# Gastrocnemius Medialis and Vastus Lateralis *in vivo* muscle-tendon behaviour during running at increasing speeds

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## ABSTRACT

This study combines *in vivo* ultrasound measurements of the Vastus Lateralis (VL) and Gastrocnemius Medialis (GM) muscles with electromyographic, kinematic and kinetic measurements during treadmill running at different speeds (10, 13 and 16 km·h$^{-1}$) to better understand the role of muscle and tendon behaviour in two functionally different muscle-tendon units. In addition, the Force-Length and Force-Velocity relationships of VL and GM were experimentally assessed by combining dynamometry and EMG data with ultrasound measurements. With increasing running speed, the operating length of the fascicles in the stance phase shifted towards smaller lengths in the GM ($P<0.05$; moving down the ascending limb of the F-L relationship) and longer lengths in the VL ($P<0.05$; moving down the descending limb) at all speeds; however, both muscles contracted close to their optimal length $L_0$, where isometric force is maximal. Whereas the length of VL SEE did not change as a function of speed, GM SEE lengthened and shortened more at higher speeds. With increasing running speed, the contribution of elastic strain energy to the positive power generated by the MTU increased more for GM (from 0.75 to 1.56 W·kg$^{-1}$) than for VL (from 0.62 to 1.02 W·kg$^{-1}$). Notwithstanding these differences, these results indicate that, at increasing running speeds, both the VL and GM muscles produce high forces at low contraction velocities, and that the primary function of both muscle-tendon units is to enhance the storage and recovery of elastic strain energy.

# INTRODUCTION

In the past two decades, advancements in ultrasonographic techniques have made it possible to investigate *in vivo* the behaviour of human muscles and tendons during locomotion [1-3]. Combining *in vivo* scanning with more standard biomechanical measurements, such as inverse dynamics techniques, has allowed studying the link between movement kinetics and muscle-tendon behaviour [4,5]. Most studies have focused on ankle function and the calf muscles[1-10]. For instance, it has been shown that the triceps surae muscle group (i.e. soleus, gastrocnemius medialis and gastrocnemius lateralis) produces forces up to 12 times body weight during running[11-12] and is the main force producer amongst all the major lower-limb muscle groups[12]. When running speed increases, the plantar flexor muscles must generate force over an increasingly shorter period of time, and this requires more forceful and rapid contractions[13]. To achieve this and meet the energy demands for sustaining running, the muscle-tendon units (MTUs) of the ankle plantar flexors work in a highly complex and tightly integrated manner[2,14]. Due to their unique design, with short muscle fibres and a long Achilles tendon, the ankle plantar flexors can exert high levels of power with minimal energy demands, as the tendon's mechanical behaviour allows the muscle to operate more isometrically, accommodating the behaviour of the MTU through a large tendon displacement[4,5]. This mechanism has a favourable effect on contractile force generation and the associated metabolic cost [4,5,15]. Modelling studies have, indeed, demonstrated that tendon strain can optimize the region where muscle fibres operate on their Force-Length (F-L) and Force-Velocity (F-V) relationships during running at increasing speeds[4,5]. Furthermore, these studies have shown that the stretching and subsequent recoiling of the Achilles tendon during running allows storing and releasing substantial amounts of elastic strain energy, which increase as a function of running speed, thus contributing more to the positive mechanical work done by the MTU of the ankle plantar-flexors [4,5]. For example, Farris & Sawicki[5] as well as Lai et al.[4] showed that the relative contribution of the Achilles tendon elastic strain energy to the positive power done by the MTU is about 65-75% at low running speeds and increases as a function of speed. It has, therefore, been postulated that this increasing elastic strain energy contribution with speed allows the muscles to undergo an increasingly smaller length change during contraction[16]. However, only few studies have so far investigated the *in-vivo* operating length and velocity of the ankle plantar flexor muscles, as well as the relative contribution of the elastic strain energy, during running at increasing speeds.

To fully understand the complex interplay between muscle and tendon behaviour during running, the contribution of other contributing MTUs with different characteristics to those of the plantar flexors should also be considered. The knee extensor muscles are of particular interest as they have longer fascicles and a stiffer and shorter tendon compared to the ankle plantar flexors[17-19], implying that the quadriceps muscle may undergo substantial length changes during locomotion[20]. Consistent with this notion are the findings of modelling studies, showing large fascicle length changes in the vastus lateralis (VL) during running, leading to hypothetical large shifts of the F-L and F-V relationships[21,22]. However, a more recent *in vivo* study[23] showed that the VL fascicles operate close to their optimum length and quasi-isometrically when running at 10 $km \cdot h^{-1}$. As a consequence, the large displacement of the VL MTU was primarily associated with changes in the length of the patellar-quadriceps tendon complex. However, no studies have so far quantified the operating length and velocity of VL or the relative contribution of its tendon complex in determining mechanical power production during running at increasing speeds.

Therefore, the present study combines *in vivo* ultrasound measurements of VL and gastrocnemius medialis (GM) muscle behaviour with electromyographic and kinetic measurements during treadmill running in order to investigate the behaviour of these two functionally different MTUs at different running speeds. In particular, we experimentally investigated: 1) the operating length and velocity of muscle fascicles and 2) the relative contribution of muscle fascicles and series elastic element (SEE) to the positive mechanical power done by the whole MTU, to better understand how they change as a function of running speed.

Based on recent literature, we hypothesised that both muscles would demonstrate small fascicle length changes, close to their optimal length, allowing the muscles to operate at a high force potential during running at increasing speeds. Therefore, we expected that the SEE would accommodate the larger part of the MTU length changes at each running speed for both GM and VL. We also expected that, for both MTUs, power would be enhanced at faster running speeds as elastic strain energy would contribute a greater proportion of the MTUs positive work during the stance phase of running.

# METHODS

## Ethical Approval

All participants received written and oral information and instructions before the study and gave their written informed consent to the experimental procedure. The experimental protocol was approved by the Ethical Committee of Liverpool John Moores University (protocol number: 18/SPS/028) and was performed in accordance with the Helsinki Declaration.

## Participants

Fifteen men (age: 24±2.4 years; body mass: 74±2.8 kg; height: 1.77±0.04 m) accustomed to endurance running participated in this study. The participants did not report any type of neuromuscular injury in the six months before the experiments.

