RESEARCH ARTICLE

Achilles tendon stiffness is unchanged one hour after a marathon

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INTRODUCTION

The Achilles tendon (AT) is one of the most ruptured and surgically treated tendons in the human body (Józsa et al., 1989). It is often injured in sports, especially in activities involving strenuous stretching of the tendon such as jumping or running (Kvist, 1994). The history of the AT is long; it appeared in our anatomy 2 million years ago. It is one of the special characteristics in the human body that facilitate our endurance running ability (Bramble and Lieberman, 2004). This poses an important question: why is the AT so prone to injuries if it has been so crucial to our survival (Malvankar and Khan, 2011)?

The AT is a big spring that acts to save energy and amplify power during the human step. Because it can experience forces up to 10 kN (Komi et al., 1992), it is difficult to imagine how it could be ruptured with a single stretch. To overcome this, a theory of overuse-induced rupture has been developed, which states that overloading of the tendon could lead to overuse injuries (Galloway et al., 1992). The idea of overuse injury as a predisposing factor to tendon ruptures is supported by the finding that 97% of ruptured tendons show signs of degenerative diseases (Kannus and Józsa, 1991).

Although the exact mechanisms of tendon ruptures are still unknown, several authors of in vitro studies have concluded that tendon ruptures are associated with a prior reduction in Young’s modulus (Schechtman and Bader, 1994; Schechtman and Bader, 2002; Wang et al., 1995; Wang and Ker, 1995; Wren et al., 2003). Young’s modulus can be thought of as a normalised stiffness because it depends on tendon material properties alone, whereas stiffness also depends on tendon cross-sectional area and length. Therefore, if a single exercise bout can overload the AT, this should be evident as a reduction in AT stiffness.

The purpose of the present study was to test the theory that physical exercise can mechanically overload the AT and reduce its stiffness. Because the mechanical properties of the AT have been previously shown to be unaltered by a short-duration exercise (Farris et al., 2011b; Mademli et al., 2006; Peltonen et al., 2010), the present study tested the effects of a long-lasting exercise. Thus, the stiffness of the AT was determined before and after a marathon. For most humans, this task represents the most strenuous single bout of exercise ever performed.

MATERIALS AND METHODS

Subjects

Twelve marathon runners participated in the study (Table 1): eight who completed a full marathon (42 km) and four who completed a half marathon (21 km). Half-marathon runners were considered to be less experienced because their target speed over a shorter distance (mean: 10.8 km h⁻¹) was the same as for full-marathon runners (mean: 10.9 km h⁻¹). All subjects were physically active with a life-long training background mostly in endurance running. Participants were informed about the procedures, benefits and possible risks involved in the study, and they all signed a written consent prior to the study. All methods were approved by the local ethical committee and the study conformed to the standards set by the Declaration of Helsinki.

Protocol overview

AT stiffness was tested on three occasions: (1) 2–4 days before the race (2 days pre-race), (2) 2 h before the race (2 h pre-race) and (3)
1 h after the race (1 h post-race). Tendon stiffness was tested twice before the race to assess test–retest repeatability. To examine whether possible changes in stiffness were linked to changes in running economy, ankle kinematics and oxygen consumption were measured during 4 min sub-maximal treadmill running in the 2 days pre-race and 1 h post-race tests.

**Testing of tendon stiffness**

Tendon mechanical testing was performed in a custom-built ankle dynamometer (University of Jyväskylä, Finland) (Fig. 1A) that operated in a passive mode, i.e. the pedal did not move. Subjects were seated with the knee fully extended, the ankle at a right angle (sole of the foot perpendicular to the shank) and the hip flexed to 60 deg (0 deg equals full extension). Their right leg was tightly anchored between the backrest and the foot pedal, where a force transducer (Precision TB5-C1, Raute, Nastola, Finland) was installed. The system allows only minimal ankle plantarflexion (typically 1–2 deg), but even the smallest heel movement was matched acoustic pad was placed under the probe to ease propagation of ultrasound waves, and the probe was secured to the leg with elastic bandages (Fig. 1A). A digital camera (InLine 250, Fastec Imaging, San Diego, USA) was placed on the ankle dynamometer’s left side to image movement of the probe during measurements. The camera’s optical axis was perpendicular to the sagittal plane over the right leg 2 cm medial to the junction separating the medial and lateral portions of the GAM. During the first session, an outline of the probe was drawn on the skin with permanent marker and this outline, together with the MTJ, were used as reference points to ease later identification of the same muscle area. An impedance-matched acoustic pad was placed under the probe to ease propagation of ultrasound waves, and the probe was secured to the leg with elastic bandages (Fig. 1A). A digital camera (InLine 250, Fastec Imaging, San Diego, USA) was placed on the ankle dynamometer’s left side to image movement of the probe during measurements. The camera’s optical axis was perpendicular to the sagittal plane and the camera was focused on the US probe’s four reflective markers that were placed on the probe handle to enable movement tracking. The camera and the US unit operated at 125 frames s⁻¹. US images were collected by the imaging unit (Aloka Alpha 10) and the digital camera’s video was recorded for later export. The system was synchronised via the AD board, which was programmed to output a transistor–transistor logic (TTL) pulse. The TTL pulse was fed into the US unit’s pulse via.

