Electromyographic Responses from the Vastus Medialis during Isometric Muscle Actions

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Introduction

The development of the linear array of electrodes has allowed for surface electromyographic (EMG) electrode placement guidelines that consider the location of the innervation zone (IZ: connection between nerve terminals and muscle fibers) and muscle fiber pennation angle. Original guidelines often suggested that electrodes be located over the largest portion of the muscle belly, because this location resulted in the greatest EMG amplitude values [13]. Electrode placements over the muscle belly can, however, result in large variations in EMG signal intensities due to interference from the IZ, which is usually near the muscle belly [1, 24, 29, 30]. Typically, electrode placements over the IZ result in lower EMG amplitude (i.e., root mean square: RMS) and greater EMG mean power frequency (MPF) values than when the electrodes are placed away from the IZ [29]. The lower EMG RMS values recorded over the IZ result from signal cancellation associated with bipolar electrode placements [21, 30]. That is, motor unit action potentials (MUAP) propagate in opposite directions from the IZ and result in MUAPs reaching each electrode simultaneously causing greater signal cancellation [29, 30]. The greater EMG MPF values recorded over the IZ are due to non-propagating MUAPs that occur at the IZ. Recent electrode placement guidelines [14, 24, 29, 30] have generally suggested avoiding the IZ due to the large variations in EMG signal intensities; however, not all guidelines [13, 14, 33] have considered the pennation angle of the muscle fibers. Misalignment of electrodes relative to the muscle fiber pennation angle can lead to over- and under-estimates of the frequency content of the EMG signal [7].

A number of studies have used the vastus medialis (VM) to examine neuromuscular function during isometric muscle actions [3, 5, 9, 10, 22, 28, 29, 31], but little is known about the effects of the IZ and muscle fiber pennation angle on EMG parameter responses during a maximal voluntary isometric contraction (MVIC) or sustained submaximal isometric muscle actions. Beck et al. [3] reported differences in absolute EMG amplitude and MPF values as a result of step incremental muscle actions at 10–90% MVIC recorded over the IZ compared to away from the IZ, but the differ-
ences disappeared when normalized to values at MVIC. Hedayatpour et al. [12] found differences between proximal, middle and distal electrode placements on the VM aligned with the muscle fiber pennation angle for the fatigue-related patterns of responses of EMG amplitude as a result of a sustained isometric muscle action at 80% MVIC. Yeung et al. [32], however, placed bipolar EMG electrodes "...at the most palpable part of the muscle belly of the VM" (p. 381) and reported a 15% decrease in MVIC, but no changes in EMG amplitude or MPF after 30 repeated MVICs. Gallina et al. [9] found that the EMG amplitude and MPF responses from the VM for electrode placements over the IZ and aligned with the muscle fiber pennation angle were less sensitive to variations in knee flexion angle than when the electrodes were placed away from the IZ. Furthermore, Nozic et al. [25] showed that there were differences in the pennation angles of the proximal (12°) vs. distal (52°) portions of the VM [25], and it has been reported that EMG parameters from electrode placements over the proximal portion of the VM were less reliable than those from placements over the distal portion. These studies [3, 9, 12, 25, 32] indicated that electrode placements on the VM can affect reliability and measured EMG parameter responses during MVIC and step incremental muscle actions, as well as the fatigue-related patterns of responses during submaximal sustained isometric muscle actions. Thus, it is important that there are standardized electrode placement guidelines for the VM to reduce the influences of the IZ and muscle fiber pennation angle on the EMG signal, as well as to allow valid comparisons of the results of previous studies. Therefore, the purpose of the present study was to: 1) examine the responses of the EMG time and frequency domain parameters from the VM when electrode placements are located over and away from the IZ during an MVIC and sustained submaximal isometric muscle action of the leg extensors; and 2) propose electrode placement guidelines for the VM that consider the location of the IZ and muscle fiber pennation angle.

Material and Methods

Subjects
9 healthy adults (7 men and 2 women, mean±SD age = 22.6±2.4 years; body mass = 82.2±14.8 kg; height = 181.3±10.5 cm) volunteered to participate in the study. All subjects were free of any knee, hip, or ankle pain. The subjects regularly participated in physical activities such as running, bicycling, and resistance training. The study was approved by the University Institutional Review Board for Human Subjects, and all subjects completed a training. The study was approved by the University Institutional

