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Differences in in-vivo muscle fascicle and tendinous tissue behaviour between the ankle plantarflexors during running

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Abstract

The primary human ankle plantarflexors, soleus (SO), medial gastrocnemius (MG) and lateral gastrocnemius (LG), are typically regarded as synergists and play a critical role in running. However, due to differences in muscle-tendon architecture and joint articulation, the muscle fascicles and tendinous tissue of the plantarflexors may exhibit differences in their behaviour and interactions during running. We combined *in-vivo* dynamic ultrasound measurements with inverse dynamics analyses to identify and explain differences in muscle fascicle, muscle-tendon unit (MTU) and tendinous tissue (SEE) behaviour of the primary ankle plantarflexors across a range of steady-state running speeds. Consistent with their role as a force generator, the muscle fascicles of the uni-articular SO shortened less rapidly than the fascicles of the MG during early stance. Furthermore, the MG and LG exhibited delays in tendon recoil during the stance phase, reflecting their ability to transfer power and work between the knee and ankle via tendon stretch and storage of elastic strain energy. Our findings add to the growing body of evidence surrounding the distinct mechanistic functions of uni- and bi-articular muscles during dynamic movements.

Keywords: running, muscle fascicles, tendon, bi-articular, muscle function

Introduction

The primary human ankle plantarflexors, soleus (SO), medial gastrocnemius (MG) and lateral gastrocnemius (LG), play an important role in supporting and accelerating the body across a wide range of running speeds [1,2]. They connect to a common Achilles tendon and together are the dominant contributors to ankle torque generation. In this respect, the SO, MG and LG are typically regarded as synergists. Nevertheless, for a variety of reasons, it is plausible that the mechanical behaviour of these muscles, as well as the muscle fascicle and tendinous tissue interactions, differ during running.

The SO is a uni-articular muscle spanning the ankle whereas the MG and LG are bi-articular muscles spanning both the ankle and knee. Previous studies have suggested that muscles which span multiple joints have distinct functions — uni-articular muscles function primarily as force and power

generators whereas bi-articular muscles modulate the moments and powers developed about the joints they span [3,4]. A previous modelling study using a joint power approach suggested that during the absorption and push-off phases of running, the gastrocnemii muscles transport mechanical power between the ankle and knee [5]. However, this study was unable to distinguish the contributions of the muscle fascicles and associated tendon. Alternatively, modelling and *in-vivo* studies have observed that during jumping, the mechanical power transported between the knee and ankle via the gastrocnemii muscles contributes to the enhanced storage of elastic strain energy [6,7]. This enhanced storage of strain energy was in part due to the delayed onset of tendon recoil of the gastrocnemii muscles compared with the SO during the propulsion phase. It remains unclear, however, whether observed functional differences between the uni- and bi-articular muscles during movements such as jumping are also evident in the *in-vivo* behaviour of the ankle plantarflexors during running.

Ultrasound studies have demonstrated that the SO and MG have differing MTU and *in-vivo* muscle fascicle behaviours during walking. Ishikawa et al. (2005) and Cronin et al. (2013) observed that the muscle fascicles of the SO have slower shortening velocities than those of the MG, and that the SO appears to play a more significant functional role in the catapult-like action of the plantarflexors [8,9]. This behaviour is consistent with a recent modelling study showing that the SO shortened at slower speeds than MG during walking and was therefore metabolically more efficient [10]. However, walking and running place different mechanical demands on the ankle plantarflexors; for example, the knee undergoes a larger joint excursion and generates a higher extensor moment during the stance phase of running compared to walking [11], which likely impacts the behaviour of the gastrocnemii. Therefore, *in-vivo* muscle fascicle and MTU behaviour during running is unlikely to mimic muscle-tendon behaviour observed for walking.

The present study combined *in-vivo* ultrasound measurements of the SO, MG and LG with an inverse dynamics analysis to identify and explain differences in *in-vivo* muscle fascicle, MTU and tendinous tissue behaviour of the ankle plantarflexors across a range of steady-state running speeds. We hypothesised that (1) the SO muscle fascicles will display a slower shortening velocity relative to the MG and LG across all speeds; and (2) compared with the SO, tendon recoil in the MG and LG will be delayed as these muscles transfer mechanical power between the knee and ankle at all running speeds.

Methods

Human experiments

Ten recreational athletes (9 males and 1 female; mean (SD) age = 27.5(5.6) years; height = 180.8(7.7) cm; body mass = 80.2(11.7) kg) with no pre-existing musculoskeletal injuries were recruited to this study, which was approved by the research ethics committees at the University of Melbourne and the University of Queensland.

