Spinal contribution to neuromuscular recovery differs between elbow-flexor and knee-extensor muscles after a maximal sustained fatiguing task

Running title: Muscle-related differences in spinal excitability recovery

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1 **ABSTRACT**

2 Data from studies of elbow-flexor (EF) or knee-extensor (KE) muscles suggest that a fatigue-

3 related decrease in motoneuron excitability only occurs in EF. It is unknown how

4 motoneuron excitability changes after sustained fatiguing maximal voluntary isometric

5 contractions (MVICs) in EF and KE in the same participants. In two sessions, eight healthy

6 men performed a 2-min MVIC of EF or KE to induce fatigue with brief MVICs before and

7 six times after the 2-min MVIC. Electromyographic responses elicited by corticospinal tract

8 stimulation at the transmastoid [cervicomedullary motor-evoked potential (CMEP)] or

9 thoracic [thoracic motor-evoked potential (TMEP)] level were recorded from EF and KE,

10 respectively. To account for muscle excitability, CMEPs and TMEPs were normalized to

11 maximal M-wave (M_{max}) elicited by peripheral nerve stimulation during each brief MVIC.

12 Immediately after the 2-min MVIC, *biceps brachii* and *brachioradialis* CMEP/M_{max} were

13 88% (SD 11%) ($P = 0.026$) and 87% (SD 12%) ($P = 0.029$) of pre-MVIC values,

14 respectively, and remained lower than PRE after 5 s of recovery [91% (SD 8%), $P = 0.036$

15 and 87% (SD 13%), $P = 0.046$, respectively]. No subsequent time points differed from PRE

16 (all $P \geq 0.253$). TMEP/M_{max} for *rectus femoris* and *vastus lateralis* were not different from

17 PRE at any time during the recovery period (all $P > 0.050$). A different recovery pattern in

18 motoneuron excitability occurred in EF as it recovered by 60 s whereas KE motoneurons

19 were unaffected by the fatiguing task. The present findings may contribute to better

20 understand muscle-specific neurophysiological differences in spinal excitability.

21

22 **Key words:** fatigue; inhibition; maximal voluntary contraction; motoneuron; spinal

23 excitability

24 **NEW & NOTEWORTHY**

25 By comparing the changes in motoneuron excitability in elbow-flexor and knee-extensor

26 muscles after sustained fatiguing maximal voluntary contractions, this study shows that

27 motoneuron recovery behavior depends on the muscle performing the exercise. A different

28 recovery pattern in motoneuron excitability occurs in elbow flexors as it recovered by 60 s

29 whereas knee extensors were unaffected by fatigue. This finding can help to increase

30 understanding of the effect of a fatigue and subsequent recovery on neural processes.

INTRODUCTION

After a 2-min sustained maximal voluntary isometric contraction (MVIC), a classical model to study fatigue, approximately one-quarter of the reduction in maximal force can be attributed to processes within the central nervous system (31). The central-mediated force loss is due to some combination of spinal and supraspinal mechanisms (9), resulting in a suboptimal activation of muscle fibers and reduced discharge rate of the motoneuron pool (2, 3). This reduction depends on fatigue-induced changes in intrinsic properties of the motoneurons (6) and the sum of the multiple inputs received by the motoneurons (14). When testing the excitability of motoneurons in humans, corticospinal tract stimulation provides the best available method to assess motoneuron excitability (21) because the corticospinal/motoneuron synapse is not modified by Ia presynaptic inhibition, unlike both the H-reflex and F-wave (25). During 2-min MVICs, this technique has been conducted by means of non-invasive electrical stimulation of the descending spinal tracts either between the mastoid processes [for elbow-flexor muscles (EF)] (6, 23, 26, 27) or over the upper thoracic spine [for knee-extensor muscles (KE)] (18). Indeed, both activate corticospinal axons in the spinal cord (37) and evidence shows that descending spinal tracts are not subject to presynaptic inhibition (29). Corticospinal tract stimulation can evoke large, short-latency and predominantly monosynaptic responses in arm and leg muscles [cervicomedullary motor-evoked potentials (CMEPs) and thoracic motor-evoked potentials (TMEPs), respectively] (33). The consequent sizes of CMEPs and TMEPs reflect motoneuron excitability when normalized to the size of the maximal M-wave (21) and their reductions indicate decreased responsiveness of the motoneuron pool to descending input.

After a 2-min MVIC, the excitability of the motoneuron pool declined in EF [e.g. (6, 10, 26, 27)] but not in KE (18). These results may occur due to different fatigue-related changes in intrinsic motoneuron properties (e.g. reduced efficacy of the motoneuronal

56   synapse; or activity-dependent changes in corticospinal axons) and descending input (34), all

57   of which can affect motoneuron excitability (33). However, motoneuron excitability after

58   sustained MVICs has been investigated while fatiguing only EF or KE, focusing on

59   neurophysiological responses to the exercise model itself, rather than on muscle-specific

60   physiological differences. Therefore, it cannot be assumed these studies' conclusions also

61   apply when the same participants perform the same fatiguing model using both EF and KE.

62   When the corticospinal responsiveness in EF and KE muscles of the same participants was

63   tested during a 2-min MVIC, motoneuron excitability was reduced in EF, but not KE (34).

64   Therefore, sustained fatiguing isometric MVICs elicit different responses in motoneuron

65   excitability in elbow-flexor and knee-extensor muscles of the same participants. However,

66   the time course of recovery of motoneuron excitability was unreported.

67   After a 2-min MVIC, voluntary force declined to 42% and 30% of baseline for EF and

68   KE, with a partial recovery over the first few minutes after exercise cessation (39).

69   Furthermore, both the excitatory (motor-evoked potential) and inhibitory (silent period)

70   responses elicited by transcranial magnetic stimulation of the corresponding motor cortical

71   area returned to baseline values within 5 s for both EF and KE (39). This suggests that the

72   full capacity of corticospinal outputs to appropriately drive motoneurons at maximal

73   voluntary force-generating capacity may have recovered to control levels within a few

74   seconds after contraction cessation. This does not preclude continued impairment of the

75   excitability of the motoneuron pool from being a possible reason for prolonged impairment in

76   the maximal voluntary force-generating capacity. However, whether time courses of recovery

77   in the motoneuron excitability in EF and KE of the same participants reflect that of the

78   functional recovery in voluntary force has not yet been elucidated. A better understanding of

79   the time course of recovery in motoneuron excitability in EF and KE muscles after fatiguing

80 exercises may be relevant to better understand muscle-specific neurophysiological

81 differences in spinal excitability and inhibition.

82   Therefore, this study investigated the effects of a 2-min MVIC on the time course of

83 recovery in the excitability of the motoneuron pool of EF and KE in the same participants.

84

85 **MATERIAL AND METHODS**

86 **Participants**

87 Based on CMEP changes observed after a 2-min MVIC with or without ischemia in 8

88 participants (6), the mean effect size of the change in the main outcome (CMEP for BB) was

89 1.3. Using this value, an α [threshold probability for rejecting the null hypothesis (type I

90 error)] at 0.05 and a β [probability of failing to reject the null hypothesis under the alternative

91 hypothesis (type II error) at 0.2], a sample size of 7 participants was considered sufficient to

92 detect meaningful changes. From 12 healthy males who participated in a series of

93 investigations [see (34, 38, 39) for further details], 4 of them chose not to participate in this

94 study because they found spinal stimulations prohibitively painful during the familiarization

95 sessions. Therefore, 8 participated in the sessions comprising this study. Of those tested, 1

96 participant was excluded from the analysis of the EF motoneuron pool due to difficulties in

97 consistently eliciting CMEP responses. Therefore, results are reported for 8 participants (age:

98 32 ± 10 years; height: 180 ± 7 cm; body mass: 75 ± 9 kg) for KE and 7 participants (age: $33 \pm$

99 11 years; height: 179 ± 7 cm; body mass: 74 ± 9 kg) for EF. Participants were instructed to

100 avoid the consumption of caffeine on the day of the experiment and avoid performing any

101 strenuous exercise during the 48 h prior to testing. The experimental protocol was approved

102 by the University of Calgary Conjoint Health Research Ethics Board (#REB14-1625). All

103 participants gave written informed consent.

104

Experimental protocol

Each participant completed one familiarization session and two experimental sessions. During the familiarization session, participants performed maximal and submaximal voluntary isometric contractions of EF and KE with and without electrical spinal and peripheral nerve stimulation. The two experimental sessions were performed in a pseudo-randomized and counter-balanced order and consisted of either a 2-min EF or KE MVIC with spinal and peripheral stimulation. Sessions were separated by between 3 and 7 days and each participant performed all tests at the same time of day to control for within-participant diurnal variation. Participants were highly motivated and instructed to perform at maximal effort until asked to relax. During the 2-min MVICs, participants received continuous visual feedback and were strongly encouraged throughout the experiments by the investigators.

