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In-Vivo Estimation and Repeatability of Force-Length Relationship and Stiffness of the Human Achilles Tendon using Phase Contrast MRI

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Abstract

Purpose—To devise a method using velocity encoded phase contrast MRI and MR-compatible dynamometry, for in-vivo estimation of elastic properties of the human Achilles tendon and to assess within-session and day-to-day repeatability of this technique.

Materials and Methods—Achilles tendon force and calcaneus-movement-adjusted displacement were measured during a submaximal isometric plantarflexion in 4 healthy subjects, 4 repeated trials each. The measured force-length (F-L) relationship was least-squares fitted to a cubic polynomial. Typical error was calculated for tendon displacement at multiple force levels, stiffness from the “linear region”, and transition point from the displacement point separating the linear and non-linear parts of the curve.

Results—Displacements of the tendon were determined up to a maximum force of 500N, with mean stiffness of 234 ± 53 N/mm, mean transition point of 2.70 ± 0.23 mm and maximum tendon displacement of 3.38 mm. Variability of tendon displacement was not dependent on the force level. Overall typical errors were 0.09 mm and 0.16 mm for within-session and between days, respectively. Typical error of transition point was 0.05 mm and 0.14 mm. Stiffness had typical errors of 47.24 N/mm and 51.95 N/mm. The tendon cross-sectional area and calcaneus displacement were found to be very significant factors in minimizing the individual differences in F-L curves.

Conclusion—The method yielded F-L relationships, stiffness and transition point values that showed good within and day-to-day repeatability. The technique compared well with the more conventional one using ultrasonography. Its reliability indicates potential for measuring tendon structural changes following an injury, disease, and altered loading.

Keywords

Velocity-encoded phase contrast MRI; Achilles tendon elastic properties; displacement; stiffness; toe region; isometric contraction

INTRODUCTION

Measurement of force-length (F-L) relationships under uniaxial mechanical testing is a common and important technique for the assessment of mechanical properties of biological tissues. This is particularly true for tendons as their elastic and time-dependent (viscous) characteristics are known to have a profound impact on the overall function of the musculoskeletal system. Not only does the tendon act as a force transmitter between the muscle and bone, its unique mechanical characteristics allow efficient energy recycling during locomotion and provide protection from muscle injury through reduction of mechanical oscillation and shock. Real-time ultrasonography has become popular for in vivo assessment of human F-L and stiffness properties of tendons (1). Recent studies using this technique have shown that the mechanical properties undergo substantial changes both with aging and disuse although the effects are partly mitigated by resistance training. Velocity encoded phase contrast magnetic resonance imaging (VE-PC-MRI) technique is potentially an alternative/supplementary in vivo technique. It provides the ability to track tissue movement using the velocity mapping of dynamically moving structures (2,3). In addition, the VE-PC-MRI technique offers added advantages of excellent soft tissue contrast including of those anatomically linked to the tendon, a large field of view (FOV) permitting the assessment of the displacement of multiple tissue structures simultaneously, and a high spatial resolution. The ability to track multiple tissue points is a particularly important advantage since it helps separate a segment of tendons for measurement of regional variability in their mechanical properties. To our knowledge, this technique has not yet been used for force-dependent length changes and stiffness measurements in human Achilles tendon. This technique therefore can be an effective alternative method for non-invasive and in vivo monitoring of tendon mechanical/structural changes applicable to normal, injured, diseased, and experimentally manipulated tendon.

The objectives of this study were to (i) establish a technique using VE-PC-MRI to estimate force-dependent length changes, stiffness, and the transition point from linear to non-linear behavior of the F-L curve in human Achilles tendon and (ii) assess its inter- and intra-exam variability. Since, to the best of our belief, this is the first time this methodology is being used to determine such parameters in humans in vivo, it is important to assess the level of within-day and day-to-day variations in the measurements and to ascertain that such variations are sufficiently low to allow reliable estimates.

MATERIALS AND METHODS

Subjects

Four healthy males (age: 29.0 ± 4.5 years, body mass: 72.8 ± 8.0 kg, height: 176.4 ± 9.9 cm, mean $\pm$ SD) volunteered for this study and signed Institutional Review Board approved consent forms. None of the subjects reported any history of muscular pathology or injury.

