In vivo behavior of the human soleus muscle with increasing walking and running speeds

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1Department of Mechanical Engineering, University of Melbourne, Parkville, Victoria, Australia; 2Centre for Sensorimotor Performance, The School of Human Movement Studies, The University of Queensland, St. Lucia, Queensland, Australia; and 3Performance Science and Innovation, Australian Institute of Sport, Belconnen, Australian Capital Territory, Australia

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Lai A, Lichtwark GA, Schache AG, Lin YC, Brown NAT, Pandy MG. In vivo behavior of the human soleus muscle with increasing walking and running speeds. J Appl Physiol 118: 1266–1275, 2015. First published March 26, 2015; doi:10.1152/japplphysiol.00128.2015.—The interaction between the muscle fascicle and tendon components of the human soleus (SO) muscle influences the capacity of the muscle to generate force and mechanical work during walking and running. In the present study, ultrasound-based measurements of in vivo SO muscle fascicle behavior were combined with an inverse dynamics analysis to investigate the interaction between the muscle fascicle and tendon components over a broad range of steady-state walking and running speeds: slow-paced walking (0.7 m/s) through to moderate-paced running (5.0 m/s). Irrespective of a change in locomotion mode (i.e., walking vs. running) or an increase in steady-state speed, SO muscle fascicles were found to exhibit minimal shortening compared with the muscle-tendon unit (MTU) throughout stance. During walking and running, the muscle fascicles contributed only 35 and 20% of the overall MTU length change and shortening velocity, respectively. Greater levels of muscle activity resulted in increasingly shorter SO muscle fascicles as locomotion speed increased, both of which facilitated greater tendon stretch and recoil. Thus the elastic tendon contributed the majority of the MTU length change during walking and running. When transitioning from walking to running near the preferred transition speed (2.0 m/s), greater, more economical ankle torque development is likely explained by the SO muscle fascicles shortening more slowly and operating on a more favorable portion (i.e., closer to the plateau) of the force-length curve.

THE HUMAN ANKLE plantar flexors, soleus (SO), medial gastrocnemius (MG) and lateral gastrocnemius (LG), play a vital role in locomotion. Together, these muscles contribute the majority of the force necessary for vertical support and forward progression of the body during walking and running (2, 15, 16, 23, 44). The muscle-tendon design of the human ankle plantar flexors favors the storage and recovery of tendon elastic strain energy over muscular work, thereby potentially improving the mechanical efficiency of human locomotion (1, 48). A long elastic tendon is connected in series with short muscle fascicles, mimicking the design of distal limb muscles in other terrestrial (4). With such a design, tendon elasticity can influence the dynamic behavior of the muscle fascicles. It is, therefore, necessary to analyze the interaction between the muscle fascicle and tendon components to fully appreciate how the ankle plantar flexors function to generate the necessary force for locomotion.

Owing to its superficial nature, much of our present knowledge about the relationship between muscle fascicles and tendon for the human ankle plantar flexors during locomotion has been derived from in vivo measurements of the MG. It has been demonstrated that the MG muscle fascicles maintain a relatively low shortening velocity throughout the stance phase of steady-state walking and running, despite substantial changes in muscle-tendon unit (MTU) length (21, 38). The majority of the MTU length change is composed of tendon stretch and recoil, which allows the muscle fascicles to generate force economically by optimizing the contractile conditions for force generation, according to the force-length (F-L) and force-velocity (F-V) properties of skeletal muscle (20, 26, 39). It has also been demonstrated that the behavior of the MG muscle fascicles is different for walking compared with running. During walking, MG muscle fascicles remain relatively isometric throughout the first one-half of stance before rapidly shortening in late stance, whereas, during running, MG muscle fascicles shorten throughout the entire stance phase (31, 38). With faster walking, the shortening velocity of the MG muscle fascicles increases significantly, potentially shifting the muscle fascicles toward less favorable force-generating regions of the F-V curve (18). The shortening velocity of the MG muscle fascicles decreases when transitioning to running at the preferred transition speed (~2.0 m/s). This observation has led some researchers to postulate that MG muscle fascicle behavior may be one of the determinants for the walk-to-run transition (18, 45).

The mechanical behaviors of the MG and SO are likely to differ. Moreover, the SO is likely to be more important for force production during locomotion due to its...
twofold greater physiological cross-sectional area compared with the MG (50). Therefore, further research is required for a more comprehensive understanding of the interaction between in vivo SO muscle fascicle and tendon behavior during walking and running across a broad range of steady-state speeds.

