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4 eccentric exercise of the elbow flexors.

5

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33 Abstract:

34 **Purpose:** Previous evidence from surface electromyograms (EMGs) suggests that exercise-
35 induced muscle damage (EIMD) may manifest unevenly within the muscle. Here we
36 investigated whether these regional changes were indeed associated with EIMD or if they were
37 attributed to spurious factors often affecting EMGs.

38 **Methods:** Ten healthy male subjects performed 3x10 eccentric elbow flexions. The subjects
39 performed a maximal voluntary contraction (MVC) immediately before (baseline) and during
40 each of the following four days after the initial exercise. At each of these five time points,
41 muscle soreness and ultrasound images from biceps brachii distal and proximal regions were
42 measured. Moreover, 64 monopolar surface EMGs were detected while 10 supramaximal
43 pulses were applied to the musculocutaneous nerve. The innervation zone (IZ), the number of
44 electrodes detecting largest M-waves and their centroid longitudinal coordinates were assessed
45 to characterize the spatial distribution of the M-waves amplitude.

46 **Results:** The MVC torque decreased (~25%; $P<0.001$) while the perceived muscle soreness
47 scale increased (~4cm; 0cm for no soreness and 10cm for highest imaginable soreness;
48 $P<0.005$) across days. The echo intensity of the ultrasound images increased at 48 h (71%), 72
49 h (95%) and 96 h (112%) for both muscle regions ($P<0.005$), while no differences between
50 regions were observed ($P=0.136$). The IZ location did not change ($P=0.283$). The number of
51 channels detecting the greatest M-waves significantly decreased (up to 10.7%; $P<0.027$) and
52 the centroid longitudinal coordinate shifted distally at 24 h, 48 h, and 72 h after EIMD
53 ($P<0.041$).

54 **Conclusion:** EIMD consistently changed supramaximal M-waves that were detected mainly
55 proximally from the biceps brachii, suggesting that EIMD takes place locally within the biceps
56 brachii.

57

58 Keywords:

59 Eccentric contractions; Muscle damage; High-density surface electromyography; Ultrasound
60 echo intensity; Neuromuscular electrical stimulation.

61

62 Abbreviations:

63 ANOVA: Analysis of variance

64 EIMD: exercise-induced muscle damage

65 EMGs: electromyograms

66 ICC: Intraclass Correlation Coefficient

67 MVC: maximal voluntary contraction

68 SD: standard deviation

69 US: ultrasound

70 **Introduction**

71 It is well-established that a single bout of eccentric exercise may lead to exercise-induced
72 muscle damage (EIMD), in particular when the activity is performed with an unaccustomed
73 intensity and duration (Hyldahl and Hubal 2014). Although EIMD can be assessed directly by
74 histological analyses (Newham et al. 1983), indirect markers such as peak torque, muscle
75 soreness and ultrasound (US) image echo intensity have been proposed to evaluate potential
76 damage following novel, eccentric exercises (Nosaka and Sakamoto, 2001; Matta et al. 2018;
77 Guilhem et al. 2016). The indirect, non-invasive assessment of EIMD is motivated by
78 documented observations of reduced capacity of generating maximal torque, a muscle soreness
79 sensation, muscle swelling, a decreased range of motion, and an increase in B-mode US gray
80 scale echo intensity of the muscle, lasting up to several days after the exercise (Warren et al.
81 1999; Hyldahl and Hubal 2014). In addition to their well-established sensitivity to EIMD
82 (Warren et al. 1999; Hyldahl and Hubal 2014), attention has been recently drawn to the
83 possibility of using these indirect, non-invasive assessment techniques to investigate whether
84 exercise-induced damage takes place locally within the muscle or not. Of a more practical
85 relevance, the non-uniform EIMD could result in an imbalance of muscle activation, potentially
86 altering the load distribution on joint structures and consequently increasing the risk of injury
87 (Hedayatpour and Falla 2002).

