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In vivo mechanical response of human Achilles tendon to a single bout of hopping exercise

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SUMMARY

Stiffness of the human Achilles tendon (AT) was determined *in vivo* before and after a single bout of hopping exercise. It was hypothesized, based on published data using *in vitro* specimens, that a reduction in AT stiffness may occur after just 1000 loading cycles at physiological stress levels. Ten healthy subjects performed two-legged hopping exercise consisting of 1150–2600 high impacts. Tendon stiffness was determined in several isometric ramp contractions [20%, 40%, 60%, 80% and 100% maximum voluntary contraction (MVC)] during which tendon elongation was measured using ultrasonography and two cameras. Tendon force was calculated by dividing measured ankle torque by magnetic resonance imaging-derived AT lever arm length. Tendon stiffness remained unchanged, being 430±200 N mm^{-1} before and 390±190 N mm^{-1} after the exercise [not significant (n.s.)]. Despite the lack of changes in stiffness, maximum tendon force during MVC was reduced from 3.5±0.6 kN to 2.8±0.7 kN (P<0.01). As the proposed decline in stiffness was not observed, it is concluded that mechanical fatigue did not take place in the AT of healthy individuals after a single bout of high-impact exercise performed until exhaustion.

Key words: Achilles tendon, stiffness, fatigue, ultrasonography.

INTRODUCTION

Both animal and human tendons have been shown to fatigue *in vitro* under continuous static or cyclic loading. *In vitro*, fatigue is often expressed as a decrease in the ultimate stress of a tendon; the stress that the tendon can bear without rupture. When rupture occurs after static loading, it is referred to as a creep rupture, and when it occurs after cyclic loading, it is known as a fatigue rupture. Creep or fatigue ruptures have been demonstrated in wallaby tendons (Wang and Ker, 1995a; Wang et al., 1995b; Ker et al., 2000) and in sheep tendons (Pike et al., 2000), fatigue ruptures in human extensor digitorum longus (EDL) tendons (Schechtman and Bader, 1994; Schechtman and Bader, 1997; Schechtman and Bader, 2002), and creep and fatigue ruptures in human Achilles tendons (AT) (Wren et al., 2003). However, ultimate stress is not a valid parameter to describe tendon fatigue in living tissue. Fortunately, most authors have concluded that the slope of the linear region of the stress–strain curve, known as Young's modulus, is an excellent indicator of tendon fatigue. At the instant of rupture, Young's modulus has been shown to decrease to approximately half of its original value (Schechtman and Bader, 2002; Wren et al., 2003).

Instead of Young's modulus, tendon stiffness is often used in *in vivo* studies to quantify tendon fatigue. The difference between stiffness and Young's modulus is that tendon stiffness depends on both material properties and dimensions of the tendon whereas Young's modulus is determined by material properties alone. In terms of stiffness, if fatigue is induced, tendon stiffness decreases, and inversely, tendon compliance increases.

An increase in tendon and aponeurosis compliance *in vivo* was first reported by Kubo and colleagues (Kubo et al., 2001a; Kubo et al., 2001b; Kubo et al., 2005) in vastus lateralis (VL) muscle after isometric contractions. By contrast, no changes in compliance have been observed after dynamic contractions in VL tendon (Kubo et al., 2001b; Kubo et al., 2005; Mademli et al., 2008; Ullrich et al., 2007) or in gastrocnemius medialis (GAM) tendon (Mademli et al., 2006). These findings suggest that the type of contraction or strain rate would determine the severity of tendon fatigue. The effect of strain rate on tendon fatigue resistance has not been investigated *in vivo* but the mode of failure in knee ligaments of primate specimens changed from a predominance of avulsion fracture at the slow rate to ligament rupture at the fast rate (Noyes et al., 1974). This indicates that high strain rates increase the likelihood of tendon rupture. Furthermore, if exercise is repeated without sufficient recovery, fatigue effects may accumulate and eventually lead to tendon overuse injuries (Kannus and Josza, 1991; Galloway et al., 1992). Therefore, this study was designed to investigate tendon mechanical responses to a single bout of exercise, and consequently, hopefully, to help to prevent tendon injuries.

In the literature, the effects of dynamic exercise on tendon properties have only been examined after isolated eccentric and concentric contractions. Thus, there is a need for a study utilizing a large number of impacts at high strain rates that occur during natural locomotion in sports and physical activities. The aim of this study was to investigate human AT fatigue by calculating tendon stiffness before and after a single bout of hopping exercise. Based on data from human specimens, it was hypothesized that Achilles tendon fatigue may be induced after as little as 1000 loading cycles (Wren et al., 2003). Tendon stiffness was chosen as an indicator of tendon fatigue since its derivative, Young's modulus, has been shown to be a valid indicator of tendon fatigue in specimen studies (Schechtman and Bader, 1994; Schechtman and Bader, 2002; Wang and Ker, 1995a; Wang et al., 1995b; Wren et al., 2003).

