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

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10

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

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

14

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31 **What is the central question of this study?**

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

35

36 **What is the main finding and its importance?**

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

40

41

42

43 Abstract

44 Determining a single compound maximal motor response (M_{MAX}) or an average
45 superimposed M_{MAX} response (M_{SUP}) are commonly used reference values in
46 experiments eliciting raw electromyographic, motor evoked potentials, H-reflexes and
47 V-waves. However, existing literature is limited in detailing the most appropriate
48 method to normalise these electrophysiological measures. Due to the accessibility of
49 assessment from a cortical and spinal perspective, the tibialis anterior is increasingly
50 used in literature and hence investigated in this study. The aims of the present study
51 were to examine the differences and level of agreement in M_{MAX}/M_{SUP} under different
52 muscle actions and contraction intensities. Following a familiarisation session, 22
53 males visited the laboratory on a single occasion. M_{MAX} was recorded under 10%
54 isometric and 25% and 100% shortening and lengthening maximal voluntary
55 contractions (MVC) at an angular velocity of $15^{\circ}\cdot s^{-1}$. M_{SUP} was also recorded during
56 100% shortening and lengthening with an average of five responses recorded. There
57 were no differences in M_{MAX} or M_{SUP} between contraction types. All variables showed
58 large, positive correlations ($P < 0.001$, $r^2 \geq 0.64$). M_{MAX} amplitude was larger ($P <$
59 0.001) at 100% shortening and lengthening intensity compared to M_{MAX} amplitude at
60 10% isometric and 25% lengthening MVC. Bland-Altman plots revealed a bias towards
61 higher M_{MAX} at the higher contraction intensities. Despite M_{SUP} being significantly
62 smaller than M_{MAX} ($P < 0.001$) at 100% MVC, M_{SUP} showed a large positive correlation
63 ($P < 0.001$, $r^2 \geq 0.64$) with all variables. It is our recommendation that M_{MAX} should be
64 recorded at specific contraction intensity but not necessarily a specific contraction
65 type.

66

67 Introduction

68 Electromyographic (EMG) signals are affected by numerous factors such as
69 preparation of the skin, electrode placement, fibre type and orientation (De Luca,
70 1997). It is therefore critical that the EMG signal is normalised to a reference value so
71 data can be interpreted meaningfully. Applying supramaximal electrical stimulation to
72 a peripheral nerve causes synchronous activation of the muscle fibres and is known
73 as the maximal motor unit response (M_{MAX} ; (Lee & Carroll, 2005). Investigations using
74 peripherally evoked measures such as the Hoffman-reflex (H-reflex) and V-wave,
75 along with cortically evoked measures such as motor evoked potentials (MEP), lateral
76 spread MEP and cervicomedullary MEP commonly use M_{MAX} as a reference value
77 (Aagaard *et al.*, 2002; Yamashita *et al.*, 2002; Kidgell & Pearce, 2010; Tallent *et al.*,
78 2012a). These spinal and corticospinal measures have been investigated under a
79 variety of conditions such as changing muscle lengths, at rest and during submaximal
80 and maximal contractions (Arányi *et al.*, 1998; Goodall *et al.*, 2009; Howatson *et al.*,
81 2011; Tallent *et al.*, 2012a). Understanding how M_{MAX} is modulated in different
82 muscles, contraction intensities and types is vital in ensuring EMG is presented in the
83 most appropriate manner.

84

85 The M_{MAX} amplitude has been shown to increase with increasing contraction intensity
86 in the tibialis anterior (Nagata & Christianson, 1995; Frigon *et al.*, 2007) and soleus
87 (Frigon *et al.*, 2007), but remain unchanged in quadriceps muscles (Linnamo *et al.*,
88 2001a) and the flexor carpi radialis (Lee & Carroll, 2005), or even decreases in the
89 quadriceps (Linnamo *et al.*, 2001b). In addition, M_{MAX} has been shown to increase
90 (Gerilovsky *et al.*, 1977; Gerilovsky *et al.*, 1989; Frigon *et al.*, 2007) and decrease

91 when recorded at longer muscle lengths (Marsh *et al.*, 1981; Kim *et al.*, 2005; Lee &
92 Carroll, 2005). Furthermore there are conflicting findings in TA with regards to how
93 M_{MAX} alters with changing length (Marsh *et al.*, 1981; Frigon *et al.*, 2007). Higher
94 contraction intensities will cause the muscle to shorten and tendon become more
95 compliant (Griffiths, 1991). A reduction in muscle length has been shown to cause an
96 increase in synchronisation and consequently an increase in M_{MAX} (Kim *et al.*, 2005).
97 Alternatively, phase cancellation of EMG will increase with increasing contraction
98 intensity and may mute the response in the muscle (Keenan *et al.*, 2006; Farina *et al.*,
99 2008). Therefore, understanding M_{MAX} response at differing contraction intensities is
100 essential from a clinical and research perspective.