Small retro-reflective markers (14 mm diameter) were placed at specific anatomical locations on the participants' trunk, arms and lower-limbs (for full details of marker locations, see Lai et al. (2015)). Participants ran at four different steady-state speeds (2 m s^{-1}, 3 m s^{-1}, 4 m s^{-1} and 5 m s^{-1}) using a self-selected foot strike pattern, cadence and stride length. Participants wore minimalist shoes (Xeroshoes, Boulder, CO, USA) to protect their feet from the surface of the instrumented treadmill and to maintain exposure of the dorsal aspect of the foot for marker placement.

Three-dimensional (3D) marker trajectories were recorded using an 8-camera, video-based, motion analysis system (Qualisys, Gothenburg, Sweden) sampling at 250 Hz. Ground reaction forces (GRFs) were recorded using two force plates embedded in an instrumented treadmill (Tandem Treadmill, Advanced Mechanical Technology Inc. (AMTI), Watertown, MA, USA) sampling at 1500 Hz. A 'stitching' algorithm was used to compute the resultant GRF, centre of pressure and free moment vectors from measurements obtained from the two force plates. Marker trajectories were filtered with a fourth-order, low-pass, Butterworth filter with a cut-off frequency of 15 Hz while the resultant GRF data were filtered using a fourth-order, low-pass, critically-damped filter operating at a cut-off frequency of 15 Hz.

A PC-based B-mode ultrasound scanner (Telemed Echo Blaster 128, Vilnius, Lithuania) recorded images at 80 Hz. A 96-element, linear, flat-profiled ultrasound probe operated at a scanning depth and width of 60 mm and a sampling frequency of 7 MHz. Ultrasound data were recorded from the right leg of each participant. Ultrasound images of the MG, LG and SO muscle fascicles were collected in two separate trials. The MG was imaged by placing the ultrasound transducer at the mid-belly of the MG whereas the SO and LG were imaged by placing the transducer at the mid-belly of

the LG. The ultrasound probe was manipulated until the deep and superficial aponeuroses and the distinct striated patterns of connective tissue defining the muscle fascicles were visible [12]. We assumed homogeneous behaviour in the muscle fascicle throughout the muscle belly [13].

Electromyographic (EMG) signals from Ag/AgCl bi-polar surface electrodes were recorded simultaneously with the ultrasound data using a wireless system (Noraxon, Scotsdale, AZ, USA) sampling at 1500 Hz. The EMG electrodes were placed on the mid-bellies of the SO, MG and LG of the right leg according to standardised recommendations [14]. A linear envelope of the raw EMG signal was calculated using the root-mean-square of a moving window (100ms). Each muscle's linear envelope was then normalised to the peak magnitude of the respective linear EMG envelope measured for running at 5 m s^{-1}. All experimental data were synchronised via a digital output signal generated by the ultrasound scanner that triggered the collection of the marker trajectories, ground reaction forces and EMG signals.

Computational simulations

OpenSim™ (v3.3) [15] was used to calculate joint angles, joint angular velocities, net joint moments and joint powers for the ankle and knee as well as MTU lengths and velocities for the SO, MG and LG. A generic 12-segment, 31-degree-of-freedom (DOF) musculoskeletal model was scaled to each participant's body anthropometry (Arnold et al., 2010). The knee and ankle were each represented as a 1-DOF hinge joint. Inverse kinematics and inverse dynamics were used to compute ankle and knee joint angles and net joint moments, respectively. Joint powers were found by multiplying the net moment at each joint by the corresponding angular velocity at each time interval. Net joint moments and joint powers were normalised to body mass.

Data processing

In-vivo muscle fascicle length and pennation angle were obtained from the ultrasound images (Fig. 1). The length of the muscle fascicle was defined as the distance between the superficial and deep aponeuroses parallel to the collagenous tissue. The pennation angle (α) was defined as the angle between the collagenous tissue and the deep aponeurosis (Fig. 1). A previously validated automatic tracking algorithm was used to quantify the muscle fascicle length and pennation angle [16]. Tracked muscle fascicle lengths and pennation angles were visually examined to ensure the automatic algorithm accurately tracked the length and angle changes. Whenever the muscle fascicle length or

pennation angle was deemed inaccurate, the two points defining the muscle fascicles were manually repositioned.

