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Title: Spatial variability of muscle activity during human walking: The effects of different EMG normalization approaches

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Spatial variability of muscle activity during human walking: the effects of different EMG normalisation approaches

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Abstract

Human leg muscles are often activated inhomogeneously, e.g. in standing. This may also occur in complex tasks like walking. Thus, bipolar surface electromyography (sEMG) may not accurately represent whole muscle activity. This study used 64-electrode high-density sEMG (HD-sEMG) to examine spatial variability of lateral gastrocnemius (LG) muscle activity during the stance phase of walking, maximal voluntary contractions (MVC) and maximal M-waves, and determined the effects of different normalisation approaches on spatial and inter-participant variability. Plantar flexion MVC, maximal electrically elicited M-waves and walking at self-selected speed were recorded in eight healthy males aged 24–34. sEMG signals were assessed in 4 ways: unnormalised, and normalised to MVC, M-wave or peak sEMG during the stance phase of walking. During walking, LG activity varied spatially, and was largest in the distal and lateral regions. Spatial variability fluctuated throughout the stance phase. Normalising walking EMG signals to the peak value during stance reduced spatial variability within LG on average by 70%, and inter-participant variability by 67%. Normalising to MVC reduced spatial variability by 17% but increased inter-participant variability by 230%. Normalising to M-wave produced the greatest spatial variability (45% greater than unnormalised EMG) and increased inter-participant variability by 70%. Unnormalised bipolar LG sEMG may provide misleading results about representative muscle activity in walking due to spatial variability. For the peak value and MVC approaches, different electrode locations likely have minor effects on normalised results, whereas electrode location should be carefully considered when normalising walking sEMG data to maximal M-waves.

Introduction

Surface electromyography (sEMG) is among the most commonly used tools in human gait research. The numerous applications of this method include the study of muscle coordination (Hug, 2011), timing of muscle activation-deactivation (Hodges and Bui, 1996) and changes in relative activity before and after an intervention (Piirainen et al., 2014). Typically a single pair of electrodes is used to obtain a bipolar sEMG signal where the common noise detected by the two electrodes is greatly reduced. However, the bipolar recording is unavoidably limited by anatomical constraints related to sEMG recordings (Farina et al., 2002; Mesin et al., 2009), and provides information about a small sub-portion of the muscle. The majority of muscles that contribute to balance and propulsion during gait, such as the quadriceps and triceps surae muscles, are very large and possess complex, variable 3-dimensional architecture along their length. Accordingly, inhomogeneous muscle activity has been observed in some conditions, such as standing (Vieira et al., 2011; Hodson-Tole et al., 2013), and might also be expected in more complex tasks like walking. Thus, results obtained using bipolar sEMG may not accurately represent whole muscle activity, especially during dynamic movements.

Data from several studies support the notion of spatial heterogeneity of muscle activity in a range of dynamic tasks including stepping up/down movements (Wolf et al., 1993) and heel raises (Kinugasa et al., 2005, 2006; Segal and Song, 2005). Importantly, the pattern of heterogeneity appears to be individual-, task- and muscle-specific. For example, during single leg heel raises, Kinugasa et al. (2005) observed heterogeneous activity in medial gastrocnemius (MG) but not lateral gastrocnemius (LG) or soleus, whereas Segal and Song (2005) observed heterogeneity in all three muscles during the same task. Furthermore, the

most active region of a muscle during a given task may depend on the level of force production required (Kinugasa et al., 2011; Hodson-Tole et al., 2013). Collectively, these findings indicate that different muscle regions may be preferentially activated for certain tasks, implying that bipolar sEMG from a single region may not give sufficient information about overall muscle activity. However, the aforementioned studies have all examined this issue in relatively simple tasks that are restricted to movement of a single joint or in a single plane. Thus, these results may not be generalisable to more complex tasks like walking.

