Aging and the effects of a half marathon on Achilles tendon force-elongation relationship

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Abstract

Purpose: We aimed to determine whether there are different changes in Achilles tendon (AT) mechanical properties in middle-aged, compared to younger runners that might indicate that tendon fatigue, induced by long distance running, is age-dependent. **Methods:** 27 middle-aged (50-67 years) and 22 younger (21-29 years) participants ran a 21km route at their own pace (mean and SD: old: $3.1 \pm 0.3 m \cdot s^{-1}$; young: $3.6 \pm 0.5 m \cdot s^{-1}$). We tested for changes in the AT force-elongation relationship using dynamometry and ultrasonography during isometric voluntary ankle plantarflexion ramp contractions, conducted 20-28h pre-run, immediately pre-run, immediately post-run and 20-28h post-run. Stride frequency and number were examined to estimate cyclic tensile loading characteristics of the tendon during running. **Results:** Muscle strength decreased significantly ($P<0.05$) in both groups immediately post-run (old: 17%; young: 11%) and recovered to baseline within 20-28h post-run. AT stiffness did not change for the younger adults, whereas the middle-aged adults showed a significant ($P<0.05$) decrease in AT stiffness (22%). However, tendon stiffness recovered to baseline 20-28h post-run. Middle-aged, compared to young adults demonstrated significantly ($P<0.05$) greater stride frequency and number, but no correlations with tendon fatigue changes were determined ($R^2 \leq 0.038$). **Conclusions:** The results suggest that the plasticity of the AT in response to short-term mechanical loading may be age-dependent and that the AT length–tension properties of middle-aged runners may be more vulnerable to change following running compared to younger athletes. However, the observed AT changes in the middle-aged runners dissipated within 20-28h post-run, suggesting that a tendon viscoelastic recovery mechanism may occur *in vivo*.

Key words tendon stiffness, tendon fatigue, running, mechanical loading, age, muscle contraction

Abbreviations

1dayPre-Run	20 to 28h before the run
1dayPost-Run	Within 20 to 28h after the run
AT	Achilles tendon
ANOVA	Analysis of variance
CSA	Cross sectional area
GM	Gastrocnemius medialis

imPre-Run	Immediately before the run
imPost-Run	Immediately after the run
MTU	Muscle-tendon unit
MVC	Maximal voluntary plantarflexion ramp contraction
SD	Standard Deviation

Introduction

Tendons play a crucial role in movement by transmitting muscle forces to the skeleton and therefore, changes in human tendon biomechanical properties could affect movement effectiveness (Ker et al. 1987; Biewener and Roberts 1989; Karamanidis and Arampatzis 2005; Lichtwark et al. 2007) and injury risk (Lavagnino et al. 2014). This may be of particular concern for older populations, who experience age-related changes in tendon composition that may affect the mechanical properties of the tendon which could eventually reduce the tendon's tolerance of mechanical loading.

Despite a lack of clarity in the literature, most *in vitro* studies in humans point towards the fact that aging can be associated with decreased tensile stiffness and ultimate failure strength of tendons and collagenous tissues (Noyes and Grood 1976; Tkaczuk 1968 Viidik 1982). In a more recent study by Lavagnino et al. (2014), using both *in vitro* animal and *in vivo* human experiments, it was shown that tendon composition and material properties diminish the ability of elongated tendons to re-establish normal tension homeostasis, making an older tendon more prone to overuse injuries after repetitive cyclic loading exercise (Lavagnino et al. 2014). This may be one explanation that older age has been identified as a predisposing factor for both overuse injuries (Huttunen et al. 2014; Jozsa et al.1989; Kvist 1994), as well as longer recovery times following tendon repair procedures (Lavagnino et al. 2013; Lavagnino et al. 2014). Specifically, Achilles tendon (AT) injuries appear to be age related, as the incidence rate of AT injury in men over 40 years is almost twice as high as in men between 18-39 years of age (Huttunen et al. 2014). An additional risk factor for AT injury appear to be activities with repetitive cyclic mechanical loading, like long distance running, as runners are one of the most susceptible populations to chronic AT injuries and AT ruptures (Brunet et al. 1990; Clement et al. 1984). During running, the AT has to cope with multiple tensile strain cycles, consisting of high strain rates and magnitudes (e.g. AT strain magnitude up to 5.5%; Lichtwark et al. 2007). Such loading profiles can lead to the tendon becoming more compliant and more susceptible to large strains, which can ultimately lead to tendon failure (Ker et al. 2000; Wang et al. 1995). These changes in the tendon force-elongation relationship are suggested to be caused by a disequilibrium between the rate of damage and the rate of repair (Ker et al., 2000), due to significant changes in expression of collagens (down regulation of Col I and up regulation of Col III and Col V), fiber angulation (increase in density of the fibers) and delamination among adjacent fibers (separation of the fibers into layers; Fung et al., 2010). Such changes in the tendon force-elongation relationship, morphology and molecular profile due to cyclic mechanical loading are collectively termed tendon fatigue (Wang et al., 1995; Fung et al., 2010; Ker et al. 2000) and the response of tendons to *in vitro* and *in vivo* cyclic mechanical loading has been well established in animal models (Fung et al., 2010; Lavagnino et al. 2014; Wang et al. 1995). Improved understanding of potential age-related differences in the response of the AT to long distance running might be important for reducing the risk of AT injuries in middle and older aged runners.

