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● *Original Contribution*

ULTRASOUND-BASED TENDON MICROMORPHOLOGY PREDICTS MECHANICAL CHARACTERISTICS OF DEGENERATED TENDONS

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Abstract—The purpose of this study was to explore the relationship between tendon micro-morphology quantified from a sonogram and tendon mechanical characteristics measured *in vivo*. Nineteen adults (nine with unilateral Achilles tendinosis) participated. A commercial ultrasound scanner was used to capture longitudinal B-mode ultrasound images from the mid-portion of bilateral Achilles tendons and a custom image analysis program was used to analyze the spatial frequency content of manually defined regions of interest; in particular, the average peak spatial frequency of the regions of interest was acquired. In addition, a dynamometer and a motion analysis system indirectly measured the tendon mechanical (stiffness) and material (elastic modulus) properties. The peak spatial frequency correlated with tendon stiffness ($r = 0.74$, $p = 0.02$) and elastic modulus ($r = 0.65$, $p = 0.05$) in degenerated tendons, but not healthy tendons. This is the first study relating the mechanical characteristics of degenerated human Achilles tendon using a non-invasive micro-morphology analysis approach. (E-mail: kulig@usc.edu) **© 2015 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Tendon mechanics, Tissue imaging, Elastic modulus, Stiffness.**

INTRODUCTION

Collagen fibers align in a bundle along the long axis of the tendon (Kannus 2000). When sonographically imaged, a tendon may reflect increased acoustic energy when the direction of the sound wave is normally incident to the long axis of the fascicles (Garcia et al. 2003; Kannus 2000). The relationship between the brightness scan (B-scan) image texture (or speckle pattern) and tissue micro-structure depends greatly on the size and spatial distribution of the acoustic tissue scatters relative to the wavelength of the incident sound wave, *i.e.*, the axial and lateral resolution of the ultrasound system (Garcia et al. 2003; Kannus 2000; Wu et al. 1992). Tendon structure observed in ultrasound images has a characteristic banded anisotropic speckle pattern with increased correlation length in the lateral dimension (Bashford et al. 2008). For a healthy tendon, this pattern is represented by a spatial frequency spectrum with a strong magnitude component and narrower spatial bandwidth around the peak spatial frequency (PSF). This pattern was not observed when imaging degenerated tendon (Bashford et al. 2008). One of the most distinguishing features of the spectra of a degenerated tendon is a diffuse spatial frequency pattern corresponding to more isotropic speckle observed in the images. A method developed by Bashford et al. (2008) for quantifying the amount of structural disorganization between persons with and without tendinosis allowed for development of classification algorithms based on linear discriminant analysis of several parameters derived from the spatial frequency spectrum. Further studies have shown some parameters to be more strongly correlated with pathology (Cassel et al. 2012; Kulig et al. 2013).

Visualization and quantification of collagen fiber alignment has been used to indicate or classify pathology in humans and equines. For example, Tuthill et al. (1999) employed a spatial frequency approach to determine a quantitative method to evaluate fatty deposits in Achilles tendons. Penteado et al. (2008) employed a Fourier transform spectroscopy technique, together with principal component analysis and clustering, to assess

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biochemical changes that occur in the degenerative process of the rotator cuff supraspinatus tendons. Chaudhury et al. (2011) used Fourier transform infrared spectroscopy to characterize the chemical and structural arrangement of tendons of the rotator cuff and to identify structural differences between tendon tears and un-injured control tendons. Similarly, a Fourier transform–second harmonic generation imaging procedure was used to differentiate collagen fiber organization between normal and injured equine tendons (Sivaguru et al. 2010).

