DIFFERENCES BETWEEN SKELETAL MUSCLE CONTRACTILE PARAMETERS ESTIMATED FROM TRANSVERSAL TENSIOMYOGRAPHIC AND LONGITUDINAL TORQUE TWITCH RESPONSE

Katja Koren[1], Boštjan Šimunič[1,2], Enrico Rejc[3,4], Stefano Lazzer[3] and Rado Pišot[1,2]

[1]University of Primorska, Science and Research Centre,
Institute for Kinesiology Research, Koper, Slovenia
[2]University of Primorska, Faculty of Education,
Department of Primary School Teaching, Koper, Slovenia
[3]University of Udine, Department of Medical and Biological Sciences, Italy
[4]University of Louisville, Department of Neurological Surgery, Louisville, Kentucky, USA

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Abstract:

Contractile properties of skeletal muscle are studied for various purposes and mainly by means of force or torque twitch responses. This study compared contractile properties estimated from isometric longitudinal and transversal vastus lateralis twitch mechanical actions using torque and tensiomyography (TMG) as assessment methods, respectively. We calculated delay, contraction, sustain, half relaxation time and peak amplitude from the maximal twitch response obtained by both methods in 19 healthy males (age 46.1±17.8 years). Results indicated a shorter delay (Δ=-23.4%; $p<.001$) and contraction time (Δ=-42.7%; $p<.001$) when calculated from the transversal tensiomyographic actions, and shorter half relaxation time (Δ=-26.2%; $p=.025$) when calculated from the longitudinal torque actions, while no difference in sustain time was found. Delay and contraction time did not correlate significantly when correlated between longitudinal and transversal actions; however, sustain time ($r=.478$; $p=.038$) and half relaxation time ($r=.608$; $p=.006$) did. In conclusion, the tensiomyography and torque gives different information reflecting that different mechanisms affect longitudinal and transversal twitch skeletal muscle deformations. Tensiomyographic response of skeletal muscle's transversal actions likely reflects more intrinsic contractile properties.

Key words: *tensiomyography, vastus lateralis, contraction time, half relaxation time*

Introduction

Skeletal muscle's contractile properties reflect the characteristics of structure and composition of muscle fibres within the muscle and have been studied to make conclusions on: muscle composition (McComas & Thomas, 1968; Dahmane, Valenčič, Knez, & Eržen, 2001; Šimunič, et al., 2011), exercise velocity and training adaptation (Maffiuletti & Martin, 2001; Paasuke, Saapar, et al., 2007), muscle resistance to fatigue (Merletti, LoConte, & Orizio, 1991; Hunter, et al., 2012), motor unit spatial distribution (Dahmane, Djordjević, Šimunič, & Valenčič, 2005), firing rate (Botterman, Iwamoto, & Gonyea, 1986), and rehabilitation of different muscle pathologies (Grabljevec, et al., 2004; Burger, Valenčič, Marinček, & Kogovšek, 1996). Till now, the gold standard for measuring contractile properties of muscles has been the distally detected muscle force/torque response (Hamada, Sale, MacDougall, & Tarnopolsky, 2000; Hill, 1953; Paasuke, Rannama, Ereline, Gapeyeva, & Oopik, 2007). However, several mechanomyographic (MMG) methods have been developed and proposed so far, like phonomyography (Maton, Petitjean, & Cnockaert, 1990), soundmyography (Orizio & Veicsteinas, 1992), acoustic myography (Barry, Geiringer, & Ball, 1985), vibromyography (Zhang, Frank, Rangayyan, & Bell, 1992), and also tensiomiography (TMG). Such alternative methods detect muscle fibre mechanical oscillations at its resonance frequency, thickening and vibration of a whole muscle belly from transversal plane of muscle actions. Several difficulties have been found for phonomyography and vibromyography (Orizio, 2002; Wong, 2001), whereas promising results were shown from TMG (Dahmane, et al., 2001; Šimunič, et al., 2011).