In previous *in vivo* human studies, various dynamic activities (such as heel drop exercise, running or hopping) have been used to examine whether changes in tendon mechanical properties occurred, that might be indicative of tendon fatigue, following repetitive cyclic loading (Farris et al. 2012; Lichtwark et al. 2013; Obst et al. 2016; Peltonen et al. 2010; Peltonen et al. 2012). However, no significant change in the AT force-elongation relationship *in vivo* has been found in three (Farris et al. 2012; Peltonen et al. 2010; Peltonen et al. 2012) of these five studies. The studies of Lichtwark et al. (2013) and Obst et al. (2016) did find an increase in strain at a given force, which could be indicative of early stage mechanical fatigue. However, these effects were much smaller than those predicted by both *ex vivo* and *in vivo* animal experiments (Fung et al. 2010; Lavagnino et al. 2014; Wang et al. 1995). This may have been due to the methodology or the nature of the loading profiles used in these studies. Crucially, these studies were conducted with predominantly young subjects (mean age under 30 years; Farris et al. 2012; Lichtwark et al. 2013; Peltonen et al. 2010). As a consequence, there is limited evidence for how the human AT in middle-aged to older runners (50 years and older) responds to a long lasting cyclic loading exercise such as long distance running. To our knowledge, one study (Mademli and Arampatzis 2008) investigated the AT fatigue response in older adults *in vivo* during cyclic submaximal isometric voluntary contractions on a dynamometer, but failed to find any significant age-related fatigue effects on the mechanical properties of the AT. However, the contraction protocol used may not have simulated the typical mechanical loading profile of a long distance run. This evidence gap is significant because older aged runners are at a higher risk for AT injury (Kvist 1994) and the finding that aging diminishes tendon fatigue quality during extended repetitive cyclic loading in *in vitro* and *in vivo* animal models (Lavagnino et al. 2014).

The purpose of this study was, therefore, to examine whether there are different changes in AT force-elongation properties between middle-aged and younger adult long distance runners following a half marathon run that might indicate that tendon fatigue, in response to repetitive cyclic loading, is age-dependent. We decided to use a half marathon run (20.5 km) at a self-selected pace, as this includes a high number of loading cycles and represents a typical training and competition distance for long distance runners. Number and frequency of strides were assessed in order to indicate the absolute amount and frequency of AT loading cycles experienced by the runners. It was hypothesized that the AT would exhibit greater mechanical changes in the middle-aged compared to the younger adult long distance runners, which would be consistent with tendon fatigue. Specifically, we expected that the AT would exhibit a more pronounced decrease in tendon stiffness for the middle-aged, compared to the younger adult long distance runners.

Methods

Subjects

The investigation was conducted with 27 middle-aged (age: 56 ± 4.7 years; height: 180.4 ± 5.9 cm; body mass: 74.6 ± 6.1 kg; mean and standard deviation (SD)) and 22 younger (age: 26 ± 2.6 years; height: 182.1 ± 6.7 cm; body mass: 74.7 ± 9.0 kg) male recreational marathon runners. The subjects had been regular runners for at least the last four and six years, for the younger and middle-aged adults respectively. All subjects participated in competitive long distance races (a minimum of two races within the last two years) and had a minimum training volume of 30 km per week (average of about 50 km per week across subjects). Exclusion criteria were any previous AT ruptures, AT injury (e.g. tendinopathy) or pain within the last 12 months, or musculoskeletal impairments in the lower limbs (e.g. ankle joint pain) determined by interview and questionnaire, that could influence the findings of the study. The study was approved by the ethical board of the German Sport University Cologne, the procedures of the study were explained to the participants, and written informed consent was obtained from all individual participants included in the study prior to the testing in accordance with the Declaration of Helsinki.

Experimental design

All subjects performed a half marathon run (20.5 km) on a flat, off-road track. The subjects were asked to run the course as fast as possible at their self-selected pace and they were accompanied and encouraged by one of the staff members, who cycled alongside them. The mechanical properties of the *triceps surae* muscle-tendon unit (MTU) of the dominant leg (preferred leg for step initiation; determined by a questionnaire) were assessed on four occasions: (a) 20 to 28h before the run (1dayPre-Run), (b) immediately before the run (imPre-Run), (c) immediately after the run (imPost-Run) and (d) within 20 to 28h after the run (1dayPost-Run). The two pre-measurements (1dayPre-Run and imPre-Run) were used in order to test the day-to-day reliability in muscle strength and tendon stiffness. The measurement 1dayPost-Run was performed to investigate if the potential tendon changes were still present one day after the run. The stride number and frequency of the individuals were examined, in order to investigate if potential changes in the AT force-elongation relationship were affected by average AT cyclic loading frequency and total number of AT loading cycles. All subjects were instructed not to perform any additional physical exercise (e.g. running) during these three days of measurements.