The mechanical characteristics of the tendon, both mechanical and material properties, are related to the arrangement of its architecture (Arya and Kulig 2010; Lichtwark and Wilson 2005; Wren et al. 2001). Mechanical properties, measured by stiffness, relate to overall deformation-resistance of the whole tendon structure, whereas material properties, measured by the elastic modules, represent the elasticity of each individual element (collagenous constituents) within the tendon. When degenerated or partially ruptured, the collagen bundle organization is disrupted, the water content in the extra-cellular matrix is increased and, with time, the ratio of collagen type III to I is also increased (van Schie et al. 2003). Degenerative tendinopathy (tendinosis) results in disruption and disorganization of tendon fibers along with progressively increasing tendon thickness and tendon cross-sectional area (Helland et al. 2013; Kongsgaard et al. 2010; Kulig et al. 2013). Given these studies and the role material properties play in mechanical properties of tendons (Heinemeier and Kjaer 2011), it is likely that a disorganized architecture (micro-morphology) would provide a stronger indicator of mechanical characteristics of the tendon than increase in thickness and cross-sectional area (macro-morphology) alone. This is especially true when tendons show early stages of degeneration, such as hypo-echocity on an ultrasound image without change in macro-morphology.

To our knowledge, there is no study relating tendon micro-morphology to its mechanical and material properties in humans. Therefore, the purpose of this study was to explore the relationship between tendon micro-morphology quantified from a sonogram and tendon mechanical characteristics measured *in vivo* in individuals with focal Achilles tendon thickening, indicating degeneration.

METHODS

Nineteen individuals, nine (aged 48.7 ± 4.4 y) with unilateral Achilles tendinosis and 10 (aged 46.8 ± 6.3 y) healthy controls were recruited for a companion study (Chang and Kulig 2015). The inclusion criteria for the tendinosis group were: (i) history of unilateral Achilles tendon pain, (ii) absence of pain in the Achilles tendon or elsewhere during walking and running at the time of participation in the study, (iii) sonographically confirmed long-standing tendinosis as focal mid-substance thickening (macro-morphology) in the anterior-posterior dimension exceeding 2 mm difference from the non-involved side and (iv) absence of any known systemic diseases or history of lower extremity surgery (our cases of Achilles tendinosis were likely of overuse origin as all participants were active and without history of systemic collagen disease [Maffulli and Kader 2002]). The inclusion criteria for the control group were the same as those for the Achilles tendinosis group with the exception of the history of unilateral Achilles pain and focal thickening on the ultrasound image.

The procedures were approved by the Health Sciences Review Board at the University of Southern California, Los Angeles. Informed consent was obtained from all patients before data collection in accordance with the Institutional Review Board's protocol.

Before commencement of the primary data collection, demographic and anthropometric data were acquired. Activity level was determined by the World Health Organization Global Physical Activity Questionnaire (GPAQ; Armstrong and Bull 2006) and the severity of Achilles tendinopathy was assessed using the Victorian Institute of Sport Assessment - Achilles (VISA-A) index (Robinson 2001).

Acquisition and analysis of the ultrasound tendon images

Longitudinal B-mode ultrasound images were acquired using a commercial ultrasound scanner (Sonoline Antares, Siemens Medical Solutions USA Inc., Malvern, PA, USA) with a linear probe (VFX 13-5, 8.9 MHz center frequency, −3 dB bandwidth 2.7 MHz) from the mid-portion of bilateral Achilles tendons at rest. Transmit foci were set at 1.0 and 1.5 cm. All other image settings were kept constant across patients. The images were stored as JPEG files with spatial sampling of 800 × 600 pixels (16 pixels/mm) on the hard drive of the ultrasound machine and exported for post-imaging quantitative analyses (ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA). All images were visually screened for indicators of tendon degeneration (*i.e.*, focal thickening, increases in cross-sectional area [CSA] and areas of hypo-echocity within the tendon).

For the analysis of tendon macro-morphology, the tendon thickness corresponding to the thickest part of tendon on the longitudinal ultrasound image, as measured with the distance plugin of the ImageJ program, and tendon CSA were calculated. The CSA was determined from the transverse image of the middle portion of each tendon, corresponding to the thickest part. The contour