Between-day repeatability of lower limb EMG measurement during running and walking

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Key words

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Abstract

There are minimal data describing the between-day repeatability of EMG measurements during running. Furthermore, there are no data characterising the repeatability of surface EMG measurement from the adductor muscles, during running or walking. The purpose of this study was to report on the consistency of EMG measurement for both running and walking across a comprehensive set of lower limb muscles, including adductor magnus, longus and gracilis. Data were collected from 12 lower limb muscles during overground running and walking on two separate days. The coefficient of multiple correlation (CMC) was used to quantify waveform similarity across the two sessions for signals normalised to either maximal voluntary isometric contraction (MVIC) or mean/peak signal magnitude. For running, the data showed good or excellent repeatability (CMC=0.87-0.96) for all muscles apart from gracilis and biceps femoris using the MVIC method. Similar levels of repeatability were observed for walking. Importantly, using the peak/mean method as an alternative to the MVIC method, resulted in only marginal improvements in repeatability. The proposed

protocol facilitated the collection of repeatable EMG data during running and walking and therefore could be used in future studies investigating muscle patterns during gait.

Introduction

Electromyography (EMG) can be used to provide insight into muscle activation during human running. Using EMG, it is possible to understand how muscle patterns change as running speed increases (Gazendam and Hof, 2007, Kyrolainen et al., 2005), how different footwear designs impact on muscle activation (Cheung and Ng, 2009) and how muscle patterns differ between running styles (Landreneau et al., 2014). EMG can also be used to quantify differences in neuromuscular control which might be associated with running injury (Baker et al., 2018, Barton et al., 2013, Smith et al., 2014), performance level (Tam et al., 2017) or metabolic energy expenditure (Kyrolainen et al., 2001, Moore et al., 2014). However, in order to interpret data from such biomechanical studies, it is important to have a precise understanding of the level of repeatability of EMG measurement in running.

Two previous studies have explored the within-session variability for a range of EMG parameters which characterise lower limb muscle activation during treadmill running (Karamanidis et al., 2004, Smoliga et al., 2010). Both studies demonstrated consistency in parameters collected from the same electrodes from data collection sessions separated by a relatively short time period (1-2 minutes). Although a useful first step, these studies do not provide insight into variability in EMG signals which results from re-application of electrodes on different measurement sessions. Such variability may arise from many factors, such as a change in electrode-skin impedance, a change in the distribution of motor units with the EMG collection volume (Merletti and Farina, 2016) or variability in synergistic muscle patterns

63 employed to perform the same task. In order to fully characterise variability in EMG
64 measurement, it is necessary quantify the consistency of EMG signals collected during
65 different measurement sessions.

66 Normalisation of EMG amplitude is required to facilitate comparison between
67 participants, muscles and measurement sessions (Besomi et al., 2020). In their consensus
68 paper, Besomi et al. (2020) identify the optimal method to be normalisation to a maximal
69 voluntary contraction, which is matched to the task in terms of joint angle/muscle length,
70 contraction type and/or joint angular velocity. In line with this idea, it has been suggested
71 that for high-velocity muscle actions, such as sprinting, amplitude normalisation should be
72 performed using a dynamic task similar in nature to the task under investigation (Ball and
73 Scurr, 2013). However, while the use of a dynamic task to normalise running EMG signals may
74 be the preferred option, there are considerable challenges to developing a laboratory
75 protocol which is sufficiently robust to ensure that all participants maximally activate each
76 muscle consistently across repeat testing sessions. As an alternative, normalisation to a
77 maximal voluntary isometric contraction (MVIC) or to the peak/mean of the dynamic signal
78 may be appropriate, especially in laboratory setting where it is difficult to perform high-
79 velocity tasks.

80 Two previous studies have sought to compare the level of repeatability between an
81 MVIC approach and a high-velocity task. Chuang et al. (2019) compared the within-session
82 repeatability of normalisation coefficients derived from MVICs, sprint cycling and sprint
83 running. Interestingly, although sprint running was associated with the largest normalisation
84 values, the MVIC data appeared to be a more consistent method of signal normalisation than
85 the other two methods, for six out of the nine muscles tested. In another study, Albertus-
86 Kajee *et al.* (2011) compared the between-day variability in the normalised EMG signal,

87 measured during maximal sprint running, between three normalisation techniques: maximal
88 sprinting, sprinting at 70% of maximum speed and MVIC. Their data showed the MVIC to be
89 the most repeatable method for three out of the six muscles studied and the maximal
90 sprinting method to be the most repeatable for the other three muscles.

91 Taken together, these two previous studies (Albertus-Kajee et al., 2011, Chuang and
92 Acker, 2019) suggest that the MVIC method could be an appropriate method for normalising
93 running EMG signals. Furthermore, the MVIC method is straightforward to implement. It is
94 therefore likely to be an appropriate methodological choice for biomechanical studies
95 exploring inter-subject differences in muscle activation (Yong et al., 2014) or the effects of an
96 intervention (Mundermann et al., 2004), which are typically performed at lower running
97 speeds. However, to date, there are no data available on the repeatability of EMG data across
98 different testing sessions at slower running speeds.

