Compound Maximal Motor Unit Response is modulated by contraction intensity, but not contraction type in Tibialis Anterior.

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Key words: eccentric contractions, electromyography, M_{MAX}, peripheral nerve stimulation.

Running Title: M_{MAX} modulation at varying contraction in intensities and types.

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What is the central question of this study?

The maximal motor unit response (M_{MAX}) is used as a reference value when quantifying electromyographic (EMG) raw signals. However, the appropriate use of M_{MAX} as a reference during varying contraction intensities and type is not clear.

What is the main finding and its importance?

M_{MAX} should be recorded at specific contraction intensity but not necessarily a specific contraction type. The guidelines presented here impact the practices of researchers and clinicians using EMG.

Abstract

Determining a single compound maximal motor response (M_{MAX}) or an average superimposed M_{MAX} response (M_{SUP}) are commonly used reference values in experiments eliciting raw electromyographic, motor evoked potentials, H-reflexes and V-waves. However, existing literature is limited in detailing the most appropriate method to normalise these electrophysiological measures. Due to the accessibility of assessment from a cortical and spinal perspective, the tibialis anterior is increasingly used in literature and hence investigated in this study. The aims of the present study were to examine the differences and level of agreement in M_{MAX}/M_{SUP} under different muscle actions and contraction intensities. Following a familiarisation session, 22 males visited the laboratory on a single occasion. M_{MAX} was recorded under 10% isometric and 25% and 100% shortening and lengthening maximal voluntary contractions (MVC) at an angular velocity of $15^{\circ -1}$. M_{SUP} was also recorded during 100% shortening and lengthening with an average of five responses recorded. There were no differences in M_{MAX} or M_{SUP} between contraction types. All variables showed large, positive correlations ($P < 0.001$, $r^2 \geq 0.64$). M_{MAX} amplitude was larger ($P < 0.001$) at 100% shortening and lengthening intensity compared to M_{MAX} amplitude at 10% isometric and 25% lengthening MVC. Bland-Altman plots revealed a bias towards higher M_{MAX} at the higher contraction intensities. Despite M_{SUP} being significantly smaller than M_{MAX} ($P < 0.001$) at 100% MVC, M_{SUP} showed a large positive correlation ($P < 0.001$, $r^2 \geq 0.64$) with all variables. It is our recommendation that M_{MAX} should be recorded at specific contraction intensity but not necessarily a specific contraction type.

Introduction

Electromyographic (EMG) signals are affected by numerous factors such as preparation of the skin, electrode placement, fibre type and orientation (De Luca, 1997). It is therefore critical that the EMG signal is normalised to a reference value so data can be interpreted meaningfully. Applying supramaximal electrical stimulation to a peripheral nerve causes synchronous activation of the muscle fibres and is known as the maximal motor unit response (M_{MAX}; (Lee & Carroll, 2005). Investigations using peripherally evoked measures such as the Hoffman-reflex (H-reflex) and V-wave, along with cortically evoked measures such as motor evoked potentials (MEP), lateral spread MEP and cervicomedullary MEP commonly use M_{MAX} as a reference value (Aagaard *et al.*, 2002; Yamashita *et al.*, 2002; Kidgell & Pearce, 2010; Tallent *et al.*, 2012a). These spinal and corticospinal measures have been investigated under a variety of conditions such as changing muscle lengths, at rest and during submaximal and maximal contractions (Arányi *et al.*, 1998; Goodall *et al.*, 2009; Howatson *et al.*, 2011; Tallent *et al.*, 2012a). Understanding how M_{MAX} is modulated in different muscles, contraction intensities and types is vital in ensuring EMG is presented in the most appropriate manner.

