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Use of transcranial magnetic stimulation to assess relaxation rates in unfatigued and fatigued knee-extensor muscles

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4 **Availability of data and material:** The data that support the findings of this study are
5 available on request from the corresponding author (JT).

6 **Code availability:** Not applicable.

7 **Authors' contributions:** GYM and JT conceived of and designed the research. GV and JT
8 performed the experiment. GV, AK and JT analyzed the data. GV, AK, GYM and JT
9 interpreted the data of the experiment. GV prepared the figures. GV and JT drafted the
10 manuscript. GV, AK, GYM and JT edited and revised the manuscript. GV, AK, GYM and
11 JT approved the final version of the manuscript.

12 **ABSTRACT**

13 We examined whether transcranial magnetic stimulation (TMS) delivered to the motor cortex
14 allows assessment of muscle relaxation rates in unfatigued and fatigued knee extensors (KE).
15 We assessed the ability of this technique to measure time course of fatigue-induced changes
16 in muscle relaxation rate and compared relaxation rate from resting twitches evoked by
17 femoral nerve stimulation. Twelve healthy men performed maximal voluntary isometric
18 contractions (MVC) twice before (PRE) and once at the end of a 2-min KE MVC and five
19 more times within 8 min during recovery. Relative (intraclass correlation coefficient; ICC_{2,1})
20 and absolute (repeatability coefficient) reliability and variability (coefficient of variation)
21 were assessed. Time course of fatigue-induced changes in muscle relaxation rate was tested
22 with generalized estimating equations. In unfatigued KE, peak relaxation rate coefficient of
23 variation and repeatability coefficient were similar for both techniques. Mean (95% CI)
24 ICC_{2,1} for peak relaxation rates were [0.933 (0.724-0.982)] and [0.889 (0.603-0.968)] for
25 TMS and femoral nerve stimulation, respectively. TMS-induced normalized muscle
26 relaxation rate was $-11.5 \pm 2.5 \text{ s}^{-1}$ at PRE, decreased to $-6.9 \pm 1.2 \text{ s}^{-1}$ ($-37 \pm 17\%$, $P < 0.001$),
27 and recovered by 2 min post-exercise. Normalized peak relaxation rate for resting twitch did
28 not show a fatigue-induced change. During fatiguing KE exercise, the change in muscle
29 relaxation rate as determined by the two techniques was different. TMS provides reliable
30 values of muscle relaxation rates. Furthermore, it is sufficiently sensitive and more
31 appropriate than the resting twitch evoked by femoral nerve stimulation to reveal fatigue-
32 induced changes in KE.

33

34 **Key words:** fatigue; knee extensors; transcranial magnetic stimulation; muscle relaxation
35 rate

36 INTRODUCTION

37 Muscle relaxation is an important component of movement control, particularly during
38 movements in which muscle activation has to switch between different contracting muscles
39 (Buccolieri et al. 2004). Muscle relaxation depends on the rate of detachment of cross-bridges
40 during the relaxation process (Houston et al. 1987) and represents the sum of all processes at
41 the level of the skeletal muscle that follow the cessation of the neural drive to the muscle
42 fibres, providing information about the intrinsic properties of muscle fibres (Dux 1993).
43 However, to date the scientific literature has emphasised muscle contraction, while muscle
44 relaxation is often overlooked (Kortman et al. 2012).

45 In humans, the properties of muscle fibres are commonly assessed by measuring the
46 resting twitch evoked by a supramaximal electrical stimulus of the peripheral nerve or
47 intramuscular nerve fibres in the relaxed muscle state (Millet et al. 2011). The characteristics
48 of the resting twitch provide information about both the speed of muscle contraction and
49 relaxation. Further, these characteristics provide insight into the force output from the muscle
50 (Todd et al. 2007). However, the relevance of this technique has been questioned since it
51 only reveals properties of the muscle at rest while muscle properties are most functionally
52 relevant during a voluntary contraction, when the central nervous system is actively driving
53 the muscle (Todd et al. 2007).

54 To overcome this issue, transcranial magnetic stimulation (TMS) delivered to the
55 motor cortex may offer a valuable alternative. TMS is a non-invasive technique that can be
56 used to excite or inhibit different cortical areas of the human brain. When single-pulse TMS
57 of sufficient intensity is delivered to the motor cortex during a voluntary contraction, it
58 induces transient excitation in both the electromyography (EMG) (i.e. motor-evoked

59 potential) and mechanical (force) responses (i.e. superimposed twitch) of the target muscle.
60 Following the motor-evoked potential, there is a period of near-silence in the EMG termed
61 the silent period. As a result of the withdrawal of voluntary drive, muscle fibres that are
62 voluntarily contracting relax and force decreases. Accordingly, it has been proposed to
63 analyze the rate of muscle relaxation during the silent period elicited by TMS delivered to
64 the motor cortex (Todd et al. 2005). This method has been applied to the finger flexors
65 (Molenaar et al. 2018), elbow flexors (Todd et al. 2005; Hunter et al. 2006; Todd et al. 2007;
66 Hunter et al. 2008; Molenaar et al. 2013), plantar flexors (McNeil et al. 2013; Yacyshyn et
67 al. 2017), and dorsiflexors (McNeil et al. 2013), either in an unfatigued or fatigued state.
68 Results have shown that TMS can be used to measure relaxation rates in the above-mentioned
69 muscle groups.

70 However, a direct comparison between TMS-induced muscle relaxation rate and the
71 relaxation rate determined from the resting twitch evoked by femoral nerve stimulation has
72 not been reported for the knee extensors (KE). It is possible that TMS-induced muscle
73 relaxation rate behaves differently for KE, when compared with other muscles, due to
74 different somatotopic organization and recruitment thresholds (Leung et al. 2018; Krishnan
75 2019), functional role (Maffiuletti et al. 2008) as well as neuromuscular aspects (Brouwer
76 and Ashby 1990; Saltin and Gollnick 2011; Vernillo et al. 2018; Temesi et al. 2019).
77 Therefore, understanding whether TMS is a valid technique that can be used for measuring
78 KE relaxation rate is important because KE is (i) responsible for knee-extensor force
79 production and therefore plays a key role during ambulatory, functional and sport activities
80 (Maffiuletti et al. 2008); and (ii) commonly used in studies investigating muscle fatigue with
81 TMS [e.g. (Sidhu et al. 2009; Goodall et al. 2012; Klass et al. 2012; Temesi et al. 2013;

82 Vernillo et al. 2018)]. Furthermore, the use of TMS, as opposed to peripheral electrical
83 stimulation, to assess muscle relaxation rate in KE would allow muscle contractile properties
84 to be examined while receiving drive from the central nervous system (Todd et al. 2007).

