



Test-retest reliability of elbow flexor contraction characteristics with tensiomyography for different elbow joint angles

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ABSTRACT

Tensiomyography (TMG) has gained popularity as a tool to quantify muscle contractile properties. However, it is unclear whether joint angle and inter-stimulus interval influence the test-retest reliability of TMG. Fifteen participants (13M/2F; 29.5 ± 7.4 y) underwent TMG recording of the biceps brachii (BB) at 10°, 45° and 90° of elbow flexion with 10 and 20 s inter-stimulus rest intervals in a randomised order on two separate days. The intra-class correlation coefficient (ICC_{2,1}) and 95% confidence interval (95%CI) were calculated for delay time (Td), contraction time (Tc), sustain time (Ts), relaxation time (Tr) and maximal displacement (Dm). Additionally, the standard error of measurement (SEM), coefficient of variation (CV) and minimal detectable change (MDC) were obtained. Across conditions the ICC_{2,1} varied for each parameter (Td:0.465–0.947, Tc:0.452–0.831, Ts:0.717–0.918, Tr:0.841–0.935, Dm:0.646–0.900). CV was low for Td (2.1–3.0%), Tc (4.4–7.7%) and Ts (5.5–6.9%) but greater for Tr (10.0–15.3%) and Dm (5.8–13.1%) across conditions. Ts (~210 ms, P < 0.001) and Dm (~17 mm, P < 0.001) were greatest at 10° and 90°, respectively, while Td was shortest (~22.9 ms, P = 0.002) at 10° compared to other angles. No differences were observed between inter-stimulus intervals or day. The findings are expected to help shape best practice methods for TMG use in field and research based settings.

1. Introduction

In sports science settings, a number of tools and techniques are used to subjectively or objectively quantify neuromuscular performance, fatigue and training adaptations. However, a limitation of many current tools is that they offer a gross overview of the characteristics and capacity of the entire working musculature. In many circumstances however, the specific contractile properties of individual muscles may provide more specific information for sports scientists and clinicians. Previously, quantification of individual muscle responses has been achieved via methods such as elastography (Lima et al., 2018) and mechanomyography (Islam et al., 2013). However, more recently, tensiomyography (TMG) has gained popularity as a time-efficient, practical and non-invasive tool to assess skeletal muscle contractile function (Krizaj et al., 2008; Macgregor et al., 2018; Martin-Rodriguez et al., 2017; Simunic, 2012; Simunic et al., 2011; Tous-Fajardo et al., 2010). TMG measures several radial deformation and time-dependent characteristics during electrically evoked muscular contractions (de Paula Simola et al., 2016a,b; Garcia-Garcia et al., 2015; Macgregor et al., 2018). TMG has also been used in studies investigating muscular imbalances (Alvarez-Diaz et al., 2016), athlete profiling (Garcia-Garcia et al., 2015), injury prevention and rehabilitation (Alvarez-Diaz et al., 2016, 2015; Garcia-Garcia et al., 2017), training adaptations (Zubac

and Simunic, 2017) and ageing (Heredia et al., 2011; Rodriguez-Ruiz et al., 2013; Simunic et al., 2018). Hence, TMG is now utilised by a variety of professionals in both field and laboratory based settings to assess changes in upper and lower body muscular function and performance.

Despite the growing use of TMG, only a handful of evidence is available regarding its reliability. The majority of these studies have been conducted in the lower limbs (Ditroilo et al., 2013; Piqueras-Sanchiz et al., 2017; Rey et al., 2012; Rodríguez-Matoso et al., 2010; Simunic, 2012; Tous-Fajardo et al., 2010) or trunk musculature (Lohr et al., 2018), with only one study using an upper limb muscle (Krizaj et al., 2008), despite the important role of upper body muscles in every day functional tasks, and sports requiring strength and/or repetitive use of the upper limbs. The results of a recent review by Martin-Rodriguez et al. (2017) collectively demonstrated ‘good-excellent’ intra-class coefficient (ICC) values for contraction time (Tc), sustain time (Ts), half-relaxation time (Tr) and maximal displacement (Dm) with TMG. Only one parameter; delay time (Td), suggested ‘low-excellent’ reliability across various musculature. Additionally, the inter-stimulus rest interval and joint angle may be important methodological considerations when testing muscle contraction characteristics using TMG. In fact, Martin-Rodriguez et al. (2017) have highlighted that using sub-optimal inter-stimulus rest intervals and joint angles may decrease the

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reliability and increase the variability of contraction characteristics. Inter-stimulus intervals ranging from 10 to 15 s are commonly used in research methodology (Carrasco et al., 2011; de Paula Simola et al., 2016a,b; Krizaj et al., 2008; Rey et al., 2012) and repeated stimulation at 0.1 Hz can result in increased twitch force amplitude over 1–2 min (Martin-Flores et al., 2017). A recent study suggests that potentiation is relevant to TMG measures but its use of submaximal stimulus intensities and a consistent testing order for different inter-stimulus intervals means that it remains unclear whether a potentiating (or fatigue) effect exists (Wilson et al., 2018). It has also been suggested that muscle contractile properties measured by TMG may be influenced by joint angle (Ditroilo et al., 2011), but this has not been systematically investigated. The length-tension relationship of muscle is such that muscle force is greatest at an optimal length and is reduced if the muscle is shorter or longer than optimal. However, it is not known whether changes in lateral excursion of the muscle, as measured by TMG, parallel length-related changes in twitch force.