The M_{MAX} amplitude has been shown to increase with increasing contraction intensity in the tibialis anterior (Nagata & Christianson, 1995; Frigon *et al.*, 2007) and soleus (Frigon *et al.*, 2007), but remain unchanged in quadriceps muscles (Linnamo *et al.*, 2001a) and the flexor carpi radialis (Lee & Carroll, 2005), or even decreases in the quadriceps (Linnamo *et al.*, 2001b). In addition, M_{MAX} has been shown to increase (Gerilovsky *et al.*, 1977; Gerilovsky *et al.*, 1989; Frigon *et al.*, 2007) and decrease when recorded at longer muscle lengths (Marsh *et al.*, 1981; Kim *et al.*, 2005; Lee & Carroll, 2005). Furthermore there are conflicting findings in TA with regards to how M_{MAX} alters with changing length (Marsh *et al.*, 1981; Frigon *et al.*, 2007). Higher contraction intensities will cause the muscle to shorten and tendon become more compliant (Griffiths, 1991). A reduction in muscle length has been shown to cause an increase in synchronisation and consequently an increase in M_{MAX} (Kim *et al.*, 2005). Alternatively, phase cancellation of EMG will increase with increasing contraction intensity and may mute the response in the muscle (Keenan *et al.*, 2006; Farina *et al.*, 2008). Therefore, understanding M_{MAX} response at differing contraction intensities is essential from a clinical and research perspective.

Changes in muscle length might also influence M_{MAX} values during shortening and lengthening contractions. It has been recommended (Zehr, 2002) that M_{MAX} is expressed relative to the specific muscle action (i.e., shortening or lengthening muscle actions). However, evidence has shown no difference between M_{MAX} amplitude when recorded during shortening and lengthening actions (Linnamo *et al.*, 2001b; Duclay & Martin, 2005). Ensuring the reference values are recorded to a standardised muscle length appears essential in the interpretation of EMG signals.

V-wave reflects the efferent neural output during voluntary muscle activation (Aagaard *et al.*, 2002). In the literature, V-wave is expressed relative to a mean M-wave (M_{SUP}) during a number of maximal contractions, (Aagaard *et al.*, 2002; Duclay & Martin, 2005; Gondin *et al.*, 2006) or maximal peak-to-peak M_{MAX} amplitude from the same number of responses (Tallent *et al.*, 2012b; Tallent *et al.*, 2013). Due to the increased potential for phase cancellation at higher contraction intensities it is unclear how these different reference values (M_{MAX} or M_{SUP}) affect the outcome and interpretation of the V-wave. Investigating how M_{MAX} is modulated under different muscle actions, and at varying contraction intensities might provide helpful methodological evidence for the use of M_{MAX} in experimental paradigms where neurophysiological parameters require normalisation.

Therefore, the aim of this study was to investigate changes in M_{MAX} under a variety of contraction modes and intensities and examine M_{SUP} during maximal shortening and lengthening contractions in the TA. The results from this study will provide guidance for researchers in the use of M_{MAX} as a reference value.

Methods

Participants

Based on previous work (Kim *et al.*, 2005) examining greater M_{MAX} amplitudes during higher contraction intensities (12%; Cohen's d = 0.45), a total of 22 participants were recruited for the study to achieve a statistical power of 0.8 with an alpha level of 0.05. Following institutional (Northumbria University) ethical approval, 22 males (mean ± SD, age 23 ± 3 years, stature 178.0 ± 7.0 cm, mass 83.1 ± 9.3 kg) volunteered to participate. After being fully briefed on the experimental protocol and screened for contraindications to the procedures, volunteers provided written informed consent.

