Homogeneity of fascicle architecture following repeated contractions in the human gastrocnemius medialis

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Abstract

This investigation sought to determine the effects of fatigue on fascicle architecture across the length of the human gastrocnemius medialis (GM). With institutional ethical approval, fifteen healthy males performed repeated isometric plantar flexion maximal voluntary contractions (MVC) until peak force fell 30% below baseline. Brightness-mode ultrasound was used to determine fascicle length and pennation angle at rest and during MVC prior to and following the fatiguing contractions. The results show a significant increase in fascicle length during MVC in the distal (2.8 mm, 8.1%) middle, (4.9 mm, 14.1%), and proximal (5.2 mm, 14.7%) regions post-fatigue compared to pre-fatigue ($p < 0.05$). There was also a significant decrease of pennation angle during MVC in the distal (3.3°, 8.8%), middle (3.9°, 9.4%), and proximal (2.9°, 6.9%) regions post-fatigue compared to pre-fatigue ($p < 0.05$). These changes, however, were not region specific. These are the first results to show that fascicle shortening within the GM remains homogeneous following fatigue, suggesting that the fascicles were fatigued in a similar pattern throughout the muscle. The significant reduction of fascicle shortening may reflect an additional strategy to maintain an optimal force output in fatigued conditions, although future work is needed to confirm this notion.

1. Introduction

Muscle architecture during isometric contractions changes as a function of the force generated by the muscle and the compliance of the tendon (Maganaris et al., 2002, Narici et al., 1996). Less force transferred from the fascicles to the tendon implies less fascicle length change, and vice versa. Fatigue, defined as a reduction in a muscle's force-generating capacity (Kawakami et al., 2000), has been shown to reduce fascicle length change between rest and contraction in the gastrocnemius medialis (GM) (Maganaris et al., 2002, Mitsukawa et al., 2009). This finding, however, only accounts for fascicle architecture in the middle region of the muscle. Recently there have been preliminary data which point towards a greater representation of faster motor units in the proximal region and slower motor units in the distal region (Hodson-Tole et al., 2013). As slower twitch muscle fibres exhibit a higher level of fatigue resistance (Vydevska-Chichova et al., 2005), fascicles in the proximal region might fatigue more rapidly than their distal counterparts. Thus, any reductions of fascicle shortening might be more pronounced in the proximal region of the muscle.

Region specific changes of fascicle architecture have been demonstrated following fatigue in animal specimens (Higham and Biewener, 2009). Indeed this was thought to be related to a higher concentration of fast twitch fibres in the affected region. It was also thought that proximal-distal variations of aponeuroses stiffness may have played a role, with fascicle shortening in the stiffer region being constrained and ultimately

shielded from the effects of fatigue (Higham and Biewener, 2009). In humans, regional variations of strain patterns in both the deep and superficial GM aponeuroses tissues have been demonstrated with magnetic resonance imaging (MRI) (Kinugasa et al., 2008), and there are indications that the compliance of aponeurotic tissues can be altered following repeated contractions (Cronin et al., 2011). Acute modifications of muscle and tendon stiffness have also been demonstrated (Ditroilo et al., 2011), which may in part be related to increases in tissue temperature (Strickler et al., 1990), which are known to occur in humans during contractions (Barcroft and Millen, 1939, Kubo et al., 2001). This raises the additional possibility of regional effects of aponeuroses, tendon, and muscle stiffness on fascicle behaviour.

A method commonly utilised in the literature to estimate changes in length of the GM series elastic element, particularly relevant in situations where MRI is not feasible, assumes that the fascicles shorten homogenously throughout the muscle (Fukunaga et al., 2001, Ishikawa et al., 2007, Ishikawa and Komi, 2007, Lichtwark et al., 2007, Mian et al., 2007). Although this assumption has been shown to be accurate in non-fatigued conditions (Maganaris et al., 1998, Shin et al., 2009), it has previously not been considered following fatigue. Potential non-uniform fascicle shortening in the GM following fatigue would thus have important implications for the modelling of muscle–tendon unit (MTU) function (Cronin and Lichtwark, 2013).