99 Previous repeatability studies, investigating walking, have sought to understand the
100 level of consistency of EMG measurement across a wide range of lower limb muscles. Most
101 of the larger superficial muscles of the lower limb have been studied, including the
102 quadriceps, hamstrings, gastrocnemius/soleus, tibialis anterior as well as the gluteal muscles.
103 However, there has been very little study of the adductor muscles. Moreover, the three
104 superficial adductor muscles do not feature in the SENIAM guidelines (Hermens et al., 2000)
105 or the more recent Atlas of Muscle Innervation Zones (Barbero et al., 2012). Together, the
106 adductor muscles comprise 13.4% of the total muscle mass of the lower extremity (Ito, 1996)
107 and it is therefore important to understand their role in the mechanics of human walking and
108 running. To facilitate such research, data are needed on the reproducibility of EMG
109 measurement from the adductor muscles.

Given the lack of previous research reporting on between-day repeatability, this study sought to characterise the reproducibility of EMG measurement of running at slower speeds. Given the potential utility of the MVIC approach, this study focused on this method of normalisation. In addition, despite removing true biological variation from with a group (Burden, 2010) normalisation to the peak/mean of the EMG signal, have been associated with higher levels of repeatability than MVIC methods (Sinclair et al., 2012). This study therefore sought to compare between-day repeatability between the MVIC, peak and mean normalisation methods. This investigation was performed on a full set of lower limb muscles, including the three superficial adductors, for running. Given the paucity of data on EMG measurement of the adductor muscles, a secondary aim of this study was to report on reproducibility during normal walking.

2 Methods

2.1 Participant characteristics

A cohort of 10 male participants, with no history of lower limb injury or surgery, participated in this study. The mean(SD) age of the participants was 30(7) years, height 1.74 (0.06) m, mass 70 (8) kg, and body mass index 23.2 (1.4) $kg \cdot m^{-2}$. The study was approved by the University Ethics Committee and all participants gave written informed consent prior to participation.

2.2 Experimental procedures

In order to characterise the between-day repeatability of dynamic EMG data, each participant visited the laboratory on two separate occasions separated by one week. During each visit, EMG data were collected from walking, running and during MVIC contractions. All EMG data were collected using a Noraxon (Scottsdale, USA) DTS system (Model 586) with Ag/AgCl pre-gelled electrodes which had an inter-electrode separation of 2 cm and an electrode diameter of 1cm. This system has an input impedance of 100 MΩ and a common mode rejection ratio of 100 dB at 50Hz. Before application of the electrodes, the skin was prepared with an abrasive gel and cleaned with an alcohol wipe. EMG data were sampled at 3000 Hz and hardware filtering used to remove frequencies above 500 Hz and below 10 Hz. With the DTS system, signals are digitised within the skin-mounted units and transmitted to a desktop computer.

EMG data were collected from the following 12 lower limb muscles: gluteus maximus, gluteus medius, vastus medialis, vastus lateralis, adductor longus, gracilis, adductor magnus, tibialis anterior, semitendinosus, biceps femoris, medial gastrocnemius and lateral gastrocnemius. All data were collected from the same limb which was selected at random. With the exception of the three adductor muscles, electrodes were placed following SENIAM guidelines (Hermens et al., 2000). In order to locate the adductor electrodes, we used an ultrasound-based protocol (Elsais et al., 2020). Using a MyLab70 (Esaote, USA) ultrasound system with a 9.23 cm probe, the borders of the three superficial adductor muscles were identified (Watanabe et al., 2009) and marked on the surface of the skin with a felt pen. The ultrasound gel was then removed, and EMG electrodes placed in the middle of the muscle belly at a predetermined point along the length of the muscle. This point was referenced to thigh length (greater trochanter to lateral epicondyle) and was 60%

of thigh length for the gracilis and adductor magnus muscles and 80% of thigh length for the adductor longus muscle. In order to determine these reference lengths, we performed a pilot study on five people, comparing EMG amplitudes from signals collected at 60, 70 and 80% of thigh length. In this pilot, we identified the position associated with the largest signal and which was therefore deemed less likely to be over the innervation zone.

Following application of the electrodes, we carried out visual inspection of the EMG signals during both a resisted isometric contraction and a typical running trial. For each muscle, we confirmed that the peak signal amplitude was at least 20 times larger than the resting EMG signal (typically <5μV). If the peak EMG signal was below this threshold, in either the static or dynamic test, then the electrode was assumed to be over the innervation zone and was repositioned at a different point along muscle belly. This process was repeated until high fidelity signals were observed for all muscles during running. In cases where it was necessary to reposition electrodes, measurements were made from appropriate anatomical landmarks to ensure consistent placement at the repeat testing session.

Before collection of the dynamic EMG data, participants performed a 5-minute warm up of running at a self-selected speed, after which they practiced both walking and running at the predetermined speeds. Data for both the walking and running trials were collected along a 32 m running track with three embedded AMTI (USA) force plates, sampling at 1200 Hz. Walking data were collected first at a predetermined speed of 1.25 ms^{-1} over a 6m section of the walkway. A minimum of 10 trials were collected which were within 5% of the predefined speed (monitored using optical timing gates) and for which appropriate contact with the force plate was made. Running data were then collected using the whole length of the running track at a speed of 3.2 ms^{-1}. This running speed was selected to be

representative of research characterising biomechanical patterns associated with running injury (Bramah et al., 2018, Ceyssens et al., 2019). Again, a minimum of 10 trials were collected which were within 5% of the target speed and for which appropriate contact with the force plate was made.