The MTU length of each plantarflexor was computed at each time interval using the distance between the scaled origin and insertion sites in the musculoskeletal model and the instantaneous joint angle. The series elastic element (SEE) represented all tendinous tissue, which included the free tendon, aponeurosis and connective tissue. SEE was defined as the difference between the model-based MTU length and the ultrasound-measured muscle fascicle length, which was rotated by the ultrasound-measured pennation angle to align with the direction of the line of force application. Each plantarflexor MTU was modelled with an individual SEE rather than a common Achilles tendon. MTU and muscle fascicle lengths were normalised by resting muscle fascicle length during static standing (l_s^m). SEE lengths were normalised by resting SEE length during static standing (l_s^t). Mean resting fascicle and SEE lengths during static standing were 0.051 ± 0.009 m and 0.282 ± 0.018 m for the SO, respectively; 0.058 ± 0.01 m and 0.481 ± 0.029 m for the MG, respectively; and 0.063 ± 0.007 m and 0.479 ± 0.026 m for the LG, respectively. MTU, muscle fascicle and SEE length changes were calculated as the change from the normalised length of each component at foot strike. MTU, muscle fascicle and SEE velocities were computed by differentiating the lengths of each component with respect to time.

Data analysis

Data were processed for five stance phases of running for each participant. The experimental data were interpolated to 200 sample points, averaged for each participant, and used to calculate a group mean. Stance phase was further separated into two periods: the first between foot strike and the time of peak ankle moment (henceforth 'ankle moment development'), and the second between the time of peak ankle moment and toe-off (henceforth 'ankle moment decline'). A two-way repeated measures ANOVA (IBM SPSS v23.0, IBM Corp., Armonk, NY, USA) was used to test whether significant main effects were present for 'muscle' (3 levels: SO, MG, LG) and 'running speed' (4 levels: 2 m s^{-1}, 3 m s^{-1}, 4 m s^{-1} and 5 m s^{-1}) and whether a significant interaction existed between these two main effects. The following outcome measures were tested: mean velocity of the MTU and muscle fascicles during ankle moment development and ankle moment decline; and peak SEE length change, timing of peak SEE recoil, and timing of peak EMG activity during the entire stance phase. When significant main effects were found, a post-hoc pairwise comparison using Fisher's least significant

difference was used to determine which muscle and which speed was significantly different. All data extracted for statistical analysis were assessed to be normally distributed using a Shapiro-Wilk normality test ($p > 0.05$). Given the number of comparisons, a conservative p-value of 0.01 was assumed.

Results

For all running speeds other than 5 m s^{-1}, the SO muscle fascicles exhibited a slower shortening velocity throughout stance compared to the MG and LG fascicles. During ankle moment development, the SO fascicles shortened significantly slower than the MG fascicles (Fig. 2, Table 1; *p = 0.001*), especially at 2 m s^{-1} and 3 m s^{-1}, where the difference in shortening velocity was on average 0.38 l_s^m s^{-1}. The SO fascicles shortened on average 0.15 l_s^m s^{-1} slower than the LG fascicles during the same period of the stance phase, but this difference did not reach statistical significance (*p = 0.022*). The SO fascicles also shortened on average 0.07 l_s^m s^{-1} slower than the MG fascicles during ankle moment decline at all speeds, but this comparison also did not reach statistical significance (*p=0.1*). There was no statistically significant difference in fascicle shortening velocity between the SO and LG during ankle moment decline (*p=0.15*) (Table 1; Fig. 2).

The reduced shortening velocities in the SO muscle fascicles during ankle moment development compared with those of the MG, and to a lesser extent LG, coincided with differences in MTU and SEE behaviour (Figs. 3-4; Table 1). During early stance, the MG and LG MTUs underwent a period of initial shortening between foot strike and approximately 20% of stance, before lengthening thereafter until the time of peak ankle moment (Fig. 3). The initial shortening was consistent with the period when the knee flexed and absorbed power (Fig. 5), and consequently, the MTU and SEE lengthening velocities were significantly greater for the SO compared to the MG (both *p < 0.001)* and LG (both *p < 0.001)* during early stance (Figs. 3-4). Similarly, during late stance, MTU shortening velocity and SEE recoil velocity were significantly greater for the SO compared to the MG (both *p < 0.001)* and LG (both *p < 0.001)*.

The SEE for SO stretched more and commenced recoiling earlier during stance phase relative to the SEEs for MG and LG (Fig. 4). The SO SEE stretched on average 0.04 and 0.046 l_s^t longer than the MG SEE (*p < 0.001)* and LG SEE (*p < 0.001),* respectively, across all speeds (Table 1). Furthermore, the SO SEE began to recoil on average 8.8% of the stance phase earlier than the MG SEE (*p < 0.001*) and

6.5% of the stance phase earlier than the LG SEE ($p < 0.001$) across all running speeds (Table 1). The SO SEE began to recoil at the start of the ankle moment decline when the ankle power transitioned from absorption to generation, whereas the MG and LG SEEs continued to stretch and only began to recoil once the knee extension moments and powers were negligible (Fig. 5). Finally, there were no significant differences in the timing of peak EMG activity between SO, MG and LG (Fig. 6; Table 1).