Orientation session
The orientation session was used to determine the location of the IZ and the pennation angle of the muscle fibers of the VM. The skin was dry shaven and cleaned with a damp cloth prior to applying a probe with 8 silver bar electrodes (5 mm × 1 mm, 10 mm interelectrode distance, Ottino Bioelectronic, Torino, Italy) over the VM on a reference line between the anterior spina iliaca superior and the anterior border of the medial ligament [29]. The length of this reference line was recorded (Table 1). The reference electrodes were placed around the subject's wrist according to the procedures described by the EMG16 User Manual [18]. The subjects were instructed to perform a submaximal isometric contraction of their leg extensors to identify their IZ. The location of the IZ was identified by the EMG channel with the minimal amplitude and phase reversal [23]. The distance of the IZ from the anterior border of the medial ligament was recorded (Table 1). The probe was then moved along the muscle until the IZ was in the center of the electrode array. The pennation angle of the muscle fibers of the VM was determined by rotating the probe around the IZ until the slopes of the 2 lines connecting the EMG waveforms from the channels above and below the IZ had symmetrical propagation of MUAPs [18]. The muscle fibers pennation angle was then recorded for each subject, and an outline of the probe was traced with a non-washable marker before removing the probe so the same electrode position could be obtained in the subsequent session.

Warm-up
All isometric leg extension testing was performed on the dominant leg based on kicking preference (all subjects were right-leg dominant), using a calibrated Cybex II dynamometer at a knee joint angle of 120°. Prior to the isometric testing, each subject performed a warm-up of five 6-s submaximal isometric muscle actions, followed by a 2-min rest period. The subjects were instructed to provide an effort corresponding to approximately 50% of their maximum during each muscle action.

Maximum voluntary isometric contraction
After completing the warm-up, each subject performed 2, 6-s MVIC trials. A 2-min rest was given after each trial. Strong verbal encouragement was provided for all trials. The MVIC was calculated for a 2-s time period corresponding to the middle 33% of each 6-s trial. The highest torque value of the 2 MVIC trials was used to calculate each 50% MVIC for the subsequent sustained isometric task.

Sustained isometric task
Each subject was instructed to perform a sustained isometric muscle action to exhaustion at 50% MVIC. Exhaustion was operationally defined as the subject being unable to maintain a torque value within ±5% of their 50% MVIC. The subjects tracked their torque production on a computer monitor placed in front

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gender</th>
<th>Reference Line (cm)</th>
<th>SENIAM Line (cm)</th>
<th>Actual IZ (cm)</th>
<th>Pennation Angle (degrees)</th>
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<td></td>
<td>3.14</td>
<td>0.62</td>
<td>1.86</td>
<td>5.97</td>
</tr>
</tbody>
</table>

Table 1 The location of the innervation zone and muscle fiber pennation angle identified by a linear array. The SENIAM [12] recommendation suggests placing the electrodes at 20% the distance between the anterior spina iliaca superior and the anterior border of the medial ligament. The SENIAM and Actual IZ were measured from the anterior border of the medial ligament on the reference line.
of them that displayed the real-time, digitalized torque signal overlaid onto a programmed template identifying their target torque value. The isometric template and real-time torque signal overlay were programmed using LabVIEW (LabVIEW version 7.1 National Instruments, Austin, TX).

Electromyographic measurements and signal processing
Surface EMG signals were recorded from the dominant VM muscle with an 8-channel linear electrode array and EMG 16 data acquisition system (EMG 16, LISIN-Prima Biomedical & Sport, Treviso, Italy). The skin over the VM was carefully abraded and cleaned with rubbing alcohol prior to securing the 8-channel linear electrode array to the VM using a double-sided adhesive strip. The adhesive strip had small holes that were cut for each silver bar electrode, and each hole was filled with 30 µl of conductive gel with a gel dispenser (AG22331, Eppendorf, Hamburg, Germany). The raw EMG signals from each electrode of the probe were recorded in a monopolar signal acquisition mode (gain × 500) and analog filtered (fourth-order Bessel, bandwidth = 10–500 Hz) with the surface EMG 16 data acquisition system. Electrode 1 corresponded to the most proximal and electrode 8 to the most distal electrode on the linear array. The monopolar EMG signals were converted to a digital form with a 12-bit analog-to-digital converter at a sampling frequency of 2048 Hz and stored on a personal computer for subsequent analyses.

7 bipolar electrode configurations (10 mm interelectrode distance) were obtained by differentiating the digitized monopolar signals forming (Fig. 1): channel 1 from electrodes 1 and 2 (30 mm proximal to the IZ), channel 2 from electrodes 2 and 3 (20 mm proximal to the IZ), channel 3 from electrodes 3 and 4 (10 mm proximal to the IZ), channel 4 from electrodes 4 and 5 (over the IZ), channel 5 from electrodes 5 and 6 (10 mm distal to the IZ), channel 6 from electrodes 6 and 7 (20 mm distal to the IZ), and channel 7 from electrodes 7 and 8 (30 mm distal to the IZ). The EMG signals were zero-meaned, and bandpass filtered (fourth-order Butterworth) at 10–500 Hz. The EMG RMS and MPF of the middle 33% (2-s epochs) of the MVIC were calculated using 0.5-s epochs for each channel. The EMG RMS and MPF values for the sustained isometric muscle action were normalized to the MVIC at each electrode location. Linear regression analyses were then used to determine the EMG RMS and MPF vs. normalized time to exhaustion relationships during the sustained isometric muscle action for all 7 channels for each subject and the composite of all subjects were compared statistically using the F-test procedures of Pedhaezur [26]. An alpha of p ≤ 0.05 was considered statistically significant for all comparisons.