## Experimental Design

Each subject participated in two experimental sessions. In the first session, the Force-Length (F-L) and Force-Velocity (F-V) relationships of VL and GM were experimentally assessed by means of isometric maximal voluntary contractions (MVC) and isokinetic tests of the knee extensors and plantar flexors, respectively. During these experiments, an ultrasound apparatus was utilised to record the fascicle length of VL and GM. The force applied to the patellar and Achilles tendon was calculated from the knee/ankle joint moment and the tendon lever arms. Finally, during each contraction mode test (isometric or isokinetic) the EMG activity of VL or GM was measured as well as that of the corresponding antagonist muscle: biceps femoris or tibialis anterior.

In the second session, the fascicle length of VL and GM, the kinematics of the body segments, the dynamics (ground reaction forces) and the EMG activity of the above two muscles were measured during running on an instrumented dual-belt treadmill at three different speeds: 10, 13 and 16 km·h$^{-1}$.

## Data Collection

### *Assessment of the F-L relationship*

For the knee extensors measurements, the participants were secured on a dynamometer (Cybex NORM, USA), fixed with a trunk and pelvic strap and the arms positioned crossed in front of the chest. For the plantar flexor measurements, the participants were secured to the same dynamometer in a prone lying position with the right knee in the anatomical position and the foot of the dominant leg fixed to the dynamometer footplate.

The F-L relationship was calculated from MVCs at various joint angles. For the knee extensors: the hip joint angle was set at 85° (0° refers to supine position) to reduce the contribution of the rectus femoris to the resultant moment of the knee extensors[23,24]; for the plantar flexors: the legs were fully extended in the anatomical position. For the knee extensors, eight MVCs of the right leg were performed from 90° to 20° of knee joint angle (0° = knee fully extended), whereas for the plantar flexors five MVCs were performed from 20° plantarflexion to 20° dorsiflexion (0° = foot at right angles to the shank) at 10° intervals.

The actual knee and ankle angles during the MVCs were measured in 2D to obtain the leverage of both muscle groups. Two dimensional kinematics were recorded on the basis of five markers for the leg: iliac spine and greater trochanter of the opposite side; lower portion of the patella (patellar tendon origin); upper anterior surface of the tibia (patellar tendon insertion); mid tibiofemoral gap (considered to represent the tibiofemoral contact point as the knee centre of rotation). Foot kinematics were based on seven surface markers: mid tibiofemoral gap point, heel, toe, medial malleolus, insertion of Achilles tendon and markers at 5 and 10 cm proximal to the calcaneal insertion. The marker positions were recorded by means of video analysis (Casio Exilim Camera) at 200 Hz and analysed with a video processing software (Tracker v4.0). The camera was positioned on the left side of the subject, at right angles to the longitudinal axis of the thigh. To eliminate any radial distortion, a rectilinear filter was applied during marker tracking on the video frames. The resultant marker trajectories were smoothed using a forward and reverse pass second order low pass Butterwoth filter (cut-off 15 Hz).

During the MVC, fascicle length changes were captured by B-mode ultrasound imaging with a 6 cm linear array probe operating at 60 Hz (Philips EPIQ 5). For VL and GM, the probe was attached to the skin approximately at 50% of the femur length and at 30% of the distance between the popliteal crease and the malleolus, respectively. In both cases, the ultrasound probe was located on the muscle belly and corrected with respect to the superficial and deeper aponeurosis, in order to have a clear image of the perimysial connective intramuscular tissue that is indicative of the muscle fascicle structure[6-10]. *In vivo* muscle fascicle length and pennation angle were calculated from the ultrasound videos (see Data Analysis).

Finally, the EMG activity of the VL and GM and the corresponding antagonist muscles, were recorded during the knee extensor and plantar flexor MVCs, respectively. Two bipolar Ag-AgCl electrodes were placed in the central region of the muscles after skin preparation (including shaving, gentle abrasion and cleaning with an alcohol-based tissue pad). The raw EMG data were recorded at 1000 Hz with a Biopac System (MP100, Biopac System, Santa Barbara, USA) together with the dynamometer data (angular velocity, moment and position). All instrumentation used in these experimental procedures were synchronised with an external manual trigger (5 V).

### Assessment of the F-V relationship

To obtain the F-V relationship, maximum isokinetic torque was recorded at the angular velocities of 30, 90, 150, 180 and 210 $\text{deg}\cdot\text{s}^{-1}$ for the plantar flexors and 45, 90, 150, 210 and 250 $\text{deg}\cdot\text{s}^{-1}$ for the knee extensors. The range of motion used during the MVC was also used during the isokinetic trials and data were analysed only in the isokinetic phase of the contraction. After a familiarization trial, the subjects performed three consecutive contractions at each velocity with two min of recovery in between. Also in these cases, the tendon force was calculated by taking into account the tendon lever arm and the influences of the antagonist muscle were subtracted, as described in the next section. All instrumentation was synchronised with an external manual trigger as above (5 V).

*Running trials*

Each athlete ran on an instrumented treadmill at three different constant speeds (10, 13 and 16 km·h$^{-1}$) for 6 min using a self- selected step frequency and step length. All participants were forefoot runners. Small retroreflective markers (14 mm diameter) were placed at specific anatomical locations on the participants' trunk, arms, and lower limbs. The marker set used in this study was the same proposed by Lai et al.[4]. However, we added another ten markers (four on the right and left knee and the other six on the right shank/foot) to measure the tendon lever arm during running as described by Rasske et al. [25] (see Data Analysis). The 3D marker trajectories were recorded using 12 Vicon cameras (Vero 2.2, Oxford Metrics, UK) sampling at 250 Hz. Ground reaction forces (GRFs), the centre of pressure and the free moment vectors were recorded using two force plates embedded in the instrumented treadmill (M-GAIT, MOTEK) sampling at 1500 Hz.

A B-mode ultrasound scanner (Telemed Echo Blaster 128, Vilnius, Lithuania) was used to record images at 60 Hz with a depth and width of 60 mm. Ultrasound data were recorded from the right VL and GM of each participant (in two separate running trials and in a randomized order) with the ultrasound probe positioned in the same location utilised during the dynamometric measurements[6-10]. Fascicle length and pennation angle were calculated with the software and procedures mentioned in the dynamometric measurements and described in the data analysis.

Finally, EMG signals from Ag-AgCl bipolar electrodes were collected simultaneously with the ultrasound, kinematic and kinetic signals using a wireless system (Biopac System) sampling at 1000 Hz. The location of the electrodes was the same used in the dynamometric measurements. All experimental data were synchronised by a digital output generated by the ultrasound scanner that triggered all instrumentation (the Vicon cameras, the treadmill ground reaction forces and the EMG signals).