85 Therefore, the aim of this study was to assess whether TMS is appropriate for
86 measuring muscle relaxation rate in KE. An important characteristic of any measurement
87 must be close agreement between consecutive measurements in one participant
88 (repeatability) and small measurement error compared with the true difference between
89 participants (reliability) (Bartlett and Frost 2008). Accordingly, we compared the
90 repeatability and reliability of peak muscle relaxation rates calculated from the falling phase
91 of the resting twitch evoked by femoral nerve stimulation and the decrease in force during
92 the period of EMG silence after delivery of TMS during KE maximal voluntary contractions
93 in healthy participants. Furthermore, in response to a sustained KE maximal voluntary
94 contraction, we assessed the ability of TMS to measure the time course of changes in the
95 muscle relaxation rate with the development of fatigue.

96

97 **METHODS**

98 **Participants**

99 Twelve healthy and physically active males (age: 31 ± 9 years; height: 179 ± 7 cm; body
100 mass: 75 ± 9 kg) volunteered for this study. Exclusion criteria for participation were injury
101 to the lower limbs during the previous six months, history of heart disease or hypertension,
102 and contraindications to TMS (Rossi et al. 2011). Participants were instructed to avoid the
103 consumption of caffeine on the day of the experiment and avoid performing any strenuous
104 exercise during the 48 h prior to testing. This study conformed to the standards set by the

105 Declaration of Helsinki, except for registration in a database. The experimental protocol was
106 approved by the University of Calgary Conjoint Health Research Ethics Board (#REB14-
107 1625). Participants were informed of the experimental protocol and all associated risks prior
108 to giving written informed consent.

109

110 **Experimental protocol**

111 Results from some of the data collected from this protocol have previously been published
112 (Vernillo et al. 2018; Temesi et al. 2019; Vernillo et al. 2019; Vernillo et al. 2020). Each
113 participant completed one familiarization session and one experimental session. During the
114 familiarization session, participants performed maximal and submaximal voluntary isometric
115 contractions of KE with and without TMS or femoral nerve stimulation. The experimental
116 session consisted of a 2-min sustained KE MVC. Before each 2-min MVC (PRE), two
117 neuromuscular evaluations (separated by 60 s) with TMS and femoral nerve stimulation (see
118 Neuromuscular evaluation section) were performed. Peak force from the second MVC of the
119 neuromuscular evaluation was always within 5% of peak force from the first MVC of the
120 neuromuscular evaluation for all participants. Mean values from the two PRE neuromuscular
121 evaluations were used for subsequent analyses. At the end of the 2-min MVC, a
122 neuromuscular evaluation was performed as an extension of the 2-min MVC (i.e. the
123 participant was not permitted to relax) (POSTimm). Additional evaluations were performed
124 5 s after relaxation (POSTrelax), as well as 1 (POST 1), 2 (POST 2), 4 (POST 4), and 8
125 (POST 8) min after the end of the 2-min MVC. The two sessions were separated by between
126 3 and 7 days and each participant performed both sessions at the same time of day to control
127 for within-participant diurnal variation.

128

129 *Force recordings*

130 All measurements were taken from the participants' right leg. Force was measured by a
131 calibrated force transducer (LC101-2K; Omegadyne, Sunbury, OH) with amplifier attached
132 to the right leg by a noncompliant strap immediately proximal to the malleoli of the ankle
133 joint. Participants were seated in a custom-built isometric ergometer in an upright position
134 with both right knee and hips at 90° of flexion and secured by chest and hip straps. The force
135 transducer was fixed to the chair such that force was measured in direct line to the applied
136 force. The force was displayed on a computer screen and participants received real-time
137 visual feedback during all voluntary contractions.

138 Because muscle relaxation was determined from the decrease in KE force during the
139 silent period, the duration of the silent period was verified to ensure it was sufficient to allow
140 for measurement of peak relaxation rates during maximal contractions. Therefore, EMG of
141 the right *vastus lateralis*, and *rectus femoris* was recorded with pairs of self-adhesive surface
142 electrodes (10-mm recording diameter; Meditrace 100; Covidien, Mansfield, MA) in bipolar
143 configuration with 30-mm interelectrode distance and reference on the *patella*. Placement of
144 EMG electrodes for *vastus lateralis* was on the distal portion of the muscle belly between the
145 apex of the greater trochanter and the superolateral border of the *patella* and for *rectus*
146 *femoris* on the distal portion of the muscle belly between the anterior superior iliac spine and
147 the superior border of the *patella* (Botter et al. 2011). The skin where electrodes were placed
148 was shaved, lightly abraded, and cleaned with isopropyl alcohol in order to achieve a low
149 impedance level (<5 kΩ). Force and EMG signals were analog-to-digitally converted at a
150 sampling rate of 2000 Hz by PowerLab system (16/35, ADInstruments, Bella Vista,

151 Australia) and octal bioamplifier (ML138; ADInstruments; common mode rejection ratio =
152 85 dB, gain = 500) with band pass filter (5-500 Hz) and analyzed offline using Labchart 8
153 software (ADInstruments).

154

155 *Transcranial magnetic stimulation*

156 The motor cortex was stimulated by a magnetic stimulator (Magstim 200²; The Magstim
157 Company Ltd, Whitland, UK) with a 110-mm double-cone coil (maximum output of 1.4 T).
158 Single stimuli were delivered to the contralateral motor cortex, producing an induced postero-
159 anterior current. Every centimetre was demarcated from the vertex to 2 cm posterior to the
160 vertex along the nasal-inion line and 1 cm laterally over the left motor cortex. Optimal coil
161 position was determined by assessing MEP responses evoked during brief isometric
162 voluntary contractions at 20% MVC and 50% maximal stimulator output. The optimal coil
163 position was where the largest motor-evoked potentials in the *rectus femoris* were elicited.
164 Optimal coil position for the session was marked on a lycra swim cap. Stimulus intensity was
165 determined by stimulus-response curve from responses during brief isometric contractions at
166 20% MVC. Four consecutive contractions were performed at 15-s intervals at each of the
167 following randomly ordered stimulus intensities: 20, 30, 40, 50, 60, 70, and 80% maximal
168 stimulator output. Optimal stimulus intensity was defined as the lowest intensity eliciting
169 maximal MEP amplitudes with minimal antagonist responses (Temesi et al. 2014). Mean
170 stimulus intensity was $63 \pm 9\%$ of maximal stimulator output.