We recently utilized a musculoskeletal modeling approach to evaluate how faster running influenced the relationship between SO muscle fascicles and tendon. We found that SO muscle fascicles remained relatively isometric during the period when the SO generated large forces, and that the utilization of tendon elastic strain energy was prioritized over muscle mechanical work to generate positive MTU work (36). Other studies have also utilized a modeling approach to investigate SO muscle fascicle behavior during walking and slow speeds of running (3, 45, 46). With faster walking, SO muscle fascicle velocity was found to increase toward less favorable shortening velocities for force generation (46). When transitioning from walking to running near the preferred transition speed, the shortening velocity of the SO muscle fascicles was found to decrease (3, 45). However, these results are based on model calculations and are yet to be verified by experiment. Thus we wish to substantiate these modeling predictions by performing direct in vivo measurements of the SO muscle fascicle and tendon behavior during walking and running.

The purpose of the present study was to combine ultrasound-based recordings of the in vivo SO muscle fascicle behavior with an inverse dynamics analysis to examine the interaction between the muscle fascicles and tendon over a range of walking and running speeds. We tested two hypotheses: first, that differences between the length changes and shortening velocities of the SO MTU and muscle fascicles will be greater than differences between the length changes and shortening between the muscle fascicles and tendon over a range of locomotion speed due to the influence of tendon elasticity; and second, that SO fascicle shortening velocity will decrease when transitioning from walking to running near the preferred transition speed (~2.0 m/s) to allow for greater, more economical muscle force production.

METHODS

Participants. Ten participants (9 men and 1 woman; age, 27.5 ± 5.6 yr; height, 180.8 ± 7.7 cm; and body mass, 80.2 ± 11.7 kg) gave informed consent to participate in the study. All participants were recreational athletes recruited from the local community. At the time of testing, no participant was suffering from any recent or preexisting musculoskeletal injury that likely affected his or her ability to walk and run. Ethical approval for the study was obtained from the relevant institutional human research ethics committee (University of Queensland, ref. no.: 2012001215).

Measurement of joint motion and ground reaction force. Three-dimensional (3D) kinematic data were collected using an eight-camera, video-based motion analysis system (Qualysis, Gothenburg, Sweden) sampling at a rate of 250 Hz. Small reflective markers (6–14 mm in diameter) were placed at specific locations on the upper and lower limbs (Table 1). Minimalist shoes (Xeroshoes, Boulder, CO) were worn by all participants rather than traditional running shoes to allow markers to be attached directly to the foot. Marker trajectories were filtered using a fourth-order, low-pass Butterworth filter with a cut-off frequency of 15 Hz.

Ground reaction force (GRF) data were collected from two force plates positioned in series in the direction of progression and embedded in an instrumented force-measuring treadmill (Tandem Treadmill, Advanced Mechanical Technology, Watertown, MA). GRF data were sampled at a frequency of 1,500 Hz. Visual 3D software (C-Motion, Germantown, MD) was used to assign the GRF to each leg based on the stride profile. In the situation in which a single leg contacted both force plates simultaneously, a built-in algorithm generated a resultant force and center of pressure vector by “stitching” together the force and center-of-pressure vectors measured from each force plate.

Table 1. Detailed description of the body segment marker locations used in this study

