Differences between vastus medialis and lateralis excitation onsets are dependent on the relative distance of surface electrodes placement from the innervation zone location

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Differences between vastus medialis and lateralis excitation onsets are dependent on the relative distance of surface electrodes placement from the innervation zone location.

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Abstract

Conflictual results between the onset of vastus medialis (VM) and vastus lateralis (VL) excitation may arise from methodological aspects related to the detection of surface electromyograms (EMGs). In this study we used an array of surface electrodes to assess the effect of detection site, relative to the muscle innervation zone, on the difference between VM and VL excitation onsets. Ten healthy males performed moderate isometric knee extension at 40% of their maximal voluntary isometric contraction. After the actual *VM-VL onset* was defined (estimated when action potentials were generated at the neuromuscular junctions of both muscles), we calculated the largest bias that the detection site may introduce in the *VM-VL onset* estimation. We also assessed whether the location often considered for positioning bipolar electrodes on each muscle leads to *VM-VL onset* estimations comparable to the actual *VM-VL onset*. Our main results revealed that a maximum absolute bias of 20.48 ms may be introduced in *VM-VL onset* estimations due to the electrodes' detection site. In addition, mean differences of ~12 ms in VM-VL onset estimations were attributable to largest possible discrepancies in the paired position of channels with respect to the innervation zone for VL and VM. When considering the classical location for positioning the bipolar electrodes over these muscles, differences error was subtle (~3.4 ms) when compared with the actual *VM-VL onset*. Nonetheless, when accounting for the effect of relative differences in electrode position between muscles is not possible, our results suggest that a systematic absolute error of ~12 ms should be considered in future studies regarding *VM-VL onset* estimations, suggesting that onset differences lower than that might not be clinically relevant.

Keywords: high-density surface electromyography; knee; muscle activation; patellofemoral pain syndrome; vastii muscles.

Introduction

Pieces of evidence suggest the loading and therefore the integrity of the patellofemoral joint depend on the timely excitation of vastus medialis (VM) and lateralis (VL). Temporal differences in VM and VL excitation, for instance, have been shown to modify the net, patellar force (Neptune et al., 2000). Even though such temporal imbalance of VM and VL excitation seems to lead to knee disorders, as the anterior knee pain (Cowan et al., 2001; Van Tiggelen et al., 2009; Voight et al., 1991), the minimal difference between the onsets of VM and VL excitation earning clinical relevance is seemingly controversial (Chester et al., 2008; Hug et al., 2015; Lankhorst et al., 2013). On one hand, a difference as small as ~5 ms between VM-VL onsets has been used to discriminate pathological from asymptomatic populations (Briani et al., 2016; Santos et al., 2008; Van Tiggelen et al., 2009). On the other hand, much larger and variable differences in VM-VL excitation onsets were reported both for health subjects (average values ranging from -61.81 ± 68.7 ms to 27.9 ± 32.92 ms; negative values mean VM being excited prior to VL) and for patients with anterior knee pain (-17.5 ± 22.89 ms to 50.56 ± 81.96 ms; Chester et al., 2008).

Conflictual results between studies may arise from methodological aspects related to the detection of surface electromyograms (EMGs). Being the difference in the onset of VM and VL excitation defined from the temporal differences in EMG amplitude, one crucial aspect is the position of bipolar electrodes in relation to the innervation zone. If the distance between the center of bipolar electrodes and the innervation zone for VM is not comparable to that for VL, action potentials travelling with equal speeds in both VM and VL would be detected at different instants. Alternatively, if the two pairs of electrodes are centered at equivalent distances from the innervation zone for both muscles, differences in conduction velocity between muscles would still affect the instant at which VM and VL action potentials would be