Neuromuscular testing protocol

Two to 3 min before each 2-min MVIC (PRE), the neuromuscular testing protocol consisted of two brief 2-3 s MVICs (separated by 60 s) with spinal and peripheral stimulation (see Neuromuscular function evaluation section). As an estimate of true MVIC force, we compared the peak forces of the two MVICs before exercise by means of a real-time display of MVIC values on a computer screen. Peak force from the second brief MVIC was always within 5% of peak force from the first brief MVIC for all participants. The neuromuscular function evaluation consisted of a brief 2-3 s MVIC with visual feedback of the force produced provided to the participants At the end of the 2-min MVIC, participants were not permitted to relax and they were required to continue their maximal effort for the first assessment post 2-min MVIC (POSTimm). Additional brief MVICs were performed 5 s after relaxation (POSTrelax) and 1 (POST 1), 2 (POST 2), 4 (POST 4), and 8 (POST 8) min after the end of the 2-min MVIC (Fig. 1).

130

****Figure 1 near here****

Force and electromyography recordings

EF force was assessed by a calibrated force transducer (2712-200 daN, Sensy, Jumet, Belgium). Participants sat upright in a chair with their right arm in a custom-built dynamometer. Both shoulder and elbow joints were at 90°, with the forearm in a supinated position. A noncompliant strap secured the wrist to the dynamometer.

KE force was measured by a calibrated force transducer (LC101-2K; Omegadyne, Sunbury, OH). Participants sat upright in a custom-built chair with the hips and right knee at 90° of flexion. A noncompliant strap secured the leg immediately proximal to the malleoli to the dynamometer.

EMG of EF [*biceps brachii* (BB) and *brachioradialis* (BR)] and KE [*rectus femoris* (RF) and *vastus lateralis* (VL)] was recorded with pairs of self-adhesive surface electrodes (10-mm recording diameter, Meditrace 100; Covidien, Mansfield, MA) in bipolar configuration with a 30-mm interelectrode distance and the reference on the medial epicondyle of the *humerus* (for EF) or the patella (for KE). Placement of EMG electrodes for BB was on the line between the medial acromion and the cubital fossa at 1/3 the distance from the cubital fossa (13) and placement for BR was over the muscle midbelly (22). Placement of EMG electrodes for RF was between the anterior superior iliac spine and the superior border of the patella, on the distal portion of the muscle belly (5) while for VL, electrodes were placed between the apex of the greater trochanter and the superolateral border of the patella, on the distal portion of the muscle belly (5). A low impedance (<5 kΩ) between electrodes was obtained by shaving and gently abrading the skin and then cleaning it with isopropyl alcohol. Force and EMG signals were converted from analog-to-digital at a

155 sampling rate of 2000 Hz by PowerLab system (16/35, ADInstruments, Bella Vista,

156 Australia) and octal bioamplifier (ML138; ADInstruments; common mode rejection ratio =

157 85 dB, gain = 500) with band pass filter (5-500 Hz) and analyzed offline using Labchart 8

158 software (ADInstruments).

159

160 ***Spinal stimulation***

161 The corticospinal tract was stimulated with single electrical stimuli of 500-μs duration via a

162 constant-current stimulator (DS7A; Digitimer, Welwyn Garden City, Hertfordshire, UK). For

163 BB and BR, CMEP responses were evoked by electrical stimulation at the transmatoid level

164 during voluntary contractions of EF. The electrical stimulus passed between two electrodes of

165 10-mm diameter (Meditrace 100) fixed to the skin over the left (cathode) and right (anode)

166 mastoid processes (37). For RF and VL, TMEP responses were evoked by electrical

167 stimulation of the descending corticospinal tract at the upper-thoracic level during voluntary

168 contractions of KE. The electrical stimulus passed between two electrodes of 10-mm

169 diameter (Meditrace 100) fixed over the thoracic spine. The cathode was placed between the

170 spinal processes of T3-T4 vertebrae and the anode ~5-10 cm above, but below the C7

171 vertebra (18). BB and RF were the main muscles of interest and stimulation intensity was

172 determined for these muscles. The stimulus intensity was determined during brief voluntary

173 isometric contractions at 50% MVIC and increased until the amplitude of BB CMEPs and RF

174 TMEPs (normalized to the corresponding M_{max}) matched approximately 50% of M_{max}

175 amplitude, since this was conducted as part of previously reported sessions (34).

176   The stimulus intensity was verified from the mean amplitude of 4 CMEPs or TMEPs.

177 Mean stimulus intensities were 151 ± 44 mA and 578 ± 125 mA in EF and KE, respectively.

178 Raw traces showing CMEPs and TMEPs before and after the 2-min MVIC and during the

179 recovery period are displayed for a single participant in Fig. 2.

180

181   ****Figure 2 near here****

182

183   ***Peripheral stimulation***

184   To evoke maximal M-wave (M_{max}) in BB, BR, RF and VL, single electrical stimuli of 200-μs

185   duration were delivered via a constant-current stimulator (DS7AH, Digitimer). For BB and

186   BR, stimuli were delivered to the brachial plexus trunk at Erb's point with a cathode

187   (Meditrace 100) in the supraclavicular fossa and a 50 × 90 mm rectangular anode (Durastick

188   Plus; DJO Global, Vista, CA) on the acromion. For RF and VL, stimuli were delivered to the

189   femoral nerve trunk via a cathode taped into the femoral triangle (Meditrace 100) and a 50 ×

190   90 mm rectangular anode (Durastick Plus) in the gluteal fold. During peripheral nerve

191   stimulation of both the brachial plexus and the femoral nerve trunk, a small gauze ball was

192   placed over the cathodes before securing it with tape in order to apply pressure over the

193   stimulation site. Single stimuli were delivered incrementally in the relaxed muscle state until

194   M_{max} and twitch amplitudes plateaued. A stimulus intensity of 130% of the intensity to elicit

195   M_{max} and maximal twitch responses was used throughout the rest of the experiment. The

196   supramaximal stimulus intensity was 153 ± 95 mA for EF and 158 ± 50 mA for KE. Raw

197   traces showing M_{max} before and after the 2-min MVIC and during the recovery period are

198   displayed for a single participant in Fig. 2.

199

200   ***Neuromuscular function evaluation***

201   The neuromuscular function evaluation consisted of a brief 2-3 s MVIC with visual feedback

202   of the force produced provided to the participants by means of a real-time display on a

203   computer screen. The participants contracted to maximal force and once maximal force was

204   attained, stimulation of the spinal tract was delivered. Once the participant returned to

205 maximal force after the induced silent period, peripheral stimulation was delivered. To avoid

206 possible contamination of the EMG signal by stimulation of either the spinal tract or

207 peripheral nerves, participants were instructed to avoid inadvertent contractions in

208 anticipation of the stimulus. They were also instructed to avoid inadvertent changes in head

209 position that may have changed the CMEP responses since changes in CMEP size may occur

210 due to movement of the electrodes relative to the point of stimulation (33).

211

212 **Data Analysis**

213 Force values were measured for the duration of the 2-min MVIC and for the brief 2-3 s

214 MVICs constituting the neuromuscular testing protocol. During the 2-min MVIC, force was

215 measured for each successive 5-s window for the entire duration of the fatiguing contraction.

216 During the brief 2-3 s MVICs, mean force was measured over the 500 ms before spinal

217 electrical stimulation.

218 Area values for M_{max}, CMEPs and TMEPs were measured between cursors marking

219 the initial deflection from the baseline to the second crossing of the horizontal axis (22). The

220 durations of the silent period after spinal electrical stimulation (SP_{CMEP} and SP_{TMEP}) were

221 measured by visually inspecting the interval from the stimulus to the return of continuous

222 voluntary EMG (32). To account for any changes in the compound muscle action potential,

223 CMEPs and TMEPs were normalized to M_{max} values (CMEP/M_{max} or TMEP/M_{max},

224 respectively) recorded during the same contraction. All data during the post 2-min MVIC

225 contractions were normalized as a percentage of the PRE evaluation except for force values

226 during the 2-min MVIC for which force data were normalized as a percentage of the PRE

227 evaluation and averaged in 5-s time windows.

228

229 **Statistical analysis**

230   Results are given as means (SD). To test differences between PRE and POSTimm, as well as

231   during the recovery time, the longitudinal analysis (muscle group × time for force and muscle

232   × time for EMG parameters) was performed using generalized estimating equations (GEE;

233   i.e. GEE under 'Generalized Linear Model' procedure in SPSS v. 26) to take into account the

234   unbalanced nature of the measurements (n = 7 for EF session and n = 8 for KE session) (20).