<table>
<thead>
<tr>
<th>Segment</th>
<th>Marker</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trunk</td>
<td>R(L)SH</td>
<td>Marker placed on the tip of the shoulder [acromio-clavicular (AC) joint]</td>
</tr>
<tr>
<td></td>
<td>C7</td>
<td>Marker placed over the spinous process of 7th cervical vertebra</td>
</tr>
<tr>
<td></td>
<td>MAN</td>
<td>Marker placed over manubrium of thoracic cage</td>
</tr>
<tr>
<td>Pelvis</td>
<td>R(L)ASI</td>
<td>Marker placed over anterior superior iliac spine (ASIS)</td>
</tr>
<tr>
<td></td>
<td>SACR</td>
<td>Marker placed at the midpoint between left and right posterior superior iliac spines (PSIS)</td>
</tr>
<tr>
<td>Thigh</td>
<td>R(L)JHAP</td>
<td>Marker located at the proximal anterior aspect of the thigh</td>
</tr>
<tr>
<td></td>
<td>R(L)JTHAD</td>
<td>Marker located at the distal anterior aspect of the thigh</td>
</tr>
<tr>
<td></td>
<td>R(L)THLD</td>
<td>Marker located at the proximal distal aspect of the thigh</td>
</tr>
<tr>
<td></td>
<td>R(L)LEPI</td>
<td>Marker over lateral epicondyle of femur</td>
</tr>
<tr>
<td></td>
<td>R(L)MEPI</td>
<td>Marker over medial epicondyle of femur</td>
</tr>
<tr>
<td>Shank</td>
<td>R(L)TIAP</td>
<td>Marker located on the proximal 1/3 of the anterior shaft of the tibia</td>
</tr>
<tr>
<td></td>
<td>R(L)TIAD</td>
<td>Marker located on the distal 1/3 of the anterior shaft of the tibia</td>
</tr>
<tr>
<td></td>
<td>R(L)TILAT</td>
<td>Marker located on the midlateral aspect of the tibia</td>
</tr>
<tr>
<td></td>
<td>R(L)LMAL</td>
<td>Marker located over the lateral malleolus</td>
</tr>
<tr>
<td></td>
<td>R(L)MMAL</td>
<td>Marker located over the medial malleolus</td>
</tr>
<tr>
<td>Foot</td>
<td>R(L)HEEL</td>
<td>Marker located on distal aspect of bisection of posterior calcaneum</td>
</tr>
<tr>
<td></td>
<td>R(L)MFS</td>
<td>Marker on superior medial midfoot</td>
</tr>
<tr>
<td></td>
<td>R(L)MFL</td>
<td>Marker on lateral midfoot</td>
</tr>
<tr>
<td></td>
<td>R(L)PI1MT</td>
<td>Marker on medial aspect of 1st metatarsophalangeal (MTP) joint</td>
</tr>
<tr>
<td></td>
<td>R(L)PI2MT</td>
<td>Marker on lateral aspect of 2nd MTP joint</td>
</tr>
<tr>
<td></td>
<td>R(L)TOE</td>
<td>Marker on distal end of 1st toe of foot</td>
</tr>
<tr>
<td>Arm</td>
<td>R(L)ARM</td>
<td>Marker located halfway laterally down the humerus</td>
</tr>
<tr>
<td></td>
<td>R(L)ELB</td>
<td>Marker located over the lateral epicondyle of the humerus</td>
</tr>
<tr>
<td></td>
<td>R(L)FOREARM</td>
<td>Marker located halfway down on the forearm</td>
</tr>
<tr>
<td></td>
<td>R(L)WR</td>
<td>Marker over the dorsal aspect of wrist</td>
</tr>
</tbody>
</table>

*Markers required for static calibration trial only. All markers were 14 mm in diameter. Note that markers were placed on both the right (R) and left (L) sides of the body.
fourth-order, low-pass, critically-damped filter with a cut-off frequency of 15 Hz was used to smooth the GRF data. We chose this filtering process so as to ensure that the GRF was filtered at the same cut-off frequency as the marker trajectories (35).

Measurement of muscle activity. Muscle electromyographic (EMG) data were recorded using a telemetered system (Noraxon, Scottsdale, AZ, USA) sampling at a frequency of 1,500 Hz. Surface electrodes were mounted on the skin overlying the muscle belly of the SO according to SENIAM (Surface Electromyography for the Non-Invasive Assessment of Muscles) recommendations (24). All signals were checked for clarity and strength during isolated dynamic ankle movements. The raw EMG data were smoothed into a linear envelope by calculating the root mean square of a moving window (100 ms). The linear envelope of the SO EMG signal measured for each locomotion condition was normalized by the peak magnitude of the EMG envelope measured for the maximum running speed condition (i.e., 5.0 m/s).

Measurement of muscle fascicle length. Ultrasound images were collected using a PC-based B-mode ultrasound scanner (Telemed Echo Blaster 128, Vilnius, Lithuania) at a sampling rate of 80 Hz. A 96-element, linear, flat-shaped ultrasound probe was used, sampling at a frequency of 7 MHz with a scanning depth and width of 60 mm. To image the SO muscle fascicles, the ultrasound transducer was positioned at the midbelly of the LG toward the mediolateral and proximodistal midlines. This site was chosen so that the transducer was aligned along the muscle fascicles and perpendicular to the deep and superficial aponeurosis to minimize errors (33). It also allowed clear striated patterns of the connective tissue to be visible across the SO superficial aponeurosis to minimize errors (33). It also allowed clear striated patterns of the connective tissue to be visible across the SO superficial aponeurosis to minimize errors (33). It also allowed clear striated patterns of the connective tissue to be visible across the SO superficial aponeurosis to minimize errors (33). It also allowed clear striated patterns of the connective tissue to be visible across the SO superficial aponeurosis to minimize errors (33). It also allowed clear striated patterns of the connective tissue to be visible across the SO superficial aponeurosis to minimize errors (33). It also allowed clear striated patterns of the connective tissue to be visible across the SO superficial aponeurosis to minimize errors (33). It also allowed clear striated patterns of the connective tissue to be visible across the SO superficial aponeurosis to minimize errors (33). It also allowed clear striated patterns of the connective tissue to be visible across the SO superficial aponeurosis to minimize errors (33).