In addition to monitoring speed, we also monitored the acceleration of the centre of mass during the running trials. This was performed using a custom MATLAB (Mathworks, USA) programme which obtained the anterior-posterior (AP) ground reaction force immediately after each trial using the C3D server (https://www.c3dserver.com/). Trials were rejected if the net AP impulse was greater than 10% of the area under the entire AP force curve. Accelerated running is known to be associated with larger hip joint powers than steady state running (Caekenberghe et al., 2013). As mechanical work must be done to accelerate the body and increase running speed, it is likely that activity in some lower limb muscles would also increase. Therefore, by excluding trials which demonstrated evidence of acceleration/deceleration, our protocol was optimised to give EMG signals likely to be consistent and repeatable across testing sessions.

A protocol for collecting MVIC data was developed which would be straightforward to implement in other laboratory settings. All MVIC data were collected after the dynamic tasks to mitigate against any risk of fatigue during the gait trials. A separate test was performed for each muscle group: gluteus maximus, gluteus medius, hamstrings, quadriceps, adductors, tibialis anterior and gastrocnemius in a random order. For each test, participants were instructed to contract maximally against a fixed resistance, provided by a strap attached to the testing plinth. Participants were given verbal encouragement to maximally contract and were instructed to hold the contraction for a minimum of three

seconds. Each test was repeated three times and a 1-minute rest given between successive repetitions (Rutherford et al., 2011).

For the gluteus maximus muscle the participant lay in a prone position with the knee of the tested limb in 90˚ of flexion and the hip in neutral. Instruction was then given to extend the hip against the strap. For the gluteus medius muscle, participants were positioned in side lying with the hip in a neutral position and instructed to abduct the hip against the strap. For the hamstring muscles, participants were positioned in a prone position with the knee in 55° flexion and the hip in a neutral position (Rutherford et al., 2011). In this position, participants were instructed to flex the knee against the strap. The quadriceps were contracted in a sitting position with the knee in 45˚ flexion position (Rutherford et al., 2011) and instruction to extend the knee against the strap.

In order to test the adductor muscles the participant was positioned in a supine lying position with the hip/knee in either neutral (adductor longus) or the hip/knee in 45˚ flexion (adductor magnus and gracilis). The decision to use different hip/knee angles for different adductor muscles was based on a data from a pilot study on 10 people which identified the position which was able to elicit highest muscle activity. In both positions, participants were instructed to adduct the limb against the fixed resistance. For the tibialis anterior, participants sat on the testing plinth with the knee in full extension and were instructed to dorsiflex the ankle and invert the foot against the fixed resistance. The MVIC for the gastrocnemius was carried out in a supported standing position (Rutherford et al., 2011) in which the participant was instructed to stand on their toes and to push up as hard as possible.

2.3 Signal processing

Raw EMG data were high pass filtered at either 20 Hz (walking) and 30 Hz (running), using an FFT filter (Figure 1). The decision to use a slightly higher filter frequency for running was made following a spectral analysis of the running EMG signals which showed evidence of higher frequency artefact with this faster movement. Dynamic EMG data were then rectified, and a linear envelope created using a 6 Hz low pass Butterworth filter (Hubley-Kozey et al., 2006). Foot contact and toe off events were identified when the vertical force measurement was greater than 20 N. Each dynamic trial was then time normalised to stance phase using these events. A period of 50% before and 50% after stance was also included so that the final data for each trial extended from -50 to 150% of stance. For each muscle, an ensemble profile was created by averaging the 10 linear envelope signals, corresponding to the separate trials (Figure 2). This average linear envelope was created for both walking and running, for each of the 12 muscles and each of the 10 participants. This data set was produced for the two different test days.

FIGURE 1 and 2 HERE

The MVIC data were processed in the same way as the dynamic EMG data for both walking and running. Specifically, for each MVIC signal, raw data were first high pass filtered at 20 Hz (walking) or 30 Hz (running), after which the signal was rectified and a linear envelope created using a 6 Hz low pass filter. A 0.1 second moving average filter was then applied (Hubley-Kozey et al., 2006) to the processed signal and the peak value identified. This process was repeated separately for each of the three separate contractions and the MVIC normalisation value taken as the maximum across the three trials for each muscle.

Three types of normalisation were implemented. Firstly, MVIC-normalised EMG profiles were created by dividing the average linear envelope by the MVIC reference value.

Secondly, mean-normalised EMG profiles were created by dividing the average linear envelope by the mean (across the whole trial: -50% to 150% of stance). Finally, peak-normalised EMG profiles were created by dividing the average linear envelope by the peak across the whole trial, to create a signal which varied between zero and one.