detected. In either or both cases, temporal differences in the variation of EMG amplitude between muscles would not be entirely attributable to differences in VM-VL excitation onset. This issue is further aggravated by the range of conduction velocity (CV) values (3–6 m/s; Andreassen and Arendt-Nielsen, 1987; Gallina et al., 2013; Methenitis et al., 2019) and by the dearth of evidence on the variation in innervation zone location in the most distal muscle region, where excitation is expected to mainly contribute to patellar lateral displacements. More specifically, for a fixed position for bipolar electrodes over VM, for example, differences in the excitation onset between VM and VL would scale with the distance between the bipolar electrodes over VL and its innervation zone location by a 1/CV factor. Thus, the farther the VL pair of electrodes may be positioned with respect to its muscle innervation zone the higher would be the bias introduced by CV on the difference in onset values between these muscles. Previous studies, from a single muscle (biceps brachii), have reported that spatial variation of electrodes placement over the muscle surface may affect the estimate of muscle onset detection up to ~70 ms (Dieterich et al., 2017; Hug et al., 2011). However, to which extent the position of electrodes affects the estimate of differences in excitation onset between VM and VL remains an open issue.

In this study we use an array of surface electrodes to assess the effect of detection site, relative to the muscle innervation zone, on the difference between VM and VL excitation onsets, henceforth termed *VM-VL onset*. With respect to the actual *VM-VL onset*, defined for when action potentials are generated at the neuromuscular junctions, we specifically ask what is the largest bias that the detection site may introduce in the *VM-VL onset* estimation. We further assess whether the skin location often considered for positioning bipolar electrodes on each muscle leads to *VM-VL onset* estimations comparable to the actual *VM-VL onset*. We expect to provide approximate, reference values for systematic errors contaminating *VM-VL onset*

estimations when accounting for the effect of relative differences in electrode position between muscles is not possible.

Methods

Participants

Ten healthy males (mean ± SD: 25.7 ± 3.8 years; 175.4 ± 6.6 cm; 73.2 ± 7.9 kg) volunteered to participate in this study. None of the participants reported muscle-skeletal injuries at the time of experiments, which commenced after the subjects provided their written, inform consent. The present study was in accordance with the latest version of the Declaration of Helsinki and approved by the University Hospital Ethics Committee (HUCFF/UFRJ No. 3.525.289).

Experimental Protocol

The volunteers were positioned on an isokinetic dynamometer (Biodex System 4, Nova York, USA), with the center of the knee coaxially aligned with the dynamometer rotation axis. The hip and the knee joints were respectively flexed at 80º and 90º, as verified with a manual goniometer (0º corresponds to full extension of the knee and hip joints). Then, we applied two maximum voluntary isometric contractions (MVIC) of knee extension, lasting 5 seconds each and with 3 minutes of rest in-between. The highest MVIC torque was considered for the submaximal contractions, wherein subjects were asked to isometrically produce a knee extension torque according to a trapezoidal profile displayed on a screen positioned 1 m in front of them. Specifically, the trapezoidal profile consisted in increasing knee torque from 0 to 40% MVIC in 5 s, holding it at 40% MVIC for 10 s, and then returning to 0% MVIC in 5 s. A

familiarization period was given to all volunteers, ensuring they could successfully follow the trapezoidal torque protocol before starting data acquisition.

Electrode placement and data acquisition

In order to ensure the parallel alignment between muscles fibers and electrodes, EMGs were first detected with a dry array of eight electrodes (1 cm inter-electrode distance; LISiN-Politecnico di Torino, Turin, Italy) and visually inspected. A line connecting the superior border of the patella and the anterior superior iliac spine was drawn on the skin, defining the femoral axis (Figure 1A). After that, one reference line was traced for each muscle with the aid of a manual goniometer, directed at 55º (for VM) and -15º (for VL) angles from the femoral axis at the superior border of the patella. The reference line was then considered for the initial positioning of the dry array, with the first electrode being placed as close as possible to the distal myotendinous junction identified from ultrasound images (10 MHz B mode, 40 mm linear transductor, 70% gain and 7 cm deep; Logiq-e; GE Healthcare, USA). The array orientation was slightly changed until the propagation of action potentials of single motor units could be clearly observed across electrodes (Merletti et al., 2001). Once the skin region leading to the detection of propagating potentials was identified, it was shaved and cleaned with abrasive paste. A linear adhesive array of sixteen silver bar electrodes (1x16, 1 cm inter-electrode distance; Spes Medica, Battipaglia, Italy) was then positioned at this location, with the reference electrode being placed over the patella. This procedure was conducted for both VM and VL, with a schematic representation of the array position over the VM muscle being shown in Figure 1A.