Experimental protocol. Participants were asked to walk and run at the following steady-state speeds: walk at 0.7, 1.4, and 2.0 m/s; and run at 2.0, 3.0, 4.0, and 5.0 m/s. All participants completed all locomotion conditions in an incremental order. For all running conditions, participants used their preferred foot strike pattern and self-selected their stride frequency and stride length. All participants selected a rear-foot strike pattern for all walking conditions; three participants maintained a rear-foot strike pattern for all the running conditions; three participants maintained a fore-foot strike pattern for all running conditions; and four participants selected a rear-foot strike pattern for running at speeds of 2.0, 3.0, and 4.0 m/s, but then shifted to a fore-foot strike pattern for running at 5.0 m/s. Simultaneous joint kinematics, ground force, EMG, and ultrasound data were captured for five complete gait cycles for each locomotion condition. The right leg was designated as the test leg for all participants, as we assumed symmetry between both legs during the gait cycle. Adequate rest time of at least 1 min was given between locomotion conditions to prevent fatigue. The total walking and running time was approximately 10 min. The collection of experimental data was synchronized via a digital output signal generated by the ultrasound scanner that triggered the recording of joint kinematics, GRF, and EMG data.

Data processing. Ankle joint angles, torques, and powers, as well as SO MTU kinematics, were calculated using OpenSim software (13). A generic 12-segment, 31-degree-of-freedom musculoskeletal model was used to represent the skeleton, as previously described by Dorn et al. (15). In particular, the ankle joint was represented as a universal joint that comprised two nonintersecting hinge joints: one for plantar flexion/dorsiflexion, and the other for inversion/eversion. Segment lengths, segment inertial properties, and MTU origin and insertion sites in the model were scaled according to distances between specific anatomical markers located on the participant and corresponding virtual markers defined in the model. Ankle joint angles were calculated using inverse kinematics, whereby an optimization routine minimized the sum of the squares of the differences between the experimentally recorded marker positions and the marker positions predicted by the model (40). A standard inverse dynamics approach was used to calculate the ankle joint torque. Ankle joint power was calculated as the net torque exerted about the ankle multiplied by the ankle angular velocity. Ankle joint torques and powers were normalized by body mass. The length of the SO MTU was calculated at each discrete time step using the origin and insertion sites of the muscle in the model and the joint orientations from inverse kinematics.

Muscle fascicle length was defined as the distance between the superficial and deep aponeurosis parallel to the lines of collagenous tissue (Fig. 1). Pennation angle ($\alpha$) was defined as the angle between the collagenous tissue and the deep aponeurosis. A previously validated automatic tracking algorithm was used to quantify changes in muscle fascicle length and $\alpha$ (10, 22). All images were visually inspected to verify that the automatic tracking algorithm accurately quantified the muscle fascicle length and $\alpha$ changes. The measurements from an image frame were deemed inaccurate if the tracking algorithm was unable to position the end points on the superficial or deep aponeuroses, or if the muscle fascicle length was not parallel to the lines of collagenous tissue. To correct the tracking error, the end points of the muscle fascicles were redefined manually.

The length of the tendon (including both the free tendon and aponeurosis) was found by subtracting muscle fascicle length projected in the direction of the line of force application from the MTU length for each time instant (21). Thus:

$$l = L_{MTU} - l_{MTU\cos\alpha}$$

where $l$ is the length of the tendon, $L_{MTU}$ is the length of the MTU, $l_{MTU}$ is the ultrasound-measured fascicle length, and $\alpha$ is the ultrasound-measured pennation angle. Muscle fascicle and tendon properties were assumed to be consistent along the length of the MTU. The muscle fascicles were also assumed to be parallel to one another. The velocities of the MTU, muscle fascicle, and tendon were calculated by differentiating their respective lengths with respect to time.

