Title: Muscle fascicle strains in human gastrocnemius during backward downhill walking

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Running title: Muscle fascicle strains during human muscle damaging exercise

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Abstract:

Extensive muscle damage can be induced in isolated muscle preparations by performing a small number of stretches during muscle activation. While typically these fibre strains are large and occur over long lengths, the extent of exercise-induced muscle damage (EIMD) observed in humans is normally less even when multiple high-force lengthening actions are performed. This apparent discrepancy may be due to differences in muscle fibre and tendon dynamics in vivo; however muscle and tendon strains have not been quantified during muscle damaging exercise in humans. Ultrasound and an infrared motion analysis system were used to measure medial gastrocnemius (MG) fascicle length and lower limb kinematics while humans walked backward, downhill for 1-hr (inducing muscle damage), and while they walked briefly forward on the flat (inducing no damage). Supramaximal tibial nerve stimulation, ultrasound, and an isokinetic dynamometer were used to quantify the fascicle length-torque (L-T) relationship pre- and 2-hr post-exercise. Torque decreased ~23% and optimal fascicle length (L_o) shifted rightward ~10%, indicating that EIMD occurred during the damage protocol even though MG fascicle stretch amplitude was relatively small (~18% of L_o) and occurred predominately within the ascending limb and plateau region of the L-T curve. Furthermore, tendon contribution to overall muscle-tendon unit stretch was ~91%. The data suggest the compliant tendon plays a role in attenuating muscle fascicle strain during backward walking in humans, thus minimising the extent of EIMD. As such, in situ or in vitro mechanisms of muscle damage may not be applicable to EIMD of the human gastrocnemius muscle.

51 Introduction

52

53 While it is known that a single bout of unaccustomed, eccentric exercise can cause muscle damage
54 (exercise-induced muscle damage, EIMD), there are limited data on the muscle mechanics that lead to
55 EIMD in humans under natural conditions. Much of our mechanistic understanding of EIMD has been
56 established from non-human experiments, in which single muscle fibres in vitro, or isolated muscles in
57 situ, were stretched under electrical activation (9, 10). Experiments of this kind usually involve a small
58 number of fibre stretches (as few as 1-10), high muscle stimulation frequencies (producing up to 100%
59 of maximal force), large stretch amplitudes (up to 60% of optimal length (L_o)), and a range of fibre
60 lengths corresponding to the descending limb of the length-tension curve (up to ~70% longer than L_o).
61 The literature indicates that the force production of fibres exposed to such protocols can be reduced by
62 ~40-80% (3-7, 24, 31, 35, 36, 59), with immediate and extensive structural damage to the sarcomeres
63 (4, 7, 51).

64

65 Although most current ideas regarding the mechanisms of EIMD (such as the 'popping sarcomere
66 hypothesis'; 40) have been derived from, or supported by, in situ or in vitro experiments, it is not clear
67 whether the nature or mechanisms of damage in these contexts are comparable to muscle damage that
68 occurs in vivo (10). For example, the force decrements observed in vivo are typically less than those
69 observed in situ or in vitro, even when the level of muscle activation is maximal (9, 41), when long
70 muscle-tendon unit (MTU) lengths are used (9, 41), or when thousands of contractions are performed
71 (26). Non-human in vivo studies have shown that multiple lengthening contractions (~20-70), produce
72 immediate reductions in force in the range of 25-55% (9, 14, 18, 57). In humans, the decrease in
73 maximum force production due to EIMD is similarly less, ranging between ~10-50%, with the
74 decrements in force typically peaking 24 to 48-hr post-exercise (26, 37-39, 41, 42). The smaller
75 magnitude and the delay in peak force decrement occurs even when thousands of contractions are

performed or if the lengthening contractions are performed at maximal intensities (26, 41). Further, the appearance of extensive structural damage to muscle fibres in vivo appears to occur ~1-4 days post-exercise rather than immediately after as observed in situ or in vitro (11, 27, 50). Thus, the characteristics of muscle damage experienced in vivo may be different from those experienced in situ or in vitro.

In addition to differences in the nature of muscle damage between isolated muscle preparations and in vivo human exercise, there may be fundamental differences in the muscle fibre dynamics during active lengthening in these contexts (10). The amplitude and rate of changes in muscle fibre length produced in situ and in vitro closely mimic the externally imposed stretches, as either the fibres have been completely removed from the muscle or the tendon has been cut. In contrast, during exercise such as walking, changes in muscle fibre length do not necessarily follow changes in MTU length due to the compliance of the in-series tendinous tissue (16, 19, 22, 29). As a consequence, it is possible that under natural conditions, both the amplitude and rate of stretch of fibres will differ from the amplitude and rate of stretch of the MTU. While it has been established that tendon plays a role in regulating muscle fibre length in vivo (13, 30, 47), the relationship between muscle fibre and tendon length changes during a bout of EIMD in humans is unknown.

A classic EIMD experimental design used to damage the human triceps surae is backward downhill walking (26, 27, 55). Compared to forward walking, where little or no muscle damage is experienced, prolonged backward, downhill walking causes substantial damage to the triceps surae, highlighted by a drop in maximal plantar flexion torque production, changes in the plantar flexion torque-angle curve and muscle soreness occurring 24 to 48 hours post-exercise (21, 26, 27, 55, 56). This damage is presumably caused by active stretch of the triceps surae muscle fibres. Interestingly, the lower limb kinematics and kinetics of backward walking are remarkably similar to those of forward walking,

however the order of events occurs in a reverse time sequence (25, 53). Hence, it could be hypothesised that muscles that generate positive work during forward walking generate negative work in backward walking and vice versa. This claim has recently been supported by simulations examining the role of the lower limb muscles during backward walking (25). Given that the soleus muscle fascicles work over the ascending limb of the length-tension curve during forward walking, it seems likely that triceps surae fascicle lengthening also occurs within the ascending limb during backward walking (48). However, length changes in the muscle fascicles and tendon of the triceps surae have never been examined in humans during backward downhill walking in vivo, nor has it been determined over which range of the length-tension relationship fascicle strain occurs.

The aim of this study was to quantify muscle fascicle and tendon dynamics of the human gastrocnemius during prolonged backward downhill walking. We hypothesised that backward downhill walking would result in muscle damage through repeated stretch of the muscle fascicles during the stance phase. We further postulated that the strains experienced by muscle fascicles would be much lower than those typical for in vitro and in situ EIMD preparations, due to the compliance of tendinous tissue that would act to buffer the fascicles from excessive stretch amplitudes. Finally, we hypothesised that fascicle lengthening during backward downhill walking would occur over a similar range of the length-tension relation as fascicle shortening during non-damaging forwards walking (i.e. the ascending limb). To test these hypotheses, we used ultrasonography to directly measure medial gastrocnemius (MG) fascicle length and changes in length during non-damaging and muscle-damaging walking trials.