Surface EMGs were acquired in monopolar derivation and amplified by a variable factor ranging, from 2,000 to 10,000 (multi-channel amplifier; 10–900 Hz anti-aliasing filter; CMRR > 100 dB; EMG-USB2, OTBioelettronica, Turin, Italy). EMGs and the torque signal provided

by the dynamometer were digitized synchronously at 2,048 samples/s using a 12-bit A/D converter. After data acquisition, signals were exported and processed off-line in Matlab (The MathWorks, Natick, Massachusetts, USA).

Figure 1

Assessing the VM-VL onset

First, single-differential EMGs were computed from two consecutive monopolar EMGs and bandpass filtered with a fourth-order Butterworth filter (15–350-HZ cut-off). The position of the innervation zones of VM and VL was then defined to be located in correspondence of the electrode providing two adjacent single-differential EMGs with a clear phase inversion of propagating, action potentials (Figure 1B; Rainoldi et al., 2004). Only single-differential EMGs detected between the innervation zone and the distal myotendinous junction were considered for further analysis (Figure 1B). The onset of muscle excitation (first detectable motor unit action potential of the task) was then estimated for each channel (i.e., pair of electrodes) using an automated algorithm (Merlo et al., 2003), separately for VM and VL. Such algorithm uses a continuous wavelet transform to detect muscle excitation intervals (on/off) and has been validated against other approaches, such as envelope-based onset estimations (Merlo et al., 2003). A visual inspection was performed to verify if the algorithm correctly identified the first VM and VL detectable activity and, when needed, the parameters of the algorithm were adjusted to avoid false positive occurrences (Dieterich et al., 2017; Merlo et al., 2003).

After computing the onset of VM and VL excitation for each channel, the onset of excitation at the innervation zone location ($\boldsymbol{t_{IZ}}$; Figure 2A) was estimated with linear regression. First, we

computed the distance (x) between each channel and the innervation zone, based on the fixed, 1 cm inter-electrode distance and on the assumption that the closest channel to the innervation zone was located 0.5 cm away from it (Figure 2A). Then, we computed $\boldsymbol{t_{IZ}}$ as the intercept of the linear regression model relating the onset values to x (cf. equation in Figure 2A). $\boldsymbol{t_{IZ}}$ was expected to provide a coarse estimation of the instant when VM ($\boldsymbol{t_{IZ}^{VM}}$) and VL ($\boldsymbol{t_{IZ}^{VL}}$) were first excited: when action potentials were generated at their neuromuscular junctions. The more distantly EMGs are detected from the innervation zone the greater the overestimation of excitation onset would be for each muscle, with the greatest possible value being provided by the most distant channel from the innervation zone ($\boldsymbol{t_{max}^{VM}}$ and $\boldsymbol{t_{max}^{VL}}$ in Figure 2B). We further computed the onset of muscle excitation considering the location commonly reported for the positioning of a pair of surface electrodes. Reference locations for bipolar recordings was defined as 4 cm superior and 3 cm medial to the superomedial patella border for VM and 10 cm superior and 7 cm lateral to the superior border of the patella for VL (Bennell et al., 2010; Boling et al., 2006; Cowan et al., 2001; Van Tiggelen et al., 2009). Consequently, the excitation onset representative of bipolar recordings from VM ($\boldsymbol{t_{bip}^{VM}}$) and VL ($\boldsymbol{t_{bip}^{VL}}$) were defined by the onset value provided by the channel in the array located most closely to reference locations just mentioned.

From these onset values we computed the difference in onset values between muscles. First, we defined the actual *VM-VL onset* as:

$$t^{VM-VL} = t_{IZ}^{VM} - t_{IZ}^{VL} \quad (1)$$

Then, with respect to this coarse estimation of the actual *VM-VL onset*, we assessed the largest, absolute estimation bias introduced by the detection site as:

$$\Delta_{max}^{VL} = |t_{IZ}^{VM} - t_{max}^{VL} - t^{VM-VL}| \quad (2)$$

$$\Delta_{max}^{VM} = |t_{max}^{VM} - t_{IZ}^{VL} - t^{VM-VL}| \quad (3)$$

where $\boldsymbol{\Delta_{max}^{VL}}$ and $\boldsymbol{\Delta_{max}^{VM}}$ respectively represent the largest errors in *VM-VL onset* estimations attributable to largest possible discrepancies in the paired position of channels with respect to the innervation zone for VL and VM. For bipolar recordings, the absolute estimation bias was computed as:

$$\Delta_{bip} = \left| t_{bip}^{VM} - t_{bip}^{VL} - t^{VM-VL} \right| \quad (4)$$