171

172 *Femoral nerve stimulation*

173 Resting muscle twitches were evoked by electrical stimulation (DS7A; Digitimer, Welwyn
174 Garden City, Hertfordshire, UK). Single pulses (1-ms duration) were delivered to the femoral
175 nerve trunk *via* a surface cathode taped into the femoral triangle (Meditrace 100) and a 50 ×
176 90 mm rectangular anode (Durastick Plus; DJO Global, Vista, CA) in the gluteal fold. During
177 femoral nerve stimulation, a small gauze ball was placed over the cathode before securing it
178 with tape in order to apply pressure over the stimulation site. Stimuli were delivered
179 incrementally in the relaxed muscle state until M-wave and twitch amplitudes plateaued. A
180 stimulus intensity of 130% of the intensity to elicit maximal M-wave and twitch amplitudes
181 was used throughout the experiment. The supramaximal stimulus intensity was 84 ± 36 mA.

182

183 *Neuromuscular evaluation*

184 The neuromuscular evaluation was previously published (Vernillo et al. 2018) and consisted
185 of a sustained contraction comprised of an MVC followed by 75% and 50% MVC for the
186 determination of the voluntary activation [i.e. the level of voluntary drive to the muscle
187 (Gandevia et al. 1995)]. TMS was delivered at each force level and participants were
188 instructed to recontract as quickly as possible to the pre-stimulus voluntary force (Mathis et
189 al. 1998). Each sustained contraction lasted approximately 9 s (~3 s per contraction intensity).
190 Immediately after the neuromuscular evaluation, a single femoral nerve electrical stimulation
191 was delivered when the muscle was relaxed. Visual feedback of the force produced, and
192 target force levels were provided to the participants by means of a real-time display on a
193 computer screen. For the purpose of the present study, only the evoked twitch and TMS
194 parameters during the 100% MVC were taken into consideration.

195

196 **Data Analysis**

197 The force traces were low-pass filtered by using a 4th order Butterworth filter with zero time-
198 lag and cut-off frequency of 10 Hz. This filtering process was necessary to remove the noise
199 of the instantaneous slope (force derivative). The duration of *vastus lateralis* and *rectus*
200 *femoris* silent periods were measured by visually inspecting the interval from the TMS
201 stimulus to the return of continuous voluntary EMG (Taylor et al. 1996).

202 Responses evoked by femoral nerve stimulation in the relaxed muscle in a potentiated
203 state were analysed for (i) amplitude of the potentiated peak twitch, (ii) time to peak
204 amplitude of the potentiated peak twitch (i.e. interval from the onset of the twitch to the peak
205 amplitude), and (iii) half-relaxation time of the potentiated peak twitch (i.e. interval between
206 the peak amplitude and the point at which force was reduced by 50%).

207 Muscle relaxation rates were calculated from the decrease in force during the silent
208 period following TMS delivery or the falling phase of the resting twitch evoked by femoral
209 nerve stimulation (Figure 1). In all instances, the peak rate of muscle relaxation was
210 calculated as the negative slope over a 10-ms interval (5 ms either side of the steepest
211 instantaneous slope) [e.g. (Todd et al. 2005; Todd et al. 2007; McNeil et al. 2013)]. To
212 account for differences in both voluntary strength and evoked twitch amplitude within and
213 between participants, normalized rates of relaxation were calculated by dividing the absolute
214 rates of relaxation by the peak force which preceded the relaxation. This value reflects the
215 relative peak relaxation rate of all knee-extensor muscles that contribute to the measured
216 force (voluntary plus evoked) and that are suppressed by the inhibitory effects of TMS (Todd
217 et al. 2005; Hunter et al. 2006; Todd et al. 2007; Hunter et al. 2008; McNeil et al. 2013;

218 Yacyshyn et al. 2017). Furthermore, time to peak relaxation was assessed as the time from
219 TMS stimulus until the moment of peak relaxation (Molenaar et al. 2013).

220

221 ****Figure 1 about here****

222

223 **Statistical analysis**

224 Absolute reliability is the variability due to random error (Ludbrook 2002) and is
225 consequently influenced by the degree to which measurements vary (with the assumption
226 that with lower variability, reliability is higher) (Vaz et al. 2013). To quantify absolute
227 reliability in the measurement error in unfatigued KE, the repeatability coefficient (RC, also
228 referred to as the smallest real difference) was determined. RC is the value below which the
229 absolute differences between two subsequent measurements would lie with 95% probability
230 (Beckerman et al. 2001; Vaz et al. 2013) and was calculated as:

231

$$RC = 2.77 \times S_w$$

232 where S_w is the within-participant standard deviation and 2.77 is obtained by multiplying $\sqrt{2}$
233 times 1.96 (Beckerman et al. 2001; Vaz et al. 2013). Furthermore, within-participant
234 variability was assessed by calculating the coefficient of variation (CV), defined as the ratio
235 of the within-participant standard deviation of the mean (Atkinson and Nevill 1998). CV for
236 all participants was calculated for all variables of interest as the within-participant standard
237 deviation divided by mean of the two measurements. The mean of all CV was considered as
238 the overall within-participant coefficient of variation. To compare both absolute and within-
239 participant reliability in unfatigued KE, paired *t*-tests were performed between peak muscle

240 relaxation rates determined via responses elicited by TMS and femoral nerve stimulation.
241 Two-way random effects, absolute agreement intra-class correlation coefficients ($ICC_{2,1}$)
242 were also calculated to determine relative reliability, defined as the size of the within-
243 participant measurement error to the inherent between-participants variability (Atkinson and
244 Nevill 1998; Vaz et al. 2013). $ICC_{2,1}$ are classified as poor (< 0.50), moderate (0.50-0.75),
245 good (0.75-0.90) and excellent (> 0.90) (Koo and Li 2016).

246 To test differences between PRE and POSTimm, as well as during the recovery time,
247 a longitudinal analysis was performed using generalized estimating equations (GEE; i.e. GEE
248 under ‘Generalized Linear Model’ procedure in SPSS v. 26) to take into account the
249 correlated nature of observations within each participant (i.e. within-participant
250 measurements) (Liang and Zeger 1986). If a significant main effect for time was observed,
251 Bonferroni’s test was used for *post-hoc* analysis. Statistical analyses were conducted using
252 IBMTM SPSSTM Statistics (version 26.0.0; IBM Corp., Somers, New York, NY) with the
253 criterion α -level set to 0.05.