Analysis of the tendon force-elongation relationship and muscle strength

The mechanical properties of the *triceps surae* MTU of the dominant leg were assessed by integrating ultrasonography (MyLab One, Esaote; Genova, Italy) and dynamometry synchronously using a custom made device (Fig. 1). The subjects were seated with their lower leg secured and with their foot on a custom made strain gauge type dynamometer (sampling frequency 1000 Hz) with the ankle and knee joints positioned at 90 degrees (thigh and foot perpendicular to the shank; see supporting data for this setup in Online Resource 1). A laser guided electrical potentiometer system was used to position the subject's foot on the force plate by setting the midpoint of the *malleolus lateralis* in line with the force plate's center of rotation (Fig. 1C). The position of the seat relative to the dynamometer was recorded by two additional electrical potentiometers, in order to reliably replicate the positions during each of the four MTU measurements (the height of the seat, distance of the seat to the force plate and position of the foot on the force plate; as showed in Fig. 1C).

Fig. 1

In order to examine muscle strength and the force-elongation relationship of the tendon during the loading phase, all subjects performed four isometric plantarflexion contractions at different force levels: a maximal voluntary plantarflexion ramp contraction (MVC), followed by three sustained contractions at 30, 50 and 80% of the maximal joint moment determined during the MVC. At every measurement session a new MVC was performed, and force levels at 30, 50 and 80% of the maximal joint moment (calculated from the new MVC) were used. All sustained contractions were guided by visual feedback of the joint moment produced by the subject on a screen. The resultant ankle joint moment was calculated using inverse dynamics. Due to aligning the axis of rotation of the ankle in line with the force plate's center of rotation, the ankle joint moment could be considered equal to the moment of the force plate (Fig. 1A). Adjustment of moments due to gravitational and compression forces was carried out for all subjects before each plantarflexion contraction. However, it is important to note that the resultant ankle joint moment is an approximation of the moment produced by the *triceps surae*, as it does not account for the moment contributions of the other synergistic agonist muscles or the antagonist dorsiflexors. For the calculation of the AT force, the resultant ankle joint moment was divided by the tendon moment arm obtained from the literature (Maganaris et al. 1998). Prior to the measurements (except imPost-Run) the subject performed ten submaximal and three maximal cyclic loading contractions to precondition the tendon (Maganaris 2003). No tendon preconditioning was done immediately after the run (imPost-Run), since this was assumed to be unnecessary after a 21km run with a great number of mechanical loading cycles.

Elongation of the tendon during the loading phase was examined by visualizing the *gastrocnemius medialis* (GM) tendon using an ultrasound probe (sample frequency: 27Hz) fixed in the longitudinal direction at the myotendinous junction of the GM. After tendon preconditioning, the resting length of the AT was determined (individually for each measurement session) using a laser system linked to electronic potentiometers, as the distance from the AT insertion to the calcaneus (identified by palpation during the first measurement one day pre run) to the adhesive tape placed on the skin close to GM myotendinous junction (Fig. 1C). The GM resting length was obtained by correcting the measured distance with the gap between the myotendinous junction and the adhesive tape (registered via electronic potentiometers), which was later used for the registration of any motion of the probe relative to the skin during the contractions (Karamanidis and Arampatzis 2005).

The elongation of the tendon during MVC was determined by digitizing the myotendinous junction in custom-made MATLAB software (MATLAB 2013b, MathWorks Inc., Natick, Massachusetts, USA). This was conducted at rest and at the three sustained (held by the subjects for three seconds) contractions, which were collected at 27Hz after a steady state was achieved at the predetermined target force level (0%, 30%, 50%, 80%). All analogue signals and ultrasound images were synchronized using a TTL signal and an input signal for the ultrasound via the EKG channel, which was specifically constructed from the manufactory in Maastricht (ESAOTE, Maastricht, The Netherlands). In the presented study, a range of ±5% of the target force was accepted (when the force was held for 3 seconds). If this was not achieved, then the specific trial was repeated. The tendon elongation at maximal force (100%) was obtained via linear extrapolation of the values at 50 and 80% of the maximal force and the corresponding elongation of the tendon during the entire loading phase. This was determined by interpolating the data between two data points of the three sustained contractions (using linear fits between two points which surrounded the given tendon force level) for all subjects. The effect of potential ankle joint angular rotation on the measured elongation of the tendon during contraction was taken into account by using the product of the ankle joint angle changes and the Achilles tendon moment arm taken from Maganaris et al. (1998). In the current study we used a potentiometer located under the heel to measure any heel lift during contraction and, thereby, calculated the changes in the ankle joint angle from rest until maximal plantarflexion moment via the inverse tangent of the ratio of the heel lift to the distance between the head of the fifth metatarsal bone and the potentiometer (Fig. 1B; see also Online Resource 1). The tendon stiffness was calculated by the divided change in force by change in elongation from 30-80% of MVC. In order to account for the differences in force levels between the four measurements, the individual given tendon force at 30 and 80% of MVC was obtained from the lowest MVC from all measurements for each subject. These two values were used to calculate the stiffness for the three other measurement sessions at the corresponding force levels.

