Physiological Measurement



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# The effect of inter-electrode distance on radial muscle displacement and contraction time of the biceps femoris, gastrocnemius medialis and biceps brachii, using Tensiomyography in healthy participants.

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**Keywords:** Tensiomyography, inter-electrode distance, muscle contraction.

**Abstract**

Context: The systematic effect of inter-electrode distance on electrically elicited radial muscle displacement (Dm) and contraction time ($T_c$) of the biceps femoris, gastrocnemius medialis and biceps brachii using Tensiomyography (TMG) is currently unavailable. Aim: To investigate the effects of inter-electrode distance (4cm, 5cm, 6cm and 7cm) on Dm and $T_c$ of the biceps femoris, gastrocnemius medialis and biceps brachii, when the current amplitude is standardised. Design: A within subject, repeated measures cross-over study. Participants: 24 participants. Results: Biceps femoris and gastrocnemius medialis Dm increased with increased inter-electrode distance (biceps femoris: $p$=0.015; gastrocnemius medialis: $p$=0.000), yet $T_c$ were not affected ($p$>0.05). Biceps brachii Dm was not affected by inter-electrode distance ($p$>0.05), yet $T_c$ became shorter with increased inter-electrode distance ($p$=0.032). Conclusion: Inter-electrode distance effects Dm but not $T_c$ in two pennate muscles (biceps femoris and gastrocnemius medialis), and $T_c$ but not Dm in one parallel muscle (biceps brachii). Optimal muscle specific inter-electrode distances were judged based on Dm measurements within the limits of this study. The following optimal inter-electrode distances are suggested; biceps femoris=6cm, gastrocnemius medialis=7cm and biceps brachii=4cm. Our findings emphasise the importance of accurate implementation and reporting of inter-electrode distance, for the reproducibility and comparability of studies using TMG.

## 1.1. Introduction

Tensiomyography (TMG) is a non-invasive technique used to quantify radial muscle belly displacement (Dm) following single twitch electrical stimuli. This technique has most commonly been used in football as an injury prevention tool since it can detect muscle stiffness, muscle damage, fatigue and bilateral muscle asymmetries [for recent review, see Macgregor et al. (2018)] (Tous-Fajardo et al., 2010, Pišot et al., 2008, Garcia-manso et al., 2011, Macgregor et al., 2016). Recently, TMG has been used in experimental studies to assess the effect of therapeutic interventions (de Hoyo et al., 2013, Pregelj and Šimunič, 2019, Zubac et al., 2019). In addition, future applications for screening, diagnosis, and monitoring post-surgical treatment response in sport have been proposed (Martin-Rodriguez et al., 2017a). Despite widespread use, as well as promising future applications, a lack of attention has been given to establishing a standardised TMG measurement protocol to increase reliability and improve measurement error (Martin-Rodriguez et al., 2017b). Importantly, there is currently no consensus regarding the optimal inter-electrode distance to use when conducting TMG measurements.

A high precision (1μm) spring-loaded sensor is used to measure Dm (mm). The sensor is positioned perpendicular to the muscle belly and compressed into the skin surface to create pre-tension for measurement sensitivity (Križaj et al., 2008). Muscle twitch is induced through a single 1ms wide, square electrical stimulus. The electrical stimulus is delivered via two surface electrodes positioned directly above the muscle belly, proximal and distal to the sensor tip. The TMG patent states that operators should use an inter-electrode distance in the range of 2cm (minimum) to 5cm (maximum) (Valenčič, 2002). Unsurprisingly, assessment of primary research studies using TMG reveals marked inter-electrode distance variability (range: from 2cm to 10cm) (Rey et al., 2012, Rodríguez-Matoso et al., 2010, Loturco et al., 2015, Dahmane et al., 2001). Further, many studies report approximate inter-electrode distances or omit the report of this information (Kersevan et al., 2002, Pišot et al., 2008, Rodríguez Ruiz et al., 2012, Ditroilo et al., 2013).

In response to each electrical stimuli, a Dm (mm) - time (ms) curve is recorded and from this data contraction time ($T_c$), delay time ($T_d$), sustain time ($T_s$) and relaxation time ($T_r$) are calculated by the TMG-S1 software (Valenčič and Knez, 1997). Possibly due to the reported reproducibility, the most commonly assessed measurements are Dm and $T_c$ (calculated as the time taken on the ascending curve between 10% and 90% of Dm, Figure 1). Martin-Rodriguez et al. (2017b) found that the relative reliability scores of Dm and $T_c$, evaluated in seven muscles reported an excellent score of ICC for Dm (0.91) and good score of ICC for $T_c$ (0.70). Further, Dm and $T_c$ demonstrated good to excellent agreement between-week (ICC; Dm=0.62, $T_c$=0.86) and between-day (ICC≥0.98) (Ditroilo et al., 2013, Šimunič, 2012).

Notwithstanding the relevance of TMG as a valid measurement technique, deliberately altering the inter-electrode distance significantly impacts the measurement of Dm, likely owing to a change in the recruitment pattern (Macgregor et al., 2018). Tous-Fajardo et al. (2010) found that increased inter-electrode distance from 3cm to 5cm increased Dm but did not affect $T_c$ of the vastus lateralis. Similarly, Wilson et al. (2018) found that increased inter-electrode distance from 5cm to 7cm increased Dm but did not affect $T_c$ of the rectus femoris. Authors also demonstrated that Dm did not increase further when inter-electrode distance was increased from 7cm to 11cm. Findings were attributed to an increase in the number of activated motor units with increased inter-electrode distance (Knaflitz, 1990, García-García et al., 2015, Sale, 1987, Vieira et al., 2016). Notably, Tous-Fajardo et al. (2010) titrated the stimulus amplitude until maximal Dm was reached, whereas Wilson et al. (2018) standardised the stimulus amplitude. When the stimulus amplitude is fixed, increased inter-electrode distance will eventually result in a decreased in muscle response because the current density within the underneath tissue decreases (i.e. becomes less focal) (Doheny et al., 2010). Recent reviews correctly refute the use of standardised stimulus amplitudes on the basis that the amplitude required to elicit maximal muscle response is not equal among all muscles or individuals (Martin-Rodriguez et al., 2017b, Macgregor et al., 2018). However, the current amplitude required to elicit a true maximum Dm is increased when inter-electrode distance exceeds the optimal value (i.e. the current amplitude-Dm curve shifts rightward). For example, Vieira et al. (2016) demonstrated that the current amplitude required to elicit maximal torque for short inter-electrode distances were smaller than that observed for longer distances. Therefore, before the titration approach can be used effectively, the optimal inter-electrode distance must be identified.

The optimal inter-electrode distance is defined in this study as the shortest electrode distance which elicits the largest Dm, when the current amplitude is standardised to elicit a submaximal muscle contraction. Non-optimal inter-electrode distances using TMG lead to methodological challenges. If the distance is too large, investigators risk reaching the maximum current amplitude available on the TMG stimulator (100mA) before maximum Dm is elicited. Furthermore, higher stimulating amplitudes increase the risk of co-activating deeper or neighbouring muscles (Macgregor et al., 2018). In this case, increases in maximum Dm can be misconstrued as an increase in the response of an individual muscle. To fall short of the optimal distance would result in an insufficient number of motor points activated within the electrical current pathway and thus a true maximum Dm would not be elicited.