Therefore, the aim of this investigation was to assess the test-retest reliability of contraction characteristics in the biceps brachii (BB) muscle using TMG. We also aimed to determine the influence of joint angle and inter-stimulus rest interval within- and between-participants over two separate testing sessions. We hypothesised that a shorter muscle length at more flexed joint angles would increase Dm and may influence other TMG measurement parameters. Additionally, we postulated that shorter inter-stimulus intervals might be subject to potentiation (increased Dm, reduced Td, Tr, Ts and Tc). The results of this study were expected to provide best practice information for sports scientists and clinicians using TMG, especially in the upper limbs to assess training, fatigue and other muscular contractile changes with training.

2. Methods

2.1. Subjects

Fifteen healthy individuals (13 males, 2 females) participated. The average (\pm SD) age, height and body mass were 29.5 ± 7.4 y, 176.9 ± 9.2 cm and 78.7 ± 14.9 kg, respectively. Participants were included in the study if they had no previous injury within the last six months to the dominant upper limb. The study was performed according to the Declaration of Helsinki and was approved by the Edith Cowan University Human Research Ethics Committee (Ethics No. 20462).

2.2. Experimental design

The contractile properties of the BB in the right arm were measured using TMG (BMC Ltd., Ljubljana, Slovenia) at rest. Participants were asked to refrain from strenuous upper body exercise 48 h prior to testing to avoid any possible lasting effects of fatigue or muscle damage. Testing was conducted on two separate days, separated by at least 48 h (range 2–7 days) at the same time of day for each participant to avoid any potential effects of circadian rhythm on performance (Fig. 1).

2.3. Procedures

During TMG recording participants sat with their shoulder in passive anterior flexion and supported at 90° by a custom designed arm bar (see Fig. 2A). During testing, the elbow was positioned at either 10° , 45° or 90° of flexion (0° equals no flexion/straight arm) in a randomised order with the forearm held in place using a Velcro strap to avoid extraneous movements of the arm. First, the middle of the muscle belly of BB was visually determined during isometric contraction with the elbow at 45° and this location was then marked with a waterproof skin marker. Square (5×5 cm) stimulating electrodes (Compex Medical AS, Ecublens, Switzerland) were placed with the inner edge 2.5 cm either

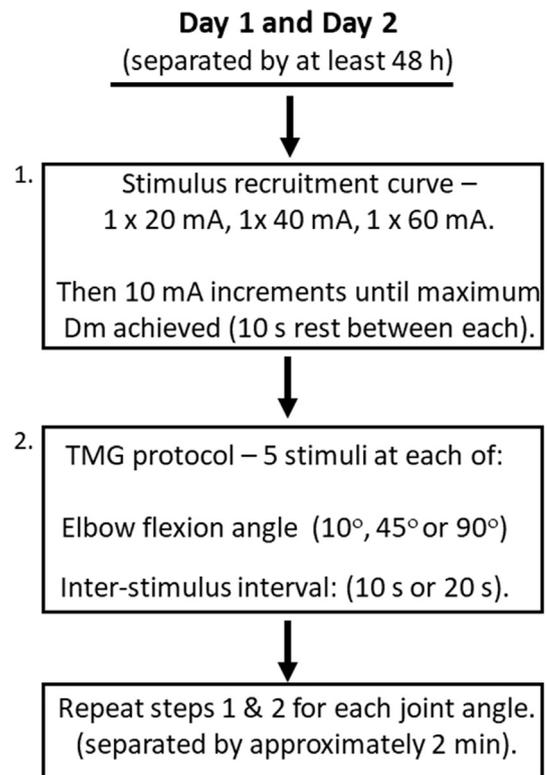


Fig. 1. Schematic overview of the testing protocol.

side of the identified muscle belly point (de Paula Simola et al., 2016a,b) at the midpoint of the line between the lateral head of the clavicle and head of the radius (Krizaj et al., 2008). Electrical stimulation was delivered by a constant current stimulator TMG-S1 device (BMC Ltd., Ljubljana, Slovenia). The TMG sensor tip with a spring constant of $0.17 \text{ N}\cdot\text{mm}^{-1}$ was placed at the marked location, and an electronic inclinometer application was used to set it perpendicular to the skin. Both the location of the electrodes and the point on the sensor (5 mm from the sensor base), were marked to: (1) ensure a consistent pressure was applied between trials and across participants, and (2) ensure consistency between conditions and days. Next, the arm was moved to the first angle to be tested. A single, 1-ms wide square-pulse was delivered at an initial intensity of 20 mA with the muscle at rest. The intensity of the stimulus was then increased by 20 mA and then 10 mA increments (above 60 mA) until a maximal contractile response (i.e., largest ‘maximal displacement’) was observed for each individual. The stimuli were delivered at intervals of 10 s. Once the maximal intensity was determined, two blocks of five stimuli were delivered with either a 10 or 20 s interval in a block randomised fashion. After approximately 2 min of rest the arm was then moved to the next angle to be tested. This process, including identification of the maximal response, was repeated for each joint angle to ensure that the stimulus intensity was individualised and thus optimal for each condition, and was re-tested on each day.