101

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

109

110 V-wave reflects the efferent neural output during voluntary muscle activation (Aagaard
111 *et al.*, 2002). In the literature, V-wave is expressed relative to a mean M-wave (M_{SUP})
112 during a number of maximal contractions, (Aagaard *et al.*, 2002; Duclay & Martin,
113 2005; Gondin *et al.*, 2006) or maximal peak-to-peak M_{MAX} amplitude from the same
114 number of responses (Tallent *et al.*, 2012b; Tallent *et al.*, 2013). Due to the increased

115 potential for phase cancellation at higher contraction intensities it is unclear how these
116 different reference values (M_{MAX} or M_{SUP}) affect the outcome and interpretation of the
117 V-wave. Investigating how M_{MAX} is modulated under different muscle actions, and at
118 varying contraction intensities might provide helpful methodological evidence for the
119 use of M_{MAX} in experimental paradigms where neurophysiological parameters require
120 normalisation.

121

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

126

127 **Methods**

128 *Participants*

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

136

137

138

139 *General Procedure*

140 Two identical trials were completed, on two consecutive days at the same time of day,
141 with the first trial used to familiarise the participants with the procedures as based on
142 previous recommendations by our laboratory (Tallent *et al.*, 2012a). All contractions
143 were performed on an isokinetic dynamometer (Cybex Norm, Cybex International, NY)
144 that was set up for ankle dorsiflexion of the dominant limb. Footedness was assessed
145 using the questionnaire from Hebbal and Mysorekar (2006). The foot was strapped
146 into an ankle adaptor and the knee was secured into a thigh stabiliser to prevent any
147 extraneous movements. The hip, knee and ankle were set at joint angles of 90, 120
148 and 90°, respectively, according to the manufacturer's instructions. Shortening and
149 lengthening contractions consisted of participants moving through a range of 30° (\pm
150 15° from the ankle at 90°) at an angular velocity of 15°·s⁻¹. Shortening and lengthening
151 contractions began at an ankle angle of 105° and 75° respectively. For shortening
152 muscle actions, participants were instructed to assist the movement of the foot
153 adaptor, and for lengthening the actions required participants to resist movement of
154 the foot adaptor. All responses (torque and EMG) were recorded as the ankle joint
155 passed through anatomical zero (90°). To ensure torque and EMG were recorded at
156 the correct angle, a trigger was set to automatically sweep as the ankle passed 90°.

157 Once secured in the isokinetic dynamometer, participants initially performed
158 shortening, lengthening and isometric MVCs. The highest torque in each muscle
159 action (shortening, lengthening and isometric) from three trials was recorded as the
160 contraction-specific MVC.

161

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

174

175 *Percutaneous Nerve Stimulation*

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

182

183 *EMG*

184 Bipolar surface EMG was recorded over the TA using electrodes (22 mm diameter,
185 model; Kendall, Tyco Healthcare Group, Mansfield, MA, USA) spaced 2 cm apart. The

186 reference electrode was placed over the medial malleolus, whilst the TA electrodes
187 were placed at one-third distance of the line between the tip of the fibula and the tip of
188 the medial malleolus (Hermens *et al.*, 2000). All sites were shaved, abraded and then
189 wiped clean with an alcohol swab prior to electrode placement. The EMG was
190 amplified ($\times 1000$), band pass filtered (10-1,000 Hz) and sampled at 5 kHz (CED Power
191 1401, Cambridge Electronic Design, Cambridge, UK). M-waves were recorded during
192 a 500 ms window, starting 50 ms before anatomical zero. Once M_{MAX} stimulator was
193 established, all further analyses were performed off-line.

