

JYX



JYVÄSKYLÄN YLIOPISTO  
UNIVERSITY OF JYVÄSKYLÄ

**This is a self-archived version of an original article. This version may differ from the original in pagination and typographic details.**

**Author(s):** Werkhausen, Amelie; Cronin, Neil; Albracht, Kirsten; Bojsen-Møller, Jens; Seynnes, Olivier R.

**Title:** Distinct muscle-tendon interaction during running at different speeds and in different loading conditions

**Year:** 2019

**Version:** Accepted version (Final draft)

**Copyright:** © 2019 the American Physiological Society

**Rights:** In Copyright

**Rights url:** <http://rightsstatements.org/page/InC/1.0/?language=en>

**Please cite the original version:**

Werkhausen, A., Cronin, N., Albracht, K., Bojsen-Møller, J., & Seynnes, O. R. (2019). Distinct muscle-tendon interaction during running at different speeds and in different loading conditions. *Journal of Applied Physiology*, 127(1), 246-253.

<https://doi.org/10.1152/jappphysiol.00710.2018>

1 **Distinct muscle-tendon interaction during running at different speeds and**  
2 **in different loading conditions**

3

4 **Amelie Werkhausen<sup>1\*</sup>, Neil J. Cronin<sup>2</sup>, Kirsten Albracht<sup>3,4</sup>, Jens Bojsen-Møller<sup>1</sup> and Olivier R.**  
5 **Seynnes<sup>1</sup>**

6 1 Department of Physical Performance, Norwegian School of Sport Sciences, Oslo, Norway

7 2 Neuromuscular Research Centre, Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland

8 3 Institute of Biomechanics and Orthopedics, German Sport University Cologne, Cologne, Germany

9 4 Department of Medical Engineering and Technomathematics, Aachen University of Applied Sciences, Aachen, Germany

10

11 **\*Corresponding author**

12 [amelie.werkhausen@nih.no](mailto:amelie.werkhausen@nih.no) Sognsveien 220, 0853 Oslo, Norway

13

14 **Running head**

15 **Muscle-tendon mechanics under differing running constraints**

16 **Abstract**

17 The interaction between the Achilles tendon and the triceps surae muscles seems to be  
18 modulated differently with various task configurations. Here we tested the hypothesis that the  
19 increased forces and ankle joint work during running under contrasting conditions (altered  
20 speed or load) would be met by different, time-dependent adjustments at the muscle-tendon  
21 level. Ultrasonography, electromyography, kinematics and ground reaction force  
22 measurements were used to examine Achilles tendon, gastrocnemius and soleus muscle  
23 mechanics in sixteen runners in four different running conditions, consisting of a combination  
24 of two different speeds (preferred and +20% of preferred speed) and two loading conditions  
25 (unloaded and +20% of body mass). Positive ankle joint work increased similarly (+13%)  
26 with speed and load. Gastrocnemius and soleus muscle fascicle length and peak velocity were  
27 not altered by either condition, suggesting that contractile conditions are mostly preserved  
28 despite the constraints imposed in this experimental design. However, at higher running  
29 speed, tendon length changes were unaltered but mean muscle electromyographic activity  
30 increased in gastrocnemius (+10%,  $P<0.01$ ) and soleus (+14%,  $P<0.01$ ). Conversely, when  
31 loading was increased, mean muscle activity remained similar to unloaded conditions but the  
32 mean velocity of gastrocnemius fascicles was reduced and tendon recoil increased (+29%,  
33  $P<0.01$ ). Collectively, these results suggest that the neuromuscular system meets increased  
34 mechanical demands by favoring economical force production when enough time is available.

35

36 **New and Noteworthy**

37 We demonstrate that muscle-tendon mechanics are adjusted differently when running under  
38 constraints imposed by speed or load, despite comparable increases in work. The  
39 neuromuscular system likely modulates the way force is produced as a function of availability  
40 of time and potential energy.

41

42 **Keywords**

43 Achilles tendon, running, load carriage, locomotion, muscle architecture

44

45

46 **Introduction**

47 Tendons play an essential role during locomotor tasks such as running (5, 22). During stance,  
48 tendons lengthen under load, uncoupling fascicle behavior from that of the muscle-tendon unit  
49 (MTU) and enabling elastic energy storage and release to accelerate the body forward during

50 push-off. This mechanism offers several advantages, allowing muscle fascicles to operate  
51 under favorable conditions for force production, recycling mechanical energy and amplifying  
52 the MTU power output during recoil (28). Importantly, the interaction between muscle and  
53 tendon can be modulated according to external constraints, as previously demonstrated with a  
54 change in running slope (21) or in the transition from walking to running (9). When running  
55 conditions demand higher rate of force development and a larger ground reaction impulse,  
56 force and work production can be adjusted thanks to greater strains of elastic elements or  
57 heightened muscle activation and consumption of metabolic energy. Although triceps surae  
58 muscles may operate at shorter lengths and at higher activation levels with increasing running  
59 speed (16, 18), a greater stretch of the elastic elements seems to preserve their relative  
60 contribution to MTU work (19). Whether the relative contributions of muscle and tendon  
61 work are modulated differently as a function of stance duration, when work requirements  
62 increase, is currently unexplored.

63 Another paradigm to study MTU behaviour in response to increased ankle joint moments  
64 involves increasing inertial load. This experimental design is complementary to the approach  
65 of increasing speed, because it also requires an increase in ankle joint moment or positive  
66 work (23) but under different time and energy constraints. Contrary to conditions imposed by  
67 increasing speed, loaded running increases the availability of potential energy, prolongs  
68 ground contact time and increases peak forces (30), implying a greater ground impulse.  
69 During jumping, Wade and colleagues showed in an elegant study how the work contribution  
70 of the ankle joint remains constant when total work is increased via additional loading,  
71 whereas it is decreased when additional work is imposed by increasing jump height (32). The  
72 authors further suggested that the use of additional elastic strain energy stored in the Achilles  
73 tendon is prioritized over muscle work during submaximal jumps with loading (32). In a  
74 similar line of thought, running with additional load may provide a paradigm to investigate  
75 neuromuscular mechanisms when availability of potential energy and time to apply force are  
76 increased.