The most frequently assessed muscles in TMG literature include the biceps femoris, gastrocnemius and the biceps brachii (Garcia-manso et al., 2011, Rey et al., 2012, Ditroilo et al., 2011, Gasparini et al., 2012, Macgregor et al., 2016, Ditroilo et al., 2013, García-Manso et al., 2012, Hunter et al., 2012, Križaj et al., 2008). To our knowledge, documentation on the systematic effect of inter-electrode distance on electrically elicited Dm and $T_c$ of the biceps femoris, gastrocnemius medialis and biceps brachii is currently unavailable. Since wide ranges of inter-electrode distance have been used in previous TMG studies, it is indispensable to identify the effects of inter-electrode distance on the most commonly assessed TMG measurements (Dm and $T_c$). It is likely that Dm data will inform the development of muscle specific optimal inter-electrode distances for TMG electrical stimulation protocols. Therefore, the aim of this study was to investigate the effects of inter-electrode distance (4cm, 5cm, 6cm and 7cm) on Dm and $T_c$ of the biceps femoris, gastrocnemius medialis and biceps brachii, when the current amplitude is standardised.

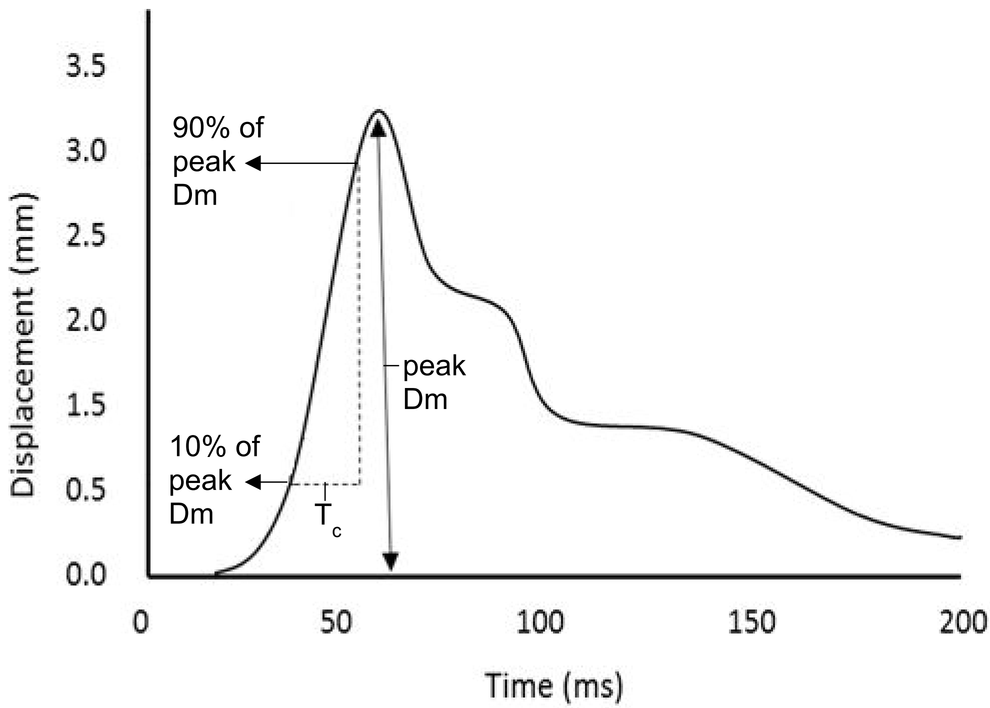



Figure 1. Typical displacement (Dm)-time curve.
Contraction time ($T_c$) is calculated as the time (ms) between 10% and 90% of peak Dm.

**2. Methods**

Experiments were conducted in accordance with the Declaration of Helsinki, within the Pain and Rehabilitation laboratory of Leeds Beckett University. This study was approved by the School of Clinical and Applied Sciences, Research Ethics Committee of Leeds Beckett University. This was a collaborative investigation conducted by two principal investigators whom collected data equally (HVW: n=12, AJ: n=12). Both investigators had received extensive training, were competent and experienced using TMG (Wilson et al., 2018, Jones et al., 2017).

### 2.1. Study design

A within subject, repeated measures study design was used whereby the effects of four independent variables (inter-electrode distance: 4cm, 5cm, 6cm and 7cm) were measured upon two dependent variables (Dm and $T_c$). This was assessed within three superficial muscles common within TMG literature (biceps femoris, gastrocnemius medialis and biceps brachii). Each participant was subject to measurements of all specified conditions (inter-electrode distance) and muscles. Participants attended the laboratory on one occasion which lasted no longer than 2-hours.

### 2.2. Participant recruitment and screening

A convenience sample of 24 unpaid healthy adult volunteers (mean±SD, age: 27.29±5.48yrs, height: 173.12±7.56cm, weight: 76.29±12.88kg, male: 16) aged between 18 and 40 years participated in our study. Participants were recruited via e-mail from a repository of participants whom had previously taken part in experiments within our laboratory, as well as via word of mouth in student lectures and staff meetings at Leeds Beckett University.

Volunteers expressing interest were provided with an information pack and given 48 hours to consider participation. Volunteers were requested not to take part in the study if they did not consider themselves healthy, took medication, had allergies to adhesive glue or had a dermatological condition(s). Volunteers who had a cardiac pacemaker or were pregnant were also requested not to participate. There was no restriction on gender, ethnicity, height or weight. Eligible volunteers were invited to attend the laboratory where they were formally screened and enrolled onto the study. Volunteers were instructed to maintain their normal diet, but to refrain from consuming caffeine for 12 hours prior to attending the study visit. Volunteers also agreed that they would refrain from exercise 72 hours prior to attending the study visit, which may result in delayed onset muscle soreness.

### 2.3. Experimental procedure

Volunteers were verbally briefed about the study and the principal investigator (HVW or AJ) screened volunteers against eligibility criteria. Eligible participants provided written consent and were reminded of their right to; request a copy of any document pertaining to them; request that a document pertaining to them was destroyed; and withdraw from the study without reason at any time. A unique identification number was used to anonymise study data.

Prior to performing measurements, TMG sensor and electrode placements were marked on the skin with a dermatological pen. Since there is no consensus regarding a standardised procedure for locating TMG sensor placement, our laboratory previously developed a standardised method (Jones et al., 2017, Wilson et al., 2018). According to this method, the sensor was located at the intersect of the transversal line denoting midway between the muscle origin (biceps femoris, ischial tuberosity; biceps brachii, scapula coracoid process) and insertion (biceps femoris, lateral femoral condyle; biceps brachii, bicep bacchii tendon estimated 10cm distal from the elbow crease); and the vertical line denoting midway between medial and lateral manually palpated muscle boarders. For the gastrocnemius medialis the sensor was located at the intersect of the transversal line denoting the widest region of muscle girth (to the naked eye) and vertical line running distal from the medial popliteal crease. Subsequently, electrode placements were marked on the skin at 2cm, 2.5cm, 3cm, and 3.5cm proximal and distal to the point of sensor placement. These distances would later achieve total inter-electrode distances of 4cm, 5cm, 6cm and 7cm, from the electrode leading edges.