Analysis of stride and velocity characteristics during running

The stride number and frequency were assessed during running using a smartphone (Samsung Galaxy Nexus, Samsung Electronics, Seoul, South Korea) attached over the lower back at the level of the lower lumbar spine (L5) of the subjects using a semi-elastic waistband. A custom made data acquisition application was used to save the smartphones' GPS (sample rate 1Hz) and 3-axis accelerometer (± 16 g range, sample rate 100Hz) data, during the run. A MATLAB program processed the recorded data and initial foot contact was identified by the minimum of the signal obtained after applying a Gaussian continuous wavelet transformation to the vertical acceleration data (method adapted from McCamley et al. (2012)). Based on the above procedure we calculated for each individual the stride frequency and total amount of strides, as well as the average and maximal (calculated over an interval of 1km) running velocity during the half marathon exercise. The parameters were acquired in order to get an indication about individual running patterns and, hence, AT loading characteristics.

Statistics

In order to examine potential differences between age groups and between the different analyzed time points (1dayPre-Run, imPre-Run, imPost-Run and 1dayPost-Run) in the mechanical properties of the *triceps surae* MTU (tendon stiffness, tendon length changes at a given force level (every 500N until a maximum of 3000N; performed from linear fits), maximal tendon elongation and maximal ankle joint moment), a two-factor repeated measures analysis of variance (ANOVA), with time point and age (middle-aged *vs.* younger; independent variable) as factors was used. For those parameters where a significant time-point effect was found, the time points imPre-Run, imPost-Run and 1dayPost-Run were calculated as a ratio to time point 1dayPre-Run and were compared between age groups and time points by a two-way repeated measures ANOVA with age as an independent factor. This was done in order to examine whether the magnitude of relative changes (in relation to baseline - 1dayPre-Run) in the significant parameters were age-dependent. When a significant time point effect or age x time point interaction was detected, a Duncan post hoc test was performed in order to examine where exactly the differences occurred. Subjects' age, height, mass, running data (half marathon tested time and weekly training volume), running velocity (average and maximal velocity) and stride number and frequency were investigated using an independent t-test to test for differences between the middle-aged and younger groups. Simple linear regression was performed in order to examine the effect of running velocity and stride number and frequency on the ratio of the tendon stiffness imPost-Run to 1dayPre-run. In order to test the day-to-day reliability in muscle strength and tendon stiffness, we additionally calculated the Intraclass Correlation Coefficient (ICC) between 1dayPre-Run and imPre-Run. The level of significance was set at α = 0.05. All statistical analyses were performed using Statistica (Release 7.1; Statsoft, Tulsa, OK, USA). All results in the text, tables and figures are presented as mean and SD.

Results

Regarding the muscle strength, there were statistically significant ($P < 0.05$) age and time point effects on maximal isometric ankle plantarflexion joint moments, with lower values for the middle-aged compared to the younger runners (Table 1). The post hoc analysis showed significantly ($P < 0.05$) lower maximal ankle joint moments immediately after the run (imPost-Run) in comparison to all other analyzed time points, independent of age (old and young), with comparable values between 1dayPost-Run and both pre-run measurements (Table 1). Accordingly, there was a statistically significant ($P < 0.05$) time point effect on the ratios of the maximal ankle joint moments with no age effect or age x time point interaction (Fig. 2). The ratio imPost-Run to 1dayPre-run of the maximal ankle joint moments was significantly ($P < 0.05$) lower than all other ratios, independent of age (Fig. 2). The ratios between imPre-Run to 1dayPre-Run and between 1dayPost-Run to 1dayPre-run of the maximal ankle joint moments were not significantly different. Hence, the values of the maximal ankle joint moments of both age groups came back to baseline, and the *triceps surae* muscle strength was recovered, 20-28h after the run (Fig. 2).

Table 1

Fig. 2

A two way ANOVA revealed a significant ($P < 0.05$) age x time point interaction on tendon stiffness (Table 1 and Fig. 2). Similar to the muscle strength, the middle-aged runners showed significantly ($P < 0.05$) lower tendon stiffness values compared to the younger runners at all analyzed time points (Table 1). Immediately after the run (imPost-Run), tendon stiffness of the middle-aged runners was significantly ($P < 0.05$) lower compared to both pre-run measurements (1dayPre-Run and imPre-Run) and the measurement 20-28h after the run (Table 1, Fig. 3). In contrast to the middle-aged runners, the younger runners showed no statistically significant differences in tendon stiffness post running in comparison to all other analyzed time points (Table 1). A high day-to-day variability in the absolute stiffness values for the younger runners was seen in the pre-run measurements (difference of 129.3 $N \cdot mm^{-1}$ between 1dayPre-Run and imPre-Run), but the mean of the individual ratios of tendon stiffness for the pre-run measurements showed a value close to 1.0 for both age groups (stiffness ratio for the middle-aged: 1.04; stiffness ratio for the younger 1.01). Furthermore, the ratios of the tendon stiffness, showed a statistically significant ($P < 0.05$) age x time point interaction, with no changes in the ratio for the young (Fig. 2). The middle-aged runners, however, showed a significantly ($P < 0.05$) lower ratio imPost-Run to 1dayPre-run compared to all other values, including the ratio imPost-Run to 1dayPre-run of the younger runners (Fig. 2). As a consequence, a significant ($P < 0.05$) relative decrease in tendon stiffness post running was present for the middle-aged in comparison to the younger runners, but by the measurement 20-28h after the run, the decrease in tendon stiffness of the middle-aged runners dissipated (1dayPre-Run; Fig. 2). Concerning our day-to-day reliability analysis in MTU mechanical properties, we found ICC values of 0.96 and 0.64 for the maximal plantarflexion moment and tendon stiffness, respectively, between 1dayPre-Run and imPre-Run.