77 The aim of the present study was therefore to investigate the behavior of the triceps surae  
78 muscles and the Achilles tendon in response to a comparable increased demand for force  
79 production when running at higher speed or with additional loading. Based on findings from  
80 other locomotor tasks (9, 15, 32), we expected greater ankle joint moment and Achilles  
81 tendon force when speed or load increased, and therefore predicted a greater stretch of the  
82 Achilles tendon in both conditions. Although joint kinematics were not expected to change in  
83 either experimental condition (4, 25), we hypothesized that the contrasting change in ground

84 contact duration imposed when speed or load increased would affect the MTU behavior  
85 differently. Namely, the time to produce ground reaction force would be reduced at higher  
86 running speed whereas it would be extended when load is added. The reduced time and the  
87 greater rate of force development required under the faster running condition were expected to  
88 induce faster fascicle shortening velocities and greater muscle activity to compensate for  
89 unfavorable conditions for force production. Contrarily, when running with load, we  
90 hypothesized that longer stance time would cause slower fascicle contraction, which would  
91 not require muscle activation to increase.

92

## 93 **Materials and Methods**

### 94 *Subjects and experimental protocol*

95 Data were collected from sixteen male distance runners (age =  $27 \pm 4$  years, height =  $1.79 \pm 0.05$   
96 m, mass =  $68 \pm 6$  kg) who ran at least 40 km per week.

97 A warm up period of five minutes barefoot running on an instrumented treadmill (M-Gait,  
98 Motekforce Link, Amsterdam, The Netherlands) was used to determine the individual  
99 preferred speed of each subject. Thereafter the subjects were asked to run at their individual  
100 preferred speed and at increased speed (+ 20% of the preferred speed), with and without  
101 additional loads (+ 20% of body weight). Loading was achieved by means of one or two  
102 adjustable weighted vests containing up to 10 kg each. Data were recorded for at least 10  
103 complete steps during each of the four conditions. Ultrasound, kinematic and kinetic data  
104 were synchronously collected from the right leg, while muscle activity was recorded from the  
105 left leg at the same time. All measurements were synchronized during acquisition with a  
106 trigger signal sent from the ultrasound apparatus. All tests were performed twice, to obtain  
107 ultrasound scans from the muscle fascicles and from the myotendinous junction. The protocol  
108 was approved by the ethical committee of the Norwegian School of Sport Sciences and all  
109 subjects gave written informed consent to participate in the study.

110

### 111 *Joint mechanics*

112 Eleven infrared cameras (Qualisys, Gothenburg, Sweden; 300 Hz) captured the three-  
113 dimensional position of 20 reflective markers, mounted on the right leg of the subjects.  
114 Reflective markers placed over relevant anatomical landmarks (right and left anterior and  
115 posterior iliac spine, medial and lateral condyles, medial and lateral malleoli, calcaneus, first,  
116 second and fifth metatarsal) were used to define the joint centers of the pelvis (3), the right  
117 knee and the right ankle. The same calibration markers were used to define local coordinate

118 systems for the body segments (pelvis, thigh, shank and foot) during a static capture. While  
119 calibration markers of the pelvis and foot were also used as tracking markers, the movement  
120 of the shank and thigh were tracked with 4-marker clusters positioned mid-way along these  
121 segments. A force plate instrumented to the treadmill measured ground reaction forces during  
122 the running trials. Initial ground contact and toe-off were defined with a threshold of 25N.  
123 Inverse kinematic and dynamic calculations were performed for the right leg using Visual 3D  
124 (C-motion, Germantown, MD). Negative and positive ankle and knee joint work were  
125 calculated by integrating the negative and positive joint power using trapezoidal integration.  
126 Individual net work was calculated as the sum of negative and positive work for both joints.  
127 Joint angle data were used to estimate MTU lengths of gastrocnemius and soleus (12).

128

### 129 *Muscle-tendon mechanics*

130 Gastrocnemius and soleus muscle fascicles and the gastrocnemius muscle-tendon junction  
131 were imaged using an ultrasound linear array transducer (LV7.5/60/96Z LS128, Telemed,  
132 Vilnius, Lithuania). The transducer was secured to the leg in a custom-made holder with self-  
133 adhesive tape to avoid probe movement. Tape was also used to rigidly attach three kinematic  
134 markers to the transducer to track its position. B-mode images with a field of view of 60 mm  
135 were collected at 80 frames s<sup>-1</sup>. To measure fascicle length and pennation angle, the  
136 transducer was placed over the gastrocnemius muscle belly and aligned with the azimuthal  
137 direction of fascicles. A semi-automated tracking algorithm was used offline to analyze  
138 fascicle lengths and pennation angles (6, 8). The image quality of the soleus scans of three  
139 subjects was insufficient for analysis. Consequently, data for this muscle are based on thirteen  
140 subjects instead of sixteen. Architectural gear ratio (AGR) was calculated as the ratio between  
141 muscle length change and fascicle length change during the stance phase, similarly to  
142 Hollville et al. (14). Muscle length change was defined as the vertical projection of fascicle  
143 length change.

144 The position of the muscle-tendon junction was tracked from the displacement of the closest  
145 visible fascicle insertion in the two-dimensional ultrasound images (Tracker 4.95;  
146 [www.physlets.org/tracker/](http://www.physlets.org/tracker/)). Applying previously obtained calibration of the image coordinate  
147 system and the transducer coordinate system, the position of the muscle-tendon junction was  
148 reconstructed into the three-dimensional coordinate system of the laboratory (20). Thus, the  
149 distance between the gastrocnemius muscle-tendon junction and a reflective marker over the  
150 osteotendinous junction on the calcaneus was defined as Achilles tendon length. The position  
151 of the osteotendinous junction of the Achilles tendon was previously identified using

152 ultrasound. The shortest perpendicular distance between the force vector of the Achilles  
153 tendon and the ankle joint center was defined as the tendon moment arm at each time point  
154 (26). Thus, Achilles tendon force was estimated by dividing the ankle moment by the moment  
155 arm of the tendon.