To conduct familiarisation measurements, participants were positioned prone with a semi-circle bolster under the ankles and arms relaxed to the side (Figure 2). Four familiarisation TMG measures were taken from the participants non-dominant biceps femoris to avoid interference with experimental data. Familiarisation measures would not be analysed; therefore, sensor and electrodes were positioned by eye. Familiarisation measures commenced from 20mA, followed by 30mA, 40mA and terminated 50mA. Current amplitude was standardised at 50mA throughout the experiment to elicit a submaximal twitch contraction, based upon a previous report that peak twitch contractions occur at stimulation amplitudes between 60 and 100mA (Macgregor et al., 2018). Participants were made aware of this information. A 2-minute wash out period was allowed prior to commencing experimental measures taken from the dominant side of the body. This was determined by the corresponding side of the body to the preferential leg used to kick a ball.

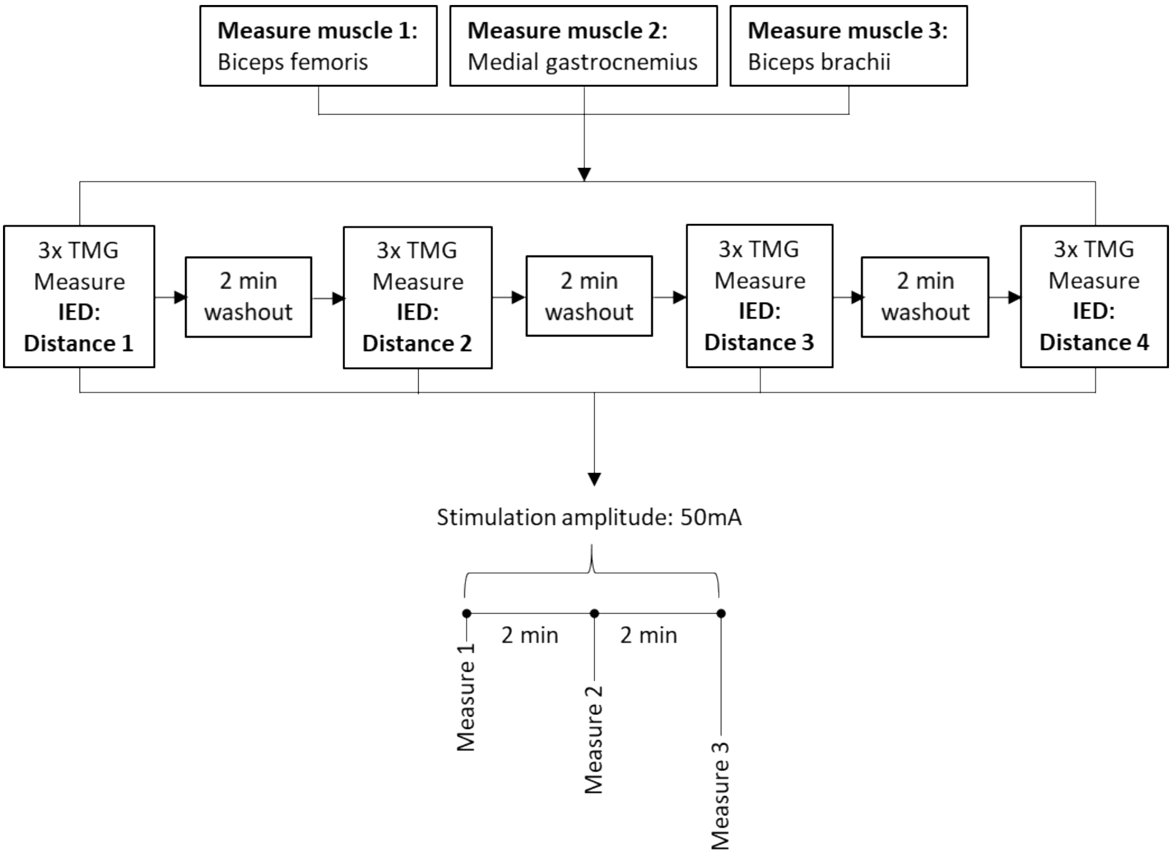
Following familiarisation, measurements were taken in sequential order of; biceps femoris, gastrocnemius medialis and finally biceps brachii. During biceps femoris and gastrocnemius medialis data collection participants were positioned prone with a semi-circle bolster under the ankles and arms relaxed to the side. However, for biceps brachii data collection participants were seated with the elbow flexed at 90° using an adjustable armrest, palm supinated with an adjustable strap across the shoulders. It was not necessary to randomise muscle testing order since muscles were not directly compared. However, it was necessary to randomise the measurement order of inter-electrode distances (4cm, 5cm, 6cm and 7cm) to limit the potential for effects of progressive error. Randomisation was achieved by initially identifying all inter-electrode distance order possibilities. The assessment of 4 inter-electrode distances generated 24 possible sequence orders, which were noted on paper tickets. Each participant was allocated to one of the possible sequence orders. Allocation concealment was achieved by enclosing each ticket within individual opaque envelopes, sealed and placed inside a second envelope. Participants were required to select one envelope which was not returned to the selection pile. Participants were not blinded to their data collection order as they could visualise the experimental process.

Two square self-adhesive electrodes (5x5cm, Med-Fit, Stockport, UK) were positioned at the first inter-electrode distance. The spring-loaded sensor was positioned in a perpendicular orientation, with the sensor tip above the skin marker denoting the muscle belly (described earlier) and compressed into the skin by ~50% of the total sensor length (judged by eye).

Figure 2. Tensiomyography set-up for measurement of the assessed muscles. A: participant position for assessment of biceps femoris and gastrocnemius medialis, B: participant position for assessment of biceps brachii, C: electrode and sensor position for all muscle assessments.


Three consecutive 1ms wide square monophasic electrical pulse stimulations were delivered to the muscle at each inter-electrode distance. Stimulus amplitude was standardised at 50mA to isolate the effects of inter-electrode distance on Dm and $T_c$. In addition, a 2-minutes inter-stimulus interval was given between measurements to limit the development of electrically evoked muscle fatigue or post activation potentiation. Although 10-seconds is popular practice in TMG literature (Rey et al., 2012, Križaj et al., 2008, Ditroilo et al., 2011, Tous-Fajardo et al., 2010), our laboratory recently demonstrated that 10s had a statistically significant effect on Dm across 10 consecutive twitch contractions (Wilson et al., 2018). Electrodes remained in situ between consecutive measures of the same inter-electrode distance to limit data variability. If necessary, the sensor was retracted back to 50% of its total length to ensure a consistent initial pressure (0.015 N/mm$^2$) according to the TMG protocol reported in previous literature (Murray et al., 2016, Macgregor et al., 2016). Figure 3 shows how this experiment was performed.

Figure 3. Experimental timeline; the effect of changing inter-electrode distance on Dm and $T_c$. Abbreviation: IED, inter-electrode distance.



### 2.4. Data analysis

Participant characteristics are presented descriptively and reported as mean (M) ± standard deviation (SD). Data was assessed for normality using a Shapiro-Wilk test. To assess the effect of inter-electrode distance (4cm, 5cm, 6cm and 7cm) on Dm and $T_c$, a within-subject simple repeated measures analysis of variance (ANOVA) was performed separately for each outcome measure (Dm and $T_c$) and each muscle (biceps femoris, gastrocnemius medialis and biceps brachii). Where significant differences were found, a pairwise comparison with Bonferroni correction for multiple comparisons was used to identify where differences occurred. Results were interpreted according to the level of statistical significance $p \leq 0.05$ and effects size reported as partial eta squared ($\eta_p^2$). Analyses were conducted with SPSS version 23.0.