126 Methods

127

128 Subjects

129 Ten male subjects (21.5 ± 1.5 yrs, 82 ± 27 kg, 1.84 ± 0.14 m) whom provided written, informed
130 consent volunteered to participate in this study. All subjects were healthy, had no history of
131 neuromuscular disease or illness and were not participating in regular, strenuous exercise. The protocol
132 was approved by a local university ethics committee and conducted according to the Declaration of
133 Helsinki.

134

135 Length-tension relationship experimental set-up

136 Length-tension data was collected immediately prior to the walking tasks as well as 2-hr after the
137 completion of the 1-hr backward walking protocol. The 2-hr wait before retesting the length-tension
138 relationship was imposed to avoid the effects of muscle fatigue (54). The data was collected according
139 to a protocol similar to that detailed in Hoffman et al. (2012). In brief, subjects had their foot attached
140 to an isokinetic dynamometer (Biodex System 3 Pro, Biodex Medical Systems, Shirley, NY, USA) that
141 rotated the ankle from the neutral ankle position to maximum dorsiflexion. Ankle joint angle position
142 was recorded from the dynamometer and the signal was low-pass filtered at 9 Hz and converted using a
143 16-bit analog-to-digital converter at 1kHz (Power 1401, Cambridge Electronic Design, UK). Plantar
144 flexion torque was also measured from the dynamometer with the signal low-pass filtered at 5 Hz and
145 analog-to-digital converted at 1 kHz (Power 1401, Cambridge Electronic Design, UK).
146 Simultaneously, MG muscle fascicle length was measured using a 96-element, multi-frequency
147 ultrasound transducer (LV7.5/60/96, Telemed, Vilnius, Lithuania) attached to a PC-based ultrasound
148 system (Echoblaster 128, UAB, Telemed, Vilnius, Lithuania). The location of the transducer on the leg
149 was determined such that it provided the clearest image of continuous MG muscle fascicles and the
150 location on the skin was marked with indelible pen. The images were captured at a mean frequency of

6 MHz, a field of view of 60 x 65 mm, a focus range of 18-26 mm in B-mode and at a frame rate of 80 frames / second using software (EchoWave II, Telemed, Vilnius, Lithuania). After the images were captured, a tracking algorithm implemented in Matlab (Mathworks, MA, USA) was used to measure muscle fascicle length during each peripheral nerve stimulation (17).

Peripheral nerve stimulation

To determine the torque produced at each muscle length or joint angle, supramaximal peripheral nerve stimulation (pulse width = 500 μs) was applied using a double pulse stimulation technique (interstimulus interval = 20 ms) to the tibial nerve with the subject at rest. Using a constant-current stimulator (DS7AH, Digitimer, UK), current was passed from a cathode (Ag-AgCl electrode, 24 mm diameter; Tyco Healthcare Group) placed on the optimal site of stimulation within the popliteal fossa to an anode (Ag-AgCl electrode, 24 mm diameter; Tyco Healthcare Group) positioned proximal to the cathode on the midline of the popliteal fossa. The stimulation evoked resting torque twitches were measured as the difference between the peak value of the twitch and the torque directly preceding the twitch. The current value used for each subject was set at 50% greater than the minimum current required to evoke the maximum torque twitch at rest.

Length-tension data collection protocol

Length-tension data was collected by applying stimulations at 12-16 pre-selected joint angles across the subject's range of motion. To minimise any thixotropic effects, the subject performed a small, brief plantar flexion contraction prior to each stimulation (46). This process was repeated 3 times randomly at each joint angle for a total of 36-48 stimulations.

174 Length-tension curve construction

175 To quantify the length-tension relationship before and after the eccentric exercise bout, we used the
176 same based method outlined in Hoffman et al. (2012) that assumes that the parallel elastic component
177 lies in parallel only with the contractile element (23, 32). The protocol and method for constructing the
178 length tension curve in this way has been shown to be consistent and reliable across different sessions
179 (23). As such, active torque was calculated as the difference between the total torque during the
180 stimulation (i.e. the peak of the twitch) and the passive torque during the contraction. To determine this
181 passive torque, the passive length-torque curve was first determined and then the passive value was
182 estimated that corresponded to the shortest fascicle length the muscle contracts to during each
183 contraction. Once active torque was calculated and then plotted against fascicle length, second order
184 exponential curves were fit for each subject according to previous physiologically appropriate models
185 (2, 43):

186 $$T_{active} = e^{-|(L^b - 1)/s|^a} \quad (1)$$

187 Where T is torque, L is fascicle length, a is roundness, b is skewness and s is width. Passive L-T curves
188 were also constructed by fitting a standard exponential expression:

189 $$T_{passive} = Ae^{k(L - L_s)} \quad (2)$$

190 Where A is curvature, k is stiffness of the curve and L_s is the slack length (or slack angle when angle-
191 torque data is fit). To determine the group mean active and passive L-T curves, the curve parameters
192 from all 10 subjects were averaged and then new curves were created using these averaged parameters.
193
194 Eccentric exercise experimental set-up

195 A four-camera, infrared motion analysis system (Qualysis AB, Gothenburg, Sweden) was used to
196 measure the kinematics of the left leg during walking. Single reflective spherical markers (diameter = 1

cm) were placed on the greater trochanter, medial and lateral femoral epicondyles, medial and lateral malleoli and the head of the 1st and 5th metatarsals while marker clusters (groups of 3-4 markers) were attached to the lateral aspect of the thigh and shank and on the dorsal aspect of the foot. The position of the markers was sampled at 200 Hz using computer software (Qualysis Track Manager, Qualysis AB, Gothenburg, Sweden) and analysed offline (Visual 3D, C-Motion Inc., Germantown, U.S.A.). At the same time, MG fascicle length was measured using the same equipment and process detailed above.

Eccentric exercise protocol

Prior to performing the muscle damaging walking protocol, subjects were required to perform a 1-min bout of forward flat walking on a motorised treadmill (Austredex, Melbourne, Australia). After this short bout, subjects walked backward at 6km/h, downhill at an angle of 13°, for one hour while they carried 10% of their body mass in a backpack. Prolonged backward downhill walking has been shown to induce muscle damage within the triceps surae (26, 27). The high speed and inclination of the treadmill were chosen to ensure that *triceps surae* muscles underwent high force eccentric contractions while walking backward.