Recent methodological advances have enabled the use of high-density sEMG (HD-sEMG), which uses arrays of small electrodes to measure activity from a large region of the muscle. To date, this method has been used in dynamic conditions including cycling (Schmitz et al., 2012), as well as to study quadriceps function in walking (Watanabe et al., 2014). However, spatial distribution of muscle activity has not been studied in the functionally important triceps surae muscles during walking. The aims of this study were: 1) to examine overall spatial variability of LG muscle activity during the mean stance phase of walking, maximal voluntary contractions (MVC) and maximal M-waves using 64-electrode HD-sEMG; 2) to examine how spatial variability in muscle activity changes as a function of time within the stance phase of walking; 3) to determine the efficacy of different sEMG normalisation approaches in reducing spatial and inter-participant variability in muscle activity during walking. The third aim was included because sEMG normalisation is commonly used to reduce inter-participant variability (Yang and Winter, 1984; Kasprisin and Grabiner, 1998) and to allow comparisons between different muscles and test sessions (see Burden, 2010). This study also investigated the effects of normalisation on spatial variability. It was hypothesised that within a given participant, spatial variability would be present in all examined conditions; that spatial variability would differ in a time-dependent manner

throughout the step cycle; and that different normalisation approaches would affect the level of spatial and inter-participant variability during walking.

Methods

Participants

The participant group consisted of eight healthy males aged between 24 and 34 (29 ± 4 years, mean $\pm$ SD; height: 176 ± 8 cm; body mass: 73 ± 10 kg). Participants were instructed to avoid heavy exercise for two days, and caffeine and alcohol for one day before testing. The study was approved by the ethics committee of the University of Jyväskylä and all participants provided written informed consent before participation.

Protocol

Participants were first seated in an ankle dynamometer (University of Jyväskylä, Finland) with knee angle fixed at 180° (full extension), and performed several sub-maximal plantar flexions as a warm up. Plantar flexion maximal voluntary contractions (MVC) and maximal electrically elicited M-waves were then recorded with ankle angle fixed at 90°. Three repetitions of each condition were performed, with rest periods of at least two minutes between MVCs and at least 10 s between electrical stimuli to avoid muscle fatigue. Participants then walked at a self-selected speed across a 10 m instrumented walkway (Raute Oy, Lahti, Finland), which was repeated three times to allow sufficient data to be averaged.

Data Collection

Surface electromyography. HD-sEMG signals were recorded with an EMG-USB 128 channel surface EMG amplifier (designed by LISiN, Politecnico di Torino and manufactured by OT Bioelectronica, Torino, Italy; input impedance > 90 MΩ; common mode rejection > 96 dB;

gain 2000; passband 10–750 Hz; sampling at 2048 Hz). Data were recorded in monopolar mode with a semi-disposable grid of 64 electrodes arranged in 5 columns of 13 electrodes (13 × 5 cm) with the proximal-medial corner electrode missing (8 mm inter-electrode distance in both directions; model ELSCH064, LISiN-OT Bioelectronica, Italy). Before electrode grid placement, the skin was shaved, abraded and cleaned with alcohol. The middle column of the electrode grid was placed over the midline of the LG muscle (determined by palpation of the muscle borders) parallel to its longitudinal axis with its distal end approximately 3–4 cm proximal to the gastrocnemius muscle-tendon junction. This position was chosen to ensure that the electrode was well within the boundaries of the LG muscle. Electrode cavities were filled with 25 μL of conductive electrolyte gel (Spes-Medica, Battipaglia, Italy). To reduce common mode interference in the monopolar sEMG signals, a reference strap (LISiN-OT Bioelectronica) was attached to each wrist. Antagonist muscle activity was recorded with a pair of circular electrodes (Ambu, Ølstykke, Denmark) placed over the tibialis anterior muscle with an inter-electrode distance of 20 mm. The antagonist sEMG signal was recorded with the same amplifier as the HD-sEMG, and only used to visually confirm the physiological nature of steps.

Electrical stimulation. Maximal M-waves were elicited by stimulating the right tibial nerve using 1-ms square wave single pulses, and participant-specific current intensities (Digitimer Stimulator DS7, Digitimer Ltd., Hertfordhire, England). First, the optimal stimulating electrode location was determined by placing an oval anode (width 4 cm; length 6 cm) above the patella and moving a circular cathode (radius 1 cm) around the popliteal fossa until a clear, repeatable LG M-wave was evoked with submaximal current intensity. The circular stimulating electrode was then attached firmly to the optimal location with tape. Supramaximal intensity for each participant was set at 1.5 times the intensity required to

elicit the maximal M-wave to ensure the maximal sEMG response. The muscle was always at rest prior to stimulation.