235   Furthermore, GEE was used to take into account the correlated nature of observations within

236   each participant (i.e. within-participant measurements) (36). GEE is considered to be robust

237   against the choice of an incorrect correlation structure (20). When significant main effects or

238   interactions were observed, Bonferroni's test was used for *post-hoc* analysis. As a measure of

239   effect size, Cohen's d (*d*) was calculated with 95% confidence intervals (CI). Values of 0.2,

240   0.5, and above 0.8 were considered *small*, *medium*, and *large*, respectively (7). Statistical

241   analysis was conducted using IBM^{TM} SPSS^{TM} Statistics (version 26, IBM Corp., Somers,

242   New York, NY). Statistical significance was set at $\alpha < 0.050$.

243

244   **RESULTS**

245   Table 1 presents values before the 2-min MVIC for maximal voluntary force, M_{max} area,

246     $CMEP/M_{max}$ for both BB and BR, $TMEP/M_{max}$ for both RF and VL, SP_{CMEP} for both BB and

247   BR, SP_{TMEP} for both RF and VL,

248

249   ****Table 1 near here****

250

251   **Force**

252   Mean force profiles for each 5-s window during the 2-min MVICs for both EF and KE are

253   presented in Figure 3 (Panel A). Force profiles during the 2-min MVICs showed a time effect

254   [χ^2 (8) = 2.941E+14, $P < 0.001$], a muscle group effect [χ^2 (1) = 8.978, $P = 0.003$], and

255 muscle group × time interaction [χ^2 (8) = 1.403E+14, P < 0.001]. The force decreased in a

256 comparable manner until 30 s. Then the difference in force between EF and KE became

257 visually appreciable from 35 s and this difference reached significance at 65 s when EF was

258 68% (SD 12%) of PRE and KE force was 59% (SD 11%) of PRE [P = 0.011, d = 0.8 (95%

259 CI -0.3-1.8)]. KE force remained significantly lower than EF until the end of the sustained

260 MVICs [mean normalized difference of PRE MVIC of 12% (SD 3%) from 65 s to 120 s].

261 Force values at the end of the 2-min MVICs were 32% (SD 7%) [P < 0.001, d = 13.7 (95%

262 CI 7.9-17.6)] and 23% (SD 5%) [P < 0.001, d = 21.8 (95% CI 13.4-27.8)] of PRE for EF and

263 KE, respectively, being also lower than those observed at POSTimm (both P < 0.001, see

264 below).

265   Figure 3 (Panel B) shows the MVIC force immediately after the 2-min contractions

266 and during recovery. MVIC force showed a time effect [χ^2 (6) = 222157.0, P < 0.001] and

267 muscle group × time interaction [χ^2 (6) = 420.3, P < 0.001], but not a muscle group effect [χ^2

268 (1) = 0.416, P = 0.519]. MVIC force at POSTimm was 48% (SD 5%) [P < 0.001, d = 14.7

269 (95% CI 8.5-18.8)] and 31% (SD 3%) [P < 0.001, d = 32.5 (95% CI 20.0-41.4)] of PRE

270 values for EF and KE, respectively. Then MVIC force remained lower than PRE values

271 through POST 2 for both EF [81% (SD 9%) of PRE values, P = 0.042, d = 3.0 (95% CI 1.3-

272 4.2)] and KE [76% (SD 17%) of PRE values, P = 0.030, d = 2.0 (95% CI 0.7-3.1)], but had

273 recovered by POST 4 [89% (SD 9%), P = 0.405, and 84% (SD 15%), P = 0.917, of PRE

274 values for EF and KE, respectively]. The decrease in MVIC force was greater in KE than EF

275 only at POSTimm [by 17%, P < 0.001, d = 4.2 (95% CI 2.2-5.7)].

276

277 ****Figure 3 near here****

278

279 **Peripheral stimulation**

13

280   M_{max} results are presented in Figure 4. A time effect [χ^2 (6) = 841.7, P < 0.001], muscle effect

281   [χ^2 (3) = 14.9, P = 0.002], and muscle × time interaction [χ^2 (7) = 60.9, P < 0.001] were

282   observed.

283   At POSTimm, M_{max} for BB increased to 150% (SD 46%) [P = 0.035, d = 1.5 (95% CI

284   0.3-2.7)] of PRE values, while no subsequent time points were different from PRE (all $P \geq$

285   0.129).

286   M_{max} for BR increased to 189% (SD 41%) [P < 0.001, d = 3.1 (95% CI 1.4-4.4)] of

287   PRE values at POSTimm. Then M_{max} for BR remained greater than PRE values through

288   POST 2 [134% (SD 21%) of PRE values, P < 0.001, d = 2.3 (95% CI 0.8-3.4)], while no

289   subsequent time points were different from PRE (P = 0.390).

290   At POSTimm, M_{max} for RF increased to 126% (SD 14%) [P < 0.001, d = 2.6 (95% CI

291   1.2-3.8)] of PRE values. Then M_{max} remained greater than PRE values through POST 1

292   [129% (SD 21%) of PRE values, P = 0.002, d = 1.9 (95% CI 0.7-3.0 while no subsequent

293   time points were different from PRE (all P = 1.000).

294   M_{max} for VL increased to 143% (SD 40%) [P = 0.022, d = 1.5 (95% CI 0.3-2.5)] of

295   PRE values at POSTimm. Then M_{max} remained greater than PRE values through POST 1

296   [118% (SD 19%) of PRE values, P = 0.001, d = 1.3 (95% CI 0.2-2.3)], while no subsequent

297   time points were different from PRE ($P \geq$ 0.119).

298   At POSTimm, the increase in M_{max} as a percentage change of PRE values was similar

299   between BB, BR, RF and VL (all $P \geq$ 0.184).

300

301   **Spinal stimulation**

302   CMEP/M_{max} for both BB and BR, as well as TMEP/M_{max} for both RF and VL are presented

303   in Figure 4. A time effect [χ^2 (6) = 24.5, P < 0.001] and muscle × time interaction [χ^2 (7) =

304   105.5, P < 0.001], but not a muscle effect [χ^2 (3) = 2.1, P = 0.543], were observed.

305   At POSTimm, CMEP/M_{max} for BB decreased to 88% (SD 11%) of PRE values [P =

306   0.026, d = 1.5 (95% CI 0.3-2.6)]. Then CMEP/M_{max} remained lower than PRE at POSTrelax

307   [91% (SD 8%) of PRE values, P = 0.036, d = 1.6 (95% CI 0.3-2.7)] while no subsequent time

308   points were significantly different from PRE (all $P \geq 0.253$).

309   CMEP/M_{max} for BR decreased to 87% (SD 12%) [P = 0.029, d = 1.5 (95% CI 0.3-

310   2.6)] of PRE values at POSTimm. Then CMEP/M_{max} remained lower than PRE at POSTrelax

311   [87% (SD 13%) of PRE values, P = 0.046, d = 1.4 (95% CI 0.2-2.5)] while no subsequent

312   time points were different from PRE (all P = 1.000).

313   TMEP/M_{max} for RF was not different from PRE at POSTimm [104% (SD 9%) of PRE

314   values, P = 1.000, d = 0.6 (95% CI -0.4-1.6)] or at any time during the recovery period (all P

315   = 1.000).

316   TMEP/M_{max} for VL was not different from PRE at POSTimm [105% (SD 10%) of

317   PRE values, P = 1.000, d = 0.7 (95% CI -0.3-1.7)] or at any time during the recovery period

318   (all P = 1.000).

319   At POSTimm, the decrease in CMEP/M_{max} for BB as a percentage of PRE values was

320   16% and 17% greater than that in TMEP/M_{max} for RF [P = 0.046, d = 1.6 (95% CI 0.4-2.7)]

321   and VL [P < 0.001, d = 1.6 (95% CI 0.4-2.7)], respectively. Similarly, the decrease in

322   CMEP/M_{max} for BR was 17% and 18% greater than that in TMEP/M_{max} for RF [P = 0.032, d

323   = 1.5 (95% CI 0.4-2.7)] and VL [P = 0.008, d = 1.6 (95% CI 0.4-2.7)], respectively.

324

325   ****Figure 4 near here****

326

327   SP_{CMEP} for both BB and BR, as well as SP_{TMEP} for both RF and VL are presented in

328   Figure 5. SP showed a time effect [χ^2 (6) = 479.4, P < 0.001] and muscle × time interaction

329   [χ^2 (7) = 105.0, P < 0.001], but not a muscle effect [χ^2 (3) = 2.3, P = 0.513].