Fig. 3

Our comparison of the tendon elongation between groups revealed a significant ($P < 0.05$) time point effect on tendon elongation values for a given tendon force up to 2000 N (examined at intervals of 500 N, Table 2). Tendons of both age groups experienced more elongation after the run (imPost-Run) compared to before and returned to baseline 20-28h after the run (1dayPost-Run). The force level of 2000 N corresponds to 29% and 37% of MVC across the younger and middle-aged participants respectively. At the higher force levels (2500 and 3000 N) there was an age x time point interaction in tendon elongation (Table 2). The middle-aged runners experienced more tendon elongation immediately after the run compared to both pre measurements and 20-28h after the run (1dayPost-Run). There was no statistically significant effect on maximal tendon elongation during MVC or resting length (Table 1).

Table 2

Half marathon tested time of the middle-aged runners was, on average, 16% slower ($P < 0.05$) than the younger runners (old: 1.54 ± 0.12h; young: 1.38 ± 0.12h) and accordingly there was a significantly lower average running velocity for the middle-aged adults (old: 3.1 ± 0.3m·s^{-1}; young: 3.6 ± 0.5m·s^{-1}; Fig. 4). Furthermore, the middle-aged runners showed significantly ($P < 0.05$) higher total number of strides (old: 9977 ± 674 strides; young: 7890 ± 1100 strides), increased stride frequency (old: 1.44 ±

0.08 strides·s^{-1}; young: 1.39 ± 0.10 strides·s^{-1}) and lower maximal velocity (old: 3.6 ± 0.6 m·s^{-1}; young: 4.2 ± 0.7 m·s^{-1}; Fig. 4). However, no significant effect of running velocities and stride number and frequency on the ratio of tendon stiffness imPost-Run to 1dayPre-run was found (average running velocity: R^2 = 0.006, *P* = 0.60; maximal running velocity: R^2 = 0.020, *P* = 0.34; total number of strides: R^2 = 0.021, *P* = 0.33; stride frequency: R^2 = 0.038, *P* = 0.20; Fig. 5). The middle-aged runners had significantly (*P* < 0.05) longer training histories (6 years *vs.* 12 years), however no differences in current weekly training volume were found (middle-aged: 47 km per week *vs.* younger: 46 km per week).

Fig. 4

Fig. 5

Discussion

The main aim of this study was to examine whether there are different changes in the AT force-elongation relationship between young and middle-aged adult long distance runners following a half marathon run, that might indicate tendon fatigue is age-dependent. We hypothesized that the AT would exhibit greater mechanical changes (reduced tendon stiffness), which would be consistent with tendon fatigue, in the middle-aged runners following a half marathon run in comparison to the younger runners.

Our results partly support this hypothesis, showing an increased tendon length at a given force for the middle-aged runners immediately after the run. Furthermore, the middle-aged runners showed clear changes in AT stiffness after the run, with an average decrease in tendon stiffness of about 22%. In contrast to this, the younger runners did not show any changes in tendon stiffness, which is in line with previous reports showing no reduction in AT stiffness *in vivo* following a long distance run in young adults (Farris et al. 2012; Lichtwark et al. 2013; Peltonen et al. 2012). This was despite a comparable reduction in maximal force production capacity of the younger runners (on average 11% reduction) compared to the middle-aged runners (17% reduction). The younger runners showed a change in tendon length immediately after the run in the toe-region of the force-elongation relationship, up to a force level of 2000N, compared to the measurements immediately before the run and 20-28h after the run. Only some slight tendencies (0.09 < P < 0.12) for greater elongation values immediately after the run were found at forces between 500-1500N, when compared to the measurement 20-28h before the run. This change in elongation could be an indication of a creep response after the 21 km run, which would be in agreement with the studies of Lichtwark et al. (2013) and Obst et al. (2016). However, in the current study we exclude the fact that, in the toe-region, any physical damage to the tendon occurred which could be interpreted as tendon fatigue. This creep response was only visible up to 29% of the maximal force production capacity, and was thus not detected in the linear portion of the force-elongation relationship. Therefore, these changes did not influence the tendon stiffness calculated at the higher regions where we did find tendon fatigue for the middle-aged subjects. Thus, the above findings suggest that *in vivo* ATs of older individuals are less capable of resisting long-lasting cyclic mechanical loading, induced by long distance running, altering their tendon mechanical properties in comparison to younger runners.