Several parameters were automatically measured from the waveform by the TMG software. The Dm provides a measure of the amount of radial deformation of the muscle. Previous studies suggest that Dm may allow inferences about muscle stiffness, fibre activation and tendon mechanics (Carrasco et al., 2011; de Paula Simola et al., 2016a,b; Hunter et al., 2012; Simunic et al., 2011). The Tc, calculated from the time between 10% and 90% of Dm, is indicative of muscle fibre type and calcium release (de Paula Simola et al., 2016a,b). Ts is the duration for which the contraction remains above 50% of Dm, and Tr is calculated as the time required to decrease from 90% to 50% of the Dm (Martin-Rodriguez et al., 2017). The Td is recorded as the latency

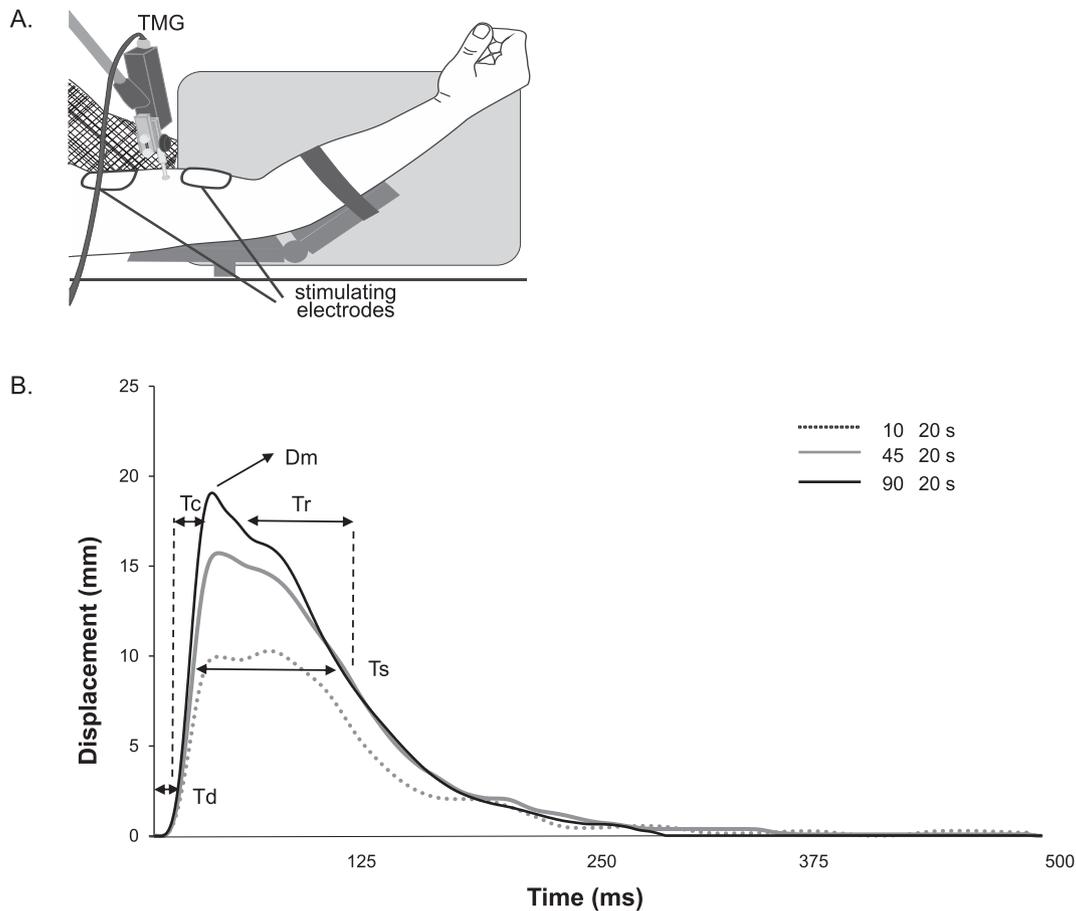


Fig. 2. (A) An example of the setup showing the placement of the stimulating electrodes, TMG sensor over the muscle belly, Velcro strap placement below the elbow and arm position for the 45° elbow flexion condition (0° equals no flexion). (B) An example TMG waveform from a single participant across all joint angles for the 20 s inter-stimulus interval condition. The measured TMG parameters; Td = delay time, Tc = contraction time, Ts = sustain time, Tr = half-relaxation time and Dm = maximal displacement, are depicted on the graph for the 90° condition only.

between the onset of the electrical stimulus and 10% of Dm. All parameters were calculated online for each trial (Stephens et al., 1975) and used for further offline analysis. A graphical representation of the TMG waveform taken from one participant across each joint angle can be found in Fig. 2B.