88

89 Imaging and electrophysiological evidence suggests the EIMD responses may manifest
90 unevenly within the muscle (Hedayatpour et al. 2008; Piitulainen et al. 2009; Guilhem et al.
91 2013; Maeo et al. 2017; Matta et al. 2019). For example, Maeo et al. (2017) reported greater
92 variations in T2-MRI after EIMD proximo-centrally within the quadriceps, suggesting that the
93 muscle distal site would be less susceptible to damage. Studies using multichannel surface
94 electromyography have also reported site-dependent changes in the electromyograms (EMGs)

95 following EIMD during voluntary contractions (Hedayatpour et al. 2008; Piitulainen et al. 2009;
96 Guilhem et al. 2013). For instance, a proximo-distal dependent decrease in the amplitude of
97 biceps brachii EMGs was observed during maximal voluntary contractions after eccentric
98 exercise (Piitulainen et al. 2009; Guilhem et al. 2013). However, during voluntary contractions,
99 other factors may contribute to local changes in the amplitude of EMGs detected from different
100 muscle sites following EIMD. First, the prolonged pain that accompanies eccentric exercise
101 has been shown to lead to regional differences in the amplitude of EMGs (Hedayatpour et al.
102 2008; Madeleine et al. 2006). Second, local changes in the amplitude of EMGs detected on the
103 same muscle have been reported even during maximal voluntary contractions and in the absence
104 of muscle damage (Miyamoto et al. 2012). Without ensuring that the exact same population of
105 motor units are excited at different time points following EIMD, any association between local
106 changes in EMG amplitude and EIMD may be misleading. Only during supramaximal nerve
107 stimulation, whereby most, if not all, motor units may be elicited (Botter and Merletti 2016),
108 would it appear possible to suppress effects on the amplitude of surface EMGs other than those
109 resulting from EIMD.

110

111 In this study, we combined supramaximal electrical stimulation of the musculocutaneous nerve
112 with high-density surface electromyography to investigate the electrophysiological topography
113 of biceps brachii EIMD. We specifically confronted the issue if EIMD leads to local changes
114 in the amplitude of M-waves detected along the biceps brachii, from one to four days after
115 eccentric exercise. We hypothesized that any local change along biceps fibers resulting from
116 EIMD would lead to a reduction in the amplitude of supramaximal M-waves detected from the
117 damaged site. Otherwise, we would expect the eccentric exercise to elicit variations in M-
118 waves' amplitude that are equally distributed over the biceps brachii muscle.

119

120 **Methods**

121 *Participants*

122 Ten healthy, young men (range values; age: 22-30 years, height: 164-193 cm, body mass: 60-
123 85 kg) volunteered to participate in the study. All participants were right-handed (self-reported)
124 and did not report any neurological or musculoskeletal disorders prior to experiments. The
125 subjects were not engaged in structured exercise sessions 12 months prior to the study, and were
126 not taking any medication or nutritional supplements during the experimental period. After
127 being informed about the experimental procedures and possible risks, all subjects provided
128 written informed consent before participating in the study. The experimental protocol was
129 conducted in accordance with the latest revision of the Declaration of Helsinki and was
130 approved by the ethics committee of our university hospital (HUCFF/UFRJ). Based on the
131 effect size estimated from our data (0.55) for changes in high-density EMGs measures over
132 time, a high statistical power was ensured (91.98%, post-hoc power analysis; Faul et al. 2007).

133

134 *Eccentric exercise and experimental protocols*

135 Maximal eccentric exercises were performed on an isokinetic dynamometer (Biodex System 4
136 Pro, Biodex, Shirley, New York). First, participants were comfortably positioned on the
137 dynamometer chair with their right shoulder flexed at 45° and right elbow coaxially aligned
138 with the dynamometer axis of rotation. They were then instructed to perform three sets of 10
139 maximal eccentric contractions of elbow flexion at an angular velocity of 30°/s (Chan et al.
140 2012) and from 110° to 0°, with 0° corresponding to full extension (Matta et al. 2018). After
141 each contraction, the elbow joint was passively returned to the initial position and subjects were
142 instructed to relax as much as possible. During the exercise, verbal encouragement was
143 provided to help subjects in attaining their maximal effort. Rest periods of 45 s between sets
144 of eccentric contractions were applied.

145

146 The study consisted of five measurement time points, conducted immediately before (baseline)
147 and 24 h, 48 h, 72 h and 96 h after the first eccentric exercise protocol. In all five sessions,
148 electrically elicited and voluntary contractions of the biceps brachii muscle were performed.
149 Four procedures for data collection were administered in the following order: (i) evaluation of
150 subjective perceived muscle soreness; (ii) acquisition of ultrasound B-mode images in two
151 different muscle sites; (iii) recording of surface EMGs from the biceps brachii with a grid of 64
152 electrodes while 10 supramaximal current pulses were applied transcutaneously to the
153 musculocutaneous nerve; (iv) two isometric, maximal voluntary elbow flexion contractions,
154 lasting 3 s each and with at least 5 min of rest between. Except for the evaluation of muscle
155 soreness, these procedures were done with the participants positioned comfortably at the
156 dynamometer chair, with their shoulder and elbow firmly fixed to the dynamometer torque
157 brace and respectively flexed at 45° and 90° (Matta et al. 2019).