For the 1-min forward walking bout, muscle fascicle length and kinematic data were collected over a 10-s period during the middle of the bout. This ensured that subjects were walking comfortably on the treadmill before data was collected and that 6-8 complete stride cycles were sampled. For the 1-hr backward downhill walking bout, data was collected similarly during the 1st and 60th minutes of the task. Measurements were also taken at 15, 30 and 45 minutes into the 1-hr walking task however these measurements did not provide any additional insights into MG fascicle behaviour during this task and thus, for clarity, they were subsequently removed from Results.

222 Data analysis

223 For every 10-s window of ultrasound data that was collected during the walking tasks, the MG fascicle
224 length, pennation angle, and the knee and ankle joint angles were determined from the average of the 6-
225 8 strides captured during that window. Muscle fascicle length and pennation angle were measured from
226 the ultrasound recording using a semi-automated algorithm that has been shown to reliably track
227 muscle fascicle length changes during gait (12). Fascicle length data was then converted and accessed
228 by Visual 3D to allow analysis between muscle parameters and kinematic variables.

229 Normative MTU length was determined using the following regression equation from previous
230 literature:

231 $$L_{MTU} = C0 + C2\beta + C4\phi \quad (3)$$

232 where C0, C2 and C4 are correlation coefficients (0.9, -0.00062 and 0.00214, respectively) determined
233 by Hawkins & Hull (20) and β and ϕ are the measured flexion joint angles of the knee and ankle,
234 respectively. To determine the estimated MG MTU length, the normative MTU length was then
235 multiplied by the shank length of each subject (20).

236 Series elastic element (SEE) length, which represents all elastic tissue in series with the muscle
237 fascicles including tendon and aponeurosis, was calculated according to a previously published model
238 (16):

239 $$L_{SEE} = L_{MTU} - L_{FAS} \times \cos\theta \quad (4)$$

240 where L_{MTU} is the MG MTU length calculated from equation 1 above, L_{FAS} is the measured fascicle
241 length and θ is the measured pennation angle relative to the line of action of the force (horizontal to the
242 image plane).

243 In Results, fascicle, MTU and SEE length are sometimes presented relative to the passive slack length.
244 The slack lengths were determined from the length-tension data collected at baseline. For fascicle slack
245 length, this value was determined from the baseline passive fascicle length-torque curve (see equation 2

246 above). MTU slack length was calculated using equation 3 above, where β was the slack angle of the
247 knee (5° flexion) and ϕ was the slack angle of the ankle determined from the baseline passive angle-
248 torque curve (see equation 2 above). SEE slack length was calculated using equation 4 above, where θ
249 is the slack pennation angle determined from the linear fit between pennation angle and fascicle length.
250 For each walking condition, fascicle, SEE and MTU stretch amplitude was calculated as the difference
251 between maximum length and minimum length while the SEE was lengthening during the stance phase
252 (i.e. while tendon force was increasing). For backward walking, this typically occurred from
253 approximately toe-strike to ~30% of the stride cycle. For forward walking, this typically occurred
254 between ~10% and ~50% of the stride cycle.
255
256 Statistical analysis
257 From the fitted L-T curves, the maximum torque (T_{max}), the muscle fascicle length at which maximum
258 torque occurs (i.e. optimal muscle length, L_o), L_s and passive parameters (k and A) were determined.
259 Dependent t-tests were used to compare these variables between baseline and two hours post-exercise
260 and were also used to compare muscle and tendon stretch parameters (e.g. fascicle, MTU, SEE stretch
261 amplitude and velocity) in forward and backward flat walking. From the ultrasound length data,
262 dependent t-tests were performed to examine how fascicle, MTU and SEE peak length during stretch
263 changed during the 1-hr backward walking exercise. The spearman ranked correlation coefficient was
264 calculated to determine the relationship between the drop in maximum torque 2-hr post-exercise and
265 the maximum fascicle stretch length during the beginning of 1-hr backward, downhill walking. All
266 group data presented in the Results are presented as means ± SD. The grey, shaded area in Figure 3A-C
267 and 5 indicates the standard error of the mean (SEM). Significant differences were established at $P \leq$
268 0.05.
269

Results

Identifying the presence of EIMD

To determine whether EIMD occurred, the shape of the active and passive L-T curves was compared between pre- and 2-hr post-exercise (Figure 1). Significant differences were found for maximum torque ($P \leq 0.05$) and optimal fascicle length ($P \leq 0.05$) where T_{max} decreased ~23% and L_o increased ~10% between pre- and 2-hr post-backward downhill walking (Table 1). However, there was no change in the shape of the passive L-T curve with no significant differences detected in passive stiffness ($P = 0.54$), passive curvature ($P = 0.19$) or slack length ($P = 0.92$; Table 1). While there was little change in the passive L-T curve, changes in the active L-T curve are consistent with the presence of EIMD.

Fascicle length working ranges relative to the L-T curve

Figure 2A provides an example of how the calculated group mean active and passive L-T curves were fit through individual L-T data points. Also shown is the average fascicle length working range while the MTU is lengthening for both backward downhill walking and forward flat walking along with horizontal error bars (SEM) indicating the variance in the minimum and maximum fascicle lengths during the stretch of the fascicles in early stance. Figure 2B shows group mean active and passive L-T curves and working fascicle ranges for backward and forward walking prior to damage occurring. For backward downhill walking, the fascicle stretched from the ascending limb to the plateau region, and in some cases to the descending limb. For forward flat walking, the fascicle shortened from the descending limb to the plateau region. This shows that the fascicle operated at lengths primarily over the ascending limb and plateau region for backward downhill walking. Furthermore, when the working ranges of backward downhill walking at the end of the 1-hr exercise bout and forward flat walking post-exercise are superimposed onto the 2-hr post-exercise L-T curve, it can be seen that the fascicles now work over a range that is at a shorter length compared to before the 1-hr exercise bout (Figure 2C).

Over the period of the 1-hr backward, downhill walking task, changes in peak length during stretch were analysed to determine if the extent of fascicle stretch was altered during the muscle damage protocol (Figure 3A-C). There was no significant change in peak length during stretch found for fascicle ($P = 0.52$), MTU ($P = 0.54$) or SEE ($P = 0.25$) between the 1st and 60th minutes of the backward, downhill walking task (Table 2). This suggests that the fascicles were not stretched to longer lengths over the duration of the muscle damage task.

To determine if there was a length dependency of the muscle damage incurred by the subjects, the drop in maximum torque experienced by each subject 2-hr post-exercise was plotted against the maximum fascicle stretch length during the start of 1-hr backward, downhill walking (Fig. 4). There was no significant correlation found between the two variables ($r = 0.13$, $P = 0.74$). As such, this suggests that the fascicle working length range had no effect on the presence and amount of muscle damage experienced by the subjects.