156

### 157 ***Muscle activity***

158 Muscle activity of gastrocnemius, soleus and tibialis anterior was measured with a wireless  
159 electromyography (EMG) system (TeleMyo DTS, Noraxon U.S.A. Inc., Scottsdale, AZ,  
160 USA) and recorded in Qualisys. Surface electrodes were placed on the muscle belly following  
161 SENIAM guidelines (13), after the recording sites were shaved and cleaned. EMG signals  
162 were collected at 1500 Hz and processed using a bandpass filter (20 – 450 Hz), rectified and  
163 low-pass filtered (10 Hz). For each subject and muscle, EMG data were normalized to the  
164 highest values recorded during the control trials (i.e. without added mass and at preferred  
165 speed).

166

### 167 ***Data processing and statistics***

168 Kinematic, kinetic, ultrasound and EMG data were acquired during ten steps and at least eight  
169 steps were included in the analysis for each subject and each condition ( $9.5 \pm 0.6$  steps). All  
170 data were visually inspected to identify and exclude steps when their pattern or excursions  
171 departed from most other steps. A bidirectional second order Butterworth filter with a 15 Hz  
172 cut-off was applied to all raw data (except EMG, see above). Velocities of relevant variables  
173 were calculated as their time differential. Data were synchronized and resampled to 101 data  
174 points per full step cycle starting with the right heel strike, to calculate means across each  
175 percentage of the step for individual subjects and conditions.

176 Descriptive statistics were calculated for main outcome variables (Achilles tendon length,  
177 fascicle velocities and muscle activity) and for other variables related to muscle-tendon  
178 behavior (MTU length, stance duration, joint kinematics and kinetics). Differences between  
179 conditions were tested with two-way repeated-measures ANOVAS (factors: speed and load)  
180 and Sidak multiple comparison tests as appropriate. For all statistical tests, alpha was set to  
181 0.05.

182

### 183 **Results**

184 The average preferred and increased running speeds were  $3.1 (\pm 0.3) \text{ m s}^{-1}$  ( $\pm 0.3$ ) and  $3.7 (\pm$   
185  $0.3) \text{ m s}^{-1}$ , respectively. Step cycle (from right foot touch-down to the following right foot

186 touch-down) and stance phase durations (from touch-down to toe-off of the right foot) were  
187 affected by load and speed conditions. When speed increased, cycle duration decreased in  
188 both the unloaded and loaded conditions (-4 to -6%). Adding load also resulted in a reduction  
189 of cycle duration at increased speed (-3%) but not at preferred speed (**Table 1**). Stance  
190 duration decreased when running speed was increased, with or without additional load. On the  
191 contrary, stance duration was longer when the subjects ran with added mass at either speed  
192 (**Figure 1A**). Stance duration did not differ between running at increased speed with load and  
193 running at preferred speed unloaded. For this reason, average stance durations for these two  
194 conditions are indicated with the same shade of grey in the figures.

195 More positive and negative work was done by the ankle joint at higher speed in both loading  
196 conditions and with increased loading in both speed conditions. The positive work performed  
197 at the knee joint also increased when subjects were loaded (irrespective of speed), whereas  
198 speed did not have a statistically significant effect on positive knee work. Ankle and knee  
199 joint net work did not change in any condition (**Table 1**).

200 Peak ankle and knee joint angles were consistent across speed and loading conditions and,  
201 accordingly, no differences were found in MTU peak lengths for gastrocnemius (interaction  $P$   
202 = 0.33, speed  $P$  = 0.11 and load  $P$  = 0.47) and soleus (interaction  $P$  = 0.33, speed  $P$  = 0.06  
203 and load  $P$  = 0.44). Likewise, the shortening magnitude of both MTUs before toe-off was  
204 similar across all speed and mass conditions ( $P > 0.12$  in all comparisons). However,  
205 maximum MTU shortening velocities increased with speed, regardless of loading conditions  
206 (**Table 1, Figure 1F**). At preferred speed, load carrying significantly reduced MTU velocities,  
207 whereas no difference was present at increased speed.

208 Mean length changes of the MTUs during a step cycle are presented for all modalities of  
209 speed and mass in **Figure 2**.

210

### 211 *Speed and load effect on tendon behavior*

212 Achilles tendon lengthening and tendon peak length were unaffected by increases in speed or  
213 load ( $P > 0.05$  in all comparisons). However, a significant increase in tendon shortening  
214 amplitude (i.e. recoil) was found when running with load (**Figure 1B**, load effect  $P$  = 0.01),  
215 independently of speed ( $P < 0.01$  at preferred and increased speed). Mean lengths of the  
216 Achilles tendon during the step cycle are presented for all modalities of speed and mass in  
217 **Figure 2**. Peak Achilles tendon force increased with speed in both loading conditions, and  
218 with loading at both speeds (**Table 1**). Achilles tendon work loops for all four conditions are  
219 presented in **Figure 3**.

220

### 221 *Speed and load effect on muscle fascicle behavior*

222 Neither speed nor mass had a substantial influence on changes in gastrocnemius (interaction  $P$   
223 = 0.55, speed  $P = 0.66$  and load  $P = 0.80$ ) or soleus ( $P = 0.60$  and  $P = 0.52$ ) fascicle length.  
224 Mean fascicle length during the stance phase, fascicle shortening and peak fascicle shortening  
225 velocity did not vary across speed and loading conditions for either muscle ( $P > 0.05$  for all  
226 variables). Loading had a main effect on mean gastrocnemius fascicle velocity during stance.  
227 Multiple comparison tests showed that mean fascicle velocity was reduced at preferred speed  
228 (post-hoc comparison  $P = 0.01$ ) but not at increased speed (post-hoc comparison  $P = 0.26$ ),  
229 whereas soleus mean shortening velocity was similar across conditions (**Table 1**). Changes in  
230 pennation angle during stance were similar under all conditions in both muscles (all  $P > 0.05$ ).  
231 Gastrocnemius AGR during stance was also unchanged when speed or load increased (**Table**  
232 **1**).