To assess muscle fascicle behaviour across the length of the GM, we used Brightness-mode (B-mode) ultrasound to image muscle fascicle length and pennation angle at distal, middle, and proximal regions at rest and during maximal voluntary contractions before and following a fatiguing protocol. It was hypothesised there would be a reduction in the magnitude of fascicle shortening following fatigue, and this would be more pronounced in the proximal region of the muscle.

2. Methods

2.1. Participants

Fifteen physically active male participants (mean ± SD: age 21.9 ± 4.1 years, height 1.80 ± 0.1 m, mass 84.0 ± 13.6 kg) were recruited. The participants were free from conditions which may have affected normal lower limb function or prevented them from completing the testing safely. All procedures were in accordance with the ethical standards of the institutional research committee. Written informed consent was obtained from all individual participants included in the study.

2.2. Testing procedures

Prior to testing, the GM measurement sites on each participant were identified, and an echo-absorptive marker placed on the surface of the skin. This created a vertical shadow across the ultrasound image to allow for monitoring of movement of the probe relative to the skin, and for accurate probe relocation following fatigue. The middle region was defined as the point at which the anatomical cross sectional area of the muscle was

maximal. Here, the position at one half of the mediolateral width was used as a measurement site, with distal and proximal regions 35 mm longitudinally from this point (Lichtwark et al., 2007).

Each participant lay in a prone position with the ankle joint angle at 90° measured with a manual goniometer. A non-elastic sling was positioned around the ball of the right foot and attached to a force gauge (Myometer, MecMesin, West Sussex, UK). The participants were fixed above the hip joint in order to minimise any extraneous movement during the contractions. The force gauge was connected to a PC and force output was recorded and displayed in real time to the experimenter. No visual feedback regarding the exerted force was provided to the participants. Three practice isometric plantar flexion maximal voluntary contractions (MVC) were performed with a rest time of 30 s between trials. Following this, 2 more MVCs were performed (also 30 s rest between trials), and the highest force output recorded (from the latter 2) was used as a baseline MVC. No muscle imaging occurred during this initial stage.

For pre-fatigue muscle architecture measurements, a clear ultrasound image was obtained (more detail below) in the middle region and fascicle characteristics were recorded at rest and during an MVC which was held for 3 s (total trial time of 4 s). This process was then repeated at the distal region, and then the proximal region (always in this order), with 30 s rest between trials. The ultrasound probe was positioned during the rest period so as to not add additional time between trials. Only 1 MVC attempt was performed during imaging at each muscle region. Following this, the participants performed a fatiguing protocol which consisted of repeated MVCs (10 s contraction, 3 s rest) until peak force output consistently fell 30% below baseline. 30 s after the last MVC of the fatiguing protocol, the same process utilised for pre-fatigue muscle architecture measurements was implemented for post-fatigue muscle architecture measurements.

2.3 Muscle architecture measurements

A PC based ultrasound system (Voluson-i, GE Medical Systems, Zipf, Austria) was used to image the muscle with a linear probe operating at a frequency of 7.2 MHz and with a field view of 35 mm in B-mode. To ensure orientation of the probe with regards to the true fascicle plane, the probe was positioned at the distal end of the GM and orientated so that the deep aponeurosis was parallel to the bottom of the image. The probe was then rotated 90° and manoeuvred along the longitudinal fascicle plane to the desired measurement site (Bénard et al., 2009). All measurements were then performed with a researcher holding the ultrasound probe securely in place. Ultrasound image sequences were recorded in a cineloop at 25 Hz and saved as a video file. Frames of interest (one at rest and one at MVC from each region, pre- and post-fatigue) were digitised using Kinovea open source video analysis software (Kinovea for Windows, Version 0.8.15, Kinovea.org). Measurements of fascicle length and pennation angle from at least 3 fascicles in the middle of each image were taken, and the average, from each image, was used for further analysis. Fascicle length was defined as the straight line orientated along the fascicle between the deep and the superficial aponeuroses and pennation angle was defined as the angle between the deep aponeurosis and the line of the muscle fascicle (Fig. 1). GM fascicles remain straight at rest (Shin et al., 2009) and errors associated with ignoring fascicle curvature during MVC (present method) are $\leqslant$6% (Muramatsu et al., 2002). If whole fascicles could not be viewed, fascicle length was

determined by linear extrapolation of the visible portion of the fascicle and the aponeurosis.