Walking and MVCs. In isometric MVC trials, torque values were recorded using a piezoelectric force transducer attached to the foot pedal of the dynamometer (Kistler; Winterhur, Switzerland). During walking trials, three dimensional ground reaction forces were recorded at 2048 Hz across the length of the 10-m walkway through the auxiliary inputs of the EMG amplifier (only the vertical force component is presented). For all trials, sEMG, force and torque signals were sampled via the same amplifier, and were thus always synchronised. The number of accepted walking cycles was 20.13 ± 3.76 (range 14–24).

Processing of sEMG

Muscle activity. The 64 monopolar sEMG signals were first organised into 59 bipolar sEMG channels along the longitudinal axis of the LG muscle, i.e. along the five electrode columns (5×12 bipolar sEMG channels, 5 x 11 channels in the most medial column). sEMG signals were then digitally band-pass filtered (20–450 Hz; 4th order Butterworth filter). Muscle activity was quantified as root mean square (RMS) amplitude for each of the 59 sEMG channels separately. RMS amplitudes were computed both for time-unnormalised data (stance phase of walking, MVC and M-wave) and time-normalised data (entire stance phase and 10% steps of the averaged stance phase). For the time-unnormalised data, RMS amplitudes were computed from "raw" sEMG signals that were only preprocessed as explained above. For walking, RMS amplitude was first calculated for the entire length of each stance phase separately, and RMS values were then averaged across trials. For MVCs, RMS amplitude was calculated within a 1000-ms epoch of the most stable part of the torque signal (smallest variation) overlapping with the peak torque. For M-waves, RMS was calculated during a fixed 30-ms epoch starting 5 ms after delivery of the electrical stimulus.

The 30-ms epoch overlapped with the main M-wave response in all participants. These analyses provided two-dimensional sEMG-RMS amplitude maps for each participant and condition. The participants' individual sEMG-RMS maps were first normalised to the maximum RMS value of the whole map, and then averaged across participants to obtain group level maps that were plotted and colour-coded (see Figure 1a; amplitude ranges are given as percentages). In addition, these sEMG-RMS maps were used to compare muscle activity distribution (i.e. spatial variability).

Normalisation of walking sEMG. Walking sEMG signals were normalised using three different normalisation approaches: (1) average rectified sEMG value during MVC, (2) average rectified value during M-wave and (3) peak sEMG value during the stance phase of walking. For MVC and M-wave, the same respective 1000-ms or 30-ms epochs were used as for RMS computation (see above). After normalisation, the walking sEMG signals were rectified, low-pass filtered at 40 Hz (4th order Butterworth filter), and re-sampled to 1000 points to enable averaging across trials. For the peak sEMG value normalisation, walking sEMG signals were first averaged across trials (as described above) and then normalised to the respective peak value for each of the 59 sEMG channels separately.

To analyse time-dependent muscle activity, the averaged walking sEMG signals were divided into 10% time epochs, and RMS was calculated channel-by-channel both for normalised and unnormalised signals for each participant. To visualise the sEMG traces during the stance phase at the group level, sEMG signals were further averaged across participants for each of the 59 sEMG channels separately.

Spatial variability in muscle activity. To compare spatial variability in muscle activity between walking, MVC and M-wave conditions, coefficients of variation of unnormalised sEMG-RMS amplitudes over all 59 sEMG channels were calculated for each participant separately (i.e. across the participants' sEMG-RMS maps; 3 task $\times$ 8 participant coefficients).

Similarly, to examine the effect of different normalisation approaches on spatial variability in muscle activity, coefficients of variation were calculated channel-by-channel for the walking sEMG signals after normalisation to either MVC, M-wave or peak sEMG within the stance phase (4 approach × 8 participant coefficients). A one-way repeated measures analysis of variance (ANOVA) was used to compare spatial variability in muscle activity (i.e. the coefficients of variation). For unnormalised sEMG, comparisons were performed between all three conditions (walk, MVC and M-wave). To determine the effect of the normalisation approaches during walking, the spatial variability of the unnormalised sEMG-RMS walking map was further compared to the three normalised walking maps (normalised to MVC, M-wave, or peak sEMG value within stance). When appropriate, Sidak post-hoc test was applied.