MTU and muscle fascicle data were normalized by the resting muscle fascicle length obtained during the static standing ($l_{MTU}^0$). Tendon length was normalized by the resting tendon length obtained during
torque increased 2.5-fold, peak ankle power increased 23-fold, and peak SO activity increased 3.5-fold. When transitioning from walking to running at the same speed (2.0 m/s), peak ankle torque increased by 40%, despite peak SO activity decreasing from 79.7 ± 17.4 to 74.8 ± 12.6%.

Distinct differences in the mechanical behavior of the SO MTU and muscle fascicles were observed during walking and running. During walking, although the MTU and muscle fascicle length curves were similar in profile, the mean length change of the MTU during the stance phase was at least twice that measured for the muscle fascicles (Fig. 4; Table 2). During running, the MTU underwent a stretch-shortening cycle, whereas the muscle fascicles actively shortened throughout the entire stance phase. As a consequence, the average change in length of the MTU was fourfold greater than that of the muscle fascicles, which in turn resulted in 30% higher peak tendon strains during running compared with walking.

The change in muscle fascicle length was influenced by locomotion speed. The mean length change in the muscle fascicles throughout stance increased significantly with faster walking ($P < 0.001$), but not with faster running ($P = 0.862$). Mean change in muscle fascicle length throughout stance for all running speeds was smaller than that for walking at 2.0 m/s (Table 2). This result was especially evident for the change in length of the muscle fascicles during the first half of stance (from foot strike to the time of peak ankle torque), where muscle fascicle length change across all running speeds varied by no more than $0.04 \pm 0.08 \lambda_{m}$, despite a progressive increase in MTU lengthening for each increment in running speed (Fig. 5A). As a consequence, peak tendon strain significantly increased ($P = 0.037$) from 6.04 ± 1.76 to 8.34 ± 3.18% when running progressed from 2.0 to 5.0 m/s (Table 2). Furthermore, the muscle fascicle length at the time of peak ankle torque became progressively shorter with increased walking speed (range: 0.99 ± 0.08 $\lambda_{m}$ for walking at 0.7 m/s to 0.89 ± 0.05 $\lambda_{m}$ for walking at 2.0 m/s) and with increased running speed (range: 1.04 ± 0.08 $\lambda_{m}$ for running at 2.0 m/s to 0.92 ± 0.11 $\lambda_{m}$ for running at 5.0 m/s) (Fig. 6A). When transitioning from walking to running at the same speed (2.0 m/s), muscle fascicle lengths at the time of peak ankle torque significantly increased ($P < 0.001$) (Fig. 6A).

The SO MTU and muscle fascicle shortening velocities were influenced by both a transition in locomotion mode (from walking to running at 2.0 m/s) and an increase in locomotion...
speed. We found that the muscle fascicle shortening velocity was less than that of the MTU during the propulsion phase of walking and running (Fig. 5B). Specifically, for both walking and running, peak MTU shortening velocity during stance was on average fivefold greater than muscle fascicle shortening velocity, with the smallest and largest differences in shortening velocities occurring when walking at 0.7 m/s (1.72 ± 0.56 \( l_s^m \)/s) and running at 4.0 m/s (9.96 ± 2.37 \( l_s^m \)/s), respectively. With faster walking, the shortening velocities of the MTU and muscle fascicles significantly increased (\( P < 0.001; \) Figs. 5B and 6B). Muscle fascicle shortening velocity also increased with faster running, while the MTU shortening velocity increased up to a running speed of 4.0 m/s before remaining at a similar magnitude between running speeds of 4.0 and 5.0 m/s. When transitioning from walking to running at the same speed (2.0 m/s), muscle fascicle shortening velocity at the time of peak ankle torque significantly decreased (\( P = 0.002 \)) from 1.04 ± 0.38 to 0.47 ± 0.39 \( l_s^m \)/s (Fig. 6B).