Muscle Force Measurements

The dominant one of the lower legs was restrained in a posterior half of a fiberglass cast with the ankle joint angle of 90° . Leg dominance was self reported by each subject. A MR-compatible interferometer (Fiberscan 2000, Luna Innovations, Blacksburg, VA) embedded on the sole of the cast measured strain induced during plantarflexion (resolution: $1 \mu\text{m}$). The strain output was digitized (200 Hz) by a data acquisition card (6024E, National Instruments, Austin, TX), and stored in a computer using custom-written data acquisition software (LabView, National Instruments, Austin, TX). The strain output was pre-calibrated with 4 known weights to estimate the resistive force applied at the ball of the foot during

isometric contraction. This process was repeated twice over the span of the entire project and the calibrated ratio remained constant (CV: 0.38%).

In order to determine the maximum voluntary contraction (MVC), the subject performed three maximum isometric plantarflexions during each imaging session, and the highest force was taken as the MVC. 40% MVC was used as the target force during dynamic PC-MRI acquisition.

Achilles tendon force was estimated following the method of Haxton (4) who calculated the ratio of the tension in the tendo-calcaneus to the resistance at the ball of the foot to be 2.67 ± 0.038 (S.E) in cadaveric limbs, whose observations were remarkably constant over his 5 samples. The author modeled the system as a first class lever in which the ankle joint acted as the fulcrum located between the applied load of the tendon and foot resistance at opposite ends.

Phase Contrast MR Imaging

The subject was then positioned inside a 3T scanner (TRIO, Siemens AG, Munich, Germany) in foot-first supine position. The experimental leg was imaged using a combination of a spinal array and a phased array torso coil.

An oblique sagittal slice of the leg bisecting the center axis of the Achilles tendon was imaged (Fig. 1). This ensured tracking of displacement at the midsection of the tendon. In order to minimize variability from changing anatomical locations, the subject-specific imaging plane was maintained in subsequent sessions.

Spoiled gradient echo 2D phase-contrast imaging was performed using velocity-encoding (VENC): 10 cm/s in the superior inferior direction, TR/TE/FA: 13.3 ms/7.5 ms/20°, slice thickness: 3 mm, receiver bandwidth: 290 Hz/pixel, 3 views per segment (VPS), 2 averages, imaging matrix: 128×256 , and FOV: 160 mm $\times$ 320 mm, retrospectively gated to acquire 22 temporal phases during 86 isometric contraction cycles. The temporal resolution was ~ 79.8 ms (TR*VPS*2).

The consistency of 86 muscle contractions was ensured using a computer-generated audio cue (interval = 1.714 s) and by projecting on the face of the magnet, the strain gauge output of the subject in real-time. The initial rise in force was used to trigger the MR scanner. The recorded force cycles were superimposed and averaged to generate one representative force curve, for F-L characterization of the tendon.

Data Processing

The PC images were smoothed with a 3-by-3 low pass filter to minimize random noise. The systematic errors from phase shading were then quantified and subtracted from the individual images of the cine sequence as described by Sinha et al. (2). Movement of the ends of the Achilles tendon during the contraction-relaxation cycle was measured iteratively from the corrected PC images given the initial pixel location and the elapsed time between consecutive images. The algorithms developed allowed for linearly interpolated sub-pixel velocity estimation, thereby achieving sub-millimeter displacement resolution.

A pixel representing muscle tissue adjacent to the Achilles tendon was tracked (Fig. 1a: upper dot) as tendon displacement instead of a pixel within the tendon since the tendon is a signal-void in MR images. Similarly, another region of interest (ROI) was placed at the tendo-calcaneus junction (Fig. 1a: lower dot) to account for the movement of the calcaneus during the cycle. Its displacement was subtracted from the observed displacement of the

proximal tendon to calculate Achilles tendon length. Both landmarks were kept constant between sessions.

Tendon cross sectional area (CSA) was calculated using an axial image at the same anatomical location where the tendon displacement was calculated (Fig. 1b, c) with the in-house developed region-growing algorithm. The mean CSA over two days was used to normalize the F-L curves by dividing the force by tendon CSA.

Force-length, Transition Point and Stiffness Measurement

The relationship between tendon elongation and force acquired from each trial was least-squares-fitted to a third-order polynomial with an added constraint that the fit increase monotonically over the entire strain interval.

While the curve was fitted to a polynomial, such F-L curves have been previously characterized in the Physiology literature as consisting of an initial “toe-region” and, at higher values of displacement as a “linear region” (5). We defined the linear region as the one in which the least-squares-fitted line had the correlation coefficient value of 0.98 (Fig. 3). Tendon stiffness (N/mm) was calculated as the slope of this line. The elastic modulus was also estimated by multiplying the tendon stiffness by the ratio of each subject’s initial tendon length to tendon CSA. The initial tendon length was defined as the distance between the two ROIs described above at rest. For a measure of “transition point”, i.e. a point separating the toe region of an F-L curve, the force level with the smallest typical error was selected and the corresponding Achilles tendon length change at that force level was defined as the transition point.