Time-dependent spatial variability in muscle activity during the stance phase of walking. To examine how spatial variability in muscle activity varied throughout the stance phase, sEMG signals obtained during walking were divided into 10% time epochs (see 'Muscle Activity'), and coefficients of variation were calculated between the 59 sEMG-RMS values both for unnormalised and normalised walking sEMG signals and for each participant separately (4 approach × 8 participant × 10 epoch coefficients). Effects of normalisation approach and time were compared using a two-way 4 (approach: no normalisation, MVC, M-wave and peak sEMG value during walking) × 10 (time: 10% steps throughout stance) repeated-measures ANOVA. When a significant interaction was detected, one-way repeated measures ANOVA was applied to examine the effects of normalisation approach and time separately. When appropriate, Sidak post-hoc test was applied.

Spatial inter-participant variability in muscle activity. Coefficients of variation of inter-participant sEMG-RMS amplitudes were first calculated for each of the 59 sEMG channels separately (59 coefficients), providing a group level spatial inter-participant variability

matrix. The mean ± SD value of this matrix is reported for each of the four conditions, and represents the level of spatial differences in LG muscle activity between participants. This analysis was done both for unnormalised and normalised walking sEMG signals.

Global inter-participant variability in muscle activity. To describe the effects of the normalisation approaches on inter-participant variability in mean LG muscle activity during the stance phase of walking (as opposed to the spatial variability calculation outlined in the previous paragraph), mean sEMG-RMS amplitude was first calculated across all 59 channels for each participant separately, and coefficients of variation were then computed between the mean EMG-RMS amplitudes for all 4 approaches (i.e. unnormalised and the 3 normalised walking sEMG signals). The same analysis was repeated in 10% steps throughout the stance phase (4 approach × 10 epoch coefficients).

Spatial correlations. Pearson's correlation coefficients were calculated between sEMG-RMS maps of (1) stance phase of walking and MVC, and (2) stance phase of walking and maximal M-wave. The calculations were performed at both the group and individual participant levels.

Distribution of muscle activity. In order to compare the distribution of sEMG-RMS amplitudes in both the proximal-distal (sEMG rows) and medial-lateral (sEMG columns) directions, sEMG-RMS amplitudes were averaged across the 12 rows and 5 columns for each condition and participant separately. A one-way ANOVA was used to compare distribution of the averaged sEMG-RMS amplitudes separately in the proximal-distal (sEMG rows) and medial-lateral (sEMG columns) directions. When appropriate, Sidak post-hoc test was applied.

In all cases, statistical significance was determined as $p < 0.05$. All statistics were done in IBM SPSS statistics (version 22, SPSS, Inc., Chicago, USA). Descriptives are given as mean ± SD. Data were tested for normal distribution and equal variances. Where necessary, data

were transformed logarithmically before computing ANOVAs, in order to fulfil the criterion of normal distribution.

Results

Spatial variability in sEMG during walking, MVCs and M-waves

Figure 1a shows group level distributions of sEMG-RMS amplitudes throughout the LG muscle during walking, MVC and M-wave. At the group level, the spatial pattern of sEMG activity during walking correlated well with the spatial pattern obtained during MVC ($r = 0.91$, $p < 0.001$), but not with the spatial pattern obtained during the M-wave ($r = -0.03$, $p = 0.80$). At the individual level, six participants showed a significant ($p < 0.05$) spatial correlation between the walking map and M-wave map ($r = 0.34 \pm 0.33$) and seven participants between the walking map and MVC map ($r = 0.69 \pm 0.12$).

The spatial variability of sEMG-RMS amplitude in LG muscle varied significantly between walking, MVC and maximal M-waves ($F = 4.19$, $p = 0.048$). Spatial variability was significantly ($p = 0.025$) lower during MVC (coefficient of variation $29 \pm 5\%$) than M-wave ($37 \pm 4\%$). However, spatial variability during walking ($31 \pm 6\%$) did not differ from MVC ($p = 0.946$) or M-wave ($p = 0.074$).

Figure 1b shows group level distributions of sEMG-RMS amplitude throughout LG during walking normalised using the peak sEMG value, MVC and M-wave approaches. Spatial variability in muscle activity during walking was significantly affected by different normalisation approaches ($F = 5.81$, $p < 0.001$). Spatial variability without normalisation was $30 \pm 6\%$, and was reduced to $9 \pm 3\%$ when normalised to the peak sEMG value during walking ($p < 0.001$), whereas normalisation to M-wave increased spatial variability to $43 \pm 9\%$ ($p = 0.037$). Spatial variability with MVC normalisation was $25 \pm 6\%$, and did not differ from the spatial variability of the unnormalised sEMG signals ($p = 0.244$).