2.4. Statistical analysis

Relative- and absolute-reliability analyses were conducted using the data collected from both testing sessions. Relative between participant reliability was assessed by calculating the two-way mixed effects, absolute agreement, single measurement intra-class correlation coefficient (ICC_{2,1}) (Koo and Li, 2016) with 95% confidence intervals (95% CI). ICCs were classified as ‘poor’ (< 0.50), ‘moderate’ (0.50–0.75), ‘good’ (0.75–0.90) and ‘excellent’ (≥ 0.90) (Lima et al., 2018). The standard error of measurement (SEM) was calculated using the formula $SEM = SD_{pooled} \sqrt{1 - ICC}$ (Atkinson and Nevill, 1998; Weir, 2005). The minimal detectable change (MDC) was calculated across participants via the formula $MDC = SEM \times 1.96 \times \sqrt{2}$ to determine the minimum difference required between trials for the change to be considered real (Weir, 2005). The within-participant coefficient of variation (CV), a measure of absolute reliability, was also calculated by using the formula $CV = SD_{pooled} / \text{mean}$ and expressed as a percentage (CV%), where the mean is the difference between the two trials, and SD_{pooled} is the standard deviation of differences of the trials. CV% values ≤ 10% were deemed to indicate low variability (Tous-Fajardo et al., 2010). Within-participant CV was first calculated individually for each participant and then averaged across all participants. All reliability statistics

were performed using Microsoft Excel (version 2015; Microsoft Corporation, Redmond, WA) and IBM SPSS Statistics v.24 (IBM, USA).

To detect differences for each outcome measure between conditions a Shapiro-Wilk test was first performed to ensure the data were normally distributed. A 3-way repeated measures analysis of variance (ANOVA) was then conducted to test for interactions between joint ANGLE (10°, 45° and 90°), REST INTERVAL (10 and 20 s) and DAY (1 and 2). Where statistical significance was detected between conditions, post-hoc *t*-tests with a Bonferroni correction were conducted. For all tests, if the sphericity was violated the Greenhouse-Geisser correction was used if the epsilon value was less than 0.75 and the Huynh-Feldt correction applied if the epsilon value exceeded this value. Alpha level was set at $P < 0.05$ and all results are displayed as mean ± SD.

3. Results

3.1. Raw scores

The average stimulation intensity used across all participants was 95.6 ± 3.1 mA with the maximal stimulator output (i.e., 100 mA) used on 81.1% of occasions. The values (mean ± SD) for each outcome measure (Td, Tc, Ts, Tr and Dm) across all conditions (joint angle, rest-interval and day) are presented in Table 1.

3.2. Relative and absolute reliability

The relative (ICC_{2,1} and SEM) and absolute (MDC and CV%) reliability of each outcome measure (Td, Tc, Ts, Tr and Dm) across all

Table 1

Displays the raw values (Mean \pm SD) for each angle, inter-stimulus interval and day. The intra-class correlation coefficient (ICC_{2,1}), 95% confidence intervals (95% CI) and standard error of measurement (SEM) indicate relative reliability. The coefficient of variation (CV) percentage and minimal detectable change (MDC) indicate absolute reliability. Td = delay time, Tc = contraction time, Ts = sustain time, Tr = half-relaxation time, Dm = maximal displacement. * # indicates a significant difference in raw (mean \pm SD) values from all other joint angles ($^{\circ}$ p < 0.001, $^{\#}$ p < 0.05), and § indicates different from 10° only (p < 0.05).