Muscle fibre and tendon dynamics prior to EIMD

Mean fascicle length during the gait cycle is shown in Figure 5 for forward flat walking and backward flat walking prior to the muscle damage protocol, as well as for during the first minute of backward downhill walking (i.e. before damage has occurred). The forward walking trace has been reversed in time to allow for comparison of reverse muscle function (53). Although the fascicle length traces appear to show a greater strain experienced by the fascicle during the initial part of the stance phase during backward downhill walking compared to backward flat walking, this difference was not significant ($P = 0.21$; Table 2). Furthermore, there were no significant differences between backward downhill walking and backward flat walking during the stance phase for MTU and SEE strain ($P =$ 0.96 and 0.6, respectively). Similarly, there was no difference in the rate of stretch during the stance

320 phase for fascicle, MTU or SEE between backward downhill walking and backward flat walking (P =
321 0.66, 0.67, 0.94, respectively; Table 2).
322
323 The amplitude of SEE stretch during the initial stance phase of backward, downhill walking was
324 compared to fascicle stretch to identify their relative contribution to total MTU stretch (Figure 3A-C).
325 The amplitude of fascicle stretch was 10.77 ± 4.18 mm (~18% L_o) while for the SEE the stretch
326 amplitude was 38.74 ± 9.41 mm. When compared to the stretch amplitude of the MTU (42.56 ± 9.56
327 mm), and accounting for the pennation angle of the muscle fascicles, the tendon contributed 91.02% to
328 the stretch of the MTU at the beginning of backward, downhill walking. Further to this, the time at
329 which maximum fascicle length occurs during the stride cycle was compared to MTU to identify
330 changes in the rate of stretch (Figure 3A-C). For MTU, peak stretch length occurred at 26 ± 3% of
331 stride time, which was significantly less than fascicle peak stretch length that occurred at 37 ± 3% of
332 stride time ($P \leq 0.05$). This indicates that the muscle fascicles continued to absorb mechanical work
333 while the MTU and SEE were shortening.
334
335 Discussion
336
337 The present study investigated human in vivo muscle mechanics of the MG before, during and after 1-
338 hr of backward, downhill walking. The 23% drop in maximum torque and 10% increase in the optimal
339 fascicle length for torque production observed 2-hr post-exercise indicate that the task induced muscle
340 damage, since most, if not all, fatigue-related force decrements typically recover within this timeframe
341 (15, 49, 54). We believe that this is the first documentation of muscle fascicle length changes during
342 exercise that induces muscle damage in humans. Importantly, damage occurred in response to low
343 amplitude muscle fascicle stretches (~18%) and fascicle lengths appeared to operate predominately on
344 the ascending limb and plateau region. While some participants reached a peak stretch on the

descending limb of the L-T curve, there was no relationship between the maximum stretch length and the amount or presence of EIMD. In contrast, the SEE experienced much larger strains (~90% of total MTU strain), which suggests that compliant tendons play a major role in minimising damage to the muscle by buffering much of the overall MTU stretch (2). This mechanism may be critical in allowing muscles to absorb mechanical work effectively without adverse muscle strains. These conditions contrast markedly with those in typical reduced muscle preparations, which suggests that mechanisms of damage revealed in vitro or in situ may not be applicable to intact human muscles with compliant tendons. This may explain why multiple stretches are required to induce substantial muscle damage in vivo.

Strains and strain rates responsible for muscle damage in vivo

To our knowledge, this is the first time that muscle fascicle length has been measured during a classic muscle damaging exercise in humans in vivo. We observed the MG muscle fascicle to stretch over an amplitude of 18% of L_o at the beginning of 1-hr backward downhill walking at lengths corresponding to the ascending limb and plateau region of the L-T curve. While EIMD occurred during this exercise protocol, the strain experienced by the fascicles was much smaller than is typically produced in situ and in vitro (4, 24, 58). It is likely that this factor contributed to the modest, 23% reduction in maximum torque 2-hr post-exercise in comparison to the large force decrements (up to ~60-80%) reported in situ and in vitro with as few as 1-10 active stretches (3-7, 58). We suggest that the extensive damage that occur in situ and in vitro is the result of muscle fibre stretches that are of an amplitude, or occur at lengths, that are unlikely to occur under natural conditions in human lower limb muscles with significant series compliance, such as the gastrocnemius muscles (8, 10).

The amplitude of stretch measured here is quite similar to an animal in vivo study by Butterfield & Herzog (9), who found that the stretch of rabbit hind limb muscle fibres was no greater than 16% of L_o.

In the present study, we observed that the tendon contributed ~90% to overall MTU length change during stretch, suggesting that the compliant tendon minimised the amplitude of stretch of the fascicle. This indicates that Achilles tendon and gastrocnemius muscle compliance is important beyond its capacity for storage and return of elastic energy for efficient locomotion (13, 16, 30, 47), and that it also has an important role in attenuating muscle stretch during eccentric contractions as has recently been demonstrated in running turkeys (47). The current experiment is not able to determine which part of the tendinous tissue is undergoing the most stretch, however high strain in comparison to the muscle fascicles is likely to occur in both the aponeurosis and Achilles tendon, similar to hopping or running (28, 29).

In addition to reducing the extent of fascicle stretch, the tendon took up most of the initial high velocity stretch and then, as it shortened, the fascicles lengthened to absorb energy. This effectively reduced the peak strain rate of the fascicles (47). The velocity of stretch is thought to play some role in the magnitude of EIMD, especially at large stretch amplitudes (6, 52). However, strain rates reported in reduced animal preparations are typically much higher (3-16 L_s/s) (6, 52) than those observed in our current experiment (~1 L_s/s). As such, our results suggest that the compliance of the tendon has an effect on buffering the both the magnitude and the rate of muscle fascicle length changes during EIMD in humans.

Muscle fascicle length ranges during EIMD in vivo

We have shown here for the first time in humans that EIMD can occur when exercising at lengths corresponding to the ascending limb or plateau region of L-T curves constructed from muscle fascicle measurements. We also found no relationship between the final length the muscle fascicle stretches to and the presence or amount of muscle damage incurred. This appears inconsistent with the mechanisms of EIMD proposed in the 'popping sarcomere hypothesis', according to which, the production of EIMD

requires sarcomeres to be stretched to muscle lengths that correspond to the descending limb of the length-tension curve (40). However, the current findings do not discount the possibility that stretching sarcomeres to very long lengths exacerbates reductions in muscle force and muscle fibre structural damage. In fact, because the lengths of sarcomeres along muscle fibres may be heterogeneous (1, 44, 45), it remains possible that some sarcomeres were stretched to sufficiently long lengths during our backward walking protocol to be consistent with the popping sarcomere hypothesis.