Figure 2 shows distribution of sEMG-RMS amplitudes in the proximal-distal (sEMG rows) and medial-lateral directions (sEMG columns). The between-participant distribution patterns were qualitatively similar for all conditions. Statistically significant differences were observed in both directions during walking (proximal-distal: $F = 5.44$, $p < 0.001$, medial-lateral: $F = 2.99$, $p = 0.032$); mean sEMG-RMS amplitude was significantly ($p = 0.001–0.026$) lower in the two most proximal rows (rows #1 and #2) of the sEMG electrode grid than in the four most distal rows (rows #9, #10, #11 and #12). In addition, rows #3, #5 and #6 showed significantly ($p = 0.005–0.014$) lower mean sEMG-RMS amplitude than row #11, and row #3 showed significantly ($p = 0.04$) lower amplitude than row #10. In the medial-lateral direction, mean sEMG-RMS amplitude was significantly ($p = 0.032$) lower in the most medial column (0.054 ± 0.01 mV) than in the most lateral column (0.071 ± 0.01 mV). A statistically significant difference was detected in the proximal-distal direction during MVC ($F = 2.02$, $p = 0.036$), although this disappeared after correction for multiple comparisons. No significant differences were detected in the mean distribution during the M-wave.

Time-dependent spatial variability in muscle activity during the stance phase of walking

Figure 3a shows group mean rectified unnormalised sEMG, as well as normalised traces for each of the 59 sEMG channels as a function of relative time during the stance phase of walking. Qualitatively, the variability appeared to be reduced with the peak sEMG value and MVC normalisation approaches, as evident from Figure 3b, where participants' individual sEMG signals are superimposed with their respective mean values. In contrast, normalisation to the M-wave appeared to increase spatial variability.

Figure 4a illustrates the evolution of spatial variability in muscle activity throughout the stance phase. A significant interaction was detected between normalisation approach and time ($F = 8.1$, $p = 0.001$). One-way repeated measures ANOVA revealed a significant effect of

time when using MVC (F = 8.7, p = 0.001), M-wave (F = 5.0, p = 0.011) and peak sEMG value (F = 26.7, p < 0.001) normalisation approaches, but not when using unnormalised sEMG signals (F = 1.6, p = 0.23). However, for the M-wave approach, the effect of time was not significant after correction for multiple comparisons. Typically, the lowest spatial variability was seen around the time of peak sEMG activity (~60–80% of total contact time; Figure 4a), as this is when the denominator in the coefficient of variation equation is greatest.

Spatial variability differed significantly between the normalisation approaches in all time epochs (F = 8.0–56.4, p < 0.01) except for the 100% epoch (F = 3.8, p = 0.069). In general, the highest spatial variability was seen when normalising to the M-wave, and the lowest when normalising to the peak sEMG value. Figure 4a indicates all significant differences between the normalisation approaches at each time epoch.

Inter-participant variability in muscle activity

Spatial inter-participant variability in muscle activity. Mean spatial inter-participant variability in muscle activity (across all 59 sEMG channels) was 25 ± 3% during unnormalised walking, 41 ± 1% with MVC normalisation, 88 ± 3% with M-wave normalisation, and 9 ± 2% with peak sEMG value normalisation. Thus, normalisation to MVC and M-wave tended to increase spatial inter-participant variability during the stance phase of walking, whereas normalisation to the peak sEMG value reduced it.

Global inter-participant variability in muscle activity. Figure 4b shows inter-participant variability of mean muscle activity in 10% time steps. Inter-participant variability in mean muscle activity during walking increased from the unnormalised level (average coefficient of variation over stance phase 15%) when normalised to MVC (47%) or M-wave (25%), whereas peak sEMG value normalisation tended to somewhat reduce it (5%). The same effect can be seen in Figure 4c, which illustrates each participant's individual mean muscle activity

(across all 59 sEMG channels) during the stance phase of walking, and the effect of the normalisation approaches.