254

255 **RESULTS**

256 **Repeatability and reliability in unfatigued knee-extensor muscles**

257 All relaxation properties showed similar CV and RC whether elicited by TMS or femoral
258 nerve stimulation (Table 1 and Figure 2). Furthermore, mean (95% CI) $ICC_{2,1}$ for peak
259 relaxation rates were 0.933 (0.724-0.982, rated moderate to excellent) for TMS-induced
260 relaxation and 0.889 (0.603-0.968, rated moderate to excellent) for resting twitches evoked
261 by femoral nerve stimulation.

262

263 ****Table 1 and Figure 2 about here****

264

265 **Force changes in fatigued knee-extensor muscles**

266 MVC force changes with fatigue are presented in Figure 3. MVC force showed a time effect
267 [χ^2 (6) = 772.7, $P < 0.001$]. MVC force decreased from 554 ± 85 N at PRE to 165 ± 55 N at
268 POSTimm ($30 \pm 10\%$ of PRE values, $P < 0.001$), and remained lower than PRE throughout
269 recovery (POST 8: 511 ± 77 N, $92 \pm 7\%$ of PRE values, $P = 0.008$).

270

271 ****Figure 3 about here****

272

273 **Resting twitch-derived parameters**

274 Potentiated peak twitch amplitude showed a time effect [χ^2 (6) = 935.8, $P < 0.001$]. The
275 amplitude decreased from 144 ± 16 N at PRE to 40 ± 12 N at POST ($28 \pm 9\%$ of PRE values,
276 $P < 0.001$), and remained lower than PRE throughout recovery (POST 8: 109 ± 16 N, $76 \pm$
277 7% of PRE values, $P < 0.001$) (Table 2).

278 Time to peak amplitude of the potentiated peak twitch showed a time effect [χ^2 (6) =
279 74.2, $P < 0.001$]. However, no time points were different than PRE ($P \geq 0.334$) (Table 2).

280 Half-relaxation time of the potentiated peak twitch showed a time effect [χ^2 (6) =
281 28.1, $P < 0.001$]. However, no time points were different than PRE ($P \geq 0.300$) (Table 2).

282 Normalized peak relaxation rate showed a time effect [χ^2 (1) = 49.3, $P < 0.001$].
283 However, no time points were different than PRE (all $P = 1.000$) (Table 2).

284

285 ****Table 2 about here****

286

287 **TMS-derived parameters**

288 For all participants at all time points, the duration of the silent period was sufficient to allow
289 for measurement of the peak relaxation rate of muscle fibres (Table 3). Time to peak
290 relaxation showed a time effect [$\chi^2(6) = 678.0, P < 0.001$]. Time to peak relaxation increased
291 from 107 ± 9 ms at PRE to 141 ± 33 ms at POSTimm ($131 \pm 28\%$ of PRE values, $P = 0.001$),
292 and recovered by POST 4 (110 ± 10 ms, $102 \pm 7\%$ of PRE values, $P = 1.000$).

293

294 ****Table 3 about here****

295

296 Absolute and normalized peak relaxation rate changes with fatigue are presented in
297 Figure 4. The absolute peak relaxation rate showed a time effect [$\chi^2(6) = 565.0, P < 0.001$].
298 Absolute peak relaxation rate decreased from $-6423 \pm 1838 \text{ N}\cdot\text{s}^{-1}$ at PRE to $-1356 \pm 394 \text{ N}\cdot\text{s}^{-1}$
299 at POSTimm ($22 \pm 6\%$ of PRE values, $P < 0.001$), and recovered by POST 8 (-6383 ± 1943
300 $\text{N}\cdot\text{s}^{-1}$, $100 \pm 15\%$ of PRE values, $P = 1.000$).

301 The normalized peak relaxation rate showed a time effect [$\chi^2(6) = 89.1, P < 0.001$].
302 Normalized peak relaxation decreased from $-11.5 \pm 2.5 \text{ s}^{-1}$ at PRE to $-6.9 \pm 1.2 \text{ s}^{-1}$ at
303 POSTimm ($63 \pm 17\%$ of PRE values, $P < 0.001$), and recovered by POST 2 ($10.1 \pm 2.0 \text{ s}^{-1}$,
304 $89 \pm 13\%$ of PRE values, $P = 0.052$).

305

306 ****Figure 4 about here****

307

308 **DISCUSSION**

309 The present study shows that the use of TMS delivered to the knee-extensor muscles can be
310 used to measure muscle relaxation rates, both in unfatigued and fatigued knee extensors, an
311 important muscle group for ambulatory and functional activities.

312

313 **Repeatability and reliability in unfatigued knee-extensor muscles**

314 Our results show that repeatability of muscle relaxation rates determined from the decrease
315 in force during the silent period following TMS delivery during MVC was similar to
316 repeatability when compared to the falling phase of the resting twitch evoked by femoral
317 nerve stimulation. This is shown by similar CV and RC in the muscle relaxation rates. The
318 mean CV for the TMS-induced normalized peak relaxation rate is similar to that previously
319 reported for finger flexors (Molenaar et al. 2018) and elbow flexors (Todd et al. 2007) in
320 healthy participants. Furthermore, RCs were also similar to that previously reported for finger
321 flexors (Molenaar et al. 2018) in healthy male participants. Relative reliability was also rated
322 moderate to excellent, as indicated by a mean $ICC_{2,1}$ value for TMS-induced peak relaxation
323 rate of 0.933 (95% CI of 0.724-0.982). Similar results have previously been reported for
324 finger flexors (Molenaar et al. 2018) in healthy male participants. Reliability refers to the
325 amount of measurement error that is deemed acceptable for the effective use of a technique;
326 and greater reliability implies that measurement differences are less likely to be due to
327 measurement errors (Atkinson and Nevill 1998). In other words, greater reliability implies a
328 greater sensitivity of the measurement in detecting true differences between participants.