**DISCUSSION**

The ankle plantar flexors, especially the SO, have been identified as the primary source of force generation and mechanical work during human walking and running (15, 16, 44). This study aimed to measure in vivo SO muscle fascicle behavior across a broad range of walking and running speeds: from slow-paced walking (0.7 m/s) through to moderate-paced running (5.0 m/s). We found that in vivo SO muscle fascicles exhibited minimal shortening compared with the MTU during locomotion, irrespective of changes in mode (i.e., walking vs. running) or steady-state speed (Fig. 5B; Table 2). With both increased walking and running speeds, we found that the SO muscle fascicles became shorter at the time of peak ankle torque, and peak SO activity also increased (Figs. 3 and 6A; Table 2). Increased muscle activity likely facilitated greater tendon stretch and recoil. Our results concur with previous experimental and modeling assertions that tendon recoil velocity is the dominant contributor to MTU shortening velocity for the ankle plantar flexors (18, 36). Furthermore, the present study provides additional in vivo evidence that when transitioning from walking to running near the preferred transition speed (2.0 m/s), SO muscle fascicles shortened more slowly and likely operated on a more favorable portion of the F-L curve, allowing higher, more economical ankle torque development.

The mechanics of walking and running are fundamentally different. Walking is often modeled as an inverted pendulum where the kinetic and gravitational potential energy of the body fluctuate out of phase, resulting in an exchange of mechanical energy (e.g., Ref. 8). In contrast, running is conceptualized as a “bouncing” gait (e.g., Refs. 14, 42), where the elastic tissues in the lower limb, primarily tendons, store and recover the mechanical energy of the body. Running compared with walking also demands larger ankle plantar flexor muscle force to generate sufficient ankle joint torque (19). Given these fundamental differences in locomotion mechanics, it is not surprising that we observed distinct differences in SO fascicle behavior during stance for walking vs. running. During walking, the SO muscle fascicle and MTU length curves had similar profiles (Fig. 4). Both the MTU and muscle fascicles shortened during early stance, lengthened moderately during midstance, and then rapidly shortened during late stance. During running, however, to generate sufficiently large ankle joint torques, the SO muscle fascicles shortened throughout the entire stance phase, despite the entire MTU undergoing a stretch-shortening cycle. These differences in behavior between walking and running as well as the magnitudes of in vivo SO muscle fascicle length change and shortening velocity measured during stance are consistent with previous ultrasound-based studies of the SO for comparable walking and running speeds (9, 30, 49). For instance, Cronin and Finni (11) reported SO fascicle length changes of 8 and 7 mm for walking at 1.4 m/s and running at 2.8 m/s, respectively. By comparison, we found SO fascicle length changes during stance of 8 mm for walking at 1.4 m/s and 8 mm for running at 3.0 m/s (Table 2).

Irrespective of locomotion mode, the SO muscle fascicles exhibited minimal shortening compared with that which occurred for the MTU. Specifically, the mean muscle fascicle length change and peak shortening velocity during stance did not exceed 0.28 ± 0.05 \( l_s^m \) and 2.01 ± 0.39 \( l_s^m \)/s, respectively, for walking, and 0.17 ± 0.07 \( l_s^m \) and 2.60 ± 0.84 \( l_s^m \)/s, respectively, for running (Fig. 5). Equivalent values for the SO MTU were 0.55 ± 0.09 \( l_s^m \) and 6.34 ± 0.80 \( l_s^m \)/s, respectively, for walking, and 0.75 ± 0.16 \( l_s^m \) and 12.05 ± 2.53 \( l_s^m \)/s, respectively, for running. As a consequence, the SO muscle fascicles contributed only 35 and 20% of the MTU length change and shortening velocity for walking and running, respectively. Similar estimates have been reported for the MG during walking and running, where muscle fascicle shortening was found to contribute between 25 and 30% of the MTU shortening velocity (38). These similar SO and MG shortening velocity contributions suggest that the mechanical behavior of these muscles may not be as distinct as first thought. Overall,
the differences between the muscle fascicles and the MTU with respect to length change and shortening velocity demonstrate the capacity of the human Achilles tendon to stretch and recoil, therefore potentially storing and recovering elastic strain energy to assist in satisfying the necessary mechanical energy demands of both walking and running (7, 21, 37).

Despite the muscle fascicles exhibiting minimal shortening compared with the behavior of the MTU, increased walking and running speed influenced the length of the SO muscle fascicles as well as their operating region on the F-L curve. With both increased walking and running speed, muscle fascicle lengths became shorter around the time when the ankle...
Fig. 3. Activity levels for each locomotion speed (see force-velocity curves due to differing muscle fascicles). We predicted shorter SO muscle fascicle lengths. Thus, to achieve greater tendon stretch and recoil (36). Moreover, if the SO $l_m$ (= 0.05 ± 0.01 m) is assumed to provide a reasonable approximation of optimal muscle fiber length with respect to the F-L relationship (Fig. 6A), then we would infer that the SO muscle fascicles remained on the plateau and upper operating regions of the ascending limb of the F-L curve for the range of locomotion speeds measured in this study. Similar findings have been reported in previous ultrasound-based (49) and modeling-based studies (3, 36) for equivalent walking and running speeds.