Discussion

The aim of this study was to identify and explain differences in *in-vivo* muscle fascicle and tendinous tissue behaviour between the ankle plantarflexors across a range of steady-state running speeds. We found that during the early portion of the stance phase, when the ankle joint moment was rapidly increasing and the ankle absorbed power, the SO muscle fascicles shortened at a slower rate than the MG and LG muscle fascicles, which coincided with increased stretching and greater lengthening velocities in the MTU and SEE of the SO compared to the MG and LG. In addition, even though the MG and LG SEEs stretched less than the SO SEE, they continued stretching for a longer portion of the stance phase. This behaviour was most likely attributable to the bi-articular nature of the gastrocnemii, which, we speculate, enabled the power generated by the knee extensors to be stored as elastic strain energy in tendon, thus delaying tendon recoil and enhancing power output at the ankle during the push-off phase of running.

Our observations that the SO muscle fascicles shortened at a lower velocity than those of the MG and LG is consistent with findings of previous *in-vivo* studies of walking [8,9]. The profiles of our MTU and muscle fascicle length changes are also qualitatively similar to results obtained from previous *in-vivo* ultrasound studies involving running [13,17,18]. Previous investigators have reported *in-vivo* muscle fascicle length changes for the SO and MG ranging from 5 to 20 mm for running at speeds ranging from 2 m s^{-1} to 5 m s^{-1}, which is consistent with our absolute peak muscle fascicle length changes of 9, 13 and 9 mm for the SO, MG and LG, respectively.

The relatively slow shortening velocity of the SO muscle fascicles coupled with a greater lengthening of the MTU and greater SEE stretch compared with the MG and LG suggests that the SO functions primarily as a force and work generator, consistent with the behaviour of a uni-articular muscle. The slower shortening velocity of the SO muscle fascicles relative to those of the MG and LG during ankle

moment development may allow the SO muscle fascicles to generate force more efficiently by shortening at a slower rate and maintaining a favourable force-velocity relationship (Fig. 2). Hence, the SO likely generated a higher force and contributed to a greater proportion of the ankle moment during running compared with the MG and LG. In addition, quasi-isometric behaviour of the muscle fascicles of the SO allowed its SEE to undergo greater stretch and recoil during active contraction and thus store and recover a greater amount of elastic strain energy. These functions are consistent with recent modelling studies that quantified muscle fibre and tendon work during running at a comparable steady-state speed [19].

In contrast, the behaviours of the muscle fascicles and SEEs of the MG and LG are consistent with their bi-articular function of transferring power and work between the ankle and knee joints. During early stance, the MTU and muscle fascicles of the MG and LG primarily shortened as the knee flexed and generated an extension moment. During the same period of stance, the ankle generated a plantarflexion moment. It is therefore likely that a portion of the positive muscular power and work generated by the active contraction of the MG and LG was absorbed by the knee extensors during early stance. Thereafter, as the ankle transitioned from absorbing to generating power and work, the MG and LG SEEs continued to stretch and store elastic strain energy until the knee moments and powers were diminished. This type of behaviour is consistent with previous suggestions that mechanical power is transported between the knee and ankle by the MG and LG [5]. However, our results further expand on the mechanisms associated with power transportation by suggesting that some of the power generated by the knee extensors is absorbed by the initial stretch of the MG and LG SEEs, and then returned later during stance, in contrast with the behaviour of the SO SEE. Our findings are also consistent with previous jumping studies, where the transfer of work and power generated by the knee extensors during the propulsion phase enhances the storage of elastic strain energy and delays the onset of elastic recoil in the SEEs of the gastrocnemii muscles [6,7]. Overall, our study adds to the body of evidence surrounding the function of the bi-articular muscles to modulate moments and powers between joints [4].

There are limitations to the *in-vivo* ultrasound and inverse dynamics approach used in this study. First, we did not directly measure or calculate the individual forces generated by the SO, MG and LG during running. Instead, our interpretations regarding the functions of the SO, MG and LG were based on the measured length changes of the MTU, muscle fascicles and SEE as well as the net

moments and powers generated by the muscles spanning the knee and ankle computed from inverse dynamics. Second, our MTU and muscle fascicle measurements may have been affected by the intermuscular transmission of force between the plantarflexors during contraction [20]. However, recent studies have shown that these forces are likely to have a minimal effect on the overall function of the plantarflexors [21]. Third, joint mechanics may have varied between the trials in which the ultrasound images of the MG, SO and LG were recorded. Fortunately, however, cross-correlation found that no differences existed in the spatio-temporal characteristics of the joint angles and torques between the trials. Fourth, the plantarflexors were modelled as individual MTUs with individual SEEs rather than a common Achilles tendon. Recent *in-vivo* and modelling studies have shown that the Achilles tendon experiences non-uniform strain during walking [22], suggesting that the plantarflexors may interact with the highly complex and intertwined Achilles tendon in an independent manner [23]. Finally, length changes of the SEE were calculated indirectly using MTU and *in-vivo* fascicle measurements and represented all tendinous tissue, which included the free tendon, aponeurosis and connective tissue. Therefore, it is possible that differences in the force-generating properties of these individual tendinous tissues [24] and the indirect nature of the SEE calculations [25] affected the overall strain exhibited by the SEE. Nonetheless, the timings of SEE stretch and recoil of the plantarflexors are unlikely to be affected by the heterogeneity of the tendinous tissues.