158

159 During the MVCs, the elbow joint was coaxially aligned with the dynamometer axis of rotation.
160 Participants were verbally encouraged to reach their maximal effort; the peak torque, averaged
161 across the two MVCs, was considered as the maximal elbow flexion torque (Chan et al. 2012).

162

163 A detailed description of the experimental procedures applied for the evaluation of muscle
164 soreness, US imaging and M-wave stimulation and detection is reported below. Each of these
165 procedures were applied separately for each experimental session.

166

167 *Muscle soreness and ultrasound imaging*

168 Subjective perceived muscle soreness of the right elbow flexors was assessed using a
169 continuous visual analogue scale, ranging from 0 (no pain) to 10 cm (worst possible pain)

170 (Matta et al. 2019; Chan et al. 2012). Subjects were asked to rate the level of perceived soreness
171 immediately after having their elbow passively extended to a full extension position (from 110°
172 to 0°) or to the maximum possible extension (Matta et al. 2018).

173

174 Ultrasound B-mode images (GE Logic, USA; 8 MHz central frequency; 6 cm depth) were
175 acquired using a 40 mm linear probe from two different muscle sites. First, the coracoid process
176 and the articular interline of the elbow joint were identified by palpation and the distance
177 between them was considered to define *reference lines* over which the ultrasound probe was
178 positioned. Three *reference lines* were drawn on the skin, perpendicularly to the muscle
179 longitudinal axis (Figure 1A). The *middle reference line* was traced 70% distally from the
180 coracoid process while the *proximal* and *distal reference lines* were respectively traced 4 cm
181 above and below the *middle reference line*. Three US images were collected on both the
182 *proximal* and *distal reference lines*, with the probe aligned parallel to the reference lines and
183 with the muscle at rest. The US images acquired at baseline were used as a reference for
184 acquiring the images during the subsequent sessions. A water-based gel was used for acoustic
185 coupling and the US acquisition configuration (e.g., time-gain compensation (TGC), gain) was
186 kept the same during all sessions.

187

188 **Figure 1**

189

190 *Positioning of stimulation and detection electrodes*

191 The musculocutaneous nerve was stimulated in monopolar derivation (Botter et al. 2009). First,
192 the nerve was identified through palpation of the skin region near the right clavicle. During
193 this procedure, participants were asked to rotate their head to the left to facilitate the nerve trunk
194 identification by an experienced researcher. A round cathode adhesive electrode (diameter 20

195 mm; Spes Medica, Battipaglia, Italy) was then placed at the skin region over the
196 musculocutaneous nerve and two short-circuited rectangular anode electrodes (size 35 x 45 mm
197 each) were positioned on the opposite side (Figure 1B). The cathode was then displaced slightly
198 from the initially identified position until the least injected current led to clearly observable
199 mechanical response of the biceps brachii muscle. Both cathode and anode electrodes positions
200 were marked on the skin.

201

202 M-waves were detected from the biceps brachii muscle with 64 electrodes arranged into 13
203 rows x 5 columns, with a missing electrode in the upper left corner (1 mm diameter; 8 mm inter-
204 electrode distance; ELSCH064R3S, OT Bioelettronica, Turin, Italy). The grid was centered at
205 the *middle reference line* and the 3rd column of electrodes was aligned parallel to the muscle
206 longitudinal axis (grid recording area of ~ 35 mm x 100 mm; Figure 1A). In this way, the 2nd,
207 7th and 12th rows of electrodes were respectively aligned with the *proximal, middle* and *distal*
208 *reference lines* (Figure 1A). The grid was fixed to the skin with a bi-adhesive foam and the
209 electrode-skin contact was ensured by filling the foam cavities with conductive paste (TEN 20
210 Conductive Paste; Weaver, Aurora, Colorado). The reference electrode was placed at the
211 olecranon. Before positioning both stimulation and detection electrodes, the skin was shaved
212 and cleaned with an abrasive paste.