329

330 **Peak relaxation rate in unfatigued knee extensors**

331 The interruption of cortical output and motoneuron activity during the TMS-induced silent
332 period implies that all KE muscle fibres that were previously contracting voluntarily (plus
333 any additional muscle fibres recruited by TMS) were now relaxing. This relaxation rate
334 reflects intrinsic contractile properties of KE rather than the ability of participants to
335 withdraw neural drive as during voluntary relaxations (Todd et al. 2007). In other words,
336 peak rate of muscle relaxation determined from the decrease in force during the silent period
337 during voluntary contractions represents only intrinsic muscle relaxation properties. During
338 complete relaxation of a muscle (i.e. during voluntary relaxation, the TMS-induced silent
339 period or the relaxation phase following a twitch), the time course of relaxation is due to an
340 interplay between the membrane-bound Ca^{2+} transport proteins and the sarcomeric proteins.
341 This interplay presents a slow phase followed by a fast (almost mono-exponential), phase
342 [for a comprehensive review see Poggesi et al. (2005)]. Previous studies reported faster mean
343 relaxation rates to the present one in healthy young men for finger flexors [-14.1 s^{-1}
344 (Molenaar et al. 2018)], elbow flexors [-13.5 s^{-1} (Hunter et al. 2006), -12.9 s^{-1} (Hunter et al.
345 2008), -14.3 s^{-1} (Molenaar et al. 2013)], and plantarflexors [-13.1 s^{-1} (Yacyshyn et al. 2017)].
346 These faster relaxation rates could be due to a greater proportion of fast-twitch muscle fibres
347 in the above-mentioned muscles compared to KE (Johnson et al. 1973).

348

349 **Peak relaxation rate in fatigued knee extensors**

350 Absolute peak relaxation rates determined from the TMS-induced decrease in MVC force
351 were affected by fatigue, slowing at the end of the 2-min MVC. After accounting for the
352 participants' force level, normalized peak relaxation rates showed similar results, declining
353 by ~37% from PRE. With the use of TMS in KE, we showed fatigue-induced slowing of

354 relaxation rate as previously reported during voluntary relaxation [e.g. (Bigland-Ritchie et
355 al. 1992)], electrically induced relaxation [e.g. (Bigland-Ritchie et al. 1983)], and TMS-
356 induced relaxation [e.g. (Todd et al. 2005; Hunter et al. 2006; Todd et al. 2007; Hunter et al.
357 2008; Molenaar et al. 2018)]. Since TMS-induced muscle relaxation rates only represent the
358 intrinsic properties of a muscle, fatigue-induced changes in relaxation rate could have been
359 due to a reduction in Ca^{2+} uptake by the sarcoplasmic reticulum (Gollnick et al. 1991).
360 Indeed, muscle relaxation is initiated by a reduction in sarcoplasmic $[\text{Ca}^{2+}]$, and the efficiency
361 of this process is dictated by three successive steps of Ca^{2+} removal: (i) dissociation of Ca^{2+}
362 from troponin C, (ii) translocation of Ca^{2+} to near the entry point of the sarcoplasmic
363 reticulum, and (iii) uptake of Ca^{2+} into the sarcoplasmic reticulum by the Ca^{2+} pump (Gordon
364 et al. 2000). When fatigue reduced KE force by ~70%, we observed a decrease in the
365 normalized peak relaxation rate. However, the normalized peak relaxation rate for the resting
366 twitch evoked by femoral nerve stimulation did not show a fatigue-induced change (from -
367 $9.4 \pm 1.4 \text{ s}^{-1}$ at PRE to $-10.5 \pm 1.7 \text{ s}^{-1}$ at POST, $P = 1.000$). Therefore, TMS-induced muscle
368 relaxation rate reveals different results than the relaxation rate determined from the resting
369 twitch evoked by femoral nerve stimulation in the fatigued KE, consistent with results
370 previously observed for fatigued elbow flexors (Todd et al. 2007). Since muscle relaxation
371 rate depends on the rate of detachment of cross-bridges during the relaxation process
372 (Houston et al. 1987), in a fatigued state TMS-induced muscle relaxation rate may be more
373 sensitive than the relaxation rate determined from the resting twitch evoked by femoral nerve
374 stimulation to an altered muscle state.

375

376 **Limitations**

377 Muscle relaxation properties can also be measured by high-frequency tetanic electrical
378 stimulation, inducing a maximal sustained contraction (de Ruiter et al. 1999). However, this
379 technique is very painful (especially in large muscle groups such as KE), making it unsuitable
380 in clinical populations such as patients with neurological disorders. Recently, Molenaar et al.
381 (2018) argued that voluntary relaxation after a finger-flexor MVC is a better representation
382 of physiological muscle relaxation than electrical stimulation. This is because in voluntary
383 motor unit recruitment, motor units are recruited according to the size principle (from small
384 to large motor units) (Henneman 1957), whereas electrical stimulation recruits motor units
385 in a nonselective, spatially fixed, and temporally synchronous pattern (from large to small
386 motor units) (Gregory and Bickel 2005; Bergquist et al. 2011; Bickel et al. 2011). However,
387 Molenaar et al. (2018) also compared TMS-induced muscle relaxation with voluntary muscle
388 relaxation and TMS was more sensitive for assessing muscle relaxation rate.

389

390 **Conclusion**

391 TMS provided suitable measures of peak relaxation rates in unfatigued KE. The use of TMS
392 for measuring muscle relaxation during MVC also seems to be sufficiently sensitive and more
393 appropriate than the resting twitch evoked by femoral nerve stimulation to reveal changes in
394 KE contractile properties that one would expect after a sustained fatiguing isometric maximal
395 contraction. Although resting twitches are deemed more practical than TMS-induced muscle
396 relaxation rates (e.g. when the equipment is unavailable or participants have
397 contraindications to the use of TMS), TMS may be useful to provide information about the
398 properties of KE in its most functionally relevant state, that is during voluntary contraction
399 (Todd et al. 2007). In other words, TMS-induced muscle relaxation rates reflect the same

400 physiological mechanisms as the relaxation rate after a single electrical twitch but examine
401 the muscle fibres when the central nervous system is driving voluntary muscle contraction.
402 Furthermore, determination of the TMS-induced muscle relaxation rate allows tracking of
403 fatigue-induced changes in intrinsic KE contractile properties without requiring the
404 interruption of ongoing contractions that potentially can alter the intrinsic muscle contractile
405 properties (Todd et al. 2005; Todd et al. 2007).

406 In conclusion, TMS-induced KE muscle relaxation is a reliable technique to measure
407 intrinsic muscle relaxation properties. The quantification of TMS-induced KE muscle
408 relaxation may help to inform research design and methodologies in TMS studies that directly
409 investigate the muscle relaxation rate of KE, which is often implicated in exercise and human
410 performance.

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556

558 **Table 1.** Repeatability and reliability of parameters related to the contractile properties of unfatigued knee-extensor muscles.