**Table 1. Anthropometric data, Achilles tendon (AT) mechanical properties and race statistics**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Mass (kg)</th>
<th>AT CSA (mm²)</th>
<th>Young’s modulus (GPa)</th>
<th>AT initial length (mm)</th>
<th>Distance (km)</th>
<th>Time (h:min)</th>
<th>Speed (km h⁻¹)</th>
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<td>172±10</td>
<td>66±11</td>
<td>67±4</td>
<td>0.78±0.33</td>
<td>256±19</td>
<td>11.2±0.3</td>
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</table>

CSA, cross-sectional area.

Fig. 1. (A) The ankle dynamometer setup. (B) Illustration of the placement of the tracking markers in the ultrasound image. GAM, gastrocnemius medialis; Sol, soleus.
input and displayed on the monitor. At the same time, ECG detection was enabled, which created a moving vertical cursor that overlaps both the US image and the TTL pulse. The US unit was synchronised by aligning the vertical cursor with the rising edge of the TTL pulse. The camera was synchronised by a flashing light signal triggered by the same TTL pulse. Maximum desynchronisation between camera and US images was the inverse of the frame rate, 1/125 = 8 ms.

AT cross-sectional area was analysed by taking transverse US images 3 cm above the heel.

Treadmill running
While running on a motorised treadmill (OJK-1, Telineyhymä, Kotka, Finland), subjects wore their race shoes and the running speed was chosen according to each subject’s target time. Two-dimensional ankle kinematics were recorded with a high-speed digital camera (InLine 250, Fastec Imaging) operating at 125 frames s⁻¹. The camera was located on the subject’s right side perpendicular to the sagittal plane. Three reflective markers were placed at different locations on the subject’s right leg: (1) the center of the knee, (2) the lateral malleolus and (3) the distal head of fifth metatarsal. Markers 1 and 2 were attached to the skin or running tights to minimise their movement. Marker 3 was placed on the running shoe.

To quantify running economy, subjects wore a breathing mask connected to an oxygen gas analyser (VMax series 229, Sensormedics, Yorba Linda, CA, USA). Steady-state oxygen consumption was achieved by running at a constant speed for 4 min. Oxygen consumption was taken as the average during the fifth minute. Running kinematics were recorded during the last minute with a 5 s video clip containing four to six strides.

Analysis of tendon stiffness
Ankle joint torque was calculated by multiplying the foot reaction force with the foot lever arm length, measured between the first metatarsal and the ankle pivot point defined as the centre of the medial malleolus. AT force was then calculated by dividing ankle torque by tendon lever arm length, measured between the pivot point and the posterior surface of the calcaneus. The placement of the foot on the pedal leads to removal of tendon slack and therefore initial tension in the AT. This initial tension varied among individuals. It was on average 0.3 kN and was subsequently subtracted from the AT force before analysis of tendon stiffness. Lever arm lengths were taken as

Table 2. Achilles tendon (AT) mechanical properties and cost of transport (COT) before and after the marathon race

<table>
<thead>
<tr>
<th>Subject</th>
<th>2 days pre-race</th>
<th>2 h pre-race</th>
<th>1 h post-race</th>
<th>AT maximum force (kN)</th>
<th>2 days pre-race</th>
<th>2 h pre-race</th>
<th>1 h post-race</th>
<th>COT (mlO₂ kg⁻¹ km⁻¹)</th>
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<td>2.3</td>
<td>119</td>
<td>116</td>
<td>109</td>
<td>226</td>
<td>306</td>
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</table>

Mean ± s.d. 2.9±0.7 3.4±0.9† 2.9±1.1 197±62 213±58† 206±59 226±11 241±22*

*Significantly different from the 2 days pre-race value (P<0.05).
†Mean values for the 2 h pre-race group include only 11 subjects and are therefore not comparable to the 2 days pre-race and 1 h post-race groups, which include all 12 subjects.
a projection along the sole of the foot when it was perpendicular to the shank. Therefore, lever arms were considered to be perpendicular to the line of force. AT stress was calculated by dividing force by tendon cross-sectional area.