Participant 8 was omitted from biceps brachii data analysis using a 7cm inter-electrode distance due to missing data. Therefore, biceps brachii data analysis using a 7cm inter-electrode distance included 23 participants (mean±SD, age: 27.29±5.48 yrs, height: 173.12±7.56cm, weight: 76.29±12.88kg, male: 16).

## 3. Results

Biceps femoris and gastrocnemius medialis Dm increased with increased inter-electrode distance ($p<0.05$) (Figures 4 and 5, Table 1). Differences between comparisons existed when inter-electrode distance was increased from 4cm to 6cm (biceps femoris, $p=0.004$; gastrocnemius medialis, $p=0.002$), and from 4cm and 5cm to 7cm in the gastrocnemius medialis ($p=0.001$, $p=0.001$ respectively). Increasing inter-electrode distance did not influence $T_c$ of either the biceps femoris and gastrocnemius medialis ($p>0.05$).

Inversely, increasing inter-electrode distance did not influence biceps brachii Dm ($p>0.05$) but $T_c$ became shorter as inter-electrode distance was increased ($p<0.05$). However, significant differences did not exist between pairwise comparisons using the Bonferroni correction ($p>0.05$) (Figure 6, Table 1).

**Table 1**. The effect of inter-electrode distance on measured variables to characterise muscle response to electrical stimulation of the biceps femoris, gastrocnemius medialis and biceps brachii.

| IED | 4cm | 5cm | 6cm | 7cm | $p$ | F | df | $\eta_p^2$ |
|---|---|---|---|---|---|---|---|---|
| Biceps Femoris | | | | | | | | |
| Dm (mm) [a] | 3.0 ± 2.4 | 3.3 ± 2.7 | 3.5 ± 2.7 | 3.6 ± 2.9 | 0.015 | 5.365 | 1.518 | 0.189 |
| 95% CI | 2.0 - 4.0 | 2.2 - 4.5 | 2.4 - 4.7 | 2.3 - 4.8 | | | | |
| $T_c$ (ms) | 28.9 ± 12.4 | 29.4 ± 14.8 | 30.1 ± 14.5 | 29.0 ± 14.2 | 0.620 | 0.595 | 3 | 0.025 |
| 95% CI | 23.6 - 34.1 | 23.2 - 35.7 | 24.0 - 36.3 | 23.1 - 35.0 | | | | |
| Gastrocnemius Medialis | | | | | | | | |
| Dm (mm) [a, b, c] | 2.5 ± 1.0 | 2.6 ± 1.0 | 2.8 ± 1.0 | 2.9 ± 0.9 | 0.000 | 12.365 | 3 | 0.350 |
| 95% CI | 2.1 - 2.9 | 2.2 - 3.0 | 2.3 - 3.3 | 2.5 - 3.3 | | | | |
| $T_c$ (ms) | 22.1 ± 2.9 | 22.1 ± 2.5 | 22.4 ± 2.2 | 22.6 ± 2.1 | 0.444 | 0.775 | 1.637 | 0.033 |
| 95% CI | 20.8 - 23.3 | 21.0 - 23.1 | 21.5 - 23.4 | 21.7 - 23.5 | | | | |
| Biceps Brachii | | | | | | | | |
| Dm (mm) | 12.9 ± 3.4 | 12.7 ± 2.9 | 12.9 ± 2.8 | 13.2 ± 3.8 | 0.653 | 0.545 | 3 | 0.024 |
| 95% CI | 11.5 - 14.4 | 11.4 - 13.9 | 11.8 - 14.2 | 11.5 - 14.8 | | | | |
| $T_c$ (ms) [d] | 29.4 ± 4.9 | 28.2 ± 4.6 | 28.6 ± 4.8 | 27.6 ± 4.2 | 0.032 | 3.540 | 2.259 | 0.139 |
| 95% CI | 27.3 - 31.5 | 26.2 - 30.1 | 26.5 - 30.7 | 25.8 - 29.4 | | | | |

Values are displayed as mean (± SD) and 95% CI. p = significance value for one-way ANOVA.

Post hoc Bonferroni analysis, $p \leq 0.05$: [a]= differences between 4cm and 6cm; [b]= differences between 4cm and 7cm; [c]= differences between 5cm and 7cm; [d]= significant main effect yet pairwise comparisons showed no significant difference between comparisons.

* Biceps femoris, n=24; gastrocnemius medialis, n=24; biceps brachii, n=23.

Abbreviations: IED, inter-electrode distance; df, degrees of freedom; F, result of the ANOVA test; $p$, significance level; $\eta_p^2$, partial eta squared; Dm, displacement; $T_c$, contraction time; mm, millimetre; ms, millisecond; CI, confidence interval.

Figure 4. Biceps femoris: mean, standard deviation and individual Dm data measured using Tensiomyography at 4 inter-electrode distances. * Significant difference between groups of mean data. (n=24).

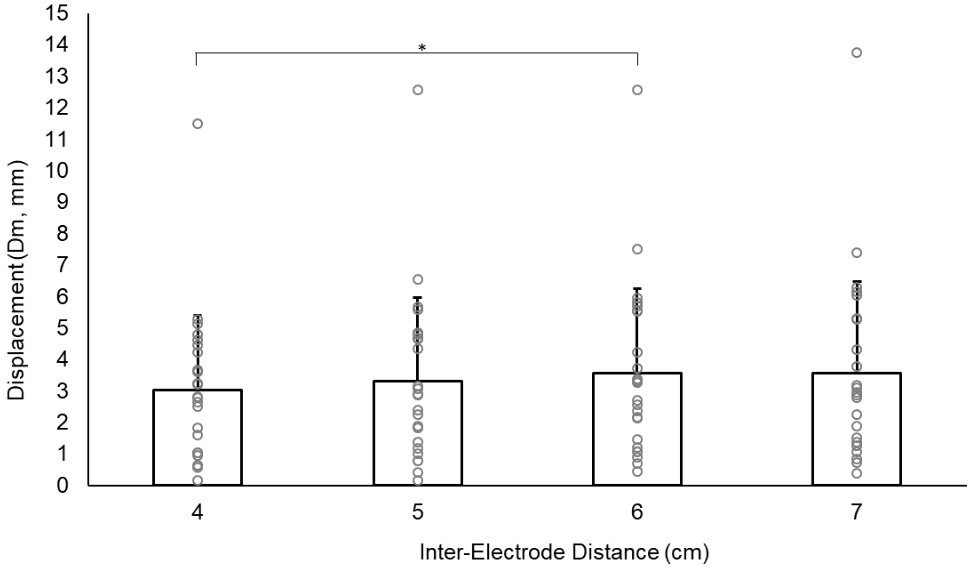



Figure 5. Gastrocnemius Medialis: mean, standard deviation and individual Dm data measured using Tensiomyography at 4 inter-electrode distances. * Significant difference between groups of mean data. (n=24).