Measures of Repeatability

Within session and day-to-day repeatability was assessed as typical error by dividing the standard deviation of the difference in measured values between the two scans by $\sqrt{2}$ (6). The repeatability was calculated for stiffness, transition point and tendon length at a force levels between 100 and 500 N with 100 N increments. 95% confidence intervals (CI) to the typical error (6) and coefficient of variation (CV) were also calculated. Data are presented as mean $\pm$ SD.

RESULTS

Achilles tendon length and force plotted over the contraction-relaxation cycle from one representative subject demonstrate their respective temporal responses (Fig. 2). First two trials were collected within the same day (a, b) while the next two (c, d) were also collected within the same day but two days after the first batch.

Figure 3 shows the tendon F-L plots from the same individual for all the four trials, with a sample straight line fit in the linear region. Across all subjects, the correlation coefficient of the third-order polynomial to the F-L data ranged from 0.9915 to 0.9998 (0.9980 ± 0.0023).

The mean range of calcaneus movement was 4.54 ± 0.29 mm, 5.12 ± 0.29 mm, 6.74 ± 0.58 mm, and 4.98 ± 0.33 mm, respectively for four subjects. The tendon displacement after subtraction of calcaneus movement was 2.87 ± 0.20 mm, 3.38 ± 0.07 mm, 2.76 ± 0.05 mm, and 2.91 ± 0.06 mm. Typical error of tendon displacement was 0.09 mm (CI: 0.08–0.12) within one scanning session and 0.16 mm (CI: 0.13–0.21) between two days. The errors at different force levels are given in Table 1.

The typical error of tendon stiffness was 47.24 N/mm (CI: 31.24–96.15, CV: 14.72%) within-session and 51.95 N/mm (CI: 34.35–105.73, CV: 17.16%) between days. The overall mean stiffness was 234 ± 53 N/mm.

The transition point (the tendon length at 400 N) was 2.70 ± 0.23 mm with a typical error and CV of 0.05 mm and 1.8 % within-session, and 0.14 mm and 4.1% between days (Table 2).

Table 3 shows tendon CSA measurements from two separate days and the mean of the two. The results illustrates that there is significant variation (~ 48 to ~ 66 mm²) in individual CSA of the tendon. However, these areas appear to remain fairly constant over the time course of these experiments for each subject. Subject-specific tendon stiffness and estimated Young's modulus are also shown in the table.

Figure 4a shows the variability in the F-L curves between the 4 subjects. Each curve was obtained from the least-squares-fitted cubic polynomial to four F-L datasets. Figure 4b is calcaneus movement adjusted F-L curves. Figure 4c shows the F-L curves after normalization to tendon CSA. As can be seen, both the corrections for the calcaneus movement as well as adjustment for the tendon CSA, lead to significantly improved uniformity of the F-L curves across the subjects.

DISCUSSION

In this study, we have measured plantarflexion force of the foot and tendon length using MR-compatible dynamometry and PC-MRI, respectively, in order to assess the tendon F-L relationship, stiffness, and transition point, and subsequently assessed the repeatability of the measurements. The Achilles tendon force was calculated from the first level moment arm ratio and plantarflexion force and the tendon length was measured at a muscle-tendon junction (Fig. 1a). Since the tendon is linked to muscle fibers with strong structural bond (5,6), it was assumed that the ROI in the muscle adjacent to the tendon accurately reflected its movement.

The casts used for immobilization of the leg were pre-calibrated to estimate the force at the ball of the foot, which in turn was multiplied with a previously reported ratio (4,7) to estimate the Achilles tendon force. Since Haxton (4) results showed remarkable consistency between his subjects, we felt this approach was a valid one. However, one needs to note that force estimation is likely one of the most significant sources of error in such experiments, and can be further compromised by factors such as variations of posture and point of exertion and measurement.