			Day 1 Mean \pm SD	Day 2 Mean \pm SD	ICC _{2,1} (95% CI)	p	SEM	CV (%)	MDC
Td (ms)	10°	10 s	22.8 \pm 1.7	22.9 \pm 1.6	0.947 (0.846, 0.982)	< 0.001	0.39	2.09	1.07
		20 s	22.8 \pm 1.8	23.0 \pm 1.6	0.884 (0.664, 0.960)	< 0.001	0.58	2.35	1.61
	45°	10 s [§]	23.6 \pm 1.4	23.6 \pm 1.3	0.840 (0.514, 0.947)	0.001	0.51	2.47	1.50
		20 s [§]	23.7 \pm 1.5	23.6 \pm 1.2	0.759 (0.263, 0.920)	0.007	0.68	2.67	1.88
	90°	10 s [§]	23.9 \pm 1.9	23.6 \pm 1.3	0.465 (-0.681, 0.823)	0.137	1.19	2.98	3.32
		20 s [§]	23.7 \pm 1.9	23.5 \pm 1.3	0.496 (-0.588, 0.834)	0.116	1.18	2.15	3.28
Tc (ms)	10°	10 s	27.1 \pm 2.8	27.5 \pm 2.4	0.791 (0.640, 0.856)	0.004	1.22	4.41	3.37
		20 s	27.9 \pm 3.8	27.3 \pm 2.4	0.665 (0.005, 0.888)	0.026	1.65	5.72	1.61
	45°	10 s	23.6 \pm 1.4	23.6 \pm 1.3	0.728 (0.167, 0.910)	0.012	1.75	6.49	4.86
		20 s	26.5 \pm 5.1	25.4 \pm 2.8	0.452 (-0.062, 0.816)	0.138	2.90	7.72	8.03
	90°	10 s	25.1 \pm 4.5	25.6 \pm 4.3	0.745 (0.226, 0.915)	0.009	2.25	7.32	6.23
		20 s	25.2 \pm 4.4	25.5 \pm 4.2	0.831 (0.489, 0.944)	< 0.001	1.80	7.03	4.99
Ts (ms)	10°	10 s	205.5 \pm 26.1	208.6 \pm 29.9	0.717 (0.139, 0.906)	0.014	14.67	6.89	40.67
		20 s	209.1 \pm 26.9	213.0 \pm 29.1	0.752 (0.255, 0.917)	0.008	13.80	6.24	38.24
	45°	10 s [#]	195.5 \pm 38.2	200.3 \pm 32.5	0.906 (0.728, 0.968)	< 0.001	10.77	6.91	29.85
		20 s [#]	196.7 \pm 36.6	199.8 \pm 29.7	0.918 (0.758, 0.972)	< 0.001	9.59	6.27	26.57
	90°	10 s [#]	178.2 \pm 31.4	178.8 \pm 28.1	0.900 (0.698, 0.967)	< 0.001	9.60	6.42	26.60
		20 s [#]	178.9 \pm 29.3	180.1 \pm 26.6	0.882 (0.644, 0.961)	< 0.001	9.79	5.50	27.13
Tr (ms)	10°	10 s	100.2 \pm 44.9	105.7 \pm 37.7	0.900 (0.711, 0.966)	< 0.001	12.65	14.28	35.08
		20 s	101.8 \pm 41.5	108.5 \pm 36.9	0.935 (0.812, 0.978)	< 0.001	9.33	12.05	25.85
	45°	10 s	105.5 \pm 47.2	112.4 \pm 47.8	0.955 (0.868, 0.985)	< 0.001	10.14	12.57	28.11
		20 s	108.2 \pm 49.9	105.9 \pm 39.3	0.919 (0.758, 0.973)	< 0.001	12.96	10.97	35.91
	90°	10 s	92.9 \pm 34.4	103.1 \pm 35.3	0.925 (0.725, 0.977)	< 0.001	9.59	9.97	26.57
		20 s	89.6 \pm 31.0	105.2 \pm 37.9	0.841 (0.446, 0.950)	< 0.001	14.04	15.26	38.91
Dm (mm)	10°	10 s	9.7 \pm 2.1	10.4 \pm 2.1	0.646 (-0.005, 0.879)	0.029	1.28	13.06	3.54
		20 s	9.9 \pm 2.2	10.3 \pm 2.3	0.762 (0.288, 0.920)	0.006	1.12	5.81	3.10
	45°	10 s [*]	12.9 \pm 3.2	13.6 \pm 3.5	0.891 (0.685, 0.963)	< 0.001	1.14	8.57	3.17
		20 s [*]	12.9 \pm 3.3	13.4 \pm 3.5	0.862 (0.594, 0.954)	< 0.001	1.28	8.62	3.55
	90°	10 s [*]	16.6 \pm 3.2	17.7 \pm 3.3	0.844 (0.620, 0.962)	< 0.001	1.31	8.65	3.63
		20 s [*]	16.5 \pm 3.4	17.6 \pm 3.0	0.900 (0.650, 0.968)	< 0.001	1.02	5.81	2.84

conditions (joint angle, rest-interval and day) are presented in Table 1. The ICC_{2,1} ranged from ‘moderate-excellent’ for all parameters except Td at 90°, and Tc 45° during the 20 s condition. The SEM was greatest for Ts and Tr, whereas the CV approached or exceeded 10% for Tr across all conditions and Dm at 10° for the 10 s condition.

3.3. Effects of joint angle and rest interval

Joint angle but not rest interval altered Dm, Ts and Td. Repeated measures ANOVA for Dm revealed a significant main effect of angle ($F_{2,28} = 47.86$, $P < 0.001$). Post-hoc analyses revealed that Dm was significantly greater at 90° (~17 mm) compared to 45° (~13 mm; $p = 0.001$), which was greater than the Dm at 10° (~10 mm; $p < 0.001$). No significant effect was observed for the interaction (angle \times rest interval \times day) ($F_{2,28} = 0.45$, $P = 0.641$), or main effects of rest ($F_{1,14} = 0.37$, $P = 0.52$) and day ($F_{1,14} = 2.97$, $P = 0.11$) (Fig. 3A).

ANOVA for Ts revealed a significant main effect of angle ($F_{2,28} = 15.83$, $P < 0.001$). Ts was significantly smaller at 90° (~179 ms) compared to 45° (~198 ms; $p = 0.023$) and 10° (~210 ms; $p < 0.001$), while Ts at 45° was also smaller than at 10° ($p = 0.046$). No significant effect was observed for the interaction (angle \times rest interval \times day) ($F_{2,28} = 0.31$, $P = 0.715$), or main effects of rest ($F_{1,14} = 2.23$, $P = 0.158$) and day ($F_{1,14} = 0.42$, $P = 0.528$) (Fig. 3B).