Because the triceps surae L-T relationship was developed from MG fascicle length and total plantar flexor torque, this L-T relationship may misrepresent the mechanical properties of the soleus or lateral gastrocnemius if their muscle properties were substantially different from those of the MG (i.e. if soleus or lateral gastrocnemius acted on a different part of the L-T curve). However, a recent study has shown that soleus fascicles shorten over the ascending limb of the soleus length-tension curve during forwards walking (48). As the kinematics and pattern of activation of muscles are similar, but in reverse, for forward and backward walking (25, 53), it seems likely that soleus fascicles also lengthen over the ascending limb during backward, downhill walking. Furthermore, it has been shown that all three triceps surae muscles operate along the ascending limb and plateau region of their length-tension curves for isometric MVCs performed across the ankle range of motion (33, 34). Thus, we believe that the MG-constructed L-T curves described here provide an accurate representation of the triceps surae length-tension relationship.

Although the prolonged backward walking protocol we employed probably caused both progressive damage and fatigue within the gastrocnemius muscle, the muscle mechanics remained largely similar across the 1-hr walking period (Figure 3A-C). There was no change in peak fascicle length during stretch, indicating no lengthening of the fascicle during the muscle damage protocol. The lack of change in mechanical behaviour could be considered surprising given that we did find a significant

shift in the optimum length and maximum force generating capacity assessed after the effects of fatigue had subsided (Fig. 2C). However, given that the forces required to walk backward remained the same across time (as the walking speed did not change), and that MTU length changes were dominated by SEE rather than fascicle length changes at the beginning of the muscle damage protocol, it is not surprising that the extent of fascicle stretch remained similar across time. In fact, the rightward shift in the length tension relationship ensured that the fascicle operated on the ascending limb of the L-T relationship throughout the task, and did not stretch to longer lengths that might result in more damage.

Conclusion

We have shown here for the first time that human gastrocnemius muscle fascicles experience relatively small strains during prolonged backward downhill walking that causes muscle damage. However, the level of EIMD produced was much less than typically observed for in situ or in vitro preparations that involve larger stretch amplitudes. We also showed that eccentric exercise does not have to be performed at fascicle lengths corresponding to the descending limb of the length-tension curve in order to induce muscle damage. Investigations are required to determine whether sarcomere heterogeneity can lead to some sarcomeres being stretched to long lengths when the fascicles operate at such short lengths. As the tendon contributed a large amount of the stretch during the backward walking protocol, we attribute the attenuation of MG fascicle stretch to tendon compliance, which obviously plays an important role in protecting such muscles from stretch during energy absorbing activities. Future studies should measure fascicle length changes during different eccentric exercise protocols in humans, in order to better understand how fascicle and tendon dynamics influence EIMD under natural conditions.

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587 injury on force and intracellular pH in rat skeletal muscles. *J Appl Physiol* 92: 93–99, 2002.

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592 **Figure legends:**

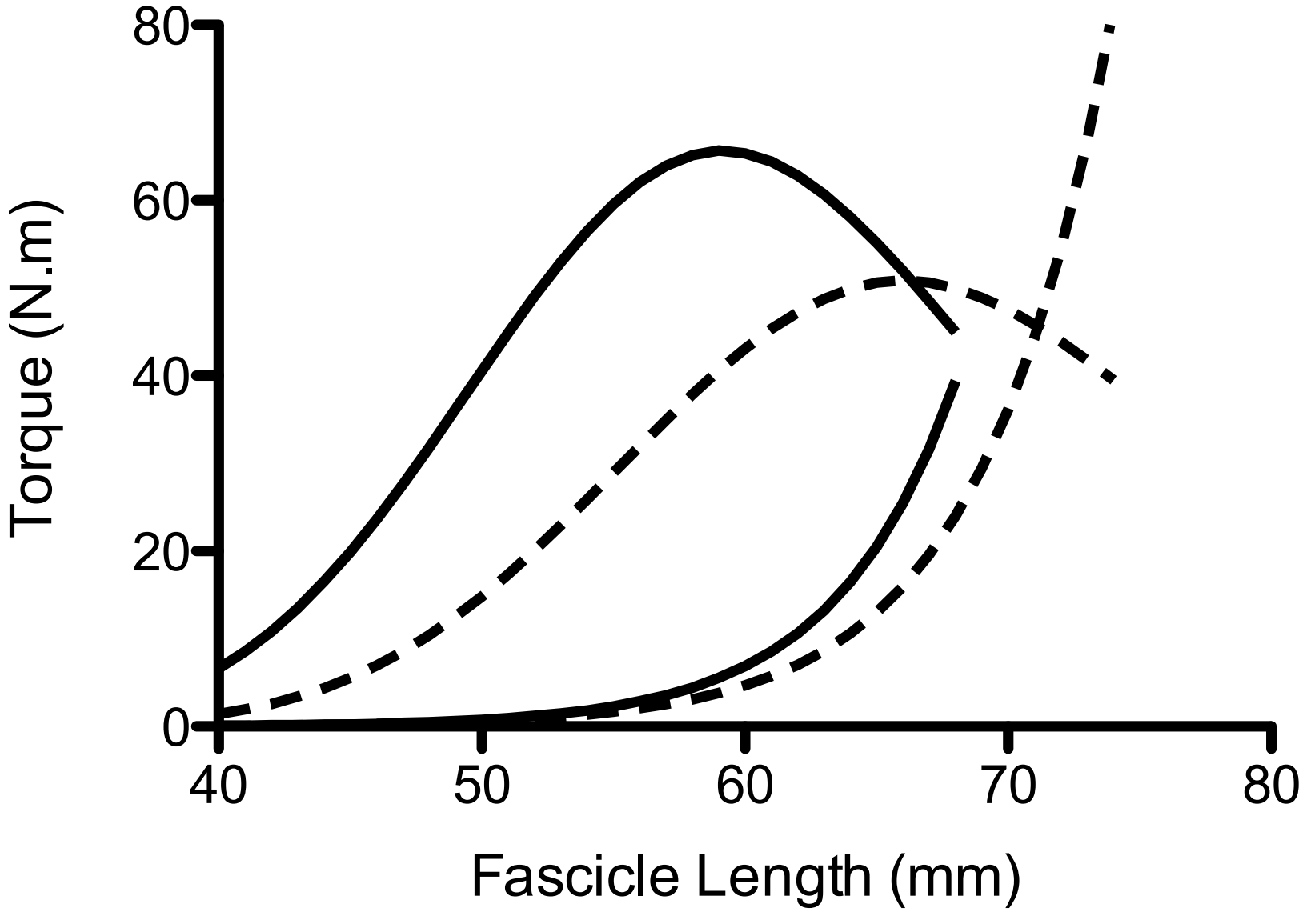
593 **Figure 1.** Mean active and passive L-T curves pre- (solid line) and 2-hr post- (dashed line) 1-hr
594 backward downhill walking. Indications of the standard deviations can be found in Table 1.