The peaks of tendon force and displacement curves nearly coincided within each trial (Fig. 2) within the temporal resolution (5 ms vs. 79.8 ms). Given that these two parameters were measured by independent methods, this consistency validates the reliability of the methods to some extent. The inter-trial differences in the force/displacement were slightly greater between two days (a, b vs. c, d) than within the same day. More pronounced day-to-day differences suggest that the muscle contractile patterns were altered significantly mostly because of the subject's altered response to the audio/visual cues. However, the F-L relationships as represented by the least-squares-fitted third-order polynomials showed convergence (Fig. 3), suggesting that the viscoelastic effect was minimal. Unlike purely elastic materials, e.g. a mechanical spring, tendons exhibit viscoelastic properties. A few prominent characteristics of viscoelasticity are: (i) The F-L curve is not linear but rather curvilinear. (ii) The F-L relationship is rate-dependent, i.e. loading rate changes the shape of the curve. (iii) Energy loss occurs during loading and unloading (hysteresis) giving rise to different F-L curves for each. (iv) In a stretched state, the energy stored dissipates over time according to a phenomenon called creep in which the unit undergoes structural change. The

variation in loading rates between repetitions that occurred in this study was insignificant given the convergence of F-L curves.

The third-order polynomial was chosen as a fitting function. Other types of functions were explored, including exponential (correlation coefficient: 0.489 ± 0.407), linear (0.920 ± 0.036), and higher degrees of polynomials, i.e. quadratic (0.991 ± 0.006), cubic (0.998 ± 0.002) and 4th order (0.940 ± 0.039). Among all other fitting functions, the cubic polynomial produced the best fit to our F-L datasets. The cubic-fitted curves demonstrated the characteristic viscoelastic curvilinear response of the Achilles tendon (Fig. 3), exhibiting an initial non-linear “toe” region, immediately followed by a steeper but fairly constant slope region. This observed behavior is similar to previously published findings of *ex vivo* (5,8) and *in vivo* ultrasound-based studies (9).

The magnitude of measurement error was similar at different force levels (Table 1), with no heteroscedasticity or systematic variability observed in the error between the force levels. Thus the mean values of 0.09 mm and 0.13 mm describe well the overall variability in Achilles tendon length within one session and between two days, respectively. However, the range for 95% CI was largest at 100 N suggesting that the measurements at low force levels are more susceptible to random errors. The day-to-day typical error was greater probably due to changes in 1) the positioning of the cast, 2) in the slice location 3) muscle activation patterns.

The identification and characterization of the toe region has not been previously reported in human *in vivo* studies. In this study the transition point was identified at a force level of 400N. The quantification of changes in the toe region provides different information from the typically reported stiffness values. For example, due to some form of clinical intervention the stiffness in a high force region may remain the same while the toe region elongates. The toe-region is associated with the presence of structural crimps, and sinusoidal shapes of collagen fibers within the tendon (5,10). Thus, we expect that changes in transition point and stiffness arise from two independent phenomena, both providing valuable information on tendon adaptation.

Stiffness variability was significantly more than that of the length and transition point (CV: 14.72% within session and 17.16% between days). This is expected considering that the slope of a line is highly sensitive to minute changes in the shape of curve. These values are also higher than those reported in two ultrasound-based studies (11,12) that measured the repeated variability (11.7% vs. 6.8%). In ultrasonography, the stiffness is measured from data retrieved from slow ramp contractions at force levels higher than 50% MVC. In the present experiments using VE-PC MRI, the values of stiffness are obtained at force levels lower than 40% to minimize fatigue over the relatively long acquisition time. Since we have found tendon length to be more susceptible to random errors at lower levels of force (Table 1), this also contributed to higher variability in the stiffness measurement. A linear region is difficult to define in a cubic function. Several methods were investigated to address this issue including determining the derivative of the fitting function. However, this approach was too sensitive to the location on the curve and quite ineffective for applying a consistent criterion across and within subjects required for assessment of stiffness repeatability. The current linear regression approach overcomes both issues. Specifically, the slope or the stiffness is not merely based on one specific point on the curve but rather based on the overall linearity of the “linear” region with a high R value. The rationale behind using 0.98 R as a criterion for drawing a line was that too high an R value failed to capture the linearity of the fit and missed the majority of the curve, and too low a value encroached into the “toe” region. While this choice was somewhat subjective, the lines drawn based on this criterion demonstrated the linear region quite well (Fig. 3).

It was found in this study that the calcaneus movement incurred during isometric contractions can be highly significant (up to 72.4% tendon displacement). Therefore, it is important to account for its movement to avoid overestimation of tendon length changes. Magnusson et al. in an ultrasound study (9) also reported that the average plantarflexion joint angular rotation measured with an electrical goniometer was 3.6 degrees which resulted in an overestimation of tendon displacement up to 30%. The importance of this issue is further underlined by the consistency of the F-L curves for the different subjects that were significantly improved (Fig. 4a vs. b) when calcaneus movement was factored in. In addition, we observed further reduction in the variability when the tendon force was normalized to CSA of the tendon (Fig. 4c).

