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RESEARCH ARTICLE

Does different activation between the medial and the lateral gastrocnemius during walking translate into different fascicle behavior?

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ABSTRACT

The functional difference between the medial gastrocnemius (MG) and lateral gastrocnemius (LG) during walking in humans has not yet been fully established. Although evidence highlights that the MG is activated more than the LG, the link with potential differences in mechanical behavior between these muscles remains unknown. In this study, we aimed to determine whether differences in activation between the MG and LG translate into different fascicle behavior during walking. Fifteen participants walked at their preferred speed under two conditions: 0% and 10% incline treadmill grade. We used surface electromyography and B-mode ultrasound to estimate muscle activation and fascicle dynamics in the MG and LG. We observed a higher normalized activation in the MG than in the LG during stance, which did not translate into greater MG normalized fascicle shortening. However, we observed significantly less normalized fascicle lengthening in the MG than in the LG during early stance, which matched with the timing of differences in activation between muscles. This resulted in more isometric behavior of the MG, which likely influences the muscle–tendon interaction and enhances the catapult-like mechanism in the MG compared with the LG. Nevertheless, this interplay between muscle activation and fascicle behavior, evident at the group level, was not observed at the individual level, as revealed by the lack of correlation between the MG–LG differences in activation and MG–LG differences in fascicle behavior. The MG and LG are often considered as equivalent muscles but the neuromechanical differences between them suggest that they may have distinct functional roles during locomotion.

KEY WORDS: B-mode ultrasound, Fascicle length, Electromyography, Locomotion, Muscle function

INTRODUCTION

The triceps surae muscle group serves an essential role in human walking, generating more than 50% of the mechanical power needed for forward propulsion and swing initiation (Neptune et al., 2001). Studies have highlighted that different activation patterns exist during walking between the monoarticular soleus and the biarticular medial gastrocnemius (MG), despite their similar role as ankle plantar flexors. Specifically, the soleus is active for a greater portion of the gait cycle (Lay et al., 2007) and has an activation pattern that is less influenced by the grade of the walking surface, when compared with the MG (Franz and Kram, 2012). The few studies that have compared the gastrocnemii have shown that the MG activation is both greater and longer in duration than the lateral gastrocnemius (LG) activation during walking (Ahn et al., 2011), with large differences in the MG to LG activation ratio between individuals (Crouzier et al., 2019). This echoes recent work showing that these two muscles share minimal common neural drive (Hug et al., 2021). Together with studies suggesting that the MG and LG may produce different ankle moments in the frontal plane (Lee and Piazza, 2008), the differences in activation suggest that these muscles may have unique functional roles during walking. Other factors, such as the muscle fascicle length and contraction velocity also contribute to a muscle's force-generating capacity. This means activation alone does not provide us with all of the information necessary to understand muscle function. Concurrent information on muscle fascicle behavior is therefore needed.

Ultrasound studies have revealed different fascicle behavior between the soleus and the MG during walking, with soleus fascicles shortening less than MG fascicles (Ishikawa et al., 2005), and exhibiting a lower shortening velocity (Cronin et al., 2013). However, we know very little about how the MG and LG differ in their fascicle behavior during locomotor tasks such as walking. Studies have reported differences in resting muscle architecture between the MG and LG, such as shorter fascicle length and greater pennation angle for the MG (Charles et al., 2019). In addition, the LG is composed of different neuromuscular compartments with specific muscle architecture and innervation (Segal et al., 1991; Wolf et al., 1993). Moreover, the Achilles tendon is composed of three subtendons arising from the three muscles of the triceps surae (Edama et al., 2015). These factors may enable different neuromechanical behaviors of the three muscles.

While muscle activation and fascicle behavior each influence force generation, understanding the interplay between these factors allows for a more comprehensive assessment of muscle function. For instance, the higher mechanical work required during incline versus level walking is associated with higher activation and a greater amount of fascicle shortening in the MG muscle (Lichtwark and Wilson, 2006). However, to date, no study has combined both electromyography (EMG) and ultrasound imaging to explore the functional differences between the MG and LG muscles during locomotor tasks. The aforementioned differences in activation between the MG and LG muscles suggest that the mechanical behavior of the fascicles may also differ, requiring caution for making inferences based on measures of only one of the two muscles. In addition, a high level of inter-individual variability

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exists in the distribution of activation between these muscles (Ahn et al., 2011; Crouzier et al., 2019), but it remains unclear whether this translates into similar inter-individual variability in fascicle behavior.

The overall aim of this study was to determine whether the differences in activation strategies between the MG and LG during walking translate into different fascicle behavior. We used an experimental approach that combined surface EMG measurements and ultrasound imaging during level and incline walking. We further aimed to interpret our data at both the population level and the individual level. Based on previous work that illustrated greater activation in the MG compared with the LG (Ahn et al., 2011; Crouzier et al., 2019), we expected the higher activation to translate into a greater amount of fascicle shortening in the MG than in the LG. Additionally, at the individual level, we hypothesized that the MG to LG activation ratio would be correlated with the difference in fascicle shortening between the two muscles.

MATERIALS AND METHODS

Participants

Twenty participants with no recent (<6 months) lower limb pain or injury were recruited. They provided informed written consent. After a quality check of the ultrasound data (see 'Ultrasound', below), 5 participants were excluded from the analysis and therefore data are reported for 15 participants (5 females and 10 males, mean±s.d. age: 25.9±3.9 years, body mass: 75.2±14.6 kg, height: 1.73±0.10 m). The study was approved by the institutional ethics review committee at The University of Queensland (approval #2013001448) and adhered to the Declaration of Helsinki.