Statistical analysis
Separate one-way (1 × 7) repeated-measures ANOVAs were performed on the absolute EMG RMS and MPF from the MVIC to determine if there were mean differences among the channels. Significant main effects for time were followed by Tukey LSD test on the simple main comparisons. The EMG RMS and MPF from the sustained isometric muscle action were normalized to the MVIC at each electrode location. Linear regression analyses were then used to determine the EMG RMS and MPF vs. normalized time to exhaustion relationships during the sustained isometric muscle action for each channel. The slope coefficients for the EMG RMS and MPF vs. normalized time to exhaustion relationships during the sustained isometric muscle action for all 7 channels for each subject and the composite of all subjects were compared statistically using the F-test procedures of Pedhaezur [26]. An alpha of p ≤ 0.05 was considered statistically significant for all comparisons.

Results
Maximal voluntary isometric contraction electromyographic measurements
Table 2 shows the absolute EMG RMS and MPF values from the 7 channels during the MVIC muscle actions. For EMG RMS, there were significant differences among channels for 15 of the 21 comparisons (Table 2). For EMG MPF, there were significant differences among channels for 12 of the 21 comparisons (Table 2).

![Fig. 1 Linear array placement depicting the distance and electrodes that create channels 1 through 7.](image)
Electromyographic root mean square and mean power frequency during maximal voluntary isometric contraction

Electrode placement recommendations for the VM suggest that bipolar arrangements should be located distal to the IZ to avoid confounding influences that may affect absolute and normalized EMG RMS and MPF values [13, 14, 29, 30]. Electrode placements located over the IZ typically result in lower EMG RMS and higher EMG MPF values compared to those away from the IZ [29, 30]. The lower EMG RMS values are a result of MUAPs that travel in opposite directions from the IZ, simultaneously reach each of the bipolar electrodes, and are cancelled by differential amplification [2]. The greater EMG MPF values over the IZ, compared to away from the IZ, are the result of non-propagating MUAPs that are only detectable over the IZ [2]. Rainoldi et al. [29] showed that for the VM, a change in electrode placement of 10 mm proximal or distal to the IZ can result in EMG amplitude changes in excess of 200%. In addition, Beck et al. [3] showed a 200% decrease in EMG amplitude and 20% increase in EMG MPF when recorded over the IZ compared to 30 mm distal to the IZ of the VM. The current findings indicated that during MVIC muscle actions, electrode placements over the IZ resulted in up to 58% lower EMG RMS and 37% greater EMG MPF values than those recorded proximal or distal to the IZ. Specifically, there were differences in the EMG RMS and MPF values for channel 4 (over the IZ) vs. channels 1, 2, 3, 6, and 7 (Table 2). Furthermore, there were differences among electrode placements proximal to the IZ (channels 1, 2, and 3), as well as those distal to the IZ (channels 5, 6, and 7). Thus, the present findings indicated that the IZ should be avoided during MVIC measurements due to large variations in EMG RMS and MPF values compared to electrode placements away from the IZ (Table 2).

The findings of the present study, in conjunction with previous studies [9, 13, 14, 29, 30], suggested that electrode placement recommendations should be developed on a muscle by muscle basis, because different muscles are characterized by a variety of factors that can affect the validity and reliability of EMG parameters measured during an MVIC including the [1, 9, 16, 20]: 1) number of IZs; 2) location of the IZ; 3) amount of movement or shift of a muscle relative to the skin surface during a muscle action; 4) amount of crosstalk from nearby muscles; 5) location of electrode placements relative to the tendinous region; and 6) muscle fiber pennation angle. The VM has only one IZ that is located at approximately the midpoint of the muscle where it begins to taper proximally, near the location where the sartorius muscle overlaps the proximal portion of the VM [19]. The proximal tapering of the VM, location of the IZ, and overlapping of the sartorius muscle result in a larger EMG recording area at the distal end of the VM than at the proximal end. The larger recording area makes distal electrode placements less likely to be over a tendinous region and affected by crosstalk from the surrounding

Fig. 2 The positive relationship between electromyographic root mean square (EMG RMS) from the vastus medialis and percent time to exhaustion during the sustained isometric muscle action at 50% MVIC. There were no significant differences (p>0.05) in slope coefficients between any of the 7 channels.

