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Is Echogenicity a Viable Metric for Evaluating Tendon Properties *In Vivo*?

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Abstract

Material properties of tissue *in vivo* present an opportunity for clinical analysis of healing progression and pathologies as well as provide an excellent research tool yielding quantified data for longitudinal and cross population studies. Echogenicity is a material's ability to reflect sound and, using ultrasound, it has been shown to increase with tendon tension *in vitro*, though this non-invasive measurement technique for determining mechanical properties has not been tested *in vivo*. The aim of this study was to establish if echogenicity, seen by the increase in image brightness, could be correlated to stress within a tissue. 18 Achilles tendons were imaged in the sagittal and transverse planes while producing a series of isometric contractions starting from rest and producing the torque equivalent of 0.5, 1.0, 1.5, and 2.0x body weights. Manual tracing identified the tendon in each of the images. The cross-sectional area determined from the transverse plane images in conjunction with the tendon force yielded the tendon stress. The echogenicity of the tendon was determined from the mean brightness change from rest to each of the contraction cases, measured from the sagittal plane images. A weak correlation existed between the echogenicity and stress ($R = 0.25$) but it was found that there was no significant change in axial area during contraction ($p = 0.683$) establishing then tendon as incompressible. Echogenicity proved to be non-functional for measuring the mechanical properties of the Achilles tendon due to the additional factors included with *in vivo* testing e.g. tendon twist and multi-axial loading.

Keywords

Ultrasound; Material Properties; Isotropy; Stress; Incompressible

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Conflict of interest statement

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Introduction

The ability to determine the functional status of an injury and the progression of healing is critical for clinicians. Mechanical properties (e.g. elastic modulus, strain) of tissue can be used as indicators of injury severity and recovery progression (Arya and Kulig, 2010; Child et al., 2010; Lichtwark et al., 2005; Schepull et al., 2007). Due to the unbiased nature of mechanical properties these data provide quantitative measures which can be compared longitudinally within subjects and across populations. Currently, there are several testing measures to acquire these mechanical properties in tendon including the invasive implantation of tantalum beads combined with x-ray imaging (Schepull et al., 2012) and elastography, which requires several large assumptions e.g. tendons are perfectly elastic and isotropic (Kongsgaard et al., 2011). A simpler, less invasive method for acquiring mechanical properties is needed for general clinical applicability.

Echogenicity is a non-invasive measurement of a material's ability to reflect sound. The attenuation of acoustical signals is characteristic of a material's mechanical properties (Yu and Boseck, 1995) and the magnitude of those properties increases when the material placed into tension (Hughes and Kelly, 1953). Tendon consists of collagen fibrils which run parallel to the direction of force application and are held together in an extracellular matrix. This structure of linked collagen fibrils allows for tendons to be stretch elastically up to 14% [Wang]. This acoustical attenuation characteristic of materials, when applied to tendon, translates to a greater ability to reflect acoustic signals when the tendon fibers are stretched during contraction. When imaged *in vitro* via ultrasound, increased tension and tendon strain resulted in less signal absorption and a greater amount of signal reflection, i.e. brightness (Duenwald et al., 2011). Additionally, mechanical property changes caused by tendon damage decreases echogenicity and has been shown as a precursor to pathology (Duenwald-Kuehl et al., 2012; Malliaras et al., 2008). In equine studies, diseased tissue was associated with decreases acoustical reflection characteristics [Crevier-Deniox et al., 2005; Garcia et al., 2003]. Therefore it is of interest to determine whether the potential exists for echogenicity of *in vivo* human tissue to provide a meaningful insight into injury mechanisms.

While previous studies working with animal and cadaveric tissue suggest the potential feasibility of using echogenicity for determining *in vivo* tendon properties, a problem still remains. A major difference between estimating tendon properties *in vitro* and *in vivo* is that the mechanical properties may change with loading rates, tendon geometry and the vector of tensile force application. Joint movement within "isometric" contraction in a dynamometer causes a lengthening of the tendon and therefore an erroneous increased strain (Arampatzis et al., 2005). Additionally, a controlled, uniaxial stress protocol used during *in vitro* tests are performed with tension being applied to the end of the tendon only, which is unrealistic since, *in vivo*, muscles connect and produce tension along the length of the tendon and not simply at the end; therefore an *in vivo* experiment is necessary.