TMG is a non-invasive, selective and simple-to-use method that allows assessment of skeletal muscle contractile properties (Dahmane, et al., 2001; Gasparini, Sabovič, Gregorič, Šimunič, & Pišot, 2012; Pišot, et al., 2008; Šimunič, et al., 2008; Valenčič & Knez, 1997; Hunter, et al., 2012). Furthermore, the majority of TMG parameters are highly reliable (Ditroilo, Smith, Fairweather, & Hunter, 2013; Križaj, Šimunič, & Žagar, 2008; Šimunič, 2012), where half-relaxation time was found to have the lowest reliability; however, still acceptable: from .77 (Tous-Fajardo, et al., 2010) to .88 (Šimunič, 2012). Specifically, in the regression-based TMG validation to an accepted standard test only two studies compared the TMG derived parameters to: (i) myosin heavy chain of human vastus lateralis muscle (Šimunič, et al., 2011) and (ii) histochemical data of seven human skeletal muscles obtained in cadavers (Dahmane, et al., 2001). To the best of our knowledge, there are no relevant data about comparing the TMG derived parameters to muscle force or torque available from *in vivo* human experiments. In the literature, only one communication analysed the relationship between peak amplitude of TMG and torque twitch responses from *in vivo* human muscle and *in vitro* toad muscle (Šimunič, 2003).

Many studies revealed a high correlation between muscle force and MMG amplitude in voluntary contractions up to 80% of maximal voluntary contraction – MVC (Esposito, Malgrati, Veicsteinas, & Orizio, 1996; Matheson, et al., 1997; Maton, et al., 1990; Orizio, Perini, & Veicsteinas, 1989; Smith & Stokes, 1993; Zwarts & Keidel, 1991), whereas higher force exertions led to dispersed results. Other, time-based, contractile parameters were never compared between any of the MMG methods and force/torque responses.

TMG was not developed to assess voluntary muscle contraction, as other MMG methods were, and therefore a direct comparison to other MMG methods is not possible. However, TMG was designed to assess twitch muscle actions and, therefore, the aim of this study was to compare contractile parameters calculated from TMG and torque twitch responses in human vastus lateralis muscle.

Methods

Participants

The participants of this study were 19 healthy males. Their basic morphological parameters are presented in Table 1. Body composition was measured by bioelectrical impedance (BIA 101, Akern Bioresearch, Italy) according to the manufacturer's instructions. All participants met the inclusion criteria and had no major skeletal, neuromuscular or cardiovascular injuries or diseases. All testing procedures were fully explained to the participants and each participant was fully informed about possible risks and the nature of the experiments, and signed the informed consent. All testing procedures conformed to the 1964 Declaration of Helsinki and were approved by the Committee for Medical Ethics of the Slovenian Ministry of Health.

Table 1. Participant's morphological data

	M±SD
N	19
Age [years]	46.1±17.8
Body height [m]	1.74±0.05
Body mass [kg]	78.4±12.7
Body mass index [kg/m²]	26.0±4.2
Fat mass [kg]	17.1±6.1

Research design

Every participant was seated in a specially designed straight-back chair, in a relaxed position. Participant's shoulders, waist and lower leg were strapped to the chair. All measurements were performed on the left leg in a cross-sectional design. During the measurements, a 60° knee flexion (0° represents fully extended knee) was assured. The measurement of torque and TMG was simultaneous. Before the beginning of the measurement the procedure was explained in detail to the participants with 5-10 test electrical pulses for the familiarization.

Electrical stimulation

Two rounded self-adhesive electrodes (Axelgaard, Pulse) were placed on the skin above the vastus lateralis (VL). To elicit twitch contraction we used a single one-millisecond pulse applied through the cathode and the anode 5 cm distally and 5 cm proximally to the measuring point, respectively. If needed, the measuring point, sensor inclination, and electrode positions were adjusted to obtain the maximal response amplitude. Single monopolar rectangular pulses of one ms duration from the electro-stimulator (TMG-S1, Slovenia) were delivered to the electrodes. The protocol consisted of a series of graded pulses, separated by 10 second pauses, from motor threshold to maximal stimulation, in steps of 5 mA. Typical motor threshold and maximal stimu-lation amplitude were around 10 mA and 100 mA, respectively.

