

30 **Abstract**

31 **Purpose:** We aimed to determine whether there are different changes in Achilles tendon (AT)
32 mechanical properties in middle-aged, compared to younger runners that might indicate that tendon
33 fatigue, induced by long distance running, is age-dependent. **Methods:** 27 middle-aged (50-67 years)
34 and 22 younger (21-29 years) participants ran a 21km route at their own pace (mean and SD: old:
35 $3.1\pm 0.3\text{m}\cdot\text{s}^{-1}$; young: $3.6\pm 0.5\text{m}\cdot\text{s}^{-1}$). We tested for changes in the AT force-elongation relationship
36 using dynamometry and ultrasonography during isometric voluntary ankle plantarflexion ramp
37 contractions, conducted 20-28h pre-run, immediately pre-run, immediately post-run and 20-28h post-
38 run. Stride frequency and number were examined to estimate cyclic tensile loading characteristics of
39 the tendon during running. **Results:** Muscle strength decreased significantly ($P<0.05$) in both groups
40 immediately post-run (old: 17%; young: 11%) and recovered to baseline within 20-28h post-run. AT
41 stiffness did not change for the younger adults, whereas the middle-aged adults showed a significant
42 ($P<0.05$) decrease in AT stiffness (22%). However, tendon stiffness recovered to baseline 20-28h post-
43 run. Middle-aged, compared to young adults demonstrated significantly ($P<0.05$) greater stride
44 frequency and number, but no correlations with tendon fatigue changes were determined ($R^2\leq 0.038$).
45 **Conclusions:** The results suggest that the plasticity of the AT in response to short-term mechanical
46 loading may be age-dependent and that the AT length–tension properties of middle-aged runners may
47 be more vulnerable to change following running compared to younger athletes. However, the observed
48 AT changes in the middle-aged runners dissipated within 20-28h post-run, suggesting that a tendon
49 viscoelastic recovery mechanism may occur *in vivo*.

50

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

52

53 **Abbreviations**

54	1dayPre-Run	20 to 28h before the run
55	1dayPost-Run	Within 20 to 28h after the run
56	AT	Achilles tendon
57	ANOVA	Analysis of variance
58	CSA	Cross sectional area
59	GM	Gastrocnemius medialis

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

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90 **Introduction**

91 Tendons play a crucial role in movement by transmitting muscle forces to the skeleton and therefore,
92 changes in human tendon biomechanical properties could affect movement effectiveness (Ker et al.
93 1987; Biewener and Roberts 1989; Karamanidis and Arampatzis 2005; Lichtwark et al. 2007) and
94 injury risk (Lavagnino et al. 2014). This may be of particular concern for older populations, who
95 experience age-related changes in tendon composition that may affect the mechanical properties of the
96 tendon which could eventually reduce the tendon's tolerance of mechanical loading.

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98 Despite a lack of clarity in the literature, most *in vitro* studies in humans point towards the fact that
99 aging can be associated with decreased tensile stiffness and ultimate failure strength of tendons and
100 collagenous tissues (Noyes and Grood 1976; Tkaczuk 1968 Viidik 1982). In a more recent study by
101 Lavagnino et al. (2014), using both *in vitro* animal and *in vivo* human experiments, it was shown that
102 tendon composition and material properties diminish the ability of elongated tendons to re-establish
103 normal tension homeostasis, making an older tendon more prone to overuse injuries after repetitive
104 cyclic loading exercise (Lavagnino et al. 2014). This may be one explanation that older age has been
105 identified as a predisposing factor for both overuse injuries (Huttunen et al. 2014; Jozsa et al.1989;
106 Kvist 1994), as well as longer recovery times following tendon repair procedures (Lavagnino et al.
107 2013; Lavagnino et al. 2014). Specifically, Achilles tendon (AT) injuries appear to be age related, as
108 the incidence rate of AT injury in men over 40 years is almost twice as high as in men between 18-39
109 years of age (Huttunen et al. 2014). An additional risk factor for AT injury appear to be activities with
110 repetitive cyclic mechanical loading, like long distance running, as runners are one of the most
111 susceptible populations to chronic AT injuries and AT ruptures (Brunet et al. 1990; Clement et al.
112 1984). During running, the AT has to cope with multiple tensile strain cycles, consisting of high strain
113 rates and magnitudes (e.g. AT strain magnitude up to 5.5%; Lichtwark et al. 2007). Such loading
114 profiles can lead to the tendon becoming more compliant and more susceptible to large strains, which
115 can ultimately lead to tendon failure (Ker et al. 2000; Wang et al. 1995). These changes in the tendon
116 force-elongation relationship are suggested to be caused by a disequilibrium between the rate of
117 damage and the rate of repair (Ker et al., 2000), due to significant changes in expression of collagens
118 (down regulation of Col I and up regulation of Col III and Col V), fiber angulation (increase in density
119 of the fibers) and delamination among adjacent fibers (separation of the fibers into layers; Fung et al.,