## Data Analysis

*Dynamometric measurements*

The total moment generated by the knee extensor and plantar flexor muscles was corrected for the gravitational force effects (determined during a passive joint rotation driven by the dynamometer) and the joint and the dynamometer axes were aligned visually during contraction. Finally, the EMG-moment relationships of the antagonist muscles (biceps femoris and tibialis anterior) were determined to estimate and account for the contribution of the antagonistic moment to the net joint moment, as described in detail previously[26].

To calculate the force applied to the tendon (patellar or Achilles), the joint moment measured with the dynamometer was divided by the tendon lever arm using a 2D approach. For the knee extensor, the lever arm was measured as the perpendicular distance from the tendon's line of action to the centre of rotation of the knee, based on the position of three markers (origin and insertion of the patellar tendon and the mid tibiofemoral gap point that was considered to represent the knee centre of rotation). The location of surface markers on these anatomical landmarks was determined by ultrasound scanning. Particularly, the surface marker over the tibiofemoral contact point was positioned in the mid space between the femur condyle and tibia plateau. This "geometric method" (see Tsapoulos et al.[27] for details) uses the tibio-femoral contact point to represent the knee centre of rotation and was used in different conditions and locomotion tasks[26-28]. In the case of plantar flexors, the lever arm was measured as the perpendicular distance from the tendon's line of action to the centre of rotation of the ankle based on the position of four markers (medial malleolus representing the ankle centre of rotation, insertion of Achilles tendon, and markers at 5 and 10 cm proximal to the calcaneus to represent the Achilles tendon line of action), as suggested by Rasske et al.[25]. The location of each marker was determined by ultrasound scanning as previously suggested by Rasske et al. [25], in order to place the markers on the correct anatomical positions.

By knowing the maximum force applied to the patellar/Achilles tendon during the MVCs and the corresponding muscle fascicle length, the F-L relationship was determined for each subject based on a second-order polynomial fit[23]. Based on this relationship, the maximal isometric force applied to the tendon ($F_{max}$) and the optimal shortening length ($L_0$, the length at which this peak occurs) were determined.

The F-V relationship was determined based on the force and fascicle velocity values during the isokinetic test and these values were fitted using the following exponential equation[29]:

$$v = \left(e^{-\frac{P}{b}} - e^{-\frac{P_0}{b}}\right) a$$

where $v$ is the fascicle velocity ($m \cdot s^{-1}$), $P$ is the force value, $P_0$ the maximal isometric force recorded during the MVC and extrapolated by the polynomial fit and $a$ and $b$ are experimentally determined constants. Constants $a$ and $b$ were obtained from the intercept ($a/b$) and slope ($1/b$) of the linearized Hill's plot of $(P_0 - P)/V$ *vs.* $P$, where $P$ is the torque developed at different velocities of shortening, $V$ is the angular velocity of shortening and $P_0$ is the maximal force of contraction (isometric, as obtained from the F-L curve) [30,31]. Finally, the intercept value on the abscissa was taken as the maximal fascicle shortening velocity ($V_{max}$).

For the ultrasound measurements, the length of muscle fascicles was defined as the distance between the deep and superficial aponeuroses. Pennation angle (α) was defined as the angle between the collagenous tissue and the deep aponeurosis[9]. A validated automatic tracking algorithm was used to quantify the muscle fascicle length and pennation angle frame by frame[32]. At the end of the auto-tracking, every frame of the tracked fascicle lengths and pennation angles was visually examined to check the algorithm accuracy. Whenever the fascicle length or pennation angle was deemed inaccurate, the two points defining the muscle fascicles were manually repositioned. For the dynamometry measurements, only the fascicle length was taken into consideration.

The raw EMG signal during the isometric contractions was filtered with a band-pass third order Butterworth filter at 20-500 Hz, whereas the onset of muscle activity was detected by a threshold that was defined as the baseline activity plus three times its standard deviation. Finally, the root-mean-square of the signal was calculated.

### Running measurements

In the last minute of each running trial, kinematics, kinetics, EMG and ultrasound data were analysed for ten stance phases for each participant. This timing was chosen to coincide with the determination of oxygen uptake. Data of each instrument were interpolated at 200 sample points. Each subject repeated the runs at 10, 13 and 16 km·h$^{-1}$ twice, once for scanning the GM muscle and a second time to scan the VL muscle.

During running, based on the kinematics and kinetics variables, a 2D inverse kinematics and inverse dynamics approach were used to compute ankle/knee joint angles and net joint moments, respectively[7]. Finally, joint powers were calculated by multiplying the net moment at each joint by the corresponding angular velocity, at each time interval. Net joint moments and joint powers were finally normalised to body mass. The calculations were performed with a custom written LabView program (v.10).

For the EMG data collected during running, the same noise reduction and onset identification procedure used for the MVC was applied. Moreover, a linear envelope of the EMG signal was calculated using the root-mean-square of a moving window (100 ms). Each muscle's linear envelope was then normalised to the peak magnitude of the respective linear EMG envelope measured during MVC and thus expressed in %RMS of MVC.

For the running measurements the MTU length of VL and GM were computed, at each instant, using the instantaneous joint angles as proposed by Hawkins and Hull[33]. The SEE length, representing free tendon and aponeurosis for each MTU, was calculated as the difference between MTU length and the ultrasound-measured muscle fascicle length, taking into account the influence of pennation angle[1]. MTU, muscle fascicle and SEE lengths were then normalised to their resting length (during static standing); velocities were then computed by differentiating the normalised lengths of each component with respect to time[4]. A reliability and sensitivity study of the ultrasound-based measurement of the VL and GM fascicle behaviour and MTU behaviour throughout the entire stance phase revealed very good reliability between single trials at all investigated speeds, with the coefficient of multiple correlations (CMC) ranging from 0.90 and 0.98. Moreover, the average root mean square difference (RMSD) values were about 5-7 mm for GM and VL fascicle lengths and around 3-4 mm for the corresponding MTUs lengths (see Supplementary Material).

The following parameters were calculated (both in GM and VL): i) average MTU length during the stance phase (upper panels in Figure 1); ii) fascicle shortening in the stance phase, taken as the peak (negative) value of the fascicle length during shortening (middle panels in Figure 1); iii) SEE strain, taken as the peak (positive) value in the stance phase; iv) SEE recoil, calculated as the mean of the maximum and minimum strain values in the late stance phase (from the maximum value of strain to the end of the stance phase; bottom panels in Figure 1).