2.4 Statistical analysis

The coefficient of multiple correlation (CMC) was used to quantify the between-day repeatability of the EMG envelopes for each of the three normalisation methods. This parameter gives a measure of waveform similarity which tends to one for identical waveforms and zero for dissimilar waveforms (Growney et al., 1997, Neter et al., 1985). Previous studies have quantified reproducibility using either an intraclass correlation coefficient (ICC) (Albertus-Kajee et al., 2011) or using the coefficient of variation (CV) (Murley et al., 2010). However, the CV can give misleading statistics when used to compare the results of different normalisation methods (Burden, 2010) as it involves dividing by the mean, which can differ between them. Furthermore, both the ICC and the CV require parameterisation of the normalised EMG signal. As the aim of this study was to quantify the similarity of normalised EMG profiles during running, the CMC was deemed a more appropriate measure, has been used previously to quantify the similarity of EMG waveforms (Kadaba et al., 1989) and is equivalent to the variance ratio (Granata et al., 2005). The following equation was used to calculate the CMC (Growney et al., 1997):

$$\mathrm{CMC} = \sqrt{\frac{\sum_i \sum_j \left(E_{ij} - \bar{E}_j\right)^2 / m(n-1)}{\sum_i \sum_j \left(E_{ij} - \bar{E}\right)^2 / (mn-1)}}$$

where E_{ij} is the EMG value (of the normalised ensemble average curve) for the i^{th} day and the j^{th} time point, $\bar{E}_j$ is the mean EMG value at time point j across all days and $\bar{E}$ is the grand mean (average over all days and time points). The summation is performed across all n days and all m time points.

The CMC was calculated individually for each muscle/participant, using the time normalised signal (-50 to 150% stance), to produce a metric quantifying signal consistency between the two testing sessions. Mean(SD), across all participants, summary statistics were then created for both the walking and the running tasks. Following recommendations of Portney and Watkins (2009), values of the CMC of between 0.5-0.75 were taken to indicate moderate repeatability, between 0.75-0.9 to indicate good repeatability and greater than 0.90 to indicate excellent repeatability. To facilitate comparison between the three normalisation methods, repeated measures ANOVA testing was used with a critical α=0.05.

3 Results

The mean CMC for running was observed to be above 0.7 for all muscles, indicating moderate to excellent repeatability (Table 1). Figure 3 shows the distribution of the CMC values for each muscle and illustrates that, in most cases, there was a relatively symmetrical distribution about the mean (provided in Table 1). This plot also shows that, for most muscles, the minimum CMC (across all participants) was above the threshold of 0.75, indicating good or excellent repeatability. However, for gracilis and biceps femoris, considerable variability was observed, with a relatively large proportion of individuals

295 exhibiting CMC values which would be considered to indicate only moderate repeatability
296 (CMC=0.5-0.75).

297 The CMC values associated with the mean and peak methods of normalisation
298 methods were, in general, slightly higher than those of the MIVC method for running (Table
299 1). The ANOVA analysis demonstrated both the peak and mean normalisation methods to
300 be associated with significantly larger CMC values ($p<0.05$) than the MVIC method for five
301 muscles: gluteus medius, vastus medialis, vastus lateralis, semitendinosus and medial
302 gastrocnemius. However, there were no significant differences between the mean and peak
303 methods. Of the two muscles demonstrating low CMCs with the MVIC method (Figure 3),
304 there were only marginal, non-significant, increases in the CMC when either the mean or
305 peak method of normalisation was used as an alternative to the MVIC. When averaged
306 across all participants, peak activity was found to exceed one for the vastus medialis, vastus
307 lateralis and medial gastrocnemius muscles. This demonstrated that, in some cases, the
308 signals collected during running were larger than those obtained during the MVIC trials.

309 CMC values for walking and running were similar across most muscles for the MVIC
310 method, with differences in the mean CMC ranging from 0.01-0.08 (Tables 1 & 2). For
311 walking, lower levels of repeatability were observed for gracilis, biceps femoris and
312 adductor longus. For these three muscles, the mean CMC was 0.75-0.76, indicating that, for
313 approximately half the participants, repeatability was only moderate. For the other muscles,
314 mean CMC values were above 0.8 indicating good or excellent repeatability across the
315 participants studied (Table 2). Similar to running, CMC values for walking were slightly
316 higher for the mean and peak methods, when compared to the MVIC method, with
317 statistically larger CMC values ($p<0.05$) observed for three muscles: vastus medialis
318 semitendinosus and medial gastrocnemius.

4 Discussion

The primary aim of this study was to quantify the between-day repeatability of surface EMG signals collected from a comprehensive set of lower limb muscles during running. The study also sought to contrast the level of repeatability between the MVIC method and the peak and mean methods and to provide insight into how EMG repeatability in running compares to walking. The data showed good or excellent repeatability for 10 of the 12 muscles studied during running and for 9 of the 12 muscles during walking. For the remaining muscles, only moderate repeatability was observed indicating differences in the EMG profiles across the different testing days. While repeatability was, in general, higher for the peak and mean methods, the magnitude of the differences in the CMC tended to be small. Furthermore, for muscles considered to have only moderate repeatability, using the peak or mean method as an alternative to the MVIC method did not lead to significant increases in the CMC.