For Td, ANOVA also revealed a significant main effect of angle ($F_{2,28} = 7.94$, $P = 0.002$). Td was significantly greater at 45° (~23.6 ms; $p = 0.005$) and 90° (~23.7 ms; $p = 0.024$) compared to 10° (~22.9 ms). No significant effect was observed for the interaction (angle \times rest interval \times day) ($F_{2,28} = 1.22$, $P = 0.310$), or main effects of rest ($F_{1,14} = 0.16$, $P = 0.692$) and day ($F_{1,14} = 0.001$, $P = 0.987$)

(Fig. 3C).

For Tc, ANOVA did not show any significant interaction effect ($F_{2,28} = 0.81$, $P = 0.457$) nor main effects of angle ($F_{2,28} = 2.74$, $P = 0.093$), rest ($F_{1,14} = 0.14$, $P = 0.714$) and day ($F_{1,14} = 0.04$, $P = 0.842$) (Fig. 3D).

Similarly, no significant interaction effect ($F_{2,28} = 3.32$, $P = 0.064$) nor main effects of angle ($F_{2,28} = 0.92$, $P = 0.411$), rest ($F_{1,14} = 0.01$, $P = 0.931$) and day ($F_{1,14} = 4.18$, $P = 0.60$) were evident for Tr (Fig. 3E).

4. Discussion

The aim of this study was to investigate the relative and absolute inter-day reliability of muscle contraction characteristics measured with TMG. Specifically, the influence of joint angle and inter-stimulus rest interval were assessed over two separate days in the BB. Collectively, the results suggest that the test-retest reliability of the TMG parameters are acceptable; however, Tr demonstrated a higher CV, and the ICC for Tc was generally ‘moderate-good’ across conditions (ICC: 0.665–0.831), but lower at 45° during the 20 s inter-stimulus interval (ICC: 0.452). Although no differences in the raw values were found for the inter-stimulus rest interval, joint angle is an important factor that should be considered. These findings are intended to shape best practice methods for the use of TMG in field-based applied, clinical and research settings when investigating muscle contractile function with fatigue, injury and return to play.

The results revealed that joint angle had a significant effect on Dm, Ts and Td. The peak radial displacement (i.e., Dm) was greatest at 90° where the BB is close to the optimal length on its length-tension curve. The larger Dm is consistent with greater sarcomere shortening, radial