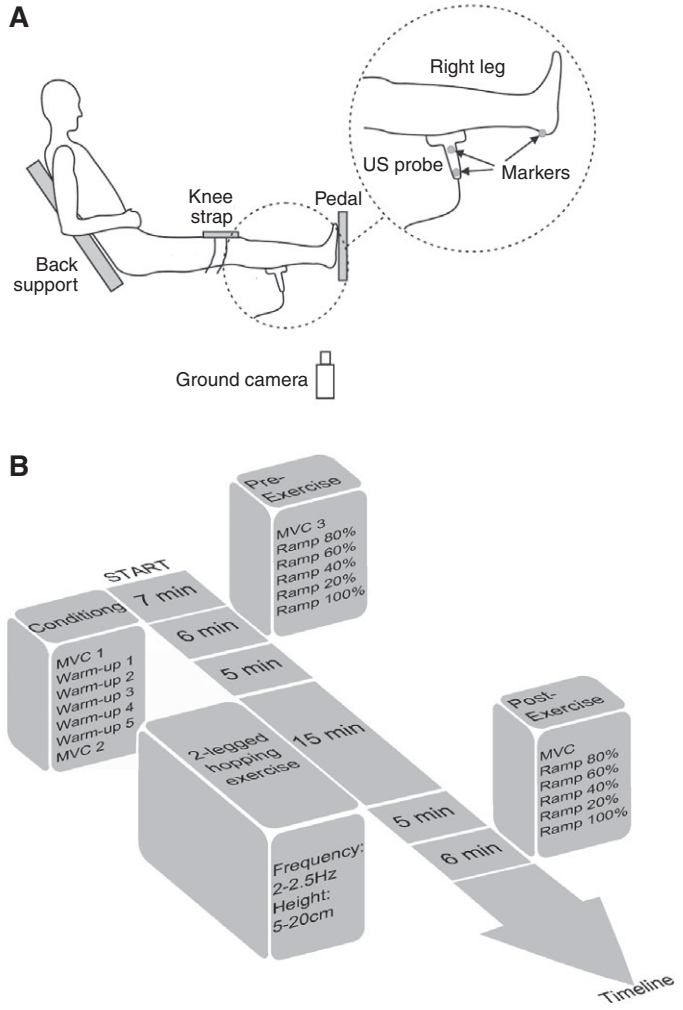

Fig. 1. (A) Illustration of the measurement setup in the ankle dynamometer. Subject was tightly anchored between the pedal and back support. Two cameras (side and ground) were set up to record heel and ultrasound probe displacement. The side camera's optical axis was perpendicular to the figure plane and therefore the camera is not visible in the figure. (B) Timeline of the current protocol. Exercise duration varied between subjects from 8 min to 22 min. MVC, maximum voluntary contraction.

MATERIALS AND METHODS

Six males and four females (N=10) participated in this study. Their mean mass, height and age were 65±10 kg, 171±8 cm and 26±3 years (Table 1), respectively. All participants were physically active and had diverse sport backgrounds. Participants were informed about the procedures, benefits and possible risks involved in the study, and they signed a written consent. All methods were approved by the ethical committee of the University of Jyväskylä. The study conformed to the standards set by the Declaration of Helsinki.

Data were acquired in an ankle dynamometer as illustrated in Fig. 1A. To measure torque, the right foot was firmly attached to a pedal with a force transducer (Precision TB5-C1, Raute, Nastola, Finland) installed at a constant distance from the pedal rotation axis. Torque was sampled with a 16-bit AD-board (Power 1401, CED Limited, Cambridge, England) at 1 kHz. Seat position was adjusted individually to obtain the following joint angles: ankle 90±3 deg., knee 180±3 deg. and hip 120±3 deg. The axis of rotation of the ankle joint was carefully aligned with the rotational axis of the pedal. Two-dimensional displacement of the myotendinous junction (MTJ) of GAM was recorded with a sampling frequency of 50 Hz using a 6 cm linear array ultrasound (US) probe (UST-5712, Aloka, Tokyo, Japan) and imaging unit (Prosound Alpha 10, Aloka, Tokyo, Japan). US images were captured on VHS tape (Panasonic, Osaka, Japan). A similar procedure has frequently been used in studies of muscle mechanics during physical activities (Arampatzis et al., 2006; Lichtwark et al., 2007). The probe was placed 2 cm medial to the position where medial and lateral gastrocnemius muscles join. The probe was supported by a custom-made cast, and secured by elastic bandages wrapped around the leg to prevent probe movement during exercise. The lack of movement of the probe was verified by marking the location of the probe on the skin prior to exercise. Movement of the US probe and heel during isometric contractions was controlled by setting two video cameras (Sony HDR-HC3, Tokyo, Japan, 576i at 50 Hz) perpendicular to each other. The ground camera captured movement of a heel marker on the posterior surface of the calcaneus, and the side camera captured the coordinates of US probe markers. An electromyography (EMG) electrode pair (Ag/AgCl, 13.2 mm^2, Blue Sensor M, Ambu A/S, Ballerup, Denmark) was placed over tibialis anterior (TA) to quantify antagonist activity during plantarflexions. The location of the electrode pair (inter-electrode distance 2 cm) was between the muscle mid belly and distal tendon. EMG signals were amplified (gain: 1000) and band-pass filtered (range: 10–1000 Hz) before being fed to the AD-board (Power 1401) for sampling at 1 kHz. All signals were synchronized by digital pulses.

The timeline and content of the experimental protocol are shown in Fig. 1B. Experiments started with tendon pre-conditioning, which is a phenomenon that is characterized by increased stretching of tendon under constant repeated loading in animals (Abrahams, 1967) and humans (Maganaris and Paul, 2002; Maganaris, 2003). To distinguish short-term tendon conditioning from long-term tendon fatigue, five isometric contractions up to 80% of maximum were performed before the measurements, because this has been shown to be adequate to remove the conditioning effect (Maganaris, 2003). Isometric maximum voluntary contraction (MVC) force was measured as the best out of three trials. Tendon stiffness was then determined pre- and post-exercise using force and length data from the best MVC and five isometric ramp contractions up to the levels of 80%, 60%, 40%, 20% and 100%, measured at this order. The time between two consecutive isometric contractions was ~1 min, and during contraction, isometric target force was maintained for 2 s to stabilize the tendon deformation (Fig. 2). It was assumed that the effects of pre-conditioning were maintained throughout the experiment. The exact time course of recovery from pre-conditioning is not clear *in vivo* but *in vitro*, Viidik reported that when static state was achieved, no elastic recovery was observed during the following 240 min in resting connective tissue (Viidik, 1973).