Experimental protocol

Following a period of familiarization, participants performed three isometric plantar flexion maximal voluntary contractions (MVCs) with 120 s rest between each. Then, participants walked on a treadmill (Nautilus Trimline T345) while we used surface EMG, B-mode ultrasound and motion capture to measure muscle activity, fascicle behavior and foot position, respectively. The last of these was done using reflective markers attached bilaterally to the calcaneus and the metatarsophalangeal joint and a 12-camera motion capture system (Flex 13, OptiTrack, Corvallis, OR, USA), operating at 120 Hz. Participants walked under two conditions in a randomized order: (i) 0% treadmill grade (level walking) and (ii) 10% treadmill grade (incline walking). In both conditions, participants walked for 60 s at their preferred walking speed (1.1±0.1 m s^{-1}), which was determined at the beginning of the protocol during level walking using standardized procedures (Dal et al., 2010). The experimental protocol was composed of eight walking trials. Participants performed two trials for each condition and repeated this twice, first to record myoelectrical activity of the MG and LG and second to measure fascicle behavior of both muscles. The recording duration was 30 s and 15 s for EMG and ultrasound trials, respectively.

EMG

We shaved, abraded and cleaned the participant's skin with alcohol to reduce the skin–electrode impedance. For the EMG trials, we placed surface electrodes (Trigno Delsys Inc., Natick, MA, USA; 10 mm inter-electrode distance) over the MG and LG muscle bellies, aligned along the direction of the muscle fascicles, determined using B-mode ultrasound imaging. Elastic bandages secured the electrodes to the skin to avoid movement artefacts. The EMG signals were amplified, digitized at 2048 Hz, band-pass filtered (20–500 Hz) and recorded in Spike2 (V7, CED Ltd, Cambridge, UK). An external trigger generated by the motion capture system was used to synchronize the motion capture data with the EMG recordings.

B-mode ultrasound

For the ultrasound trials, we positioned two linear ultrasound probes (5–8 MHz, 60 mm field-of-view, LV8-5L60N-2, ArtUS, Telemed, Vilnius, Lithuania) over the MG and LG muscle bellies, at the same location where the EMG electrodes were placed in the previous trial. Although the fascicle plane may change during walking, we optimized the probe orientation to be aligned with the muscle fascicle plane during static standing and secured the probes with elastic bandages. Ultrasound data were captured at 120 Hz (Echo Wave II 3.7.1, Telemed). An external trigger generated by the motion capture system was used to synchronize the motion capture with the two ultrasound systems.

Data analysis

3D motion capture

Motion capture data were labeled, gap filled and smoothed with a 10 Hz low-pass second-order Butterworth filter (Motive, OptiTrack, Corvallis, OR, USA). Using custom-written scripts in Matlab (R2018b, The Mathworks, Natick, MD, USA), we identified heel-strike and toe-off based on foot vertical velocity, as described previously (O'Connor et al., 2007). Heel-strike and toe-off were used to determine the timing of each gait cycle for each individual and split the data into stance and swing phases for further analyses.

EMG

EMG and ultrasound analyses were conducted in Matlab R2018b. The EMG data analysis considered 15 gait cycles per trial. First, the signals were band-pass filtered using a second-order Butterworth filter (20–500 Hz). We visually checked all raw EMG data to detect movement artefacts or noise. In two trials for two different participants, we processed only 6 and 12 cycles instead of 15 because of movement artefacts. Then, we rectified and low-pass filtered (12 Hz) the EMG signal measured during the MVC trials and the maximal value was considered as the maximal EMG amplitude (EMG_{max}). The rectified EMG signals from 15 gait cycles were low-pass filtered at 12 Hz to determine the EMG envelope and then normalized to EMG_{max}. Finally, we interpolated the data from each gait cycle to 100 data points.

Ultrasound

Where required, we optimized the ultrasound image properties, such as brightness and contrast, post-data collection in the Echo Wave II software. Then, the image quality was checked to exclude videos whose quality was not sufficient (e.g. when fascicles were not clearly visible or when the fascicles moved entirely out of the imaging plane). After this quality check, we excluded five participants and we left out one trial for the MG and LG of another participant. We processed five gait cycles of ultrasound data per trial using a validated (Cronin et al., 2011; Gillett et al., 2013) semi-automated tracking algorithm (Ultratrack; Farris and Lichtwark, 2016), combined with manual corrections. We manually selected a region of interest surrounding the entire muscle belly and two regions of interest for each aponeurosis on the initial frame. Then, we drew a fascicle that represented the average fascicle orientation in the mid-region of the muscle belly and two straight lines in the inner limits of each aponeurosis. We used this to assess the changes in

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fascicle length and pennation angle, similar to previous methods (Dick and Wakeling, 2017; Aeles et al., 2018). The algorithm then tracked the fascicle and aponeuroses in sequential frames by implementing an affine flow model. Key frames were implemented at each heel-strike to help the algorithm account for tracking drift. Manual changes to the tracking were made where required, which was in most trials, mostly during shortening of the fascicles. The same investigator (R.H.) processed all videos to exclude inter-investigator variability (Aeles et al., 2017b) and the final tracking accuracy in all videos was then confirmed by another experienced operator (J.A.). Muscle fascicle length was calculated as the distance between the superficial and deep aponeuroses, along the fascicle orientation (Aeles et al., 2018). In limited cases, the fascicle extended outside the field of view. When this occurred, extrapolation was used to extend the fascicle and aponeuroses outside the image's field of view. The angle between the tracked muscle fascicle and the deep aponeurosis was defined as the pennation angle (Bolsterlee et al., 2015). We subsequently low-pass filtered all ultrasound data at 12 Hz, and calculated instantaneous fascicle velocity as the derivative of fascicle length with respect to time. Data from each gait cycle were interpolated to 100 data points. We normalized fascicle length and fascicle velocity to the mean fascicle length at heel-strike during level walking (L_{HS}; mean±s.d. group value: 55.4±7.6 mm and 65.3±10.0 mm for the MG and LG, respectively). The change in pennation angle was expressed as the absolute difference of the mean pennation angle at heel-strike during level walking (mean±s.d. group value: 20.9±3.0 deg and 13.6±2.3 deg for the MG and LG, respectively). For further reference, positive values indicate fascicle lengthening and negative values indicate fascicle shortening. Similarly, positive pennation angle values indicate an increase in pennation angle.