In summary, we found that the primary human ankle plantarflexors exhibited differences in *in-vivo* muscle fascicle, MTU and tendinous tissue behaviour during running. The slower shortening velocities of the uni-articular SO muscle fascicles reflected its function as a force generator, whereas more rapid shortening in the muscle fascicles and the delayed onset of the SEE recoil of the bi-articular MG and LG reflected these muscles' abilities to transfer power and work between the knee and ankle joints. We suggest that differences in MTU architecture and joint articulation help to explain the observed variations in MTU, muscle fascicle and tendinous tissue behaviour of the SO, MG and LG during running.

Perspective

Historically, the ankle plantarflexors have been considered to work as synergists with their primary function being to generate joint torques about the ankle. However, recent developments in *in-vivo* measurement techniques and musculoskeletal modelling have raised questions regarding their synergistic function, particularly in relation to whether differences in MTU architecture and joint

articulation influence muscle-tendon behaviour and interactions, for example, by maximising power output through the storage and utilisation of tendon strain energy. As more experimental and modelling data come to light, through advances in tendon research [26] and continued investigation on other purportedly synergistic muscles such as the vasti and rectus femoris (e.g. [27]), it is hoped that the distinct mechanistic functions of uni- and bi-articular muscles will be further illuminated.

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Conflict of interest

No conflicts of interest, financial or otherwise, are declared by the author(s).

Author contribution

A.L., A.S. and M.P. designed the study; A.L and G.L. performed the experiments and analysed the data; and all authors drafted, edited and revised the manuscript.

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Figure and table captions

Figure 1: Exemplar ultrasound images of the (A) medial gastrocnemius (MG), (B) lateral gastrocnemius (LG), and (C) soleus (SO). The length of the muscle fascicle (yellow lines) was calculated as the distance between the superficial and deep aponeuroses (red dotted lines) parallel to the collagenous tissue. The pennation angle (α) was calculated as the angle between the line of collagenous tissue and the deep aponeuroses.

Figure 2: Muscle fascicle length changes and velocities for the SO (black solid line), MG (blue dotted line) and LG (red dash-dotted line) for running at four steady-state speeds. Muscle fascicle lengths and velocities were normalised to their corresponding resting fascicle lengths during static standing (l_s^m). Muscle fascicle length changes were calculated as the change from the normalised muscle fascicle lengths at foot strike. Negative and positive velocities denote shortening and lengthening, respectively. The vertical dashed and dotted lines denote the time of peak ankle moment and the time of toe-off, respectively. The images at the top of the figure indicate the configuration of the musculoskeletal model at foot strike, time of peak ankle moment, and toe-off for each running speed. The shaded regions represent ±1SD of the fascicle length changes and velocities for the respective muscles.

Figure 3: Muscle-tendon unit (MTU) length changes and velocities for the SO (black solid line), MG (blue dotted line) and LG (red dash-dotted line) for running at four steady-state speeds. MTU lengths and velocities were normalised to their corresponding resting fascicle lengths during static standing (l_s^m). MTU length changes were calculated as the change from the normalised MTU lengths at foot strike. Negative and positive velocities denote shortening and lengthening, respectively. The vertical dashed and dotted lines denote the time of peak ankle moment and the time of toe-off, respectively. The images at the top of the figure indicate the configuration of the musculoskeletal model at foot