There is a need for a minimally invasive and simple measure to establish the mechanical properties of tendon during the healing progression to be used for longitudinal and cross subject analysis. Therefore the goal of this study is to correlate the minimally invasive

technique of echogenicity to the *in vivo* stress within the Achilles tendon. We hypothesize there will be a linear increase in ultrasound image brightness of the tendon with increased stress which would suggest echogenicity is a functional tool for mechanical property assessment of tendons.

Methods

Echogenicity and stress measurements were taken from 9 healthy subjects (age 25 ± 5.6 , height $172.0 \text{ cm} \pm 5.1$, mass $68.7 \text{ kg} \pm 10.8$) with no history of Achilles tendon rupture or tendinopathy. Each subject read and signed an informed consent approved by the University of Delaware institutional review board. Retro-reflective markers were placed on the lateral and medial malleoli of the subjects to establish the joint center. The subjects knelt in a Biodex 3 System (Biodex Medical Systems, Shirley, New York) with their ankles and knees fixed at 90° and hips at 0° . The moment arm of the Achilles tendon was determined using a method similar to Manal and colleagues (Manal et al., 2010). A LogiQ P6 ultrasound [GE medical systems, Fairfield, CT] with a ML6-15 transducer was used to measure the distance from the skin's surface to the center of the Achilles tendon. Retro-reflective markers were placed at the center of the transducer and the distance from the markers to the head of the transducer was measured. The transducer was placed on the ankle to align the markers on the transducer with those fixed to the malleoli. 3D motion data [Qualysis Motion Capture System, Gothenburg, Sweden] were recorded during the ultrasound collection and a triggering device was used to establish a time point in the motion capture data at which the ultrasound image was taken. The Achilles tendon moment arm was calculated as the distance between the 2 sets of markers less the distance to the head of the transducer and the distance from the skin's surface to the center of the tendon, measured on the ultrasound images. This was repeated for the opposite leg. The desired forces through the Achilles tendon were 0.5, 1.0, 1.5, and 2.0 body weights. The desired tendon forces and moment arms were used to calculate the torque equivalent for each force, as torque was the measure provided as visual feedback to the subject.

The subject's positioning remained the same from the moment arm collection to the contraction collection. Prior to the subject eliciting any contraction, the passive torque of the subject's ankle at 90° was recorded. This was added onto the calculated desired torques to ensure only active contraction was accounting for the stress in the tendon. As previously stated, the knee was placed at 90° , which increases slack in the gastrocnemius and greatly limits the gastrocnemii's contribution to the ankle torque during plantar flexion. Since the gastrocnemii were not the major contributor to the ankle moment, ultrasound images were taken at the soleus myotendinous junction (MTJ). The ultrasound transducer was held at a fixed angle and position on the skin's surface along the longitudinal axis approximately 6 to 8 cm proximal from the calcaneus. Tendons were imaged at 15 MHz and 80% image brightness gain for all trials. An image was first taken with no contraction and then the subject was prompted to contract to a torque of 0.5x BW using visual feedback to match the applied torque to the desired. When that value was reached, an image was recorded and the subject was instructed to contract until the next torque was achieved. This process was repeated for 1.0, 1.5 and 2.0x BW. Three (3) trials of the contraction protocol were performed. The transducer was removed and replaced onto the ankle between each trial. The

subject was given sufficient time to recover between trials to avoid fatigue. Axial images were taken with the same protocol described previously but at a distance approximately 3cm proximal from the calcaneus insertion point. Due to the curvature of the skin's surface around the Achilles tendon, an Aquaflex gel pad (Parker Laboratories, Fairfield, NJ) was used to provide a full field-of-view of the tendon.

Despite efforts to fix the ankle into a particular angle, slight motion of the ankle was inevitable. Using the method described by Arampatzis and colleagues we accounted for any undesired rotation of the ankle. We collected a baseline ultrasound image of the soleus MTJ with the ankle at 90°. While maintaining the transducer location, we passively changed the angle of the footplate of the Biodex to place the ankle into 92° of plantar flexion and collected another image. The process was repeated for 94°, 96° and 98° of plantar flexion. The change in MTJ displacement per degree was calculated for each subject. During the contraction collection, the ankle joint rotation was tracked with motion capture. The change in joint angle during the contraction protocol was associated with a MTJ displacement during the passive motion into plantar flexion. The resulting tendon length change caused by the minor foot movement was subtracted from the overall tendon length change (Arampatzis et al., 2005).