Discussion

The main findings of this study were that during walking, LG muscle activity varied spatially, and activity was largest in the distal and lateral regions of the muscle. Spatial variability in sEMG activity fluctuated throughout the stance phase of walking, and was generally lowest around the time of peak muscle activity. Of the three normalisation approaches that were tested, spatial and inter-participant variability were smallest when normalising LG walking sEMG signals to the peak sEMG value during the stance phase, since this approach yields the highest normalised output. Inter-participant variability was largest with the MVC approach, and spatial variability was largest when normalising to maximal M-wave. These findings have important implications concerning the limitations of sEMG for walking studies, and the optimal approach to normalise walking EMG data.

Spatial variability in sEMG activity during walking, MVCs and M-waves. Previous studies have shown that in some muscles and some individuals, region-specific muscle activity is evident in standing (Hodson-Tole et al., 2013), as well as relatively simple dynamic tasks such as stepping up and down and calf raises (Segal et al., 1991; Wolf et al., 1993; Kinugasa et al., 2005). More recently, region-specific activity was also reported in rectus femoris during walking (Watanabe et al., 2014). In all three of the examined conditions in the present study, LG activity was spatially distributed, and was greater in the distal and lateral regions during walking and MVCs.

Nonuniform muscle activity can be explained both by physiological and anatomical factors.

First, motor units are distributed into 'functional compartments' within a muscle, and these compartments are recruited selectively for different tasks (Wolf et al., 1993; Nichols, 1994; Vieira et al., 2010). Indeed, the human LG muscle contains two main nerve branches and numerous sub-branches (Segal et al., 1991), so functional compartmentalisation is somewhat expected in this muscle (Vieira et al., 2010), and could explain why some portions of LG are more active than others during the stance phase of walking.

Second, sEMG amplitude is affected by numerous anatomical factors, such as fibre distribution, orientation and depth with respect to the recording electrodes (Merletti et al., 1999a, 1999b; Farina and Merletti, 2001; Farina et al., 2001; Rainoldi et al., 2004). For example, subcutaneous tissue is known to act as a low-pass filter (Solomonow et al., 1994; Farina and Merletti, 2001), although variations in its thickness probably had minimal influence on our results obtained from normal-weight participants. Another significant anatomical factor that may increase spatial variability is the innervation zones, corresponding to the areas where individual neuromuscular junctions are concentrated (i.e. end-plate region), and can be located by identifying the origin of action potential propagation with sEMG electrode arrays (Masuda and Sadoyama, 1986) or grids (Masuda and Sadoyama, 1988). The location of the main innervation zone varies between individuals (Masuda, 1985) and muscles (Rainoldi et al., 2004), and may be scattered in compartmentalised muscles (English et al., 1993; Vieira et al., 2010). Therefore, their influence on spatial variability depends on their locations. Bipolar sEMG signals show a clear reduction in amplitude and substantial changes in power spectrum when recorded over the innervation zone (Roy et al., 1986; Farina et al., 2002). Innervation zones shift with respect to the sEMG electrodes during dynamic movements (Martin and MacIsaac, 2006) and even during isometric contractions with increasing force (Piitulainen et al., 2009). The position of the innervation zone(s) is

particularly important given the inevitable movement between muscle and sEMG electrodes in walking. Nonetheless, it should be noted that innervation zone location may have a smaller effect in pennate muscles like LG (Mesin et al., 2011). In any case, taking into account the physiological and anatomical complexity of the LG muscle, spatial variability is logical.

The mean sEMG maps for the stance phase of walking and MVC (Figure 1) were well correlated, so normalisation of walking sEMG signals to MVC resulted in a spatially homogeneous pattern. Thus, when using the MVC normalisation approach, sEMG electrode location on the skin surface over LG muscle may be less important. Normalising to the peak sEMG value within the stance phase led to the smallest spatial variability in muscle activity, which is unsurprising as information from the exact same task and sEMG signals was used in the normalisation. Nonetheless, some spatial variability can be expected, e.g. due to shift of the muscle with respect to the recording electrodes during the stance phase. Our results indicate that if no normalisation is used, the maximal effect of electrode position on sEMG amplitude may be ~40% in LG muscle. In the case of normalisation to MVC and the peak value within stance, our data suggest that changes in electrode position would have a relatively small effect, altering sEMG amplitude by up to ~15–20% on average (Figure 1b).