330   At POSTimm, SP_{CMEP} for BB increased to 144% (SD 20%) [$P < 0.001$, $d = 3.1$ (95%

331   CI 1.4-4.4)] while no other time points were different from PRE (all $P \geq 0.249$).

332   SP_{CMEP} for BR increased to 148% (SD 12%) [$P < 0.001$, $d = 5.7$ (95% CI 3.1-7.5)] of

333   PRE values at POSTimm. Then SP_{CMEP} for BR remained greater than PRE at POSTrelax

334   [125% (SD 13%) of PRE values, $P < 0.001$, $d = 2.7$ (95% CI 1.1-3.9)] while no subsequent

335   time points were different from PRE (all $P = 1.000$).

336   At POSTimm, SP_{TMEP} for RF increased to 153% (SD 28%) [$P < 0.001$, $d = 2.7$ (95%

337   CI 1.2-3.8)] of PRE values. SP_{TMEP} for RF remained greater than PRE through POST 1

338   [116% (SD 13%) of PRE values, $P = 0.008$, $d = 1.7$ (95% CI 0.5-2.8)] while no subsequent

339   time points were different from PRE (all $P = 1.000$).

340   SP_{TMEP} for VL increased to 148% (SD 17%) [$P < 0.001$, $d = 4.0$ (95% CI 2.1-5.4)] of

341   PRE values at POSTimm. Then SP_{TMEP} for VL remained greater than PRE through POST 1

342   [113% (SD 11%) of PRE values, $P = 0.018$, $d = 1.7$ (95% CI 0.5-2.7)] while no subsequent

343   time points were different from PRE (all $P \geq 0.447$).

344   At POSTimm, the increase in SP as a percentage change of PRE values was similar

345   between BB, BR, RF and VL (all $P = 1.000$).

346

347   ****Figure 5 near here****

348

349   **DISCUSSION**

350   Despite a similar and gradual recovery of voluntary force for both elbow-flexor and knee-

351   extensor muscles after a sustained maximal isometric voluntary contraction, the present study

352   showed that time courses of recovery in the motoneuron excitability of the two muscle

353   groups in the same participants differs, i.e. it did not reflect the functional recovery in

354   maximal voluntary force. Therefore, this study is the first to describe that responses at the

355 motoneuron level recovered differently in elbow-flexor and knee-extensor muscles after an

356 intense fatiguing task in the same participants. Specifically, only the excitability of the

357 motoneuron pool of *biceps brachii and brachioradialis* was reduced and responses to

358 corticospinal tract stimulation for *biceps brachii* and *brachioradialis* required 5 to 60 s to

359 return to pre-exercise levels.

360

361 **Motoneuron excitability and fatigue**

362 Compared with baseline, maximal force decreased by 69% in KE and by 52% in EF when

363 assessed immediately after the 2-min MVIC (i.e. POSTimm). This observation is in line with

364 previous studies (11, 18, 27, 39) and confirms the fatiguing nature of the 2-min MVIC.

365 Furthermore, although MVIC force declined at the end of the 2-min MVIC for both EF and

366 KE, M_{max} of BB, BR, RF and VL increased in size as previously observed after a 2-min EF

367 (6, 10, 39) or KE (39) MVICs. Although the neurophysiological mechanisms of the increased

368 M_{max} following a sustained maximal isometric contraction remain unclear, our result suggests

369 that excitation had not failed, at least not at the sarcolemmal level.

370 During the brief MVIC performed as an extension of the 2-min MVIC, CMEP/M_{max}

371 for BB was smaller compared to the PRE values. This decrease is consistent with previous

372 studies examining responses of motoneuron pools of BB to corticospinal stimulation at the

373 end of 2-min MVICs either by means of conditioned [i.e. the corticospinal stimulation was

374 delivered in the silent period following a conditioning transcranial magnetic stimulation pulse

375 (26, 27)] or unconditioned [i.e. when the corticospinal stimulation was delivered in isolation

376 (6, 27, 34)] CMEPs. Evidence suggests the depression of the responses to the corticospinal

377 tract stimulation may reflect changes in the motoneurons, consequently becoming less

378 excitable to a given input (6, 27) as our group recently observed during a 2-min EF MVIC

379 (34). The concomitant fatigue-induced lengthening of SP_{CMEP} may also suggest a decrease in

380   excitability of the motoneuron pool. However, we cannot completely rule out the lengthening

381   of SP_{CMEP} to a slowing of the conduction velocity of the repeatedly-activated muscle fibers

382   (see below) (4, 28). Several possible mechanisms may have contributed to the decreased

383   excitability of the motoneuron pool. For instance, repetitive activation of motoneurons can

384   lead to an insufficient release of neurotransmitters, in particular monoamine

385   neurotransmitters such as serotonin and norepinephrine, from the synaptic vesicles, thus

386   compromising synaptic efficacy (12). This level of neuromodulatory input to motoneurons

387   has been suggested to account for some of the decrease in motoneuronal excitability

388   immediately after exercise (10, 30). Besides intrinsic changes of the motoneuron properties

389   with repetitive activity and through neurotransmitters, the excitability of the motoneuron pool

390   could have also been modulated by afferent feedback. Synaptic input received by the

391   motoneuron during fatiguing contractions comprises concurrent increases in excitatory (i.e.

392   descending drive and muscle spindle) and inhibitory (i.e. group Ib, group III and IV and

393   Renshaw cell) afferent feedback (31). The inhibitory influence of group Ib afferents (Golgi

394   tendon organs) and Renshaw cells should not have played a substantial role since a

395   diminished activity is generally observed with fatigue (9). Furthermore, the excitatory

396   influence of muscle spindles is unlikely to have played a major role in reducing the

397   excitability of the motoneuron pool with muscle fatigue since tendon vibration during a

398   prolonged fatiguing muscle contraction showed no effects on conditioned CMEP size (26).

399   Conversely, an increased firing of group III and IV muscle afferents is a well-accepted

400   explanation for the observed reduction in the excitability of the motoneuron pool (31).

401   Indeed, during a prolonged fatiguing muscle contraction, group III-IV afferents become

402   increasingly excited (6), presumably mediating an increase in the motoneuronal

403   afterhyperpolarization period which reduces the likelihood for neuronal discharge (24).

404   The size of TMEP/M_{max} for both RF and VL responses did not change at POSTimm,

405   in agreement with Temesi et al. (34) who showed that TMEP/M_{max} responses did not change

406   from 5 to 115 s of a 2-min MVIC. Furthermore, as previously shown for VL (18), the present

407   study observed that although TMEP/M_{max} responses did not change after the 2-min MVIC,

408   SP_{TMEP} for both RF and VL increased in duration. While this may be seen as a potential

409   indicator of decreasing motoneuron excitability, Kennedy et al. (18) argued that it may also

410   owe to a slowing of the conduction velocity of the repetitively-activated muscle fibers,

411   ultimately manifesting as increased TMEP duration (4, 28). Moreover, changes in voluntary

412   descending drive can affect motoneuron excitability, likely creating a confounding

413   interpretation of the results because measuring motoneuron excitability during changing

414   levels of descending drive would result in the evoked response reflecting changes both in

415   motoneuron excitability and level of the voluntary descending drive. Therefore, by only

416   analyzing TMEP/M_{max} responses it can be hard to isolate the true contribution of spinal

417   mechanisms (8). To control the ongoing descending drive on measures of motoneuron

418   excitability, the technique elicits CMEPs or TMEPs during the silent period that follows a

419   transcranial magnetic stimulation pulse upon the motor cortex during a voluntary contraction

420   (27). The resultant CMEP or TMEP responses may better reflect the excitability of

421   motoneurons when they are not being acted upon by descending drive and not actively firing.

422   When this technique was used during a submaximal 10-min KE contraction at a constant

423   level of integrated EMG (8), TMEP/M_{max} responses in RF were reduced. Future studies

424   should employ the above mentioned technique to study changes at the level of the

425   motoneurons for a KE sustained MVIC, as previously shown in EF (27).

426

427   **Motoneuron excitability during recovery**

428   After a 2-min MVIC, the excitatory (motor-evoked potential) or inhibitory (silent period)

429   responses elicited by transcranial magnetic stimulation of the corresponding motor cortical

430   area quickly returned to baseline values for both EF and KE (39). Findings from the present

431   study showed that only CMEP/M_{max} decreased at the end of the 2-min MVIC and remained

432   lower than PRE 5 s after contraction cessation. Thus, only spinal motoneurons innervating EF

433   became less responsive with fatigue. Moreover, CMEP/M_{max} returned to pre-exercise values

434   by 1 min after contraction cessation (at POST 1), in line with a previous study that found that

435   CMEP/M_{max} depression was evident when tested 2-5 s after a 2-min MVIC (10). Other

436   studies performed the first post-exercise contractions either 15 s (6) or 30 s (27) after the end

437   of a 2-min MVIC, failing to observe a reduction from control values. Thus, motoneuron

438   excitability in EF recovers rapidly after a 2-min MVIC, suggesting that the fatigue-related

439   decrease in the motoneuron excitability could be underestimated if measured with any delay.

