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A reliable method to quantify tendon structure using B-mode ultrasonography

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Summary

Structure is an important clinical marker of tendon health; however, current standards utilize qualitative scores that are not strongly reliable. Therefore, the purpose of this study was to establish the reliability of an image processing technique that quantifies tendon collagen structure using B-mode ultrasound images. Longitudinal images of the Achilles tendon were collected in 12 healthy-young adults and intra- and interrater reliability was assessed over multiple image selections and multiple days. Intraclass correlation coefficients were strong ($r > 0.71$) for all comparisons. These findings demonstrate that quantitative assessments of tendon structure using B-mode ultrasonography are reliable.

Keywords

tendon structure; collagen; organization; reliability; quantitative

Introduction

Achilles tendinopathy is characterized by painful, non-inflammatory swelling of the tendon that limits patient function and mobility.[1–5] One of the most common musculoskeletal injuries in physically active populations,[2,4] tendinopathy leads to altered tendon structure, reduced mechanical properties, decreased organization and uniformity of the collagen fibers, and vascular infiltration.[6] Quantifying these changes provides essential information regarding the disease progression and response to treatments. Invasive measurements are utilized in animal studies to rigorously quantify tendon structure and function in response to injury. For example, cellular and macroscale changes are characterized using histology[6,7] and crossed polarizer imaging,[8] respectively.

Ultrasound assessments are often used clinically to evaluate tendon status in patients with symptomatic tendinopathy. Clinical assessments combine qualitative measures of tendon structure and neovascularization with quantitative measures of tendon thickness and cross-sectional area to grade tendinopathy severity.[9] While tendon thickness and cross-sectional area are reliable measurements, qualitative assessments of both structure and

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neovascularization are more variable amongst observers.[9] Ultrasound techniques such as shear wave elastography and micro-morphology have been used to correlate mechanical properties with measured changes in tendon structure.[10,11] In both techniques, gross changes in the structure of the tendon explain changes in tendon stiffness. However, the reliability of these techniques is not well described for human tendon and the clinical relevance of these measures in tendon is not entirely clear.

Non-invasive imaging that reliably quantifies tendon structure would provide researchers and clinicians the tool needed to prospectively track tendinopathy progression and develop potential biomarkers of tendon health. While there are several imaging paradigms to consider, we identified one that can be performed in a clinical setting without the need for special equipment or training. A previous group developed a computational analog to crossed polarizer imaging in order to quantify tendon structure in large tendons.[12] This imaging technique is well-documented and has been successfully implemented in small animal models. While this ultrasound technique is strongly correlated with crossed polarizer imaging, the reliability of analyzing images of human tendon has not been reported.

Therefore, the purpose of this study was to assess the intra- and interrater reliability of a non-invasive ultrasound imaging technique that quantifies tendon structure. We hypothesized that quantifying collagen organization using this computational analog of crossed polarizing imaging is more reliable than current standards for grading tendon structure.[9]

Materials and Methods

Study design and image acquisition:

Twelve healthy young adults (BMI: 23.8 ± 2.4; Age: 25± 2; Sex: 5 Males) with no signs or symptoms of Achilles Tendinopathy participated in this IRB approved study to establish the reliability of this measurement of collagen organization in vivo through high frequency ultrasound. All subjects provided written consent before participation. Longitudinal B-mode ultrasound images of the right Achilles tendon (n = 12) mid-substance - approximately 4 cm proximal to the insertion into the calcaneus - were acquired by a single investigator (O1) using an 18 MHz probe (L18–10L30H-4, SmartUs, TELEMED, Milan, Italy). Subjects lay prone with the ankle resting comfortably off the end of a treatment table. A series of continuous ultrasound images were acquired to generate a video of the complete imaging process, from which two observers (01 and O2) independently selected images for analysis. Image acquisition settings were kept constant across all patients (Dynamic Range: 72dB; frequency: 18 MHz; gain: 47 dB). Intra- and interrater reliability was assessed by calculating the collagen organization of the three observers' (O1, O2, and O3) independently identified regions of interest of the Achilles tendons over multiple days on multiple tendon images (Fig. 1). These regions of interest were exported and processed using custom scripts (MATLAB, MathWorks, Natick, MA) to quantify the collagen organizational structure of the Achilles tendon.