120 2010). Such changes in the tendon force-elongation relationship, morphology and molecular profile
121 due to cyclic mechanical loading are collectively termed tendon fatigue (Wang et al., 1995; Fung et al.,
122 2010; Ker et al. 2000) and the response of tendons to *in vitro* and *in vivo* cyclic mechanical loading has
123 been well established in animal models (Fung et al., 2010; Lavagnino et al. 2014; Wang et al. 1995).
124 Improved understanding of potential age-related differences in the response of the AT to long distance
125 running might be important for reducing the risk of AT injuries in middle and older aged runners.

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127 In previous *in vivo* human studies, various dynamic activities (such as heel drop exercise, running or
128 hopping) have been used to examine whether changes in tendon mechanical properties occurred, that
129 might be indicative of tendon fatigue, following repetitive cyclic loading (Farris et al. 2012; Lichtwark
130 et al. 2013; Obst et al. 2016; Peltonen et al. 2010; Peltonen et al. 2012). However, no significant
131 change in the AT force-elongation relationship *in vivo* has been found in three (Farris et al. 2012;
132 Peltonen et al. 2010; Peltonen et al. 2012) of these five studies. The studies of Lichtwark et al. (2013)
133 and Obst et al. (2016) did find an increase in strain at a given force, which could be indicative of early
134 stage mechanical fatigue. However, these effects were much smaller than those predicted by both *ex*
135 *vivo* and *in vivo* animal experiments (Fung et al. 2010; Lavagnino et al. 2014; Wang et al. 1995). This
136 may have been due to the methodology or the nature of the loading profiles used in these studies.
137 Crucially, these studies were conducted with predominantly young subjects (mean age under 30 years;
138 Farris et al. 2012; Lichtwark et al. 2013; Peltonen et al. 2010). As a consequence, there is limited
139 evidence for how the human AT in middle-aged to older runners (50 years and older) responds to a
140 long lasting cyclic loading exercise such as long distance running. To our knowledge, one study
141 (Mademli and Arampatzis 2008) investigated the AT fatigue response in older adults *in vivo* during
142 cyclic submaximal isometric voluntary contractions on a dynamometer, but failed to find any
143 significant age-related fatigue effects on the mechanical properties of the AT. However, the contraction
144 protocol used may not have simulated the typical mechanical loading profile of a long distance run.
145 This evidence gap is significant because older aged runners are at a higher risk for AT injury (Kvist
146 1994) and the finding that aging diminishes tendon fatigue quality during extended repetitive cyclic
147 loading in *in vitro* and *in vivo* animal models (Lavagnino et al. 2014).

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

160

161 **Methods**

162 *Subjects*

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

176

177 *Experimental design*

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

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193 *Analysis of the tendon force-elongation relationship and muscle strength*