MVC was measured independently before and after the exercise and all ramp contraction target levels (%) were calculated accordingly. A practical consequence of this is that tendon stiffness was determined at a lower absolute force level after the exercise. As demonstrated by *in vivo* stress–strain curves (Lichtwark and Wilson, 2005), stiffness is independent of the force in the linear region. Therefore, a constant effort level was preferred over a constant force level (which could not have been guaranteed anyway because tendon force was calculated and not measured).

Two-legged hopping (feet together) on a contact mat (custom-made, capacitor switch) was performed until exhaustion. Loading was focused on the triceps surae muscles. Subjects were instructed to hop at their preferred frequency (range: 2.0–2.5 Hz) and strongly

Table 1. Subject anthropometric data and tendon mechanical properties before the exercise

Subject	Gender	Mass (kg)	Height (cm)	CSA (mm^2)	Lever arm (cm)	Maximum stress (MPa)	Maximum strain (%)	Young's modulus (GPa)
1	M	69	173	38	5.4	101	4.1	3.2
2	M	69	173	49	4.6	65	12.4	0.5
3	F	50	157	29	4.3	101	5.4	4.0
4	F	59	165	39	4.6	58	5.8	1.0
5	M	66	171	57	5.2	76	5.8	2.1
6	M	65	176	48	5.2	55	4.5	2.4
7	M	70	178	49	5.3	82	6.5	1.6
8	F	69	168	35	4.3	119	4.5	2.3
9	M	85	183	61	5.4	65	4.5	1.8
10	F	52	163	30	4.6	81	8.6	1.4
Mean		65.4	171	43	4.9	80	6.2	2.0
s.d.		10.0	8	11	0.4	21	2.6	1.0
c.v.		0.15	0.04	0.25	0.09	0.26	0.41	0.50

CSA, cross-sectional area; s.d., standard deviation; c.v., coefficient of variation.

activate their calf muscles to keep the heels off the ground during the contact phase. During exercise, flight time ranged from 0.2 s to 0.4 s (coinciding with a jump height of 5–20 cm), and the total number of jumps ranged between 1150 and 2600 (Table 2).

Magnetic resonance images

Magnetic resonance images (MRI) of the lower right leg were taken one week before the exercise. Lever arm length and tendon cross-sectional area (CSA) were analyzed from MRI, which were acquired in axial [repetition time (TR)=4500.00, echo time (TE)=10.90, flip angle=90.00, matrix=256×256 and slice thickness=7.00 mm] and sagittal orientations (TR=100.00, TE=3.70, flip angle=60.00, matrix=256×256 and slice thickness=5.00 mm). CSA was determined by manually outlining the AT in axial images along the tendon from the calcaneus to the GAM MTJ. The smallest CSA (located 1.7–5.1 cm from the calcaneus) was used for later calculations (Kongsgaard et al., 2005). The smallest CSA has the greatest stress concentration, and failures usually begin there during cyclic fatigue tests (Wren et al., 2003). From sagittal images, AT lever arm length was determined as the perpendicular distance from the midpoint of the superior surface of the trochlea of the talus to the line of action of AT, a method that is similar to that previously used by Maganaris (Maganaris, 2001) and Finni et al. (Finni et al., 2006). Ankle angle was 90 deg. (sole of the foot perpendicular to shank) (Fig. 3).

Data analysis and processing

AT force ($\Delta\mathbf{F}$) was calculated by dividing measured ankle torque by MRI-derived AT lever arm length. To yield $\Delta\mathbf{F}$, initial tendon force before contraction was subtracted from maximum tendon force at the end of the plateau phase of each isometric contraction as shown in Fig. 2. It was assumed that all plantar flexion force was transmitted through the AT and that the lever arm length remained constant. It is known that AT moment arm does not necessarily remain constant during muscle activity but variations are mainly caused by large joint rotations (Rugg et al., 1990; Maganaris et al., 2000), which were not observed here (<5 deg.), and therefore a constant moment arm is often used (e.g. Fukunaga et al., 1996). A TA activity correction method (Mademli et al., 2004) was applied to the measured force values if necessary. If TA EMG activity was observed during isometric plantarflexion, a counter-movement (dorsiflexion) was performed to produce similar TA EMG activity. Dorsiflexion torque was then added to the plantarflexion torque to estimate true AT force. Seven out of ten subjects produced ankle plantar flexion without TA activity, and for the remaining three subjects, the correction was always less than 5%. Tendon stress was calculated by dividing tendon force by the smallest tendon CSA deduced from MRI.

Tendon elongation (ΔL) was also determined between initial and maximum tendon length during each isometric contraction and the mean of the initial tendon lengths from all trials was calculated. Tendon length was determined as the two-dimensional distance between the AT insertion at the proximal portion of the calcaneus and the MTJ of GAM muscle (Fig. 4). To measure tendon length correctly, heel and MTJ displacements were transformed to a common coordinate system (CS). This was because the heel moved within the laboratory CS whereas the MTJ moved in the US probe CS. The principle of different coordinate systems is presented in Fig. 4. A similar method, although in three-dimensional space, has previously been used by Lichtwark and Wilson (Lichtwark and Wilson, 2005). On this occasion two-dimensional analysis was considered adequate, because all movement could be restricted to a single plane. Kinematics and US videos were captured and

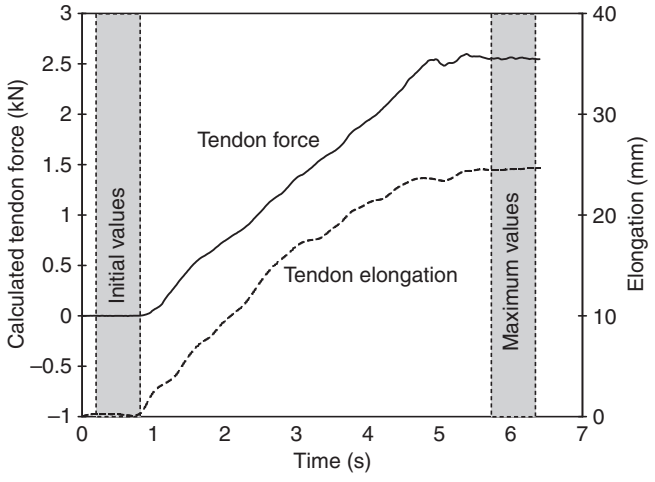

Fig. 2. Example of a single 80% ramp contraction (before exercise). Calculated tendon force (solid line) and measured elongation (broken line) are drawn over time. Achilles tendon force ($\Delta\mathbf{F}$) and tendon elongation (ΔL) were calculated as the difference between initial and maximum values that were taken as the mean over the grey shaded area.

