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Quantification of collagen fiber orientation in human tendons with the coefficient of variation of echogenicity

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ABSTRACT

The grayscale distribution on the ultrasonic images of tendons may be reduced with alignment of collagen fibers, because ultrasound signal intensity changes with alterations in tendon collagen fiber orientation due to acoustic anisotropy in the tendons. The purpose of this study was to investigate changes in the coefficient of variation (CV) of echogenicity in the Achilles tendon during passive dorsiflexion (the angle task) and isometric plantar flexion (the contraction task). Achilles tendon transverse ultrasonic images were collected from 14 healthy individuals every 10° from 20° to −20° (positive values for plantar flexion) in the angle task and every 10% maximum voluntary contraction (MVC) from 0% to 70% MVC in the contraction task. The CV of echogenicity was measured in each image. In addition, relative changes in the measured variables between the former half (20–0° in the angle task, 0% to 30% MVC in the contraction task) and the latter half (0° to −20° in the angle task, 40% to 70% MVC in the contraction task) of each task were compared. The CV of echogenicity decreased with increases in the dorsiflexion angle and intensity of isometric contractions. Furthermore, relative changes in the CV of echogenicity were greater at more dorsiflexed positions in the angle task and at lower torque levels in the contraction task. These results suggested that decreases in the CV of echogenicity were partially related to the alignment of the tendon collagen fibers with tendon stretching.

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1. Introduction

The mechanical property of tendon affects muscle function and sport performance (Arampatzis et al., 2006; Kubo et al., 2015) and depend on morphological characteristics and collagen fiber orientation (e.g., Connizzo et al., 2013). To date, several studies have used ultrasonography and magnetic resonance imaging to investigate the cross-sectional areas and stiffness in human tendons *in vivo* (e.g., Kubo et al., 2009). Previous findings obtained using histopathological techniques (Fredberg and Stengaard-Pedersen, 2008; Khan et al., 1999; Maffulli et al., 2000) revealed the degeneration and disordered arrangement of collagen fibers in symptomatic tendons. On the other hand, collagen fiber orientation in animal and human cadaver tendons has been quantified using the polarizer light method and second-harmonic generation microscopy (Lake et al., 2009; Miller et al., 2012; Williams et al., 2005). However, difficulties are associated with applying these techniques to clinical practice *in vivo*. Therefore, a quantitative technique to non-invasively assess collagen fiber orientation in human tendons *in vivo* is essential for obtaining a better understanding of the mechanisms underlying tendinopathy and preventing tendon injuries.

Ultrasonography has been widely applied to evaluate pathological changes and healing processes in tendons *in vivo*. The quantification of echogenicity (related to acoustic impedance) was shown in terms of brightness (gray scale) of the ultrasonic images (Nicoll et al., 1992). The echogenicity of tendons generally depends on the density and arrangement of collagen fibers within tendons (Goss et al., 1979). Normal tendons are hyperechoic, because collagen fibers of tendon arrange longitudinally and pack densely. On the other hand, the echogenicity of animal and human tendons reduced with tendinopathy (Crevier-Denoix et al., 2005; Docking et al., 2015; Ohberg et al., 2001), and thus the echogenicity of rat tendon increased during healing process (Chamberlain et al., 2013). An ultrasonographic tissue characterization technique (raw ultrasonographic images were analyzed with a custom-designed algorithm that quantifies the three-dimensional stability of echo patters and distribution on consecutive images) has recently been proposed to evaluate the internal structure of human tendons *in vivo* (van Schie et al., 2010). However, this technique is limited to semi-quantitative assessment of tendon structures and requires custom built tracking and data collection devices besides the ultrasonic device. Previous studies attempted to investigate

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changes in collagen fiber orientation in tendons using descriptive statistics such as skewness, kurtosis, and entropy calculated from grayscale histograms on ultrasonic images of tendons (Collinger et al., 2010; Wearing et al., 2013). However, few studies have quantified collagen fiber orientation in human tendons from any variables related to echogenicity *in vivo*.

Using ultrasonography, the echogenicity of tendons changes with the angle of incidence of the ultrasound beam, and this characteristic is referred to as acoustic anisotropy (Bleakney and White, 2005; Crass et al., 1988; Lehtinen et al., 1994). When collagen fibers within tendons are aligned parallel to the tendon axis and perpendicular to the ultrasound beam, ultrasonic images of tendons appear hyperechoic and the grayscale distribution on images would be even. Conversely, when the arrangement of collagen fibers is disordered, the grayscale distribution would be uneven. Previous studies demonstrated that the angular deviation of tendon collagen fibers decreased with tendon stretching (Connizzo et al., 2013; Lake et al., 2009; Miller et al., 2012). Based on these findings, it is likely that the dispersion of echogenicity, *i.e.*, the coefficient of variation (CV) (SD/mean) of echogenicity of each pixel on an ultrasonic image, indicates the orientation (arrangement) of collagen fibers in tendons.

Previous studies using ultrasonography demonstrated that tendons are stretched during passive stretching (Herbert et al., 2011; Morse et al., 2008) and isometric contractions (Kubo et al., 2001; Magnusson et al., 2001). During passive stretching and isometric contractions, the orientation of collagen fibers in tendons are probably aligned parallel to the tendon axis. The purpose of this study was to investigate changes in the CV of echogenicity in the Achilles tendon during passive dorsiflexion and isometric plantar flexion. We hypothesized that the CV of echogenicity decreases with tendon stretching.