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

205 *Fig. 1*

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

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

237 The elongation of the tendon during MVC was determined by digitizing the myotendinous junction in
238 custom-made MATLAB software (MATLAB 2013b, MathWorks Inc., Natick, Massachusetts, USA).
239 This was conducted at rest and at the three sustained (held by the subjects for three seconds)
240 contractions, which were collected at 27Hz after a steady state was achieved at the predetermined
241 target force level (0%, 30%, 50%, 80%). All analogue signals and ultrasound images were
242 synchronized using a TTL signal and an input signal for the ultrasound via the EKG channel, which
243 was specifically constructed from the manufactory in Maastricht (ESAOTE, Maastricht, The
244 Netherlands). In the presented study, a range of $\pm 5\%$ of the target force was accepted (when the force
245 was held for 3 seconds). If this was not achieved, then the specific trial was repeated. The tendon
246 elongation at maximal force (100%) was obtained via linear extrapolation of the values at 50 and 80%
247 of the maximal force and the corresponding elongation of the tendon during the entire loading phase.
248 This was determined by interpolating the data between two data points of the three sustained
249 contractions (using linear fits between two points which surrounded the given tendon force level) for
250 all subjects. The effect of potential ankle joint angular rotation on the measured elongation of the
251 tendon during contraction was taken into account by using the product of the ankle joint angle changes
252 and the Achilles tendon moment arm taken from Maganaris et al. (1998). In the current study we used a
253 potentiometer located under the heel to measure any heel lift during contraction and, thereby,
254 calculated the changes in the ankle joint angle from rest until maximal plantarflexion moment via the
255 inverse tangent of the ratio of the heel lift to the distance between the head of the fifth metatarsal bone
256 and the potentiometer (Fig. 1B; see also Online Resource 1). The tendon stiffness was calculated by the
257 divided change in force by change in elongation from 30-80% of MVC. In order to account for the
258 differences in force levels between the four measurements, the individual given tendon force at 30 and
259 80% of MVC was obtained from the lowest MVC from all measurements for each subject. These two
260 values were used to calculate the stiffness for the three other measurement sessions at the
261 corresponding force levels.

262

263 *Analysis of stride and velocity characteristics during running*

264 The stride number and frequency were assessed during running using a smartphone (Samsung Galaxy
265 Nexus, Samsung Electronics, Seoul, South Korea) attached over the lower back at the level of the
266 lower lumbar spine (L5) of the subjects using a semi-elastic waistband. A custom made data

267 acquisition application was used to save the smartphones' GPS (sample rate 1Hz) and 3-axis
268 accelerometer (± 16 g range, sample rate 100Hz) data, during the run. A MATLAB program processed
269 the recorded data and initial foot contact was identified by the minimum of the signal obtained after
270 applying a Gaussian continuous wavelet transformation to the vertical acceleration data (method
271 adapted from McCamley et al. (2012)). Based on the above procedure we calculated for each individual
272 the stride frequency and total amount of strides, as well as the average and maximal (calculated over an
273 interval of 1km) running velocity during the half marathon exercise. The parameters were acquired in
274 order to get an indication about individual running patterns and, hence, AT loading characteristics.

275

276 *Statistics*

277 In order to examine potential differences between age groups and between the different analyzed time
278 points (1dayPre-Run, imPre-Run, imPost-Run and 1dayPost-Run) in the mechanical properties of the
279 *triceps surae* MTU (tendon stiffness, tendon length changes at a given force level (every 500N until a
280 maximum of 3000N; performed from linear fits), maximal tendon elongation and maximal ankle joint
281 moment), a two-factor repeated measures analysis of variance (ANOVA), with time point and age
282 (middle-aged vs. younger; independent variable) as factors was used. For those parameters where a
283 significant time-point effect was found, the time points imPre-Run, imPost-Run and 1dayPost-Run
284 were calculated as a ratio to time point 1dayPre-Run and were compared between age groups and time
285 points by a two-way repeated measures ANOVA with age as an independent factor. This was done in
286 order to examine whether the magnitude of relative changes (in relation to baseline - 1dayPre-Run) in
287 the significant parameters were age-dependent. When a significant time point effect or age x time point
288 interaction was detected, a Duncan post hoc test was performed in order to examine where exactly the
289 differences occurred. Subjects' age, height, mass, running data (half marathon tested time and weekly
290 training volume), running velocity (average and maximal velocity) and stride number and frequency
291 were investigated using an independent t-test to test for differences between the middle-aged and
292 younger groups. Simple linear regression was performed in order to examine the effect of running
293 velocity and stride number and frequency on the ratio of the tendon stiffness imPost-Run to 1dayPre-
294 run. In order to test the day-to-day reliability in muscle strength and tendon stiffness, we additionally
295 calculated the Intraclass Correlation Coefficient (ICC) between 1dayPre-Run and imPre-Run. The level

296 of significance was set at $\alpha = 0.05$. All statistical analyses were performed using Statistica (Release 7.1;
297 Statsoft, Tulsa, OK, USA). All results in the text, tables and figures are presented as mean and SD.