Table 2. Effect of exercise on mechanical properties of tendon

		Stiffness (N mm^{-1})		Initial length (cm)		Maximum force (kN)	
Subject	Jumps	Pre	Post	Pre	Post	Pre	Post
1	1200	571**	678*	22.1	22.2	3.8	3.1
2	1500	118**	160**	22.5	21.6	3.2	2.8
3	1150	690**	650**	18.6	18.9	3.3	2.9
4	2200	231*	183*	20.3	20.3	2.7	1.2
5	1700	610**	569*	19.4	19.7	4.4	3.4
6	2100	650**	334	22.3	22.4	3.4	2.5
7	1500	337*	339*	22.7	22.5	4.0	3.7
8	2500	429**	355**	19.9	19.9	4.1	3.4
9	2400	453**	433**	24.2	24.0	4.0	3.1
10	2600	208**	188*	20.5	20.7	2.4	2.2
Mean	1885	430	390	21.2	21.2	3.5	2.8**[a]
s.d.	542	200	190	1.7	1.6	0.6	0.7
c.c.	0.29	0.47	0.49	0.08	0.07	0.26	0.18

**P<0.01, *P<0.05, for correlation coefficient (c.c.), **[a]P<0.01, different from pre value.

digitized with Vicon Motus (v. 8.5, Vicon, Oxford, England) software. Tendon strain was calculated by dividing tendon elongation by tendon initial length.

Stiffness calculations and statistics

Tendon stiffness was deduced by using the least-squares method to find the line of best fit for $\Delta\mathbf{F}$ *vs* ΔL pairs from isometric contractions as shown in Fig. 5. Thus, the method takes into account the random variability in tendon force and length measurements because it is based on several pairs of data points. Young's modulus was calculated by replacing tendon force with tendon stress and elongation with strain. To determine stiffness (and Young's modulus) within the linear region, stress levels of 10 MPa or above were accepted for analysis (Schechtman and Bader, 2002; Wang and Ker, 1995a). 10 MPa stress usually corresponded to 10–20% of MVC level. The non-parametric Wilcoxon test was used to test for statistical differences and linear correlation between post/pre ratios of selected parameters was tested by calculating Pearson's correlation coefficients (c.c.). The level of significance was set to P<0.05. In tables, mean, standard deviation (s.d.) and coefficient of variation (c.v.=s.d./mean) are reported. In figures, all values are means ± s.d.

RESULTS

The main results of the study are summarized in Table 2. Subjects performed 1150–2600 hops before exhaustion. Neither AT stiffness nor initial tendon length changed due to exercise. Tendon stiffness was 430±200 N mm^{-1} before and 390±190 N mm^{-1} after the exercise [not significant (n.s.)]. Despite the lack of changes in tendon stiffness, there was a significant reduction in maximum force production capacity from 3.5±0.6 kN to 2.8±0.7 kN (P<0.01).

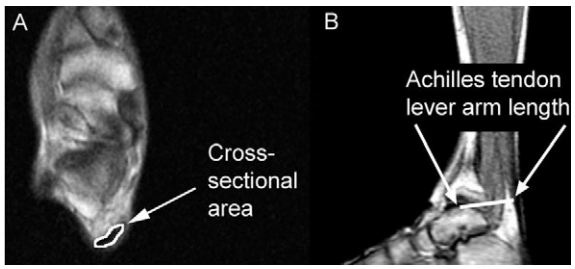

Fig. 3. (A) Achilles tendon cross-sectional area and (B) lever arm length were calculated from magnetic resonance images. The lever arm length was defined as the perpendicular distance from the midpoint of the superior surface of the trochlea of the talus to the line of action of Achilles tendon. Ankle angle was 90 deg. (sole of the foot perpendicular to shank).

A negative correlation was found between post/pre ratio of initial tendon length and post/pre ratio of stiffness (–0.622, P=0.037). Interpretation of this is that although neither stiffness nor initial length was significantly changed as a group, possible individual change in stiffness was related to initial length change, with the negative sign meaning that decrease in stiffness was observed together with tendon lengthening (creep). Stiffness also tended to decrease with increasing number of jumps but the correlation was non-significant (–0.527, P=0.118). There was no significant correlation between post/pre ratios of maximum tendon force and stiffness (0.423, P=0.223).

Fig. 5 demonstrates a single ramp-derived stiffness against stiffness that was deduced from several $\Delta\mathbf{F}$ *vs* ΔL pairs over the linear strain region. Stiffness was independent of the method, being 117 N mm^{-1} (R=0.98, P<0.01) when derived from single ramp contraction and 118 N mm^{-1} (R=0.97, P<0.01) when derived from $\Delta\mathbf{F}$ *vs* ΔL pairs. This observation was repeated in all cases when ramp stiffness was calculated.

The individual tendon CSAs are plotted as a function of the proximal distance from the calcaneus in Fig. 6. Mean AT CSA was 43±11 mm^2 and lever arm length 4.9±0.4 cm (Table 1).