It is possible that the lower levels of repeatability observed in gracilis and biceps femoris during running may have resulted from relative movement between the innervation zone and electrode. Such relative movement will affect the amplitude of the EMG signal (Merletti and Muceli, 2019, Rainoldi et al., 2000). Similarly, it is also possible that there were small differences in the positioning of the electrode relative to the innervation zone between testing sessions, which would also impact on signal magnitude and influence repeatability. While we took steps to reposition electrodes if signals were low, we did not use an array EMG technique to precisely locate the position of the innervation zone for each muscle. Furthermore, although data are available on innervation zone position during isometric contraction (Barbero et al., 2012), there are minimal data to describe how the

343 innervation zones moves with respect to an overlying electrode during movements typical
344 of running. Given this limitation, it is essential that future research is undertaken, using
345 array EMG techniques (Besomi et al., 2019), to fully map the position and relative
346 movement of the innervation zone across all superficial lower limb muscles during
347 movements associated with walking and running. Such research will lead to improved
348 guidelines and optimise positioning of EMG electrodes for gait measurement.
349 In addition to relative movement between the innervation zone and electrodes, a
350 range of other factors may underlie the increased variability in gracilis and biceps femoris.
351 Such factors include compression of subcutaneous tissue (e.g. from overwrapped bandage),
352 a change in the distance between the electrodes and fascia and variability in the properties
353 of the electrode-tissue interface (e.g. skin-electrode impedance). In addition, small
354 differences in the orientation of electrodes with respect to the underlying muscle fibres
355 could lead to between-day variability in EMG signals. It is therefore imperative that all such
356 factors are carefully controlled and that electrodes are placed consistently, at a location
357 which is away from the innervation zone. With such experimental rigour, it will be possible
358 to understand whether the variability, observed in this study, is due to differences in muscle
359 synergies across different testing sessions.
360
361 **4.1 Comparison with previous research**
362 Albertus-Kajee *et al.* (2011) investigated the between-day repeatability of the
363 quadriceps, gastrocnemius and hamstring muscles at maximal speed sprinting. In general,
364 our data appear to suggest slightly higher repeatability at our slower running speed of 3.2
365 ms^{-1} for the vastus lateralis and lateral gastrocnemius but lower repeatability for biceps
366 femoris. However, direct comparison cannot be made as Albertus-Kajee *et al.* (2011) used

367 an intraclass correlation coefficient to quantify consistency of mean EMG amplitude
368 between different testing sessions whereas we used a CMC to characterise waveform
369 similarity between sessions. In another study, Taborri et al. (2018) reported on the
370 repeatability of synergy-based signal factorisation from EMG data in running. With this
371 approach, the aim is to understand coordinated muscle action by identifying a small number
372 of muscle synergies. Similar to the findings of the current study, their data suggested a high
373 level of between-day repeatability from surface EMG measurement in running. However,
374 direct comparison is not possible as they did not report on individual muscles as the aim was
375 to quantify the repeatability of muscle synergies.
376 Kadaba et al. (1989) reported on the between-day repeatability of EMG profiles for
377 10 muscles during walking using the peak method. This earlier work reported CMC values of
378 between 0.66-0.88 which are slightly lower than our data for the peak method (Table 2).
379 Interesting, Kadaba et al. (1989) also observed lower mean CMC values for adductor longus
380 and the medial/lateral hamstrings. This lower repeatability of the biarticular muscles
381 appears consistent with the data of the current study (Table 2) and, as explained above, may
382 reflect relative movement between the electrode and the innervation zone during dynamic
383 movement. More recent studies have reported on between-day repeatability in walking for
384 a smaller number of muscles. For example, good repeatability has been observed for the
385 gastrocnemius and tibialis anterior with the MIVIC method (Murley et al., 2010) and for the
386 vastus medialis, biceps femoris and tibialis anterior using normalisation to the peak of a
387 separate dynamic task (Lyytinen et al., 2016). Building on this research, the current study is
388 the first to report on the repeatability of a full set of lower limb muscles, including the three
389 superficial adductors, during walking.
390 **4.2 Method of normalisation**

391 A secondary objective of this study was to understand how the type of normalisation
392 could affect repeatability of the gait EMG profile. Some researchers have used either the
393 peak (Reeves et al., 2019) or mean (Shiavi et al., 1987) dynamic methods for normalising
394 EMG data collected during gait. However, by virtue of dividing by the magnitude of the
395 signal under investigation, these two approaches remove true biological variation from the
396 group (Allison et al., 1993, Burden, 2010), retaining only information on the temporal profile
397 of the EMG signal. Given this limitation, peak/mean approaches should be limited to studies
398 which are designed to compare amplitude within a person and muscle in the same testing
399 session (Besomi et al., 2020). In contrast, normalisation to an MVIC is appropriate for a
400 wider range of experimental designs, e.g. comparison of amplitude between
401 participants/muscles and across different testing sessions (Besomi et al., 2020). Our finding,
402 of only small differences in reproducibility between the MVIC and peak/mean methods,
403 supports the use of MVIC to normalise EMG in running and is in line with previous
404 recommendations (Burden, 2010) that MIVC methods can be used reliably.
405 For this study, we chose to investigate the MVIC method of normalisation, rather
406 than use a task which was matched to running in terms of joint angle/muscle length,
407 contraction type and/or joint angular velocity (Besomi et al., 2020). Our motivation was to
408 create and test a protocol which would be feasible across different laboratory settings.
409 Previous researchers have advocated the use of maximal sprinting (Albertus-Kajee et al.,
410 2011) or cycling (Chuang and Acker, 2019) to normalise EMG signals during running.
411 However, it is not clear whether such movements are associated with changes in muscle
412 length similar to those of slower speed running. Interestingly, a recent study investigating
413 medial gastrocnemius and vastus lateralis during running (Monte et al., 2020) identified a
414 quasi-isometric behaviour, characterised by minimal length change in the muscle. While