Achilles and patellar tendon forces were calculated as the net ankle or net knee moment divided by the tendon lever arm, where the moment arm was estimated as suggested by Rasske et al.[25]. The contribution of the antagonist moment (biceps femoris and tibialis anterior) to the net joint moment, was taken into account (as described above) and subtracted from that of the agonist muscles.

As proposed by Farris & Sawicki[5] the force attributable to GM and VL series elastic elements ($F_{SEE}$) can be estimated by multiplying the “overall” tendon force by the relative PCSA of these muscles according to the literature (15.9% of the PCSA of plantar flexors for GM and to and 34% of the PCSA of the knee extensors for VL[34,35]). To estimate the force acting along the GM and along the VL muscle fascicles, the corresponding $F_{SEE}$ was divided by the cosine of their pennation angle, as proposed by Lichtwark & Wilson[9]. Finally, the MTU force of those two muscles was assumed to be equal to $F_{SEE}$, as suggested by Farris & Sawicki[5].

The power output of the GM and VL MTU, of the GM and VL muscle fascicles and of the GM and VL SEE was then calculated as the product of their respective forces and velocities, where the velocity was the first derivative of the length changes[5]; the positive power (during the propulsive phase) was then estimated for each running trial: positive power for the fascicle ($P_{fas}^{+}$), for the SEE ($P_{SEE}^{+}$) and for the MTU ($P_{MTU}^{+}$). The interplay between these power values is indicative of the fascicle and tendon interaction[5] where the most efficient scenario corresponds to $P_{fas}^{+} = 0$, a condition in which the fascicles are contracting isometrically and all $P_{MTU}^{+}$ is supplied by $P_{SEE}^{+}$ (i.e. by SEE recoil).

Force and velocity variations with fascicle operating length of GM and VL during the active state of the stance phase (at the three running speeds) are plotted in Figure 2 over the F-L and F-V curves as obtained during the dynamometric measurements. Fascicle length and fascicles velocity values were obtained from the analysis of the ultrasound scans. As proposed by Bohm et al.[23], force was normalised to the maximum force obtained during the maximal isometric knee extension and plantar flexors contractions ($F/F_{max}$); fascicle length and fascicle velocity were normalized to the experimentally determined optimal fascicle length ($L/L_0$) and maximum shortening velocity ($V/V_{max}$), respectively. Therefore, the estimated forces corresponding to the fascicle lengths and velocities measured during running represent values at the activation level the contracting muscles exhibited during each running task.

### Statistical analyses

A one-way ANOVA for repeated measures was conducted to test the possible differences among running speeds. The following outcome measures were tested (during the stance phase): mean length of MTU; fascicles shortening; SEE strain and recoil; average EMG activity (linear envelope); mean values of fascicle operating length, $F_{max}$ and $V_{max}$; the mechanical power production of each component in both muscles (VL and GM). When significant main effects were found, a post-hoc pairwise comparison using Fisher's least significant difference was used to determine the effect of speed. The alpha level was set to $P < 0.05$ and statistical analysis was performed with SPSS (v24.0). All data extracted for statistical analysis were normally distributed (Shapiro-Wilk normality test, $P > 0.05$).

## RESULTS

### F-L and F-V relationships

The F-L and F-V curves as obtained during the dynamometric measurements are reported in Figure 2. The values of $L_0$, $F_{max}$ and $v_{max}$ derived from these relationships were 9.57±1.66 cm, 5107±882 N and 122.3±14.5 cm·s$^{-1}$ for the VL and 5.37±1.01 cm, 1019±177 N and 107.4±11.3 cm·s$^{-1}$ for the GM, respectively. The operating length of the fascicles, the force and the velocity of GM and VL during the active state of the stance phase (at the three running speeds) are also plotted in Figure 2 over the F-L and F-V curves. The average length of both muscles during the stance phase changed significantly as a function of running speed (Figure 2A: VL, Figure 2C: GM) (main effect: $P<0.001$). However, the length changes (L) in the muscles occurred in opposite directions: in respect to their corresponding optimal length ($L_0$,), VL fascicle length increased significantly (main effect: $P<0.01$) while GM fascicle length decreased significantly (main effect: $P<0.001$) with running speed. For each muscle, the comparisons revealed significant differences between all running speeds. However, at all speeds, both muscles operated close to their optimal length, the relative values ($L/L_0$) being

1.03±0.05, 1.06±0.06 and 1.1±0.06 for VL and 0.92±0.05, 0.88±0.07, 0.83±0.07 for GM, at 10, 13 and 16 km·h$^{-1}$, respectively.

Contraction velocity of VL and GM fascicles showed significant differences as a function of running speed (Figure 2B: VL, Figure 2D: GM) (main effect: $P<0.001$). Contraction velocity decreased for VL when the speed increased (main effect: $P<0.05$), whereas it increased for GM (main effect: $P<0.01$). Similar to fascicle length changes, the comparison of velocities for each muscle between running speeds showed significant differences ($P<0.05$) between all conditions. For both muscles, contraction velocity (V) was lower than their corresponding maximum values, with the relative values ($V/V_{max}$) being: 3.1±3, 1.8 ±3.5, -2.8±3.8 % for VL and 5±4.8, 8.1±6.7, 12±7 % for GM, at 10, 13 and 16 km·h$^{-1}$, respectively.

## Muscle and tendon parameters during running

Figure 3 shows the mean MTU length values, the fascicle shortening, the SEE strain and recoil for both muscles in each running trial. VL MTU length, as well as VL SEE strain and recoil, showed no significant differences between running speeds, whereas an increase in VL fascicle shortening (main effect: $P < 0.001$) was observed at each speed. The average length of the GM MTU increased with running speed (main effect: $P < 0.001$). The same was the case for the GM fascicle shortening (main effect: $P < 0.001$) and GM SEE strain and recoil (main effect: $P < 0.001$), with significant differences between all three speeds for the latter.

## EMG activity

Figure 4 shows the average EMG values of VL and GM in all running conditions during the stance phase of the gait cycle. The mean EMG values of VL and GM showed significant differences as a function of speed (main effect: $P<0.001$ and $P<0.01$ for GM and VL, respectively), with significant differences found for each muscle between all running speeds.