233

### 234 *Speed and load effect on muscle activity*

235 Running at higher speed increased mean muscle activity of the gastrocnemius and soleus  
236 during stance, in both loading modalities (interaction  $P < 0.01$  for gastrocnemius and  $P = 0.04$   
237 for soleus, speed  $P < 0.01$  for both muscles). Post-hoc comparisons indicated that mean EMG  
238 during the stance phase increased with speed with or without loading. There was no main  
239 effect of loading on muscle activity ( $P = 0.61$ ) (**Figure 1D, E**). There were no interaction  
240 effects for time integral activity (reflecting the total amount of muscle activity) but we  
241 observed a main effect of loading for gastrocnemius ( $P < 0.01$ ) and soleus ( $P = 0.04$ ).  
242 Additionally, speed did not affect time integral activity of the gastrocnemius, whereas it was  
243 increased for soleus ( $P = 0.04$ ). Activity of the antagonist tibialis anterior peaked during the  
244 swing phase in all conditions. However, the mean activity of this muscle during the stance  
245 phase increased with speed in both loading conditions, whereas load carrying did not affect  
246 tibialis anterior activity. The activity of all three muscles during the whole step cycle is  
247 presented in **Figure 4**.

248

### 249 **Discussion**

250 The present study examined the effect of increased mechanical demand of running on human  
251 triceps surae muscle-tendon behavior, when either speed or loading was altered. Based on the  
252 different availability of potential energy and time to produce force imposed by the two  
253 conditions, it was hypothesized that plantarflexion force would be produced via distinct

254 adjustments of muscle-tendon interaction. Consistent with predictions from the literature,  
255 higher running speed reduced the stance duration while stance was prolonged with loading. In  
256 addition, both conditions required more mechanical work at the ankle joint (as previously  
257 shown in 23). Kinematic data showed little variation, which resulted in similar MTU stretch  
258 and shortening amplitudes across all conditions. However, the analysis of muscle and tendon  
259 mechanics also showed that the greater force requirements were met by different muscle-  
260 tendon behavior in the speed and loading conditions.

261 When speed was increased, the strain patterns of the Achilles tendon and muscle fascicles  
262 remained similar. The higher force produced during a shorter stance duration is therefore  
263 attributed to the greater muscle activity of the gastrocnemius and soleus. Additional loading  
264 did not affect tendon stretch either, but greater recoil was observed. Overall fascicle behavior  
265 remained similar, but in contrast to the increased speed condition, a reduction in mean fascicle  
266 shortening velocity was observed in the gastrocnemius when running at preferred speed with  
267 load. Hence, the increased work and impulse produced with added loading may be attributable  
268 to more favorable contractile conditions, and possibly result from a greater return of elastic  
269 energy due to the configuration of the running task.

270

### 271 *Speed and load effect on muscle fascicle behavior and activity*

272 In the present study, gastrocnemius and soleus fascicle peak shortening velocities and  
273 operating lengths were similar between loading conditions. Unchanged AGR and angular  
274 excursion of fascicles during stance further suggest that loading had little effect on contractile  
275 conditions. Yet gastrocnemius mean shortening velocity was reduced when running at  
276 preferred speed with loading, which may be associated with the longer duration of the stance  
277 phase. The inconsistency of the effect of loading on mean fascicle velocity across speeds  
278 suggests that a shorter stance phase duration may abolish the possibility to reduce contractile  
279 velocity when running with load. Regardless of running speed, unaltered or reduced  
280 contraction velocities observed with increased loading constitute advantageous conditions for  
281 increased force production while limiting the need to increase the mean level of activation.  
282 Further investigation is required to explain the different adjustments of gastrocnemius fascicle  
283 velocity when loading is added at different speeds. However, differences between the present  
284 results and a similar experiment on an animal model (25) are noteworthy. The initial stretch of  
285 fascicles measured in guinea fowl running with a similarly heavy load did not occur in  
286 humans in this study. Although the loading was similar, relative to body mass (22% vs 20%  
287 of body mass), load may have been higher relative to the gastrocnemius muscle force for the

288 guinea fowl than for humans (i.e. because of different force/bodyweight ratios), or the relative  
289 tendon stiffnesses of guinea fowls may have been higher due to calcifications. If this were the  
290 case, it can be speculated that a similar fascicle stretch as in the guinea fowls could have been  
291 observed in humans at loads higher than in this study. Finally, loading conditions and  
292 contractile behavior may simply differ between humans and guinea fowls, because of the foot  
293 posture of the birds.

294 Despite the reduced ground contact duration at higher running speed, fascicles maintained the  
295 same shortening velocities (peak and mean), lengths and AGR seen at preferred speed. This  
296 may seem counterintuitive but is in line with previous work examining gastrocnemius (9,  
297 personal communication) and soleus (18, personal communication) fascicles within similar  
298 speed ranges. However, *in silico* data have shown that fascicle shortening velocities are higher  
299 at higher running speeds (3.5 - 8 m/s) than those used in this study (3.1 and 3.7 m/s) (7).  
300 Hence, the present data may either indicate that fascicles only maintain their contraction  
301 velocities within certain speed ranges or that our methods lack the resolution to detect smaller  
302 differences.

303 The unchanged operating length of fascicles in this study contrasts with the shift towards  
304 shorter operating lengths observed by others in the gastrocnemius (16) and soleus (18)  
305 muscles at higher running speeds. This inconsistency may again partly be due to differences  
306 in speed increment between previous studies (e.g. 33% between 3 and 4 ms<sup>-1</sup> (18)) and our  
307 protocol (20%, between 3.1 and 3.7 ms<sup>-1</sup>). Despite the good reliability of the method to track  
308 fascicle behavior (11), small changes induced within our speed conditions may have gone  
309 undetected due to insufficient sensitivity of ultrasound measurements. Nonetheless, the  
310 advantage conferred to the triceps surae muscles by operating towards the top of the  
311 ascending limb of the force-length relationship seems to be maintained at the speeds used in  
312 the present protocol.