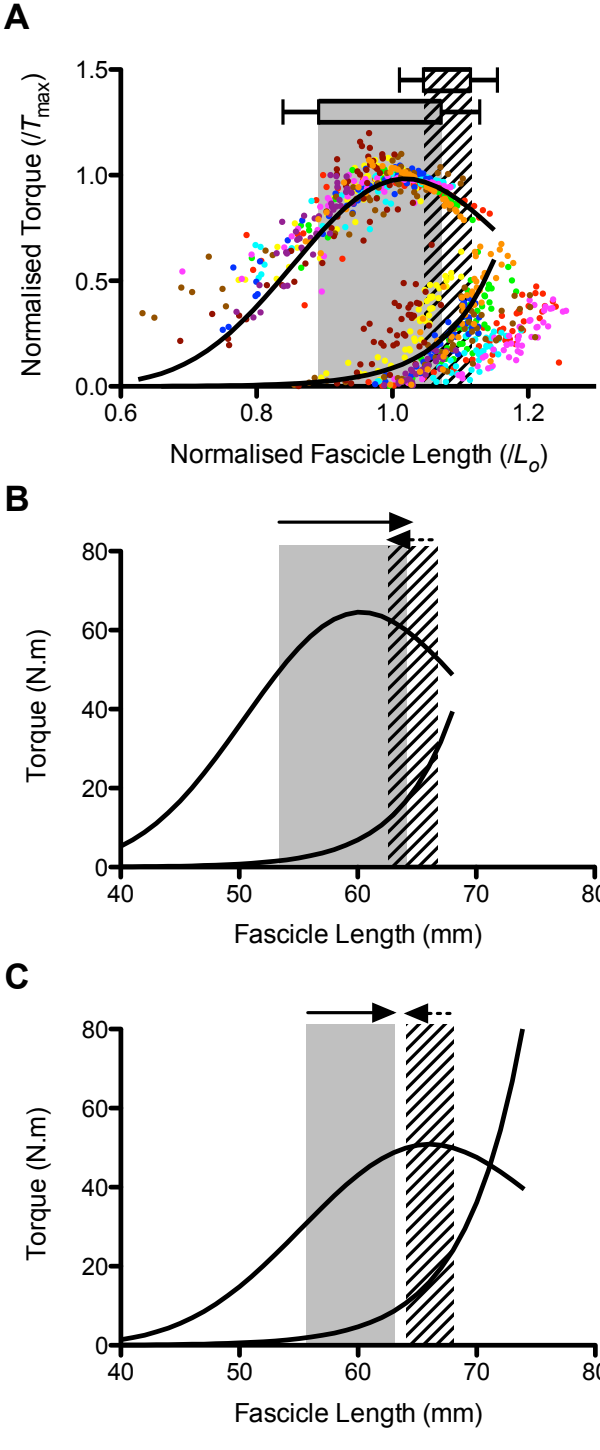
595

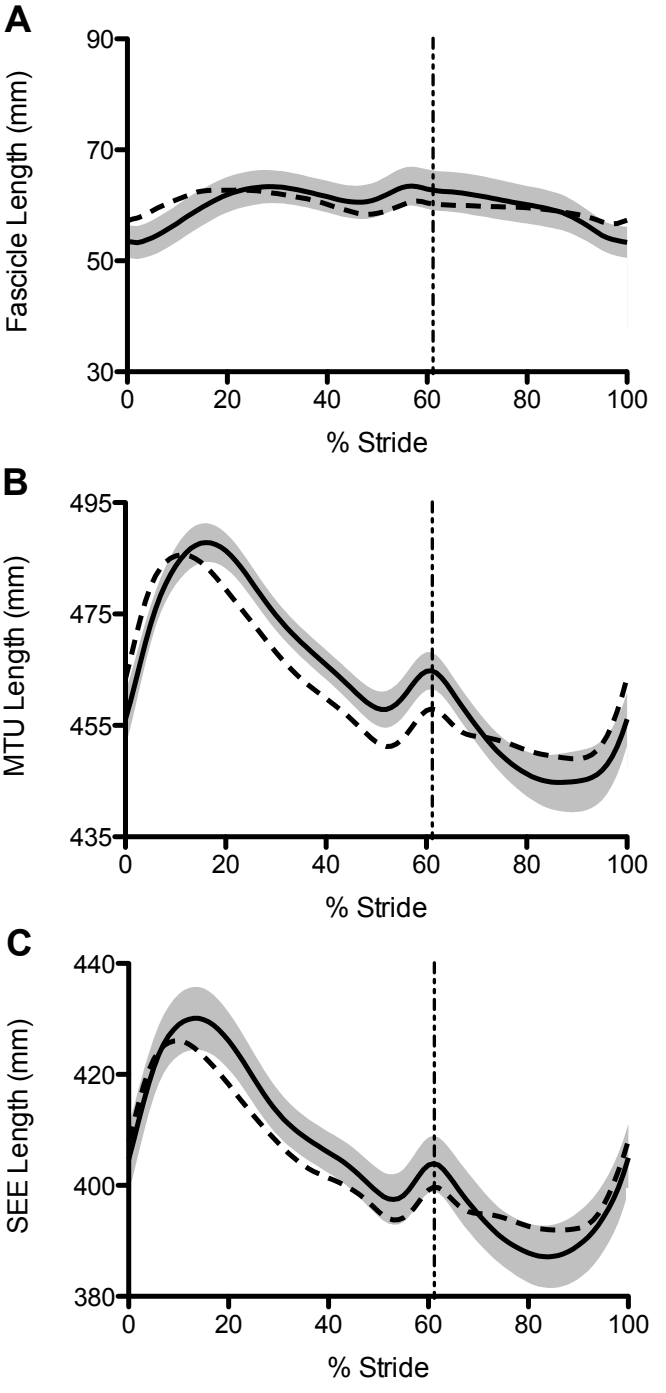
596 **Figure 2.** Group mean active and passive length-torque (L-T) curves with working fascicle length
597 ranges. *A)* An example of how the calculated group mean L-T curves were fit through individual
598 subject L-T data points (each colour represents an individual subject). Fascicle length is normalised to
599 the calculated optimum length and torque is normalised to the calculated maximum torque.
600 Superimposed onto the L-T curves are the fascicle length ranges during the initial part of the stance
601 phase during forward flat walking prior to the muscle damaging exercise (black and white diagonal
602 shading) and during the first minute of backward downhill walking (grey shading). The horizontal error
603 bars indicate the SEM of the group mean minimum or maximum fascicle length. *B)* Pre- and *C)* 2-hr
604 post 1-hr backward downhill walking L-T curves with working fascicle length ranges. Arrows indicate
605 the direction of stretch (backward downhill walking; solid arrow) or shortening (forward walking;
606 dotted arrow) that occurs relative to the L-T curve.