[FIG. 1]

Ultrasound image of a longitudinal section of human GM muscle, (A) the superficial aponeurosis, (B) the deep aponeurosis, (C) striations of fat and connective tissue between the fascicles, (D) digitised markers representing fascicle length and pennation angle, (E) shadow running vertically through the image resulting from an echo absorptive marker placed on the surface of the skin.

2.4 Statistical analysis

In order to assess test–retest reliability of our ultrasound measurements, fascicle length and pennation angle was measured in the middle region of the GM at rest and during MVC 5 min before pre-fatigue measurements. This data (referred to as baseline) was then compared to pre-fatigue (rest and MVC respectively) measurements from the middle region. Reliability was assessed by calculating the coefficient of variation (CV) and intraclass correlation coefficient (ICC3,1) (Atkinson and Nevill, 1998, Weir, 2005). The typical error (TE) of the measurements was also calculated to assess whether any changes in muscle architecture were real or due to measurement error (Hopkins, 2004).

The muscle region (distal, middle, and proximal) and fatigue state (pre- and post-fatigue) were considered as two independent factors. The effects of these two factors on fascicle length at rest and during MVC and on pennation angle at rest and during MVC were assessed using 2 × 3 repeated measures analysis of variance (RM ANOVA).

As a different MVC was required for ultrasound measurements at each region of the muscle, it was necessary to ensure MVC force output was consistent across the repeated contractions. A 2 × 3 RM ANOVA was thus used to examine the effects of muscle region (distal, middle, and proximal) and fatigue state (pre-and post-fatigue) on MVC force output. Post hoc analysis with Bonferroni adjustments were used following all RM ANOVA analyses where applicable.

All statistical analyses were completed with the SPSS software statistical package (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp). Assumptions of normality were checked and confirmed for all variables. Data are presented as means ± standard deviation (SD). Statistical significance was set at $p < 0.05$.

3. Results

3.1 Reliability

Test–retest reliability data and typical error values for baseline and pre-fatigue measurements of fascicle length and pennation angle are presented in Table 1. All reliability measurements were seen to have a CV of <5% and an ICC3,1 of ⩾0.9, indicating high reliability (Atkinson and Nevill, 1998).

[TABLE 1]

3.2 Force output

Isometric plantar flexion MVC force output when making ultrasound measurements at various regions of the GM pre- and post-fatigue is shown in Table 2. There was no significant effect of muscle region on force output. There was however a significant main effect of fatigue state on force output ($p = 0.001$), with average force when imaging the 3 regions of the GM being 38.1% lower post-fatigue compared to pre-fatigue. There was no significant interaction effect between muscle region and fatigue state.

[TABLE 2]

3.3 Fascicle length at rest pre- and post-fatigue

Fascicle length data from all regions for all measurement points can be seen in Table 3. There was no significant main effect or interaction of muscle region or fatigue state on fascicle length at rest.

[TABLE 3]

3.4. Fascicle length during MVC pre and post fatigue

There was no significant main effect of muscle region on fascicle length during MVC. There was however a significant main effect of fatigue state on fascicle length ($p = 0.029$), with post hoc analysis showing fascicles were longer in distal (2.8 mm, 8.1%), middle (4.9 mm, 14.1%), and proximal (5.2 mm, 14.7%) regions post-fatigue compared to pre-fatigue. There was no significant interaction effect between muscle region and fatigue state on fascicle length during MVC. Changes in fascicle length between rest and MVC (delta values) at all regions pre-and-post fatigue are presented in Fig. 2A.

[FIG 2]

3.5. Pennation angle at rest pre- and post-fatigue

Pennation angle data from all regions for all measurement points is shown in Table 3. A significant main effect was found when comparing pennation angles of the different muscle regions at rest ($p = 0.019$). Post hoc analysis revealed smaller pennation angles in the distal region compared to the proximal region ($p = 0.027$). There was also a significant main effect of fatigue state on pennation angle at rest, with larger pennation angles post-fatigue compared to pre-fatigue ($p = 0.031$). Post hoc analysis revealed a significant increase in the middle region (1.6°, $p = 0.039$) of the GM from pre- to post-fatigue, whilst the distal and proximal regions remained unaffected. There was no significant interaction effect between muscle region and fatigue state on pennation angle at rest.