313 While fascicle operating range was preserved when running at increased speed, mean muscle  
314 activity of gastrocnemius and soleus increased, regardless of the loading condition. The  
315 observation of increased muscle activity at higher running speed is consistent with previous  
316 reports (17, 18), and reflects a higher magnitude of activation. Despite the shorter stance  
317 phase, a faster rate of muscle activation also resulted in an increase in total amount of activity  
318 (i.e. time integral EMG) of the soleus. Conversely, when additional load was added to the  
319 runners at preferred speed, the longer duration of the stance phase resulted in an increase in  
320 time integral EMG but mean EMG activity was reduced. The post-hoc analysis further  
321 indicated that mean EMG increased with loaded running at higher speed, confirming that time

322 availability is a critical factor driving the modulation of muscle activity when the mechanical  
323 demand increases. The advantage of the greater time availability may be linked to the way  
324 mechanical resonance of the system was altered when adding mass. In vitro data suggest that  
325 spring-like limb behavior during locomotion may be naturally regulated, by matching muscle  
326 strain patterns to the resonance frequency of the MTU (29). Hence, by increasing body mass  
327 without increasing running speed, the neuromuscular system may adopt an activation pattern  
328 in resonance with a lower natural frequency, maximizing force production and utilization of  
329 elastic energy.

330 With the exception of mean gastrocnemius fascicle shortening velocity decreasing with  
331 loading at preferred running speed, and the greater time integral of soleus EMG with loading  
332 at faster running speed, the adjustments in fascicle behavior and muscle activity observed  
333 between conditions were similar overall (e.g. peak fascicle velocity, fascicle operating length  
334 or mean EMG amplitude), for the gastrocnemius and soleus. Hence, different adjustments  
335 reflecting anatomical specificities of these muscles were largely missing here, although they  
336 may appear with larger increments in speed or load.

337 Collectively, the findings discussed above indicate that the system tends to meet an increased  
338 mechanical demand by modulating contraction velocity or muscle rate of activation as a  
339 function of time availability.

340

#### 341 ***Speed and load effect on tendon behavior***

342 Contrary to our expectations, tendon strain pattern was not affected when running speed  
343 increased, while a simulation study, conversely, predicted an increased elastic contribution to  
344 the positive work of the soleus and gastrocnemius MTUs when running speed increased from  
345 2.1 to 9 ms<sup>-1</sup> (19). However, the same study also suggested that the contribution of elastic  
346 strain energy of the gastrocnemius MTU would remain unchanged at intermediate speeds  
347 (19). Our Achilles tendon length results support the latter, by showing that neither tendon  
348 strain nor recoil changed at speeds lower than 4 ms<sup>-1</sup>. This unchanged tendon behavior is  
349 consistent with the unchanged fascicular behavior and suggests a rather constant contribution  
350 of elastic energy within the studied range of speeds.

351 On the other hand, loading affected the Achilles tendon behavior through an increased recoil  
352 (but not stretch) amplitude, irrespective of the speed conditions. The apparent disagreement  
353 between the unaltered tendon stretch during the first part of the stance phase and the changes  
354 in recoil during the push-off is surprising and may have been caused by several factors.  
355 Firstly, methodological issues may have limited the precision of Achilles tendon length

356 measurements (24, 31). To assess this possibility, we measured the inter-day variability of  
357 tendon strain measurements on a separate set of data collected for another project (n = 10,  
358 unpublished), using the same protocol as for the control running condition of the present  
359 study. We found a coefficient of variation of 10%, which in regards to sought differences in  
360 strain below 10% likely limited the sensitivity of our measures. Yet the reason why it would  
361 have affected tendon stretch and recoil differently remains unexplained. Aside from  
362 methodological explanations, the effective stiffness of the aponeurosis may have changed due  
363 to the influence of transverse strain. Farris and colleagues (10) established that a considerable  
364 amount of strain takes place transversely in the gastrocnemius aponeurosis during isometric  
365 contractions, which concurrently reduced longitudinal strain in the Achilles tendon. In  
366 addition, aponeurosis stiffness seemingly increases proportionally with contraction force  
367 because of radially expanding muscle fascicles (1, 2), and possibly also increases with MTU  
368 length (27). In the present case, a higher aponeurosis stiffness may have limited longitudinal  
369 tendon strain, in particular at the high Achilles tendon forces occurring at long MTU length  
370 measured at mid-stance when running with load. The hypothesis of additional energy being  
371 stored through transverse strain is also compatible with the unchanged joint kinematics and  
372 muscle operating conditions observed under loading. This may be seen as an advantageous  
373 way to increase tendon work when required, while allowing the subjects to run with the same  
374 joint coordination as in unloaded conditions and at preferred speed. Finally, the mismatched  
375 changes in tendon stretch and recoil with loading may also have been caused by the longer  
376 duration of the ground contact in this condition. By allowing a larger proportion of the tendon  
377 recoil to take place before the onset of the swing phase, the greater contact time would result  
378 in an increased impulse. This hypothesis would additionally be consistent with the observed  
379 faster velocities of tendon recoil.

380 Regardless of the factors explaining the lack of change in tendon stretch, the greater  
381 magnitude of tendon recoil arguably occurred while force was still being transmitted.  
382 Although certain methodological simplifications (as suggested by Matijevich et al. (24)) may  
383 give the impression of a continued tendon recoil while slackness has in fact been reached, we  
384 contend that this is not the case here. Firstly, tendon shortening continues after toe-off, when  
385 there is no longer any tension. Secondly, measurements obtained with shear wave  
386 elastography indicate that the gastrocnemius MTU is only slack beyond an ankle  
387 plantarflexion angle of 25°, which exceeds the angular range measured for this joint during  
388 running. We acknowledge, however, that tension and slackness levels cannot be inferred from

389 the present data. For this reason, interpreting the larger recoil measured when running with  
390 load (e.g. in relation to energy return) can only be done with caution.

391

### 392 ***Conclusion***

393 The present study shows distinct triceps surae muscle-tendon interaction in response to  
394 increased requirements for force and work at the ankle joint during running when speed or  
395 load increased. When ground contact time could be prolonged (i.e. with load), fascicle  
396 contractile velocity was preserved or lower and force was produced over a longer period of  
397 time. When running at increased speed and with shorter contact times, additional force was  
398 produced by greater muscle activation. These findings indicate that during running, the  
399 neuromuscular system meets increased mechanical demands by favoring economical force  
400 production when enough time is available.