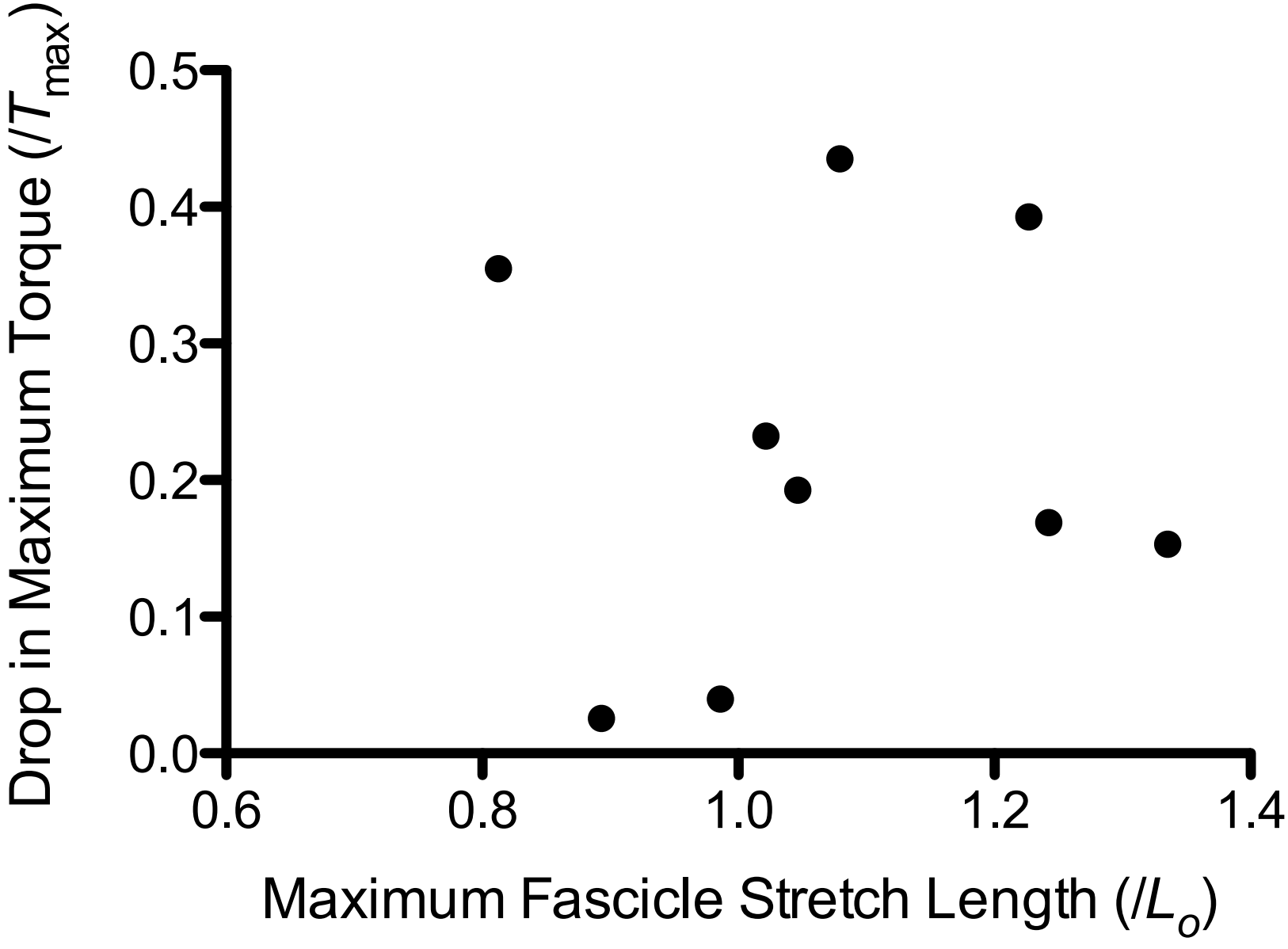
607

608 **Figure 3.** Group mean stride traces of *A)* fascicle, *B)* muscle-tendon unit (MTU) and, *C)* series elastic
609 element (SEE) length at the beginning (solid line) and at the end of 1-hr backward downhill walking
610 (dashed line). Grey shading indicates the standard error of the mean (SEM) for the stride length trace at
611 the beginning of 1-hr backward downhill walking. As the variability is similar across both conditions,
612 only one SEM is displayed for reader clarity. The vertical line indicates the stance phase (left) and
613 swing phase (right) for the stride cycle.
614
615 **Figure 4.** Drop in maximum torque (T_{max}) 2-hr post-exercise plotted against the maximum fascicle
616 stretch length experienced during the beginning of 1-hr backward downhill walking for each individual
617 subject. Fascicle length is normalised to the calculated optimum length and torque is normalised to the
618 calculated maximum torque. If a strong positive correlation between the amount of muscle damage
619 (drop in torque) and the operating length of the fascicle (maximum stretch length) is found then this
620 may suggest a length dependency to the muscle damage experienced during the backward walking
621 protocol.
622
623 **Figure 5.** Group mean fascicle length stride traces prior to muscle damage occurring during forward
624 flat walking (dashed line), backward flat walking (dotted line) and the first minute of backward
625 downhill walking (solid line). Grey shading indicates the SEM of backward downhill walking. As the
626 variability is similar across all conditions, only one SEM is displayed for reader clarity. The forward
627 flat walking trace has been reversed (toe-off (TO) to heel-strike (HS)) to match the pattern of backward
628 walking (toe-strike (TS) to heel-off (HO)) as indicated by the horizontal arrows. The vertical line
629 indicates the stance phase (left) and swing phase (right) for the stride cycle.
630


80
60
40
20
0
Torque (N.m)
40
50
60
70
80
Fascicle Length (mm)