TMG measurements

Highly sensitive (1 μm) TMG displacement sensor (G40 digital-optical comparator, TMG-BMC Ltd., Slovenia) was placed perpendicular to the skin overlying the VL muscle belly. TMG measuring point was defined at 30% of femur length above the patella on the lateral side (Šimunič, et al., 2011). The tip of the sensor was pressed against the skin with an initial contact pressure of 77 N·mm^{-2}.

Torque measurements

An analogue force transducer (TSD121C, BIOPAC Systems Inc., USA) was mounted 38.0±2.3 cm distally from the axis of the knee rotation on the tibia. Force analogue output was sampled at a frequency of 2 kHz using a data acquisition system (MP100, BIOPAC Systems Inc., USA) connected with a personal computer. Force records were filtered (digital Butterworth filter, 50 Hz cut-off frequency).

Contractile parameters estimation

From every TMG and force twitch response, the maximal amplitude (Am), delay time (Td), contraction time (Tc), sustain time (Ts), and half relaxation time (Tr) were extracted (Figure 1). The maximal amplitude (Am) was defined as the peak amplitude of the twitch response; Td was defined as the time between the electrical stimulus and the time twitch response reached 1% of the Am; Tc was defined as the time twitch amplitude rises from 1% to 99% of the Am; Ts was defined as the time period within which twitch amplitude was above 50% of its Am; Tr was defined as the time needed for twitch amplitude to fall from 99% to 50% of its Am. Indexes $_D$ and $_T$ stand for displacement and torque twitch response, respectively. Only responses with the highest Am were taken into account for further analysis.

Statistical analysis

All statistical analyses were conducted using a software package SPSS for Windows and Microsoft Excel programme. Data are presented as means with standard deviations if not reported otherwise. All extracted parameters passed the normality test using Shapiro-Wilk procedure. One-way repeated measures ANOVA was used to establish differences in all contractile parameters between both methods and furthermore the effect size was used to report practical difference in units of standard deviations. Pearson's correlation coefficient was used to establish the relationship between the same contractile parameters detected with both methods. Statistical significance was set at .05.

Results

When the amplitude of electrical current increased, displacement reached its maximal values in all participants, and in 15 participants (in 79%) torque also reached its maximal values. Four participants did not reach the maximal torque because of discomforts due to electrical stimulation. Figure 1 presents TMG and torque-stacked twitch responses recorded in one representative participant (Figure 1A and B), as well as the graphic definition of the analysed parameters (Figure 1C). It can be noted that the peak displacement occurred substantially earlier (about 33 ms) in TMG (Figure 1A) than in torque (Figure 1B) recordings.

Table 2 presents average values for contractile parameters calculated from maximal twitch responses obtained by both methods. Thus, Td_D and Tc_D were 23.4% and 42.7% shorter (p<.001) than Td_T and Tc_T, respectively, whereas Tr_T was 26.2% shorter (p=.025) than Tr_D. There were no differences between Ts_D and Ts_T. By using Pearson's correlation

