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1 **Acute effects of conventional vs. wide-pulse neuromuscular electrical stimulation on**
2 **quadiceps evoked torque and neuromuscular function**

3

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14

15 **Running title:** Acute effects of conventional vs. wide-pulse NMES

16

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ABSTRACT

26 **Purpose:** The effectiveness of a neuromuscular electrical stimulation (NMES) program is
27 proportional to the level of evoked torque, which can be achieved with either conventional or
28 wide-pulse stimulations. The aim of this study was to compare evoked torque, objective
29 fatigability and related peripheral and central alterations, as well as changes in central nervous
30 system (CNS) excitability induced by an acute session of conventional vs. wide-pulse NMES.

31 **Methods:** Seventeen young men underwent three 20-min NMES sessions: conventional (0.2
32 ms/50 Hz), wide-pulse at 50 Hz (1 ms/50 Hz) and wide-pulse at 100 Hz (1 ms/100 Hz).
33 Neuromuscular measurements (i.e., maximal voluntary contraction, voluntary activation,
34 evoked responses to femoral nerve stimulation and CNS excitability) were performed on the
35 right quadriceps femoris muscle before and after each NMES session. CNS excitability was
36 measured using transcranial magnetic, thoracic and transcutaneous spinal cord stimulations.

37 **Results:** The level of evoked torque was not significantly different between conventional and
38 wide-pulse protocols applied at the maximal tolerable current intensity. All NMES protocols
39 induced objective fatigability (~14% decrease in maximal voluntary contraction torque, $p <$
40 0.001) associated with peripheral (decrease in doublet torque and potentiated M-wave
41 amplitude, $p = 0.002$ and $p < 0.001$, respectively) but not central (unchanged voluntary
42 activation, $p = 0.79$) alterations. However, these acute changes did not differ between NMES
43 protocols and none of the NMES protocols modified markers of CNS excitability.

44 **Conclusion:** These results may allow to conjecture that chronic effects and treatment
45 effectiveness could be comparable between conventional and wide-pulse NMES.

46

47

NEW & NOTEWORTHY

48 This study is the first to investigate the level of evoked torque generated by conventional and
49 wide-pulse NMES protocols applied to the quadriceps muscle at the maximal tolerable current
50 intensity, as well as NMES-induced objective fatigability and related peripheral and central
51 alterations, with a particular emphasis on changes in CNS excitability. A similar level of
52 evoked torque and objective fatigability was observed between NMES protocols, with
53 peripheral but no central alterations (including CNS excitability).

54

55 **Keyword:** NMES; fatigability; corticospinal excitability; spinal excitability; motoneuronal
56 excitability

57

58 **Abbreviations:** ANOVA, analysis of variance; CNS, central nervous system; CONV,
59 conventional NMES (0.2 ms/50 50 Hz); Db100, doublet evoked by 100-Hz paired pulses;
60 EMG, electromyography; EPT, evoked peak torque; MEP, motor evoked potential; M_{POT} ,
61 potentiated M-wave; M_{SUP} , M-wave evoked during submaximal contractions, MVC, maximal
62 voluntary contraction; NMES, neuromuscular electrical stimulation; PRE, before the 20-min
63 NMES protocol; POST, after the 20-min NMES protocol; RF, rectus femoris; SD, standard
64 deviation; SR, spinal reflex; TMEP, thoracic motor evoked potential; TTI, torque-time
65 integral; VA, voluntary activation; VL, vastus lateralis; VM, vastus medialis; WP50, wide-
66 pulse NMES applied at 50 Hz; WP100, wide-pulse NMES applied at 100 Hz; 20% MVC-
67 EMG, EMG recorded during submaximal contractions at 20% MVC.

INTRODUCTION

69 Neuromuscular electrical stimulation (NMES), which consists of evoking contractions
70 by applying an electrical current - mainly over the muscle belly but also occasionally over the
71 nerve trunk - via surface electrodes, is commonly used in rehabilitation for the
72 recovery/preservation/improvement of muscle mass and function ¹. The conventional use of
73 NMES in clinical practice, mainly targeting the quadriceps femoris, usually encompasses
74 narrow pulses (0.2-0.5 ms) delivered at a frequency of 30-50 Hz ². Patients are encouraged to
75 attain their maximal tolerable current intensity, as achieving the highest level of evoked
76 torque is a key factor for optimizing the effectiveness of a NMES program ³⁻⁵. Indeed, Lai et
77 al. ³ reported a linear dose-response relationship between NMES effectiveness and evoked
78 torque, with training-induced strength gains of 24% and 48% for evoked torque levels of 25%
79 and 50% of maximal voluntary contraction (MVC), respectively. However, the ability to
80 increase current intensity is limited by the associated discomfort ⁶ and the maximal stimulator
81 output ⁷. Thus, it is of clinical importance to appraise NMES protocols that can maximize
82 evoked torque.

83 Current charge (i.e., current intensity \times pulse duration \times stimulation frequency) is a
84 strong predictor of NMES-evoked torque ^{8,9}. Increasing current intensity is limited by the
85 associated discomfort ^{6,10}. Similarly, evoked knee extension torque increases logarithmically
86 with increasing stimulation frequency until 50-60 Hz, but then reaches a plateau ^{8,11}.
87 Therefore, increasing pulse duration could be considered as an alternative strategy to
88 maximize evoked torque ^{8,12,13}. Indeed, the use of wide pulses (~1 ms) seems to increase the
89 level of evoked torque with respect to conventional narrow pulses. For example, Liebano et
90 al. ¹⁴ observed a trend for a 5.5% greater maximal evoked torque with 1-ms compared to 0.4-
91 ms pulses. While this difference between the two pulse durations was not significant, likely
92 because of a relatively small number of subjects (i.e., 19 young women), our team recently