The range of tendon displacements measured in this study fell within the range of those reported by ultrasound-based studies, particularly ones (7,9,13) published by Maganaris et al. (11.1 mm), Magnusson et al. (10.7 mm), and Reeves et al. (12.1 mm) at 100% MVC. The first two investigators accounted for calcaneus movement. Our method produced the tendon displacement values of up to 3.38 mm at 40% MVC. Objective comparison of these values across studies, however, is a little difficult since there are differences in the segment of the tendon structures analyzed. While these investigators measured tendon displacement at the gastrocnemius myotendinous junction thereby measuring the load-displacement characteristics of the Achilles tendon and aponeurosis combined, we confined our analysis to the Achilles tendon alone (Fig. 1a) whose elastic properties may likely be different (14,15).

One of the main limitations of this study was related to the relatively low temporal resolution achievable with PC-MRI, which led to a limited number of F-L data pairs. Given this constraint, the development of a new PC-MRI sequence that can improve the temporal resolution while maintaining data acquisition time would be desirable. While higher temporal resolution can be achieved by alternate spiral acquisition (16) or the use of steady-state velocity encoding sequences (17), there is typically a compromise on spatial resolution or image quality.

In conclusion, PC-MRI and MR-compatible dynamometry provide excellent repeatability of Achilles tendon F-L curves and transition point which reflects the toe region. However, tendon stiffness as measured from sub-maximal force levels showed CV of around 15% and therefore should be reported with caution. This study further underlines the importance of accounting for tendon CSA and calcaneus motion when between-subject differences in F-L relationships are assessed.

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REFERENCES

1. Fukashiro S, Itoh M, Ichinose Y, Kawakami Y, Fukunaga T. Ultrasonography gives directly but noninvasively elastic characteristic of human tendon in vivo. *Eur J Appl Physiol Occup Physiol*. 1995; 71:555–557. [PubMed: 8983925]
2. Sinha S, Hodgson JA, Finni T, Lai AM, Grinstead J, Edgerton VR. Muscle kinematics during isometric contraction: development of phase contrast and spin tag techniques to study healthy and atrophied muscles. *J Magn Reson Imaging*. 2004; 20:1008–1019. [PubMed: 15558560]
3. Drace JE, Pelc NJ. Measurement of skeletal muscle motion in vivo with phase-contrast MR imaging. *J Magn Reson Imaging*. 1994; 4:157–163. [PubMed: 8180454]

4. Haxton HA. Absolute muscle force in the ankle flexors of man. *J Physiol.* 1944; 103:267–273. [PubMed: 16991644]
5. Proske U, Morgan DL. Tendon stiffness: methods of measurement and significance for the control of movement. A review. *J Biomech.* 1987; 20:75–82. [PubMed: 3558432]
6. Hopkins WG. Measures of reliability in sports medicine and science. *Sports Med.* 2000; 30:1–15. [PubMed: 10907753]
7. Reeves ND, Maganaris CN, Ferretti G, Narici MV. Influence of 90-day simulated microgravity on human tendon mechanical properties and the effect of resistive countermeasures. *J Appl Physiol.* 2005; 98:2278–2286. [PubMed: 15705722]
8. Almeida-Silveira MI, Lambertz D, Perot C, Goubel F. Changes in stiffness induced by hindlimb suspension in rat Achilles tendon. *Eur J Appl Physiol.* 2000; 81:252–257. [PubMed: 10638386]
9. Magnusson SP, Aagaard P, Dyhre-Poulsen P, Kjaer M. Load-displacement properties of the human triceps surae aponeurosis in vivo. *J Physiol.* 2001; 531:277–288. [PubMed: 11179410]
10. Benjamin M, Ralphs JR. Tendons and ligaments--an overview. *Histol Histopathol.* 1997; 12:1135–1144. [PubMed: 9302572]
11. Hansen P, Aagaard P, Kjaer M, Larsson B, Magnusson SP. Effect of habitual running on human Achilles tendon load-deformation properties and cross-sectional area. *J Appl Physiol.* 2003; 95:2375–2380. [PubMed: 12937029]
12. Kubo K, Morimoto M, Komuro T, Tsunoda N, Kanehisa H, Fukunaga T. Influences of tendon stiffness, joint stiffness, and electromyographic activity on jump performances using single joint. *Eur J Appl Physiol.* 2007; 99:235–243. [PubMed: 17106717]
13. Maganaris CN, Paul JP. Tensile properties of the in vivo human gastrocnemius tendon. *J Biomech.* 2002; 35:1639–1646. [PubMed: 12445617]
14. Arruda EM, Calve S, Dennis RG, Mundy K, Baar K. Regional variation of tibialis anterior tendon mechanics is lost following denervation. *J Appl Physiol.* 2006; 101:1113–1117. [PubMed: 16728516]
15. Paxton JZ, Baar K. Tendon mechanics: the argument heats up. *J Appl Physiol.* 2007; 103:423–424. [PubMed: 17446408]
16. Asakawa DS, Nayak KS, Blemker SS, et al. Real-time imaging of skeletal muscle velocity. *J Magn Reson Imaging.* 2003; 18:734–739. [PubMed: 14635159]
17. Grinstead J, Sinha S. In-plane velocity encoding with coherent steady-state imaging. *Magn Reson Med.* 2005; 54:138–145. [PubMed: 15968674]