Finally, to ascertain the validity of our approach for the onset estimation of $\boldsymbol{t_{IZ}}$, we calculated the conduction velocity using two different methods: i) the inverse of the linear regression coefficient (see equation in Figure 2A); ii) the validated, maximum likelihood, multichannel method (Farina et al., 2001; Farina et al., 2004). In the latter case, conduction velocity values were considered only after verifying the peak of the cross-correlation function between EMGs for each muscle and participant was higher than 0.8 (Farina et al., 2004).

Figure 2

Statistics

Parametric analysis was considered for inferential statistics, after ensuring the data normality (Shapiro-Wilk test; $P > 0.26$ for all cases) and homoscedasticity (Bartlett's test; $P > 0.20$ for all cases). To assess the statistical significance of the estimation bias associated with the different detection sites tested, the mean values of $\boldsymbol{\Delta_{max}^{VL}}$, $\boldsymbol{\Delta_{max}^{VM}}$ and $\boldsymbol{\Delta_{bip}}$ were compared with the positive bound of the 95% confidence interval defined by the standard deviation of $\boldsymbol{t^{VM-VL}}$ values. Moreover, the one-way repeated measures ANOVA was applied to assess differences in estimation bias among the different detection sites. The paired samples T-test was used to compare the conduction velocity values provided by the two methods (i.e., the inverse of the

linear regression coefficient and the maximum likelihood multichannel), separately for VM and VL. When a significant main effect was detected, the Tukey's test was used for post-hoc comparisons. All statistical analysis were carried out by Statistica (Version 10, StatSoft Inc., Tulsa, USA) and the level of significance was $P < 0.05$.

Results

Propagation of action potentials in both VM and VL muscles was clearly observed for all participants. Nonetheless, the number of single-differential EMGs providing propagating potentials varied between muscles and participants (from 4 to 6 channels for VM and 5 to 8 for VL). Differences between onsets computed for EMGs detected by consecutive channels in relation to $\boldsymbol{t_{IZ}}$ are illustrated for two participants and for both muscles in Figure 2B. Each of the two participants provided a different number of channels for which onset values could be computed: 4 and 5 channels respectively for the VM and VL muscles for participant A and 6 and 8 channels for VM and VL for participant B. Consequently, the maximal bias in the estimation of excitation onset for both muscles varied between participants (cf. black and grey traces in Figure 2B).

The different combinations of channels considered for estimating *VM-VL onsets* (eq. 2-4) provided significantly greater onset values with respect to the actual *VM-VL onset* (eq. 1; mean ± standard deviation; -127.2 ± 1.88 ms). Specifically, the mean values of $\boldsymbol{\Delta_{bip}}$ (3.4 ± 0.76 ms), $\boldsymbol{\Delta_{max}^{VL}}$ (12.8 ± 1.35 ms) and $\boldsymbol{\Delta_{max}^{VM}}$ (10.1 ± 0.94 ms) exceeded the upper bound of the 95% confidence interval for $\boldsymbol{t^{VM-VL}}$ (1.2 ms; Figure 3A). One-way ANOVA further revealed a significant effect of channels' location on the estimation of *VM-VL onset* ($F = 31.81$; $P < 0.001$). Pairwise analysis indicated significant differences for $\boldsymbol{\Delta_{max}^{VL}}$ and $\boldsymbol{\Delta_{max}^{VM}}$ when compared with

$\boldsymbol{\Delta_{bip}}$ (Tukey's post-hoc test; $P < 0.001$ for both cases), though not between $\boldsymbol{\Delta_{max}^{VL}}$ and $\boldsymbol{\Delta_{max}^{VM}}$ ($P = 0.08$).