## Kinetic parameters

Figure 5 shows the average joint power at the level of knee and ankle during the stance phase at each running velocity (upper panels). Joint power parameters could be divided into two different phases: negative (absorption) or positive (propulsion). During the absorption phase the mean values of knee joint power were -6.25±0.88, -6.43±0.81, -6.53±0.77 W·kg$^{-1}$, whereas during the propulsion phase were 2.3±0.45, 3.29±0.51, 3.39±0.54 W·kg$^{-1}$, at 10, 13 and 16 km·h$^{-1}$, respectively. Significant differences were observed between all running speeds ($P<0.05$ and $P<0.01$ for negative and positive phase, respectively). The mean absorption values of ankle were -3±0.23, -4.51±0.37, -7.12±0.44

$W \cdot kg^{-1}$, whereas during the propulsion phase were 5.40±0.88, 7.38±0.96, 9.72±0.91 $W \cdot kg^{-1}$, at 10, 13 and 16 $km \cdot h^{-1}$, respectively. Significant differences were observed between all running speeds ($P<0.001$ for both stance phases).

Increasing running speed from 10 to 16 $km \cdot h^{-1}$ resulted in an increase in average positive power produced by the MTU ($P_{MTU}^{+}$) in both muscles (middle panel of Figure 5) (VL: $P<0.05$ and GM: $P<0.001$) and significant differences were observed among speeds. $P_{SEE}^{+}$ increased in GM as a function of speed ($P<0.01$), whereas no significant difference was observed for VL. For both muscles, $P_{fas}^{+}$ showed significant differences as a function of speed ($P<0.05$).

The relative contribution of the fascicles in determining $P_{MTU}^{+}$ was different between muscles (lower panel in Figure 5). For VL, 45% of the $P_{MTU}^{+}$ could be attributed to the fascicle behaviour (no significant changes among speeds), whereas for GM, only 35% of the positive power was generated by the fascicle (no significant changes among speeds). As a consequence, tendon elastic power contributed 55% and 65% to the positive power done by the MTU for VL and GM, respectively (as an average, at all investigated speeds).

## DISCUSSION

In the present study, the fascicle and SEE behaviours of two functionally different MTUs (Vastus Lateralis in the knee extensors and Gastrocnemius Medialis in the ankle plantar flexors) were analysed during running at increasing speeds. Our data showed that, with increasing running speeds, both muscles operate at high contractile force potential and low velocity regions, confirming fully our first hypothesis. However, our second hypothesis is only partly confirmed as the SEE behaviour at increasing running speeds differed between the two MTUs.

With faster running speeds, the GM muscle fascicle operating range shifted towards smaller lengths, on the ascending limb of the F-L relationship. This muscle fascicle shortening allows the SEE to provide more elastic energy during the propulsion phase and therefore to produce more positive power as speed increases. In contrast, the operating length range of the VL muscle fascicles shifted towards longer lengths, on the descending limb of the F-L curve, with faster running speeds. However, the VL fascicle length changes are much smaller than those in the GM fascicles (with no major differences as a function of speed), and there are no significant changes in either SEE recoil or the positive mechanical power done by the SEE. Notwithstanding the lack of advantage in terms of elastic energy recovery from the VL SEE, the quasi-isometric behaviour of VL fascicles during running at increasing speeds has the benefit of economical muscle force production during the propulsion phase.

Data reported in this study confirm the findings of the modelling study of Lai et al. [4] and the hypothesis formulated by Bohm et al. [23] for the GM MTU and extend these considerations to the *in vivo* behaviour of the VL MTU at different running speeds.

As shown by our data, increasing running speed affected differently the mechanical output of the two muscles. Contrary to our second hypothesis, the mechanical power provided by the SEE during the propulsive phase of running increased as a function of speed in the GM only, while it remained relatively constant for VL. On the other hand, the relative contribution of tendon elastic strain energy to the positive work done by the MTU was found to be rather constant as a function of speed in both muscles and to amount to about 65% for GM and 55% for VL.

A likely explanation for the lack of length changes in VL SEE at faster speeds (and therefore greater loading) is the high stiffness of the patellar-quadriceps tendon complex [17-19] in combination with the anatomical position of this structure distally to the point of ground reaction force application. These characteristics are consistent with a SEE suited more for effective contractile force transmission distally and joint position control, rather than elastic strain energy storage and release[36]. Furthermore, the architecture of the knee extensor MTU (longer muscle fascicles compared to the in-series shorter tendon) is suited more for dissipation of the mechanical energy during the first instant of the shock-absorption phase (see Figure 5). Indeed, when the speed increased, the ankle plantar flexors absorbed most of the mechanical power, allowing the knee extensors to work with little energy changes as a function of speed (see Figure 5). Therefore, the small changes in the negative phase of power may not have been sufficient to further increase the elongation and consequent recoil in the VL SEE. Indeed, as indicated by several authors [4,12,21,22] during the first part of stance, VL absorbs mechanical energy whereas in the second phase of stance (propulsion phase) its function is to stabilize the body. In the propulsion phase, the back thigh muscles (especially the hamstrings) play the most important role in force development (e.g. Farris & Sawicki[5]). Therefore, it can be expected that the contribution of VL to force production decreases as a function of speed, allowing the hip and the hamstrings to provide more force as running speed increases.

The important differences in the SEE behaviour between GM and VL MTUs result in a different mechanical output for the MTU. During the energy absorption phase of running, both MTUs store elastic strain energy, but as showed in Figure 5, the power generated by GM is far larger than that of VL. Consequently, it is possible that the SEE of VL absorbs and dissipates mechanical power, where the SEE of the GM works more as an energy saving mechanism. One other possible contributing mechanism for the SEE behaviour of GM is proximal-distal power transfer between joints, since this is a bi-articular muscle[37]. Therefore, further studies should include multiple levels of analysis for the SEE.

## VL muscle-tendon behaviour

This is the first study that experimentally investigated the operating length and velocity of the human VL fascicles during level running at increasing speeds. Our data provide evidence that the VL fascicles operate close to their optimum working length ($L_0$) with small length changes during the stance phase, where the muscle is active and generates force and power. Furthermore, the same muscle fascicle behaviour was observed with increasing speed.

As highlighted by our data, at increasing speeds, the VL muscle was activated in the initial stance phase, and this is associated with the function of the muscle to decelerate and support the body mass [12]. In agreement with Bohm et al.[23], during the active state, the MTU showed the largest elongation (Figure 1), whereas the fascicles operated with significantly smaller changes around their optimal length ($L_0$). Indeed, as shown by Figure 1, the main length changes of the VL MTU were primarily associated with changes of the series-elastic elements of the VL (patellar and quadriceps tendon complex). This SEE behaviour, allows the fascicles to take advantage of the high force-length-velocity potentials during the active phase of the stance phase where the VL muscle generates force[23,38].