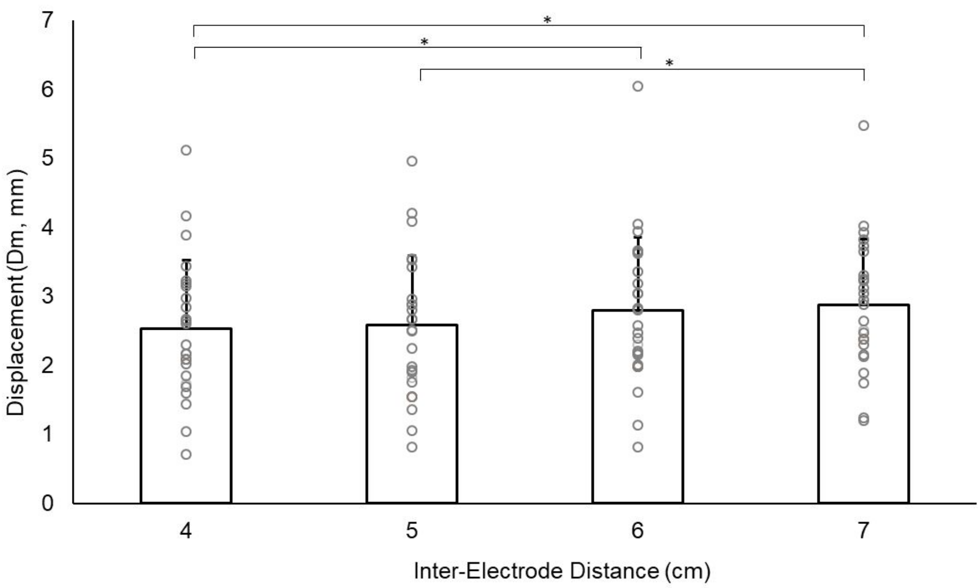
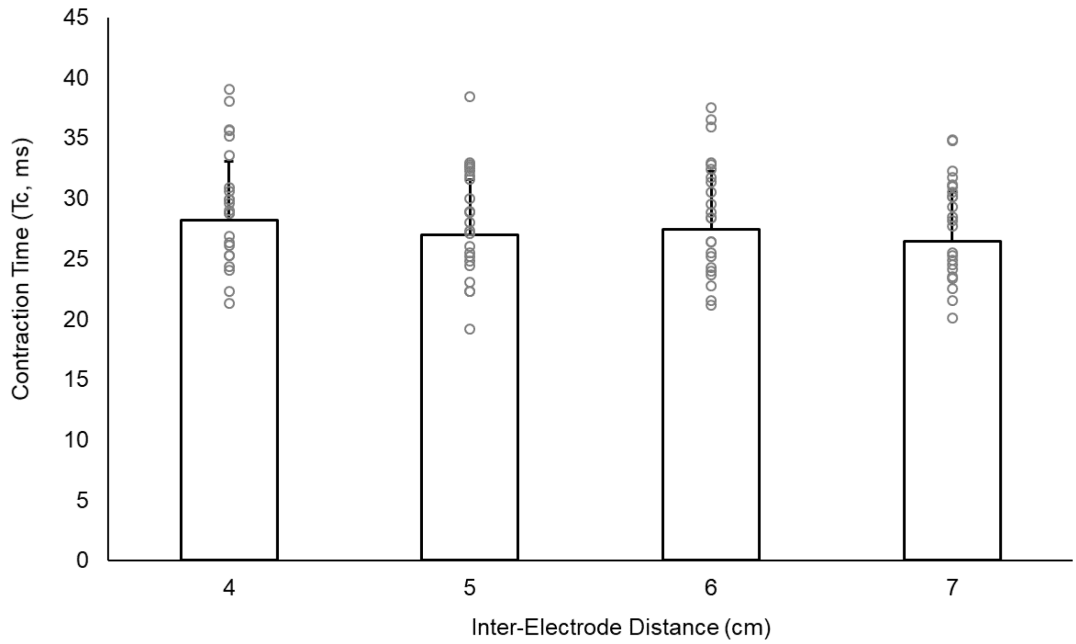


Figure 6. Biceps brachii: mean, standard deviation and individual $T_c$ data measured using Tensiomyography at 4 inter-electrode distances. * Significant difference between groups of mean data. (n=23).



## 4. Discussion

In this study we investigated the effects of four inter-electrode distances (4cm, 5cm, 6cm and 7cm) on Dm and $T_c$ of the biceps femoris, gastrocnemius medialis and biceps brachii, using a standardised current amplitude. Presented data revealed that: (1) biceps femoris Dm increased when the distance between electrodes increased from 4cm to 6cm but did not alter $T_c$; (2) gastrocnemius medialis Dm increased when inter-electrode distance increased from 4cm to 6cm, 4cm to 7cm and 5cm to 7cm, but $T_c$ did not alter; (3) biceps brachii Dm did not change with increased inter-electrode distance. The inter-electrode distance appeared to affect biceps brachii $T_c$, yet differences did not exist between pairwise comparisons. Collectively, these results indicate that the inter-electrode distance critically affects measurements of Dm and $T_c$, with potential, significant implications for the optimisation of muscle specific inter-electrode distances using TMG.

### 4.1. Electrode position, muscle displacement and contraction time

Inter-electrode distance appears to be an important factor determining the magnitude of electrically elicited, muscle response. This is particularly important to consider when using TMG to investigate therapeutic interventions and future applications in clinical practice. In the current study, Dm of the biceps femoris and gastrocnemius medialis increased with increased inter-electrode distance. As hypothesised, the most likely reason accounting for this key result is that the number of stimulated motor fibres increased until maximum Dm was achieved, when the current amplitude was standardised (Vieira et al., 2016, Knaflitz, 1990, García-García et al., 2015). This is an important finding since a wide range of inter-electrode distances are reported in previous TMG literature. Findings of the current study extend previous work from our laboratory that demonstrated similar results in the rectus femoris (Wilson et al., 2018). For comparison, electromyography studies were considered. In a previous study, Vieira et al. (2011) identified that fibers of motor units activated during standing are localised along the longitudinal axis of the human gastrocnemius medialis muscle. Later, authors demonstrated an in increase in maximal extension torque with increased inter-electrode distance (Vieira et al., 2016). The authors proposed that the number of motor fibres within the current pathway using shorter inter-electrode distances was likely a fraction of that within the current pathways of longer distances.

Our findings suggest that the largest change in Dm occurs when inter-electrode distance increases by ≥2cm, for example from 4cm to 6cm (biceps femoris and gastrocnemius medialis) and from 4cm or 5cm to 7cm (gastrocnemius medialis). Motor units within these muscles are scattered with wide distributions along the proximo-distal muscle regions (Botter et al., 2011, Saitou et al., 2000, Laskowski and Sanes, 1987). It is likely that Dm reflects the summation of multiple active motor units, within the same motor territory in proximity to stimulating electrodes. Therefore, the wide distribution of motor points within the proximo-distal region of each muscle can in part, explain why 2cm appeared significant.