The cross-sectional area and brightness of the tendons were determined using ImageJ (National Institutes of Health, USA). The cross-sectional area and longitudinal images of the tendon were manually traced on a hi-resolution touch-screen display [Wacom Technology Corporation, Vancouver, WA]. The ultrasound image was collected in gray scale and each pixel had a white count which ranged from 0 to 255. ImageJ provided a histogram of the pixel white count within the area selected, or tendon in the case of this study, along with an overall average white count. The average value of the tendon area traced in the sagittal plane determined the brightness. Due to brightness being transducer location dependent, the relative change in image brightness between the no contraction image and the subsequent images from the isometric case determined the tendon echogenicity. The stress during each isometric contraction was determined using Equation 1:

$$\sigma = \frac{\left(\frac{\tau}{MA}\right)}{A} \quad \text{Equation 1}$$

Where σ is the tendon stress, τ is the torque produced at the joint, MA is the moment arm and A is the cross-sectional area of the tendon. A regression of percent brightness change versus stress determined the correlation between stress and echogenicity. Tracking the movement of the soleus MTJ throughout the contractions not only identified a consistent collection location, but also allowed for the determination of tendon stiffness defined as tendon force (N) divided by change in tendon length, or elongation (mm).

Results

A weak correlation exists between the percent change in tendon brightness and stress applied ($R = 0.25$). Although there was a positive slope associated with the image brightness and the stress (0.14), 7 of the 18 cases decreased in brightness following the baseline case of

no contraction. There was a significant motion of the tendon fibers ($p < 0.001$) within the region of interest demonstrated by the tendon elongation, even after the length change of 0.16(0.08)cm at 2 BW caused by an average ankle rotation of 4.4°(0.82) was taken into account. The cross sectional area of the tendon had no significant change with increasing stress ($p = 0.683$). Similarly, the stiffness also did not change through the isometric contraction range ($p = 0.982$).

Discussion

The poor correlation between the change in ultrasound image brightness and the stress within the tendon prevents echogenicity from being a viable metric for determining mechanical properties of the Achilles tendon *in vivo*. A power analysis with a medium effect size ($\beta = 0.9$) was performed which determined the number subjects was enough to establish the significance of this correlation. While previous *in vitro* studies demonstrated the feasibility of using increased echogenicity as a measure to track strain within a tissue, these measurements were performed using a uniaxial force (i.e. the tendon is stretched at the ends and without rotation). *In vivo* measurements are subject to additional variables. The Achilles tendon has spiraling, non-linear, fibers which causes tendon twist under tension (Doral et al., 2010). The 3D twisting of the tendon fibers while using 2D ultrasound may lead to the imaging of new fibers as the original fibers move out of plane. Tendon elongation also caused different portions of the tendon to be imaged. As the subject contracted, the tendon moved an average of 9.2mm through the region of interest at 2BW. The twisting and elongation add variability disrupting the tendon stress to brightness correlation.

The directional component of force applied to the tendon varied compared to the *in vitro* cases. The soleus contributed most of the force through the Achilles and the soleus attaches along the inferior portion of the tendon. Rather than pulling from the proximal end along the longitudinal axis, the soleus pulls along the entire attachment and in the line of action of the muscle fibers, which are not parallel to the tendon fibers. In some subjects this resulted in a widening of the tendon in the sagittal plane and altered the region of interest imaged, adding variability into the brightness measurement unrelated to fiber tension.

The mechanical properties of the Achilles tendon in this study were similar to the findings of previous researchers. The tendon stiffness at 2BW was 146.2 N/mm which matched the results of Schepull and colleagues (143 N/mm) and Neugebauer and colleagues (162 N/mm) (Neugebauer and Hawkins, 2012; Schepull et al., 2007). The average stress at 2BW (1349N) averaged 27.7MPa, a similar finding to Magnusson and colleagues reporting 36.5MPa at 2641N. To better compare the mechanical properties to previous research, the standard error of measurement (SEM) were calculated for the area (0.02 cm²) and the elongation (0.09 mm). The SEM applied to the values found would lead to a stress to range from 26.1 to 28.1 MPa due to error, but these values are still comparable to previous findings. Similarly, the SEM of elongation accounted for a stiffness range of 144.4 to 147.7 N/mm which also within the range of reported tendon stiffness.