DISCUSSION

The main finding of this study was that neither stiffness nor initial length of the GAM tendon changed after exhaustive high-impact hopping exercise. Despite this, a significant reduction was observed in maximum isometric plantarflexion force (Table 2). The results suggest a lack of acute mechanical changes in connective tissue regardless of diminished muscle force production capacity. The findings are in agreement with previous *in vivo* studies of human tendons that have not found an increase in tendon compliance after static or cyclic exercise (Mademli et al., 2006; Mademli et al., 2008; Ullrich et al., 2007) or after cyclic exercise alone (Kubo et al., 2001b; Kubo et al., 2005).

By contrast, some reports demonstrate that isometric contractions were able to increase VL tendon compliance (Kubo et al., 2001a; Kubo et al., 2001b; Kubo et al., 2005). This indicates that AT might be more fatigue resistant than VL tendon. Although some mechanical properties of human tendons are similar (Wren et al., 2001), fatigue resistance may be one property that varies. For

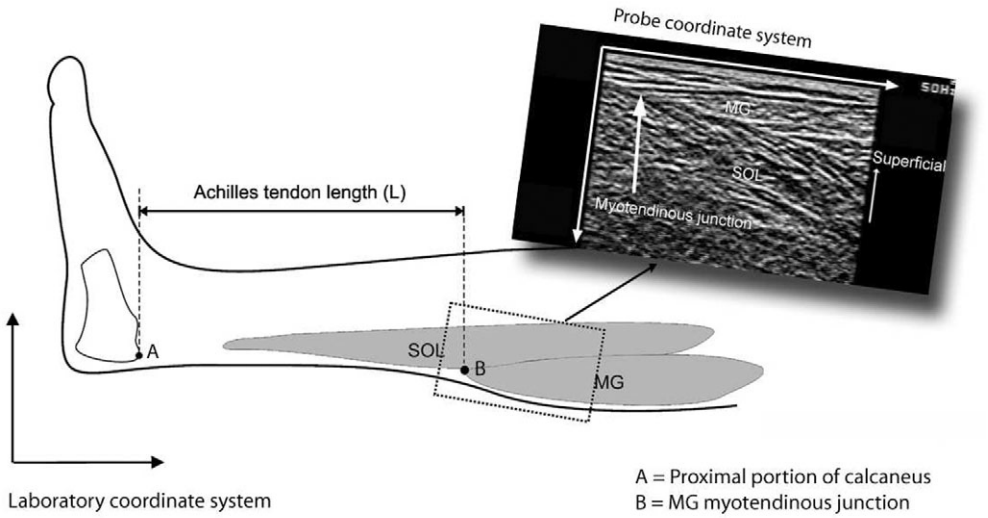

Fig. 4. Schematic representation of two coordinate systems. Achilles tendon length was determined as the distance between insertion (A) and myotendinous junction (B). Coordinates of myotendinous junction were transformed from the probe coordinate system to the laboratory coordinate system. Note that the vertical axis of the ultrasound (US) image is flipped to match image orientation in the US imaging unit. SOL=soleus, MG=medial gastrocnemius.

example, high-stressed animal tendons showed higher fatigue resistance than low-stressed tendons despite equivalent Young's modulus (Ker et al., 2000; Pike et al., 2000). The possibility that AT is more fatigue resistant than VL tendon has to be at least considered. Duration of loading could also be important. Total loading time was 500 s (10 s times 50 reps) in the isometric experiment by Kubo et al. (Kubo et al., 2005) whereas it was roughly the same in the current hopping task (0.25 s times 1885 reps). This suggests that longer duration isometric contractions could have more damaging potential. Interestingly, Wang et al. (Wang et al., 1995b) showed the opposite; fatigue ruptures occur at shorter duration than would be predicted from creep ruptures alone. Similar results were reported by Noyes et al., who reported that connective tissue ruptures dominate over bone fractures at high strain rates (Noyes et al., 1974). Nonetheless, natural movement rarely includes long duration loadings, and hence the current finding that AT was not fatigued after a single bout of hopping exercise is relevant with respect to human natural locomotion.

Wren et al. reported that *in vitro* tendons can be ruptured at physiological stress levels (40–60 MPa) after 1000 cycles or less if 8% initial strain level is exceeded (Wren et al., 2003). To estimate this, we assumed that maximal hopping stress roughly equals MVC stress. This was based on observations by Fukashiro et al. (Fukashiro et al., 1995), who deployed a buckle force transducer technique to demonstrate that peak tendon force (3.8 kN) is very similar to the current MVC force (3.5 kN) during low-intensity hopping (7 cm) such as here (5–20 cm). Therefore, calculation of maximum hopping strain yields an average of 5%. This is not enough to exceed the level set by Wren et al. (Wren et al., 2003), but considering that strain in free tendon is higher (even double) than strain in tendon and aponeurosis combined (which was measured in the present study) (Magnusson et al., 2003; Finni et al., 2003), there is a good chance that the 8% limit was exceeded in the free tendon. Overall, calculations suggest that the stress and strain conditions set by Wren et al. (Wren et al., 2003) to rupture AT with less than 1000 cycles were probably met in the present study.