401

### 402 ***Acknowledgements***

403 We thank three anonymous reviewers for their particularly insightful comments.

404 **References**

- 405 1. **Arellano CJ, Gidmark NJ, Konow N, Azizi E, and Roberts TJ.** Determinants of  
406 aponeurosis shape change during muscle contraction. *J Biomech* 49: 1812-1817, 2016.
- 407 2. **Azizi E, and Roberts TJ.** Biaxial strain and variable stiffness in aponeuroses. *J*  
408 *Physiol* 587: 4309-4318, 2009.
- 409 3. **Bell AL, Brand RA, and Pedersen DR.** Prediction of hip joint centre location from  
410 external landmarks. *Hum Mov Sci* 8: 3-16, 1989.
- 411 4. **Brughelli M, Cronin J, and Chaouachi A.** Effects of Running Velocity on Running  
412 Kinetics and Kinematics. *The Journal of Strength & Conditioning Research* 25: 2011.
- 413 5. **Cavagna GA, Saibene FP, and Margaria R.** Mechanical work in running. *J Appl*  
414 *Physiol* 19: 249-256, 1964.
- 415 6. **Cronin NJ, Carty CP, Barrett RS, and Lichtwark G.** Automatic tracking of medial  
416 gastrocnemius fascicle length during human locomotion. *J Appl Physiol* 111: 1491-1496,  
417 2011.
- 418 7. **Dorn TW, Schache AG, and Pandy MG.** Muscular strategy shift in human running:  
419 dependence of running speed on hip and ankle muscle performance. *J Exp Biol* 215: 1944-  
420 1956, 2012.
- 421 8. **Farris DJ, and Lichtwark GA.** UltraTrack: Software for semi-automated tracking of  
422 muscle fascicles in sequences of B-mode ultrasound images. *Comput Methods Programs*  
423 *Biomed* 128: 111-118, 2016.
- 424 9. **Farris DJ, and Sawicki GS.** Human medial gastrocnemius force-velocity behavior  
425 shifts with locomotion speed and gait. *PNAS* 109: 977-982, 2012.
- 426 10. **Farris DJ, Trewartha G, McGuigan MP, and Lichtwark GA.** Differential strain  
427 patterns of the human Achilles tendon determined in vivo with freehand three-dimensional  
428 ultrasound imaging. *J Exp Biol* 216: 594-600, 2013.
- 429 11. **Gillett JG, Barrett RS, and Lichtwark GA.** Reliability and accuracy of an  
430 automated tracking algorithm to measure controlled passive and active muscle fascicle length  
431 changes from ultrasound. *Comput Methods Biomech Biomed Engin* 16: 678-687, 2013.
- 432 12. **Hawkins D, and Hull ML.** A method for determining lower extremity muscle-tendon  
433 lengths during flexion/extension movements. *J Biomech* 23: 487-494, 1990.
- 434 13. **Hermens HJ, Freriks B, Disselhorst-Klug C, and Rau G.** Development of  
435 recommendations for SEMG sensors and sensor placement procedures. *J Electromyogr*  
436 *Kinesiol* 10: 361-374, 2000.
- 437 14. **Hollville E, Nordez A, Guilhem G, Lecompte J, and Rabita G.** Interactions  
438 between fascicles and tendinous tissues in gastrocnemius medialis and vastus lateralis during  
439 drop landing. *Scand J Med Sci Sports* 0: 2018.
- 440 15. **Huang T-wP, and Kuo AD.** Mechanics and energetics of load carriage during human  
441 walking. *J Exp Biol* 217: 605, 2014.
- 442 16. **Ishikawa M, and Komi PV.** The role of the stretch reflex in the gastrocnemius  
443 muscle during human locomotion at various speeds. *J Appl Physiol* 103: 1030-1036, 2007.
- 444 17. **Kyröläinen H, Avela J, and Komi PV.** Changes in muscle activity with increasing  
445 running speed. *J Sports Sci* 23: 1101-1109, 2005.
- 446 18. **Lai A, Lichtwark GA, Schache AG, Lin Y-C, Brown NAT, and Pandy MG.** In  
447 vivo behavior of the human soleus muscle with increasing walking and running speeds. *J Appl*  
448 *Physiol* 118: 1266-1275, 2015.
- 449 19. **Lai A, Schache AG, Lin Y-C, and Pandy MG.** Tendon elastic strain energy in the  
450 human ankle plantar-flexors and its role with increased running speed. *J Exp Biol* 217: 3159-  
451 3168, 2014.
- 452 20. **Lichtwark GA, and Wilson AM.** In vivo mechanical properties of the human  
453 Achilles tendon during one-legged hopping. *J Exp Biol* 208: 4715-4725, 2005.