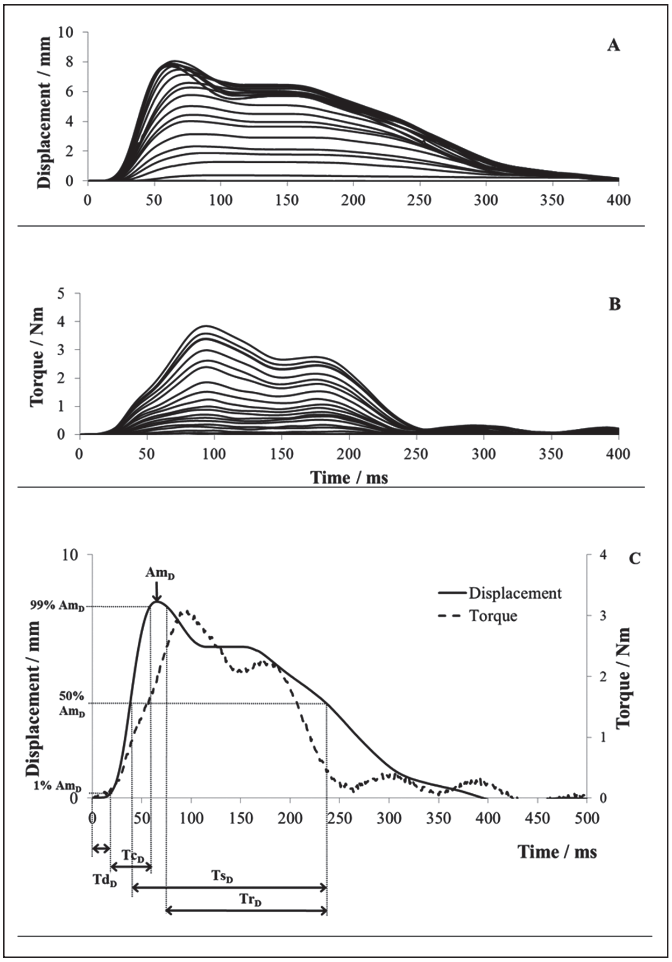

Figure 1. Stacked tensiomyographic (A) and torque (B) twitch responses from above threshold to maximal response where contractile parameters were calculated as defined in (C).

Table 2. Inter-method differences and Pearson's correlation coefficients of four time parameters calculated from tensiomyographic (TMG) and torque maximal twitch response

Methods/ Parameter	TMG	Torque	p_{DIFF}	ES_{DIFF}	Pearson's correlation coefficients	p_R
Delay time / ms	14.1±1.4	18.4±2.4	<.001	1.47	.084	.732
Contraction time / ms	38.6±5.0	67.4±4.5	<.001	1.88	.231	.340
Sustain time / ms	117.3±61.2	106.7±22.0	.204	/	.478	.038
Half relaxation time / ms	84.2±55.7	62.1±20.7	.025	/	.608	.006

ES – effect size

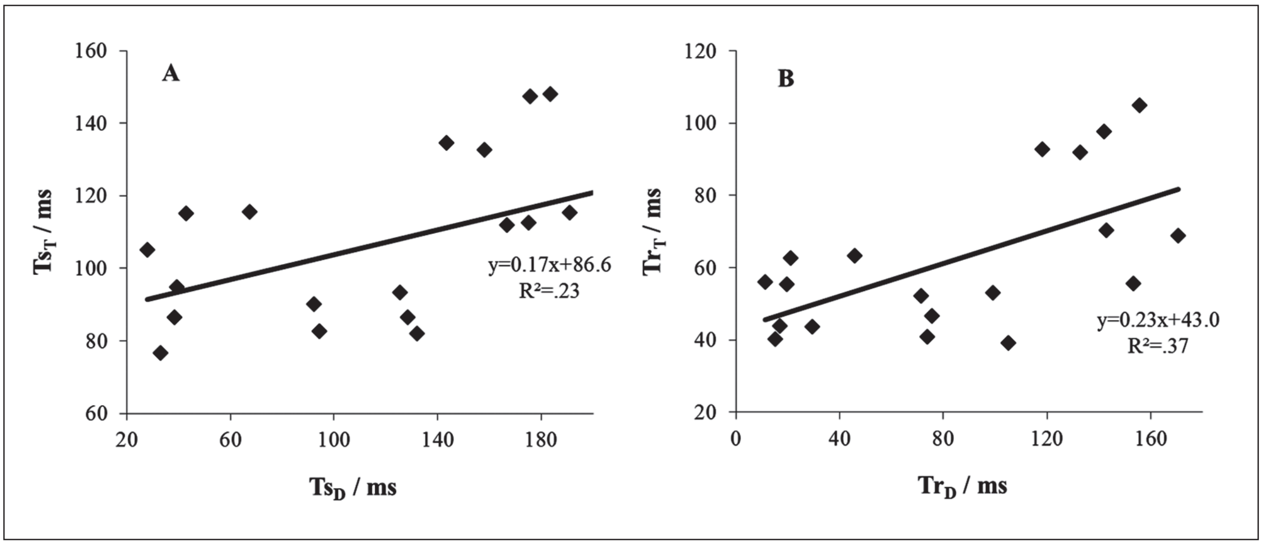

Figure 2. Inter-method correlations of (A) sustain time (Ts) and (B) half relaxation time (Tr).

coefficients, we did not find significant correlations either between Td_D and Td_T or between Tc_D and Tc_T. However, inter-method correlation was positive for the other two parameters, Ts and Tr (Figure 2).