2. Methods

2.1. Subjects

Fourteen healthy males (age: 25.8 ± 5.0 yrs, height: 170.9 ± 4.5 cm, body mass: 67.5 ± 10.0 kg, mean ± SD) volunteered for this study. Exclusion criteria included having a history of injury and/or surgery on the Achilles tendon, and affecting systematic diseases. All subjects were fully informed of the procedures to be utilized, as well as the purpose of the study. Written informed consent was obtained. This study was approved by the Ethics Committee for Human Experiments, Department of Life Science (Sports Sciences), The University of Tokyo.

2.2. Experimental tasks

The following two tasks were performed by each subject: 1) passive ankle dorsiflexion (the angle task) and 2) isometric plantar flexion (the contraction task). The legs of each subject were randomly allocated to the angle task and the contraction task. During both tasks, subjects lay in the prone position, and their foot was secured to a footplate connected to a dynamometer (Myolet, Asics, Japan) with their knee fully extended.

1) *Angle task:* The ankle joint was fixed at 20, 10, 0, −10, and −20° (0° was the neutral anatomical position at which the sole of the foot was 90° to the tibia; positive values for plantar flexion). Subjects were instructed to fully relax during this task. Unfortunately, we measured the electromyogram of plantar flexor muscles during the angle task. In this task, however, we measured passive torque in each ankle angle. The measured passive torque gradually increased with increases in dorsiflexion angle. Any subjects did not show fluctuation of the passive torque, and the measured passive torque values during the angle task were similar to the previously reported ones using similar manner to our study (e.g., Morse et al., 2008). If the subjects did not relax (*i.e.*, contracted) during this task, the measured passive torque–angle curves would be differed from those in the previous studies. Therefore, we judged that the subjects were relaxed during this task. The joint angle order was randomized to avoid any systematic effects. There was at least a 1-min rest period between two consecutive attempts.
2) *Contraction task:* The ankle joint was set at 15°, at which passive torque was almost zero (Muraoka et al., 2004). After a standardized warm-up, subjects performed two or three maximal voluntary isometric contractions (MVC) for plantar flexion. The peak torque was recorded in every trial, and the mean MVC value was used to determine the target torque during the contraction task. After a sufficient rest period, at least for 10 min after the completion of the MVC measurement, subjects performed isometric contractions at 0%, 10%, 20%, 30%, 40%, 50%, 60%, and 70% of MVC. During contractions, subjects were encouraged to maintain the target force displayed on an oscilloscope (DS-6612, IWATSU, Tokyo, Japan). The order of the measurement of each torque level was randomized in order to avoid any systematic effects. At least a 2-min rest period was permitted between two consecutive attempts in order to avoid the effects of fatigue.

2.3. Ultrasonic images of the Achilles tendon

Transverse ultrasonic images of the Achilles tendon were obtained using B-mode ultrasonography (SSD-6500, Aloka, Japan) with a 7.5 MHz linear transducer (UST-5410, Aloka, Japan). The transducer was positioned perpendicular to the longitudinal axis of the Achilles tendon 30 mm proximal to the calcaneus. Settings (gain, focus, and time gain compensation) were held constant throughout the experimental period. In both tasks (see below), ultrasonic images were collected approximately 30 s after reaching the target ankle angles and exerted torque levels (Fig. 1). The echogenicity of the Achilles tendon was measured using open-source image analysis software (Image J, NIH, Bethesda, MD) (Kubo et al., 2012). In measurements of echogenicity, an oval-shaped region of interest (ROI) that included as much of the Achilles tendon as possible, but avoided the surrounding tissue was selected. The grayscale of each pixel in the ROI was graded from 0 (black) to 255 (white) and was represented as a grayscale histogram obtained through Image J. Mean echogenicity and the CV of echogenicity were calculated from grayscale histograms. Measurement of the echogenicity of the tendon was performed three times for the same image, and the average value of the three measurements was

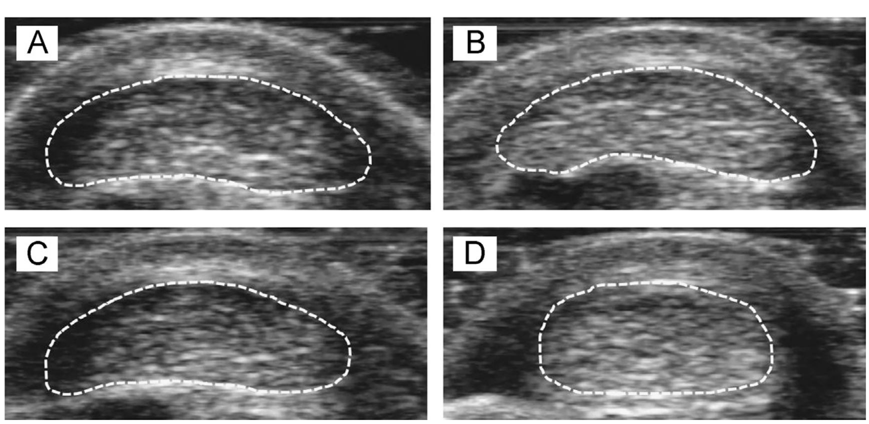

Fig. 1. Typical ultrasound images of the Achilles tendon in the angle task (A and B) and contraction task (C and D). Dashed lines in the image represent the outline of the Achilles tendon. A: −20°, B: 20°, C: 0% MVC, D: 70% MVC.

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