- 454 21. **Lichtwark GA, and Wilson AM.** Interactions between the human gastrocnemius  
455 muscle and the Achilles tendon during incline, level and decline locomotion. *J Exp Biol* 209:  
456 4379-4388, 2006.
- 457 22. **Lichtwark GA, and Wilson AM.** Is Achilles tendon compliance optimised for  
458 maximum muscle efficiency during locomotion? *J Biomech* 40: 1768-1775, 2007.
- 459 23. **Liew BXW, Morris S, and Netto K.** The effects of load carriage on joint work at  
460 different running velocities. *J Biomech* 49: 3275-3280, 2016.
- 461 24. **Matijevich ES, Branscombe LM, and Zelik KE.** Ultrasound estimates of Achilles  
462 tendon exhibit unexpected shortening during ankle plantarflexion. *J Biomech* 72: 200-206,  
463 2018.
- 464 25. **McGowan CP, Duarte HA, Main JB, and Biewener AA.** Effects of load carrying on  
465 metabolic cost and hindlimb muscle dynamics in guinea fowl (*Numida meleagris*). *J Appl*  
466 *Physiol* 101: 1060-1069, 2006.
- 467 26. **Obst SJ, Barber L, Miller A, and Barrett RS.** Reliability of Achilles Tendon  
468 Moment Arm Measured In Vivo Using Freehand Three-Dimensional Ultrasound. *J Appl*  
469 *Biomech* 33: 300-304, 2017.
- 470 27. **Raiteri BJ, Cresswell AG, and Lichtwark GA.** Muscle-tendon length and force  
471 affect human tibialis anterior central aponeurosis stiffness in vivo. *PNAS* 2018.
- 472 28. **Roberts TJ, Marsh RL, Weyand PG, and Taylor CR.** Muscular Force in Running  
473 Turkeys: The Economy of Minimizing Work. *Science* 275: 1113-1115, 1997.
- 474 29. **Robertson BD, and Sawicki GS.** Unconstrained muscle-tendon workloops indicate  
475 resonance tuning as a mechanism for elastic limb behavior during terrestrial locomotion.  
476 *PNAS* 112: E5891-E5898, 2015.
- 477 30. **Silder A, Besier T, and Delp SL.** Running with a load increases leg stiffness. *J*  
478 *Biomech* 48: 1003-1008, 2015.
- 479 31. **Stosic J, and Finni T.** Gastrocnemius tendon length and strain are different when  
480 assessed using straight or curved tendon model. *Eur J Appl Physiol* 111: 3151-3154, 2011.
- 481 32. **Wade L, Lichtwark G, and Farris DJ.** Movement Strategies for Countermovement  
482 Jumping are Potentially Influenced by Elastic Energy Stored and Released from Tendons. *Sci*  
483 *Rep* 8: 2300, 2018.
- 484

485 **Figure 1.** Group mean values ( $\pm$  s.d.) during the stance phase for key variables that vary with loading or speed  
486 (preferred (PS) compared to increased speed (IS)): stance phase duration (A), Achilles tendon (AT) shortening  
487 (B), AT velocity (C), gastrocnemius (GM) EMG (D), soleus (SOL) EMG (E) and muscle-tendon unit (MTU)  
488 velocity (F). \* $P < 0.05$  for main effects of mass, §  $P < 0.05$  for main effect of speed, and #  $P < 0.05$  for interaction  
489 effect

490 **Figure 2.** Instantaneous lengths of the gastrocnemius and soleus muscle-tendon unit (MTU) (A-B), fascicles (C-  
491 D) and the Achilles tendon (AT) (E) during a whole step cycle for running at two different speeds (preferred  
492 speed and preferred speed + 20%) and two loading conditions (unloaded and loaded with 20% of body mass).  
493 Data are time normalized to 101 points and displayed as group means. The shaded area represents the stance  
494 phase of the conditions BM IS as dark grey, BM PS and AM IS as medium grey, and AM PS as light grey.

495 **Figure 3.** Achilles tendon (AT) work loops during the stance phase of running unloaded and loaded (with 20% of  
496 body mass) at preferred and faster speed (preferred speed + 20%).

497 **Figure 4.** Electromyographic activity of gastrocnemius medialis (GM) (A), soleus (SOL) (B) and tibialis anterior  
498 (TA) (C) during a whole step cycle for running at two different speeds (preferred speed - PS and increased speed  
499 - IS) and mass (body mass - BM and added mass - AM) conditions. Time series are normalized to 101 points and  
500 EMG values are normalized to the maximum activity during unloaded running at preferred speed. Data are  
501 displayed as group means. The shaded area represents the stance phase of the conditions BM IS as dark grey,  
502 BM PS and AM IS as medium grey, and AM PS as light grey.

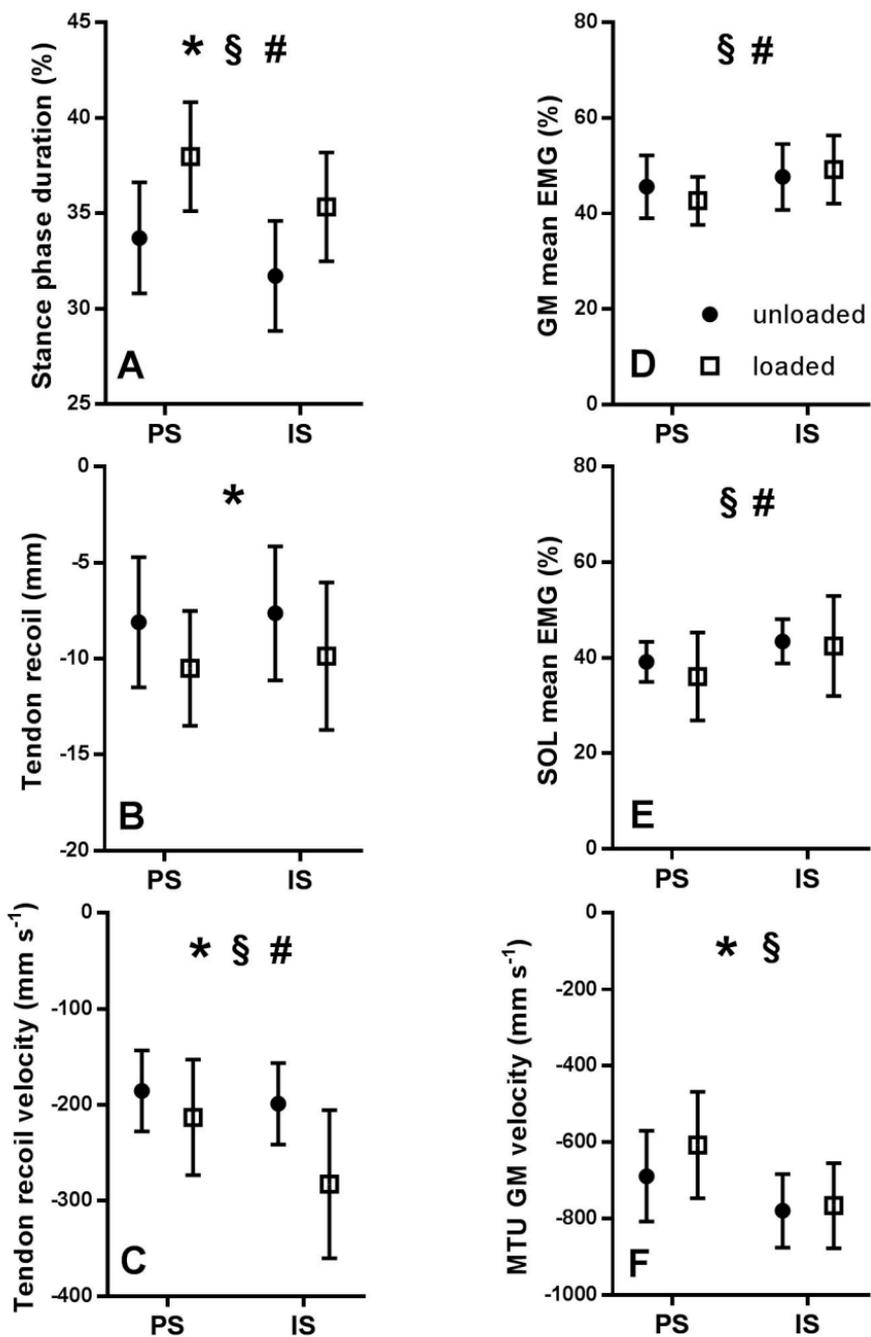
**Table 1.** Durations of step cycle and stance phase, positive work performed at the ankle and the knee, peak shortening velocities of the Achilles tendon, muscle-tendon units and fascicles, and AT forces, when running unloaded or loaded, at preferred speed or increased speed. Velocities were normalized to the respective mean length during stance.