93 demonstrated a significantly greater maximal evoked torque for wide-pulse (45% MVC)
94 compared to conventional NMES (40% MVC) on a large population ⁷, these results being
95 observed when using both a conventional frequency (50 Hz) and a higher frequency (100 Hz).
96 However, previous studies comparing narrow- and wide-pulse stimuli delivered to the
97 quadriceps femoris muscle at the maximal tolerable current intensity measured evoked torque
98 during a single NMES train ^{7,14}. Indeed, comparisons of conventional vs. wide-pulse NMES
99 repeated trains were previously investigated for the triceps surae muscle at submaximal
100 current intensities ^{15,16}. Thus, it remains to be determined whether (i) the use of wide-pulses at
101 the maximal tolerable current intensity would lead to a greater evoked torque throughout a
102 full NMES session consisting of multiple trains delivered to the quadriceps femoris muscle,
103 and if so, (ii) greater objective fatigability (i.e., decrease in MVC torque induced by a NMES
104 session) would be observed with respect to conventional NMES with narrow pulses.
105 Objective fatigability is an important limiting factor of NMES, especially in clinical
106 populations who may show a premature and exaggerated decrease in maximal voluntary force
107 due to the particular motor unit recruitment ¹⁷. This NMES-induced objective fatigability has
108 usually been found to be related to peripheral alterations (i.e., neuromuscular propagation and
109 contractile failure) ^{15,18}.

110 Besides the possibility of a greater evoked torque throughout a full NMES session,
111 wide-pulse NMES is well known for favouring the activation of central pathways at low
112 current intensities ^{19,20}. For instance, wider pulses are known to depolarize sensory axons
113 more effectively – due to their longer strength-duration time constant and lower rheobase than
114 motor axons ²¹ – therefore leading to a greater transmission of afferent volleys to the central
115 nervous system (CNS). As a result, it has been demonstrated that afferent volleys induced by
116 electrical stimuli can modulate CNS excitability ²², in the same way as when afferent volleys
117 are mechanically induced ²³. For instance, it was reported that a single session of wide-pulse

118 NMES increased motor evoked potential (MEP) amplitude in response to transcranial
119 magnetic stimulation ²⁴⁻²⁶. However, these findings were mainly obtained with nerve trunk
120 stimulation, low current intensities (i.e., 15% of maximal M-wave ²⁷⁻³⁰) and long-duration
121 NMES sessions (i.e., up to 120 min ³¹⁻³⁴), which differ from NMES clinical practice
122 recommendations ². In addition, the effect of wide-pulse NMES on CNS excitability may be
123 frequency-dependent. For example, Mang et al. ³⁰ reported that a 100-Hz stimulation
124 frequency was more effective than 10 and 50 Hz in increasing MEP amplitude. It remains
125 unknown whether the use of wide-pulses could similarly impact CNS excitability when
126 NMES is applied at the maximal tolerable current intensity over the muscle belly (i.e., as
127 usually recommended for rehabilitation purposes ³⁵). Overall, the changes in cortical, spinal
128 and motoneuronal excitability after a single session of such NMES protocols remain to be
129 determined ³⁶, by further considering the possibility that such alterations could be frequency-
130 dependent ³⁷⁻³⁹.

131 The aims of the present study were therefore to compare the acute effects of a single
132 session of conventional vs. wide-pulse NMES at low- and high-frequency on 1) the level of
133 evoked torque, 2) the amount and origin of NMES-induced objective fatigability, and 3) CNS
134 excitability (i.e., cortical, spinal and motoneuronal excitability). We adhered to clinical
135 practice recommendations by delivering NMES to the quadriceps femoris muscle belly at the
136 maximal tolerable current intensity. We hypothesised that 1) a greater level of evoked torque
137 would be generated by wide-pulse NMES protocols, 2) this higher torque would lead to
138 greater objective fatigability as well as changes in CNS excitability compared to conventional
139 NMES, and 3) the high-frequency protocol would induce greater objective fatigability and
140 changes in CNS excitability than low-frequency NMES.

141

MATERIALS & METHODS

142 **Subjects**

143 Twenty-seven healthy men were initially screened for eligibility. Subjects were excluded if
144 their maximal tolerable current intensity exceeded the maximal stimulator output (100 mA)
145 during a first visit dedicated to inclusion and familiarization to the study procedures.
146 Seventeen subjects (age: 27 ± 6 years, mass: 69 ± 5 kg, stature: 177 ± 7 cm) were ultimately
147 included in the study. They were recreationally active and reported no history of neurological
148 or musculoskeletal impairment. None of them had previously engaged in systematic NMES
149 training. Subjects were asked to refrain from strenuous and unaccustomed physical activity
150 for 24 h before testing to minimize the risk of neuromuscular fatigue or muscle damage. The
151 study protocol was approved by the local ethics committee and was in accordance with the
152 latest update of the Helsinki Declaration (except for registration in a database). All subjects
153 gave their written informed consent before participation.

154

155 **Experimental design**

156 Subjects visited the laboratory on four separate occasions, including a familiarization visit
157 followed by three experimental sessions. The first visit aimed to familiarize subjects with
158 neuromuscular testing and with each NMES protocol (i.e., progressive increments of current
159 intensity up to the maximal tolerable current intensity). As illustrated on Figure 1, the
160 experimental sessions included neuromuscular testing (see *Neuromuscular testing*) that was
161 conducted before (PRE) and immediately after (POST) standardized NMES protocols (see
162 *NMES protocols*). The experimental sessions were performed in a random order and at the
163 same time of the day with 5 to 10 days between sessions. All experimental sessions were
164 conducted on the right quadriceps femoris muscle under isometric conditions. To investigate

165 NMES-induced objective fatigability, changes in MVC torque were quantified, and the
166 underlying central (i.e., voluntary activation (VA) measured using the interpolated twitch
167 technique) and peripheral (i.e., neuromuscular propagation failure estimated with M-wave and
168 contractile failure estimated with evoked doublet) alterations were further investigated. To
169 determine NMES-induced changes in CNS excitability, we investigated MEPs with
170 transcranial magnetic stimulation (i.e., corticospinal excitability⁴⁰), thoracic motor evoked
171 potentials (TMEPs) with thoracic electrical stimulation (i.e., motoneuron excitability⁴¹) and
172 spinal reflexes (SRs) with transcutaneous spinal cord stimulation (i.e., excitability of
173 monosynaptic responses between Ia afferents and motor neurons⁴²).