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Image analysis:

Collagen organization was quantified using a custom-written algorithm that has been utilized in small animal studies and described in great depth elsewhere.[12] Briefly, a rectangular region of interest (Fig. 2A) was selected to isolate the visible length of the free Achilles tendon (3cm probe scanning width). The edges of the tendon were avoided to prevent issues resulting from edge artifact. Tendon collagen fascicles appear hyperechoic under ultrasound compared to the hypoechoic non-collagenous matrix between the fascicles, giving the tendon a banded appearance distinctly different from other visible structures (Fig. 2B). The alignment of these bands was assessed by applying a linear kernel over groups of pixels in the image through a convolution matrix at varying angles between 0 and 180 degrees. A power series function determined the angle at which maximum echo intensity occurred and determined to be the primary direction of the fibers. This maximum echo intensity occurred when the angle of the convolution was the same as the angle of hyperechoic fiber in a particular kernel. The distribution of local collagen alignment is plotted in a histogram (Fig. 2C) showing the number of fascicles aligned at different angles around the mean angle. The circular standard deviation (CSD) is calculated by determining the number of collagen fascicles that were not aligned in the primary fiber direction and the magnitude of the difference between their alignment and the mean angle. CSD and collagen organization are then, by definition, inversely proportional: the higher the CSD, the less organized the structure of the tendon.

Reliability:

Intra- and interrater reliability of this collagen organization measurement was assessed by three investigators on images independently selected by two investigators (O1 and O2) (Fig. 1). Prior to making these selections, investigators defined criteria for a candidate image: 1 - clear bands spanning the length of the image to ensure the fascicles were in plane, 2 - minimal artifact as a result of probe liftoff or poor contact, 3 - the probe is stationary in several frames preceding and following the image to avoid movement artifact. From these selections, 24 ultrasound images, 12 selected by the two investigators, were generated to be analyzed.

Three investigators selected regions of interest from each of the 24 tendon images on multiple days. Each investigator selected regions of interest that contained the largest amount of the Achilles tendon visible for collagen analysis without including any structures that were not part of the tendon. Investigators were blinded to the subject identification code and made these selections in randomized sequences. Regions of interest were selected on back-to-back days resulting in 144 unique selections to be analyzed. The computational analog of crossed polarizer imaging was applied on these images that generated CSD values.

Intraclass Correlation (ICC) coefficients (r) were calculated using a two-way mixed-effects model[13] to establish intra- and interrater reliability of measurements of collagen organization (CSD). ICC coefficients were calculated by comparing the CSD values resulting from the image and region of interest selections in three conditions (Fig. 1): 1 - between-image intrarater reliability; 2 - between-day intrarater reliability; and 3 - interrater reliability. With two sets of images analyzed by three investigators, each intra- and interrater

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reliability assessments were performed on six and four combinations, respectively. ICC r values range between 0 and 1 with several specific ranges of 0.9–1.0, 0.68–0.90, 0.36–0.67, and 0–0.36 describing very strong, strong, moderate, and poor correlations, respectively.[14] We established an *a priori* r value of 0.68 as a clinically-relevant threshold for reliability when analyzing collagen organization.

Results

Inter- and intrarater reliability was strong or very strong for all comparisons ($r \geq 0.71$, $p < 0.001$, Tables 1 and 2). Intrarater reliability on the same set of images was between 0.88 and 0.95 for all three observers. Intrarater reliability on two sets of images independently selected by O1 and O2 was between 0.71 and 0.84 for all three observers. Interrater reliability on the two sets of images assessed by the three observers on the same day was 0.85 and 0.88 for image sets 1 and 2, respectively. These interrater reliability measurements were repeated for measurements from the following day and found similarly strong r values of 0.85 and 0.87. CSD measurements averaged 6.94 with a coefficient of variation of 7% between all images and observers (Tables 1 and 2).