559 Values are means (95% confidence interval).

	Stimulation site	RC	CV	ICC_{2,1}
Normalized peak relaxation rate	Motor cortex	1.8 s ⁻¹ (0.7-3.0)	5.6% (3.0-8.0)	0.933 (0.724-0.982)
	Femoral nerve	1.5 s ⁻¹ (0.7-2.2)	5.9% (2.6-9.2)	0.889 (0.603-0.968)
Time to peak relaxation	Motor cortex	9.5 ms (5.6-13.6)	3.3% (1.8-4.7)	0.891 (0.619-0.968)
Potentiated peak twitch amplitude	Femoral nerve	15.9 N (2.1-29.8)	3.9% (0.5-7.2)	0.828 (0.437-0.950)
Time to peak amplitude	Femoral nerve	3.2 ms (0.6-5.7)	1.3% (0.3-2.3)	0.932 (0.772-0.980)
Half-relaxation time	Femoral nerve	10.0 ms (2.2-17.7)	4.7% (1.4-8.0)	0.893 (0.645-0.969)

560 RC, repeatability coefficient; CV, coefficient of variation; ICC_{2,1}, two-way random effects, absolute agreement intra-class

561 correlation coefficients.

562

563 **Table 2.** Characteristics of the potentiated resting twitch evoked by femoral nerve stimulation before (PRE) and at the end of the
564 2-min MVC. After the sustained contraction, a neuromuscular function evaluation was performed as an extension of the 2-min
565 MVC (POSTimm) and additional evaluations were performed after 5 s of relaxation (POSTrelax) and 1 (POST 1), 2 (POST 2), 4
566 (POST 4), and 8 (POST 8) min after the end of the 2-min MVC. Values are means \pm SD (min/max). For differences between time-
567 points ‡, $P < 0.001$.

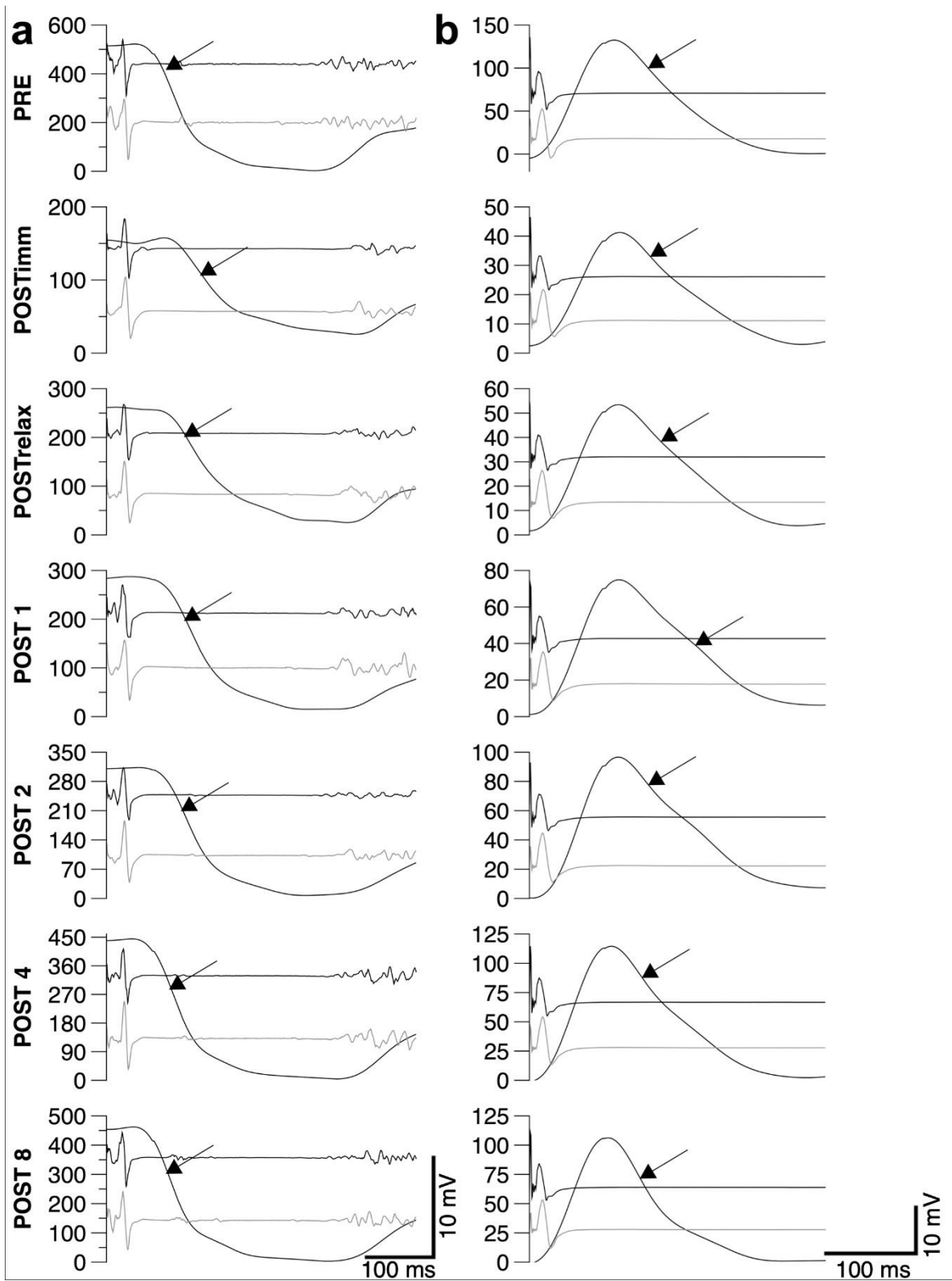
Variable	PRE	POSTimm	POSTrelax	POST 1	POST 2	POST 4	POST 8
Potentiated peak twitch amplitude (N)	144 \pm 16 (115/182)	40 \pm 12‡ (17/67)	50 \pm 17‡ (23/88)	84 \pm 28‡ (44/139)	111 \pm 27‡ (63/161)	120 \pm 18‡ (94/143)	116 \pm 19‡ (86/144)
Time to peak amplitude (ms)	88 \pm 6 (79/99)	88 \pm 5 (79/94)	92 \pm 10 (79/94)	92 \pm 6 (80/102)	90 \pm 4 (79/97)	86 \pm 5 (75/94)	81 \pm 4 (72/87)
Half-relaxation time (ms)	72 \pm 12 (58/93)	72 \pm 13 (50/92)	72 \pm 15 (51/101)	74 \pm 13 (58/99)	74 \pm 13 (56/101)	69 \pm 11 (51/82)	65 \pm 10 (51/87)
Normalized peak rate of relaxation (s ⁻¹)	-9.4 \pm 1.4 (-7.1/-11.3)	-10.5 \pm 1.7 (-8.2/-13.6)	-10.1 \pm 1.5 (-7.4/-12.3)	-8.9 \pm 1.3 (-6.8/-10.4)	-8.9 \pm 1.4 (-6.8/-11.2)	-9.4 \pm 1.6 (-7.7/12.4)	-11.2 \pm 1.3 (-9.3/-13.8)