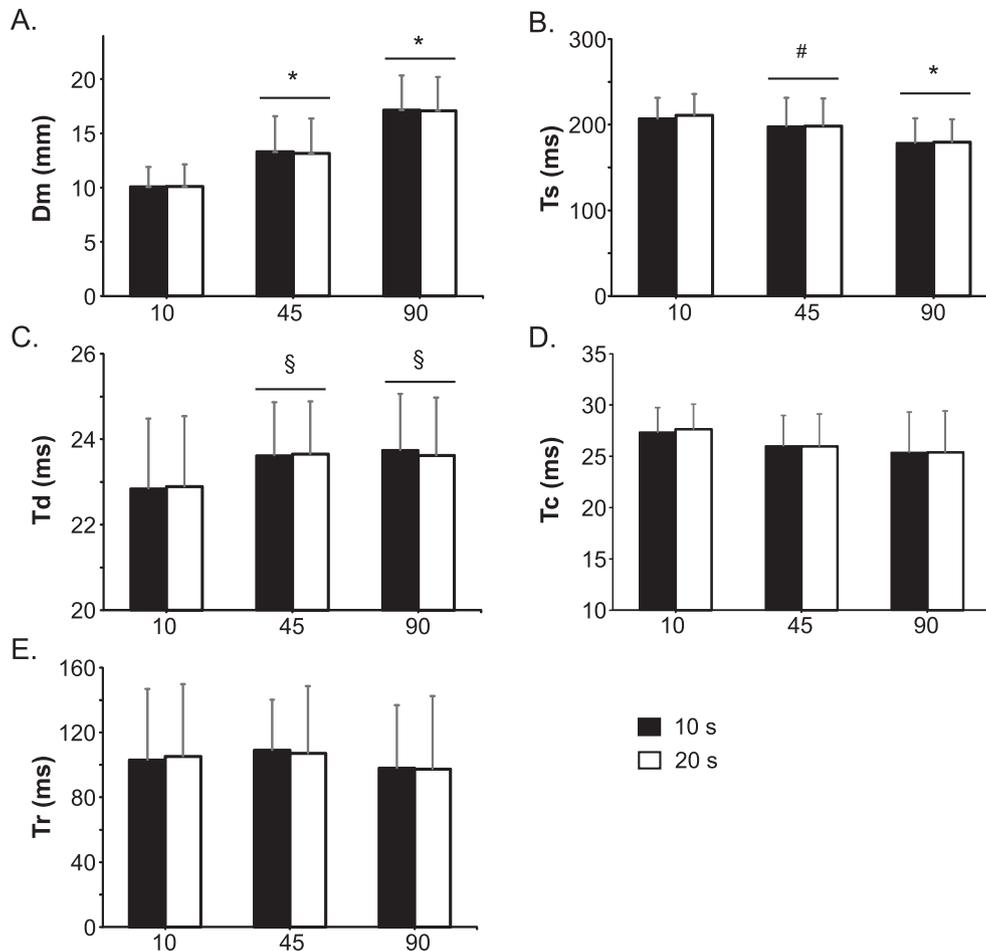


Fig. 3. Shows the effect of joint angle and inter-stimulus interval for (A) Dm, (B) Ts, (C) Td, (D) Tc and (E) Tr. All data are presented as mean \pm SD collapsed across both experimental days. Data for the 10 s inter-stimulus intervals are shown as black columns and for 20 s intervals as white columns. * indicates a significant difference from all other joint angles ($p < 0.001$), # indicates significantly different from all joint angles ($p < 0.05$) and § indicates different from 10° only ($p < 0.05$). No differences were observed between inter-stimulus intervals.

bulging and an increased cross-sectional area of muscle underlying the sensor in this position during the contraction (Wakeling and Randhawa, 2014). Conversely, when the joint was more extended, Dm decreased. Two factors that may contribute to this decrease are the longer-than-optimal sarcomere lengths, and a decrease in compliance of the elastic component of the muscle-tendon unit. Although the Dm has previously been used to describe muscle stiffness properties (Carrasco et al., 2011; de Paula Simola et al., 2016a,b; Hunter et al., 2012; Simunic et al., 2011), it is not clear which aspect of muscle fibre and/or tendon stiffness it reflects. Specifically, when testing across joint angles the twitch force will vary according to muscle length, thus, any relationship between Dm and the stiffness of tissues in series with the muscle fibres will be complex. Although it is possible that the sensor location over the muscle differs during flexion/extension, this approach has also been used by Ditroilo et al. (2011) and additionally, the stimulus intensity was maximal at each joint angle.

Interestingly, Ts was also affected by joint angle, but was increased rather than decreased with longer muscle lengths. The duration of Td was significantly less at 10°. As Td is the time between stimulus onset and 10% of Dm, the reduced duration of Td may partly result from the overall smaller maximal radial displacement (Dm). At this stage it is unclear whether other mechanisms may also potentially contribute to this observation. While the electromechanical delay preceding the production of force is quicker at longer muscle lengths, the initial movement of the muscle fibres is not influenced by joint angle (Lacourpaille et al., 2013). An overall greater maximal radial

displacement in a flexed position could also have resulted in an increased duration to reach 50% Dm. However, the opposite was observed, which could be explained by a slowing of the temporal characteristics of twitches (measured as force output) on the descending limb of the muscle's length-tension curve (Stephens et al., 1975). Conversely, no differences were observed for Tc or Tr across joint angles. The underlying mechanism that prevents Tc and Tr being affected by joint angle is at this stage unknown. These findings suggest that, analogous to measurements of muscle force, joint angle should be controlled during TMG assessments.

Of further interest, the use of short or long rest intervals (i.e., 10 or 20 s) between stimuli did not affect any muscle contraction characteristics. In neurological studies, trains of moderate to high stimulation frequencies (20–100 Hz) have been shown to cause an initial potentiation of force and subsequent muscle fatigue (Dreibati et al., 2010) and with monitoring over several minutes single twitches at 0.1 Hz increase in amplitude (Martin-Flores et al., 2017). However, fatigue or potentiation is unlikely to be problematic in practical TMG testing settings where single pulse stimulation is employed usually once every 10 s (de Paula Simola et al., 2016a,b; Piqueras-Sanchiz et al., 2017; Rey et al., 2012; Tous-Fajardo et al., 2010) or greater (Carrasco et al., 2011) and is performed a limited number of times. Thus, a rest interval of 10 s appears to be sufficient when stimulation is repeated five times as in the current study, and may be a more time effective method of TMG assessment, especially when testing multiple muscle sites or large cohorts of individuals.

