quantitatively evaluate tendon quality. With further development of this methodology, clinicians might be able to evaluate location-specific changes in tendon quality noninvasively and monitor healing tendons in patients undergoing treatment. Furthermore, the most effective inflammatory medications or exercise modifications in physical therapy could be administered by understanding the etiology of the changes in tendon quality (e.g., fatty infiltration and hypercellularity).

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Conflicts of interest

There are no conflicts of interest.

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