such behaviour would support the use of normalisation with an MVIC, it is important to acknowledge that longitudinal muscle lengthening and shortening will have a considerable effect on the amplitude of EMG signals and therefore may affect repeatability. Further research is therefore needed to fully understand muscle length change behaviour across a full set of lower limb muscles during running and the potential for such length changes to impact on between-session repeatability.

We acknowledge that our approach of creating a linear envelope, with a 6 Hz low pass filter, will smooth the data (Figure 2) and may reduce the variability in the temporal profile of the unnormalised EMG profile. It is possible that this reduced variability may have contributed to the similar levels of variability between the different normalisation approaches. Nevertheless, it is common practice to use this processing technique and meaningful information can be extracted from EMG data following the creation of a linear envelope. Therefore, given the high levels of reproducibility demonstrated in this study, we would advocate the use of MVIC methods for running at slower speeds and for walking.

4.3 Methodological limitations

There are several limitations to this study which should be acknowledged. Firstly, as outlined above, we did not locate the position of the innervation zone for each muscle nor did we investigate the potential for movement between the innervation zone and the electrode during the two movements studied. If the innervation zone moves under the electrode during data collection, then this will lead to geometrical artefact which can strongly alter the amplitude of the recorded signal. Such artefact is likely to reduce between-day repeatability of EMG signals and may explain the lower CMCs from the biceps

femoris and gracilis. However, the finding of good or excellent repeatability for the other muscles studied, suggest that the corresponding electrodes were placed away from the innervation zone. Nevertheless, there is a need for further research, using array EMG (Merletti and Muceli, 2019), to map the position of the innervation zones for the three superficial adductors and to quantify the relative movement of the innervation zones during running across a full set of lower limb muscles.

Another potential limitation is that our recorded EMG signals could have been contaminated with crosstalk from neighbouring muscles. While we did not specifically investigate the potential for crosstalk across all 12 muscles, our protocol for placing electrodes over the three adductor muscles involved the use of ultrasound to identify muscle borders (Elsais et al., 2020). Furthermore, in our earlier study, we provided evidence that the adductor EMG electrodes maintained a position which was at least 5 mm within the muscle boundary across a range of hip flexion–extension angles and different contraction levels (Elsais et al., 2020), thereby minimising the potential for crosstalk. Nevertheless, given that electrodes were positioned in the same location on the two different testing sessions, it is unclear whether our finding of high repeatability for most muscles studies, was influenced by the presence of crosstalk.

We did not use a dynamometer to control the position the limb during MVIC testing or measure joint torque in each MVIC test. This decision was made because of the relatively large number of muscles studied. However, we did carefully control joint angles for each test and instructed participants to contract maximally against a fixed resistance. Given our findings of good repeatability, this protocol is associated with consistent MVIC-normalised EMG signal data and should be easy to replicate in other laboratories or clinical settings. A final potential limitation was that it was not possible to objectively assess whether

participants were fully activating their muscles during the MVIC tests. This would have required the use of an electrical stimulation technique (Lewek et al., 2004) and was deemed beyond the scope of this investigation.

In conclusion, we have described a protocol for creating MVIC-normalised EMG signals during running which demonstrated high levels of between-day repeatability for 10 out of the 12 muscles studied. This protocol requires the use of ultrasound to position the three adductor electrodes, careful monitoring of speed and acceleration during dynamic trials but relatively straightforward procedures for MVIC testing. We suggest that this protocol may be appropriate for future studies investigating muscle activation patterns during non-maximal speed running. However, we acknowledge that further research is required to investigate the potential effect of relative movement between the innervation zones and electrodes.

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Tables

Table 1: Repeatability data for the running task. Mean(SD) values are presented for the coefficient of multiple correlation (CMC) across the 10 participants for each muscle and normalisation technique. In addition, for the MIVIC and mean-normalised methods, the average (across the 10 participants) peak value of the normalised EMG signal is presented.

	MVIC		**Mean**		**Peak**
	Mean(SD) CMC	Average peak (%MVIC)	Mean(SD) CMC	Average peak (%Mean)	Mean(SD) CMC
Gluteus maximus	0.94(0.02)	80	0.94(0.02)	355	0.95(0.02)
Gluteus medius	0.91(0.06)	92	0.96(0.02)	356	0.96(0.02)
Vastus medialis	0.91(0.07)	126	0.97(0.02)	368	0.97(0.01)
Vastus lateralis	0.94(0.03)	138	0.97(0.01)	349	0.97(0.01)
Adductor longus	0.83(0.11)	36	0.88(0.06)	221	0.85(0.11)
Gracilis	0.70(0.11)	72	0.74(0.12)	244	0.73(0.12)
Adductor magnus	0.91(0.04)	95	0.93(0.02)	284	0.93(0.03)
Tibialis anterior	0.87(0.08)	66	0.92(0.05)	235	0.92(0.05)
Semitendinosus	0.87(0.07)	71	0.92(0.05)	319	0.92(0.05)
Biceps femoris	0.77(0.17)	59	0.83(0.12)	256	0.82(0.12)
Medial gastrocnemius	0.95(0.03)	27	0.97(0.01)	342	0.98(0.01)
Lateral gastrocnemius	0.96(0.02)	93	0.96(0.02)	346	0.97(0.01)