Figure 1. Sagittal (a) and axial (b) MR images of the lower leg from one representative subject. The prescribed sagittal slice location is shown in the axial image (b) with a white dashed line. The upper and lower dots (a) denote the anatomical locations used to measure the tendon and calcaneus displacement, respectively during contraction-relaxation cycle. The subject-specific location of the origin and insertion of AT and the slice orientation were kept constant between sessions. Magnified view of (b) showing the segmented CSA of the tendon (c).

Figure 2.

Subject 1 tendon displacement and force profiles during an isometric contraction-relaxation cycle. First two trials (denoted by a, b) were collected within a same day while the next two (denoted by c, d) were also collected within a same day but two days after the first batch.

Figure 3.

Subject 1 F-L relationships after least-squares fitting of the cubic polynomial to each trial set. A sample line is shown from which the stiffness and transition point were calculated. Coefficient correlation values are also shown to assess the quality of the fit.

Figure 4.

(a) Intersubject variability of average F-L curves using the tendon displacement only. (b) Intersubject variability after calcaneus movement was subtracted. (c) Intersubject variability after normalized to subject-specific tendon CSA.

TABLE 1

Typical error and 95% confidence intervals (CI) in tendon length at different force levels within one scanning session and between two different measurement days. At each force level the typical error has been calculated based on 8 pairs of measurement from 4 subjects.

F(N)	Within session				Day-to-day			
	Typical error	Lower CI	Upper CI	CV%	Typical error	Lower CI	Upper CI	CV%
100	0.14	0.09	0.28	9.19	0.22	0.14	0.44	11.32
200	0.11	0.07	0.23	5.26	0.18	0.12	0.36	7.15
300	0.07	0.04	0.13	2.60	0.15	0.10	0.30	5.33
400	0.05	0.03	0.10	1.83	0.14	0.09	0.29	4.05
500	0.09	0.06	0.19	2.39	0.16	0.10	0.32	4.40
Total	0.09	0.08	0.12	4.30	0.16	0.13	0.21	8.26

TABLE 2

Mean values, typical error with 95% confidence intervals (CI) and coefficient of variation (CV) of Achilles tendon stiffness and transition point (tendon length change at 400N).

	Stiffness (N/mm)		Transition point (mm)	
	Within session	Day-to-day	Within session	Day-to-day
Mean value	233.9 ± 52.9	233.9 ± 52.9	2.70 ± 0.23	2.70 ± 0.23
Typical error	47.24	51.95	0.05	0.14
Lower CI	31.24	34.35	0.03	0.09
Upper CI	96.15	105.73	0.10	0.29
CV%	14.72	17.16	1.83	4.05

TABLE 3

Summary of Achilles tendon CSA, tendon stiffness, and Young's modulus.

	Subject 1		Subject 2		Subject 3		Subject 4	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Area (mm ²)	66.3	64.9	46.2	51.4	61.7	63.5	54.0	55.1
Mean (mm ²)	65.6 ± 1.0		48.8 ± 3.7		62.6 ± 1.2		54.6 ± 0.8	
Stiffness (N/mm)	254.61 ± 50.96		224.25 ± 65.66		265.61 ± 39.22		191.23 ± 32.57	
Modulus (MPa)	225.11 ± 45.06		271.12 ± 79.38		263.07 ± 38.84		220.65 ± 37.58	