174 *Neuromuscular testing.* Testing began with the determination of femoral nerve stimulation
175 intensity. Then, after a standardized warm-up (i.e., ten 3-5 s submaximal isometric
176 contractions of progressively increasing intensity until reaching 90% of estimated MVC),
177 baseline torque generating capacity was assessed. One MVC without stimulation followed by
178 two MVCs with superimposed (i.e., 100-Hz paired stimuli delivered at maximal torque) and
179 post-MVC femoral nerve stimulation (i.e., 100-Hz paired stimuli ~2 s following the MVC and
180 single twitch separated by 3 s) in potentiated relaxed muscles were performed. The 100-Hz
181 paired stimuli allowed estimation of VA using the interpolated twitch technique whereas the
182 single stimulation allowed the associated EMG response to be obtained (potentiated M-wave;
183 M_{POT}). During MVCs, subjects were instructed to contract their muscles as strongly as
184 possible for ~4 s with a progressive torque build-up. A 1-min resting period separated the
185 MVCs. Afterwards, the EMG associated with 20% MVC (20% MVC-EMG) was determined
186 by performing a 5-s contraction at 20% MVC using visual feedback displayed on a computer
187 monitor. Series of contractions at the target EMG were then performed to set the stimuli at
188 appropriate intensities (see below). Baseline CNS excitability was then assessed with five sets
189 of measurements each including four stimuli delivered in a random order every ~5 s (i.e., to

190 record MEP, TMEP, SR and M-wave responses; see Figure 1). Each set lasted ~20 s, and was
191 separated by rest periods of 20 s. The NMES protocol started 1 min following the last set.

192 As NMES-induced CNS excitability changes can begin to recover within seconds after
193 exercise cessation⁴³, these assessments were distributed between the last six NMES-evoked
194 contractions to avoid any recovery effect at POST (Figure 1B). During the 30-s resting period
195 of NMES protocols, a set of stimuli was delivered during contractions targeting 20% MVC-
196 EMG as determined at PRE. Immediately after the NMES protocols, torque generating
197 capacity was assessed using one MVC with femoral nerve stimulation delivered on the MVC
198 plateau and ~2 and 5 s after MVC in potentiated relaxed muscles.

199 *NMES protocols.* Three NMES protocols were performed in a random order: narrow-pulse
200 (0.2 ms) NMES applied at 50 Hz (CONV), wide-pulse (1 ms) NMES applied at 50 Hz
201 (WP50), and wide-pulse (1 ms) NMES applied at 100 Hz (WP100). The current was delivered
202 with a commercially-available device (BioStim 2.1, Mazet Santé, Electronique du Mazet, Le
203 Mazet Saint Voy, France), which provides biphasic, symmetrical, rectangular pulses. Two 2-
204 mm-thick self-adhesive electrodes were placed over the right quadriceps femoris muscle
205 (Figure 2A). One electrode, measuring 198 cm² (180 × 110 mm, axion GmbH, Leonberg,
206 Germany), was placed on the distal third of thigh length over the vastus lateralis (VL) and
207 vastus medialis (VM) muscles. A small part of the electrode was trimmed to allow EMG
208 electrodes placement. The other electrode, measuring 85 cm² (125 × 86 mm, axion GmbH),
209 was placed 5-7 cm below the inguinal ligament. Each 10-s stimulation train included a ramp-
210 up of 2 s, a steady tetanic phase of 7 s and a ramp-down of 1 s. Each train was followed by a
211 30-s rest period and a total of 30 NMES trains were delivered during each protocol (Figure 1).
212 Current intensity was gradually increased by the subjects to their maximally-tolerated level
213 during a test train of 30 s that was delivered at the beginning of the NMES protocol. Then,
214 subjects were allowed to consistently adjust the current intensity to their maximum tolerable

215 level until the 25th train of each protocol. The maximal tolerated current intensity varied
216 between 27 and 100 mA. Current intensity remained constant from the 25th train to the end of
217 the NMES protocol to maintain a stable setup for CNS excitability assessments. For each
218 NMES protocol, current intensity and the associated discomfort were assessed after each
219 block of 5 trains.

220

221 **Instrumentation**

222 *Torque and EMG recordings.* Subjects were seated in an isometric dynamometer (ARS
223 dynamometry; SP2 Ltd., Ljubljana, Slovenia) with the hips and the knees at 90° and 60° of
224 flexion (with 0° referring to the extended neutral position), respectively. The leg was attached
225 to the chair by a noncompliant strap just proximal to intermalleolar axis. Trunk and pelvis
226 were also securely strapped to the chair to prevent compensatory movements during
227 contractions. The knee extension torque signal was low-pass filtered (20 Hz) and analog-to-
228 digital converted at a sampling rate of 2 kHz by a PowerLab system (16/35; ADInstruments,
229 Bella Vista, Australia). EMG activity from the right VL, VM and rectus femoris (RF) muscles
230 was recorded with pairs of self-adhesive surface electrodes (Melitracen 100; Covidien,
231 Mansfield, MA) in bipolar configuration, with electrodes placed 30 mm apart. The reference
232 electrode was placed on the ipsilateral patella. Before electrode placement (Figure 2A), the
233 skin was shaved, abraded, and cleaned with isopropyl alcohol to limit impedance. EMG
234 signals were amplified with an octal bio-amplifier (ML138; ADInstruments), band-pass
235 filtered (5–500 Hz), and analog-to-digital converted at a sampling rate of 2 kHz by the
236 PowerLab system.