298

299 **Results**

300 Regarding the muscle strength, there were statistically significant ($P < 0.05$) age and time point effects
301 on maximal isometric ankle plantarflexion joint moments, with lower values for the middle-aged
302 compared to the younger runners (Table 1). The post hoc analysis showed significantly ($P < 0.05$)
303 lower maximal ankle joint moments immediately after the run (imPost-Run) in comparison to all other
304 analyzed time points, independent of age (old and young), with comparable values between 1dayPost-
305 Run and both pre-run measurements (Table 1). Accordingly, there was a statistically significant ($P <$
306 0.05) time point effect on the ratios of the maximal ankle joint moments with no age effect or age x
307 time point interaction (Fig. 2). The ratio imPost-Run to 1dayPre-run of the maximal ankle joint
308 moments was significantly ($P < 0.05$) lower than all other ratios, independent of age (Fig. 2). The ratios
309 between imPre-Run to 1dayPre-Run and between 1dayPost-Run to 1dayPre-run of the maximal ankle
310 joint moments were not significantly different. Hence, the values of the maximal ankle joint moments
311 of both age groups came back to baseline, and the *triceps surae* muscle strength was recovered, 20-28h
312 after the run (Fig. 2).

313 *Table 1*

314 *Fig. 2*

315

316 A two way ANOVA revealed a significant ($P < 0.05$) age x time point interaction on tendon stiffness
317 (Table 1 and Fig. 2). Similar to the muscle strength, the middle-aged runners showed significantly ($P <$
318 0.05) lower tendon stiffness values compared to the younger runners at all analyzed time points (Table
319 1). Immediately after the run (imPost-Run), tendon stiffness of the middle-aged runners was
320 significantly ($P < 0.05$) lower compared to both pre-run measurements (1dayPre-Run and imPre-Run)
321 and the measurement 20-28h after the run (Table 1, Fig. 3). In contrast to the middle-aged runners, the
322 younger runners showed no statistically significant differences in tendon stiffness post running in
323 comparison to all other analyzed time points (Table 1). A high day-to-day variability in the absolute
324 stiffness values for the younger runners was seen in the pre-run measurements (difference of 129.3
325 $\text{N}\cdot\text{mm}^{-1}$ between 1dayPre-Run and imPre-Run), but the mean of the individual ratios of tendon

326 stiffness for the pre-run measurements showed a value close to 1.0 for both age groups (stiffness ratio
327 for the middle-aged: 1.04; stiffness ratio for the younger 1.01). Furthermore, the ratios of the tendon
328 stiffness, showed a statistically significant ($P < 0.05$) age x time point interaction, with no changes in
329 the ratio for the young (Fig. 2). The middle-aged runners, however, showed a significantly ($P < 0.05$)
330 lower ratio imPost-Run to 1dayPre-run compared to all other values, including the ratio imPost-Run to
331 1dayPre-run of the younger runners (Fig. 2). As a consequence, a significant ($P < 0.05$) relative
332 decrease in tendon stiffness post running was present for the middle-aged in comparison to the younger
333 runners, but by the measurement 20-28h after the run, the decrease in tendon stiffness of the middle-
334 aged runners dissipated (1dayPre-Run; Fig. 2). Concerning our day-to-day reliability analysis in MTU
335 mechanical properties, we found ICC values of 0.96 and 0.64 for the maximal plantarflexion moment
336 and tendon stiffness, respectively, between 1dayPre-Run and imPre-Run.

337 *Fig. 3*

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339 Our comparison of the tendon elongation between groups revealed a significant ($P < 0.05$) time point
340 effect on tendon elongation values for a given tendon force up to 2000 N (examined at intervals of 500
341 N, Table 2). Tendons of both age groups experienced more elongation after the run (imPost-Run)
342 compared to before and returned to baseline 20-28h after the run (1dayPost-Run). The force level of
343 2000 N corresponds to 29% and 37% of MVC across the younger and middle-aged participants
344 respectively. At the higher force levels (2500 and 3000 N) there was an age x time point interaction in
345 tendon elongation (Table 2). The middle-aged runners experienced more tendon elongation
346 immediately after the run compared to both pre measurements and 20-28h after the run (1dayPost-Run).
347 There was no statistically significant effect on maximal tendon elongation during MVC or resting
348 length (Table 1).

