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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 2.0 × 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 the 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.

1. 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 and Wilson, 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 (Hughs 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, 2006). 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-Denoix 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

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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 that 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.

2. Methods

Echogenicity and stress measurements were taken from 9 healthy subjects (age **25** ± 5.6, height 172.0 cm ± 5.1, mass 68.7 kg ± 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°(Fig. 1). 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

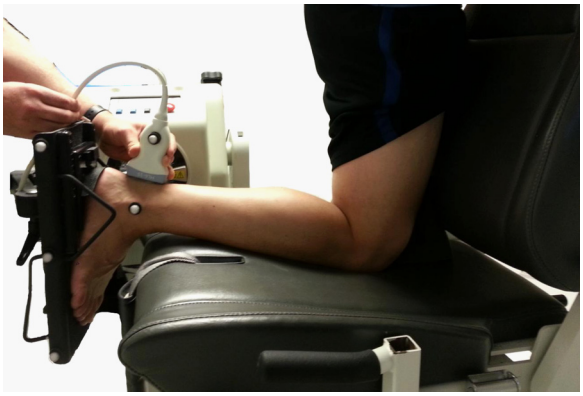

Fig. 1. The setup for the collection of moment arm data. This is the same setup for the collection of the brightness and stress data with a change in probe position and/or orientation. Note the ankle and knee at 90° and markers placed on the malleoli and at the center of the ultrasound transducer.

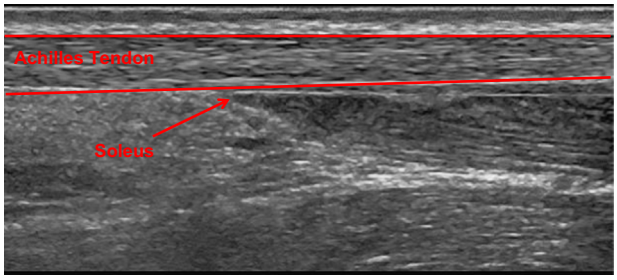

Fig. 2. The Achilles tendon outlined in red. Note: The soleus MTJ is used as a reference for ultrasound transducer placement. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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 6–8 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 0.5 × 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 2.0 × BW. Three (3) trials of the contraction protocol were performed. The transducer was removed a 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 3 cm 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 (Fig. 2). The stress during each isometric contraction was determined using Eq. 1:

$$\boldsymbol{\sigma} = \frac{(\boldsymbol{\tau}/\mathrm{MA})}{\boldsymbol{A}} \tag{1}$$

Where $\boldsymbol{\sigma}$ is the tendon stress, $\boldsymbol{\tau}$ 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).

3. Results

A weak correlation exists between the percent change in tendon brightness and stress applied ($R=0.25$) (Fig. 3). 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