A
Normalised Torque (/T_{max})
1.5
1.0
0.5
0.0
0.6
0.8
1.0
1.2
Normalised Fascicle Length (/L_o)
B
Torque (N.m)
80
60
40
20
0
40
50
60
70
80
Fascicle Length (mm)
C
Torque (N.m)
80
60
40
20
0
40
50
60
70
80
Fascicle Length (mm)


A
Fascicle Length (mm)
90
70
50
30
0
20
40
60
80
100
% Stride
B
MTU Length (mm)
495
475
455
435
0
20
40
60
80
100
% Stride
C
SEE Length (mm)
440
420
400
380
0
20
40
60
80
100
% Stride


Drop in Maximum Torque (/T_{max})
0.5
0.4
0.3
0.2
0.1
0.0
0.6
0.8
1.0
1.2
1.4
Maximum Fascicle Stretch Length (/L_o)

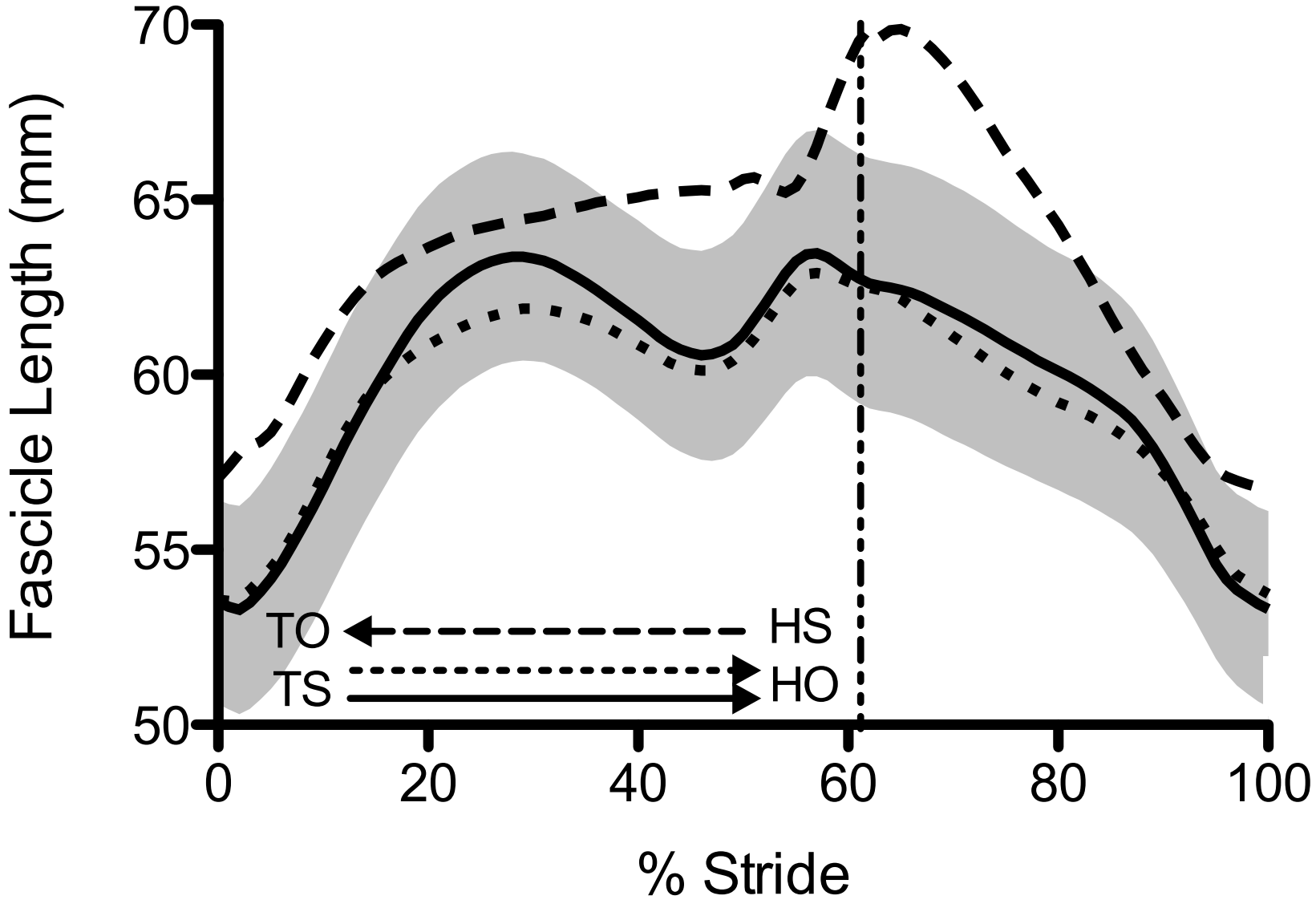

Fascicle Length (mm)
70
65
60
55
50
0
20
40
60
80
100
% Stride
TO
HS
TS
HO

Table 1. *Active and passive length-torque curve parameters pre- and 2-hr post 1-hr backward downhill walking.*

	T_{max} (N.m)*	L_o (mm)*	k	A	L_s (mm)
Pre	65.69 ± 7.88	59.13 ± 6.23	0.22 ± 0.07	0.18 ± 0.01	58.41 ± 5.33
2-hr Post	50.89 ± 9.51	66.01 ± 8.71	0.20 ± 0.06	0.17 ± 0.04	60.19 ± 7.56

T_{max}, maximum torque; L_o, optimal fascicle length; k, stiffness; A, curvature; L_s, slack fascicle length. Values are mean ± SD. * indicates significant difference between pre- and 2-hr post exercise ($P \leq 0.05$).

Table 2. *Medial gastrocnemius fascicle, muscle-tendon unit and series elastic element stretch amplitude, maximum stretch velocity and peak length during stretch during backward flat walking, the start of, or the end of 1-hr backward downhill walking.*

	Stretch Amplitude (mm)		Maximum Stretch Velocity (L_s/s)		Peak Length During Stretch (mm)	
	BFW	Start BDW	BFW	Start BDW	Start BDW	End BDW
Fas	9.61 ± 4.05	10.77 ± 4.18	1.30 ± 0.39	1.34 ± 0.45	64.17 ± 8.74	63.00 ± 8.70
MTU	42.32 ± 10.14	42.56 ± 9.55	6.73 ± 1.63	6.58 ± 1.97	487.9 ± 31.4	485.7 ± 38.9
SEE	38.30 ± 10.56	38.74 ± 9.41	6.51 ± 1.34	6.54 ± 1.71	429.3 ± 30.6	425.4 ± 38.1

Fas, fascicle; MTU, muscle-tendon unit; SEE, series elastic element; BFW backward flat walking; BDW backward downhill walking; L_s/s, fascicle slack lengths / sec. All values are mean ± SD.