194

195 *Torque*

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

202

203 *Statistics*

204 A one-way ANOVA was used to detect differences between M_{MAX} at 10% isometric
205 MVC, 25%, 100% and M_{SUP} at 100% shortening and lengthening MVC. Where
206 necessary, LSD *post-hoc* analysis was used to make pairwise comparisons with 95%
207 CI (SPSS, v20.0, Chicago, Illinois, USA). Coefficient of determination and the limits of
208 agreement (Bland & Altman, 1986) with 95% CI were also calculated between the
209 variables (GraphPad Software Inc, La Jolla, CA, USA). Correlation coefficients were

210 determined as 0.0–0.1 = trivial, 0.10–0.3, small, 0.3–0.5 = moderate, 0.5–0.7 = large,
211 and 0.7–0.9 = very large (Hopkins, 2009). Effect sizes (η^2) were defined as: 0.2 trivial,
212 0.21–0.6 = small, 0.61–1.2 = moderate, 1.21–1.99 = large; > 2.0 = very large.

213

214 **Results**

215 Isometric contractions were conducted at an average of $8.28 \pm 3.21\%$ (target = 10%)
216 of isometric MVC, shortening at $26.1 \pm 3.66\%$ (target = 25%), $95.6 \pm 11.8\%$ (target
217 = 100%) of shortening MVC and lengthening at $27.1 \pm 4.12\%$ (target = 25%), $96.2 \pm$
218 9.97% (target = 100%) of lengthening MVC. There was no significant difference ($P >$
219 0.05) between lengthening and shortening contraction intensities, showing
220 contractions were conducted at the same relative intensity.

221

222 Figure 1 shows individual and average $M_{\text{MAX}}/M_{\text{SUP}}$ amplitudes during varying
223 isometric, shortening and lengthening contractions intensities and a representative
224 trace. The ANOVA revealed there were significant differences in M_{MAX} amplitude
225 between conditions ($F_{(6)} = 6.96$; $P < 0.001$; $\eta^2 = 0.25$). *Post Hoc* analysis showed 10%
226 isometric M_{MAX} MVC was significantly lower than 25% shortening M_{MAX} ($P = 0.03$; 95%
227 CI; -0.03 to -0.69 mV), 25% lengthening M_{MAX} ($P = 0.03$; 95% CI; -0.05 to -0.64 mV),
228 100% shortening M_{MAX} ($P < 0.01$; 95% CI; -0.40 to -1.32 mV), 100% lengthening M_{MAX}
229 ($P < 0.01$; 95% CI; -0.37 to -1.32 mV). M_{MAX} was significantly higher at 100%
230 shortening ($P = 0.02$; 95% CI; 0.11 to 1.03 mV) and 100% lengthening ($P = 0.02$; 95%
231 CI; 0.08 to 1.03) compared to 25% lengthening M_{MAX} .

232

233 All M_{MAX} amplitudes were significantly ($P < 0.001$) correlated across intensities (r^2 ,
234 ≥ 0.64). The highest correlations were between contraction types at the same intensity
235 with M_{MAX} (100% MVC $r^2 = 0.87$; 25% MVC $r^2 = 0.86$). Bland-Altman plots showed a
236 bias towards higher M_{MAX} values at higher contraction intensities (Figure 2). There was
237 no bias between shortening and lengthening contractions. Similarly, isometric M_{MAX}
238 and M_{SUP} showed no bias.

239

240 **Discussion**

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

251

252 It has been recommended when using M_{MAX} as a reference value, it should be
253 recorded under the same contraction intensity as the variable being investigate (Zehr,
254 2002). The results in this study indicated that with increased contraction intensity the
255 peak-to-peak M_{MAX} amplitude increased. Previous work has shown similar results in
256 contraction intensities ranging from 40-80% isometric MVC in TA (Nagata &

257 Christianson, 1995) and 10-30% isometric MVC in TA and the soleus (Frigon *et al.*,
258 2007). Although this effect is reported previously and supported by the current study,
259 the exact mechanisms for this remains unclear. Frigon *et al.* (2007) suggested that
260 M_{MAX} increased at higher contraction intensities because the muscle length has be
261 shown to be up to 28% shorter at the same joint angle (Griffiths, 1991); and thus, could
262 improve the synchronisation of the action potential. However, contrary to our findings,
263 other authors have reported no change (Linnamo *et al.*, 2001a; Lee & Carroll, 2005)
264 or even a decrease (Linnamo *et al.*, 2001b) in M_{MAX} with increasing contraction
265 intensities. The high degree of variability between subjects might explain why the
266 literature offers little consistency (Lee & Carroll, 2005). In addition, phase cancellation
267 has shown to reduce the EMG response at the muscle during higher contraction
268 intensities (Keenan *et al.*, 2006; Farina *et al.*, 2008). If the responses in EMG are
269 muted at higher contraction intensities then the lack of change in M_{MAX} amplitude
270 appears to be associated with the limitations in surface EMG recording (Farina *et al.*,
271 2014).