237 *Femoral nerve stimulation.* Single and paired rectangular electrical pulses of 1 ms (400-V
238 maximal output voltage) were delivered via a constant-current stimulator (DS7R; Digitimer,

239 Hertfordshire, UK) to the right femoral nerve. A 30-mm diameter surface cathode (Meditrace
240 100) and a 50 × 100 mm anode (Durastick Plus; DJO Global, Vista, USA) were taped to the
241 skin onto the femoral triangle and in the gluteal fold (Figure 2B). Electrical stimuli were first
242 delivered at 50 mA and then the intensity was increased with 10-mA increments until knee
243 extension peak twitch torque and maximal M-wave responses were observed. The resulting
244 stimulation intensity was then further increased by 30% (supramaximal intensities: 116 ± 46
245 mA, 127 ± 42 mA and 121 ± 52 mA for CONV, WP50 and WP100 sessions, respectively; p =
246 0.22) and maintained throughout the sessions for the interpolated twitch technique and for
247 evoking the M-wave during the 20% MVC-EMG contractions (M_{SUP}), that was used to
248 normalize all the other evoked EMG responses.

249 *Thoracic stimulation.* A constant-current stimulator (DS7R) was used to evoke TMEP by
250 delivering a single rectangular electrical pulse of 1 ms between surface electrodes (Meditrace
251 100) positioned between the spinous processes of T1 and T2 (anode) and between T3 and T4
252 (cathode) (Figure 2B). The stimulation intensity was adjusted to elicit a VL TMEP amplitude
253 of 20% VL M_{SUP} during an ongoing 20% MVC-EMG contraction. Mean stimulation
254 intensities were 452 ± 191 mA, 473 ± 195 mA and 471 ± 209 for CONV, WP50 and WP100
255 sessions, respectively (p = 0.43).

256 *Transcutaneous spinal cord stimulation.* Subjects were asked to maintain a symmetrical
257 position of the upper limbs and their head as immobile as possible in a neutral position. Single
258 rectangular electrical pulses of 1 ms were delivered via a constant-current stimulator (DS7R).
259 A 68 × 125 mm anode (axion GmbH) was placed over the midline of the abdomen between
260 the xiphoid process of the sternum and the umbilicus and a 50 × 50 mm cathode (Durastick
261 Plus) was placed on the midline of the back between the spinous processes of L1 and L2
262 (Figure 2B). To confirm that the responses were caused by activation of the sensory fibers, a
263 rectangular double-pulse stimulation (inter-pulse interval: 50 ms) was delivered to test

264 homosynaptic depression of the evoked responses⁴². This phenomenon was confirmed in the
265 present study as the responses associated to the second transcutaneous spinal cord stimulation
266 were noticeably smaller than those following the first stimulus for all NMES protocols ($4.0 \pm$
267 1.6 and 0.7 ± 0.6 mV, respectively). Stimulus intensity was adjusted to evoke VL SR with an
268 amplitude of 20% of VL M_{SUP} during an ongoing 20% MVC-EMG contraction. Mean
269 stimulation intensities were 115 ± 33 , 116 ± 29 and 120 ± 30 mA for CONV, WP50 and
270 WP100 sessions, respectively ($p = 0.43$).

271 *Transcranial magnetic stimulation.* Single-pulse transcranial magnetic stimulation ~~with a 1-~~
272 ~~ms duration~~ was delivered to the left motor cortex via a concave double-cone coil using a
273 magnetic stimulator (Magstim 200²; The Magstim Company Ltd., Whitland, UK). The
274 optimal coil location was established for each visit as the position that elicited the largest
275 MEP in the VL muscle during a low-level voluntary contraction (20% MVC). This position
276 was marked on a swim cap that was secured to the scalp. Subsequently, transcranial magnetic
277 stimulation intensity was adjusted to evoke VL MEP with an amplitude of 20% of VL M_{SUP}
278 during an ongoing 20% MVC-EMG contraction. Mean stimulation intensities were 48 ± 13 ,
279 49 ± 14 and $48 \pm 12\%$ of maximum stimulator output for CONV, WP50 and WP100 sessions,
280 respectively ($p = 0.73$).

281 *Discomfort assessment.* Subjects were asked to place a vertical mark on a horizontal line (100
282 mm) to rate the level of discomfort. The scale ranged from 0 to 100 mm, with the zero-value
283 representing “no discomfort” and the 100 mm value representing “worst possible discomfort”.
284 The mean discomfort was calculated for each NMES protocol by averaging the five
285 discomfort assessments.

286

287 **Data analysis**

288 *Voluntary and evoked torque.* Objective fatigability was quantified as the PRE-to-POST
289 decline in MVC torque induced by each NMES session. MVC torque was determined as the
290 highest voluntary torque recorded during the three MVCs. The amplitude of the doublets
291 evoked by 100-Hz paired pulses (Db100) was measured and VA was calculated as follows:

$$292 \quad VA = [1 - [\text{superimposed Db100} \times (\text{torque at stimulation} / \text{maximal torque before} \\ 293 \quad \text{stimulation}) / \text{Db100}] \times 100$$

294 ⁴⁴. Db100 and VA were measured from the trial where the MVC torque was the highest before
295 the superimposed paired stimuli were delivered. Alterations associated to objective
296 fatigability were considered as PRE-to-POST decreases of Db100 and M_{POT} (i.e., peripheral
297 alterations), and VA (i.e., central alteration).