Presented data demonstrates less population variability in gastrocnemius medialis measurements (95% CI 2.1-3.3), compared to the bicep femoris (95% CI 2.0-4.8). Electrode proximity to terminal nerve branches, i.e. to muscle motor points, can explain this difference. For a given current intensity, close electrode proximity to the muscle motor point increases the sensitivity and amplitude of the muscle response to electrical stimulation (Davis et al., 1990, Laskin et al., 1993, Wheeler et al., 2002). In this study, electrodes were positioned 3.5cm proximal and distal to the muscle belly. The muscle belly of the biceps femoris was located at 50% of the muscle length, and of the gastrocnemius medialis at the widest region of muscle girth (~30% of the muscle length). Despite wide inter-individual variability in motor point locations, on average motor points of the biceps femoris and gastrocnemius medialis are located at approximately 33% and 25% of the muscle length respectively, proximal of the muscle origins (Botter et al., 2011). Therefore, in this study it is likely that electrodes spanned the motor point of the gastrocnemius medialis but not the biceps femoris. Alternatively, differences in muscle responses could be influenced by the subcutaneous layer thickness which differs between the gastrocnemius medialis (average ± SD, 7mm ± 2mm) and bicep femoris (12mm ± 4mm) (Botter et al., 2011). In fact, the effect of participant intrinsic factors on TMG measurements could explain the evident outlier within our data (Figures 4 & 5). In addition to subcutaneous layer thickness, other influencing factors include; muscle excitation threshold, muscle stiffness, fascia thickness, fibre composition, intramuscular connective tissue elasticity, motor neuron orientation and skin conductivity (Knaflitz, 1990, Pišot et al., 2008, Križaj et al., 2008, Tous-Fajardo et al., 2010).

Biceps brachii Dm varied minimally across inter-electrode distances, which could be explained with consideration of the size of stimulating electrodes, relative to the muscle width ($cm^2$ muscle coverage). This study used 5cm wide electrodes to stimulate all muscles, yet the biceps brachii is, on average, ~3cm wide (n=50, cadaveric measurement)(Joshi et al., 2014). It is possible that biceps brachii measurements reflect co-contraction of the biceps brachii and adjacent muscles within a ~1cm medial and lateral proximity. In comparison the biceps femoris and gastrocnemius medialis are on average ~5cm (n=12, in vitro measurement) and ~6cm (n=8, in vitro measurement) wide respectively (Fiorentino and Blemker, 2014, Bolsterlee et al., 2017). Together, this can elucidate why the largest confidence intervals in Dm measurements are associated with the biceps brachii (95% CI 11.4-14.8mm), compared with the biceps femoris (95% CI 2.0-4.8mm) and gastrocnemius medialis (95% CI 2.1-3.3mm).

A competing explanation for Dm plateau demonstrated in biceps brachii measurements is that the stimulation amplitude, relative to the size of the muscle, was greater at the biceps brachii compared with the biceps femoris and gastrocnemius medialis. In this study, the standardised current amplitude (50mA) was considered submaximal for all assessed muscles (Tous-Fajardo et al., 2010, Alvarez-Diaz et al., 2014, Alentorn-Geli et al., 2015, Alvarez-Diaz et al., 2016). Evidence from our laboratory suggests that 77mA (range 39–99mA) is the mean current amplitude required to elicit maximum biceps brachii Dm (n=29, unpublished data). In a larger muscle, the rectus femoris, 66mA (range 32–90mA) is the mean current amplitude required to elicit maximum Dm (n=62, unpublished data). Therefore, in this study 50mA stimulated the biceps brachii at approximately 65% of maximum. Based on similar muscle architecture to the rectus femoris, the biceps femoris and gastrocnemius medialis were stimulated at approximately 75% of maximum. One could argue the possibility that 50mA elicited a maximal response in some participants, and consequently the plateau in biceps brachii Dm reflects a “ceiling effect”. However, the mean data ranges are comparable between the small parallel and larger pennate muscles (biceps brachii, range 39–99mA; rectus femoris, range 32–90mA). Therefore, it is unlikely that stimulus amplitude resulted in the plateau of biceps brachii Dm as a plateau in biceps femoris and gastrocnemius medialis Dm was not found.

In addition to those noted above, a further factor that could influence Dm and $T_c$ findings, but has not yet been explored in TMG literature, is the muscle fibre pennation. It is understood that the pennation angle of muscle fibres determines the muscle mechanical capabilities (Lieber and Fridén, 2000). Relative to the force-generating axis, muscle fibres extend either at an angle, i.e. pennate muscles (biceps femoris and gastrocnemius medialis), or in parallel, i.e. parallel muscles (biceps brachii) (Lieber and Fridén, 2000). Consequently, pennate muscles have a greater number of fibres that exist within a given volume of muscle, which maximises the capacity for muscle force production, compared to parallel muscles (Enoka, 2015). In comparison, parallel muscles have a greater number of muscle fibres arranged in-series which maximises the capacity for muscle shortening velocity. It is understood that action potentials propagate along muscle fibres arrange in-series and cannot propagate across muscle fibres arranged in-parallel (Mortimer and Bhadra, 2004). Based on this, it is likely that increased inter-electrode distance captures a greater number of muscle fibres arranged in-parallel in pennate muscles, and in-series in parallel muscles. Muscle fibre pennation may therefore explain why increased electrode distance increased biceps femoris and gastrocnemius medialis Dm yet $T_c$ varied minimally, and the opposite was true for the biceps brachii ($T_c$ became shorter and Dm varied minimally). Importantly, pairwise comparisons between electrode distances did not demonstrate differences in biceps brachii $T_c$, which may be a function of adjacent muscle co-contraction as described earlier. Nevertheless, it is evident that muscle pennation plays an important role in the effect of inter-electrode distance on Dm and $T_c$. Further, it appears that electrode distance effects the muscle fibre activation but not cross-bridge formation or cycling kinetics. This agrees with previous investigations, discussed earlier, that reported identical findings in the rectus femoris and vastus medialis (Tous-Fajardo et al., 2010, Wilson et al., 2018).

### 4.2. What is the best electrode configuration to consider in TMG applications?

Maximisation of Dm is the chief criteria for determining the optimal inter-electrode distance in TMG applications. In this study, optimal inter-electrode distance was defined as the distance that elicited the largest Dm, using the shortest inter-electrode distance, when a standardised submaximal current amplitude was used. Before conducting this study the inter-electrode distance able to recruit as many motor neurones as possible was unknown (Martin-Rodriguez et al., 2017b). The optimal distance for the biceps femoris is judged based on the presented increase in Dm from 4cm to 6cm inter-electrode distance, which did not increase further from 6cm to 7cm. Similarly, judgements for the gastrocnemius medialis are based on the increased in Dm from 4cm and 5cm to 7cm. On this basis, optimal stimulation is obtained when the inter-electrode distance is set at 6cm and 7cm for TMG measurements taken from the biceps femoris and gastrocnemius medialis respectively. In accord with the definition of optimal inter-electrode distance presented earlier, our results suggest that 4cm can be accepted as the optimal inter-electrode distance for the biceps brachii since Dm was not affected by altered electrode distance. Distances <4cm and >7cm were not investigated, therefore optimal inter-electrode distances reported for the biceps brachii and gastrocnemius medialis should be used until further evidence is available.

### 4.3. Limitations and perspectives

Data was collected by two principal investigators which increased the potential for variability in the execution of the experimental protocol. To reduce inter-investigator variability, two equally competent and experienced investigators were selected. Participants were not blinded to the assignment sequence orders. Yet, the effects of this were negatable because TMG measures involuntary muscle contraction and is therefore independent of conscious or volitional effort. Lastly, subcutaneous fat thickness was not measured which is postulated to effect Dm and $T_c$ measurements. Future studies should consider accounting for these factors to homogenise the sample population.