Data reduction

From the processed EMG data, we extracted peak and average EMG amplitude during stance and during the whole cycle. For fascicle length, we calculated the amount of fascicle lengthening during stance (L_{length}) by subtracting the minimal fascicle length during early stance from the subsequent maximal fascicle length during stance (Fig. 1). This method takes into account the brief fascicle shortening that occurs directly following heel-strike, evident in most participants. We also determined the amount of fascicle shortening (L_{short}) following this initial lengthening period by taking the difference between the maximal fascicle length during the lengthening period and the fascicle length at toe-off. Similarly, the change in pennation angle was calculated for the lengthening period (β_{length}) and for the shortening period (β_{short}) by subtracting the maximal and the minimal values during each of these phases. The peak fascicle shortening velocity was also extracted during the shortening period (V_{short}). We analyzed 30 cycles per condition (15 per trial) for EMG and 10 cycles per condition (5 per trial) for ultrasound. We first extracted these parameters from each cycle and then averaged the resulting values over all cycles within a trial. Finally, we averaged the two trial values.

Statistics

Statistical analyses were performed in Statistica v8.0 (Statsoft, Tulsa, OK, USA). All data were confirmed to be normally distributed using a Kolmogorov–Smirnov test. We used a two-way repeated-measures ANOVA (factor: muscle [MG, LG] and condition [level, incline]) to determine whether EMG amplitude (peak and average) and fascicle behavior (L_{length}, L_{short}, β_{length}, β_{short} and V_{short}) differed between muscles and between conditions.

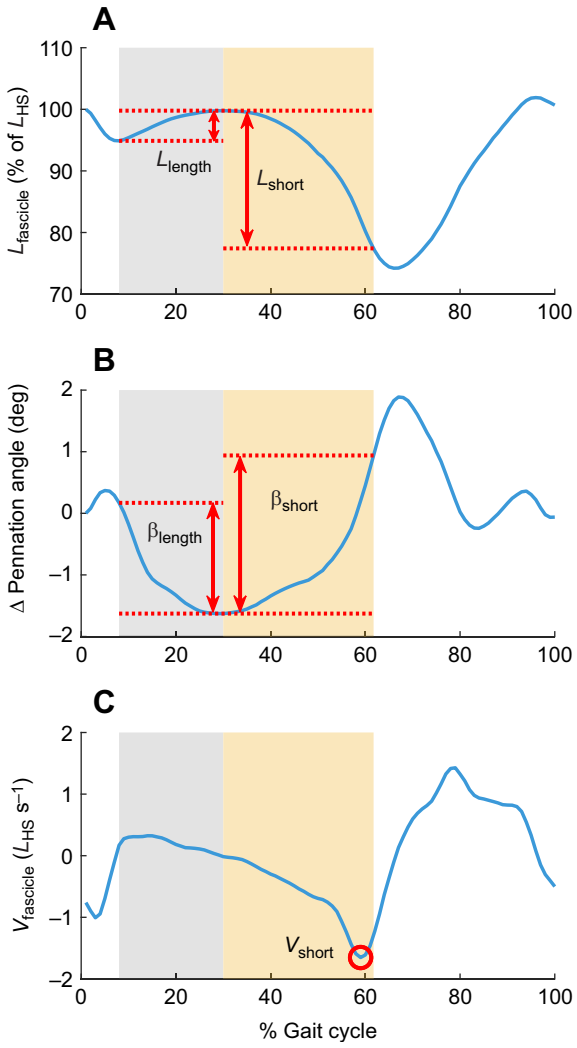

Fig. 1. Illustration of the methods used to calculate ultrasound parameters. Representative fascicle length ($L_{fascicle}$; A), change in pennation angle (B) and fascicle velocity ($V_{fascicle}$; C) data (mean group pattern) for the lateral gastrocnemius (LG) during level walking (blue curves), through the lengthening period (gray area) and the shortening period (orange area). $L_{fascicle}$ and $V_{fascicle}$ were normalized to the fascicle length at heel-strike during level walking (L_{HS}). Heel-strike occurs at 0% on the *x*-axis. The end of the orange area corresponds to the toe-off event, which we considered as the end of the shortening period. The red horizontal dotted lines represent the minimum or the maximum value within a period. The red arrows indicate the extracted parameters, i.e. the amount of fascicle lengthening (L_{length}) and fascicle shortening (L_{short}) (A), and the pennation angle decrease (β_{length}) and increase (β_{short}) (B). The red circle in C corresponds to the peak shortening velocity (V_{short}).