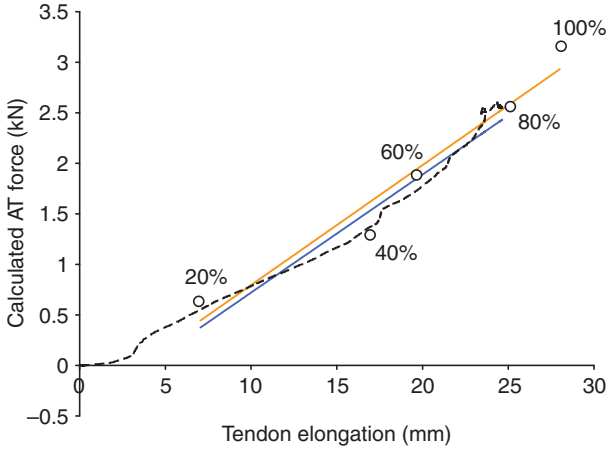

Fig. 5. Pre-exercise data example that compares a single 80% ramp contraction (broken line) with Achilles tendon force ($\Delta\mathbf{F}$) *vs* tendon elongation (ΔL) pairs (open circles). Determining stiffness by fitting a straight line to $\Delta\mathbf{F}$ *vs* ΔL pairs yielded a stiffness of 118 N mm^{-1} (orange line, R=0.97, P<0.01). Repeating the fit for 80% ramp contraction yielded a stiffness of 117 N mm^{-1} (blue line, R=0.98, P<0.01).

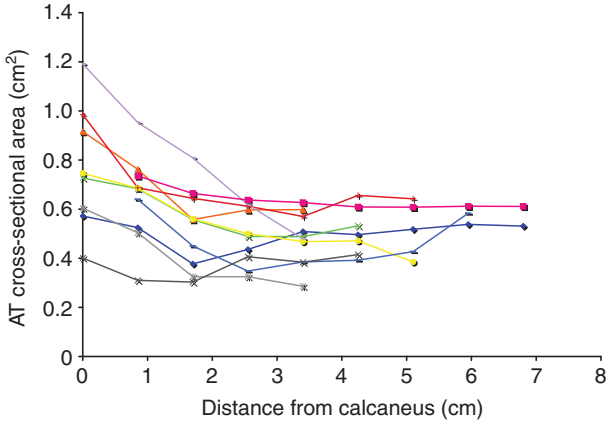

Fig. 6. Individual Achilles tendon (AT) cross-sectional areas as a function of proximal distance from the calcaneus. Distance 0 cm corresponds to the first magnetic resonance image slice where AT is visible. Each subject is presented by a separate colour.

Wren et al. are the only ones to demonstrate such short times to rupture (Wren et al., 2003). Also, they are the only ones to report induced rupture for human AT. Cycle times to rupture at comparable stress levels were much higher in tendons of sheep (Pike et al., 2000) and wallaby (Wang et al., 1995b), and also in human EDL tendons (Schechtman and Bader, 1997). The reason for different results could be methodological and due to difficulties in clamping techniques (Ker et al., 2000). Wren et al. note that the AT is especially hard to work with and easily leads to non-uniform strain (Wren et al., 2003). Furthermore, what makes comparison difficult is that there is not always a clear indication of tendon strain during fatigue tests (Pike et al., 2000; Wang et al., 1995b). Another disadvantage of cadaver tendons is that they are usually extracted from older donors and therefore suffer from possible tissue degradation and decreased stiffness due to ageing [for a review of ageing, see Reeves (Reeves, 2006).

AT mechanical properties

Calculated AT force is prone to error in ankle moment arm length and assumptions of how force is distributed among plantar flexor tendons. It has been demonstrated that tendon moment arm changes due to ankle rotation and muscle contraction (Rugg et al., 1990; Maganaris et al., 2000) and that plantar flexion torque is not transmitted by AT only but has a contribution from deep plantar flexors (Finni et al., 2006; Finni et al., 2000). These possible sources of error are acknowledged here but no compensatory calculations are made, because not enough data is available to reliably estimate the amount of correction that should be used. In any case, a review of the forces in this study shows that they are in good agreement with directly measured tendon forces *in vivo*. Mean maximum isometric force (3.5 kN) was higher than 1–3 kN in walking (Finni et al., 1998), approximately equal to 4 kN in hopping (Fukashiro et al., 1995) and lower than 9 kN in running at 16 km h^{-1} (Komi et al., 1992).

Tendon mechanical properties, such as stiffness or Young's modulus, also rely on the ability to determine tendon elongation or strain. The mean maximal tendon strain in this study (6.2%) is within the range given by others (Magnusson et al., 2003; Maganaris and Paul, 2002; Lichtwark and Wilson, 2005). Mean tendon stiffness (430 N mm^{-1}) matches previously reported values under similar conditions by Rosager et al. (Rosager et al., 2002) but is somewhat higher than reported during one-legged hopping (Lichtwark and Wilson, 2005) and lower than calculated for free tendon during isometric contractions (Magnusson et al., 2003). Maganaris and Paul used a different approach to estimate the contribution of gastrocnemius to the net torque and yielded a stiffness of 150 N mm^{-1} (Maganaris and Paul, 2002). Previous reports on Young's modulus have varied around 0.8–1.2 GPa (Magnusson et al., 2003; Rosager et al., 2002; Maganaris and Paul, 2002; Lichtwark and Wilson, 2005) and are lower than the current mean (2.0 GPa). Differences could be induced by different methodology, because Lichtwark and Wilson used ultrasonography instead of MRI (Lichtwark and Wilson, 2005), and Magnusson et al. measured CSA at a constant distance (2 cm proximal) from calcaneus instead of using the smallest value (Magnusson et al., 2003). Both groups ended up with a higher tendon CSA, and this could explain their lower value for Young's modulus.

Previous *in vitro* reports of Young's modulus have determined a range 0.7–1.1 GPa for human specimens (Schechtman and Bader, 1994; Johnson et al., 1994; Wren et al., 2001) but the comparison to *in vivo* is not straightforward. The strain value in this study includes free tendon and aponeurosis, and strain of free tendon has previously been shown to be much higher than for aponeurosis in active contractions (Finni et al., 2003; Magnusson et al., 2003). Therefore, strain in this study might be an underestimate (and Young's modulus an overestimate) compared with *in vitro*.