As the unnormalised M-wave map was poorly correlated with the walking map, normalising to maximal M-wave increased spatial variability of the normalised walking sEMG signals by 45%. This indicates that use of the maximal M-wave normalisation approach is more prone to effects of electrode positioning, and could alter sEMG amplitude by up to 57% during walking (Figure 1b), where muscles move with respect to the recording electrodes (Cronin and Finni, 2013). Sources of error arising from sEMG electrode position can only be

counteracted by using HD-sEMG, and thus large effects on sEMG signals may be expected when using a single pair of sEMG electrodes.

Time-dependent spatial variability throughout the stance phase of walking. As shown in Figure 4, compared to the unnormalised sEMG signals, spatial variability in muscle activity at different time epochs of the stance phase was generally reduced (on average by 70%) when normalising to the peak value within the stance phase. This was particularly evident in the late stance phase (60–80% of stance), as this is when the peak sEMG consistently occurred in all 59 sEMG channels. Normalising to MVC had little effect on reducing spatial variability at different time epochs, whereas normalising to M-max actually increased spatial variability (on average by 45%) at all time epochs. Thus, the peak value approach is recommended in cases where minimising spatial variability is important. However, as noted by Burden (2010), this approach also impairs the ability to make certain comparisons between muscles, individuals and tasks reliably, as it does not provide any indication of actual muscle activation level. In such cases, normalising to MVC may be a suitable alternative, since it was generally comparable to the peak sEMG approach, and has the advantage of providing at least an approximate indication of muscle activation (see also Burden et al., 2003). Compared to the other examined methods, normalising to maximal M-wave was inferior at reducing spatial variability in muscle activity. It is important to note that short analysis windows in sEMG amplitude estimation can increase its variation, e.g. RMS amplitude. For this reason, spatial variability obtained from 10% steps (< 100 ms windows) of the stance phase may not be directly comparable to the results obtained using the entire stance phase (~500–800 ms windows). However, the analysis windows for MVC (1000 ms) and M-wave (30 ms incorporating the main M-wave response) can be considered sufficient, and are thus likely to have had minimal effects on the comparisons between normalisation approaches.

It should be noted that normalised EMG amplitudes were quite similar between the MVC and peak value approaches (Figure 3b). This somewhat surprising result may be attributable to a relatively large role of LG activity in walking, a relatively low level of LG activity during MVC, or even an inability to fully activate this muscle during MVC. Although normalisation to MVC has been shown to yield lower values for triceps surae muscles than those in the present study (e.g. Franettovich et al., 2010), a recent study also reported similarly high levels of normalised activity in MG muscle (Murley et al., 2014). Thus we do not believe that this finding invalidates our use of MVC as a normalisation method. Nonetheless, future studies may benefit from combining LG MVC measurement with a measure of activation level (e.g. interpolated twitch technique) to control for the possibility of submaximal activation.

Inter-participant variability in muscle activity. As with spatial variability in muscle activity, normalising to the peak value within the stance phase led to the smallest inter-participant variability in mean muscle activity across all 59 sEMG channels. This finding is generally consistent with previous data from lower limb muscles (Yang and Winter, 1984; Burden et al., 2003), although there remains active debate about the optimal normalisation method to minimise inter-participant variability (see Burden et al., 2003). However, in our data, differences between the peak value within stance approach and the unnormalised data were only evident around 70–80% of the stance phase, which, as noted above, is due to the fact that peak EMG occurred at this time. Both the MVC and maximal M-wave methods yielded larger inter-participant variability. These data collectively suggest that when a study aim is to minimise inter-participant variability, walking sEMG data should be normalised to the peak value within the stance phase, as suggested previously by Burden (2010). This may be particularly relevant in longitudinal studies where electrodes need to be replaced, since the

presence of spatial variability will introduce additional variation in any sEMG parameter if re-placement errors occur. However, as noted above, normalisation to the peak value does not enable some comparisons to be made, e.g. relative sEMG activity before and after a training intervention.

Study limitations. These results may not be generalisable to other triceps surae muscles, since differences between the muscles of this group are evident in muscle architecture and size (Kinugasa et al., 2005; Ward et al., 2009), and thus potentially in spatial muscle activity. In this study LG was chosen because of its large, relatively flat skin surface that is beneficial for HD-sEMG recordings, as well as its importance in human walking. LG was considered more suitable than medial gastrocnemius, which often exhibits distal curvature that can cause signal loss. Moreover, practical limitations meant that only one electrode grid could be used at one time. Crosstalk from neighboring muscles was also not taken into account in this study. However, the degree of crosstalk was likely minimal due to the use of small, spatially selective electrodes that were always at least 2 cm from the border of MG. Furthermore, Campanini et al. (2007) used a 12 electrode grid to examine crosstalk during walking, and concluded that crosstalk was minimal and constant across muscle regions in LG.