3.6. Pennation angle at MVC pre- and post-fatigue

A significant main effect was found when comparing pennation angles of the different muscle regions during MVC ($p = 0.002$). Post hoc analysis revealed smaller pennation angles in the distal region compared to the middle and proximal regions ($p = 0.003$, $p = 0.024$ respectively). There was also a significant main effect of fatigue state on pennation angle during MVC ($p = 0.047$), with post hoc analysis revealing pennation angles were smaller in distal (3.3°, 8.8%), middle (3.9°, 9.4%), and proximal (2.9°, 6.9%) regions post-fatigue compared to pre-fatigue. There was no significant interaction effect between muscle region and fatigue state on pennation angle during MVC. Changes in pennation angle between rest and MVC (delta values) at all regions pre- and post-fatigue are presented in Fig. 2B.

4. Discussion

The present investigation sought to determine the effects of fatigue on human GM fascicle behaviour. Here we show that fascicle shortening was homogeneous pre- and post-fatigue, while being significantly reduced at all regions of interest following the repeated contractions.

Previous examination of GM fascicle architecture during non-fatigued isometric plantar flexion MVC showed fascicle shortening to be uniform at distal, middle, and proximal regions (Maganaris et al., 1998). The current results support these findings, however, fascicle lengths at rest and during MVC (pre-fatigue) were longer in all regions than Maganaris et al. (1998)). As the percentage change between rest and MVC in the present study (pre-fatigue), (43.2%, 47.2%, and 44.1% in the distal, middle, and proximal regions respectively), is in line with Maganaris et al. (1998)) (46%, 48%, and 45.5% respectively), this is likely a result of the participants, on average, possessing longer muscle fascicles. The present results do show regional differences in pennation angles, with smaller pennation angles in the distal region compared to the proximal region at rest, and smaller pennation angles in the distal region compared to the middle and proximal regions during MVC, pre- and post-fatigue. This demonstrates proximal-distal variations in pennation angles inherently found in human GM muscle, previously shown with MRI (Shin et al., 2009). The data of Maganaris et al. (1998)) do not show these regional differences, as the fascicle measurement sites were located closer to the middle region of the muscle, where pennation angles are not significantly different.

The relative uniformity of fascicle behaviour post-fatigue in our results was surprising and suggests that the fascicles were fatigued in a homogeneous pattern, regardless of any potential regional differences in muscle fibre type. The fatiguing protocol may have been sufficient to induce fatigue in the slower twitch fibres as well as the fast twitch fibres, thus bringing the distal fascicles in line with their proximal counterparts. An assessment of fascicle shortening throughout the time course of a fatiguing protocol or additional measures of myoelectric activity in different muscle regions may shed further light on this. The present findings additionally suggest that regional variations of aponeuroses strain patterns as shown by Kinugasa et al. (2008)) are not reflected in heterogeneous fascicle behaviour. Previous MRI results demonstrating uniform fascicle shortening in the GM in non-fatigued conditions (Shin et al., 2009) corroborate this notion, however, our findings are the first to show this following fatigue.

The reduction of fascicle shortening in our study post-fatigue supports the findings of Mitsukawa et al. (2009)) who showed the same phenomenon in the middle region of the GM. In their investigation, the change of fascicular geometry over time significantly correlated with a decrease in plantar flexion MVC torque, whilst no such relationship was found for the synergistic soleus muscle. The authors suggest therefore that fatigue caused a decline in GM force output which was reflected by the change in fascicle architecture. Although we did not monitor fascicle shortening within the soleus and therefore cannot rule out its contribution to the decline in plantar flexion MVC force, the change of fascicle behaviour in our study must have been a consequence of fatigue and likely also reflects a decline in GM force production. In other words, a decrease of fascicle force generation resulted in less force being transferred through the tendon, less tendon elongation, and subsequently less fascicle shortening.

This may have been related to some of the conventional mechanisms of muscular fatigue, such as impaired excitation–contraction coupling, or reduced cross-bridge cycling, which limited the fascicles in their ability to elongate the tendon with the same magnitude as before (Place et al., 2009, St Clair Gibson and Noakes, 2004). However, according to force–length relationships detailed by Maganaris (2003)), the increase of fascicle length in the middle region of the muscle in our study during MVC (from 29.9 to 34.8 mm) would mean the fascicles were closer to their optimal lengths for force production (39 mm). In addition, the decrease of pennation angle in the middle region of the muscle (from 41.6° to 37.7°) would have put the pennation angles closer to their optimal angle for force production (34.6°) (Manal et al., 2006). Is it possible that this could be an additional mechanism utilised by the central nervous system to help maintain an optimal force output in fatigued conditions?