Discussion

Clinical evaluations of tendinopathy rely on qualitative measurements of tendon structure made by physicians and biomedical technicians.[9] While advanced imaging modalities quantify the structure of tendon via collagen organization,[15–18] such techniques are invasive and cannot be utilized in patient populations. Recent advances in ultrasound image analysis[12] provide a non-invasive platform for quantifying tendon structure, but the reliability of this technique has not yet been documented. Therefore, the purpose of this study was to assess the intra- and interrater reliability of a non-invasive ultrasound imaging technique that quantifies tendon structure. This is the first study to establish the reliability of this non-invasive technique for quantifying tendon structure, in which we found that measurements of collagen organization strongly correlated within and between observers ($r \geq 0.71$, $p < 0.001$).

Measurements of collagen organization made using ultrasound imaging were similar to prior reports in the small animal literature. In our study of Achilles tendons of recreationally-active young adults, we calculated a CSD 95% confidence interval of 5.3 – 8.2. Mouse and rat models measured CSD using this technique and found 95% confidence intervals of 4.6 – 5.2.[12,19] These slightly lower values from small animal studies may be explained by several technical and biologic factors. Higher probe frequencies of 55 and 40 MHz were utilized to study the smaller structures of mouse[12] and rat[19] tendons, respectively. However, due to the larger tendons in humans, a transducer frequency of 18MHz was suitable to clearly visualize the striated pattern of the Achilles tendon.[20] Small animal studies control for variability by having genetically similar animals that all have a short and homogenous life, limiting the likelihood of asymptomatic tendon damage experienced by humans throughout a lifetime of loading. Increases in CSD have been observed in small animal models in response to injury and healing through both crossed polarizer imaging[21] and the computational analog used in this study.[22] These changes in CSD were shown to be correlated with several mechanical

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properties[21] such as dynamic modulus and peak strain. These measures directly impact a tendon's ability to effectively transfer load and are particularly important when assessing a patient's functional outcomes after injury and treatment.

Our findings build confidence in the potential clinical utility of quantifying tendon collagen organization using B-mode ultrasonography. Unlike current clinical measures of tendon structure that are qualitative and less reliable,[9] this computational analog of crossed polarizer imaging does not require special training or experience to generate reliable measures of collagen organization. In addition, current clinical assessments rely on discrete scores from 0 to 4, making it difficult to track small changes in tendon health. Establishing a reliable, continuous-scale metric, like collagen organization, provides clinicians the ability to track changes in tendon structure, which may lead to a new biomarker of tendon health.

Characterizing tendon structure has several advantages over other ultrasonography techniques that characterize the material properties of tendon.[23,24] Images used for quantifying the CSD of tendon can be acquired with a standard clinical ultrasonography system. In addition, this optical-based analysis could potentially be applied to existing ultrasound images, which would allow investigators to leverage large data sets of medical images. Shear-wave elastography and other modalities require special equipment and can be sensitive to the amount of pressure the user applies to the tissue being imaged.[23] Ultrasound propagation speed is strongly correlated with tendon tension but has poor reproducibility due to experimental set-up considerations.[25] While these other ultrasound techniques provide exciting opportunities to characterize tendon mechanics and material properties, they do not quantify tendon structure, which is a key clinical consideration for assessing tendon health.[9]

This study was affected by several limitations. Images were acquired during a single session by a biomechanical engineer instead of a radiologist or sports medicine physician. We decided to acquire a series of images during a single visit because prior work demonstrates that similar images of the Achilles tendon can be acquired over multiple sessions by multiple investigators.[9] We then asked two blinded observers to select a single image from each series of images that looked to be the highest quality. Our strong r values within and between observers and selected images suggests that this ultrasound analysis paradigm is robust and reliable. We decided to study healthy Achilles tendon due to its superficial and easy to image structure. However, this ultrasound technique has been utilized in small animal studies linking tendon damage with structure, suggesting robustness for characterizing tendons of varying size and injury status. Regions of interest that were analyzed in the current study were limited by the 3cm width of the ultrasound transducer. Although tendon properties vary along the length of the tendon,[26] tendinopathies are often localized to small portions of the tissue that would easily fit within this scanning constraint.[27] Similar to other ultrasound techniques, B-mode ultrasound images are sensitive to probe pressure. Due to study constraints the amount of pressure applied to the probe was not able to be quantified; however, the examiner acquired all images while maintaining contact with the medial and lateral aspects of the tendon to ensure the pressure of the probe was not noticeably deforming the tendon. Animal models have shown increases in tensile loads to decrease CSD[12] but the effects of transverse compression have not been studied.