General Procedure

Two identical trials were completed, on two consecutive days at the same time of day, with the first trial used to familiarise the participants with the procedures as based on previous recommendations by our laboratory (Tallent *et al.*, 2012a). All contractions were performed on an isokinetic dynamometer (Cybex Norm, Cybex International, NY) that was set up for ankle dorsiflexion of the dominant limb. Footedness was assessed using the questionnaire from Hebbal and Mysorekar (2006). The foot was strapped into an ankle adaptor and the knee was secured into a thigh stabiliser to prevent any extraneous movements. The hip, knee and ankle were set at joint angles of 90, 120 and 90°, respectively, according to the manufacturer's instructions. Shortening and lengthening contractions consisted of participants moving through a range of 30° (± 15° from the ankle at 90°) at an angular velocity of $15°\cdot s^{-1}$. Shortening and lengthening contractions began at an ankle angle of 105° and 75° respectively. For shortening muscle actions, participants were instructed to assist the movement of the foot adaptor, and for lengthening the actions required participants to resist movement of the foot adaptor. All responses (torque and EMG) were recorded as the ankle joint passed through anatomical zero (90°). To ensure torque and EMG were recorded at the correct angle, a trigger was set to automatically sweep as the ankle passed 90°. Once secured in the isokinetic dynamometer, participants initially performed shortening, lengthening and isometric MVCs. The highest torque in each muscle action (shortening, lengthening and isometric) from three trials was recorded as the contraction-specific MVC.

The M_{MAX} was recorded at 10% of isometric, 25% and 100% shortening and lengthening MVC. A 10% isometric contraction is often used to stabilise the H-reflex in the TA (Griffin & Cafarelli, 2007; Tallent et al., 2012a), and consequently this was considered the resting M_{MAX} value. The simulation intensity for eliciting M_{MAX} was set at 150% above a plateau in peak-to-peak M_{MAX} amplitude. This was recorded through an increasing stimulation intensity at 10% isometric MVC and verified during 25% shortening and lengthening contractions. Establishing M_{MAX} took around 64 gradually increasing intensity pulses at 10% isometric MVC. M_{SUP} was calculated from the average of 5 traces at 100% shortening and lengthening MVC, whilst M_{MAX} during a maximal contraction was recorded as the greatest peak-to-peak amplitude of the 5 contractions. The order of contraction intensity (10%, 25%, 100%) and type (shortening and lengthening) was randomised.

Percutaneous Nerve Stimulation

Searching for optimal site of stimulation began below the head of the fibula, over the peroneal nerve. A 1 ms electrical stimulation was administrated using a 40 mm diameter cathode/anode arrangement (Digitimer DS7AH, Welwyn Garden City, Hertfordshire, UK). Once the optimal site was located, the sight was marked with semi-permanent ink. The cathode/anode was strapped to the participants leg for the entirety of the experiment.

EMG

Bipolar surface EMG was recorded over the TA using electrodes (22 mm diameter, model; Kendall, Tyco Healthcare Group, Mansfield, MA, USA) spaced 2 cm apart. The reference electrode was placed over the medial malleolus, whilst the TA electrodes were placed at one-third distance of the line between the tip of the fibula and the tip of the medial malleolus (Hermens *et al.*, 2000). All sites were shaved, abraded and then wiped clean with an alcohol swab prior to electrode placement. The EMG was amplified (×1000), band pass filtered (10-1,000 Hz) and sampled at 5 kHz (CED Power 1401, Cambridge Electronic Design, Cambridge, UK). M-waves were recorded during a 500 ms window, starting 50 ms before anatomical zero. Once M_{MAX} stimulator was established, all further analyses were performed off-line.

Torque

To ensure that participants reached the required target torque level, real time feedback was provided on a computer monitor positioned 1 m away. Live feedback was displayed on the monitor of the dynamometer to provide feedback on target forces to achieve during each condition. The torque signal was sampled at 5 kHz, extracted from the dynamometer and synchronized with the EMG signal and analysed off line (Signal v3.0, Cambridge Electronics, Cambridge, UK).