568

569 **Table 3.** Comparison of the time to peak relaxation and the silent period evoked after delivery of the transcranial magnetic
570 stimulation during maximal voluntary contractions. The neuromuscular evaluation was performed before (PRE) and at the end of
571 the 2-min MVC. After the sustained contraction, a neuromuscular function evaluation was performed as an extension of the 2-min
572 MVC (POSTimm) and additional evaluations were performed after 5 s of relaxation (POSTrelax) and 1 (POST 1), 2 (POST 2), 4
573 (POST 4), and 8 (POST 8) min after the end of the 2-min MVC. Values are means \pm SD (min/max). For differences between time-
574 points †, $P < 0.01$; ‡, $P < 0.001$.

Variable	PRE	POSTimm	POSTrelax	POST 1	POST 2	POST 4	POST 8
Time to peak relaxation (ms)	107 \pm 9 (90/120)	141 \pm 33 [†] (60/179)	143 \pm 15 [‡] (128/169)	129 \pm 12 [‡] (111/148)	121 \pm 11 [‡] (105/137)	110 \pm 10 (97/128)	105 \pm 7 (95/118)
<i>Rectus femoris</i> silent period (ms)	275 \pm 58 (168/365)	313 \pm 52 [‡] (221/398)	277 \pm 64 (178/375)	267 \pm 64 (159/356)	270 \pm 64 (172/350)	275 \pm 61 (182/365)	263 \pm 64 (188/354)
<i>Vastus lateralis</i> silent period (ms)	277 \pm 61 (166/364)	319 \pm 53 [‡] (219/414)	277 \pm 67 (147/375)	267 \pm 62 (166/369)	273 \pm 54 (192/363)	269 \pm 65 (164/373)	266 \pm 65 (183/375)

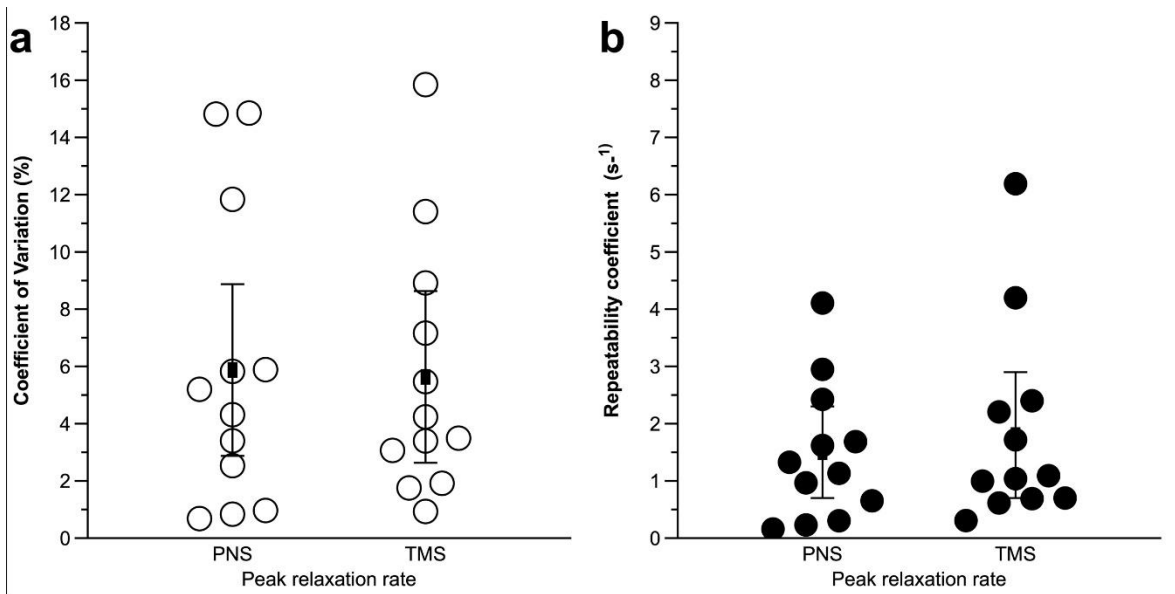
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577 **Figure 1.** Peak muscle relaxation rates before the 2-min maximal MVC (PRE) and at the end
578 of the 2-min MVC. After the sustained contraction, a neuromuscular function evaluation was
579 performed as an extension of the 2-min MVC (POSTimm) and additional evaluations were
580 performed after 5 s of relaxation (POSTrelax) and 1 (POST 1), 2 (POST 2), 4 (POST 4), and
581 8 (POST 8) min after the end of the 2-min MVC. Peak muscle relaxation rates were calculated
582 from the decrease in force during the silent period during maximal voluntary contractions
583 (Panels A), and from the falling phase of the resting twitch evoked by femoral nerve
584 stimulation (Panels B). Stimuli were delivered at time 0 ms. Peak rate of relaxation was
585 calculated as the negative slope over a 10-ms interval (5 ms either side of the steepest
586 instantaneous slope). To account for differences in both voluntary strength and evoked twitch
587 amplitude, normalized rates of relaxation were calculated by dividing the absolute rates of
588 relaxation by the peak force which preceded the relaxation. EMG traces for *rectus femoris*
589 (black traces) and *vastus lateralis* (grey traces) show muscular responses evoked by TMS
590 (Panels A) and femoral nerve stimulation (Panels B). Force and EMG traces are from a single
591 participant (33-year-old man). Arrows indicate the time at which the peak relaxation rate
592 occurred. Different scales have been used for y-axes for illustrative purposes.