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Our data show that tendon organization can be reliably quantified by analyzing longitudinal B-mode ultrasound images of the Achilles tendon. This non-invasive and reliable measurement of tendon structure can be utilized on existing image sets acquired using standard ultrasonography equipment. Further, prospectively studying tendinopathy progression may establish this imaging technique as a biomarker of tendon health that could be easily integrated into standard-of-care imaging protocols for patients being treated for tendon pain.

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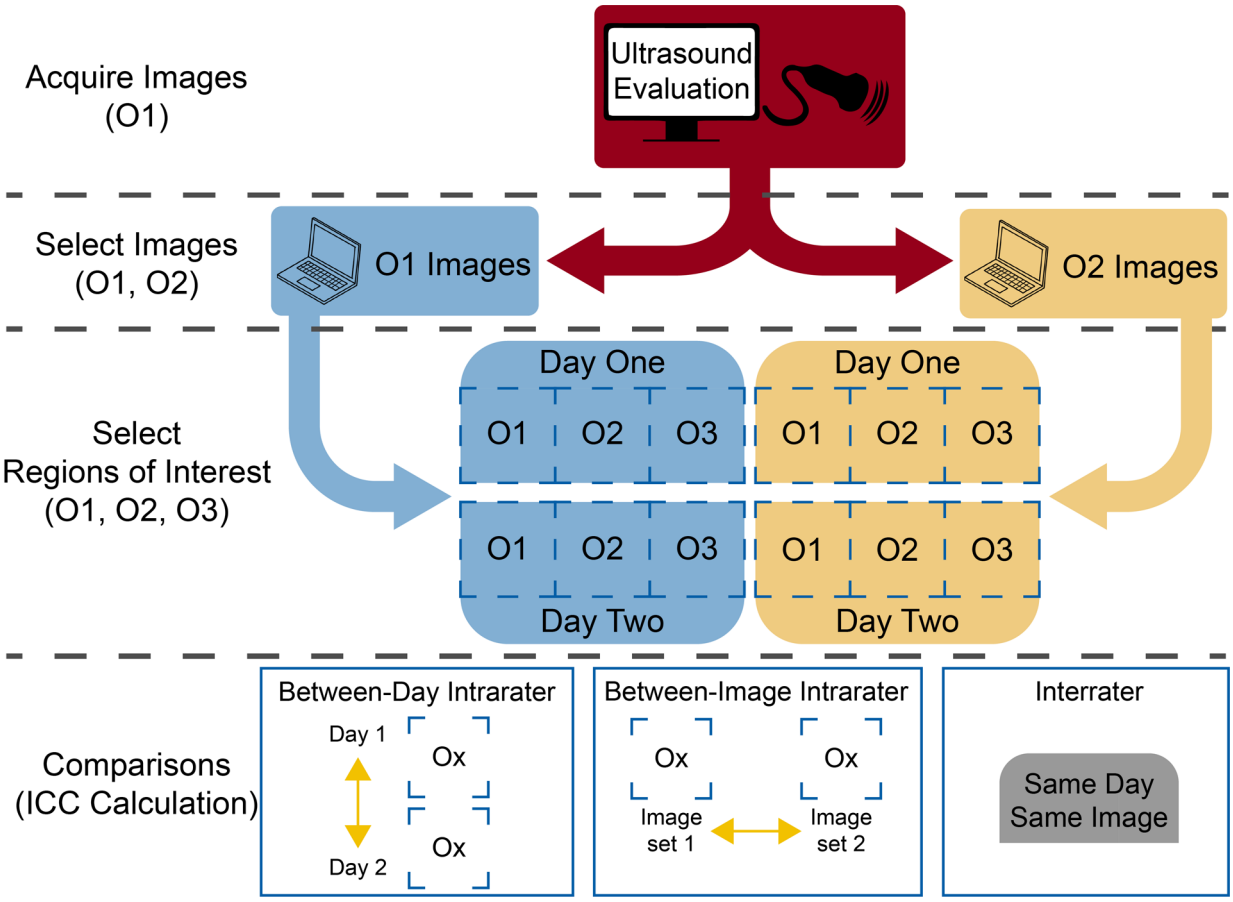


Figure 1.