Fig. 3 The negative relationship between electromyographic mean power frequency (EMG MPF) from the vastus medialis and percent time to exhaustion during the sustained isometric muscle action at 50% MVIC. There were no significant differences (p>0.05) in slope coefficients between any of the 7 channels.

Location of the innervation zone and pennation angle for the vastus medialis

The reference line between the anterior border of the medial ligament and the anterior spina iliaca superior was 50.89 ± 3.14 cm. The IZ was 11.56 ± 1.86 cm proximal to the anterior border of the medial ligament (Table 1). The mean muscle fiber pennation angle was 52.78±5.97° (Table 1).

Discussion

Electromyographic root mean square and mean power frequency during maximal voluntary isometric contraction

There were significant negative relationships for normalized EMG RMS vs. normalized time to exhaustion for the composite of all subjects for all channels (Fig. 2). Furthermore, there were no significant differences among slope coefficients for any of the channels (Fig. 2).

There were significant negative relationships for normalized EMG MPF vs. normalized time to exhaustion for the composite of all subjects for all channels (Fig. 3). Furthermore, there were no significant differences among slope coefficients for any of the channels (Fig. 3).
muscles, including the sartorius. In addition, it has been suggested [9, 20, 30] that the VM shifts proximally during a muscle action which can cause proximal electrode placements to move over the IZ and result in lower EMG RMS and greater EMG MPF values than electrode placements distal to the IZ [9, 20, 30]. Gal- lina et al. [9] reported that when performing an MVIC, a small proximal shift in the IZ of the VM resulted in 50–75 % lower EMG RMS and 25 % greater EMG MPF when electrode placements were located 10 mm proximal to the IZ compared to those 10 mm distal to the IZ. It has also been suggested that the muscle fibers of the VM have different pennation angles distal and proximal to the IZ, with the distal portion being angled at approximately 50 ° and the proximal at approximately 35–40 ° [9, 16, 20, 24, 29, 31]. In the present study, the average pennation angle for the VM was 53 ° with a range of 43–62 °, which was in agreement with previous studies that used MRI, dissection, muscle mapping and linear array to measure the VM pennation angle [1, 15, 27, 29]. It is thus recommended that when recording EMG parameters from the VM during an MVIC without a linear array, electrode placements should be located between 10 and 30 mm distal to the midpoint of the muscle to avoid the IZ, and oriented at 53 ° to approximate the pennation angle of the muscle fibers.

Electromyographic root mean square and mean power frequency patterns of responses during sustained submaximal isometric muscle actions

Fatiguing submaximal isometric muscle actions are typically characterized by increases in EMG RMS and decreases in EMG MPF [23]. These fatigue-related neuromuscular responses have been attributed to decreases in muscle pH caused by the buildup of metabolic byproducts (hydrogen ions, inorganic phosphate, potassium, and ammonia), which alter muscle contractility resulting in greater muscle activation to maintain a constant force or torque [4, 17]. In addition to a decrease in muscle pH [4], fatigue-induced decreases in EMG MPF have also been associated with increases in extracellular potassium, which results in a progressive loss of membrane excitability [8]. In the present study, increases in EMG RMS and decreases in EMG MPF occurred for all electrode placements (channels 1, 2, 3, 4, 5, 6, and 7) and there were no differences among the slope coefficients for the normalized EMG RMS and EMG MPF vs. time relationships for the composite of all subjects (Fig. 2,3). These findings were in agreement with those of Silva et al. [31], who reported similar EMG RMS and EMG MPF vs. time relationships during submaximal isometric muscle actions at 50 % MVIC of the leg extensors for the composite of all subjects. Thus in the present study, increases in EMG RMS and decreases in EMG MPF from the VM were 53 ° with a range of 43–62 °, which was in agreement with those of Silva et al. [31], who reported similar MPF vs. time relationships during submaximal isometric muscle actions for the composite of all subjects. Nevertheless, there were no differences among the slope coefficients for the normalized EMG RMS and EMG MPF vs. time relationships, for all electrode placements (channels 1, 2, 3, 4, 5, 6, and 7) and for the composite of all subjects.

Summary

Based on the findings of the present study, it is recommended that during an MVIC muscle action, bipolar electrode placements should be located 10–30 mm distal to the midpoint of the VM to avoid the IZ and oriented at 53 ° to approximate the muscle fiber pennation angle. Although the EMG RMS and EMG MPF vs. time relationships were not affected by electrode placement, this placement is also recommended during sustained submaximal muscle actions so that MVICs can be measured to normalize the data. The current findings indicated that single EMG RMS and EMG MPF measurements from MVICs were affected by electrode placements relative to the IZ, while the EMG RMS and EMG MPF vs. time relationships during the sustained isometric muscle action were not.

Conflict of interest: The authors have conflict of interest to declare.

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