We used statistical parametric mapping (SPM) with a two-way repeated-measures ANOVA to compare the EMG profiles between muscles and conditions. To assess the inter-individual variability in muscle activation distribution, we used descriptive statistics (mean±s.d.) of the normalized EMG amplitude ratios calculated as MG/(MG+LG) (Crouzier et al., 2019). Finally, to test whether the differences in activation between muscles translated to differences in fascicle behavior, we performed correlations for both conditions between the difference in MG–LG average EMG amplitude during the lengthening period and the difference in MG–LG fascicle length and pennation angle changes during the same lengthening period. We ran similar correlations for the shortening period. A Bonferroni

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Table 1. Normalized myoelectrical activity in the medial and lateral gastrocnemius during level and incline walking

	Level walking		Incline walking	
EMG parameters	MG	LG	MG	LG
Peak EMG amplitude (% of EMG_{max})	45.0±13.2	34.3±14.1*	68.3±18.1‡	62.0±19.3*,‡
Average EMG amplitude during stance (% of EMG_{max})	16.2±4.8	11.0±3.5*	22.4±6.6‡	18.1±5.2*,‡
Average EMG amplitude during whole cycle (% of EMG_{max})	11.2±2.9	7.9±2.6*	15.1±4.1‡	12.3±3.4*,‡

MG, medial gastrocnemius; LG, lateral gastrocnemius; EMG, electromyography; EMG_{max}, EMG amplitude during maximal isometric voluntary contraction. Values were normalized to maximal isometric contraction and are reported as means±s.d. *Significant difference from MG. ‡Significant difference from level walking. n=15.

correction for multiple comparisons was applied. The level of significance was set at P<0.05.

RESULTS

Myoelectrical activity

There was a main effect of muscle and condition on peak EMG amplitude (muscle: P=0.049; condition: P<0.001), average EMG amplitude during stance (muscle and condition: P<0.001), and average EMG amplitude during the whole cycle (muscle and condition: P<0.001), with no significant muscle×condition interactions (all P≥0.616). Specifically, MG peak EMG was higher than LG peak EMG, regardless of the condition (Table 1). Similarly, average EMG amplitude during stance and during the whole cycle was higher for the MG than for the LG, regardless of the condition (Fig. 2). All EMG parameters, i.e. peak and average EMG amplitude during stance and during the whole cycle, were higher during incline walking than during level walking, in both the MG and LG.

The SPM analysis revealed a main effect of muscle from 18% to 36% of the gait cycle (P<0.001) and a main effect of condition from 25% to 56% (P<0.001), from 64% to 78% (P<0.001) and from 89% to 92% (P=0.023) of the gait cycle. There was no significant interaction between muscle and condition. Specifically, the MG had higher EMG amplitude than the LG from 18% to 36% of the gait cycle and the EMG amplitude was higher for the incline condition than for the level condition during the 25–56%, 64–78% and 89–92% phases of the gait cycle.

Muscle fascicle behavior

Inspection of the time-varying profiles of muscle fascicle behavior (Figs 3 and 4) revealed that the MG and LG fascicles, after a brief shortening in most of the individuals, lengthened after heel-strike (hereafter referred to as ‘fascicle lengthening period’). Following this, the fascicles shortened and rotated to steeper pennation angles until toe-off (hereafter referred to as ‘fascicle shortening period’).

There was a main effect of muscle on L_{length} (P=0.017) and β_{length} (P<0.001), but no main effect of condition (both P≥0.193) nor a muscle×condition interaction (both P≥0.490). Specifically, the MG fascicles lengthened less than the LG fascicles, regardless of the condition (Fig. 3A,B, Table 2). Similarly, the pennation angle decreased less for the MG than for the LG, regardless of the condition (Fig. 3C,D).

There was a main effect of condition on L_{short} (P<0.001) with no main effect of muscle (P=0.309) nor a muscle×condition interaction (P=0.414). Specifically, L_{short} was higher during incline walking than during level walking, regardless of the muscle (Fig. 3A,B, Table 2). Furthermore, for β_{short}, there was a main effect of muscle (P=0.038) and condition (P=0.009), but there was no significant muscle×condition interaction (P=0.532). Specifically, the MG muscle underwent a greater increase in pennation angle than the LG, regardless of the condition. Moreover, the increase in pennation angle was greater during incline walking than during level walking, regardless of the muscle (Fig. 3C,D).