Mitsukawa et al. (2009)) recorded electromyographic (EMG) activity of the soleus and the GM and found that although it decreased in both, there was no significant difference between the two, despite the change of fascicle architecture in the GM and not in the soleus. The authors suggested that surface EMG may not be able to fully evaluate the magnitude of neural drive during repeated maximal contractions in fatigued muscle. Nordlund et al. (2004)) assessed intramuscular EMG activity in the GM and soleus over nine bouts of 10 isometric plantar flexion MVCs lasting for 2 s with 1 s rest (10 s interval between bouts) and found central drive to the GM decreased whilst there was no change in central drive to the soleus. Muscle architecture was not assessed. Neither of these findings can wholly support nor reject the above notion, and hence the possibility of this being a central nervous system mechanism to optimise force production during fatigue remains unknown. Future studies integrating twitch interpolation techniques, examination of fascicle length changes, and measures of muscle force are needed for further investigation.

Muscle fascicle architecture during isometric contractions can be affected by tendon stiffness. Previous findings have shown tendon creep to occur during 5 isometric plantar flexion MVCs, which led to an increase of fascicle shortening (Maganaris et al., 2002). Although the protocol used by Maganaris et al. (2002)) was different from ours (1 s contraction with 1 s rest), longer duration isometric contractions have also been shown to induce tendon creep in the GM MTU (Mademli and Arampatzis, 2005). It is likely, therefore, that the 5 MVCs performed before ultrasound measurements in our study did induce tendon creep. However, Maganaris et al. (2002)) found no further changes after the fifth contraction, which suggests that any effect of tendon creep on fascicle

architecture in our study was the same for pre- and post-fatigue measurements, and can thus be eliminated as a cause of the difference in fascicle length between pre- and post-fatigue MVCs. This is supported by the high concordance between the baseline (used to assess reliability) and pre-fatigue fascicle measurements.

Additionally, in the present results, there was an increase in pennation angle (1.4°) at rest in the middle region of the muscle following fatigue. It is possible that an increase in blood volume or intracellular and extracellular fluid in the affected area, which has been shown to occur following isometric exercise (Sejersted et al., 1984), caused this increase. On the other hand, as the typical error of pennation angle measurements between baseline and pre-fatigue was 1.5°, this may have been due to measurement error rather than a real change (Atkinson and Nevill, 1998, Hopkins, 2004). This is supported by the fact there were no changes in resting fascicle length following fatigue.

The use of ultrasonography to measure fascicle characteristics has its limitations. Muscles and tendinous tissue for example are known to move in three-dimensional planes (Azizi and Roberts, 2009, Iwanuma et al., 2011) and if the plane of a contraction shifts from the field of view of two-dimensional ultrasound, the same muscle fascicles may not be visualised throughout the entire contraction (Klimstra et al., 2007). The ultrasound probe in our experiment was also re-positioned (with an echo absorptive marker) at each measurement site following fatigue. Therefore, the same fascicles may not have been directly imaged across conditions. However, our results show that force output was consistent when imaging the GM at different regions during different MVCs, the repeated measures of fascicle length and pennation angle demonstrated high reliability, and all of the fascicle length changes were greater than the typical error of the same measurements between baseline and pre-fatigue. Furthermore, previously outlined MRI and ultrasound data showing uniform fascicle shortening in non-fatigued conditions is in line with our findings (Maganaris et al., 1998, Shin et al., 2009). We are therefore confident in our results.

In summary, we have demonstrated that fascicle shortening in the human GM remains homogeneous during isometric MVC pre- and post-fatigue, despite being significantly reduced as a result of repeated contractions. The relative uniformity of fascicle behaviour suggests that the fascicles were fatigued in a uniform manner throughout the muscle, and lends support to the accuracy of GM MTU modelling implemented in fatigued conditions. The reduction of fascicle shortening may reflect an additional mechanism utilised by the central nervous system to maintain optimal fascicle force in fatigued conditions, although future investigations will be needed to confirm or reject this idea.