Intra- and inter-individual variation

Fig. 5 illustrates typical intra-individual variation, whereby some $\Delta\mathbf{F}$ *vs* ΔL pairs lie outside the predicted line. These random variations were treated by determining tendon stiffness as the slope of the best-fit line to several $\Delta\mathbf{F}$ *vs* ΔL pairs. To see how this method compares with stiffness derived from a single ramp contraction, some ramps were analyzed point-to-point. An example of the comparison is shown in Fig. 5; pre-exercise stiffness that was determined from $\Delta\mathbf{F}$ *vs* ΔL pairs was 118 N mm^{-1} (R=0.97, P<0.01) while stiffness determined from 80% ramp was 117 N mm^{-1} (R=0.98, P<0.01). The former method was used because it allows analysis of more contractions and hence decreases the possible effect of random error. The methodological origin of trial-to-trial variation could be in the image tracking, because MTJ cannot always be unambiguously defined, especially after exercise when muscles sometimes become swollen and the visual appearance of the US image changes. Physiological variation could be caused, for example, by different activation of plantar flexor antagonists.

Tables 1 and 2 indicate that while the correlation values for each line fit to determine stiffness are significant, there is still high inter-individual variation in Young's modulus (c.v.=0.50). This does not necessarily reflect such a high variety in tendon mechanical properties. More likely, it is due to the high variation in tendon maximal strain (c.v.=0.41). As mentioned earlier, free tendon has much higher strain, and therefore individuals with a higher proportion of free tendon tend to exhibit higher GAM tendon strains.

Conclusions

Even though the maximal force production capacity of young and physically active individuals was reduced (–20%) after a single bout of high-impact hopping exercise (1150–2600 cycles), GAM tendon was not fatigued as evidenced by its unchanged stiffness. As a high-stressed tendon, AT might be more fatigue resistant than a number of other tendons in the human body. Although results agree with many previous *in vitro* studies (Pike et al., 2000; Schechtman and Bader, 1997; Wang et al., 1995b,) they disagree with the one and only study conducted with human ATs (Wren et al., 2003). It is possible that clamping problems, often associated to *in vitro* experiments, could cause premature failure and thus different conclusions about tendon fatigue resistance between *in vitro* and *in vivo*. The current observation that tendon was not fatigued seems natural; it proves that the body is able to protect tendon from damage even during high-impact exercise.

LIST OF ABBREVIATIONS

AT	Achilles tendon
CS	coordinate system
CSA	cross-sectional area
EDL	extensor digitorum longus muscle
EMG	electromyography
GAM	gastrocnemius medialis muscle
MRI	magnetic resonance imaging
MTJ	myotendinous junction (most distal part of GAM muscle under US probe)
SOL	soleus muscle
TA	tibialis anterior muscle
US	ultrasound/ultrasonography
VL	vastus lateralis muscle
$\Delta\mathbf{F}$	tendon force
ΔL	tendon elongation

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REFERENCES

Abrahams, M. (1967). Mechanical behavior of tendon *in vitro*. *Med. Biol. Eng. Comput.* **5**, 433-443.

Arampatzis, A., Karamanidis, K., Stafilidis, S., Morey-Klapsing, G., DeMonte, G. and Brüggemann, G. (2006). Effect of different ankle- and knee-joint positions on gastrocnemius medialis fascicle length and EMG activity during isometric plantar flexion. *J. Biomech.* **39**, 1891-1902.

Finni, T., Komi, P. V. and Lukkariniemi, J. (1998). Achilles tendon loading during walking: application of a novel optic fiber technique. *Eur. J. Appl. Physiol.* **77**, 289-291.

Finni, T., Komi, P. V. and Lepola, V. (2000). *In vivo* human triceps surae and quadriceps femoris muscle function in squat jump, counter movement jump and hopping. *Eur. J. Appl. Physiol.* **83**, 416-426.

Finni, T., Hodgson, J. A., Lai, A. M., Edgerton, V. R. and Sinha, S. (2003). Nonuniform strain of human soleus aponeurosis-tendon complex during submaximal voluntary contractions *in vivo*. *J. Appl. Physiol.* **95**, 829-837.

Finni, T., Hodgson, J. A., Lai, A. M., Edgerton, V. R. and Sinha, S (2006). Muscle synergism during isometric plantarflexion in achilles tendon rupture patients and in normal subjects revealed by velocity-encoded cine phase-contrast MRI. *Clin. Biomech.* **21**, 67-74.

Fukashiro, S., Komi, P. V., Järvinen, M. and Miyashita, M. (1995). *In vivo* Achilles tendon loading during jumping in humans. *Eur. J Appl. Physiol.* **71**, 453-458.

Fukunaga, T., Roy, R. R., Shellock, F. G., Hodgson, J. A. and Edgerton, V. R. (1996). Specific tension of human plantar flexors and dorsiflexors. *J. Appl. Physiol.* **80**, 158-165.

Galloway, M. T., Jokl, P. and Dayton, O. W. (1992). Achilles tendon overuse injuries. *Clin. Sports Med.* **11**, 771-782.

Johnson, G. A., Tramaglini, D. M., Levine, R. E., Ohno, K., Choi, N. and Woo, S. L. (1994). Tensile and viscoelastic properties of human patellar tendon. *J. Ortho. Res.* **12**, 796-803.

Kannus, P. and Josza, L. (1991). Histopathological changes preceding spontaneous rupture of a tendon: a controlled study of 891 patients. *J. Bone Joint Surg.* **73**, 1507-1525.

Ker, R. F., Wang, X. T. and Pike, A. V. L. (2000). Fatigue quality of mammalian tendons. *J. Exp. Biol.* **203**, 1317-1327.

Komi, P. V., Fukashiro, S. and Järvinen, M. (1992). Biomechanical loading of Achilles tendon during normal locomotion. *Clin. Sports Med.* **11**, 521-531.