Statistics

A one-way ANOVA was used to detect differences between M_{MAX} at 10% isometric MVC, 25%, 100% and M_{SUP} at 100% shortening and lengthening MVC. Where necessary, LSD *post-hoc* analysis was used to make pairwise comparisons with 95% CI (SPSS, v20.0, Chicago, Illinois, USA). Coefficient of determination and the limits of agreement (Bland & Altman, 1986) with 95% CI were also calculated between the variables (GraphPad Software Inc, La Jolla, CA, USA). Correlation coefficients were determined as 0.0–0.1 = trivial, 0.10–0.3, small, 0.3–0.5 = moderate, 0.5–0.7 = large, and 0.7–0.9 = very large (Hopkins, 2009). Effect sizes (η2) were defined as: 0.2 trivial, 0.21–0.6 = small, 0.61–1.2 = moderate, 1.21–1.99 = large; > 2.0 = very large.

Results

Isometric contractions were conducted at an average of 8.28 ± 3.21% (target = 10%) of isometric MVC, shortening at 26.1 ± 3.66% (target = 25%), 95.6 ± 11.8% (target = 100%) of shortening MVC and lengthening at 27.1 ± 4.12% (target = 25%), 96.2 ± 9.97% (target = 100%) of lengthening MVC. There was no significant difference ($P > 0.05$) between lengthening and shortening contraction intensities, showing contractions were conducted at the same relative intensity.

Figure 1 shows individual and average M_{MAX}/M_{SUP} amplitudes during varying isometric, shortening and lengthening contractions intensities and a reprehensive trace. The ANOVA revealed there were significant differences in M_{MAX} amplitude between conditions ($F_{(6)}= 6.96$: $P<0.001$; η2 = 0.25). *Post Hoc* analysis showed 10% isometric M_{MAX} MVC was significantly lower than 25% shortening M_{MAX} ($P = 0.03$; 95% CI; −0.03 to −0.69 mV), 25% lengthening M_{MAX} ($P = 0.03$; 95% CI; −0.05 to −0.64 mV), 100% shortening M_{MAX} ($P < 0.01$; 95% CI; −0.40 to −1.32 mV), 100% lengthening M_{MAX} ($P < 0.01$; 95% CI; −0.37 to −1.32 mV). M_{MAX} was significantly higher at 100% shortening ($P = 0.02$; 95% CI; 0.11 to 1.03 mV) and 100% lengthening ($P = 0.02$; 95% CI; 0.08 to 1.03) compared to 25% lengthening M_{MAX}.

All M_{MAX} amplitudes were significantly ($P < 0.001$) correlated across intensities (r^2, ≥0.64). The highest correlations were between contraction types at the same intensity with M_{MAX} (100% MVC $r^2 = 0.87$; 25% MVC $r^2 = 0.86$). Bland-Altman plots showed a bias towards higher M_{MAX} values at higher contraction intensities (Figure 2). There was no bias between shortening and lengthening contractions. Similarly, isometric M_{MAX} and M_{SUP} showed no bias.

Discussion

It has been reported that EMG should be normalised to M_{MAX} under the same muscle action and contraction intensity (Zehr, 2002; Duclay & Martin, 2005). This study offers further insight into the influence that contraction conditions may affect the amplitude of M_{MAX} amplitude. Specifically, the main findings were, 1) there was no difference between M_{MAX} amplitudes when recorded at like-intensities during shortening and lengthening contractions; 2) M_{MAX} was influenced by intensity of the contraction, with an increase and systematic bias to an increase M_{MAX} during higher intensity contractions; and 3) M_{MAX} at 100% MVC was greater compared to M_{SUP} at 100% MVC. However, M_{SUP} was not different to M_{MAX} at 10% MVC, showing little systematic bias and was strongly correlated ($r^2 \geq 0.64$).