In the literature, it is well established that during cyclic mechanical loading at a given stress, tendon strain continuously increases and tendon stiffness decreases, with a much faster respective increase and decrease just before rupture (Beaumont 1989; Fung et al. 2010; Wang et al. 1995). We suggest that in the current study, AT fatigue changes occurred in the middle-aged, but not in the younger runners. Even though, the current younger runners demonstrated a decrease in the maximal force production capacity after a half marathon run consisting of on average 7890 AT loading cycles on the examined leg (indicated by the total number of strides), no change in tendon stiffness was seen for the younger runners. The middle-aged runners completed the run by using a higher total number of strides and an increased stride frequency compared to the younger runners (old: 9977 ± 674 strides *vs.* young: 7890 ± 1100 strides; 1.44 ± 0.08 strides$\cdot s^{-1}$ *vs.* 1.39 ± 0.10 strides$\cdot s^{-1}$). Thus, one might argue that our observed age-related differences in the AT force-elongation relationship might be related to differences in the frequency and amount of cyclic tensile loading cycles on the tendon between age groups. However, we did not find a significant effect of stride number and frequency on the ratio of the tendon stiffness imPost-Run to 1dayPre-run (Fig. 5). Therefore, it appears that the current changes in tendon properties were related to internal changes within the tendon, rather than stride pattern differences between the age groups.

Although not statistically significant, we cannot exclude the possibility that our observed fatigue changes in tendon stiffness were partly related to slight difference in resting length post running (4.7

mm difference between imPre and imPost for the middle-aged runners). However, because the average reduction in tendon stiffness after the half marathon run in the middle-aged adults was reasonably high (on average 22%), this cannot be fully explained by potential changes in tendon resting length post running. Moreover, in order to account for a potential effect of the slight difference in resting length on AT mechanical properties, we additionally calculated the normalized tendon stiffness (examining of the slope of the force-strain, instead of the force-elongation relationship) and our main findings did not change. A possible explanation for the tendon fatigue found in the middle-aged, but not the young runners, may be alterations in viscoelastic and cellular properties in aging tendons, such as a decrease in cell density and altered cell shape (Lavagnino et al. 2013). These alterations have been shown to diminish the cellular contraction rate and thus, the ability of elongated tendons to re-establish normal tensional homeostasis (Lavagnino et al. 2013). Such age-related changes may be predisposing factors for overuse injuries of tendons (Kaux et al. 2011) and thus, might lead to tendon fatigue changes.

Despite the tendon stiffness of the middle-aged runners decreasing on average by 22% immediately after the run, the fatigue changes dissipated 20-28 hours after the exercise. The middle-aged runners showed a tendon stiffness ratio of 0.98 for the measurements 20-28h after the run to 20-28h before the run. In other words, the results of the current study show that the tendons of middle-aged adults are capable of recovering from acute changes in tendon length-tendon properties within 20 to 28 hours after a half marathon run. Due to this fast recovery, we suggest that the tendons of the middle-aged runners in the present study did not reach a critical level of AT fatigue at the micro or nano structure level. The recovery of our observed changes in the AT force-elongation relationship might be driven by cell-based tendon contraction and viscoelastic recovery, with expected short-term (minutes) and long-term (hours) components (Dahners et al. 1986; Gardner et al. 2012; Lavagnino et al. 2014). Although these recovery mechanisms are negatively affected by age (Bjornsson et al. 2011), they might have played a role in the fast recovery of the tendon within 24h. Future studies are needed to support the existence of these suggested changes and recovery mechanisms in tendons *in vivo*.

Regarding our method for assessing the AT mechanical properties *in vivo*, a number of points should be highlighted. One might argue that a 90 degree knee joint angle (instead of full extension), could lead to the GM tendon being tensed at a different starting length, leading to tendon slack (Reeves et al., 2005). However, this measurement error is likely to be small in our stiffness calculation, as we measure the slopes in the linear region (between 30-80% of maximal force) of the force-elongation relationship. Additionally, it could be argued that by using a 90 degree knee joint angle, the contribution of the GM muscle to the resultant ankle joint moment during MVC is reduced (Arampatzis et al. 2006), since the GM muscle operates on the ascending limb of the force-length relationship. This drawback may affect our generated tendon length-tension properties. However, it is important to note, that by using this configuration, we could more tightly fix the limbs in the dynamometer and reduce the soft tissue deformation, which decreases the inevitable ankle joint angular rotation during contraction in comparison to a fully extended knee joint (current study: less than 5 degrees ankle joint changes during MVC; extended knee: average changes of 14-18 degrees at the ankle joint; see e.g.: Arampatzis et al. 2005; Karamanidis et al. 2005). Accordingly, we found similar maximal tendon strain values (around 4.5% at baseline for the young adults), measured at the GM myotendinous junction during MVC, compared to reported GM tendon strain values from fully extended knee joints (Karamanidis and Aramptatis 2005; Mademli and Arampatzis 2008; Mademli et al. 2006; Stenroth et al. 2012). Further, it is mandatory to address the fact that, in the current measurement setup, we estimated the AT force, but examined tendon displacement via ultrasound at the myotendinous junction of the GM, instead of the free AT, affecting our tendon stiffness values in absolute terms. This drawback, and the fact that different parts of the tendon and/or aponeurosis show different mechanical properties are, potentially, the reasons that the strain measured at the free AT is approximately two to three times higher compared to the *gastrocnemii* tendon, at given ankle joint torque or AT force levels (Farris et al., 2013; Lichtwark et al., 2013; Obst et al. 2016). Therefore, the *triceps surae* tendon and aponeurosis cannot be considered a single tendon that extends uniformly during plantarflexor contraction. However, when we assessed tendon stiffness in a more dorsiflexed position (ankle joint angle of 85 degrees) and thereby increased the force potential of the plantarflexor muscles due to a rightwards shift in the force-length relationship of the contractile element of the *gastrocnemii* (and *soleus* muscle), we found no significant differences in tendon stiffness between the two different ankle joint angles (see additional data in Online Resource 1). In addition, it has to be pointed out that while analyzing the more distal myotendinous junction of the *soleus* by ultrasound seems to be possible in most young adults, it is particularly problematic and in some cases even impossible to detect and analyze the displacement of myotendinous junction of the s*oleus* in many middle-aged or older individuals during contraction.