272

273 Our results support previous findings that showed no difference in M_{MAX} under
274 shortening and lengthening contractions (Linnamo *et al.*, 2001b; Duclay & Martin,
275 2005) when measured at the same joint angle. Supramaximal stimulation of the
276 peripheral nerve at shorter muscle lengths improves the synchronisation of the action
277 potential (Kim *et al.*, 2005). With an enhanced synchronisation of the action potential
278 there is an increase in M_{MAX} (Kim *et al.*, 2005). However, the varying pennation angle
279 of muscles might explain why not all studies have found increases in M_{MAX} at shorter
280 muscle lengths (Gerilovsky *et al.*, 1977; Gerilovsky *et al.*, 1989; Frigon *et al.*, 2007).

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

296

297 Unlike MEP's, H-reflex and EMG signals, V-wave is expressed relative to an M_{SUP}
298 (Aagaard *et al.*, 2002; Duclay & Martin, 2005; Gondin *et al.*, 2006) or M_{MAX} (Tallent *et*
299 *al.*, 2012b; Tallent *et al.*, 2013). In this study, there was no difference in M_{MAX} at a low
300 intensity contraction ($\leq 25\%$) and M_{SUP} . This would suggest that EMG/V-waves
301 recorded during an MVC could be expressed relative to a low intensity M_{MAX}
302 contraction. There was also good level of agreement between M_{SUP} and M_{MAX} at low
303 intensity contractions suggesting these values could be used interchangeably,

304 although in the interest of rigor, it would be sensible to use a single well controlled
305 M_{MAX} measure to normalise all conditions.

306

307 **Conclusion**

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

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438 Competing interests

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

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447 Author Contributions

448 Experiments were performed at Northumbria University. JT, SG and GH designed

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450 and interpreted the data; JT, SG, DK, RD and GH drafted or revised the final

451 manuscript. All authors approved the final version of the manuscript and agree to be

452 accountable for all aspects of the work. All persons listed qualify for authorship.