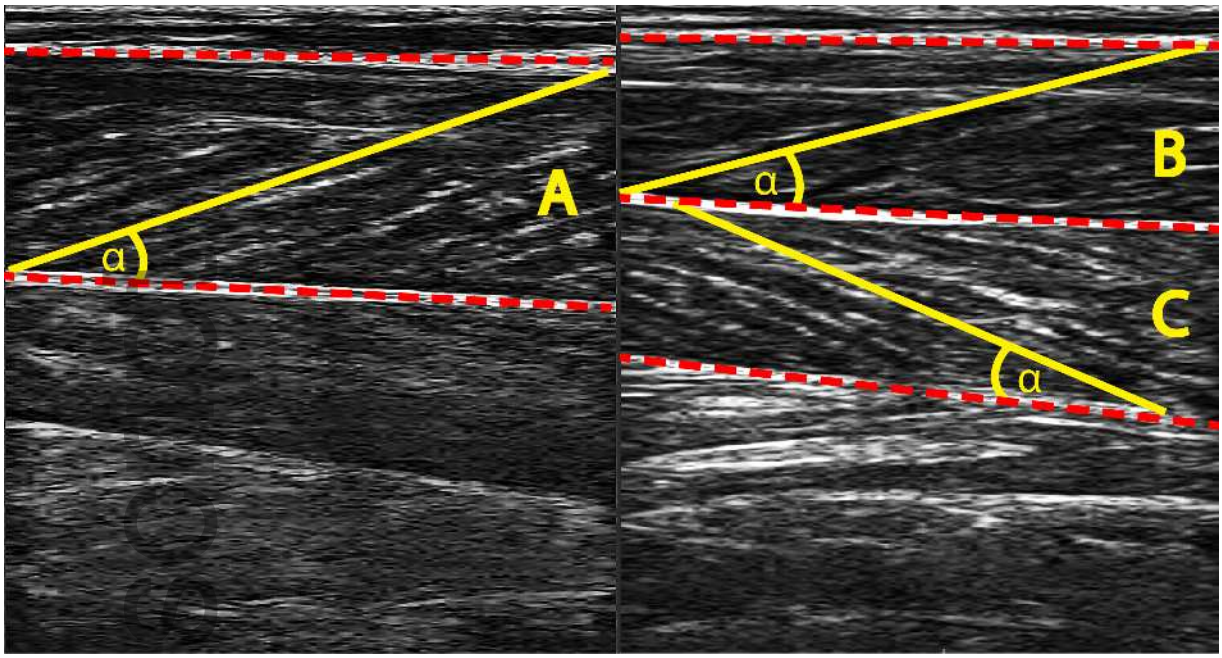
strike, time of peak ankle moment, and toe-off for each running speed. The shaded regions represent ±1SD of the MTU length changes and velocities for the respective muscles.

Figure 4: Series elastic element (SEE) length changes and velocities for the SO (black solid line), MG (blue dotted line) and LG (red dash-dotted line) for running at four steady-state speeds. SEE lengths and velocities were normalised to their corresponding resting SEE lengths during static standing (l_s^t). SEE length changes were calculated as the change from the normalised SEE lengths at foot strike. Negative and positive velocities denote shortening and lengthening, respectively. The vertical dashed and dotted lines denote the time of peak ankle moment and the time of toe-off, respectively. The images at the top of the figure indicate the configuration of the musculoskeletal model at foot strike, time of peak ankle moment, and toe-off for each running speed. The shaded regions represent ±1SD of the SEE length changes and velocities for the respective muscles.

Figure 5: Joint angles, joint angular velocities, net moments and powers calculated at the ankle and knee for running at four steady-state speeds. Net joint moments and joint powers were normalised to body mass.

Figure 6: EMG linear envelope for the SO (black solid line), MG (blue dotted line) and LG (red dash-dotted line) measured for the stance phase of running for four steady-state speeds. EMG activity was normalised to the peak EMG activity measured for running at 5 m s^{-1}. The vertical dashed and dotted lines denote the time at which peak ankle moment was developed and the time at which toe-off occurred, respectively. The images at the top of the figure indicate the configuration of the musculoskeletal model at foot strike, time of peak ankle moment, and toe-off for each running speed.

Table 1: Spatial-temporal parameters illustrating muscle fascicle, MTU and SEE behaviour of the ankle plantarflexors during the stance phase of running at four steady-state speeds. These parameters were used to test statistical significance across muscles and running speeds. When significant main interactions were found, a post-hoc pairwise comparison was performed to determine which muscle and speed was significantly different. Negative and positive velocities denote shortening and lengthening, respectively.



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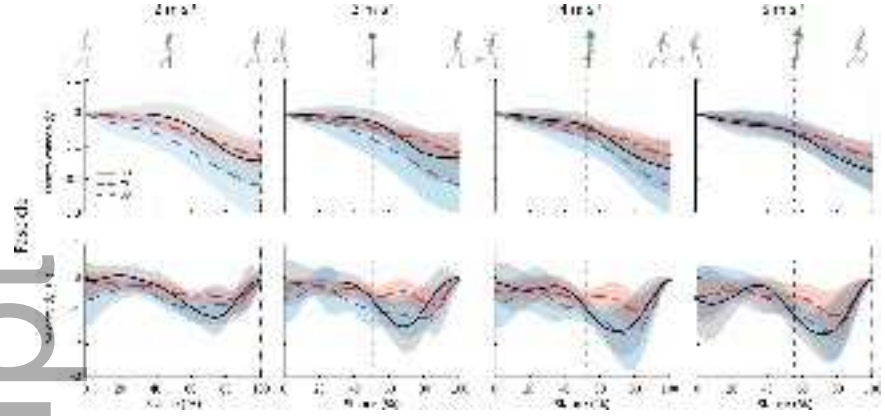