To the authors' knowledge, this is the first study to investigate the effect of inter-electrode distance on Dm and $T_c$ of the biceps femoris, gastrocnemius medialis and biceps brachii. Presented results demonstrate that inter-electrode distance effects; Dm but not $T_c$ in two pennate muscles (biceps femoris and gastrocnemius medialis); and $T_c$ but not Dm in a parallel muscle (biceps brachii). In addition, optimal muscle specific inter-electrode distances were judged based on Dm measurements, identified as; 6cm, 7cm and 4cm for the biceps femoris, gastrocnemius medialis and biceps brachii respectively. Findings of this study emphasise the importance of accurate implementation and reporting of inter-electrode distance for reproducibility and comparability of studies using TMG. Future research should consider validating the findings presented in this study, using electrodes that do not exceed the muscle width and in physiologically homogenous sample populations. In addition, investigations should be extended to more superficial muscles measurable using TMG.

**References**


ALENTORN-GELI, E., ALVAREZ-DIAZ, P., RAMON, S., MARIN, M., STEINBACHER, G., RIUS, M., SEIJAS, R., ARES, O. & CUGAT, R. 2015. Assessment of gastrocnemius tensiomyographic neuromuscular characteristics as risk factors for anterior cruciate ligament injury in male soccer players. *Knee Surgery, Sports Traumatology, Arthroscopy,* 23**,** 2502-2507.

ALVAREZ-DIAZ, P., ALENTORN-GELI, E., RAMON, S., MARIN, M., STEINBACHER, G., BOFFA, J. J., CUSCÓ, X., ARES, O., BALLESTER, J. & CUGAT, R. 2014. Effects of anterior cruciate ligament injury on neuromuscular tensiomyographic characteristics of the lower extremity in competitive male soccer players. *Knee Surgery, Sports Traumatology, Arthroscopy,* 24**,** 2264–2270.

ALVAREZ-DIAZ, P., ALENTORN-GELI, E., RAMON, S., MARIN, M., STEINBACHER, G., RIUS, M., SEIJAS, R., BALLESTER, J. & CUGAT, R. 2016. Comparison of tensiomyographic neuromuscular characteristics between muscles of the dominant and non-dominant lower extremity in male soccer players. *Knee Surgery, Sports Traumatology, Arthroscopy,* 24**,** 2259–2263.

BOLSTERLEE, B., D'SOUZA, A., GANDEVIA, S. C. & HERBERT, R. D. 2017. How does passive lengthening change the architecture of the human medial gastrocnemius muscle? *Journal of Applied Physiology,* 122**,** 727-738.

BOTTER, A., OPRANDI, G., LANFRANCO, F., ALLASIA, S., MAFFIULETTI, N. A. & MINETTO, M. A. 2011. Atlas of the muscle motor points for the lower limb: implications for electrical stimulation procedures and electrode positioning. *European Journal of Applied Physiology***,** 2461.

DAHMANE, R., VALENCIC, V., KNEZ, N. & ERZEN, I. 2001. Evaluation of the ability to make non-invasive estimation of muscle contractile properties on the basis of the muscle belly response. *Medical & Biological Engineering & Computing,* 39**,** 51-55.

DAVIS, G. M., SERVEDIO, F. J., GLASER, R. M., GUPTA, S. C. & SURYAPRASAD, A. G. 1990. Cardiovascular responses to arm cranking and FNS-induced leg exercise in paraplegics. *Journal of Applied Physiology,* 69**,** 671-677.

DE HOYO, M., ALVAREZ-MESA, A., SANUDO, B., CARRASCO, L. & DOMINGUEZ, S. 2013. Immediate effect of kinesio taping on muscle response in young elite soccer players. *J Sport Rehabil,* 22**,** 53-8.

DITROILO, M., HUNTER, A. M., HASLAM, S. & DE VITO, G. 2011. The effectiveness of two novel techniques in establishing the mechanical and contractile responses of biceps femoris. *Physiological Measurement,* 32**,** 1315-1326.

DITROILO, M., SMITH, I. J., FAIRWEATHER, M. M. & HUNTER, A. M. 2013. Long-term stability of tensiomyography measured under different muscle conditions. *Journal of Electromyography and Kinesiology,* 23**,** 558-563.

DOHENY, E. P., CAULFIELD, B. M., MINOGUE, C. M. & LOWERY, M. M. 2010. Effect of subcutaneous fat thickness and surface electrode configuration during neuromuscular electrical stimulation. *Medical engineering physics,* 32**,** 468-474.

ENOKA, R. M. 2015. *Neuromechanics of Human Movement-5th Edition*, Human Kinetics.

FIORENTINO, N. M. & BLEMKER, S. S. 2014. Musculotendon variability influences tissue strains experienced by the biceps femoris long head muscle during high-speed running. *Journal of biomechanics,* 47**,** 3325-3333.

GARCÍA-GARCÍA, O., CANCELA-CARRAL, J. M. & HUELIN-TRILLO, F. 2015. Neuromuscular Profile of Top-Level Women Kayakers Assessed Through Tensiomyography. *The Journal of Strength & Conditioning Research,* 29**,** 844-853.

GARCÍA-MANSO, J. M., RODRÍGUEZ-MATOSO, D., SARMIENTO, S., DE SAA, Y., VAAMONDE, D., RODRÍGUEZ-RUIZ, D. & DA SILVA-GRIGOLETTO, M. E. 2012. Effect of high-load and high-volume resistance exercise on the tensiomyographic twitch response of biceps brachii. *Journal of Electromyography and Kinesiology,* 22**,** 612-619.

GARCIA-MANSO, J. M., RODRIGUEZ-RUIZ, D., RODRIGUEZ-MATOSO, D., DE SAA, Y., SARMIENTO, S. & QUIROGA, M. 2011. Assessment of muscle fatigue after an ultra-endurance triathlon using tensiomyography (TMG). *Journal of Sports Sciences,* 29**,** 619-625.

GASPARINI, M., SABOVIC, M., GREGORIC, I., SIMUNIC, B. & PISOT, R. 2012. Increased fatigability of the gastrocnemius medialis muscle in individuals with intermittent claudication. *European Journal of Vascular and Endovascular Surgery,* 44**,** 170-176.

HUNTER, A. M., GALLOWAY, S. D., SMITH, I. J., TALLENT, J., DITROILO, M., FAIRWEATHER, M. M. & HOWATSON, G. 2012. Assessment of eccentric exercise-induced muscle damage of the elbow flexors by tensiomyography. *Journal of Electromyography and Kinesiology,* 22**,** 334-341.

JONES, A., HIND, K., WILSON, H., JOHNSON, M. I. & FRANCIS, P. 2017. A standardised protocol for the assessment of lower limb muscle contractile properties in football players using tensiomyography. *Advances in Skeletal Muscle Function Assessment,* 1**,** 13.

JOSHI, S., JOSHI, S., SONTAKKE, Y. & MITTAL, P. 2014. Some details of morphology of biceps brachii and its functional relevance. *Journal of the Anatomical Society of India,* 63**,** 24-29.

KERSEVAN, K., VALENCIC, V., DJORDJEVIC, S. & IMUNIC, B. 2002. The muscle adaptation process as a result of pathological changes or specific training procedures. *Cellular & Molecular Biology Letters,* 7**,** 368.

KNAFLITZ, M. M., R. DE LUCA C. J. 1990. Inference of motor unit recruitment order in voluntary and electrically elicited contractions. *Journal of Applied Physiology,* 68**,** 1657-1667.

KRIŽAJ, D., ŠIMUNIČ, B. & ŽAGAR, T. 2008. Short-term repeatability of parameters extracted from radial displacement of muscle belly. *Journal of Electromyography and Kinesiology,* 18**,** 645-651.