440   This consideration is further reinforced by a recent study showing how post-fatigue

441   assessments should be initiated immediately following task cessation because spinal

442   mechanisms substantially recover within 30 s of recovery (1).

443

444   **Differences between extensor and flexor motoneuron pool**

445   Rather than limb-specific differences in the behavior of motoneuron pool excitability, the

446   observed results could reflect differences between flexor (i.e. BB and BR) and extensor (i.e.

447   RF and VL) muscles. With activation of group III and IV afferents during 2-min MVICs of

448   both the elbow extensors (23) and EF (6), inhibition of the motoneuron pool has been

449   observed (although smaller CMEP/M_{max} reflecting reduced intrinsic excitability due to

450   repetitive activation cannot be ruled out). However, CMEP/M_{max} responses during the

451   subsequent recovery period differed between elbow extensors and flexors under ischemic

452   conditions. Indeed, CMEP/M_{max} elicited in the elbow extensors did not recover during the

453   first 2 min of recovery (23); whereas in EF, CMEP/M_{max} recovered within 15 s of the end of

454   the sustained contraction (6). These observations suggest that the effects of group III and IV

455   afferents differ among motoneuron pools. In the lower limbs, TMEP/M_{max} responses evoked

456   in VL did not change after a 2-min MVIC (18). Similarly, in the present study, the

457   excitability of the motoneuron pool of the extensor muscle (i.e. RF and VL) was maintained

458   after the 2-min MVIC. Conversely, excitability of the motoneuron pool for the flexor muscle

459   (i.e. BB and BR) decreased by ~12%. Given that inhibition of the motoneuron pool has been

460   demonstrated in the proximal muscles of the upper limb [i.e. both elbow flexors (6) and

461   extensors (23)] but not in KE [i.e. VL (18) or RF and VL in the present study], there is

462   insufficient evidence to suggest that the changes reported in the study may be due to

463   functional (i.e. flexor *versus* extensor) muscle differences. Instead, the above-mentioned

464   results could suggest that upper- *versus* lower-limb differences determined the behavior of

465   motoneuron pool excitability and, therefore, a different balance of fatigue-related changes in

466   the intrinsic motoneuron properties (as well as in sensory and descending input) of different

467   limbs.

468

469   **Limitations**

470   Although our study provides evidence that fatigue and recovery of motoneuron excitability

471   depends on the muscle performing the exercise in young men, women exhibit different

472   fatigue characteristics than men (15) and are generally less fatigable than men for sustained

473   isometric contractions (16). Nevertheless, recent evidence shows no effect of sex on

474   motoneuron excitability after an isometric sustained contractions (41). Furthermore, healthy

475   aging causes changes in the intrinsic properties of the motoneurons such that there is a

476   decrease in both the number of motoneurons (35), the excitability of motoneurons (19), and

477   the maximal firing rate of motor units (17). Nevertheless, fatiguing intermittent maximal

478   isometric KE contractions showed no effect on motoneuron excitability in older males (40).

479   However, whether the same results we observed apply to older males has yet to be

480   determined. Consequently, we can only generalize our findings to young adults.

481

482   **Conclusion**

483   The present study is the first to show for the same participants that a diminished output from

484   spinal motoneurons after a sustained maximal isometric exercise model occurs for the elbow-

485   flexor but not the knee-extensor muscles. Specifically, while excitability of *rectus femoris*

486   and *vastus lateralis* motoneurons was not altered by a fatiguing 2-min MVIC, reduced

487   excitability of spinal motoneurons was observed in *biceps brachii* and *brachioradialis* with

488   rapid recovery (within 60 s). Therefore, spinal contribution to neuromuscular fatigue and

489   subsequent recovery may differ for elbow-flexor and knee-extensor muscles. The present

490   findings may contribute to better understand muscle-specific neurophysiological differences

491   in spinal excitability and inhibition. Indeed, elucidating the neurophysiological mechanisms

492   underlying muscle-specific adaptations in spinal excitability and inhibition can be important

493   for interpreting alterations in the properties of the nervous system associated with aging and

494   disease.

495

505

506   **DISCLOSURES**

507   No conflicts of interest, financial or otherwise, are declared by the authors.

508

509   **AUTHOR CONTRIBUTIONS**

510   JT and GYM conceived of and designed the research. GV, JT and MM performed the

511   experiment. GV, JT, MM and RLK analyzed the data. GV, JT, MM, RLK, and GYM

512   interpreted the data of the experiment. GV prepared the figures. GV and JT drafted the

513   manuscript. GV, JT, MM, RLK, and GYM edited and revised the manuscript. GV, JT, MM,

514   RLK, and GYM approved the final version of the manuscript.

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616

TABLE AND FIGURE CAPTIONS

Table 1. Participants' control values before the fatiguing contraction (i.e. 2-min maximal voluntary contraction). Data are presented as mean (standard deviation) and ranges and were recorded during brief (2-3 s) maximal voluntary contractions.

Figure 1. The fatigue protocol performed in two separate sessions for both elbow-flexor and knee-extensor muscles. Each protocol began with a neuromuscular function evaluation before (PRE) the fatiguing contraction [2-min sustained maximal voluntary isometric contraction (MVIC), represented by the black trapezoid). The neuromuscular function evaluation required participants to perform a brief (~2-3 s) MVIC (white bars). Once maximal force was attained, either transmastoid or thoracic stimulation was delivered. When the participant returned to maximal force after the silent period induced by the spinal stimulus, peripheral stimulation (i.e. femoral nerve or brachial plexus electrical stimulation) was delivered. At the end of the 2-min MVIC, the same neuromuscular function evaluation was performed as an extension of the 2-min MVIC (POSTimm) and additional evaluations were performed after 5 s of relaxation (POSTrelax) and 1 (POST 1), 2 (POST 2), 4 (POST 4) and 8 (POST 8) min after the end of the 2-min MVIC. Time 'zero' corresponds to the beginning of the recovery period.

Figure 2. Single-participant data of raw electromyographic (EMG) responses. Responses were evoked in the *biceps brachii* (Panel A) and *brachioradialis* (Panel B) by transmastoid stimulation (CMEP) and peripheral nerve stimulation to the brachial plexus trunk at Erb's point [M_{max}, (Panels E and F)]. Responses were also evoked in the *rectus femoris* (Panel C) and *vastus lateralis* (Panel D) by thoracic stimulation (TMEP) and peripheral nerve stimulation to the femoral nerve trunk [M_{max}, (Panels G and H)]. CMEP, TMEP and M_{max} are
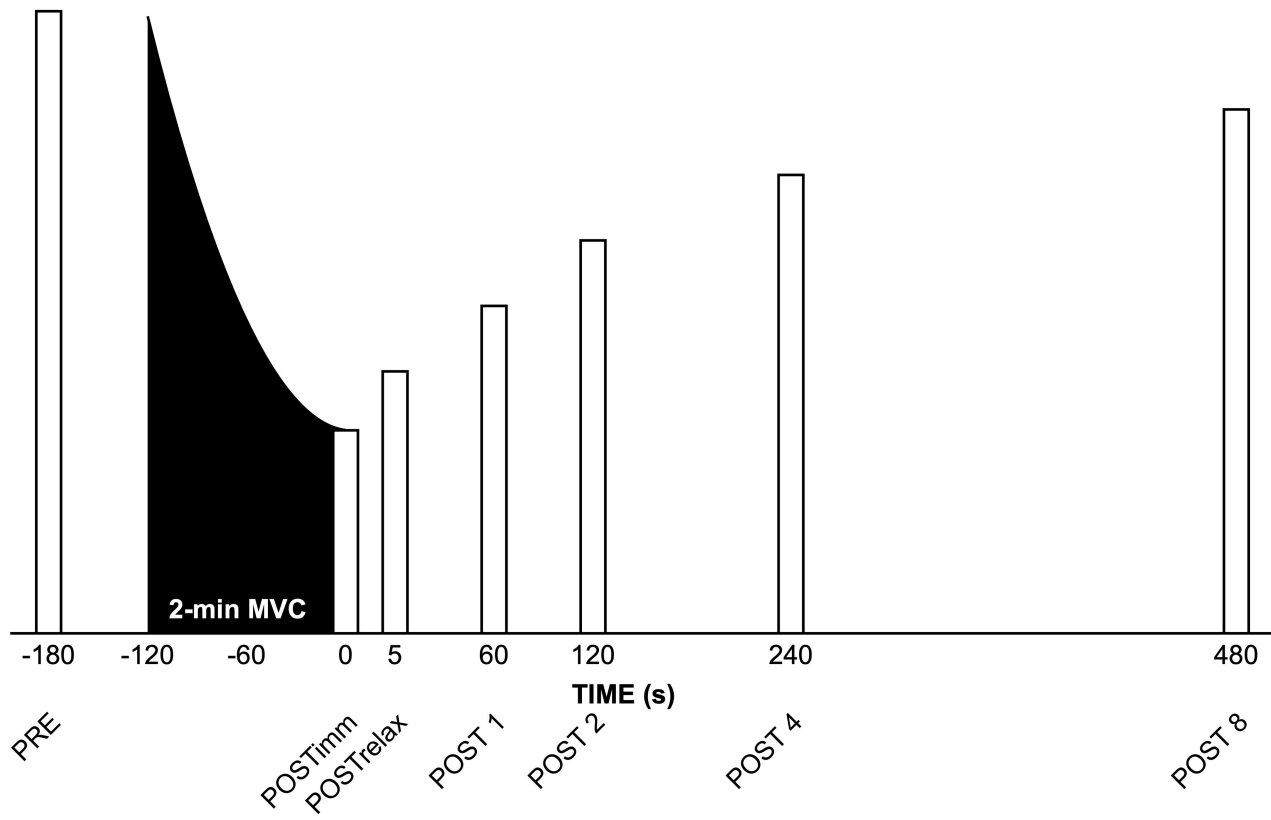
642   highlighted by the shaded areas. Stimuli were delivered at time 0 ms (represented by the