With increasing running speed, the VL fascicles operate at longer lengths on the descending limb of the F-L curve (see Figure 3), however, the favourable fascicle length conditions (isometric behaviour during stance) seem to be the result of adjusted muscle activation. In all running conditions, VL muscle EMG activity was increased in the first part of the stance phase where the MTU is lengthened. This coordinated time course between EMG activity and MTU elongation provides evidence that time-adjusted muscle activation contributes to the minimisation of fascicle length changes during running at increasing speed. As a consequence, the MTU could operate at a high force-generating potential and absorb substantial power during the first phase of the stance, due to SEE strain (Figures 2 and 5, upper panel). On the contrary, during the propulsion phase the activation of VL muscle is much lower (10-20% of the EMG activity at MVC) (Figure 4), but sufficient to allow the VL muscle fascicles to remain quasi-isometric also in the second part of the stance phase. It is possible that the SEE recoil allows the muscle to maintain a rather constant length, while the SEE accommodates the larger part of the MTU length change. This speculation is supported by the pattern of mechanical power production during the propulsion phase in our tests, which show that about half of the positive power provided by VL is derived from SEE and that VL SEE elongation did not change significantly over the speeds tested (Figure 5, lower panel).

Therefore, the favourable fascicle operating conditions observed in the present study may have functional importance for human running at increasing speeds, because less active muscle volume would be required for a certain mechanical demand, allowing a reduction in metabolic cost during locomotion [4,14,39]. Since during the propulsion phase the VL does not contribute to the positive power generated (Figure 5, upper panel), the knee extensors do not need to provide additional energy and a simple energy exchange within the MTU close to the optimal length ($L_0$) of the muscle fascicles may allow for a minimisation of the activation level and duration of muscle activity during the stance phase, which improves the economy of muscle force generation[23,29].

## GM muscle-tendon behaviour

This is the first study experimentally investigating the operating length and velocity of the human GM fascicles during level running at increasing speeds. The results indicate that the GM fascicle length continued to shorten (albeit moderately) throughout the period of high force development, irrespective of running speed.

A previous modelling study investigating the behaviour of the GM MTU during running at increasing speed found similar results to ours, with the fascicles operating isometrically, at smaller lengths as a function of running speed[4]. Furthermore, GM fascicle length change has also been observed to be small during running in experimental studies but at lower running speeds[3,5,10]. In the present study, the GM fascicles shortened relatively little during the stance phase of running (Figure 3), but more than those of VL. Operating on the ascending limb of the F-L curve may be a mechanism to lessen the likelihood of muscle damage caused by active eccentric contractions [40,41]. Similar to the VL fascicles, the GM fascicles operated quasi-isometrically and close to their optimal length ($L_0$, see Figure 2) allowing the GM muscle to develop large contractile forces. These muscle forces increase the stretch and recoil of the tendon, thereby facilitating greater storage and recovery of tendon elastic strain energy.

We found that the GM elastic strain energy provided a greater relative contribution to the positive power done by the MTU compared to the positive power provided by the muscle fascicles and that this contribution increased as a function of speed in absolute terms but remained almost constant in relative terms (Figure 5). This result is consistent with previous studies suggesting that muscle fibres in distal limb muscles, such as the ankle plantar-flexors, contract isometrically to facilitate greater storage and recovery of tendon elastic strain energy at fast locomotion speeds[42].

The greater storage and recovery of elastic strain energy, however, is coupled with an unfavourable shift of the muscle fascicles on their force-length relationship. In fact, although the GM muscle fascicles work with small and almost isometric changes, their operating regions shift down on the ascending limb of the F-L curve. It is likely that the observed increase in EMG activity with increasing running speeds reflects a greater volume of active muscle recruited in an effort to counteract these unfavourable contractile mechanics conditions.

Regarding the contribution of the SEE in the GM MTU, our data are in agreement with those of previous studies that estimated the relative contribution of tendon elastic strain energy to the positive work done by the MTU for the ankle plantar-flexors during running [5]: SEE strain energy was found to contribute 60% to the MTU positive work for the gastrocnemius when running at 7.2 $km \cdot h^{-1}$. Our data confirm these findings and extend them to a wider range of speeds supporting the idea that elastic strain energy contributes a greater proportion of the MTU propulsive power developed by the ankle plantar flexors compared to their muscle fascicles during running.

## Limitations

There are certain limitations to the *in vivo* ultrasound and inverse dynamics approach used in this study that require consideration. Firstly, we did not directly measure or calculate the individual forces generated by the GM and VL MTUs during running. Instead, our interpretation regarding the function of the two muscles was based on the measured length changes of the MTU, muscle fascicles and SEE, as well as on the net moments and powers generated by the muscles spanning the knee and ankle, computed from inverse dynamics. Secondly, we assumed negligible inter-muscular force transmission between the individual muscles comprising the ankle plantar flexor and knee extensor groups. In support of this assumption are the findings of Tijs et al.[43], who have shown that non-myotendinous forces are likely to have a minimal effect on the overall function of muscles. Thirdly, the geometric approach used in this study modelled the MTUs with individual SEEs rather than with a common Achilles or patellar tendon. Finally SEE length changes were indirectly calculated, rather than measured, using MTU and *in vivo* fascicle measurements. Such SEE length estimates represent all connective tissue structures in-series with the muscle fascicles, including the aponeurosis and free tendon.

A modelling study has previously indicated that the aponeuroses and the free tendon, despite being anatomically in-series, may not be mechanically in-series, meaning that the forces acting along them during contraction might differ [44]. However, previous studies have shown that, at comparable speeds, 70% of MTU power is attributable to changes in SEE [5], and 66% of MTU power is attributable to Achilles tendon changes[4]. This striking similarity indicates that, irrespective of whether the aponeurosis and the tendon are mechanically in-series or not, the aponeurosis is quite stiff, as also considered by others[45] and consequently, most of the plantar flexor SEE deformation likely resides in the free Achilles tendon. Therefore, the strain elastic energy estimates for the plantar flexor (Figure 5) would reflect mostly the Achilles tendon contribution. However, a recent paper by Zelik and Franz[46] indicated that the deformation of the plantar flexor SEE component, as calculated in the present study, and that of the free Achilles tendon may be different.