LASKIN, J. J., ASHLEY, E. A., OLENIK, L. M., BURNHAM, R., CUMMING, D. C., STEADWARD, R. D. & WHEELER, G. D. 1993. Electrical stimulation-assisted rowing exercise in spinal cord injured people. A pilot study. *Spinal Cord,* 31**,** 534.

LASKOWSKI, M. B. & SANES, J. R. 1987. Topographic mapping of motor pools onto skeletal muscles. *Journal of Neuroscience,* 7**,** 252-260.

LIEBER, R. L. & FRIDÉN, J. 2000. Functional and clinical significance of skeletal muscle architecture. *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine,* 23**,** 1647-1666.

LOTURCO, I., GIL, S., DE SOUZA LAURINO, C. F., ROSCHEL, H., KOBAL, R., ABAD, C. C. C. & NAKAMURA, F. Y. 2015. Differences in muscle mechanical properties between elite power and endurance athletes: A comparative study. *The Journal of Strength & Conditioning Research,* 29**,** 1723-1728.

MACGREGOR, L. J., DITROILO, M., SMITH, I. J., FAIRWEATHER, M. M. & HUNTER, A. M. 2016. Reduced Radial Displacement of the Gastrocnemius Medialis Muscle After Electrically Elicited Fatigue. *Journal of sport rehabilitation,* 25**,** 241-247.

MACGREGOR, L. J., HUNTER, A. M., ORIZIO, C., FAIRWEATHER, M. M. & DITROILO, M. 2018. Assessment of Skeletal Muscle Contractile Properties by Radial Displacement: The Case for Tensiomyography. *Sports Medicine,* 48**,** 1607–1620.

MARTIN-RODRIGUEZ, S., ALENTORN-GELI, E., TOUS-FAJARDO, J., SAMUELSSON, K., MARIN, M., ALVAREZ-DIAZ, P. & CUGAT, R. 2017a. Is tensiomyography a useful assessment tool in sports medicine? *Knee Surg Sports Traumatol Arthrosc,* 25**,** 3980-3981.

MARTIN-RODRIGUEZ, S., LOTURCO, I., HUNTER, A. M., RODRIGUEZ-RUIZ, D. & MUNGUIA-IZQUIERDO, D. 2017b. Reliability and Measurement Error of Tensiomyography to Assess Mechanical Muscle Function: A Systematic Review. *J Strength Cond Res,* 31**,** 3524-3536.

MORTIMER, J. T. & BHADRA, N. 2004. Peripheral nerve and muscle stimulation. *Neuroprosthetics: Theory and Practice***,** 638-682.

MURRAY, A. M., JONES, T. W., HOROBEANU, C., TURNER, A. P. & SPROULE, J. 2016. Sixty seconds of foam rolling does not affect functional flexibility or change muscle temperature in adolescent athletes. *International journal of sports physical therapy,* 11**,** 765.

PIŠOT, R., NARICI, M., ŠIMUNIČ, B., BOER, M., SEYNNES, O., JURDANA, M., BIOLO, G. & MEKJAVIĆ, I. 2008. Whole muscle contractile parameters and thickness loss during 35-day bed rest. *European Journal of Applied Physiology,* 104**,** 409-414.

PREGELJ, S. & ŠIMUNIČ, B. 2019. Effects of 8-week electrical muscle stimulation on the muscle contractile properties in adolescent girls. *Annales Kinesiologiae,* 9**,** 105-120.

REY, E., LAGO-PEÑAS, C. & LAGO-BALLESTEROS, J. 2012. Tensiomyography of selected lower-limb muscles in professional soccer players. *Journal of Electromyography and Kinesiology,* 22**,** 866-872.

RODRÍGUEZ-MATOSO, D., RODRÍGUEZ-RUIZ, D., SARMIENTO, S., VAAMONDE, D., DA SILVA-GRIGOLETTO, M. E. & GARCÍA-MANSO, J. 2010. Reproducibility of muscle response measurements using tensiomyography in a range of positions. *Revista Portuguesa de Cardiologia,* 3**,** 81-86.

RODRÍGUEZ RUIZ, D., QUIROGA ESCUDERO, M. E., RODRÍGUEZ MATOSO, D., SARMIENTO MONTESDEOCA, S., LOSA REYNA, J., SAÁ GUERRA, Y. D., PERDOMO BAUTISTA, G. & GARCÍA MANSO, J. M. 2012. The tensiomyography used for evaluating high level beach volleyball players. *Revista Brasileira de Medicina do Esporte,* 18**,** 95-99.

SAITOU, K., MASUDA, T., MICHIKAMI, D., KOJIMA, R. & OKADA, M. 2000. Innervation zones of the upper and lower limb muscles estimated by using multichannel surface EMG. *Journal of human ergology,* 29**,** 35-52.

SALE, D. G. 1987. Influence of exercise and training on motor unit activation. *Exercise and Sport sciences Reviews,* 15**,** 95-151.

ŠIMUNIČ, B. 2012. Between-day reliability of a method for non-invasive estimation of muscle composition. *Journal of Electromyography & Kinesiology,* 22**,** 527-530.

TOUS-FAJARDO, J., MORAS, G., RODRIGUEZ-JIMENEZ, S., USACH, R., DOUTRES, D. M. & MAFIULETTI, N. A. 2010. Inter-rater reliability of muscle contractile property measurements using non-invasive tensiomyography. *Journal of Electromyography and Kinesiology,* 20**,** 761-766.

VALENČIČ, V. 2002. *Method for selective and non-invasive detection of skeletal muscles contraction process* [Online]. Google Patents. Available: http://www.google.com/patents/WO2002074167A1?cl=en [Accessed 1 October 2015].

VALENČIČ, V. & KNEZ, N. 1997. Measuring of skeletal muscles' dynamic properties. *Artificial organs,* 21**,** 240-242.

VIEIRA, T. M., POTENZA, P., GASTALDI, L. & BOTTER, A. 2016. Electrode position markedly affects knee torque in tetanic, stimulated contractions. *European Journal of Applied Physiology***,** 335–342.

VIEIRA, T. M. M., LORAM, I. D., MUCELI, S., MERLETTI, R. & FARINA, D. 2011. Postural activation of the human medial gastrocnemius muscle: are the muscle units spatially localised? *The Journal of physiology,* 589**,** 431-443.

WHEELER, G. D., ANDREWS, B., LEDERER, R., DAVOODI, R., NATHO, K., WEISS, C., JEON, J., BHAMBHANI, Y. & STEADWARD, R. D. 2002. Functional electric stimulation–assisted rowing: increasing cardiovascular fitness through functional electric stimulation rowing training in persons with spinal cord injury. *Archives of physical medicine and rehabilitation,* 83**,** 1093-1099.

WILSON, H. V., JOHNSON, M. I. & FRANCIS, P. 2018. Repeated stimulation, inter-stimulus interval and inter-electrode distance alters muscle contractile properties as measured by Tensiomyography. *PLoS One,* 13**,** e0191965.

ZUBAC, D., PARAVLIĆ, A., KOREN, K., FELICITA, U. & ŠIMUNIĆ, B. 2019. Plyometric exercise improves jumping performance and skeletal muscle contractile properties in seniors. *Journal of musculoskeletal neuronal interactions,* 19**,** 38-49.