However, in order to actually examine if different heads of the *triceps surae* show differences in elongation after a half marathon run between age groups, future studies are needed, possibly examining the influence of a fatiguing protocol on the free AT in middle-aged or older adults. By using the current inverse dynamics approach to calculate AT forces during plantarflexion contraction, we were not able to consider muscle activation and, therefore, we could not account for the contribution of all the other co-activating ankle plantarflexors and antagonist dorsiflexors in our joint kinetic analysis. It is presently difficult to accurately assess *in vivo* tendon force noninvasively, and without concomitant estimation of *in vivo* tendon force we can only speculate on the significance of the observed age-related findings in tendon mechanical properties. However, we believe that this drawback potentially affects our results in absolute terms more than the validity of the corresponding comparative data. Finally, the AT moment arms were taken from the literature (Maganaris et al. 1998), which might lead to erroneous results in the individual calculation in tendon force and hence tendon stiffness. While this drawback could potentially influence our comparison between middle-aged and younger runners, the main focus was the intra individual comparison before and after the run and this may not significantly affect the main findings of the current study.

In conclusion, our results suggest that the ATs of middle-aged adult long distance runners are less capable of resisting prolonged cyclic mechanical loading, significantly altering their tendon force-elongation relationship (increase of tendon elongation and decrease in AT stiffness). However, the fatigue changes observed in the tendon of the middle-aged adult long distance runners in this study dissipated within 20-28h, demonstrating the ability of the human tendon *in vivo* to rapidly recover, which might indicate the existence of both a short term viscoelastic recovery mechanism and a cell mediated recovery mechanism in tendons *in vivo*. Further investigations are necessary to examine the plasticity of human AT in response to short-term mechanical loading in the elderly and which recovery mechanisms are involved in this relatively fast dissipation of the observed fatigue changes of the tendon. Such knowledge may, in the future, contribute to the reduction of AT injuries, especially in middle-aged to older age groups.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Tables

Table 1 Mean (± SD) *triceps surae* muscle-tendon unit mechanical properties assessed at all time points for the middle-aged and younger adult long distance runners.

	Middle-aged (n = 27)				Young (n = 22)			
	1dayPre Run	imPre Run	imPost Run	1dayPost Run	1dayPre Run	imPre Run	imPost Run	1dayPost Run
Joint Moment $_{max}$ ($N \cdot m \cdot kg^{-1}$)[*]	2.9 (0.6)	2.9 (0.5)	2.4[1,2,4] (0.7)	2.8 (0.6)	3.5 (0.6)	3.6 (0.5)	3.1[1,2,4] (0.6)	3.5 (0.6)
Resting length (mm)	235.0 (33.1)	235.3 (29.0)	230.6 (32.7)	236.0 (27.1)	223.2 (21.3)	224.2 (21.7)	228.2 (22.7)	224.5 (19.8)
Tendon Elongation$_{max}$ (mm)	8.9 (1.9)	9.2 (1.9)	9.0 (2.2)	8.9 (1.9)	10.1 (2.2)	9.9 (1.9)	9.8 (2.0)	10.0 (2.1)
Tendon Stiffness ($N \cdot mm^{-1}$)[*]	983.1 (233.1)	978.6 (310.6)	769.0[1,2,4] (298.0)	940.6 (284.1)	1390.4 (597.2)	1261.1 (541.6)	1171.8 (375.6)	1331.2 (475.9)

1dayPreRun, 20-28h before the half marathon run; imPreRun, immediately before the half marathon run; imPostRun, immediately after the half marathon run; 1dayPostRun, 20-28h after the half marathon run

[*]: Significant age effect ($P < 0.05$)

[1]: Significant difference to 1dayPre-Run ($P < 0.05$)