643   continuous vertical lines) before the 2-min MVIC (PRE), at the end of the 2-min MVIC

644   (POSTimm), after 5 s of relaxation (POSTrelax), and 1 (POST 1), 2 (POST 2), 4 (POST 4)

645   and 8 (POST 8) min after the end of the 2-min MVIC. Arrows indicate the time at which the
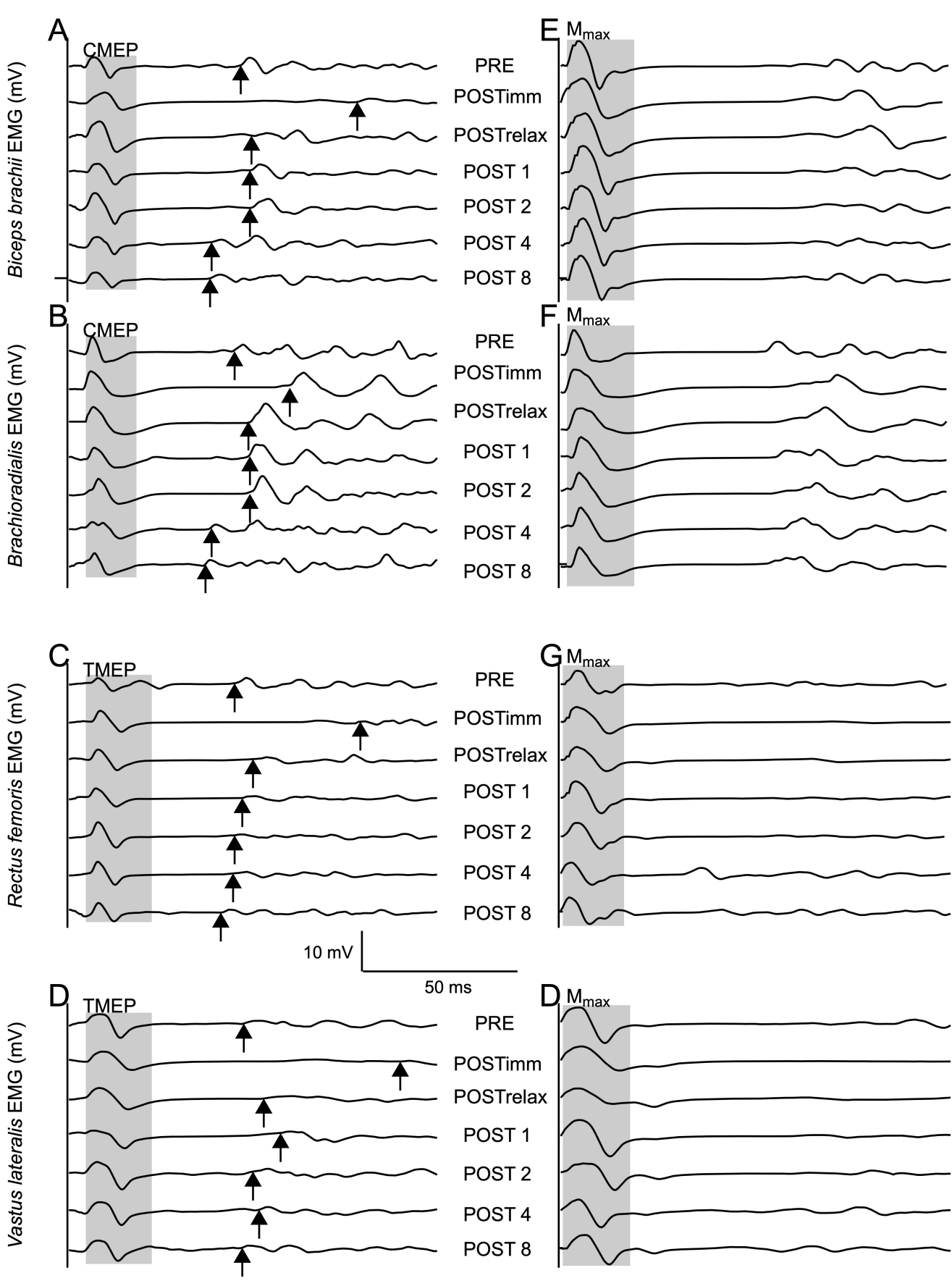
646   silent period after CMEP and TMEP ended.

647

648   **Figure 3.** Panel A: Means and standard deviations of force values (as percentage of the PRE

649   values) of the elbow flexors (EF) and knee extensors (KE) muscles during the 2-min

650   sustained maximal voluntary isometric contraction (MVIC). Each point represents a 5-s

651   window. Significant differences between EF and KE were observed during the second half of

652   the 2-min MVIC (as indicated by the shaded area, $P < 0.05$). For differences within muscle

653   relative to the PRE 2-min MVIC: ‡, $P < 0.001$. At sign (@) denotes within muscle

654   differences between the end of the 2-min MVICs and POSTimm: $P < 0.05$. For differences

655   between muscles within the same time-points: $, $P < 0.001$. Panel B: Changes in force after

656   the sustained 2-min MVIC for elbow flexors (EF, $n = 7$) and knee extensors (KE, $n = 8$). At

657   the end of the 2-min MVIC a neuromuscular function evaluation was performed as an

658   extension of the 2-min MVIC (POSTimm) and additional evaluations were performed after 5

659   s of relaxation (POSTrelax) and 1 (POST 1), 2 (POST 2), 4 (POST 4) and 8 (POST 8) min

660   after the end of the 2-min MVIC. The shaded box indicates the sustained 2-min MVIC and

661   time 'zero' corresponds to the beginning of the recovery period. Values are means and

662   standard deviations and expressed as a percentage of the PRE evaluation. For differences

663   between time-points within the same muscle *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$. For