298 The torque-time integral (TTI) of each NMES-evoked contraction was quantified for
299 the entire 10-s stimulation period and evoked peak torque (EPT) for each NMES-induced
300 contraction was defined as the highest torque reached during each train and was expressed as
301 a percentage of MVC torque (Figure 3A). A first analysis of TTI and EPT was focused on the
302 first NMES-evoked contraction. A second analysis of these parameters was conducted for
303 blocks of 5 NMES-evoked contractions (block 1: contraction 1-5, block 2: 6-10, block 3: 11-
304 15, block 4: 16-20, block 5: 21-25 and block 6: 26-30). Finally, a global analysis was done on
305 the total TTI (i.e., sum of TTI of the 30 NMES-evoked contractions) and the mean EPT (i.e.,
306 mean EPT of the 30 NMES-evoked contractions).

307 *EMG.* The 20% MVC-EMG was determined by calculating the average root mean square
308 EMG of VL from the 20% MVC. To assist subjects in visualizing and maintaining the
309 ongoing 20% MVC-EMG contraction, the VL root mean square EMG was smoothed with a
310 400-ms time window. For all tested muscles, M_{POT} , M_{SUP} , SR, TMEP and MEP peak-to-peak
311 amplitudes were also measured and then averaged from the five sets (Figure 3B). SR, TMEP

312 and MEP were consistently expressed as a percentage of M_{SUP} . Comparing changes in MEP
313 and TMEP (MEP/TMEP ratio) allowed us to distinguish neural changes between supraspinal
314 and spinal levels³⁶. If we assume that SR and H reflex are two neurophysiological responses
315 involving the same spinal pathway⁴⁵, SR amplitude can be influenced by motoneuron
316 excitability as well as by the efficacy of synaptic transmission (i.e., pre-synaptic inhibition,
317 homosynaptic post-activation depression)^{42,46}. As stimulation of the descending tracts directly
318 activates large-diameter axons (not subjected to pre-synaptic inhibition), TMEP amplitude
319 may be used as a proxy of motoneuron excitability⁴⁷. Therefore, comparing SR and TMEP
320 (SR/TMEP ratio) should allow us to distinguish whether potential changes in spinal
321 excitability were pre-synaptic (i.e., efficacy of Ia-afferent – α -motoneuron pathway) or post-
322 synaptic (i.e., motoneuron excitability) in origin.

323

324 **Statistical analysis**

325 Statistical analyses were performed with JAMOVI software (version 1.1.9;
326 <https://www.jamovi.org/download.html>). Shapiro-Wilk and Levene tests were used to
327 examine data normality and homogeneity of variances, respectively. All data are presented as
328 mean values \pm SD. Statistical significance was set at $p < 0.05$.

329 One-way repeated measure ANOVAs were performed to compare the intensities of
330 each stimulation technique (i.e., femoral nerve, transcutaneous spinal cord, thoracic and
331 transcranial magnetic stimulation) between NMES protocols. One-way repeated measure
332 ANOVAs were also used to evaluate differences between NMES protocols at PRE for all
333 neuromuscular variables (i.e., MVC torque, Db100, VA, M_{POT} , SR, TMEP, MEP, SR/TMEP
334 and MEP/TMEP). One-way repeated measure ANOVAs were conducted to compare the TTI
335 and EPT of the first NMES-evoked contraction, and the total TTI and mean EPT of the whole

336 NMES session between NMES protocols. As discomfort data were not normally distributed, a
337 Friedman test was used to compare mean discomfort levels between NMES protocols. Two-
338 way repeated measure ANOVAs (block of 5 NMES-evoked contraction [1 vs. 2 vs. 3 vs. 4 vs.
339 5 vs. 6] × NMES protocol [CONV vs. WP50 vs. WP100]) were performed to analyse the
340 evolution of current intensity, TTI and EPT during and between NMES protocols. Two-way
341 repeated measure ANOVAs (time [PRE vs. POST] × NMES protocol [CONV vs. WP50 vs.
342 WP100]) were also used for all neuromuscular variables. Tukey *post hoc* tests were
343 performed when the ANOVAs revealed a significant effect. Effect size is only presented for
344 significant findings as partial eta-squared (η^2_p) with values representing small ($\eta^2_p \geq 0.01$),
345 medium ($\eta^2_p \geq 0.06$), and large ($\eta^2_p \geq 0.14$) effects ⁴⁸.

346

RESULTS

347 **Neuromuscular electrical stimulation protocols**

348 Current intensity increased progressively during the first four blocks of contractions ($p \leq$
349 0.03) and then plateaued after block 4 ($p = 0.22$; Figure 4A). Current intensity was higher for
350 CONV compared to WP50 and WP100 ($p < 0.001$), with no difference between WP50 and
351 WP100 ($p = 0.58$; Figure 4A). For the first NMES-evoked contraction, TTI and EPT were
352 similar between NMES protocols (TTI: 546 ± 260 Nm.s for CONV, 614 ± 419 Nm.s for
353 WP50 and 462 ± 294 Nm.s for WP100; EPT: $26.8 \pm 14.1\%$ MVC for CONV, $32.0 \pm 17.6\%$
354 MVC for WP50 and $25.9 \pm 13.7\%$ MVC for WP100; $p = 0.11$ and $p = 0.23$, respectively). For
355 the whole NMES session, TTI and EPT were similar between NMES protocols ($p = 0.19$ and
356 $p = 0.23$, respectively). Block 6 showed lower TTI than the other blocks ($p < 0.001$), except
357 block 1 ($p = 0.12$). Block 1 showed lower EPT compared to blocks 3 and 4 ($p = 0.009$), and
358 EPT of block 6 was also lower than the other blocks ($p < 0.001$), excepted block 1 ($p = 0.52$)
359 (Figure 4B and 4C). Total TTI, mean EPT, as well as for mean discomfort (CONV: $64.8 \pm$
360 28.1 mm; WP50: 66.3 ± 28.6 mm; WP100: 70.8 ± 26.8 mm) were similar between NMES
361 protocols (all $p \geq 0.17$; Figure 5).