612

613 **Table 2:** Repeatability data for the walking task. Mean(SD) values are presented for the
614 coefficient of multiple correlation (CMC) across the 10 participants for each muscle and
615 normalisation technique. In addition, for the MIVIC and mean-normalised methods, the
616 average (across the 10 participants) peak value of the normalised EMG signal is presented.

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	MVIC		**Mean**		**Peak**
	Mean(SD) CMC	Average peak (%MVIC)	Mean(SD) CMC	Average peak (%Mean)	Mean(SD) CMC
Gluteus maximus	0.93(0.04)	22	0.94(0.04)	538	0.96(0.01)
Gluteus medius	0.93(0.03)	44	0.95(0.02)	469	0.95(0.02)
Vastus medialis	0.90(0.05)	25	0.95(0.03)	439	0.95(0.04)
Vastus lateralis	0.93(0.05)	36	0.97(0.01)	452	0.93(0.06)
Adductor longus	0.75(0.09)	9	0.78(0.03)	293	0.83(0.09)
Gracilis	0.76(0.08)	20	0.83(0.07)	304	0.83(0.09)
Adductor magnus	0.87(0.11)	64	0.89(0.09)	384	0.89(0.09)
Tibialis anterior	0.92(0.03)	34	0.94(0.03)	341	0.94(0.02)
Semitendinosus	0.80(0.09)	31	0.88(0.06)	373	0.88(0.06)
Biceps femoris	0.76(0.15)	23	0.78(0.14)	456	0.77(0.15)
Medial gastrocnemius	0.90(0.03)	57	0.93(0.01)	531	0.97(0.02)
Lateral gastrocnemius	0.94(0.03)	38	0.96(0.01)	571	0.96(0.01)

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Figures

Figure 1: Example raw EMG signals (after high pass filtering at 30 Hz) for a participant during one running trial. EMG data have been scaled for visualisation purposes and the number in brackets provides peak signal amplitude (μV) across the trial for each muscle.