The inter-day reliability of TMG parameters was generally, but not uniformly high. Ts (ICC: 0.717–0.918) and Tr (ICC: 0.841–0.955) displayed ‘moderate-excellent’ relative reliability across all conditions. These results are similar to those of the previous research in the lower limb muscles (de Paula Simola et al., 2016a; Ditroilo et al., 2011, 2013; Simunic, 2012; Wilson et al., 2018) and in the BB muscle (Krizaj et al., 2008). However, a limitation of these comparisons is that only one other study (Wakeling and Randhawa, 2014) reported the type of ICC formulas used and thus, the magnitude of correlation may be different depending on the type of analysis conducted (Lahey et al., 1983). In our study, the reliability of Dm was also classified as ‘good-excellent’ at 90° and 45° (ICC: 0.844–0.900) however, Dm only displayed ‘moderate-good’ reliability at 10° (ICC: 0.646–0.762). In other studies the ICC for Dm has been generally reported as > 0.900 (Carrasco et al., 2011; de Paula Simola et al., 2016a; Ditroilo et al., 2011; Krizaj et al., 2008; Rodríguez-Matoso et al., 2010; Simunic, 2012; Tous-Fajardo et al., 2010). Specifically, these investigations were in the lower limb musculature, using knee flexion angles of 30–60° for quadriceps and hamstring assessment (de Paula Simola et al., 2016a; Rey et al., 2012; Simunic, 2012), and a ‘neutral’ position for the gastrocnemius (Ditroilo et al., 2013). It is unclear what joint angle was used for the upper limb study by Krizaj et al. (2008). Therefore, a direct comparison between investigations is difficult. However, in support of joint specific differences in reliability, Ditroilo et al. (2011) demonstrated that the relative reliability of the Dm and Tc was reduced in the biceps femoris as the knee joint became more flexed, which is in contrast to our findings. Additionally, we found that Td displayed ‘good-excellent’ relative reliability at 10° and 45°, but was considerably lower at 90° of flexion. ‘Good-excellent’ reliability of Td has been reported in the majority of other studies (Carrasco et al., 2011; de Paula Simola et al., 2016a; Rey et al., 2012; Simunic, 2012), with only Ditroilo et al. (2013) demonstrating ‘moderate’ reliability. Considering that the Td represents the electrical conduction time of the signal and initial onset of contraction the ‘poor’ reliability reported in our study at 90° of flexion is somewhat surprising given that no apparent differences in reliability were observed for all other outcome measures at 90°. However, it is important to note that this lower reliability derived from a large difference between sessions for one participant with no apparent cause, such that exclusion of this participant puts the ICC into the ‘good-excellent’ range (90°, 10 s: ICC = 0.810, 90°, 20 s: ICC = 0.826). While sarcolemmal conduction velocity can be decreased by fatigue or after damaging exercise (Kouzaki et al., 2016; Piitulainen et al., 2010), it is unclear how and why changes in muscle length in the absence of muscle fatigue or damage can affect the duration and reliability of Td. Nonetheless, the length-tension relationship at larger joint angles may play an important role during the onset of contraction. Additionally, a similar observation was noted for Tc, with a single, different participant displaying a large difference between sessions. When this participant was excluded from the analysis the ICC for Tc increased from 0.452 to 0.875 for the 45° 20 s condition. Collectively, the results suggest that muscle specific testing parameters should be established in upper and lower limbs even for muscles that have similar functional roles at the joint (i.e., elbow and knee flexors).

The variability of measurement is an important factor to consider when using TMG to test within and between subject differences. The intra-participant CV was found to be low for Td, Tc and Ts (CV = 2.2–7.7%), which is similar to the values reported in other studies (de Paula Simola et al., 2016a; Simunic, 2012; Tous-Fajardo et al., 2010). The intra-participant variability for Tr and Dm across all conditions was generally higher (CV = 5.8–15.3%), which has also been similarly reported by de Paula Simola et al. (2016) and Ditroilo et al. (2013). However, given that values under 10% are considered acceptable, our results indicate that only the Dm at 10° should be interpreted with caution. Once again this observation is in contrast to the low CVs at longer muscle lengths observed by Ditroilo et al. (2011) in the biceps femoris. In addition, the CV for Tr approached or exceeded 10% across

all conditions and coupled with the large MDC values required in order to detect meaningful differences, provides less confidence when trying to investigate changes in Tr between and within individuals.

To our knowledge this is the first study to investigate (1) the effects of joint angle and inter-stimulus interval on muscle contractile function in the upper limb using TMG, and (2) to report the reliability of TMG parameters under these conditions. We suggest that other aspects outside the scope of this study should also be considered in future research. Firstly, the maximal stimulator output was used on the majority of occasions, however it is unclear if this was actually the maximal muscle contraction response achievable. Secondly, modelling of muscle shape changes during twitch contractions and their relationship to TMG measures may help interpretation of these measures in terms of underlying muscle and tendon biomechanics. Lastly, investigations to establish muscle specific contractile differences are warranted to provide a comprehensive understanding of normal contractile function for comparison to injury, ageing and disease related changes.

Collectively, the findings suggest that TMG is a reliable tool to assess muscle contractile function in the BB. Dm, Ts and Td are influenced by joint angle, with reliability at longer muscle lengths decreasing for Dm and Ts, and increasing for Td. Therefore, joint angle should be considered when conducting TMG assessment in the BB. Additionally, Tr and Tc should be interpreted with caution due to the higher intra- and inter-subject variation and lower overall relative reliability, respectively. Short or longer inter-stimulus intervals do not influence the contractile responses in the BB muscle and thus, 10-s inter-stimulus intervals may be time efficient without compromising reliability. TMG should be considered by practitioners as a reliable tool which may provide important information about contractile function during fatigue, post-injury or in athlete profiling in field and research based applications.

Conflict of interest

The authors declare they have no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jelekin.2019.02.002>.

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