213

214 *Stimulation protocol and surface EMG recordings*

215 Ten biphasic, rectangular current pulses (100 μ s per phase; 1 pps) were applied to evoke
216 supramaximal M-waves from the biceps brachii (Rehastim Science Mode, Hasomed,
217 Germany). For all experimental sessions, the stimulation intensity was set at 20% over the
218 maximal current level, which was identified at the baseline session with a staircase current
219 profile, separately for each subject. Specifically, the current intensity was gradually increased

220 (steps of 2 mA) until no clear increment of the M-wave peak-to-peak amplitude could be
221 visually appreciated (Piitulainen et al. 2011); this level was defined as the maximal stimulation
222 intensity (mean \pm S.D.: 41.4 ± 8.2 mA). For each current intensity, two biphasic, rectangular
223 current pulses (100 μ s per phase; 1 pps) were applied.

224

225 Monopolar surface EMGs were amplified (200 V/V gain, 10-900 Hz bandwidth amplifier,
226 common-mode rejection ratio >100 dB; EMG-USB2; OT Bioelettronica, Turin, Italy) and
227 digitized at 2,048 samples/s using a 12-bit A/D converter with 5 V dynamic range. Offline
228 synchronization with stimulation instants was ensured through an output trigger signal issued
229 by the stimulation device and sampled synchronously with the EMGs.

230

231 *Ultrasound image and EMG processing*

232 The echo intensity was considered for the analysis of the US images (Matta et al. 2019). First,
233 all images were digitized in .jpeg format (US image size: 499 x 318 pixels, calibration factor =
234 0.012 cm/pixel) and a region of interest was selected using a custom made Matlab script (The
235 MathWorks Inc., Natick, Massachusetts). The region of interest size was the same across
236 subjects (95 x 150 pixels; ~ 2 cm²) and it was positioned to span as much of the biceps brachii
237 as possible without containing any surrounding fascia. The echo intensity of the region of
238 interest was then computed as the mean value of the greyscale histogram distribution (0: black
239 and 255: white) and the average value across the three images collected from each region (i.e.,
240 proximal and distal), was considered for further analyses.

241

242 The spatial distribution of M-waves peak-to-peak amplitude detected from the biceps brachii
243 was quantified for each subject, separately for each experimental session. Initially, raw EMGs
244 were visually inspected to identify bad channels due to electrode-skin contact problems or

245 power line interference. Low-quality monopolar signals were rarely observed (97 out of 3200
246 signals; 10 subjects x 64 electrodes x 5 sessions) and were replaced with the linear interpolation
247 of the neighbouring channels. Monopolar EMGs were then band-pass filtered with a fourth-
248 order Butterworth filter (15-350 Hz cut-off frequencies) and the stimulation artifact was
249 removed by offline blanking (3 ms starting from the trigger pulse; Piitulainen et al. 2011). After
250 that, single-differential (bipolar) EMGs were calculated as the algebraic difference between
251 monopolar EMGs detected by consecutive rows of electrodes. M-wave templates were
252 obtained by triggering and averaging EMGs over 30 ms epochs (Pinto et al. 2018), across the
253 10 stimulation pulses and separately for each channel and experimental session (Figure 2A).
254 Finally, the innervation zone was identified visually as broadly described in the literature
255 (Gallina et al. 2013; Piitulainen et al. 2009). For each column of electrodes, a single innervation
256 zone was identified, corresponding to the location from where action potentials with opposed
257 polarity arose and propagated toward the fibers' endings; i.e., the endpoint electrodes in the
258 column (Figure 2A; cf. Fig 2 in Gallina et al. 2013). This procedure provides half a channel
259 resolution for IZ identification (Gallina et al. 2013; Piitulainen et al. 2009). Innervation zones
260 were clearly identified for all cases and their median position across columns was considered
261 as the single position value for each experimental session and subject.