AT length was calculated as the distance between the MTJ of the GAM muscle and the bony superior and posterior surface of the heel. Initial position of the heel was taken when the foot was properly placed on the pedal. Possible heel displacement in the superior–inferior direction during muscle contraction was measured with a position sensor located under the heel. MTJ displacement was analysed from the US images with software that exploits pyramidal implementation of Lukas–Kanade feature tracking (Bouguet, 2001). The software requires the user to place nine tracking points over the area of interest, as shown in Fig. 1B. The tracking points were placed just superior to the MTJ along the aponeurosis separating the GAM and the soleus, but still on the side of the GAM. We have found that this placement yields the most repeatable results. The tracking algorithm has been previously shown to have a repeatability of 98% (Magnusson et al., 2003). Each trial was tracked twice and the resulting coordinates were averaged. The average was then taken as the MTJ coordinates in the US probe CS. Coordinate transformation between two CSs is always possible if both CSs are defined at all instants of time, i.e. their origins and axes are known. A rigid video calibration object was used to determine the laboratory CS, which did not change over time, and the CS of the US probe was given by the four reflective markers placed on the probe handle (Fig. 1A). The origin of the US image relative to the probe markers was determined by placing an echogenic marker on the image origin and measuring its distance from the markers. Because all movement could be restricted to the sagittal plane, analysis was made in two dimensions and it was estimated that this introduced a ~0.2 mm systematic error to tendon elongation and can thus be neglected. A similar procedure has also been used in three-dimensional space (Gerus et al., 2011; Lichtwark and Wilson, 2005). Tendon elongation was calculated by subtracting initial tendon length at the onset of force production and strain was calculated by dividing tendon elongation by initial length.

Three AT force–elongation curves were normalised in time and then averaged, resulting in one force–elongation curve per subject per testing session (Fig. 2B). AT stiffness was calculated as the slope of the least-squares line of the ascending limb of the force–elongation curve between 10 and 80% of MVC force. Young’s modulus was determined the same way, but from the stress–strain curve.

Analysis of running economy
Cost of transport (COT) was expressed in millilitres of oxygen per kilogram per kilometre (mlO₂kg⁻¹km⁻¹), which is known to be relatively independent of running speed (Cavagna and Kaneko, 1977).

Ankle kinematics were recorded because they were thought to be related to possible AT stiffness changes. Digitised joint coordinates were filtered with a Butterworth low-pass filter (8 Hz) and ankle angle was calculated as the angle between the shank segment (from the centre of the knee to the lateral malleolus) and the foot segment (from the lateral malleolus to the distal head of the fifth metatarsal). Landing and take-off were determined from the video. All four to six contacts were then time normalised and averaged to yield a single ankle angle trace per subject.

To define landing technique, the angle at ground contact was offset corrected and deviations in ankle angle were determined relative to this zero value: a positive angle corresponded to dorsiflexion and a negative angle to plantarflexion. Subjects were categorised into three groups: forefoot contact (FFC), mid-foot contact (MFC) and heel contact (HC). The categorisation was based on the ankle angle deflection (relative to ground contact) at the moment corresponding to 0.225 times the interval from the ground contact to the maximum dorsiflexion deflection (illustrated by circles in Fig. 3). If the deflection was more than +1 deg (dorsiflexion), the subject was an FFC runner; if the deflection was less than –1 deg (plantarflexion), the subject was an HC runner; and if the deflection was between –1 and +1 deg, the subject was an MFC runner. Categorisation was carried out before and after the marathon.

Statistics
Data were tested for normality using the Kolmogorov–Smirnov test and the non-parametric Wilcoxon’s signed-rank test was used to test differences between sessions and groups accordingly. Spearman’s linear correlation coefficient was also calculated between selected parameters. All runners were treated as one group because full and half marathons were considered to be equally stressful for each individual relative to his/her training background. (In comparisons, full- and half-marathon runners behaved statistically similarly.) Data are presented as means ± s.d.

RESULTS
All subjects finished the marathon close to their target time. The mean running speed during the race was not significantly different from the mean running speed on the treadmill (P=0.115; Table 1).

AT mechanical properties
All 12 subjects were tested 2 days before and 1 h after the marathon (Table 2). Mean AT stiffness did not change statistically between the 2 days pre-race test (197±62 Nmm⁻¹) and the 1 h post-race test (206±59 Nmm⁻¹; N=12, P=0.312). Tendon stiffness was measured at the same absolute force level before and after the race, because there was no statistical difference between MVC in the 2 days pre-race and 1 h post-race tests: 2.9±0.7 versus 2.9±1.1 kN, respectively (P=0.638). Young’s modulus of the AT was 0.78±0.33 GPa (Table 1).