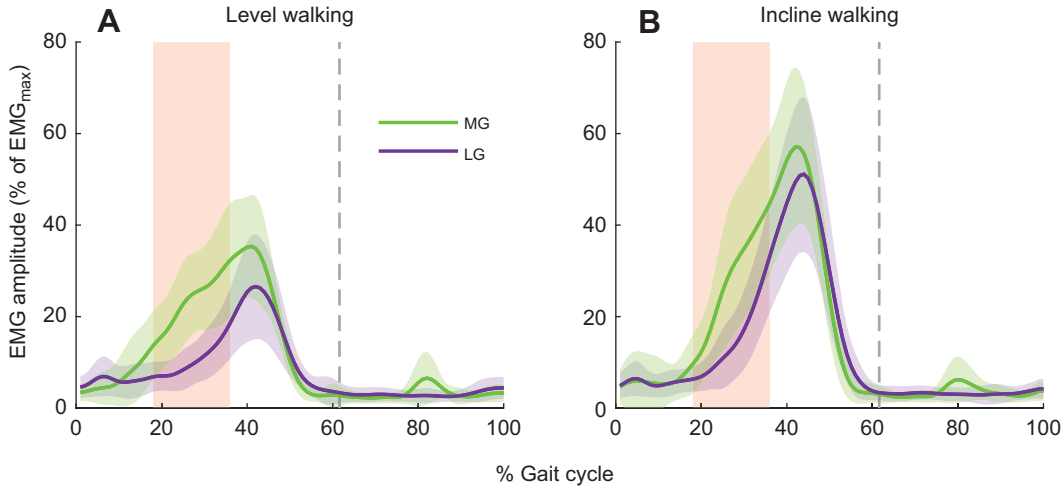

Fig. 2. Average time-varying electromyography (EMG) patterns measured during walking. EMG amplitude data during level walking (A) and incline walking (B) are presented as the mean (thick line) and standard deviation (shaded area) of all participants. EMG amplitude was normalized to that measured during a maximal isometric voluntary contraction (EMG_{max}). Heel-strike occurs at 0% on the x-axis. The dashed vertical lines correspond to the average timing of toe-off during the gait cycle. The red area represents the period of the gait cycle where the statistical parametric mapping indicates a significant difference in EMG amplitude between muscles (18–36% of the gait cycle). Peak EMG and average EMG amplitude measured during stance and during the whole cycle were: (i) significantly higher for the medial gastrocnemius (MG) than for the LG, regardless of the condition and (ii) significantly higher for incline walking (B) than for level walking (A), regardless of the muscle. n=15.

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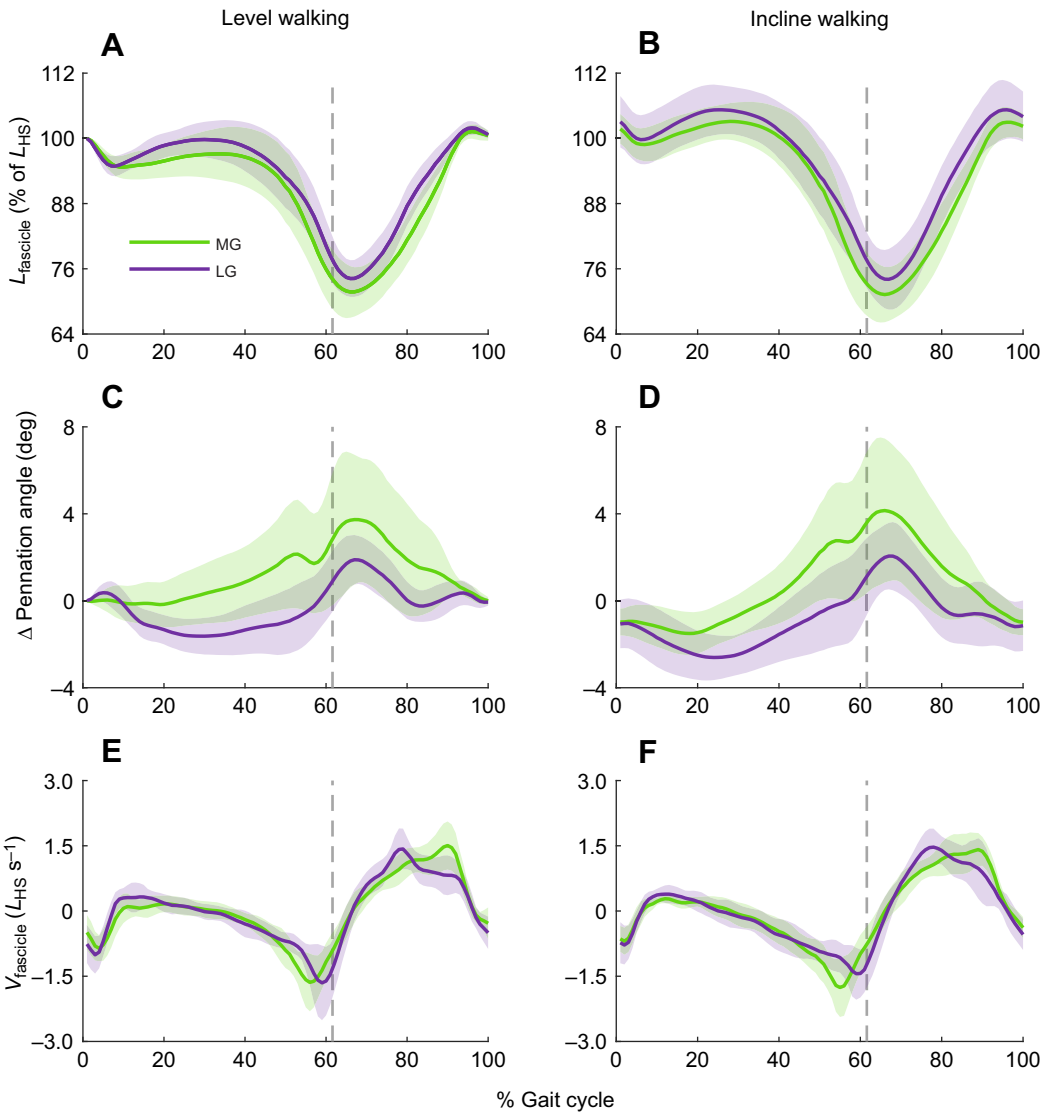

Fig. 3. Average time-varying fascicle behavior measured during walking. $L_{fascicle}$ (A,B), change in pennation angle (C,D) and $V_{fascicle}$ (E,F) data during level (left) and incline (right) walking are presented as the mean (thick line) and standard deviation (shaded area) of all participants. $L_{fascicle}$ and $V_{fascicle}$ were normalized to L_{HS} during level walking. Heel-strike occurs at 0% on the *x*-axis. The dashed vertical lines correspond to the average timing of toe-off during the gait cycle. During the lengthening period, fascicle lengthening and the decrease in pennation angle were significantly lower for the MG than for the LG, regardless of the condition. During the shortening period, there was a greater increase in pennation angle for the MG than for the LG. Moreover, during the shortening period, fascicle shortening and the change in pennation angle were higher for incline walking (right panels) than for level walking (left panels), regardless of the muscle. n=15.