It has been recommended when using M_{MAX} as a reference value, it should be recorded under the same contraction intensity as the variable being investigate (Zehr, 2002). The results in this study indicated that with increased contraction intensity the peak-to-peak M_{MAX} amplitude increased. Previous work has shown similar results in contraction intensities ranging from 40-80% isometric MVC in TA (Nagata &

Christianson, 1995) and 10-30% isometric MVC in TA and the soleus (Frigon *et al.*, 2007). Although this effect is reported previously and supported by the current study, the exact mechanisms for this remains unclear. Frigon *et al.* (2007) suggested that M_{MAX} increased at higher contraction intensities because the muscle length has be shown to be up to 28% shorter at the same joint angle (Griffiths, 1991); and thus, could improve the synchronisation of the action potential. However, contrary to our findings, other authors have reported no change (Linnamo *et al.*, 2001a; Lee & Carroll, 2005) or even a decrease (Linnamo *et al.*, 2001b) in M_{MAX} with increasing contraction intensities. The high degree of variability between subjects might explain why the literature offers little consistency (Lee & Carroll, 2005). In addition, phase cancellation has shown to reduce the EMG response at the muscle during higher contraction intensities (Keenan *et al.*, 2006; Farina *et al.*, 2008). If the responses in EMG are muted at higher contraction intensities then the lack of change in M_{MAX} amplitude appears to be associated with the limitations in surface EMG recording (Farina *et al.*, 2014).

Our results support previous findings that showed no difference in M_{MAX} under shortening and lengthening contractions (Linnamo *et al.*, 2001b; Duclay & Martin, 2005) when measured at the same joint angle. Supramaximal stimulation of the peripheral nerve at shorter muscle lengths improves the synchronisation of the action potential (Kim *et al.*, 2005). With an enhanced synchronisation of the action potential there is an increase in M_{MAX} (Kim *et al.*, 2005). However, the varying pennation angle of muscles might explain why not all studies have found increases in M_{MAX} at shorter muscle lengths (Gerilovsky *et al.*, 1977; Gerilovsky *et al.*, 1989; Frigon *et al.*, 2007).

Furthermore, it is expected that an increase in M_{MAX} at shorter muscle lengths should be associated with decreased duration of M_{MAX}, although this is not consistently observed (Frigon *et al.*, 2007). In our study, M_{MAX} was recorded during shortening and lengthening muscle contractions and importantly, electrical stimulation was delivered at the same joint angle, with the assumption that the muscle was at the same length. Furthermore, current data also showed a strong positive correlation, and a good level of agreement, between M_{MAX} during shortening and lengthening muscle actions. Thus, it appears that EMG signals do not necessarily need to be expressed relative to a contraction specific M_{MAX}, rather, the joint angle should be consistent (Nagata & Christianson, 1995; Kim *et al.*, 2005; Frigon *et al.*, 2007). A high level of agreement and a strong correlation was found between shortening and lengthening muscle actions, despite lengthening muscle actions generating a higher level of absolute torque. The differences in M_{MAX} at an 'absolute' torque might explain why there is a small discrepancy between shortening and lengthening M_{MAX} at the same relative intensity.

Unlike MEP's, H-reflex and EMG signals, V-wave is expressed relative to an M_{SUP} (Aagaard *et al.*, 2002; Duclay & Martin, 2005; Gondin *et al.*, 2006) or M_{MAX} (Tallent *et al.*, 2012b; Tallent *et al.*, 2013). In this study, there was no difference in M_{MAX} at a low intensity contraction (≤ 25%) and M_{SUP}. This would suggest that EMG/V-waves recorded during an MVC could be expressed relative to a low intensity M_{MAX} contraction. There was also good level of agreement between M_{SUP} and M_{MAX} at low intensity contractions suggesting these values could be used interchangeably, although in the interest of rigor, it would be sensible to use a single well controlled M_{MAX} measure to normalise all conditions.