Figure 3

Both the linear regression (Figure 2) and the multichannel methods provided equivalent estimates of conduction velocity for VM and VL. Statistical analysis was applied after discarding two subjects for VL and one subject for VM, given the peak of the cross-correlation function between consecutive EMGs was lower than 0.8 (Farina et al., 2004). Group results for conduction velocity values obtained with the remaining data are shown in Figure 3B. Conduction velocity estimates obtained with the linear regression method amounted to 4.40 ± 0.73 m/s for VM and to 4.99 ± 1.17 m/s for VL and were statistically similar to those obtained with the maximum likelihood multichannel method for VM (4.30 ± 0.46 m/s) and VL (4.98 ± 0.87 m/s; paired samples T-test; $P = 0.56$ for VM; $P = 0.96$ for VL).

Discussion

In the present study, we investigated the effect of surface electrodes detection site, in relation to the innervation zone, on the estimate of differences between VM and VL excitation onsets. After defining the actual *VM-VL onset* (eq.1 in Methods) and testing different combinations of EMGs channels for estimating *VM-VL onsets* (eq. 2-4 in Methods), we observed that a maximum bias of 20.48 ms may be introduced in *VM-VL onset* estimations due to the electrodes' detection site (Figure 3A). In addition, our results revealed that a systematic error of ~12 ms (mean value of $\boldsymbol{\Delta_{max}^{VL}}$ and $\boldsymbol{\Delta_{max}^{VM}}$) should be considered in *VM-VL onset* estimations when differences in electrode position relative to innervation zone between muscles are disregarded. Interestingly, such small systematic error found in the present study becomes

remarkably relevant when considering that lower values of difference between VM-VL excitation onsets has been observed as clinically meaningful in investigations regarding the etiology of patellofemoral pain syndrome, the effectiveness of therapeutic interventions or training programs (Bennell et al., 2010; Dieter et al., 2014; Hedayatpour and Falla, 2014; Karst and Williet, 1995; Santos et al., 2008; Van Tiggelen et al., 2009; Voight et al., 1991; Witvrouw et al., 2000). For instance, when investigating whether the eccentric loading would modify the relationship between the excitation onsets of these muscles, differences of ~4 ms between VM relative to VL were observed in healthy subjects at baseline, whereas ~16 ms were identified during post eccentric exercise sessions (Hedayatpour and Falla, 2014). Moreover, significant timing differences of less than 10 ms have been observed when comparing VM-VL excitation onsets of asymptomatic group with patellofemoral pain syndrome patients (Santos et al., 2008; Voight et al., 1991). Therefore, to ascertain whether such minimal differences between VM and VL onsets should be solely attributable to muscle excitation onset or may be biased by methodological aspects, the systematic error introduced by the positioning of surface electrodes in relation to innervation zone between muscles should be scrutinized.

In the present study the actual *VM-VL onset* was calculated as $\boldsymbol{t^{VM-VL}}$ (cf. eq.1 in Methods). It is worth noting, however, that a precisely estimation of the actual *VM-VL onset* would demand the knowledge of the exact site where the actions potentials were generated in the innervation zone at both muscles. Based on the fixed, 1 cm inter-electrode distance, we estimated the excitation onset of VM (t_{IZ}^{VM}) and VL (t_{IZ}^{VL}) assuming that the neuromuscular junction was located 0.5 cm away from the closest channel to the innervation zone (Figure 2A). Considering that the neuromuscular junction could be located within this 0.5 cm range (e.g., at 0.4 cm from the closest channel to the innervation zone), it is possible that we slightly overestimated the excitation onsets of VM and VL, affecting the results of the actual *VM-VL onset* obtained in the

present study. However, the conduction velocities of the action potentials estimated with our method (see equation in Figure 2A) were similar with the values obtained using the validated, maximum likelihood, multichannel method (Farina et al., 2001; Farina et al., 2004; Figure 3B), indicating that our approach for the onset estimation of t_{IZ}^{VM} and t_{IZ}^{VL} was valid. Another point one could raise is that the bias in the estimation of *VM-VL onset* could be predicted as a function of conduction velocity and of where the innervation zone in each muscle is located. However, by doing so, we believe the applied validity of our estimates would be limited, giving we are unaware of how variable the location of innervation zone in each muscle is. Indeed, previous studies assessing the location of the innervation zone did not provide figures for the distribution of innervation zone location in the distal region of the two muscles (Gallina and Vieira; Saitou). Those that did quantify the distribution of innervation zone location, were limited to the muscle central region (REFs) and not to the distal muscle region, where muscle forces are expected to mainly affect lateral displacements of the patella rather than to contribute to knee extension.