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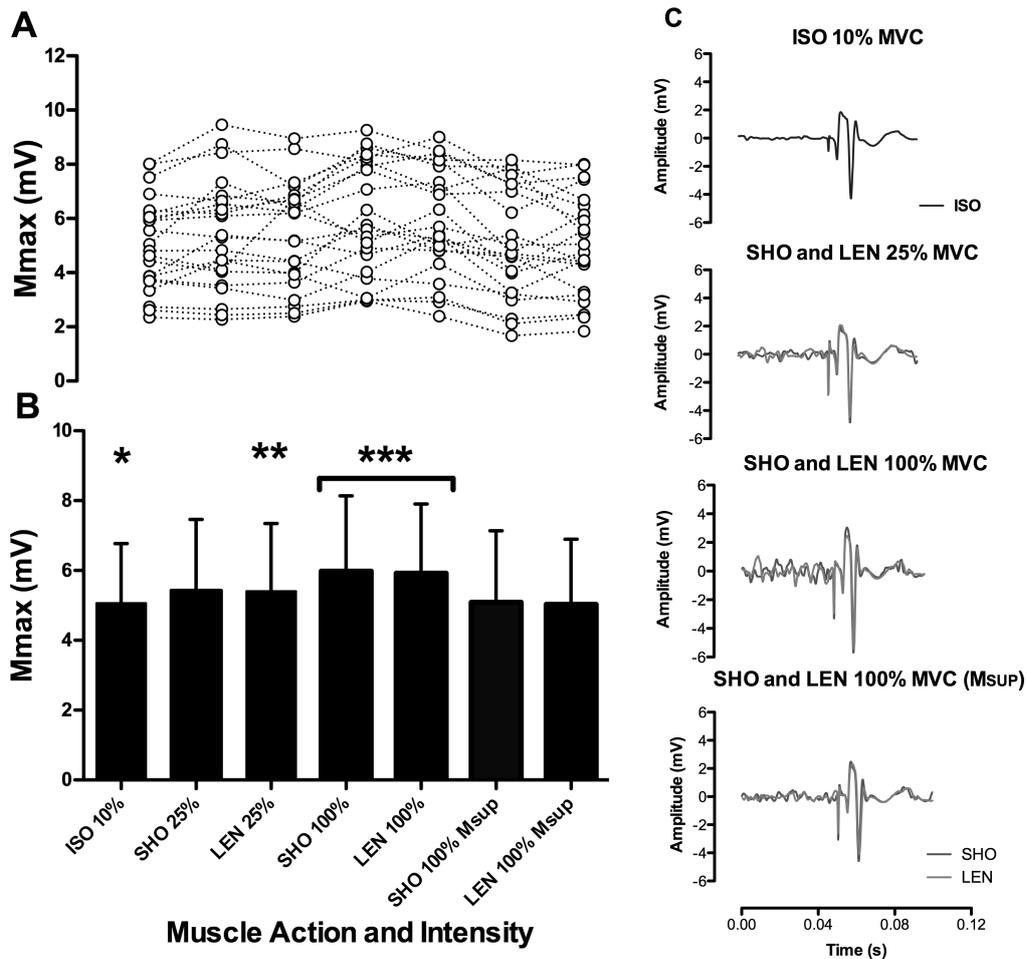
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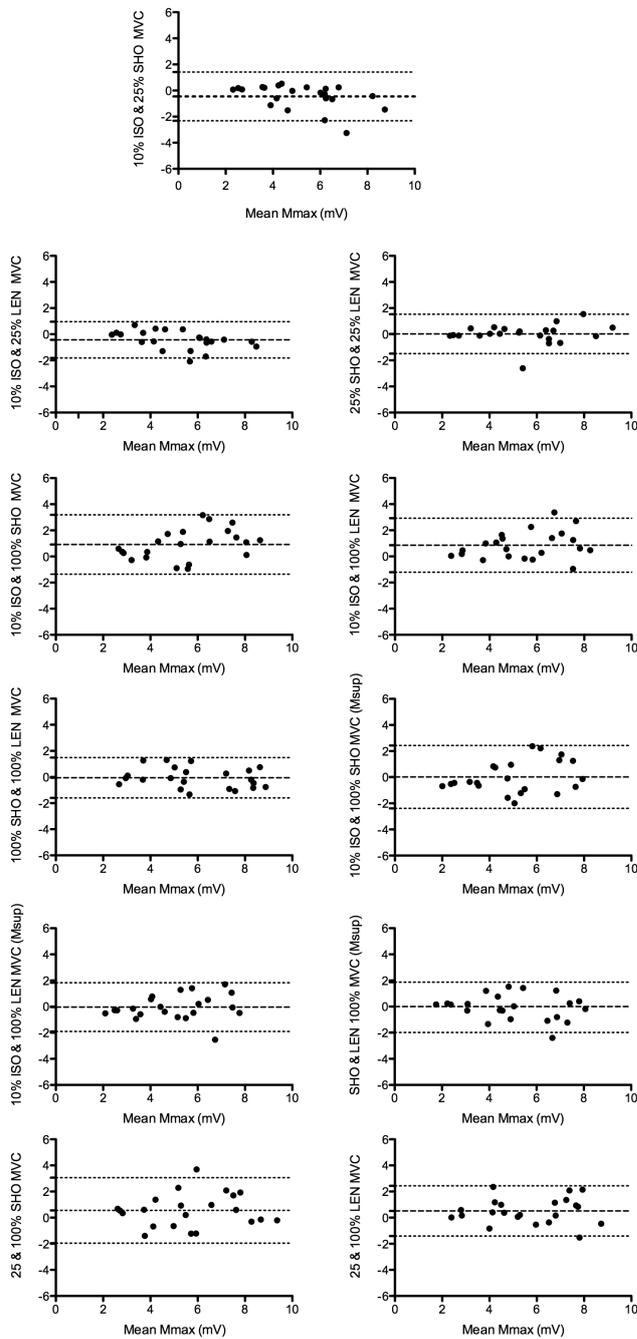
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463 **Figure 1.** Clear dots represent individual responses at different M_{MAX} contraction464 intensities and contraction types (**A**). Bars represent mean M_{MAX} and M_{SUP} responses465 (mean \pm SD) (**B**). Representative trace from a single participant of M_{MAX} recorded at466 10% ISO, SHO and LEN 25% and 100% MVC, SHO and LEN M_{SUP} (**C**). ISO =467 Isometric, SHO = Shortening, LEN = lengthening; *denotes significantly ($P < 0.05$)468 different from 25% and 100%, SHO and LEN MVC M_{MAX} ; **denotes significantly469 different from 100% SHO and LEN M_{MAX} ; ***denotes significantly different from SHO470 and LEN M_{SUP} .

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