Faster locomotion also increased the muscle fascicle shortening velocity and shifted the operating regions of the SO muscle fascicles on the F-V curve. For example, when comparing slow-paced running at 2.0 m/s with moderate-paced running at 5.0 m/s, the peak muscle fascicle shortening velocity during stance significantly increased from 1.59 ± 0.43 to 2.60 ± 0.84 $l_m$/s (Fig. 5B; $P < 0.003$). Even at the fastest running speed measured in this study (5.0 m/s), our estimates of the peak SO muscle fascicle velocity are much lower than the approximate value of the muscle’s maximum shortening velocity of 10–12 $l_m$/s reported by others [assuming the resting $l_m$ is a reasonable approximation of the optimal muscle fiber length (17, 53)]. Therefore, even though muscle fascicle shortening velocity increased with faster walking and running, it is possible that the range of shortening velocities recorded remain on relatively favorable force-generating regions of the F-V curve. Similar F-V operating regions have been predicted in modeling-based studies for the SO (27, 36) and observed in ultrasound-based studies for both the SO (11) and the MG (18, 31, 38). Consequently, the tendon recoil velocity following stretch during the first half of stance provides the majority of the MTU shortening velocity. Our observations in this study are consistent with previous assertions that distal limb muscles function relatively isometrically during faster locomotion speeds to facilitate greater tendon stretch and recoil, and hence increased storage and recovery of elastic strain energy (5, 14).

Previous studies have proposed that impaired force-generating conditions in the ankle plantar flexors with respect to the F-V relationship may be one of the mechanisms that trigger the transition from walking to running at the preferred transition speed (~2.0 m/s) (18, 45). In the present study, we found that, developed peak torque (Fig. 6A). Larger tendon strains with faster walking and running were likely attributable to the combined effect of increased muscle activity, higher ankle joint torques (and presumably SO muscle force), and reduced muscle fascicle lengths. Thus, to achieve greater tendon stretch (and subsequent recoil), it is necessary for the fascicles to operate at shorter lengths. This behavior is consistent with our recent modeling study (36), investigating the influence of increased running speed on the utilization of elastic strain energy in the Achilles tendon. We predicted shorter SO muscle fascicles with faster running, which resulted in a leftward shift along the F-L curve and thus increased tendon stretch and recoil (36).
as walking speed increased from 0.7 to 2.0 m/s, muscle fascicle shortening velocity at the time of peak ankle joint torque increased from 0.00 ± 0.12 to 1.04 ± 0.38 \( \text{m/s} \) (Fig. 5B). To generate sufficient ankle torque (i.e., SO muscle force), SO activity also progressively increased (Fig. 3; Table 2). When transitioning from walking to running at 2.0 m/s, the SO muscle fascicle shortening velocity at the time of peak ankle joint torque significantly decreased by 0.57 ± 0.78 \( \text{m/s} \). Concurrently, muscle fascicle length at the time of peak ankle joint torque significantly increased by 0.15 ± 0.13 \( \text{m} \). Again, if the \( l_f^0 \) is assumed to represent the optimal fiber length, then an increase in SO fascicle length during running resulted in a rightward shift on the F-L curve toward more favorable force-generating conditions (Fig. 6A). Thus, when transitioning from walking to running at the same speed, the SO muscle fascicles shortened more slowly and operated on a more favorable portion (i.e., closer to the plateau) of the F-L curve, allowing higher ankle torques (i.e., SO muscle force) to be developed for an equivalent amount of SO muscle activity (Fig. 3). This behavior in turn would facilitate greater tendon stretch and recoil. Similar reductions in the SO muscle fascicle shortening velocity in conjunction with increased muscle force production for running compared with walking around the preferred transition speed have been found in modeling-based studies (3, 45). Moreover, previous in vivo ultrasound-based studies have found similar reductions in muscle fascicle velocity for MG (18). Hence, our results support previous assertions that ankle plantar flexor performance may play a role in the transition from walking to running around the preferred transition speed. Nevertheless, we cannot be certain that our measured decrease in SO muscle fascicle shortening velocity when transitioning from walking to running at the same speed represents a meaningful shift on the F-V curve, and so other explanations, such as overexertion of the ankle dorsiflexor muscles (29), unfavorable ankle angular velocities (28), improved coordination of the actions of the knee extensor and ankle plantar flexor muscles (47), and the dynamic interplay between the stance and swing phases (32), should also be considered.