[2]: Significant difference to imPre-Run ($P < 0.05$)

[4]: Significant difference to 1dayPost-Run ($P < 0.05$)

Table 2 Mean (± SD) tendon elongation values assessed at different force levels during the loading phase at all time points for the middle-aged and younger adult long distance runners

	Middle-aged (n = 27)				Young (n = 22)			
Tendon force (N)	1dayPre Run	imPre Run	imPost Run	1dayPost Run	1dayPre Run	imPre Run	imPost Run	1dayPost Run
500	1.7	1.7	1.9[1,2,4]	1.6	1.7	1.6	1.9[2,4]	1.6
	(0.54)	(0.50)	(0.57)	(0.47)	(0.42)	(0.47)	(0.50)	(0.34)
1000	3.4	3.4	3.8[1,2,4]	3.3	3.4	3.2	3.7[2.4]	3.2
	(1.07)	(0.99)	(1.10)	(0.93)	(0.84)	(0.95)	(1.00)	(0.67)
1500	4.9	5.0	5.3[1,2,4]	4.8	5.1	4.7	5.5[2,4]	4.8
	(1.35)	(1.44)	(1.35)	(1.20)	(1.26)	(1.40)	(1.41)	(0.97)
2000	6.0	6.1	6.5[1,2,4]	5.9	6.3	6.0	6.8[2,4]	6.1
	(1.42)	(1.62)	(1.52)	(1.30)	(1.47)	(1.59)	(1.56)	(1.22)
2500	6.7	6.8	7.2[1,2,4]	6.5	7.2	6.8	7.5	7.0
	(1.34)	(1.60)	(1.59)	(1.27)	(1.70)	(1.61)	(1.53)	(1.46)
3000	7.3	7.4	7.8[1,2,4]	7.1	7.8	7.5	8.0	7.6
	(1.50)	(1.66)	(1.65)	(1.43)	(1.82)	(1.59)	(1.57)	(1.54)

1dayPreRun: 20-28h before the half marathon run; imPreRun: immediately before the half marathon run; imPostRun: immediately after the half marathon run; 1dayPostRun: 20-28h after the half marathon run

[1]: Significant difference to 1dayPre-Run ($P < 0.05$)

[2]: Significant difference to imPre-Run ($P < 0.05$)

[4]: Significant difference to 1dayPost-Run ($P < 0.05$)

Figure Captions

Fig. 1 Diagram of the used custom made strain gauge type dynamometer. The subjects were seated with their lower leg secured and their foot on the dynamometer, with the ankle and knee joints positioned at 90 degrees (see Fig. 1C). A laser guided potentiometer system was used to position the midpoint of the *malleolus lateralis* in line with the force plate's center of rotation and to calculate the tendon's resting length (ATresting length) between the most proximal point of the tuber calcanei and an adhesive tape placed on the skin close to GM myotendinous junction. The position of the seat relative to the dynamometer (horizontal and vertical position) was recorded by two additional potentiometers. The axis of rotation of the ankle was aligned with the force plate's center of rotation, therefore the ankle joint moment (MAnkle) was equal to the moment of the force plate (MPlate), which is the product of FPlate (resultant force of the force plate) and rPlate (moment arm of the force plate; see Fig. 1A). The ankle joint changes ($\Delta\alpha$) during the contractions were calculated via the inverse tangent of the ratio of the heel lift (calculated by potentiometer POTHeel) to the distance between MET5 (position of the head of fifth metatarsal bone) and POTHeel (see Fig. 1B)

Fig 2 Mean (and SD) ratio (the time points imPre-Run, imPost-Run and 1dayPost-Run were calculated as a ratio to time point 1dayPre-Run) of the muscle strength (Joint Moment_{max}) and tendon stiffness, calculated for the different time points to the baseline measurement for the middle-aged and younger adult long distance runners. (Rpre-r: ratio immediately before the run to 20-28h before the run; Rpost-r1: ratio immediately after the run to 20-28h before the run; Rpost-r2: ration 20-28h after the run to 20-28h before the run

$^{\#}$: Significant different to all other ratios for young and old ($P < 0.05$)

^{1}: Significant different to Rpre-r ($P < 0.05$)

^{3}: Significant different to Rpost-r2 ($P < 0.05$)

Fig 3 Force-elongation relationship (mean and SD) of the tendon for the different time points for the middle-aged (A) and younger (B) adult long distance runners. (1dayPre-run: 20-28h before the run; imPre-Run: immediately before the run; imPost-Run: immediately after the run; 1dayPost-Run: 20-28h after the run)

Fig 4 Remodelled half marathon running track and normalized running velocity examined in 1 km intervals (running velocity was normalized to the fastest interval for each subject independently) for an middle-aged (A) and younger (B) adult long distance runner

Fig 5 Relationship between total number of strides and average stride frequency during the half marathon run and the ratio of tendon stiffness immediately after the run to 20-28h before the run for the middle-aged (A) and younger (B) adult long distance runners. No significant effect of stride number and frequency on tendon stiffness ratio was found ($R^2 \leq 0.038$, $P \geq 0.20$)