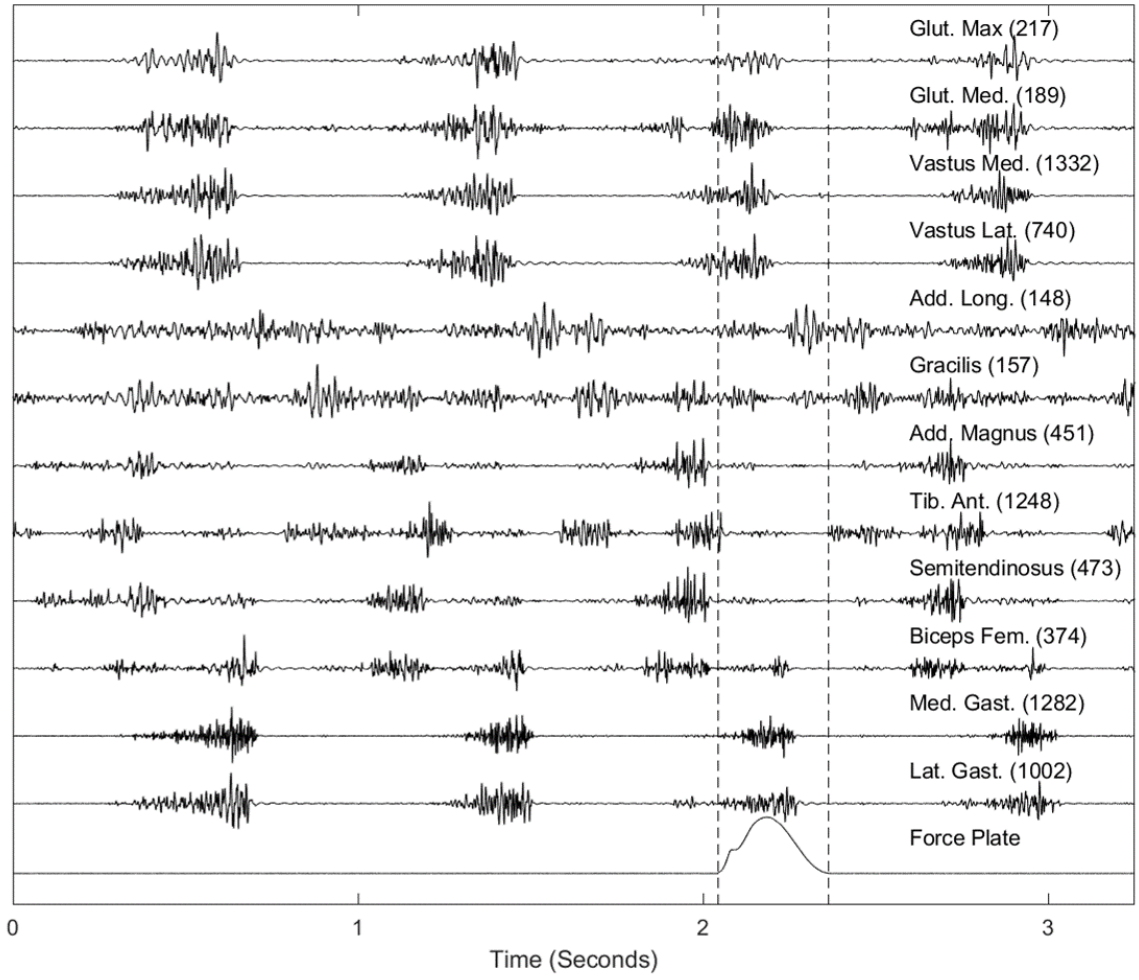

Figure 2: Linear envelope profiles created by ensemble averaging 10 trials across a single testing session for the same participant as shown in Figure 1. Ensemble data have been scaled so that the peak value is the same for all muscles. The shaded area shows the SD across the 10 trials.

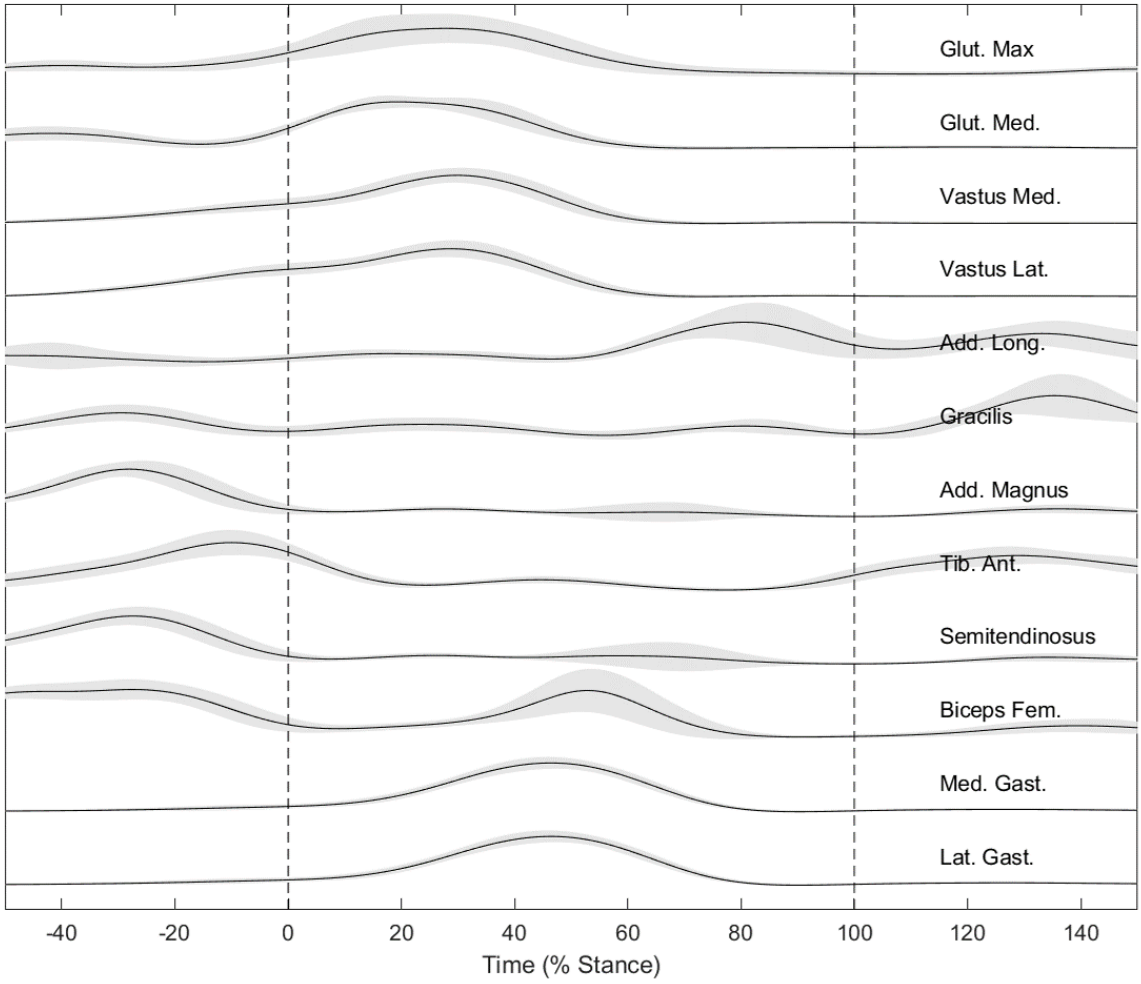

Figure 3: Box plots illustrating the distribution of the CMC across the 10 participants for each of the 12 muscles. On each box, the central mark indicates the median, the bottom and top edges indicate the 25th and 75th percentiles and the whiskers extend to the most extreme data points. Outlier are shown using the '+' symbol.