Conclusions

During walking, LG muscle exhibited nonuniform activity, whereby the distal and lateral muscle regions were most active. The level of spatial variability in sEMG activity varied throughout the stance phase of walking, and was generally lowest in the period of the highest sEMG activity. Normalising walking sEMG signals to the peak sEMG value during the stance phase reduced spatial variability within the LG muscle, throughout the stance phase

and between individuals. Normalising to MVC reduced spatial variability but increased inter-participant variability. Normalising to M-wave generally produced the greatest spatial variability across the mean stance phase and at different time intervals within it. In LG, unnormalised single channel sEMG recordings may provide misleading results about representative whole muscle activity in walking due to extensive spatial variability, particularly in studies where electrodes need to be replaced between measurements. Whilst the peak value within the stance phase was the most effective at reducing variability among the examined normalisation approaches, for comparisons between tasks and muscles, or when using HD-sEMG specifically to study spatial variability, normalising to MVC may be more applicable. For both approaches, different electrode locations would be expected to have a minor effect on the normalised results, whereas electrode location should be carefully considered when planning to normalise walking sEMG data to the maximal M-wave.

Disclosures

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Figure legends

Figure 1. Group level maps of spatial distribution of sEMG **(a)** during walking (mean of the stance phase), MVC and M-wave, and **(b)** walking when normalised to peak sEMG during the stance phase, sEMG-RMS amplitude during MVC or M-wave. Spatial correlation values between unnormalised walking and MVC and M-wave maps are shown on the maps in the upper row, and ranges of EMG-RMS amplitudes across all sEMG channels are superimposed on the unnormalised and normalised walking maps. EMG-RMS values are normalised to the maximum RMS value within each participant's individual map prior to averaging. The reported ranges represent the amount of spatial variation in sEMG activity within each map.

Figure 2. Distribution of sEMG-RMS amplitudes in proximal-distal (sEMG rows, left panels) and medial-lateral directions (sEMG columns, right panels) of the LG muscle during walking (top), MVC (middle) and M-wave (bottom). The gray traces are participants' individual mean values across the EMG rows or columns. Black traces represent group mean values. * = $p < 0.05$, rows 1 and 2 compared to rows 9–12, and column 1 to column 2. † = < 0.05, rows 3, 5 and 6 compared to row 11. # = $p < 0.05$, row 3 compared to row 11.

Figure 3. Group level (n = 8) averaged unnormalised and normalised sEMG signals during the stance phase of walking. Values were normalised to sEMG-RMS amplitude of MVC, M-wave or peak sEMG value during the stance phase itself. **(a)** Group averages for individual EMG channels of the HD-sEMG grid are shown as separate traces. **(b)** Group averages for the individual EMG channels are superimposed (gray traces) with the mean of all sEMG channels (black traces). In the lowest panel, participants' individual averaged force traces (gray) during the stance phase are superimposed on the group mean value (black trace).

Figure 4. Evolution of spatial variability (a), inter-participant variability in mean muscle activity (b), and each participant's (n = 8) mean LG muscle activity (c) during the stance phase of walking. **(a and b)** Coefficients of variation in sEMG activity are shown as a function of contact time for unnormalised sEMG signals, as well as after normalising to MVC, M-wave and peak sEMG. Significant effects of time on spatial variability are shown for each normalisation approach at the bottom of panel a (# = $p < 0.05$ relative to 60% epoch, * = $p < 0.05$ relative to 70%, † = $p < 0.05$ relative to 80% using peak sEMG value normalisation; ¤ = $p < 0.05$ relative to 70% using MVC normalisation). At the top of panel a, significant differences ($p < 0.05$) between a given normalisation approach and all other approaches are indicated with corresponding symbols for each time epoch separately. If only

two approaches differed from each other, their symbols are indicated with *vs*. **(c)** Mean muscle activity of each participant (gray traces) averaged across all 59 sEMG channels; unnormalised and normalised sEMG signals are shown. Superimposed black traces represent group mean muscle activity.