Commented [TV1]: Basta que nao seja distal! Verificar e procurar

In order to compare the actual *VM-VL onset* with the common location in the literature for positioning a pair of surface electrodes, we considered two adjacent channels from the linear array of electrodes that provided a bipolar *VM-VL onset* estimation. Although the results from bipolar *VM-VL onset* exceeded the upper bound of the 95% confidence interval of the actual *VM-VL onset* ($\boldsymbol{\Delta_{bip}}$ in Figure 3A), the error obtained from the pair of electrodes was significantly smaller than the other detections sites tested ($\boldsymbol{\Delta_{max}^{VL}}$ and $\boldsymbol{\Delta_{max}^{VM}}$). Specifically, it was observed an absolute error of 3.4 ms, which is subtle when compared with the largest, absolute estimation bias introduced by the detection site. As previously discussed, considering the neuromuscular junction may not be located exactly at 0.5 cm from the closest channel to the innervation zone, the t_{IZ}^{VM} and t_{IZ}^{VL} might be overestimated in this study and, hence, the error between the bipolar *VM-VL onset* and the actual *VM-VL onset* could be even lower. However,

when accounting for the effect of relative differences in electrode position between muscles is not possible, we suggest that a systematic absolute error of ~12 ms should be considered regarding *VM-VL onset* estimations.

Regarding the differences between VM and VL excitation onsets, our results demonstrated that for asymptomatic subjects the VM excitation onset precedes the onset of VL during moderate level of isometric knee extension (40% MVC). These results corroborate previous findings with other tasks (Boling et al., 2006; Hedayatpour and Falla, 2014). Although we may not assert that differences in muscle excitation onset would affect the balance of knee muscle forces (Hug et al., 2015), the prior excitation of VM in relation to VL seems to be an important mechanism to keep the patella integrity (Neptune et al., 2000). This idea is reinforced by evidence showing that in conditions of patellofemoral dysfunction the opposite may occur, i.e., delayed onset of VM in relation to VL (Boling et al., 2006; Van Tiggelen et al., 2009). Specifically, our results revealed a difference value of -127.2 ms between VM and VL excitation onsets, which is not in agreement with the values reported in the literature even considering the systematic error of bipolar estimation calculated here (~3.4 ms; Figure 3A). However, the results from bipolar configuration are considerably variable among studies, with average *VM-VL onset* values ranging from -61.81 ± 68.7 ms to 27.9 ± 32.92 ms (Chester et al., 2008). The interindividual variability of VM and VL neural strategies (Avrillon et al., 2021), the diversity of EMGs processing to define the VM and VL excitation onsets, and the large variety of tasks involved (e.g., stair ascent/descent, patella tendon reflex and knee extension) may explain such marked difference among studies (Chester et al., 2008; Wong, 2009).

Commented [TV2]: Leo, voce sabe o que isso quer dizer? Eu nao. Vc leu esse trabalho? Ou deixa assim ou por favor faça o que o revisor pediu: discuta!

Investigating the methodological bias of surface electrodes placement relative to innervation zone location on the *VM-VL onset* has research, practical implications. First, the bipolar