We found no main effect of muscle (P=0.927), condition (P=0.793) or muscle×condition interaction (P=0.689) on V_{short} (Fig. 3E,F).

Relationship between myoelectrical activity and muscle fascicle behavior

Fig. 4 shows the individual time-varying profiles for both EMG and fascicle behavior for all participants during level walking. The EMG time-varying profiles revealed a high amount of variability between participants in terms of both shape and amplitude. For example, across participants, the MG/(MG+LG) ratio of peak EMG amplitude ranged from 44.3% to 68.6% with a mean (±s.d.) value of 57.6±8.0%. To determine whether these inter-individual differences translated into different muscle fascicle behavior, we assessed the relationship between the MG–LG differences in average EMG amplitude during the lengthening or the shortening period and the respective MG–LG differences in fascicle behavior, i.e. L_{length}, L_{short}, β_{length} and β_{short} during level walking and incline walking. Out of the eight correlations, only one significant negative correlation between MG–LG differences in average EMG amplitude during lengthening and MG–LG differences in β_{length} during level walking was observed (R^2=0.42, P=0.009). This correlation revealed that the greater the bias of activation to the MG during stance, the lower the bias of change in pennation angle to the MG. Of note, there was no correlation between MG–LG differences in average EMG amplitude during lengthening and the MG–LG differences in L_{length} (R^2=0.14, P=0.177 and R^2=0.17, P=0.127 for level and incline walking, respectively).

DISCUSSION

We combined EMG and ultrasound measurements to determine whether the observed differences in activation between the MG and

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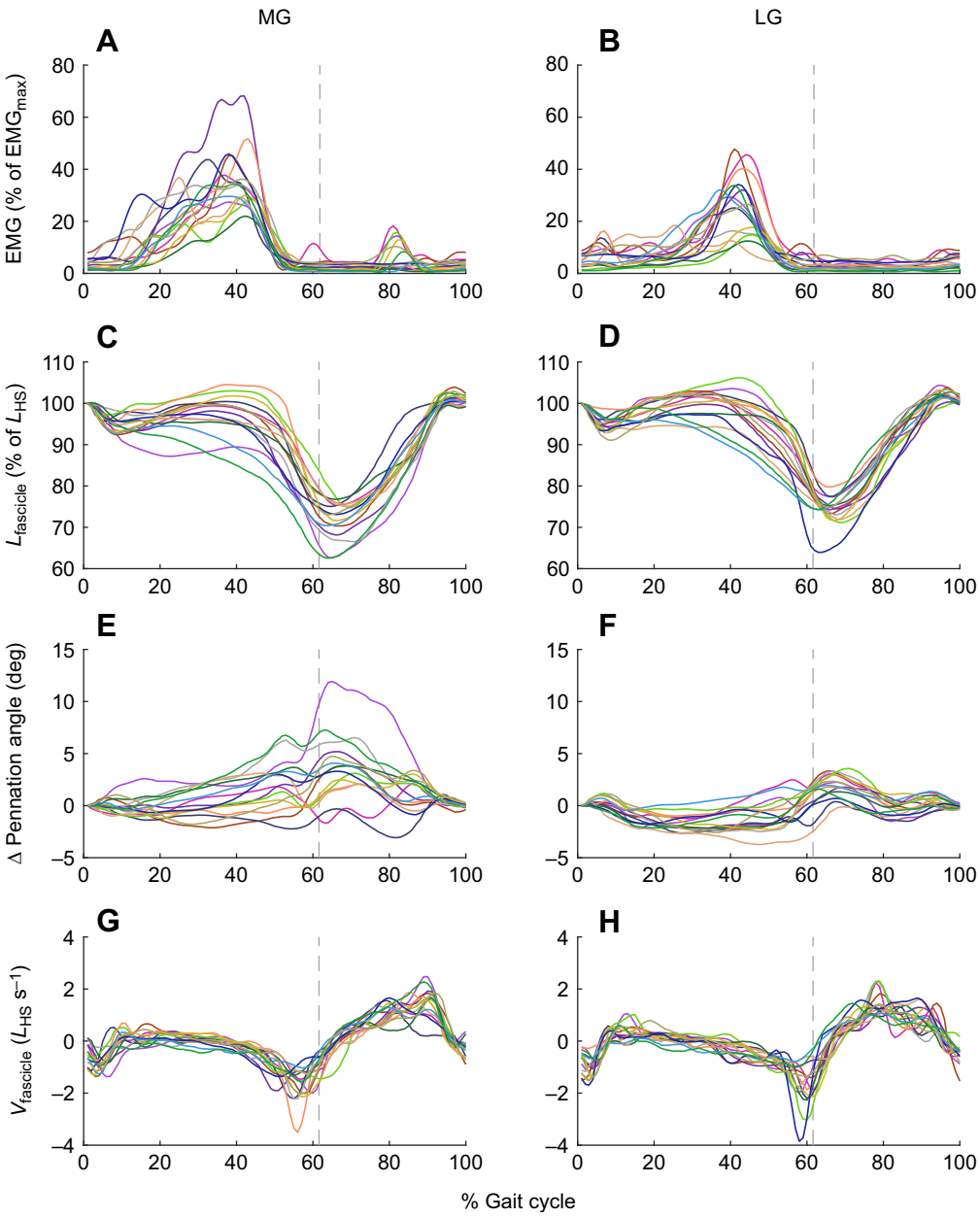