349 *Table 2*

350

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

356 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:
357 4.2 ± 0.7 m·s⁻¹; Fig. 4). However, no significant effect of running velocities and stride number and
358 frequency on the ratio of tendon stiffness imPost-Run to 1dayPre-run was found (average running
359 velocity: R² = 0.006, P = 0.60; maximal running velocity: R² = 0.020, P = 0.34; total number of strides:
360 R² = 0.021, P = 0.33; stride frequency: R² = 0.038, P = 0.20; Fig. 5). The middle-aged runners had
361 significantly (P < 0.05) longer training histories (6 years vs. 12 years), however no differences in
362 current weekly training volume were found (middle-aged: 47 km per week vs. younger: 46 km per
363 week).

364 *Fig. 4*

365 *Fig. 5*

366

367 **Discussion**

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

373

374 Our results partly support this hypothesis, showing an increased tendon length at a given force for the
375 middle-aged runners immediately after the run. Furthermore, the middle-aged runners showed clear
376 changes in AT stiffness after the run, with an average decrease in tendon stiffness of about 22%. In
377 contrast to this, the younger runners did not show any changes in tendon stiffness, which is in line with
378 previous reports showing no reduction in AT stiffness *in vivo* following a long distance run in young
379 adults (Farris et al. 2012; Lichtwark et al. 2013; Peltonen et al. 2012). This was despite a comparable
380 reduction in maximal force production capacity of the younger runners (on average 11% reduction)
381 compared to the middle-aged runners (17% reduction). The younger runners showed a change in
382 tendon length immediately after the run in the toe-region of the force-elongation relationship, up to a
383 force level of 2000N, compared to the measurements immediately before the run and 20-28h after the
384 run. Only some slight tendencies (0.09 < P < 0.12) for greater elongation values immediately after the
385 run were found at forces between 500-1500N, when compared to the measurement 20-28h before the

386 run. This change in elongation could be an indication of a creep response after the 21 km run, which
387 would be in agreement with the studies of Lichtwark et al. (2013) and Obst et al. (2016). However, in
388 the current study we exclude the fact that, in the toe-region, any physical damage to the tendon
389 occurred which could be interpreted as tendon fatigue. This creep response was only visible up to 29%
390 of the maximal force production capacity, and was thus not detected in the linear portion of the force-
391 elongation relationship. Therefore, these changes did not influence the tendon stiffness calculated at the
392 higher regions where we did find tendon fatigue for the middle-aged subjects. Thus, the above findings
393 suggest that *in vivo* ATs of older individuals are less capable of resisting long-lasting cyclic mechanical
394 loading, induced by long distance running, altering their tendon mechanical properties in comparison to
395 younger runners.

396

397 In the literature, it is well established that during cyclic mechanical loading at a given stress, tendon
398 strain continuously increases and tendon stiffness decreases, with a much faster respective increase and
399 decrease just before rupture (Beaumont 1989; Fung et al. 2010; Wang et al. 1995). We suggest that in
400 the current study, AT fatigue changes occurred in the middle-aged, but not in the younger runners.
401 Even though, the current younger runners demonstrated a decrease in the maximal force production
402 capacity after a half marathon run consisting of on average 7890 AT loading cycles on the examined
403 leg (indicated by the total number of strides), no change in tendon stiffness was seen for the younger
404 runners. The middle-aged runners completed the run by using a higher total number of strides and an
405 increased stride frequency compared to the younger runners (old: 9977 ± 674 strides *vs.* young: $7890 \pm$
406 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
407 age-related differences in the AT force-elongation relationship might be related to differences in the
408 frequency and amount of cyclic tensile loading cycles on the tendon between age groups. However, we
409 did not find a significant effect of stride number and frequency on the ratio of the tendon stiffness
410 imPost-Run to 1dayPre-run (Fig. 5). Therefore, it appears that the current changes in tendon properties
411 were related to internal changes within the tendon, rather than stride pattern differences between the
412 age groups.