electrodes placement currently recommended in the literature to assess differences between VM and VL excitation onsets seems to be reasonable. However, when the relative electrodes position with respect to the innervation zone location is unknown, differences between VM and VL onsets up to 12 ms maybe not considered to advance on inferences about the effect of a treatment or training, or differences among distinct populations, for example. Second, distinct experimental setups (e.g., interelectrode distance) may affect the estimate of VM-VL onset. It might occur due to the relationship between interelectrode distance and surface electrodes pick-up volume (termed as surface EMGs sensitivity; Vieira and Botter, 2021). Basically, the pick-up volume represents the muscle excitation area beneath bipolar surface electrodes, and its detection volume increases with the interelectrode distance. In the particular case of large muscle with fibers oriented parallel to the skin (such as VM and VL), detecting EMGs from surface electrodes aligned parallel to its fibers, would result in EMGs collected from the same group of fibers despite variations in the interelectrode distance (Vieira and Botter, 2021). Therefore, increasing the distance between electrodes, for example, it is likely that the onset estimated by bipolar electrodes, regardless of where it is centered along the vastii distal fibers, would provide an onset estimate similar to the actual VM-VL onset. In the present study, however, we estimated VM and VL onset considering 1 cm interelectrode distance due to its common applicability in the literature. Nonetheless, as stated previously, increasing the sensitivity of surface EMGs (i.e., increasing the distance between two electrodes) would be an alternative to minimize the systematic error contaminating VM-VL onset estimation due to surface electrodes positioning. It is important to note also that the present study has some limitations. The reference values of systematic error stated here are valid for EMGs detected from a specific portion of VM and VL muscles (i.e., distal region) and not for the whole muscle. It has been suggested that distinct regions of VM, for instance, can be modulated differently by the nervous systems (Cabral et al., 2018), and present distinct motor unit conduction velocities

(Hedayatpour et al., 2008), which it will likely affect the estimate of VM and VL onsets. In addition, the bias introduced by electrodes positioning with respect to innervation zone on *VM-VL onset* estimation, were calculated for a low/moderate knee extension torque level (40% MVC), which might to be suitable for knee patients population (REF). Therefore, future studies might investigate whether electrodes placement affect the estimate of VM-VL onset during different knee extension torque levels or other tasks (e.g. ballistic contraction) which is more likely to be applicable to other populations, as sports athletes.

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Figure captions

Figure 1:

Linear array of surface electrodes positioning and raw EMGs. A, shows a schematic representation of a linear adhesive array of electrodes placement according to vastus medialis distal fibers orientation. Innervation zone is indicated with dashed lines on the channel 5. B, shows seven monopolar and six single-differential EMGs channels (channel 1 closest to patella). Single motor unit action potential propagation can be visualized in a short epoch of single-differential EMGs (expanded view in grey box). Grey trace indicates the innervation zone location identified where a clear phase inversion of action potential propagation can be visualized along the EMGs channels.

Figure 2:

Four raw single-differential EMGs considered from vastus medialis muscle is represented on the left upper corner (A). Innervation zone is indicated with a distance of 0.5 cm from the closest EMGs channel that detected the initial propagating, action potential (channel 4 in A). On the left bottom corner (A) we demonstrated the linear regression equation used to computed the onset of muscle excitation of each EMGs channels (black dots) according to their distance from innervation zone. Variables of the linear regression model are indicated as *y* (onset of muscle excitation of each EMGs channel), *x* (distance of EMGs channel from innervation zone), *CV* (conduction velocity) and t_{IZ} (onset of excitation at the innervation zone location). In B, we presented a representative result of the onset of muscle excitation computed for each EMGs channel collected from vastus medialis and vastus lateralis with respect to their distance from innervation zone. $\boldsymbol{t_{max}^{VM}}$ and $\boldsymbol{t_{max}^{VL}}$ indicate the greatest possible values of excitation onset bias due to the distance of EMGs channels from innervation zone location of two participants.

Figure 3:

Panel A shows the group results (mean (black circles) and standard deviation (whiskers)) of VM-VL onset absolute bias computed from different detections site (Δ_{bip}, Δ_{max}^{VL} and Δ_{max}^{VM}) in relation to the actual VM-VL onset (t^{VM-VL}). Δ_{bip}, differences of VM-VL onset excitation estimated from a common location for positioning bipolar electrodes over the VM and VL in relation to the actual VM-VL onset. Δ_{max}^{VL} and Δ_{max}^{VM} represent the largest errors in VM-VL onset estimations attributable to largest possible discrepancies in the paired position of channels with respect to the actual VM-VL onset. B, group results (mean (black dots and white squares) and standard deviation (whiskers)) of VM and VL motor unit

action potential conduction velocity estimated from two methods: inverse of the linear regression coefficient and maximum likelihood multichannel. Asterisks denotes statistical significance ($P < 0.05$).