Fig. 1

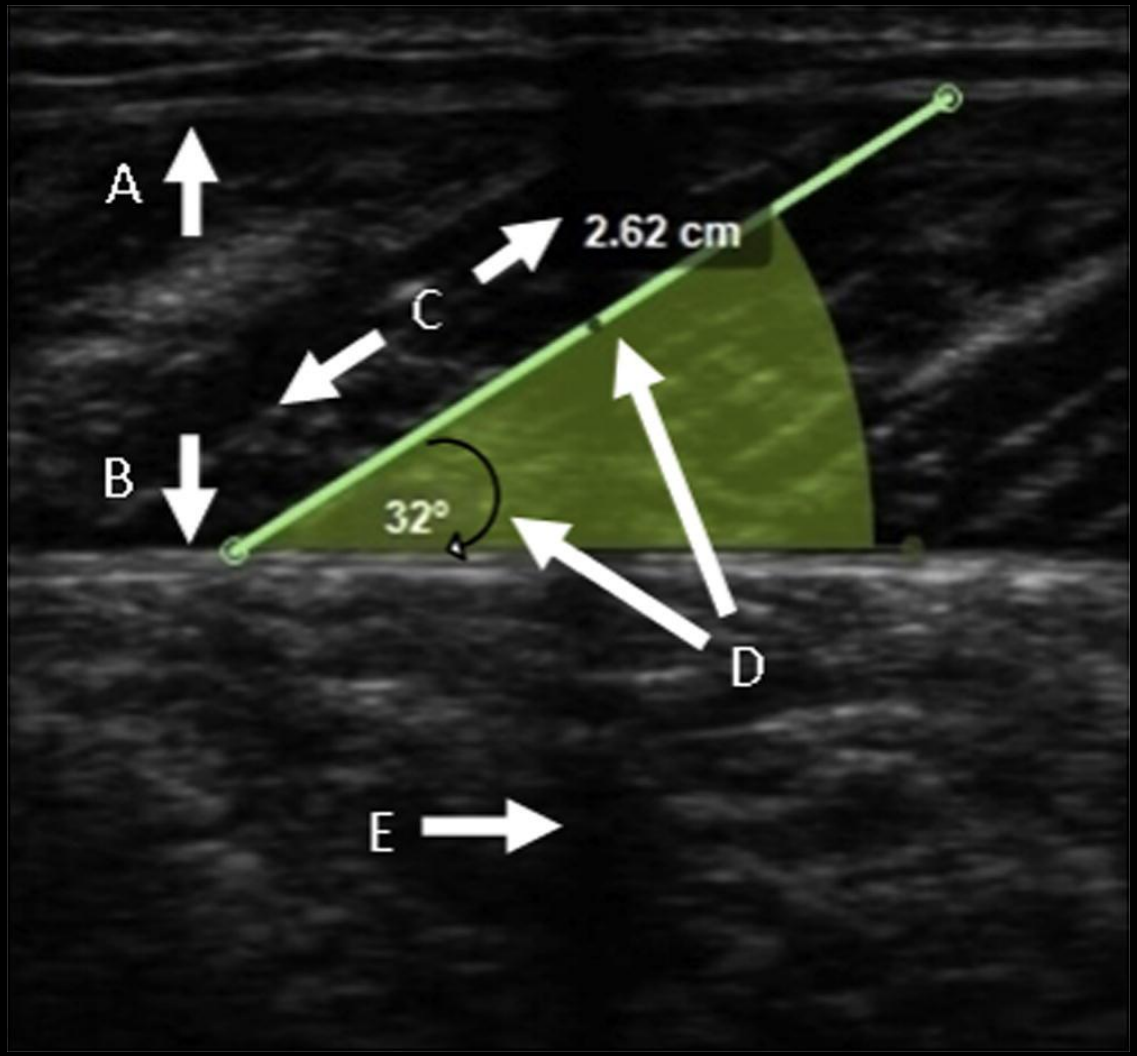

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Fig. 2

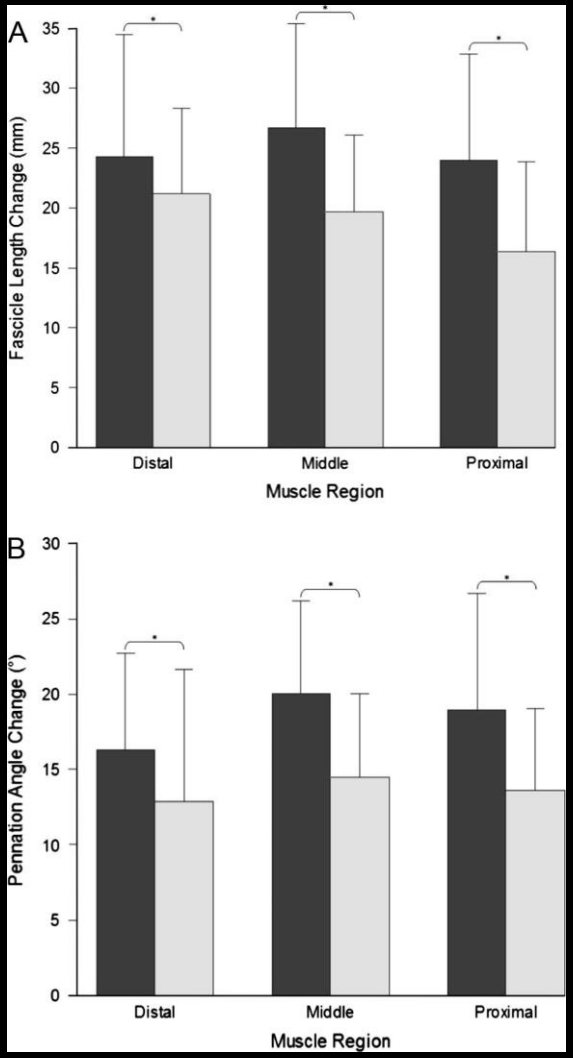

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Table 1

Test–retest reliability data and typical error values for baseline and pre-fatigue measurements of fascicle length and pennation angle.

	CV (%)	**$ICC_{3,1}$**	**TE**
Fascicle length			
Rest	2.9	0.98	1.9 mm
MVC	4.7	0.91	2.1 mm
Pennation angle			
Rest	4.1	0.91	1.5°
MVC	3.9	0.96	1.8°

Coefficient of variation (CV), intraclass correlation coefficient ($ICC_{3,1}$), typical error (TE), maximal voluntary contraction (MVC).

Table 2

Isometric plantar flexion MVC force output when making ultrasound measurements at various regions of the GM pre- and post-fatigue. Values are mean ± SD, *n* = 15 participants, *significant reduction post-fatigue compared to pre-fatigue ($p < 0.05$, with Bonferroni correction).

	Pre-fatigue (N)	**Post-fatigue (N)**
Distal	415.1 ± 158.1	248.9 ± 74.9*