## Conclusions and Future Perspectives

Here we show for the first time that during running at increasing speeds: 1) the plantar-flexors SEE behaviour impacts positively on the mechanical power produced, and 2) both plantar-flexor and knee extensor muscle fascicles contract quasi-isometrically and close to their optimal length $L_0$, thus producing high forces economically. Future studies should investigate the link between muscle-tendon behaviour during running and the associated metabolic cost.

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## Competing of Interests

All author declared no conflict of interest. No funding has been received for this study

## Author Contributions

AM, CM, PZ and VB contributed to conception and design of the study. AM recorded and analysed the data under the supervision of CM, PZ and VB. AM, CM, PZ and VB contributed to the interpretation of the data, wrote and critically revised the manuscript. All authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work. All persons included as an author qualify for authorship, and all those who qualify for authorship are listed.

# REFERENCES

1. Fukunaga T, Kubo K, Kawakami Y, Fukashiro S, Kanehisa H, Maganaris C. In vivo behaviour of human muscle tendon during waling. *Proc R Soc Lond B* 2001; 268: 229-233
2. Ishikawa M, Pakaslahti J, Komi PV. Medial gastrocnemius muscle behavior during human running and walking. *Gait Posture* 2007; 25: 380–384.
3. Lichtwark GA, Bougoulias K, Wilson AM. Muscle fascicle and series elastic element length changes along the length of the human gastrocnemius during walking and running. *J Biomech* 2007; 40: 157-164.
4. Lai A, Schache AG, Lin YC, Pandy MG. Tendon elastic strain energy in the human ankle plantar-flexors and its role with increased running speed. *J Exp Biol* 2014; 217: 3159-3168.
5. Farris DJ & Sawicki GS. Human medial gastrocnemius force-velocity behavior shifts with locomotion speed and gait. *Proceedings of the National Academy of Sciences, 2012;* 109: 977-982.
6. Werkhausen A, Cronin NJ, Albracht K, Bojsen-Møller J, & Seynnes OR. Distinct muscle-tendon interaction during running at different speed and in different loading conditions. *Journal of Applied Physiology,* 2019; 127: 246-253.
7. Lai A, Lichtwark GA, Schache AG, Pandy MG. Differences in in vivo muscle fascicle and tendinous tissue behaviour between the ankle plantarflexors during running. *Scand J Med Sci Sports* 2018; DOI: 10.1111/sms.13089.
8. Cronin NJ, Carty CP, Barrett RS, Lichtwark G. Automatic tracking of medial gastrocnemius fascicle length during human loco- motion. *J Appl Physiol* 2011; 111: 1491-1496.
9. Lichtwark GA, Wilson AM. In vivo mechanical properties of the human Achilles tendon during one-legged hopping. *J Exp Biol* 2006; 208: 4715-4725.
10. Cronin NJ, Finni T. Treadmill versus overground and barefoot versus shod comparisons of triceps surae fascicle behaviour in human walking and running. *Gait Posture* 2013; 38: 528-533.
11. Komi PV. Relevance of in vivo force measurements to human biomechanics. *J. Biomech.* 1990*;* 23: 23-34.
12. Dorn TW, Schache AG, Pandy MG. Muscular strategy shift in human running: dependence of running speed on hip and ankle muscle performance. *J Exp Biol* 2012; 215: 1944-1956.
13. Weyand PG, Sandell RF, Prime DNL, Bundle MW. The biological limits to running speed are imposed from the ground up. *J. Appl. Physiol.* 2010; 108: 950-961.

14. Roberts TJ. The integrated function of muscles and tendons during locomotion. *Comp Biochem Physiol* 2002; 133: 1087-1099.
15. Roberts TJ, Azizi E. Flexible mechanisms: the diverse roles of biological springs in vertebrate movement. *J. Exp. Biol. 2011;* 214: 353-361.
16. Alexander RM. Tendon elasticity and muscle function. *Comp. Biochem. Physiol.* 2002; 133: 1001-1011.
17. O’Brien TD, Reeves ND, Baltzopoulos V, Jones DA, Maganaris CN. Mechanical properties of the patellar tendon in adults and children. *J Biomech* 2010; 216: 631-642.
18. Arampatzis A, Stafilidis S, Demonte G, Karamanidis K, Morey-Klapsing G, Bruggermann GP. Strain and elongation of the human gastrocnemius tendon and aponeurosis during maximal plantarflexion effort. *J. Biomech.* 2005; 38: 833–841.
19. Stafilidis S, Karamanidis K, Morey-Klapsing G, Demonte G, Bruggermann GP, Arampatzis A. Strain and elongation of the vastus lateralis aponeurosis and tendon *in vivo* during maximal isometric contraction. *Eur. J. Appl. Physiol.* 2005; 94: 317–322.
20. Sawicki GS, Lewis CL, Ferris DP. It pays to have a spring in your step. *Exerc. Sport Sci. Rev.* 2009*;* 37: 130.
21. Arnold EM, Hamner SR, Seth A, Millard M, Delp SL. How muscle fiber lengths and velocities affect muscle force generation as humans walk and run at different speeds. *J. Exp. Biol.* 2013; 216: 2150–2160
22. Santuz A, Ekizos A, Janshen L, Baltzopoulos V, Arampatzis A. On the Methodological Implications of Extracting Muscle Synergies from Human Locomotion. *Int. J. Neural Syst.* 2017; 27: 1750007
23. Bohm S, Marzilger R, Mersmann F, Santuz A, Arampatzis A. Operating length and velocity of human vastus lateralis muscle during walking and running. *Scientific Reports* 2018; 8: 5066.
24. Herzog W, Abrahamse SK, Lter Keurs HE. Theoretical determination of force-length relations of intact human skeletal muscles using the cross-bridge model. *Pflugers Arch* 1990; 416: 113–119.
25. Rasske K, Thelen DG, Franz JR. Variation in the human Achilles tendon moment arm during walking. *Comput. Methods Biomech. Biomed. Engin* 2017; 20: 201-205.
26. Kellis E, Baltzopoulos V. Muscle activation differences between eccentric and concentric isokinetic exercise. *Med. Sci. Sports Exer*. 1998; 30: 1616-1623.
27. Tsaopoulos DE, Baltzopoulos V, Maganaris CN. Human patellar tendon moment arm length:

measurement considerations and clinical implications for joint loading assessment. *Clin Biomech* 2006; 21: 657–667.