Excluding one subject who did not participate in the 2 h pre-race test and analysing repeatability between the 2 days pre-race and 2 h pre-race tests revealed that AT stiffness was highly correlated (r=11, r=0.844, P<0.01) and that there were no statistical differences between the results: 203±61 Nmm⁻¹ in the 2 days pre-race test versus 213±56 Nmm⁻¹ in the 2 h pre-race test (P=0.320). Within this group, AT MVC force was higher (N=11, P<0.001) in the 2 h pre-race test (3.4±0.9 kN) than in the 2 days pre-race test (2.9±0.9 kN), probably indicating learning. It then decreased back to 3.0±1.0 kN at 1 h after the race (P<0.01).

Running economy and landing technique
Mean COT was 226±11 mlO₂kg⁻¹km⁻¹ in the 2 days pre-race test, and significantly higher in the 1 h post-race test at 241±22 mlO₂kg⁻¹km⁻¹ (N=12, P<0.05). Change in stiffness was not correlated with change in COT (R²=0.262, P=0.410).

Six out of 12 subjects changed their landing technique from FFC to either MFC or HC following the race. From the remaining six, three did not change their landing technique (they maintained FFC or HC) and three went from HC to MFC. There were no statistical differences in any of the measured parameters between the group of runners who were initially FFC runners and the rest.

DISCUSSION
The most important finding of the present study was that AT stiffness was not influenced by the marathon, and thus the theory of overuse-
induced mechanical changes in the AT was not supported. The second important finding was that day-to-day repeatability of AT stiffness was good, suggesting that this is a valid method for studying tendon mechanical changes over time. It is not known what happened immediately after the race, because AT stiffness was deduced after a 1 h delay. However, it is unlikely that the results would have been different if tendon stiffness had been measured immediately after the race, because AT mechanical properties have been previously shown to remain unchanged immediately after fatiguing concentric contractions (Mademli et al., 2006), hopping to exhaustion (Peltonen et al., 2010) and 30 min of running (Farris et al., 2011b).

### AT fatigue resistance

Several studies have shown that AT stiffness is unchanged after an acute bout of exercise (Farris et al., 2011b; Mademli et al., 2006; Peltonen et al., 2010). On the contrary, patella tendon stiffness has been shown to decrease after 50 cycles in which 3 or 10 s MVC contractions were alternated with 3 or 10 s rest periods (Kubo et al., 2001a; Kubo et al., 2001b; Kubo et al., 2005). Stress-in-life, the maximum physiological stress that a tendon experiences based on its muscle’s cross-sectional area, has been shown to affect tendon fatigue quality. Tendons that have high stress-in-life tend to be more fatigue resistant (Ker et al., 2000; Pike et al., 2000). Therefore, the usually higher tensile stress of the AT (70–80 MPa) (Lichtwark and Wilson, 2005; Peltonen et al., 2010) compared with that of the patella tendon (30–40 MPa) (Hansen et al., 2006; Westh et al., 2008) suggests that the AT may be more fatigue resistant, which could explain the discrepancy in the literature between tendons.

AT ruptures have been demonstrated at physiological stress levels (40–60 MPa) after only 1000 loading cycles where 8% initial strain was exceeded (Wren et al., 2003). This is in vast contrast to the present results and warrants attention. Because AT force during

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**Fig. 3.** Mean ankle angle traces of four to six running steps before (solid line) and after (broken line) the marathon. Positive angle corresponds to dorsiflexion and negative to plantarflexion (relative to ground contact). Circles indicate the moment when ankle angle was measured for categorisation: FFC, forefoot contact; MFC, mid-foot contact; HC, heel contact.
running is typically 3–4kN (Komi et al., 1992) and mean AT cross-sectional area was 67 mm² in the present study, the tensile stress during running probably varied between 45 and 60 MPa. The AT strain during running is reported to be 3.5% (Farris et al., 2011a), which is probably an underestimation because the AT would not be very good at storing elastic energy during running, which is one of its major functions (Alexander and Bennet-Clark, 1977). Nonetheless, 3.5% is given for the portion of the AT that attaches to the MTJ muscle, which could be half of the strain in the free AT directly above the heel (Finni et al., 2003; Magnusson et al., 2003). Therefore, the conditions set by the study of Wren and colleagues (Wren et al., 2003) to rupture AT after 1000 loading cycles are probably met during a marathon, which consists of peak forces of several kiloNewtons that are repeated approximately 20,000 times. Because several other studies have shown that it takes considerably more than 1000 cycles to rupture a tendon (Pike et al., 2000; Schechtman and Bader, 2002; Wang et al., 1995), it is likely that donor age, tissue degeneration or clamping problems influenced the early AT rupture after 1000 cycles, and that the living human AT is not as prone to injuries as cadaver tendons are.