Ultrasound evaluations of continuous longitudinal imaging of the tendon were performed by a single investigator (O1). Single images of the tendon were independently selected by two investigators (O1 and O2). Regions of interest from each image were independently selected by three investigators (O1, O2, and O3) on back to back days. These selections were analyzed to generate the quantitative measure of collagen organization: circular standard deviation (CSD). Intra- and interrater reliability was calculated using a two-way mixed-effects intraclass correlation (ICC) model.

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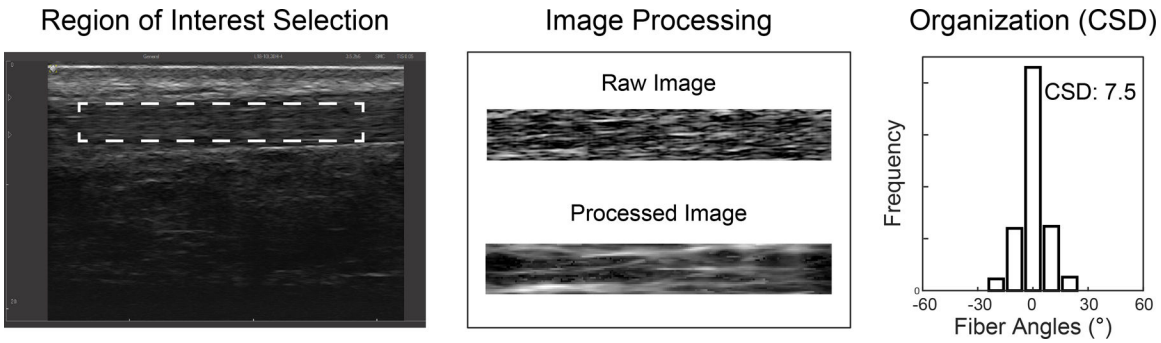


Figure 2.

Rectangular regions of interest that encompassed as much of the free Achilles tendon as possible were independently selected by three investigators *(left)*. Selections were processed computationally to identify the fascicles of the tendon (*middle*). Collagen fascicle alignment was calculated based on the deviation from the mean direction of all identified fascicles, termed the circular standard deviation (CSD).

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Table 1.

Intrarater reliability (r) and CSD (95% confidence intervals)

	ICC Coefficients (*r*)			
	Day 1/Day 2		O1 Images/O2 Images	
	O1 Images	O2 Images	Day 1	Day 2
O1	0.93 ($p < 0.001$)	0.94 ($p < 0.001$)	0.77 ($p < 0.001$)	0.83 ($p < 0.001$)
O2	0.88 ($p < 0.001$)	0.93 ($p < 0.001$)	0.71 ($p < 0.001$)	0.76 ($p < 0.001$)
O3	0.91 ($p < 0.001$)	0.95 ($p < 0.001$)	0.84 ($p < 0.001$)	0.84 ($p < 0.001$)
	95% Confidence Intervals (CSD)			
	O1 Images		O2 Images	
	Day 1	Day 2	Day 1	Day 2
O1	5.30 – 7.34	5.54 – 7.57	6.55 – 7.52	6.87 – 7.59
O2	5.66 – 6.79	5.43 – 6.80	6.22 – 7.38	6.69 – 7.51
O3	6.48 – 7.77	6.75 – 7.80	7.55 – 8.15	7.20 – 7.94

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Table 2.

Interrater reliability (r) and CSD (95% confidence intervals)

	ICC Coefficients (*r*)	
	O1 Images	**O2 Images**
Day 1	0.88 ($p < 0.001$)	0.88 ($p < 0.001$)
Day 2	0.85 ($p < 0.001$)	0.87 ($p < 0.001$)
	95% Confidence Intervals (CSD)	
	O1 Images	**O2 Images**
Day 1	5.86 – 7.25	6.84 – 7.62
Day 2	5.80 – 7.32	6.98 – 7.62