With faster running, we observed a progressive increase in SO muscle fascicle shortening velocities at the time of peak ankle joint torque (Fig. 6). It is possible that, for running speeds beyond those measured in this study (i.e., >5.0 m/s), muscle fascicles will continue to shorten at faster rates and thus shift further down the F-V relationship, away from favorable force-generating conditions. This prediction could explain the observations from previous modeling and experimental studies that peak ankle plantar flexor force production during stance was greatest for running speeds of 6–7 m/s (15, 34). With faster running, ground contact times progressively decrease, which reduces the period of time the ankle plantar flexors have to generate sufficient force. Therefore, it has been speculated that the F-V relationship of the lower limb muscles may be a critical factor in limiting maximum sprinting speed (43, 51, 52). However, contrary to this premise, our recent modeling study predicted that SO muscle fascicle shortening velocity did not continue to increase for running speeds beyond 5.0 m/s, but instead maintained a relatively isometric behavior and operated at favorable force-generating conditions on the F-V curve (36). We proposed that the relatively isometric behavior of the SO muscle fascicles as running speed approached maximum sprinting was due to the ability of the elastic Achilles tendon to stretch and recoil. Future in vivo measurements of the muscle fascicles in the plantar flexors as well as of other major extensor muscles are needed to substantiate these propositions for running speeds up to maximum sprinting.

The ultrasound-based approach used in this study to record SO muscle fascicle behavior during walking and running was associated with some limitations that should be acknowledged. First, the sampling frequency of our ultrasound unit was limited to 80 Hz. At running speeds higher than 5.0 m/s, a sampling rate of 80 Hz would not be sufficient to provide the bandwidth needed to comprehensively investigate subtle changes in muscle fascicle behavior (12). We were, therefore, unable to investigate running speeds approaching maximum sprinting. Second, muscle fascicle measurements may vary according to the location of the ultrasound probe. Previous experimental studies conducted on the gastrocnemius have demonstrated the potential for muscle fascicle changes to differ along the entire length of a muscle (54) or across different compartments of a muscle (25). However, other studies have reported uniform changes in muscle fascicle length to occur for the gastrocnemius during isometric contractions (41) and during walking and running (37). Future research should investigate whether all of the SO muscle fascicles behave in a homogeneous manner during walking and running. Despite these limitations, the magnitudes and profiles of the SO muscle fascicle lengths and velocities measured for walking and slow running are consistent with results obtained from previous ultrasound- and modeling-based studies of the SO. Third, fibers within the physiological tendon can twist and curve (and hence may not strain from point to point), which would cause our measurements of tendon strains to be overestimated. Regardless, our key finding that the magnitudes of tendon strain increase as a result of higher muscle force generation with faster locomotion would remain unchanged. Finally, our ultrasound measurements of muscle fascicle lengths are based on the assumption that muscle fascicles act in the same two-dimensional plane as the ultrasound image for the duration of the stance phase. Due to the 3D curvature of muscles, muscle fascicles likely rotate in and out of the measurement plane during a contraction, resulting in a possible overestimation of muscle fascicle length change (33). However, by measuring the global movement of the muscle, we were able to compare the MTU and muscle fascicle length changes and shortening velocities. Even substantial errors in these measurements would not alter our main findings regarding the relationship between MTU and muscle fascicle behavior with changes in locomotion mode and steady-state speed.

**Conclusion.** The present study investigated the dynamic interaction between SO muscle fascicles and tendon with a change in locomotion mode (i.e., walking vs. running) and steady-state speed. Irrespective of locomotion speed, the muscle fascicles exhibited minimal shortening compared with the overall behavior of the MTU due to the contribution of the stretch and recoil of the elastic Achilles tendon. While increased muscle activity resulted in shorter SO muscle fascicle lengths during faster walking and running, the muscle fascicles remained on the plateau and upper regions of the ascending limb of the F-L relationship. When transitioning from walking to running at a speed close to the preferred walk-to-run transition, the ankle muscles developed higher, more economical plantar flexor torque, most likely as a result of the SO muscle...
fascicles shortening more slowly and operating on a more favorable portion of the F-L curve.

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

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