Middle	411.5 ± 165.0	264.5 ± 84.9*
Proximal	417.8 ± 159.9	257.3 ± 80.3*

Maximal voluntary contraction (MVC), *gastrocnemius medialis* (GM).

Table 3

Fascicle length and pennation angle at rest and during MVC pre- and post-fatigue at various regions of the GM. Values are mean ± SD, *n* = 15 participants, *significant difference post-fatigue compared to pre-fatigue, #significant difference compared to the distal region ($p < 0.05$, with Bonferroni correction).

	Pre-fatigue		**Post-fatigue**	
	Rest	**MVC**	**Rest**	**MVC**
Fascicle length, mm				
Distal	56.1 ± 13.0	31.9 ± 8.6	55.9 ± 10.2	34.7 ± 11.3*
Middle	56.6 ± 11.7	29.9 ± 5.9	54.4 ± 11.3	34.8 ± 9.6*
Proximal	54.2 ± 13.4	30.3 ± 6.8	51.8 ± 14.0	35.5 ± 9.7*
Pennation angle, °				
Distal	21.1 ± 3.9	37.4 ± 8.2	21.2 ± 4.0	34.1 ± 9.5*
Middle	21.6 ± 4.5	41.6 ± 8.4#	23.2 ± 4.7*	37.7 ± 8.9*#
Proximal	22.9 ± 5.8#	41.8 ± 10.2#	25.3 ± 7.3#	38.9 ± 11.0*#

Maximal voluntary contraction (MVC), *gastrocnemius medialis* (GM).