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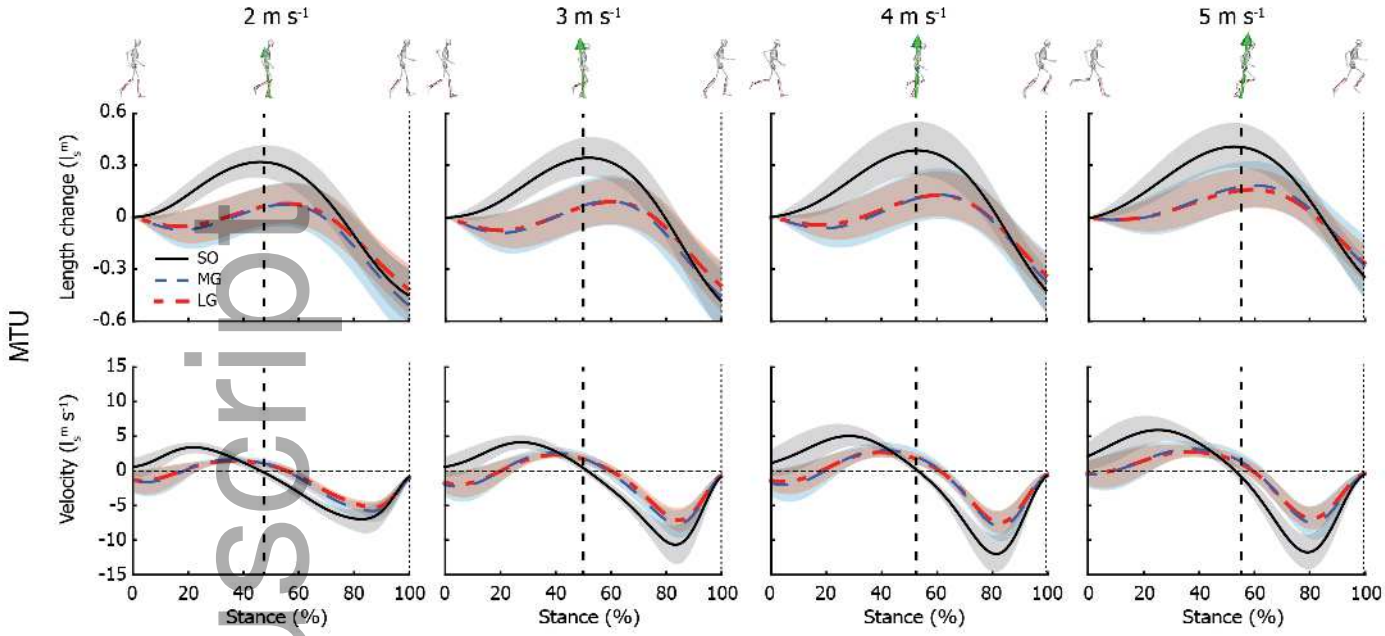

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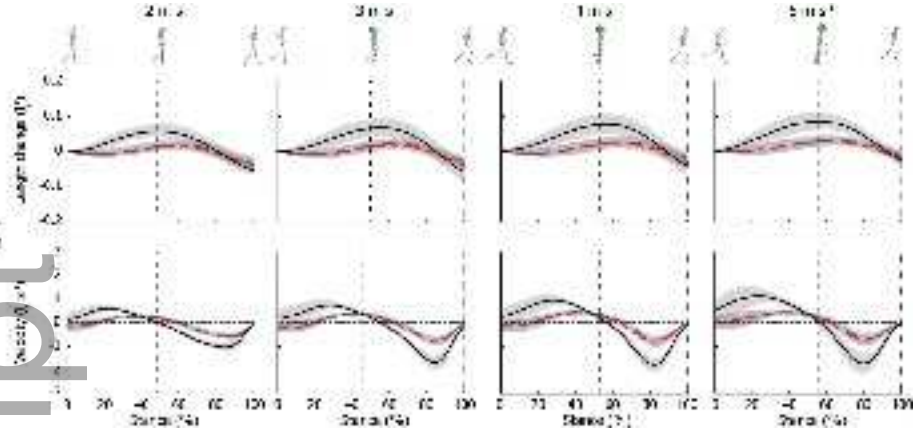