28. Baltzopoulos V. A videofluoroscopy method for optical distortion correction and measurement of knee-joint kinematics. *Clin Biomech* 1995; 10: 85–92.
29. Hill AV. The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. B* 1938; 126: 136-195.
30. Ferri A, Scaglioni G, Pousson M, Capodaglio P, Van Hoecke J, Narici MV. Strength and power changes of the human plantar flexors and knee extensors in response to resistance training in old age. *Acta Physiol Scand* 2003; 177: 69-78.
31. Csapo R, Maganaris CN, Seynnes OR, Narici MV. On muscle, tendon and high heels. *J Exp Biol* 2010; 213: 2582-2588.
32. Gillett JG, Barrett RS, Lichtwark GA. Reliability and accuracy of an automated tracking algorithm to measure controlled passive and active muscle fascicle length changes from ultrasound. *Comput Methods Biomech Biomed Engin* 2013; 16: 678-687.
33. Hawkins D, Hull ML. A method for determining lower extremity muscle-tendon lengths during exion/extension movements. *J Biomech* 1990; 23: 487–494.
34. Kurokawa S, Fukunaga T, Fukashiro S. Behavior of fascicles and tendinous structures of human gastrocnemius during vertical jumping. *J Appl Physiol* 2001; 90:1349–1358.
35. Bekenecker P, Raiteri B, Hahn D. Patella tendon moment arm function considerations for human vastus lateralis force estimates. *J. Biomech*. 2019; 27: 225-231.
36. Ker RF, Alexander RMcN, Bennett MB. Why are mammalian tendons so thick? *Journal of Zoology*, 1998: 216: 309–324.
37. Prilutsky BI, Zatsiorsky V. Tendon action of two-joint muscles: transfer of mechanical energy between joints during jumping, landing and running. *J. Biomech.* 1994; 27: 25-34
38. Nikolaidou ME, Marzilger R, Bohm S, Mersmann F, Arampatzis A. Operating length and velocity of human M. vastus lateralis fascicles during vertical jumping. *R. Soc. Open Sci.* 2017; 4: 170185.
39. Fletcher JR and MacIntosh BR. Running Economy from a Muscle Energetics Perspective. *Front. Physiol.* 2017; 8: 433. doi: 10.3389/fphys.2017.00433.
40. Lieber RL & Fridén J. Muscle damage is not a function of muscle force but active muscle strain. *J. Appl. Physiol.* 1993; 74: 520-526.
41. Proske U & Morgan DL. Tendon stiffness: methods of measurement and significance for the control of movement. A review. *J. Biomech.* 1987; 20: 75-82.
42. Biewener AA, Roberts TJ. Muscle and tendon contributions to force, work, and elastic energy

savings: a comparative perspective. *Exerc. Sport Sci Rev* 2000; 28: 99-107.

43. Tijs C, van Dieën JH, Maas H. No functionally relevant mechanical effects of epimuscular myofascial connections between rat ankle plantar flexors. *J Exp Biol*. 2015; 218: 2935-2941.
44. Epstein M, Wong M, Herzog W. Should tendon and aponeurosis be considered in series? *J Biomech.* 2006; 39: 2020–2025.
45. Magnusson SP, Narici MV, Maganaris CN, Kjaer M. Human tendon behaviour and adaptation, in vivo. *J Physiol.* 2008, 586: 71-81.
46. Zelik KE, Franz JR. It's positive to be negative: Achilles tendon work loops during human locomotion. *PlosOne* 2017; 12: e0179976.

## FIGURES LEGEND

**Figure 1.** Mechanical behaviour of Vastus Lateralis (left panels) and Gastrocnemius Medialis (right panels) muscle-tendon unit, muscle fascicle and series elastic elements during the stance phase while running at three steady-state speeds: solid line: 10 km·h$^{-1}$; thick dashed line: 13 km·h$^{-1}$; thin dashed line: 16 km·h$^{-1}$. MTU, muscle fascicle and SEE lengths were normalised to their corresponding resting lengths during static standing. Negative and positive length values denote shortening and lengthening, respectively.

**Figure 2.** Operating length (upper panels) and velocity (lower panels) of Vastus Lateralis (left) and Gastrocnemius Medialis (right) muscle fascicles (mean and standard deviation) during the stance phase of running onto the normalised force-length and force-velocity curve. Force is normalised to the maximum force obtained during the maximal isometric knee extension and plantar flexors contractions; fascicle length and fascicle velocity are normalized to the experimentally determined optimal fascicle length and maximum shortening velocity, respectively. Black dots: 10 km·h$^{-1}$; grey dots: 13 km·h$^{-1}$; white dots: 16 km·h$^{-1}$; * $P < 0.05$; § $P < 0.01$; # $P < 0.001$.

**Figure 3.** Average change of MTU, fascicle shortening, SEE strain and SEE recoil values for Vastus Lateralis (left panels) and Gastrocnemius Medialis (right panels) as a function of running speed. Values are means ± SD and are dimensionless (relative lengths, normalised for the condition of static standing). Black column: 10 km·h$^{-1}$; dark grey column: 13 km·h$^{-1}$; light grey column: 16 km·h$^{-1}$. Significantly different from 10 km·h$^{-1}$ (*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$); significant difference between 13 and 16 km·h$^{-1}$ (#: $P<0.05$; ##: $P<0.01$; ###: $P<0.001$).

**Figure 4.** EMG linear envelope for Gastrocnemius Medialis (upper panels) and Vastus Lateralis (lower panels) measured in the stance phase when running at three steady-state speeds (solid line: 10 km·h$^{-1}$; thick dashed line: 13 km·h$^{-1}$; thin dashed line: 16 km·h$^{-1}$). EMG activity was normalised to the peak EMG activity measured during the MVC.

**Figure 5.** Upper panels: average net joint power (normalised to body mass) calculated at the knee (left) and ankle (right) level when running at three steady-state speeds (solid line: 10 km·h$^{-1}$; thick dashed line: 13 km·h$^{-1}$; thin dashed line: 16 km·h$^{-1}$). Middle panels show the absolute value of the mechanical power done by the SEE (black columns) and by the muscle fascicles (white columns) for VL (left panel) and GM (right panel). The sum of the two components represent the total mechanical power of the MTUs. Lower panels show the relative contribution of the SEE (black columns) and muscle fascicles (white columns) in determining the mechanical power done by the MTU for VL (left panel) and GM (right panel).