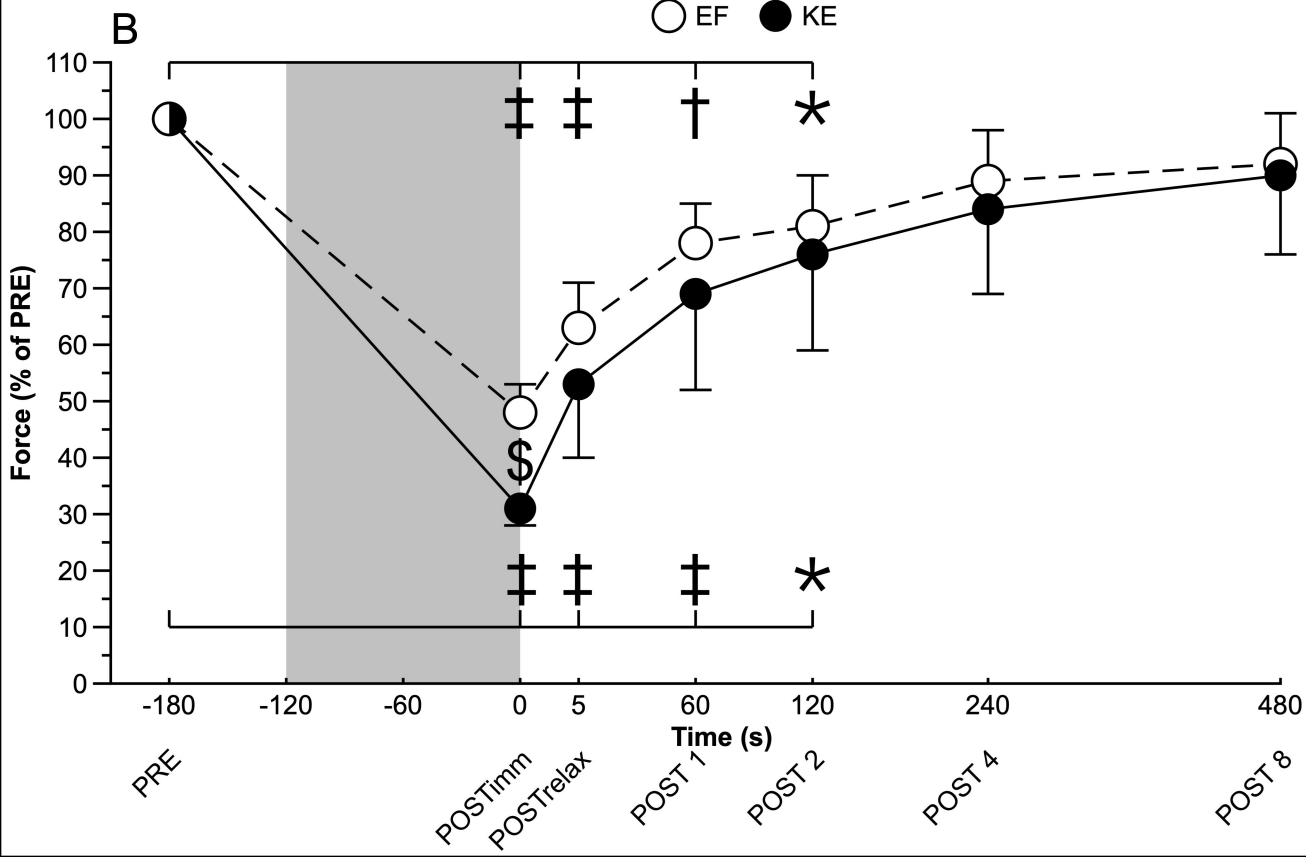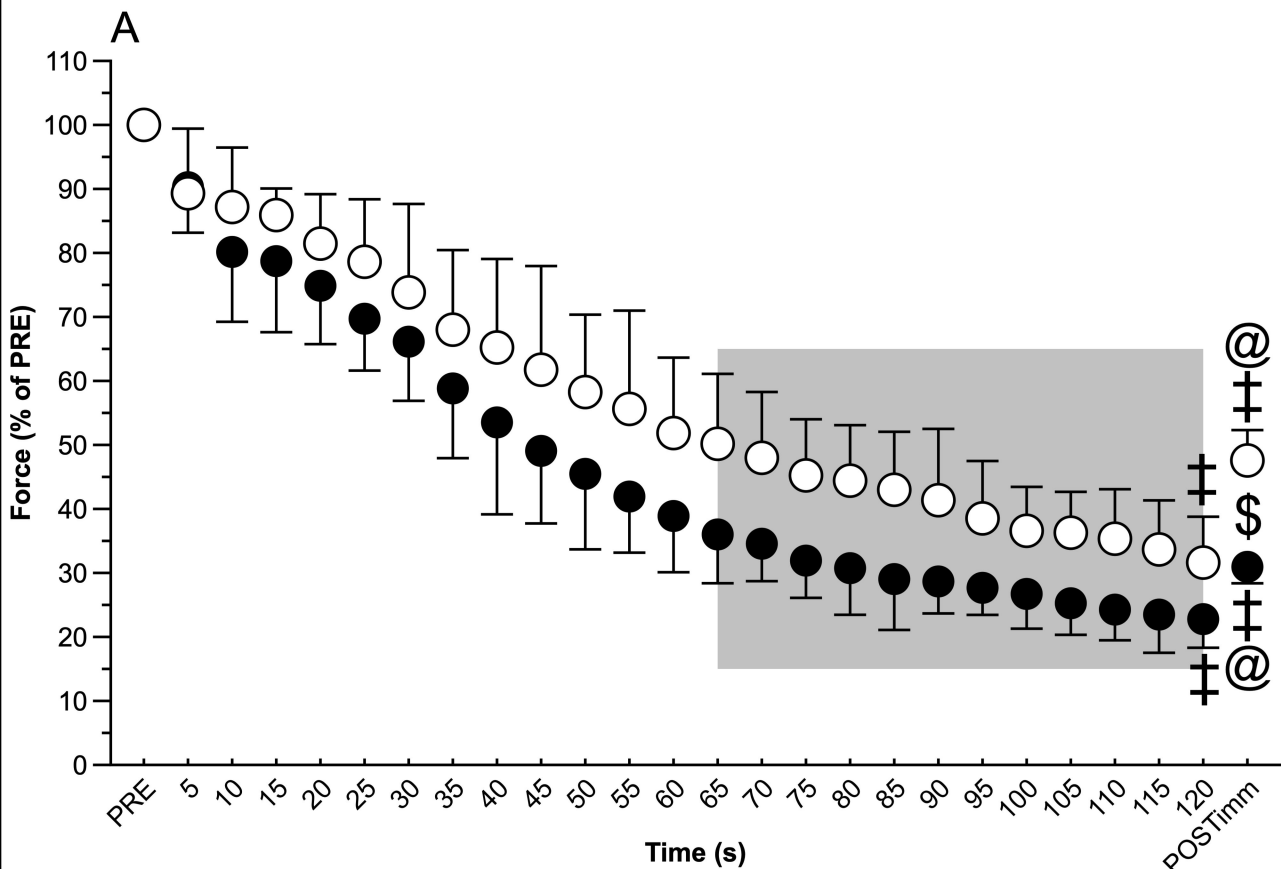664   differences between muscles within the same time-points $, $P < 0.001$.

665

666 **Figure 4.** Changes after the 2-min maximal voluntary isometric contraction (MVIC) in the

667 maximal M-wave (M_{max}) and spinal motor-evoked potentials [either as cervicomedullary

668 motor-evoked potentials (CMEP/M_{max}) in *biceps brachii* and *brachioradialis* ($n = 7$), or as

669 thoracic motor-evoked potentials (TMEP/M_{max}) in *rectus femoris* and *vastus lateralis* ($n = 8$)]

670 normalized to M_{max}. At the end of the 2-min MVIC a neuromuscular function evaluation was

671 performed as an extension of the 2-min MVIC (POSTimm) and additional evaluations were

672 performed after 5 s of relaxation (POSTrelax) and 1 (POST 1), 2 (POST 2), 4 (POST 4) and 8

673 (POST 8) min after the end of the 2-min MVIC. The shaded box indicates the sustained 2-

674 min MVIC and time 'zero' corresponds to the beginning of the recovery period. Values are

675 means and standard deviations and expressed as a percentage of the PRE evaluation. For

676 differences between time-points within the same muscle: *, $P < 0.05$; †, $P < 0.01$; ‡, $P <$

677 0.001. For differences between muscles within the same time-points: *biceps brachii* was

678 different than *rectus femoris* and *vastus lateralis* (# < 0.05); *brachioradialis* was different