An interesting finding from this study was the incompressible nature of the Achilles tendon. Tendon is typically modeled as transversely isotropic and incompressible (Garcia et al.,

2000; Kou et al., 2001), but these are numerical assumptions. This study showed that as the tendon is put in isometric tension *in vivo*, the resultant axial stress does not compress it, demonstrated by the axial area not significantly decreasing with increasing tension. This information can be used in estimates of mechanical parameters using other non-invasive techniques such as ultrasound elastography. These techniques find the shear modulus (μ) of the tendon but desire the more applicable Young's modulus (E), which has been correlated to healing progression (Schepull et al., 2007). Knowing tendon is incompressible is one key assumption in using the estimation $E=3\mu$.

Movement of the probe during the contractions can vary the brightness measurement. To ensure ultrasound probe movement was not a factor, a reliability test was performed. Longitudinal images were taken with the subject at rest, the subject then contracted and relaxed and an image was taken again to verify the brightness was the same in both cases. This was repeated several times on several subjects to confirm scan repeatability. The ICC (3,1) of brightness yielded a 0.995 indicating the probe location and tendon brightness was not changed by contraction.

This study demonstrated that additional factors associated with *in vivo* measurements, including tendon fiber arrangement and motion; do not allow for echogenicity as a feasible metric for determining Achilles tendon mechanical properties. Future studies utilizing echogenicity would be advised to be selective in which tendons are measured and ensure the joint involved is able to be immobilized. The twist of the Achilles during contraction and the various attachment points along the tendon led to different tendon fibers being measured, adding variability in brightness. Selecting a superficial tendon, such as the flexor tendons of the fingers, which have fixed uniaxial loading driven by proximal muscle attachments combined with minimal twisting during contraction may yield more favorable results (Lee et al. 2005). Even if the proper tendon is selected and the joint is immobilized, the tendon will still move relative to the ultrasound transducer due to muscle contraction and this must be a known limitation when using echogenicity.

Despite a weak correlation between brightness and stress, other tendon properties were shown which are applicable to additional, non-invasive measurement techniques. The finding of tendon incompressibility during axially tension provides a key component for translating the shear modulus, found through a non-invasive measure such as elastography, into the more clinically meaningful measure, Young's modulus.

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Figure 1.

The setup for the collection of moment arm data. This is the same setup for the collection of the brightness and stress data with a change in probe position and/or orientation. Note the ankle and knee and 90° and markers placed on the malleoli and at the center of the ultrasound transducer.

Figure 2.

The Achilles tendon outlined in red. Note: The soleus MTJ is used as a reference for ultrasound transducer placement.

Figure 3.

The correlation between echogenicity, in terms of % brightness change) and stress on the Achilles tendon. Note: The lack of correlation ($R = 0.25$) indicates stress does not impact the brightness of the tissue.

Figure 4.

Anatomical differences of the Achilles tendon (traced in red) between two subjects at 0 BW and 1.0 BW. The red arrows indicate the line of action of the muscles fibers, which is not in parallel to the tendon fibers. Note the tendon of subject A widens with contraction while the tendon of subject B maintains a similar width.

Table 1

The mean(SD) area, elongation and stiffness of the tendon during isometric contractions.

BW (%)	Area (cm ²)	Elongation (mm)	Stiffness (N/mm)	Echogenicity
0	0.46 (0.07)	-	-	99.66 (10.03)
50	0.47 (0.07)	2.52 (1.23)	133.7 (42.0)	100.49% (2.55%)
100	0.48 (0.08)	4.75 (2.10)	141.9 (49.0)	101.71% (4.27%)
150	0.49 (0.08)	7.22 (2.39)	140.1 (64.7)	102.42% (5.48%)
200	0.49 (0.08)	9.22 (2.05)	146.2 (100.3)	102.36% (6.45%)
p-value	0.683	> 0.001	0.982	

BW indicates the percent body weight being transmitted through the Achilles tendon. Echogenicity is being reported as the absolute brightness (in white count from 0 – 255) at 0 BW and subsequent percent change. Note the lack of significant change in area with increasing force applied to the tendon.