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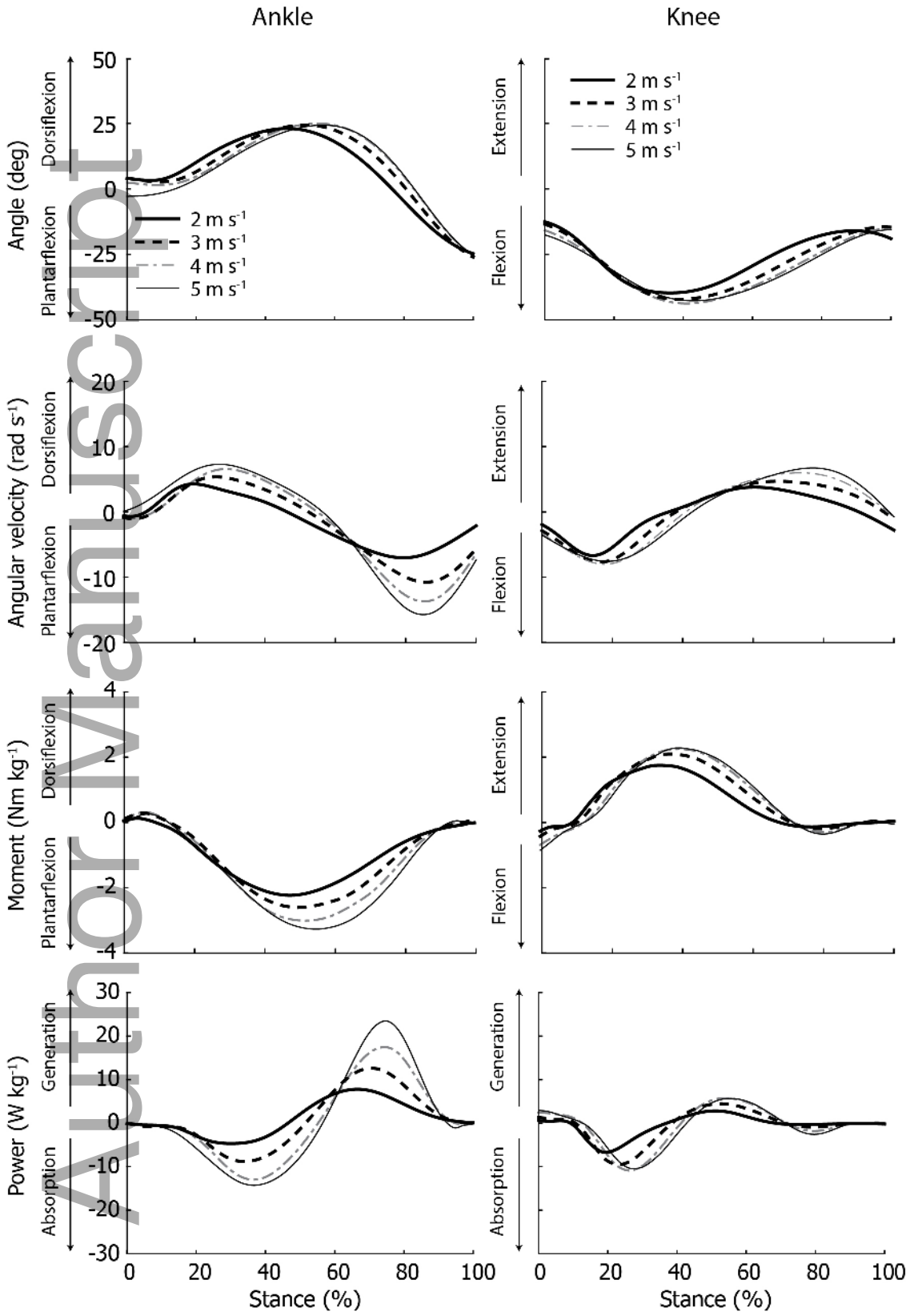

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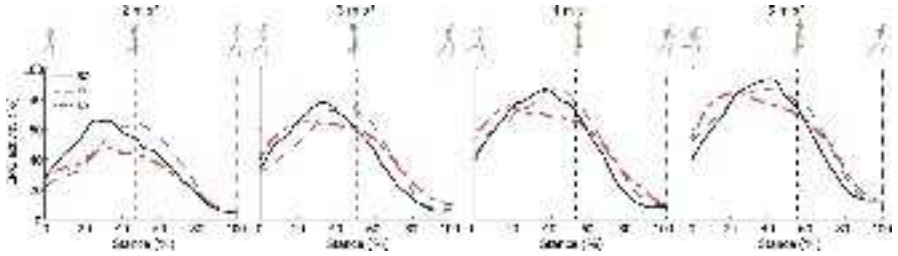

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