Discussion and conclusions

This manuscript highlights, for the first time, the differences in twitch dynamics of torque and TMG mechanical responses. A previous publication (Šimunič, Križaj, Narici, & Pišot, 2010) presented recruitment dependence of twitch parameters on the stimulation amplitude and method (TMG or torque) being applied.

Šimunič et al. (2011) have demonstrated that Tc estimated from TMG twitch response is linearly and positively correlated to MHC-I proportion in human vastus lateralis. Distally detected muscle torque twitch response is also frequently used to analyse muscle contractile properties (Paasuke, Saapar, et al., 2007). However, this last approach has some limitation, as reported by Allen, Lee, and Westerblad (1989). In particular, the twitch stimulation might not release sufficient intracellular Ca^{2+} to uncover enough actin-myosin binding sites for the development and transmission of representative twitch force. Furthermore, twitch force exerted by a contracted muscle belly has to be transmitted through connective tissue to be measured by an external force transducer. Usually, there is already a slack of exerted muscle force present before the connective tissue is completely stretched. Therefore, representative muscle belly twitch force could not be measured unless longer lasting tetanic stimulation is applied (Hoyle, 1983; Kawakami & Lieber, 2000). Interestingly, TMG twitch response seems to be less affected by viscoelastic properties of muscle belly itself, connective tissue, joint mechanics, and surrounding tissues (Šimunič, 2003). In fact, using a second-order damped mathematical model (Šimunič, 2003), we calculated that the effect of mechanical damping and viscosity was 4.6 times greater when twitch response was transmitted in longitudinal (torque) than in transversal directions (displacement). This finding was an upgrade to previous mathematical representations of tendinous and skeletal muscle systems with the first-order system that acknowledge only elasticity. That clearly motivated us to demonstrate differences in time-based contractile parameters measured by both methods.

In 15 out of 19 participants displacement as well as torque twitch responses reached the maximal amplitude when electrical impulse was gradually increased. In four participants torque twitch response did not reach its maximal values because of the discomfort due to electrical stimulation. This did not interfere with the data. Our previous publication revealed that Tc, Td and Tr, when measured by TMG or as a torque, were stimulation amplitude dependent; however, only at very low stimulation amplitudes that elicit responses approximately up to 50% of peak values (Šimunič, et al., 2010). The motor units recruitment order plays an important role in justifying the stimulation amplitude. Some experiments support reversal motor unit activation order – according to the size principle (Heyters, Carpentier, Duchateau, & Hainaut, 1994; Trimble & Enoka, 2001), while others are rather conflicting (Binder-Macleod, Halden, & Jungles, 1995; Feiereisen, Duchateau, & Hainaut, 1997; Knaflitz, Merletti, & DeLuca, 1990). Just recently, Rodriguez-Falces and Page (2013) demonstrated that VL motor units were orderly recruited for femoral nerve stimulation, but followed no particular order for direct quadriceps stimulation in comparison to other muscles, where nerve size principle was followed.

Therefore, when we compared Tc extracted from the maximal or highest responses obtained by two methods, we could clearly see a significant difference: Tc ranged from 30.8 to 50.2 ms and from 54.3 to 72.4 ms for displacement and torque, respectively. This finding supports our discussion from the previous paragraph that shift-to-the-right in torque twitch response is present (with the effect size equalling 1.88) and furthermore suggests that displacement, as a measure of a mechanical twitch response, carries more intrinsic information about muscle contraction when elicited using electrical twitches. Similarly, Td showed significantly lower values for displacement in comparison to the torque twitch response (with the effect size equalling 1.47). Adding Td and Tc gives us time to peak estimation where we found that Am_D was reached 33.1 ms (39%; p<.001) earlier than Am_T.