Validity of tendon data

Because the initial tension (approximately 0.3 kN) is subtracted from the tendon force, the maximum tendon force (3.4 kN) in the present study is probably a slight underestimation, but it is still comparable to previously reported values, e.g. 1–3 kN in walking (Finni et al., 1998), 4 kN in hopping (Fukashiro et al., 1995) and 9 kN in sprint running (Komi et al., 1992). In the present study, the point of application of force was assumed to be under the first metatarsal. This is true only at the time of the maximum force. We have verified with pressure insoles that the force vector travels from the fifth metatarsal towards the first metatarsal when the force builds up, thus increasing the foot lever arm. However, tendon lever arm length also increases during application of force (Rugg, 1990) and these changes may cancel each other out in gear ratio calculations. Thus we have used constant lever arm lengths. Even if a small error is introduced, it is likely to be the same before and after the marathon and does not influence the main result of the present study, which was that the AT stiffness is unchanged after a marathon. The mean stiffness and Young’s modulus in the present study (197 N mm⁻¹ and 0.78 GPa) were consistent with those previously reported under a similar dynamometer setup (Maganaris and Paul, 2002) and during one-legged hopping (Lichtwark and Wilson, 2005). The Young’s modulus was also comparable to values previously reported for human (Schechtman and Bader, 2002) and animal (Pollock and Shadwick, 1994) tendons in vitro.

Running technique and economy

Nine out of 12 subjects changed their landing technique after the marathon, probably to compensate for neuromuscular fatigue. Changes in running technique did not equate to a more efficient way of transportation; on the contrary, their running was less economical after the race, as evidenced by their increased COT. This is in line with previous observations and no single reason for decreased running economy has been identified (Hausswirth et al., 2003). It was presumed that if the AT was fatigued, the utilisation of elastic energy would be reduced, which could influence COT in running. However, the AT was not fatigued and changes in COT and stiffness were not correlated. A similar observation has been made previously, when AT elongation was directly measured during a 30 min run, and no changes in elongation were found (Farris et al., 2011b). However, we did find evidence for calf muscle fatigue, because the MVC force decreased after the race from the 2h pre-race test. Therefore, subjects probably changed their running technique as a result of muscle fatigue.

Implications for human movement

A large body of evidence suggests that physical activity does not reduce the stiffness of the AT, but rather a lack of physical activity does. A typical training effect, regardless of whether training is plyometric or isometric resistance training, is an increase in AT stiffness (Burgess et al., 2007), although the effect may be invariant to training background as runners and non-runners were found to have similar AT stiffness (Rosager et al., 2002). This may indicate that it is advantageous to have a certain optimal stiffness for achieving efficient muscle output during cyclic movements (Lichtwark and Barclay, 2010). In contrast to physical activity, it is well known that physical inactivity, such as 90 days of bed rest (Reeves et al., 2005) or aging-related reductions in exercise frequency (Narici and Maganaris, 2006), decreases AT stiffness. When the training data are supplemented with the finding that neither long-lasting (present study) nor short-duration exercise (Farris et al., 2011b; Mademli et al., 2006; Peltonen et al., 2010) causes a reduction in AT stiffness, the theory of overuse-induced mechanical changes in the human AT is not strongly supported by the published literature.

Returning to the question posed in the introduction about why the AT is so prone to injuries, the present study indicates that even long-lasting physiological loading, like that incurred during a marathon, does not cause notable reduction in the AT stiffness. Thus it may be that running itself does not predispose the AT to injuries. Rather, a combination of a rapid increase in stress, a quick crossover to new sporting activities without a training period, poor technique and/or improper footwear could play a role that has not yet been identified. The present study also found that subjects increased their oxygen consumption and changed their landing technique after a marathon, both of which were probably related to fatigue of the muscles rather than the tendon.

LIST OF ABBREVIATIONS

AT Achilles tendon
COT cost of transport
CS coordinate system
FFC forefoot contact
GAM gastrocnemius medialis
HC heel contact
MFC mid-foot contact
MTJ myotendinous junction
MVC maximum voluntary contraction
TTL transistor–transistor logic
US ultrasound/ultrasonography

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