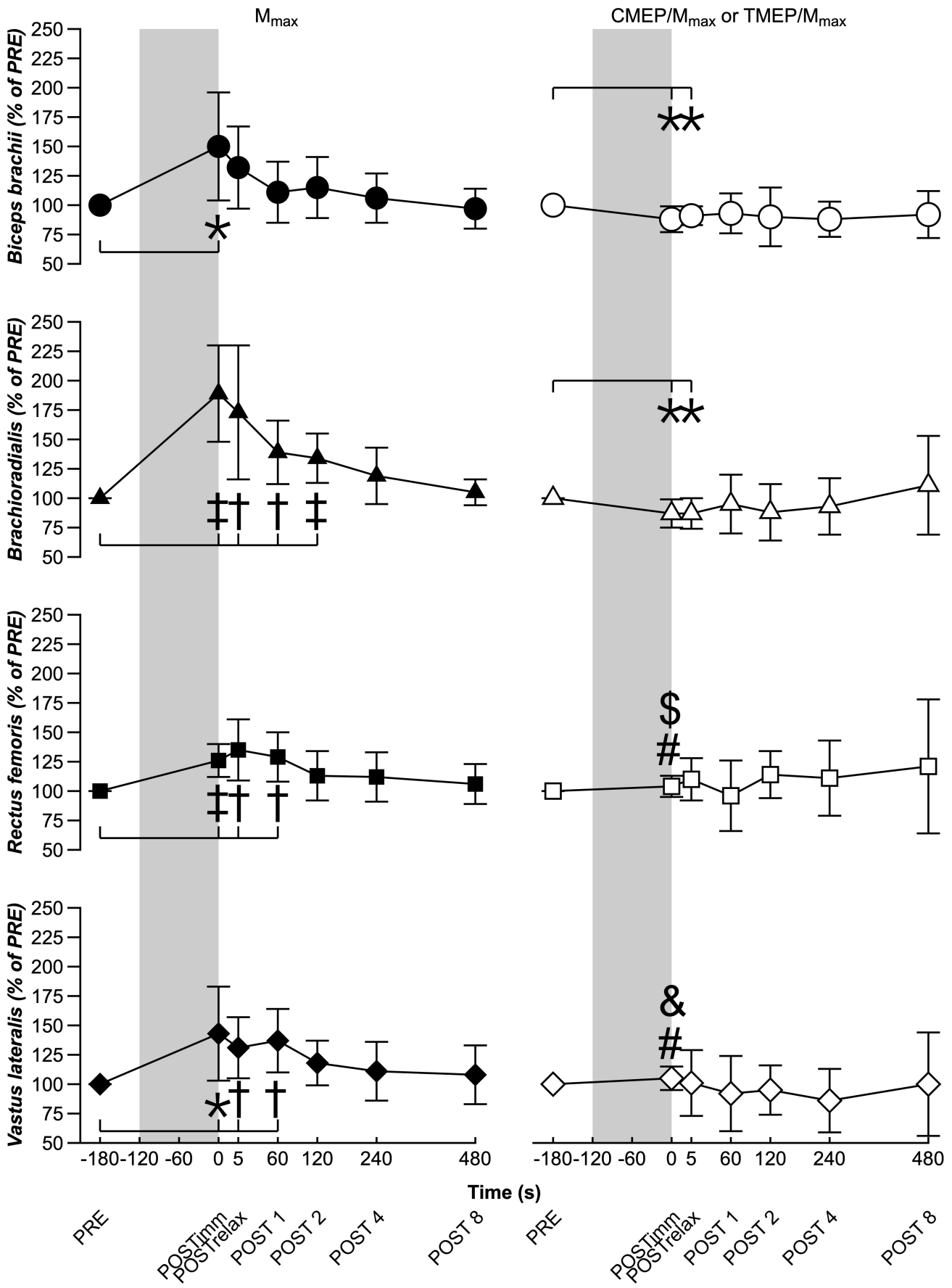679 than *rectus femoris* ($\$ < 0.001$) and *vastus lateralis* (& < 0.01).

680

681 **Figure 5.** Changes after the 2-min maximal voluntary isometric contraction (MVIC) in silent

682 period duration after transmastoid stimulation delivered to either the *biceps brachii* or the

683 *brachioradialis* ($n = 7$) and thoracic stimulation delivered to either the *rectus femoris* or the

684 *vastus lateralis* ($n = 8$). At the end of the 2-min MVIC a neuromuscular function evaluation

685 was performed as an extension of the 2-min MVIC (POSTimm) and additional evaluations

686 were performed after 5 s of relaxation (POSTrelax) and 1 (POST 1), 2 (POST 2), 4 (POST 4)

687 and 8 (POST 8) min after the end of the 2-min MVIC. The shaded box indicates the sustained

688 2-min MVIC and time 'zero' corresponds to the beginning of the recovery period. Values are

689 means and standard deviations and expressed as a percentage of the PRE evaluation. For
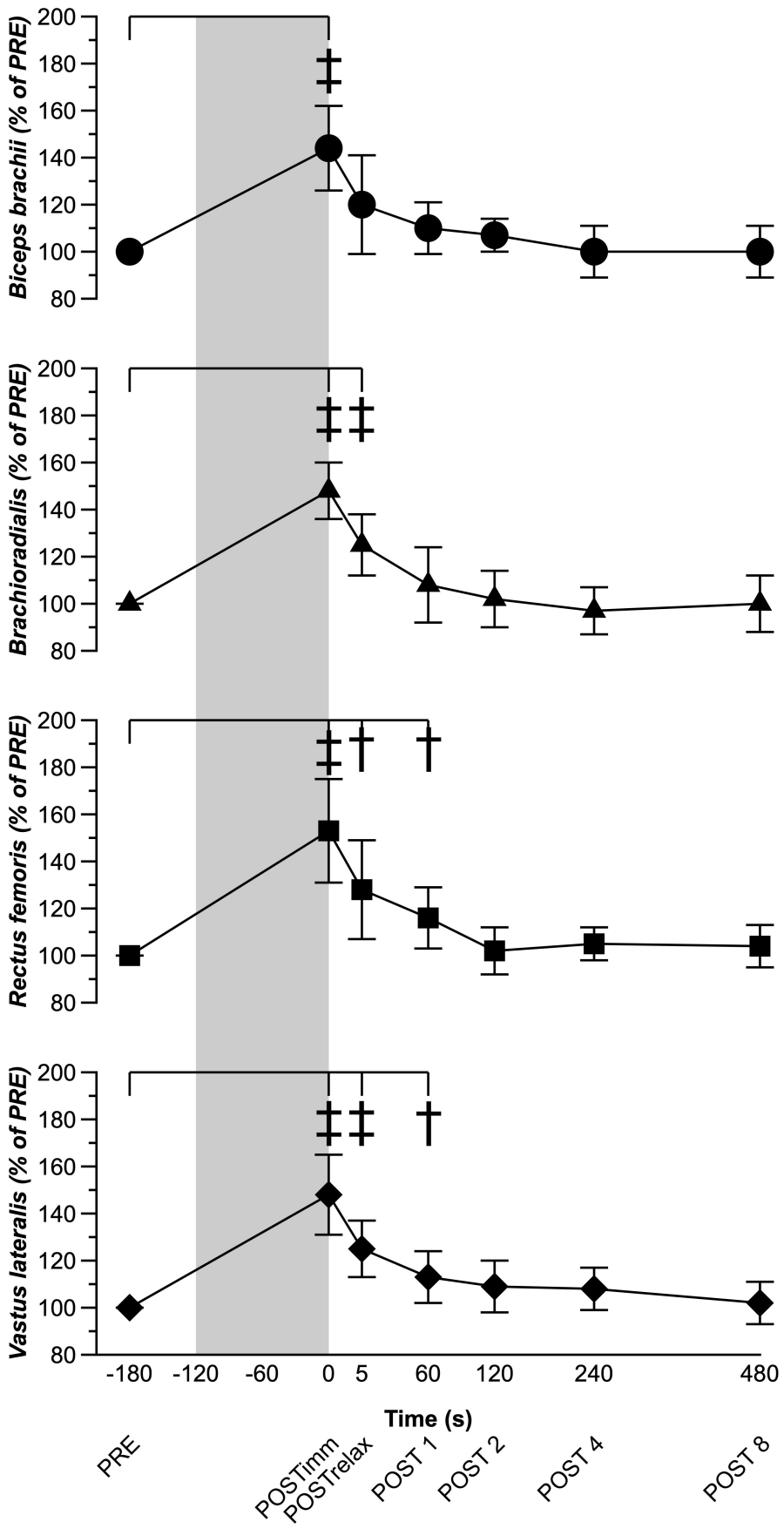
690   differences between time-points within the same muscle: *, $P < 0.05$; †, $P < 0.01$; ‡, $P <$

691   0.001.

2-min MVC

TIME (s)

-180   -120   -60   0   5   60   120   240   480

PRE   POSTimm   POSTrelax   POST 1   POST 2   POST 4   POST 8

Variable	EF ($n = 7$)		KE ($n = 8$)	
MVC (N)	285 (SD 44) Range: 244-377		590 (SD 85) Range: 481-679	
	BB ($n = 7$)	BR ($n = 7$)	RF ($n = 8$)	VL ($n = 8$)
M_{max} area (mV·s)	0.095 (SD 0.023) Range: 0.062-0.131	0.047 (SD 0.018) Range: 0.025-0.072	0.034 (SD 0.019) Range: 0.007-0.056	0.081 (SD 0.017) Range: 0.058-0.104
CMEP area (mV·s)	0.055 (SD 0.015) Range: 0.038-0.076	0.032 (SD 0.022) Range: 0.010-0.069		
TMEP area (mV·s)			0.026 (SD 0.014) Range: 0.007-0.047	0.042 (SD 0.020) Range: 0.023-0.078
SP_{CMEP} (ms)	55 (SD 4) Range: 49-60	55 (SD 6) Range: 49-66		

SP$_{TMEP}$ (ms)		57 (SD 4)	60 (SD 6)
		Range: 52-63	Range: 50-68

EF, elbow flexors; KE, knee extensors; BB, *biceps brachii*; BR, *brachioradialis*; RF, *rectus femoris*; VL, *vastus lateralis*; MVC, isometric maximal voluntary contraction; M$_{max}$, maximal M-wave; CMEP, cervicomedullary motor-evoked potential; TMEP, thoracic motor-evoked potential; SP$_{CMEP,}$ silent period after transmastoid electrical stimulation; SP$_{TMEP,}$ silent period after thoracic electrical stimulation.