Fig. 4. Individual time-varying EMG and fascicle behavior estimated during level walking. EMG amplitude (A,B), $L_{fascicle}$ (C,D), change in pennation angle (E,F) and $V_{fascicle}$ (G,H) data for each participant (represented by different colors) for the MG (left) and LG (right). EMG amplitude was normalized to EMG_{max}; $L_{fascicle}$ and $V_{fascicle}$ were normalized to L_{HS} during level walking. Heel-strike occurs at 0% on the *x*-axis. The dashed vertical lines correspond to the average timing of toe-off during the gait cycle. Approximately one-third of the participants exhibited a second MG EMG burst during the swing phase (A) similar to previous reports (Hug et al., 2019). This pattern did not occur in the LG (B). *n*=15.

the LG during walking translate into different fascicle behavior. We found that the MG was more active than the LG but, in contrast to our hypothesis, this did not translate into greater MG fascicle shortening during the stance phase. However, we observed less MG fascicle lengthening during early stance, when compared with the LG. Additionally, the inter-individual variability in the distribution of activation between the MG and LG did not explain the variability in fascicle behavior between individuals.

The higher muscle activation for the MG than for the LG, consistent with previous reports (Ahn et al., 2011; Crouzier et al., 2019), was greatest during early stance, which coincides with the period where between-muscle differences in fascicle behavior were most apparent (Fig. 2). Specifically, we observed that the MG underwent less active lengthening than the LG during early stance by approximately 2.3% of L_{HS} (2.0 mm), corresponding to more than 30% of the total lengthening during stance. These differences in fascicle behavior that occurred early in the gait cycle are likely related to the highly tuned interaction between the gastrocnemii and their partially independent Achilles subtendons, which are able to undergo non-uniform displacement (Franz et al., 2015). During the early stance phase of walking, the MG and LG actively resist lengthening while the whole muscle–tendon unit (MTU) lengthens (Farris and Sawicki, 2012; Lichtwark and Wilson, 2006). During late stance, muscle activation decreases and the muscle–tendon interaction enables the series-elastic element to undergo rapid shortening (Lichtwark and Wilson, 2006). The smaller difference between the MG and LG activation during late stance combined with the rapid shortening of the series-elastic element likely explains why we did not observe a muscle difference in fascicle shortening or fascicle shortening velocity.

The higher muscle activation during early stance enables the MG fascicles to remain in a more isometric state, compared with the LG fascicles. It is well established that this isometric behavior of the plantar flexor muscle fascicles enables their tendon to stretch and store elastic strain energy, which is subsequently released in late push-off (Farris and Raiteri, 2017; Fukunaga et al., 2001; Ishikawa

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Table 2. Muscle fascicle parameters for the medial and lateral gastrocnemius during level and incline walking

	Level walking		Incline walking	
Muscle fascicle parameter	MG	LG	MG	LG
L_{length} (% of L_{HS})	4.6±3.1	6.9±3.5*	5.9±3.0	7.4±2.3*
β_{length} (deg)	−1.3±0.9	−2.1±0.8*	−1.2±0.5	−1.8±0.6*
L_{short} (% of L_{HS})	−24.2±3.2	−24.0±3.4	−30.9±4.7‡	−28.9±5.1‡
β_{short} (deg)	3.9±1.8	3.2±1.1*	5.4±2.5‡	4.1±1.2*,‡
V_{short} (L_{HS} s^{-1})	−2.1±0.6	−2.1±0.8	−2.2±0.6	−2.1±0.4

MG, medial gastrocnemius; LG, lateral gastrocnemius; L_{length}, amount of fascicle lengthening; β_{length}, change in pennation angle during the fascicle lengthening period; L_{short}, amount of fascicle shortening; β_{short}, change in pennation angle during the fascicle shortening period; V_{short}, peak shortening velocity; L_{HS}, fascicle length at heel-strike during level walking. Values are reported as means±s.d. *Significant difference from MG. ‡Significant difference from level walking. n=15.

et al., 2005; Lichtwark and Wilson, 2006). While a similar catapult-like mechanism occurs in the LG, our results suggest that it may occur to a lesser extent because the lower activation results in a reduced ability to maintain the LG fascicles isometrically. Thus, the MG muscle–tendon interaction may be better tuned to store and return elastic strain energy for effective push-off. These findings are inconsistent with results from a modeling study, which predicts that, during walking, MG muscle fibers undergo alternating periods of negative and positive work to act like a spring, whereas LG muscle fibers behave more isometrically to function in a strut-like manner (Lai et al., 2019). Our *in vivo* data displayed smaller active length changes in the MG fascicles, corresponding more to strut-like behavior, and a greater active lengthening in the LG, likely resulting in more negative work production and corresponding to spring-like behavior. This discrepancy may, in part, arise from differences between measured muscle activation and model-predicted muscle excitation for the MG and LG, and from the use of generic data in Lai et al. (2019). Nonetheless, the combination of experimental data with predictions from neuro-musculoskeletal models provides a powerful and promising approach to understand human and animal locomotor function.

In addition to muscle activation, differences in tissue properties may also contribute to the reduced lengthening of the MG fascicles during walking. For example, the MG muscle has, on average, a higher passive shear modulus (Le Sant et al., 2017; Lindemann et al., 2020) and a larger volume (Crouzier et al., 2018) than the LG, likely providing more resistance to lengthening. Moreover, the subtendon stiffness seems to match the muscle passive stiffness with a stiffer MG subtendon compared with the LG subtendon (Yin et al., 2021). The nervous system may choose to activate the MG more than the LG to enhance the catapult-like mechanism of the stiffer MTU and larger muscle and thus to decrease the overall activation cost (Biewener and Roberts, 2000; Crouzier et al., 2018; Crowninshield and Brand, 1981). Furthermore, it has been suggested that the MG and LG may have different roles in the frontal plane, with the MG having a greater inversion moment arm (Lee and Piazza, 2008). These differences may lead to a higher exploitation of the MG than the LG to contribute the necessary inversion in the second part of the stance phase (Arnold et al., 2014) and to allow an efficient push-off. Taken together, the differences in tissue properties and anatomy between the MG and LG MTUs and the bias of activation towards the larger MG likely results in a greater use of elastic energy – enabling more economical walking.