362

363 **Objective fatigability**

364 MVC torque, Db100, VL M_{POT} and VA data are presented in Table 1. At PRE, MVC torque,
365 Db100, VL M_{POT} and VA were similar between NMES protocols (all $p \geq 0.07$). MVC torque,
366 Db100 and VL M_{POT} were reduced from PRE-to-POST (all $p \leq 0.002$; $\eta^2_p \geq 0.46$; Figure 6A).
367 VA was not changed after NMES sessions ($p = 0.79$; Figure 6A).

368

369 **Central nervous system excitability measurements**

370 We only present the evoked EMG responses for the VL muscle, as we set stimulation
371 intensities targeting evoked responses of 20% of VL M_{SUP} during a contraction performed at
372 20% MVC-EMG determined on the VL. Yet, as can be seen in Supplementary data
373 (<https://figshare.com/s/c84a6b315575e81364b3>), VM and RF behaved similarly. TMEPs
374 were obtained from 16 subjects due to excessive discomfort for one subject.

375 Data on CNS excitability are presented in Table 2. At PRE, SR, TMEP, MEP,
376 SR/TMEP and MEP/TMEP were similar between NMES protocols (all $p \geq 0.45$). All evoked
377 EMG responses were not changed after NMES protocols (all $p \geq 0.20$, Figure 6B).

378

DISCUSSION

379 The purpose of the present study was to compare 1) evoked torque, 2) objective
380 fatigability and related peripheral and central alterations, as well as 3) changes in CNS
381 excitability, induced by an acute session of conventional vs. wide-pulse NMES. Contrary to
382 our hypothesis, the level of evoked torque was not significantly different between the NMES
383 protocols. NMES induced peripheral, but not central alterations, that did not differ between
384 protocols, i.e. objective fatigability was similar for conventional and wide-pulse NMES. None
385 of the NMES protocols modified markers of CNS excitability.

386

387 **Neuromuscular electrical stimulation session**

388 Contrary to our hypothesis, we did not observe a significant difference in the level of evoked
389 torque between conventional and wide-pulse protocols (i.e., approximately 27% vs. 29% of
390 MVC) when considering only the first NMES-evoked contraction. This result is in accordance
391 with the findings of Liebano et al.¹⁴ who failed to demonstrate a significantly greater
392 maximal evoked torque with 1-ms compared to 0.4-ms pulse duration trains in 19 subjects,
393 yet they interpreted these results as a trend that could lead to significant differences with
394 additional subjects. However, we recently reported a significantly greater maximal evoked
395 torque for WP50 and WP100 than for CONV NMES (i.e., approximately 45% vs. 40% of
396 MVC) for a single NMES train in 50 subjects⁷ that partially supports the hypothesis that wide
397 pulses may lead to greater evoked torque than narrow pulses. Indeed, there is a high
398 intersubject variability for maximal evoked torque (see Fig. 1 of Espeit et al.⁷), which may
399 mask some differences between conditions that could only arise when considering a large
400 population. Moreover, the weak level of evoked torque measured for the first NMES-evoked
401 contraction in the present study (< 30% of MVC) is in accordance with previous observations

402 ^{49,50}. This may reflect a certain reluctance of subjects to increase current intensity at the
403 beginning of a NMES session and the necessity of an accommodation to NMES before
404 initiating a training program.

405 When the entire NMES sessions was considered in the present study, we did not
406 observe any difference between conventional and wide-pulse protocols for the mean level of
407 evoked torque (i.e., ~37% MVC), in agreement with the 25 to 45% MVC range classically
408 observed in the literature ^{18,49-52}. However, subjects required higher intensities to achieve the
409 maximal tolerable level with conventional than wide-pulse NMES protocols, in line with
410 previous reports in healthy young ⁷ and old ⁵³ adults. As the evoked torque is proportional to
411 current charge (i.e., the product of current intensity and pulse duration), lower current
412 intensities are usually needed with longer pulse durations ⁸. The lower current intensity with
413 wide-pulse NMES reduces the likelihood of reaching the maximal stimulator output, leading
414 to a smaller proportion of subjects limited by this practical issue ⁷.

415

416 **Objective fatigability**

417 The three NMES protocols induced similar levels of objective fatigability. These results
418 corroborate previous findings reporting similar losses in MVC torque after conventional vs.
419 wide-pulse NMES at low- and high-frequency delivered at submaximal (and constant) current
420 intensity to the triceps surae muscles ^{15,54,55} or to the tibial nerve ⁵⁶. In the same way, similar
421 MVC torque losses have been reported after wide-pulse low-frequency vs. wide-pulse high-
422 frequency NMES sessions applied to the triceps surae muscles at an intensity evoking 30% of
423 MVC ⁵⁵ and to the tibial nerve at an intensity evoking 10% of MVC ⁵⁶. Yet, the observed
424 reduction of ~14% MVC was lower than that usually reported (-20 to -30%) after a
425 conventional NMES session at maximal tolerable current intensity on the quadriceps femoris

426 ^{18,49,51,52}. An explanation for this lower MVC torque decrease could be the different knee
427 angle used here (60° of flexion) vs. in the aforementioned studies (~90°). Fouré et al. ⁵⁰
428 demonstrated in fact that NMES at short muscle length (50° of knee flexion) induced less
429 objective fatigability and muscle damage compared to long muscle length (100° of knee
430 flexion). Objective fatigability could mainly be ascribed to the occurrence of peripheral
431 alterations, as evidenced by decreases in Db100 and M_{POT}, with no or little implication of
432 central factors (i.e., unchanged VA). Neuromuscular propagation failure (M_{POT} reduction of
433 ~16%) and contractile failure (Db100 reduction of ~6%) have already been commonly
434 reported following quadriceps ^{18,51} and triceps surae NMES ^{15,55,57}, despite contrary isolated
435 results showing unchanged M-wave and potentiated peak twitch after low-frequency NMES
436 of the triceps surae ⁵⁸. Peripheral alterations could be explained by the repeated activation of
437 the same muscle fibers arising from the spatially-fixed motor unit recruitment imposed by
438 NMES ⁵⁹. The unchanged voluntary activation has already been observed after conventional
439 maximal-intensity NMES of the quadriceps ^{18,51} and wide-pulse low-intensity NMES applied
440 to the triceps surae muscle belly ¹⁵, probably as a consequence of the little CNS involvement.
441 Again, conflicting results (voluntary activation failure) have been observed after low- ⁵⁸ and
442 high- ⁵⁷ frequency NMES of the triceps surae. However, a possible effect of frequency on the
443 origin of objective fatigability was discarded by Papaiordanidou et al. ⁵⁵, who reported similar
444 objective fatigability associated with unchanged voluntary activation and decreased doublet
445 torque following four NMES protocols entailing narrow- or wide-pulses at low or high
446 frequency. Altogether, the aetiology and the amount of objective fatigability was similar
447 between NMES protocols, with peripheral but no central alterations.