Kongsgaard, M., Aagaard, P., Kjaer, M. and Magnusson, S. P. (2005). Structural Achilles tendon properties in athletes subjected to different exercise models and in Achilles tendon rupture patients. *J. Appl. Physiol.* **99**, 1965-1971.

Kubo, K., Kanehisa, H., Kawakami, Y. and Fukunaga, T. (2001a). Effects of repeated muscle contractions on the tendon structures in humans. *Eur. J Appl. Physiol.* **84**, 162-166.

Kubo, K., Kanehisa, H., Kawakami, Y. and Fukunaga, T. (2001b). Influences of repetitive muscle contractions with different modes on tendon elasticity *in vivo*. *J. Appl. Physiol.* **91**, 277-282.

Kubo, K., Kanehisa, H. and Fukunaga, T. (2005). Influences of repetitive drop jump and isometric leg press exercises on tendon properties in knee extensors. *J. Strength Cond. Res.* **19**, 864-870.

Lichtwark, G. A. and Wilson, A. M. (2005). *In vivo* mechanical properties of the human Achilles tendon during one-legged hopping. *J. Exp. Biol.* **208**, 4715-4725.

Lichtwark, G. A., Bougoulias, K. and Wilson, A. M. (2007). Muscle fascicle and series elastic element length changes along the length of the human gastrocnemius during walking and running. *J. Biomech.* **40**, 157-164.

Mademli, L., Arampatzis, A., Morey-Klapsing, G. and Brüggemann, G. (2004). Effect of ankle joint position and electrode placement on the estimation of the antagonistic moment during maximal plantarflexion. *J. Electromyogr. Kinesiol.* **14**, 591-597.

Mademli, L., Arampatzis, A. and Walsh, M. (2006). Effect of muscle fatigue on the compliance of the gastrocnemius medialis tendon and aponeurosis. *J. Biomech.* **39**, 426-434.

Mademli, L., Arampatzis, A. and Walsh, M. (2008). Age-related effect of static and cyclic loadings on the strain-force curve of the vastus lateralis tendon and aponeurosis. *J. Biomech. Eng.* **130**, 011007.

Maganaris, C. N. (2001). Force–length characteristics of *in vivo* human skeletal muscle. *Acta Physiol. Scan.* **172**, 279-285.

Maganaris, C. N. (2003). Tendon conditioning: artefact or property? *Proc. Biol. Sci.* **270**, 39-42.

Maganaris, C. N. and Paul, J. P. (2002). Tensile properties of the *in vivo* human gastrocnemius tendon. *J. Biomech.* **35**, 1639-1646.

Maganaris, C. N., Baltzoboulos, V. and Sargeant, A. J. (2000). *In-vivo* measurement-based estimation of the human Achilles tendon moment arm. *Eur. J. Appl. Physiol.* **83**, 363-369.

Magnusson, S. P., Hansen, P. and Aagaard, P. (2003). Differential strain patterns of the human gastrocnemius aponeurosis and free tendon, *in vivo*. *Acta Physiol. Scand.* **177**, 185-195.

Noyes, F. R., DeLucas, J. L. and Torvik, P. J. (1974). Biomechanics of anterior cruciate ligament failure: an analysis of strain-rate sensitiviy and mechanism of failure in primates. *J. Bone Joint Surg. Am.* **56**, 236-253.

Pike, A. V. L., Ker, R. F. and Alexander, R. M. (2000). The development of fatigue in high- and low-stressed tendons of sheep (*ovis aries*). *J. Exp. Biology.* **203**, 2187-2193.

Reeves, N. D. (2006). Adaptation of the tendon to mechanical usage. *J. Musculoskelet. Neuronal Interact.* **6**, 174-180.

Rosager, S., Aagaard, P., Dyhre-Poulsen, P., Neergaard, K., Kjaer, M. and Magnusson, S. P. (2002). Load-displacement properties of the human triceps surae aponeurosis and tendon in runners and non-runners. *Scand. J. Med. Sci. Sports* **12**, 90-98.

Rugg, S. G., Gregor, R. J., Mandelbaum, B. R. and Chiu, L. (1990). *In vivo* moment arm calculations at the ankle using magnetic resonance imaging (MRI). *J. Biomech.* **23**, 495-497.

Schechtman, H. and Bader, D. L. (1994). Dynamic characterization of human tendons. *Proc. Inst. Mech. Eng. Part H. J. Eng. Med.* **208**, 241-248.

Schechtman, H. and Bader, D. L. (1997). *In vitro* fatigue of human tendons. *J. Biomech.* **30**, 829-835.

Schechtman, H. and Bader, D. L. (2002). Fatigue damage of human tendons. *J. Biomech.* **35**, 347-353.

Ullrich, A. C., Mademli, L. and Arampatzis, A. (2007). Effects of submaximal and maximal long-lasting contractions on the compliance of vastus lateralis tendon and aponeurosis *in vivo*. *J. Electromyogr. Kinesiol.* **19**, 476-483.

Viidik, A. (1973). Functional properties of collagenous tissues. *Int. Rev. Connect Tissue Res.* **6**, 127-215.

Wang, X. and Ker, R. (1995a). Creep rupture of wallaby tail tendons. *J. Exp. Biol.* **198**, 831-845.

Wang, X., Ker, R. and Alexander, R. (1995b). Fatigue rupture of wallaby tail tendons. *J. Exp. Biol.* **198**, 847-852.

Wren, T. A. L., Yerby, S. A., Beaupré, G. S. and Carter, D. R. (2001). Mechanical properties of the human achilles tendon. *Clin. Biomech.* **16**, 245-251.

Wren, T. A. L., Lindsey, D. P., Beaupré, G. S. and Carter, D. R. (2003). Effects of creep and cyclic loading on the mechanical properties and failure of human Achilles tendons. *Ann. Biomed. Eng.* **31**, 710-717.