Sustain time (Ts) was not different when estimated using the two methods and, consequently, Tr_T was found to be shorter to compensate for longer Td_T and Tc_T. Shorter Tr_T was also found by Šimunič et al. (2010) in human biceps brachii and also by Šimunič (2003) in isolated toad gastrocnemius muscle. Šimunič (2003) also demonstrated that Tr of force twitch is shorter when gastrocnemius tendon was included serially within a measured skeletal muscle system than when it was not. This demonstrates the role of the elastic energy stored in the tendon during the contracting phase that is later released during relaxation phase thus pulling sarcomeres to a resting position much quicker.

Interestingly, during the contraction phase we did not find any correlation either between Td_D and Td_T or between Tc_D and Tc_T. This finding supports the view that different mechanisms affect the propagation of twitch response in longitudinal and transversal directions, even though the muscle volume stays intact at contraction (Hill, 1948). It has been well documented (Dahmane, et al., 2001, 2005; Šimunič, et al., 2011) that Tc_D is correlated to the proportion of type 1 muscle fibers and to the MHC-I proportion. Given that lower viscoelastic properties allow quicker mechanical energy release in transversal direction, the result is a higher rate of muscle belly thickening than of its elongation. It is important to notice that we calculated contractile parameters differently than in other TMG studies (Šimunič, et al., 2011; Dahmane, et al., 2001, 2005), where the authors calculated Td as the time interval from the electrical impulse application to the achieved 10% of Am, Tc as the time interval from 10% to 90% of Am and Tr as the time from 90% to 50% Am. Therefore, direct comparisons of absolute values were not possible. By using a different technique, we followed an accepted definition for the calculation of contractile parameters that is valid for force/torque twitch signals.

TMG detects contractile properties directly on the skin above the skeletal muscle being observed, as all the other MMG methods. There is little effect of muscle/tendon tissue itself or surrounding tissue on the propagation of the mechanical twitch response in transversal direction. A previous study (Orizio, 1993) stated that the transversal (MMG) signal provides information on the intrinsic muscle mechanical activity. Although MMG is influenced by many factors of muscle morphology (Orizio, 1993) and physical milieu, such as intramuscular pressure, muscle stiffness, and osmotic pressure (Ouamer, Boiteux, Petitjean, Travens, & Sales, 1999), MMG reveals non-propagative la-teral thickening and vibration and as such can provide us with some notable advantages over electromyography (Xie, Guo, & Zheng, 2010) and, as we have demonstrated, also over torque. Thus, it was shown (Barry & Cole, 1988) that high velocity of the MMG response might be explained by the fact that the vibration of the actin-myosin coupling caused increased vibration of the myosin heads and/or turbulence of the intracellular or extracellular fluid mediums that is transmitted non-propagative to the skin surface to be detected by an MMG sensor. Therefore, such high sensitivity found in transversal muscle actions suggested that MMG may also provide information regarding motor unit firing rates (Akataki, Mita, Watakabe, & Itoh, 2003; Bichler, 2000; Bichler & Celichowski, 2001).

In conclusion, this study clearly demonstrated that the contractile parameters estimated from the TMG response are shorter during contraction phase, which is a proof of them being more related to intrinsic muscle properties. This is additionally supported by our previous study (Šimunič, et al., 2011) where we presented a multiple regression model that reliably estimated myosin heavy chain proportion from Td, Tc, and Tr TMG parameters. Although muscle mechanics has its origins in the measurements of force and torque responses, an alternative approach, such as TMG, gives us a complementary information on muscle contraction dynamics that offers several opportunities in the fields of research, diagnostics, rehabilitation, sport training, and ergonomics.

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Correspondence to:
Katja Koren, B.Sc.
Science and Research Centre of Koper
Institute for Kinesiology Research, University of Primorska
Garibaldijeva 1, SI-6000 Koper, Slovenia
Phone: +386 05 66 37 700
Fax: +386 05 66 37 710
E-mail: Katja.Koren@zrs.upr.si

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