448

449 **Central nervous system excitability measurements**

450 Contrary to our hypothesis, we did not observe any significant changes in corticospinal
451 excitability (unchanged MEP) for all NMES protocols applied at maximal tolerable current
452 intensity. Despite some evidence suggesting that conventional NMES may increase MEP^{60,61}
453 and pulse duration may not influence changes in corticospinal excitability²⁷, the lack of
454 changes for MEPs was even more unexpected after wide-pulse NMES. This was particularly
455 true for wide-pulse NMES at 100 Hz, as this frequency has been demonstrated to be the most
456 effective for increasing corticospinal excitability³⁰. Indeed, increases in corticospinal
457 excitability were largely reported for the tibialis anterior after a session of wide-pulse NMES
458 applied to the common peroneal nerve at submaximal current intensities^{24,26} and also for hand
459 muscles following radial/ulnar nerve stimulation at weak current intensities^{31,33}, despite
460 conflicting findings⁶². We speculate that the discrepancies between the present and previous
461 studies are due to experimental procedures. For instance, the application of wide-pulse NMES
462 to the muscle belly rather than to the nerve trunk is known to minimize the involvement of the
463 CNS^{63,64}. Moreover, to the best of our knowledge, no changes in MEP have ever been
464 reported after a NMES session applied at the maximal tolerable current intensity, as
465 submaximal intensities were used in previous studies (e.g. current intensity evoking 15% of
466 M-wave or a visible contraction)^{30,33}. In a systematic review, Chipchase et al.²² suggested
467 that current intensity appeared to have the strongest relation with the modulation of
468 corticospinal excitability, as 65% of experiments using current above motor threshold
469 reported increased MEP whereas 42% of studies reported decreased MEP after stimulations
470 below motor threshold. Furthermore, previous studies focusing on the time-course of MEP
471 following NMES of the tibialis anterior and first dorsal interosseus muscles, reported an
472 increase that began to be significant after 24 and 45 min of wide-pulse NMES with a duty
473 cycle of 50%, respectively^{30,32}. All the studies using wide-pulse NMES sessions shorter than
474 30 min failed to observe changes in MEP^{38,65,66}. Based on these considerations, it seems that

475 applying NMES to the quadriceps muscle following clinical practice guidelines to
476 improve/restore muscle function ⁶⁷ – as done here – may not influence corticospinal
477 excitability. The use of longer NMES sessions, higher duty cycles and submaximal current
478 intensities, as usually done in stroke rehabilitation ^{68,69}, should probably induce cortical
479 plasticity. Yet, NMES has been shown to favour sensorimotor activation, as revealed by
480 functional magnetic resonance imaging investigations considering 5- and 13-min NMES
481 protocols applied to the quadriceps ⁷⁰ or triceps surae ⁷¹ muscles.

482 Because MEP depends on the synaptic relays in corticospinal projections at the
483 cortical level as well as at the spinal level ⁷², MEP should also be interpreted in light of
484 changes at the spinal level ³⁶. Therefore, the present neuromuscular testing procedures
485 allowed to investigate more accurately the potential changes at the cortical, spinal and
486 motoneuronal levels. In addition to reporting unchanged corticospinal excitability, we did not
487 observe any change of cortical, spinal and motoneuronal excitability after conventional and
488 wide-pulse NMES. At the spinal level, unchanged H-reflex has been found after conventional
489 NMES applied over the triceps surae muscle belly ⁵⁷ or to the tibial nerve ⁷³. Conflicting
490 results have been observed after wide-pulse NMES at 20 Hz to 100 Hz, with no changes or
491 decreases in H-reflex amplitude ^{73,74}. Interestingly, Dideriksen et al. ³⁷ found that conventional
492 NMES applied at 100 Hz on the radial nerve substantially increased H-reflex amplitude, yet
493 this result was not observed with a lower stimulation frequency (30 Hz), suggesting a
494 frequency-dependent changes in spinal excitability after NMES. These results were recently
495 confirmed with wide-pulse NMES applied on the tibial nerve, with 20-Hz and 100-Hz trains
496 leading to decreased and increased H-reflex amplitude, respectively ^{38,39}. However, it should
497 be acknowledged that CNS activation has been shown to differ between wide-pulse NMES
498 applied over the muscle belly and the nerve trunk ^{63,64}, making such comparison difficult.