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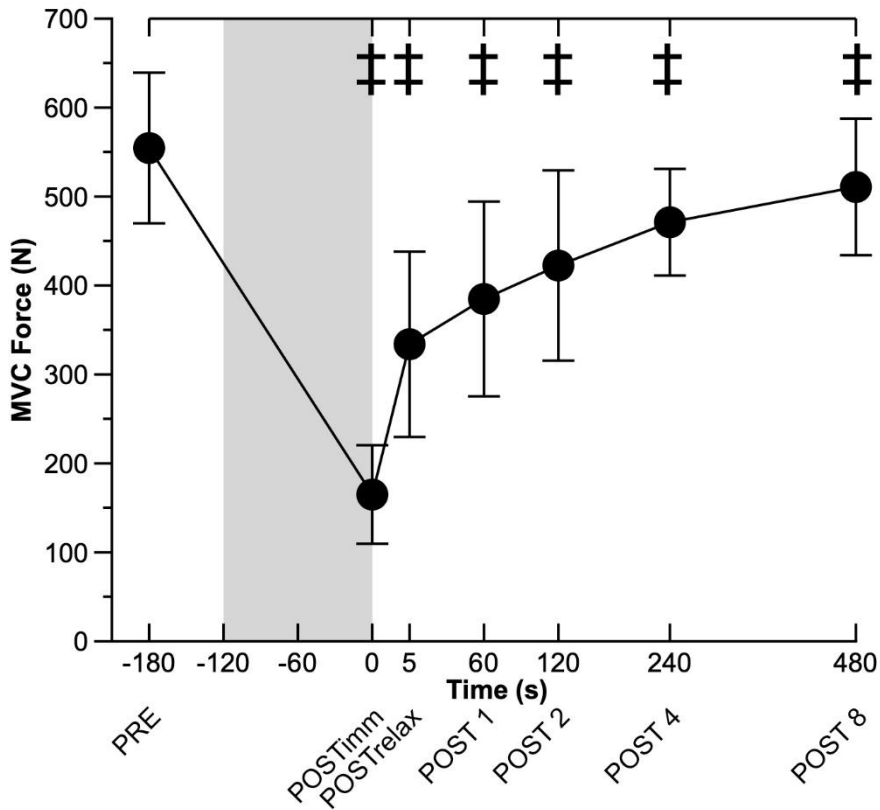


595

596 **Figure 2.** Comparison of coefficient of variation (Panel A), and repeatability coefficient
 597 (Panel B) of peak muscle relaxation rates determined from the falling phase of the resting
 598 twitch evoked by femoral nerve stimulation (PNS), and the decrease in force during the silent
 599 period during maximal voluntary contractions (TMS). Empty circles represent individual
 600 data, black squares means, and error bars 95% confidence intervals. Different scales have
 601 been used for y-axes for illustrative purposes.

602

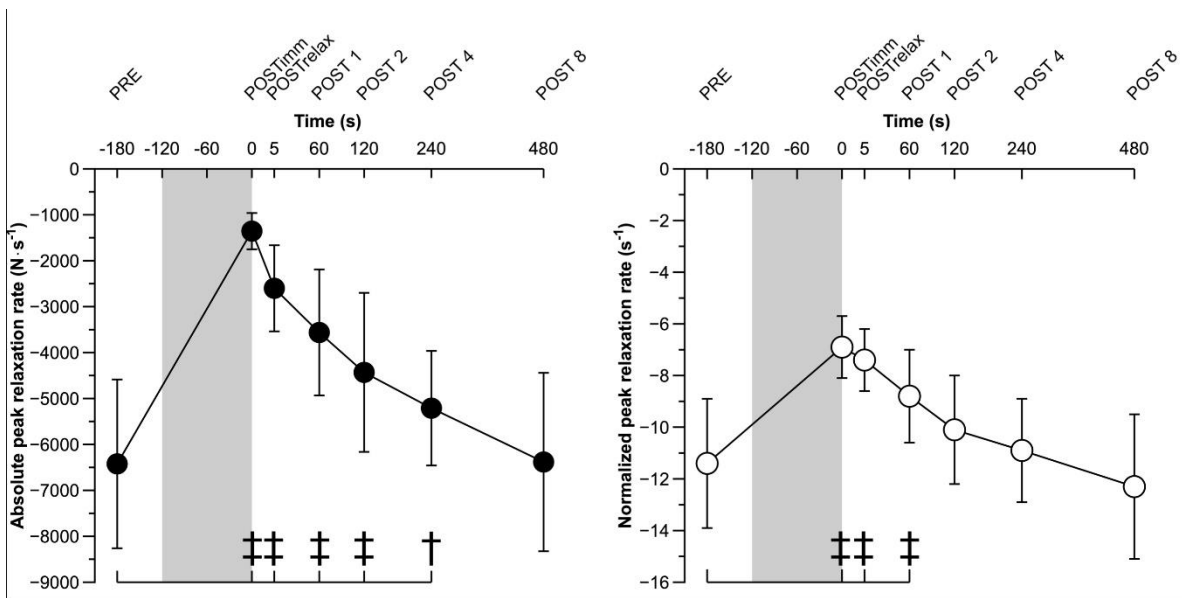
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604

605 **Figure 3.** Changes in maximal voluntary contraction (MVC) force. The neuromuscular
 606 function evaluation was performed before (PRE) and at the end of the 2-min MVC. After
 607 the sustained contraction, a neuromuscular function evaluation was performed as an
 608 extension of the 2-min MVC (POSTimm) and additional evaluations were performed after
 609 5 s of relaxation (POSTrelax) and 1 (POST 1), 2 (POST 2), 4 (POST 4), and 8 (POST 8)
 610 min after the end of the 2-min MVC. The shaded box indicates the sustained 2-min MVC
 611 and time 'zero' corresponds to the beginning of the recovery period. Values are means \pm
 612 SD. For differences between time-points ‡, $P < 0.001$.

613



614

615 **Figure 4.** Changes in absolute and normalized peak relaxation rates (as determined from the
 616 TMS-induced decrease in force) during maximal voluntary contractions. The neuromuscular
 617 function evaluation was performed before (PRE) and at the end of the 2-min MVC. After the
 618 sustained contraction, a neuromuscular function evaluation was performed as an extension
 619 of the 2-min MVC (POSTimm) and additional evaluations were performed after 5 s of
 620 relaxation (POSTrelax) and 1 (POST 1), 2 (POST 2), 4 (POST 4), and 8 (POST 8) min after
 621 the end of the 2-min MVC. The shaded box indicates the sustained 2-min MVC and time
 622 'zero' corresponds to the beginning of the recovery period. Values are means \pm SD. For
 623 differences between time-points †, $P < 0.01$; ‡, $P < 0.001$.

624