		Unloaded		Loaded		ANOVA results		
		Preferred speed	Increased speed	Preferred speed	Increased speed	Interacti on effect	Main effect speed	Main effect load
<b>Duration [s]</b>	<b>Cycle</b>	0.68 ± 0.04	0.66 ± 0.05*	0.68 ± 0.05	0.64 ± 0.05* <sup>§</sup>	0.01	<0.01	0.02
	<b>Stance</b>	0.23 ± 0.02	0.21 ± 0.02*	0.26 ± 0.02 <sup>§</sup>	0.23 ± 0.02* <sup>§</sup>	<0.01	<0.01	<0.01
<b>Duty factor [%]</b>		34 ± 3	32 ± 3*	38 ± 3 <sup>§</sup>	35 ± 3* <sup>§</sup>	0.03	<0.01	<0.01
<b>Work [J]</b>	<b>positive</b>	55 ± 13	62 ± 15*	62 ± 18 <sup>§</sup>	69 ± 20* <sup>§</sup>	0.95	<0.01	<0.01
	<b>negative</b>	14 ± 8	16 ± 10	17 ± 9 <sup>§</sup>	19 ± 10 <sup>§</sup>	0.61	0.14	<0.01
<b>net</b>	<b>Ankle</b>	32 ± 14	36 ± 16*	37 ± 18 <sup>§</sup>	43 ± 20* <sup>§</sup>	0.25	<0.01	<0.01
	<b>Knee</b>	27 ± 11	29 ± 12	31 ± 11 <sup>§</sup>	31 ± 11	0.13	0.14	<0.01
<b>Peak shortening velocity [mms<sup>-1</sup>]</b>	<b>GM MTU</b>	0.45 ± 0.27	0.43 ± 0.26*	0.53 ± 0.30 <sup>§</sup>	0.50 ± 0.38*	0.06	<0.01	0.01
	<b>SOL MTU</b>	0.40 ± 0.34	0.34 ± 0.31*	0.48 ± 0.41 <sup>§</sup>	0.45 ± 0.49*	0.09	<0.01	<0.01
	<b>GM fasc.</b>	1.68 ± 1.06	1.82 ± 1.22	1.87 ± 1.41	2.00 ± 1.23	0.76	0.68	0.15
	<b>SOL fasc.</b>	1.96 ± 1.55	1.99 ± 1.74	1.89 ± 1.12	2.53 ± 1.84	0.47	0.10	0.47
<b>Mean shortening velocity [mms<sup>-1</sup>]</b>	<b>GM fasc.</b>	1.03 ± 0.66	1.34 ± 0.91	0.99 ± 0.85 <sup>§</sup>	1.18 ± 0.75	0.29	0.05	0.03
	<b>SOL fasc.</b>	0.78 ± 0.86	0.98 ± 1.21	0.64 ± 0.81	1.02 ± 1.41	0.97	0.24	0.26
<b>AGR during stance</b>	<b>GM</b>	1.16 ± 0.06	1.16 ± 0.08	1.17 ± 0.10	1.17 ± 0.10	0.61	0.92	0.27
<b>Force [N]</b>	<b>AT</b>	4336 ± 931	4644 ± 1037*	4501 ± 1029 <sup>§</sup>	4896 ± 1059* <sup>§</sup>	0.21	<0.01	<0.01
<b>Impulse [Ns]</b>		469 ± 59	452 ± 61*	560 ± 76 <sup>§</sup>	529 ± 75* <sup>§</sup>	<0.01	<0.01	<0.01

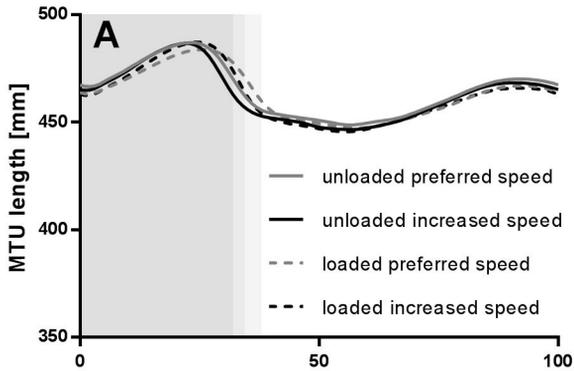
Data for work, shortening velocities of gastrocnemius (GM) and soleus (SOL) muscle-tendon units (MTU) and fascicles, and force were obtained during the stance phase. Values are mean ± SD. \* Significantly different from the preferred speed condition with the same load; <sup>§</sup> Significantly different from the unloaded condition at the same speed.

load ↑

speed ↑



## Gastrocnemius



## Soleus