499

500 **Limitations**

501 Some limitations should be acknowledged for the present study. First, five subjects were
502 limited by the maximal stimulator output (100 mA), as they attained this current intensity
503 between the 21st and 25th NMES-evoked contractions. The impact on our results could
504 however be considered negligible, as current intensity remained constant from the 25th
505 NMES-evoked contraction to the end of the NMES protocol to maintain a constant setup for
506 CNS excitability assessments. Second, the number of MVC and evoked responses might have
507 been insufficient to ensure reliability ⁷⁵. However, a low number of MVCs was often used in
508 this type of studies, especially after NMES to avoid recovery ⁵⁴. It was also recently
509 demonstrated that five stimuli produce reliable MEP recordings within one session and our
510 time constraints (~20 s) prevented additional trials to be performed ⁷⁶. Third, the lack of
511 change in evoked EMG responses may be due to the choice to evoke them during contraction,
512 especially with contractions targeting a constant EMG level. As this procedure attempts to
513 hold motoneuron output constant, it is likely to minimise changes in CNS excitability.
514 However, this procedure was necessary to minimize stimulation intensity and the associated
515 discomfort, especially for thoracic stimulation. Fourth, there are differences between SR and
516 H reflex. Transcutaneous spinal cord stimulation evokes SR in several muscles and both sides
517 that may influence each other. Facilitation from close synergists, disynaptic inhibition from
518 antagonists, or influence from the contralateral side could affect SR amplitude (Minassian et
519 al., 2007; Hostoetter et al., 2019). Finally, changes in CNS excitability induced by wide-pulse
520 NMES have been regularly investigated in a subpopulation of so-called “responders” ^{38,39}. A
521 frequency-dependent modulation of spinal excitability was reported in “responders”, whereas
522 unchanged H-reflex amplitude was observed in “non-responders” ³⁸. This may explain the
523 variability observed in this study, which led to a lack of significant changes in CNS
524 excitability despite a concomitant increase of 16% and 14% of SR and TMEP after wide-

525 pulse NMES at 100 Hz. However, it was not possible to distinguish “responders” and “non-
526 responders” in the present study, as this classification is often based on the observation of
527 extra-torque, which requires a constant and low-current intensity⁷⁷.

528

529 **Perspective**

530 The acute effects of the three tested NMES protocols on neuromuscular function were
531 comparable. Regardless of pulse duration and stimulation frequency, NMES-evoked torque
532 and objective fatigability were similar, and CNS excitability was unchanged after 20 min of
533 quadriceps NMES. Therefore, based on these results, the use of quadriceps NMES at the
534 maximal tolerable current intensity (such as done in clinical practice) with either narrow- or
535 wide-pulses may supposedly lead to comparable treatment effectiveness. Yet, it remains to be
536 demonstrated in healthy and especially clinical populations. For instance, CNS excitability
537 changes should be more expected in patients with neurological conditions, such as stroke or
538 spinal cord injury^{68,69}.

539

540

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544

545

CONFLICT OF INTEREST STATEMENT

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

547

548

AUTHOR CONTRIBUTIONS

549 L.E., J.G., G.Y.M., V.R., N.A.M., and T.L. conceived and designed research; L.E., E.L. and
550 C.G.B. performed experiments; L.E. analyzed data; L.E., J.G., G.Y.M., V.R., N.A.M., and
551 T.L. interpreted results of experiments; L.E. prepared figures; L.E., C.G.B., J.G., G.Y.M.,
552 V.R., N.A.M., and T.L. drafted manuscript; L.E., E.L., C.G.B., J.G., G.Y.M., V.R., N.A.M.,
553 and T.L. approved the final version of manuscript.

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751

FIGURE CAPTIONS

752 **Figure 1.** Experimental session design: neuromuscular testing (in white) at PRE (panel A)
753 and POST (panel B), and NMES protocol (in grey). MVC, maximal voluntary contraction;
754 NMES, neuromuscular electrical stimulation.

755 **Figure 2.** Illustration of electrodes placement. Location of EMG and NMES electrodes (panel
756 A). Location of anode (A, red) and cathode (C, blue) electrodes for femoral nerve stimulation
757 (1), thoracic stimulation (2) and transcutaneous spinal cord stimulation (3) (panel B).

758 **Figure 3.** Example for a representative subject of evoked torque traces for each NMES
759 protocol (narrow-pulse 50 Hz (CONV), wide-pulse 50 Hz (WP50) and wide-pulse 100 Hz
760 (WP100) (panel A) and EMG traces from vastus lateralis in response to femoral nerve (M-
761 wave evoked during submaximal contractions, M_{SUP}), transcutaneous spinal cord (spinal
762 reflex, SR), thoracic (thoracic motor evoked potential, TMEP) and transcranial magnetic
763 (motor evoked potential, MEP) stimulations (overlays of five sets of measurements) (panel
764 B).

765 **Figure 4.** Evolution of current intensity (panel A), torque-time integral (TTI; panel B) and
766 evoked peak torque (EPT, panel C) during narrow-pulse 50 Hz (CONV, grey lines), wide-
767 pulse 50 Hz (WP50, blue lines) and wide-pulse 100 Hz (WP100, red lines) NMES protocols.
768 CONV higher than WP50 and WP100: # $p < 0.001$. Block higher than the preceding block: *
769 $p < 0.05$ and ** $p < 0.01$. Block lower than the 2nd, 3rd, 4th and 5th blocks: \$ $p < 0.001$. Blocks
770 lower than the 3rd and 4th blocks: £ $p < 0.01$. MVC, maximal voluntary contraction.

771 **Figure 5.** Total torque-time integral (TTI) and mean evoked peak torque (EPT) for narrow-
772 pulse 50 Hz (CONV), wide-pulse 50 Hz (WP50) and wide-pulse 100 Hz (WP100) NMES
773 protocols. Columns, error bars and squares indicate mean, SD and individual data,
774 respectively.

775 **Figure 6.** PRE-to-POST changes in objective fatigability (maximal voluntary contraction
776 (MVC) torque, 100-Hz doublet torque (Db100), vastus lateralis potentiated M-wave (M_{POT})
777 and voluntary activation (VA); panel A) and in central nervous system excitability
778 measurements (vastus lateralis spinal reflex (SR), thoracic motor evoked potential (TMEP)
779 and motor evoked potential (MEP); panel B) induced by each NMES protocol (CONV, WP50
780 and WP100). Columns, error bars and squares indicate mean, SD and individual data,
781 respectively. Individual values of subjects with out-of-scale data are presented as red squares.