Despite differences in muscle activation between level and incline walking, we did not find any difference in the amount of lengthening between these conditions. There are at least three possible explanations. First, incline walking predominantly influences peak activation (25–56% of the gait cycle), when the fascicles have already stopped lengthening. This may explain the difference in fascicle shortening but not in fascicle lengthening between level and incline walking. Second, incline walking limits the ankle plantarflexion immediately after heel-strike and increases the following ankle dorsiflexion. This, in turn, increases MTU length during stance (Lichtwark and Wilson, 2006), which, by stretching the tendon, increases the tension in the tendon. This process likely supports fascicle lengthening despite the higher activation. Finally, the increased MTU length during stance caused by incline walking likely results in greater energy storing potential in the tendon. Thus, at the muscle level, the higher mechanical demand for incline walking is achieved via higher activation and greater fascicle shortening but with a similar amount of fascicle lengthening.

We found that the greater MG than LG activation was only true ‘on average’, as 4 out of the 15 participants exhibited either a balanced MG–LG peak activation (MG to LG peak activation ratio between 49% and 51%) or an activation biased towards the LG (MG to LG ratio <49%). A study performed on 85 participants observed similar inter-individual variability, repeatable between days, despite the group data also revealing that the MG was activated more than the LG (Crouzier et al., 2019). However, the MG–LG activation ratios seem more biased towards the MG in that study as only 3 out of 85 participants had a MG to LG ratio <49% and 4 out of the 85 participants had a MG to LG ratio between 49% and 51%. This discrepancy is likely due to the higher walking speed in our study (1.1 m s^{-1}) compared with that in Crouzier et al. (2019) (0.9 m s^{-1}). Indeed, when walking speed increases, the MG–LG activation ratios tend to be closer to 50% (Ahn et al., 2011). Regardless, our results show that the MG–LG differences in activation level are not related to the inter-individual variability in MG–LG differences in fascicle behavior. Several factors could explain the absence of correlations, including different tendon and muscle mechanical properties across participants. For instance, a large variability in subtendon stiffness (Yin et al., 2021) and Achilles tendon twist (Edama et al., 2015; Knaus and Blemker, 2021) has been observed between individuals. It is therefore possible that activation varies between participants to account for known differences in mechanical and architectural properties of the muscle and tendon (Aeles et al., 2017a), and enable similar movement kinetics and kinematics during motor tasks.

Some limitations in the experimental approach used in this study need to be considered. First, the ultrasound measurements were made in 2D with a limited field of view whereas muscle is a 3D complex object with non-uniform deformations (Rana and Wakeling, 2011). While it is possible that there are 3D shape changes that are affected by the activation, our analysis focused on the primary movement plane of the muscle fascicles. Moreover, to track fascicles in the same plane during locomotion is challenging. To limit the impact of this issue, we tracked the average movement of the fascicles within the field of view instead of a single fascicle

and we followed guidelines to minimize errors in fascicle tracking (Aeles et al., 2017b; Aggeloussis et al., 2010; Bolsterlee et al., 2016). Finally, EMG and ultrasound measurements were performed on the same day but during different walking trials. It is challenging to maintain both EMG electrodes and the ultrasound probe at appropriate locations for simultaneous recordings on an individual muscle. However, we averaged the data over 30 (EMG) and 10 (ultrasound) cycles, and are confident that the EMG and ultrasound patterns are representative of the walking conditions.

Conclusion

In this study, we found that, during walking, the higher activation of the MG was associated with less fascicle lengthening than for the LG. This enabled the MG fascicles to remain more isometric and may enhance the catapult-like muscle–tendon interaction in the MG, compared with the LG, and decrease the overall activation cost. At the individual level, we found no relationship between the MG–LG differences in activation and the between-muscle differences in fascicle behavior, which may be linked to potential inter-individual variability in muscle and tendon properties of the gastrocnemii. Our results highlight that slightly different neuromuscular behavior may be provided by these two synergist muscles that are often considered as equivalent muscles with the same function. These findings show that we cannot derive information from experimental measurements on one muscle to infer the behavior or the function of its synergist muscles. Empirical data from *in vivo* experiments that combine EMG and B-mode ultrasound in multiple muscles will provide insights for the evaluation of neuro-musculoskeletal models and simulations.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: F.H., T.J.M.D.; Methodology: R.H., J.A., N.Y.K., R.F., F.H., T.J.M.D.; Software: R.H., J.A., F.H.; Validation: R.H., J.A., N.Y.K., R.F., F.H., T.J.M.D.; Formal analysis: R.H., J.A.; Investigation: N.Y.K., R.F., F.H., T.J.M.D.; Resources: T.J.M.D.; Data curation: R.H., J.A., N.Y.K., R.F., T.J.M.D.; Writing - original draft: R.H., J.A.; Writing - review & editing: R.H., J.A., F.H., T.J.M.D.; Visualization: R.H.; Supervision: J.A., F.H., T.J.M.D.; Project administration: T.J.M.D.; Funding acquisition: F.H., T.J.M.D.

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Data availability

The EMG and ultrasound data are available from figshare: https://doi.org/10.6084/m9.figshare.14251130.v4

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