413

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

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

428

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

442

443 Regarding our method for assessing the AT mechanical properties *in vivo*, a number of points should
444 be highlighted. One might argue that a 90 degree knee joint angle (instead of full extension), could lead
445 to the GM tendon being tensed at a different starting length, leading to tendon slack (Reeves et al.,

446 2005). However, this measurement error is likely to be small in our stiffness calculation, as we measure
447 the slopes in the linear region (between 30-80% of maximal force) of the force-elongation relationship.
448 Additionally, it could be argued that by using a 90 degree knee joint angle, the contribution of the GM
449 muscle to the resultant ankle joint moment during MVC is reduced (Arampatzis et al. 2006), since the
450 GM muscle operates on the ascending limb of the force-length relationship. This drawback may affect
451 our generated tendon length-tension properties. However, it is important to note, that by using this
452 configuration, we could more tightly fix the limbs in the dynamometer and reduce the soft tissue
453 deformation, which decreases the inevitable ankle joint angular rotation during contraction in
454 comparison to a fully extended knee joint (current study: less than 5 degrees ankle joint changes during
455 MVC; extended knee: average changes of 14-18 degrees at the ankle joint; see e.g.: Arampatzis et al.
456 2005; Karamanidis et al. 2005). Accordingly, we found similar maximal tendon strain values (around
457 4.5% at baseline for the young adults), measured at the GM myotendinous junction during MVC,
458 compared to reported GM tendon strain values from fully extended knee joints (Karamanidis and
459 Arampatzis 2005; Mademli and Arampatzis 2008; Mademli et al. 2006; Stenroth et al. 2012). Further, it
460 is mandatory to address the fact that, in the current measurement setup, we estimated the AT force, but
461 examined tendon displacement via ultrasound at the myotendinous junction of the GM, instead of the
462 free AT, affecting our tendon stiffness values in absolute terms. This drawback, and the fact that
463 different parts of the tendon and/or aponeurosis show different mechanical properties are, potentially,
464 the reasons that the strain measured at the free AT is approximately two to three times higher compared
465 to the *gastrocnemii* tendon, at given ankle joint torque or AT force levels (Farris et al., 2013; Lichtwark
466 et al., 2013; Obst et al. 2016). Therefore, the *triceps surae* tendon and aponeurosis cannot be
467 considered a single tendon that extends uniformly during plantarflexor contraction. However, when we
468 assessed tendon stiffness in a more dorsiflexed position (ankle joint angle of 85 degrees) and thereby
469 increased the force potential of the plantarflexor muscles due to a rightwards shift in the force-length
470 relationship of the contractile element of the *gastrocnemii* (and *soleus* muscle), we found no significant
471 differences in tendon stiffness between the two different ankle joint angles (see additional data in
472 Online Resource 1). In addition, it has to be pointed out that while analyzing the more distal
473 myotendinous junction of the *soleus* by ultrasound seems to be possible in most young adults, it is
474 particularly problematic and in some cases even impossible to detect and analyze the displacement of
475 myotendinous junction of the *soleus* in many middle-aged or older individuals during contraction.

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

491

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

503

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509

510 **Conflict of interest**

511 The authors declare that they have no conflict of interest.

512

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622	Tables

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624 **Table 1** Mean (\pm SD) *triceps surae* muscle-tendon unit mechanical properties assessed at all time

625 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·m·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·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)

626

627 1dayPreRun, 20-28h before the half marathon run; imPreRun, immediately before the half marathon

628 run; imPostRun, immediately after the half marathon run; 1dayPostRun, 20-28h after the half marathon

629 run

630 *: Significant age effect ($P < 0.05$)

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

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

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

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

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

643

644 1dayPreRun: 20-28h before the half marathon run; imPreRun: immediately before the half marathon
 645 run; imPostRun: immediately after the half marathon run; 1dayPostRun: 20-28h after the half marathon
 646 run

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

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

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

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659 **Figure Captions**

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

673

674 **Fig 2** Mean (and SD) ratio (the time points imPre-Run, imPost-Run and 1dayPost-Run were calculated
675 as a ratio to time point 1dayPre-Run) of the muscle strength ($\text{Joint Moment}_{\text{max}}$) and tendon stiffness,
676 calculated for the different time points to the baseline measurement for the middle-aged and younger
677 adult long distance runners. (Rpre-r: ratio immediately before the run to 20-28h before the run; Rpost-
678 r1: ratio immediately after the run to 20-28h before the run; Rpost-r2: ration 20-28h after the run to 20-
679 28h before the run

680 #: Significant different to all other ratios for young and old ($P < 0.05$)

681 ¹: Significant different to Rpre-r ($P < 0.05$)

682 ³: Significant different to Rpost-r2 ($P < 0.05$)

683

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

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

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

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