Conclusion

The results from this study show that M_{MAX} is not altered by shortening or lengthening contraction type, but is modulated with changes in contraction intensity. Possible mechanisms may be due to the shortened muscle lengths at the higher contraction intensities. M_{MAX} should be used relative to task specific contraction intensities and it is vital that it is recorded under consistent reproducible conditions. No differences were seen between M_{MAX} at low intensity contractions and M_{SUP} at 100% MVC. There was also low systematic bias and strong correlations suggesting V-wave can be expressed relative to M_{MAX} recorded during low intensity contractions or M_{SUP} at 100% MVC. It is our recommendation that M_{MAX} should be recorded at specific contraction intensities but not necessarily a specific contraction type. However, consistency of M_{MAX} recording throughout the experiment is vital.

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Competing interests

The authors have no competing interests to declare, financial or otherwise.

Funding

No funding was received for this study.

Acknowledgements

No acknowledgements

Author Contributions

Experiments were performed at Northumbria University. JT, SG and GH designed the study protocol; JT and SG acquired the data; JT, SG, DK, RD and GH analysed and interpreted the data; JT, SG, DK, RD and GH drafted or revised the final manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons listed qualify for authorship.

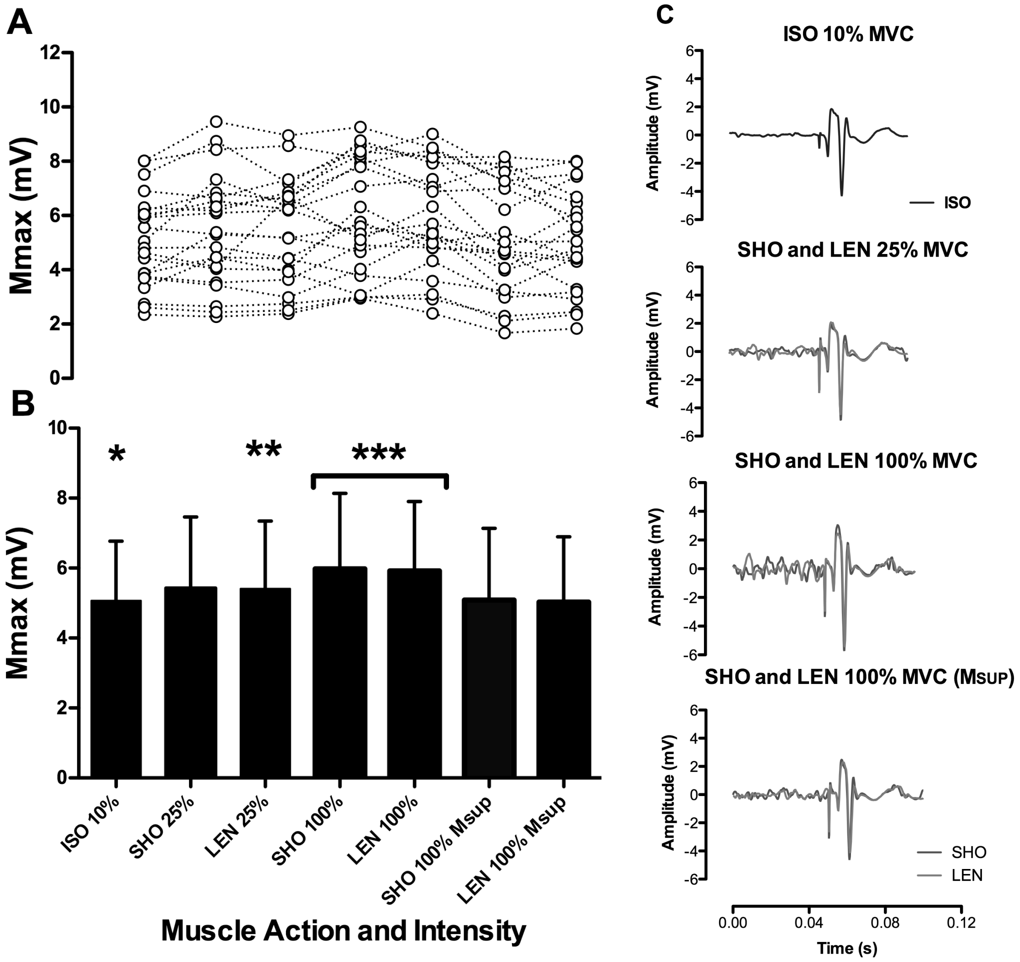

Figure 1. Clear dots represent individual responses at different M_{MAX} contraction intensities and contraction types (**A**). Bars represent mean M_{MAX} and M_{SUP} responses (mean ± SD) (**B**). Representative trace from a single participant of M_{MAX} recorded at 10% ISO, SHO and LEN 25% and 100% MVC, SHO and LEN M_{SUP} (**C**). ISO = Isometric, SHO = Shortening, LEN = lengthening; *denotes significantly ($P < 0.05$) different from 25% and 100%, SHO and LEN MVC M_{MAX}; **denotes significantly different from 100% SHO and LEN M_{MAX}; ***denotes significantly different from SHO and LEN M_{SUP}.

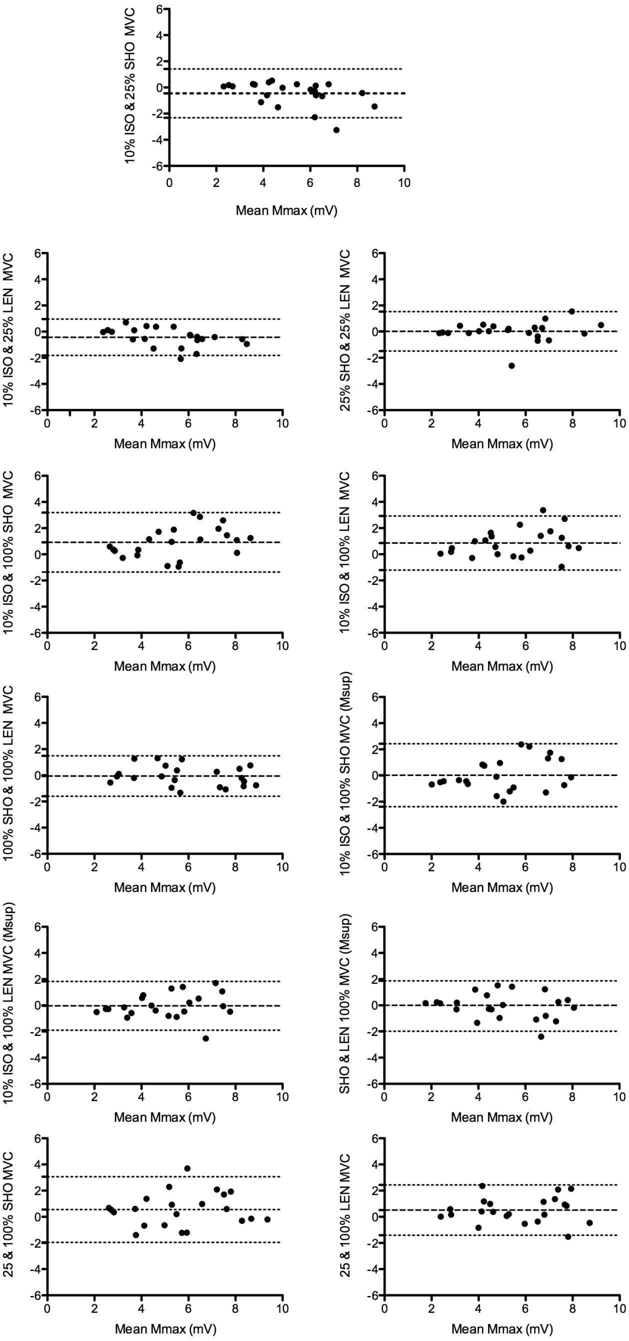

Figure 2. Bland-Altman plots for M_{MAX} and M_{SUP} (mV) across varying contraction intensities and type. Dashed line indicated change in mean with 95% confidence intervals. Dots represent individual responses.