262

263

Figure 2

264

265 M-wave peak-to-peak amplitude was computed for each of the 59 single-differential channels,
266 providing topographic maps for the biceps brachii muscle (Figure 2B). The number of
267 electrodes detecting relatively large M-waves, termed as *segmented channels*, and the region
268 where these electrodes were located (the longitudinal and transverse coordinates of *segmented*
269 *channels'* centroid) were computed for each amplitude map. *Segmented channels* were

270 identified from the M-waves as those that presented a peak-to-peak amplitude greater than 70%
271 of the maximum amplitude across the grid (Vieira et al. 2010), and the centroid coordinates
272 were calculated as the weighted average of *segmented channels* across columns (X) and rows
273 (Y) (Figure 2B):

274

$$275 \quad X = \frac{1}{A} \sum_{n=1}^N a_n x_n$$

276

$$277 \quad Y = \frac{1}{A} \sum_{n=1}^N a_n y_n$$

278

$$279 \quad A = \sum_{n=1}^N a_n$$

280

281 where N is the total number of *segmented channels* for each subject, A is the sum of all peak-
282 to-peak amplitude values of *segmented channels* in the map and a_n is the peak-to-peak
283 amplitude of *segmented channel* with coordinates x_n and y_n . The centroid longitudinal
284 coordinate (Y) indicates where the EMG amplitude was recorded as the strongest along the
285 muscle and was retained for further analysis (Figure 2B).

286

287 *Statistical analysis*

288 A reliability study was conducted on an additional group of three men (age: 25, 27 and 34 years,
289 height: 177, 179 and 185 cm, body mass: 74, 78 and 80 kg). The same experimental procedures
290 were applied to this group at five consecutive measurement time points, with the exception of
291 eccentric exercise. The Intraclass Correlation Coefficient (ICC) and the coefficient of variation
292 (CoV) were considered to assess the inter-day reliability (test-retest) of the following variables:
293 (i) MVC peak torque; (ii) echo intensity, separately for proximal and distal regions; (iii) average

294 amplitude value of all channels of the grid (resulting in a single value for each measurement
295 time point). The ICC values were calculated using the two-way mixed effects model and
296 absolute agreement definition (Koo et al. 2016) and interpreted by thresholds (poor: 0.00–0.39;
297 fair: 0.40–0.59; good: 0.60–0.74; excellent: 0.75–1.00) (Cicchetti and Sparrow 1981). The
298 averaged CoV across subjects was calculated for each variable and interpreted as acceptable if
299 $CoV < 12\%$, intermediate if $12\% < CoV < 20\%$ or unacceptable if $CoV > 20\%$ (Balshaw et al.
300 2017).

301
302 After ensuring data normality (Shapiro-Wilk normality test; $P > 0.06$) and homoscedasticity
303 (Bartlett's test; $P > 0.08$ for all cases), a parametric analysis was considered for inferential
304 statistics. The one-way repeated measures ANOVA was applied to compare the main effect of
305 time on MVC peak torque, perceived muscle soreness, IZ longitudinal position, number of
306 *segmented channels* and centroid longitudinal coordinate. The two-way repeated measures
307 ANOVA was applied to compare the main and interaction effect of the time and the two regions
308 tested (proximal and distal) on the echo intensity. The Greenhouse-Geisser correction was used
309 for the centroid longitudinal coordinate analysis, since the sphericity assumption in the
310 repeated-measures ANOVAs was violated (Mauchly's test; $P = 0.012$). When a significant main
311 effect was detected, the Bonferroni's post-hoc test was used for paired comparisons. All
312 analyses were carried out with Statistica (Version 10, StatSoft Inc., Tulsa, USA) and the level
313 of significance was set at 5%.

314

315 **Results**

316 For all variables used to examine the difference between days, the average ICC was always
317 higher than 0.89, indicating excellent reliability. Specifically, the ICC values (95% confidence
318 interval) were 0.89 (0.55-0.99) for MVC peak torque, 0.94 (0.76-0.99) for echo intensity at

319 proximal region, 0.97 (0.88-0.99) for echo intensity at distal region, and 0.97 (0.88-0.99) for
320 average amplitude value of the grid. Additionally, the averaged CoV values were $4.5 \pm 2.7\%$
321 (mean \pm standard deviation) for MVC peak torque, $9.4 \pm 4.7\%$ for echo intensity at proximal
322 region, $8.8 \pm 1.4\%$ for echo intensity at distal region and $9.9 \pm 2.9\%$ for average amplitude value
323 of the grid, indicating acceptable values for all variables.

324

325 A main effect of time was found for both peak torque and perceived muscle soreness (ANOVA;
326 $P < 0.001$ for both cases). The Bonferroni's post-hoc test revealed that the MVC torque
327 significantly decreased at 24 h (mean \pm S.D.: 50 ± 12 Nm), 48 h (50 ± 12 Nm), 72 h (50 ± 11
328 Nm) and 96 h (52 ± 11 Nm) with respect to baseline (66 ± 10 Nm; Figure 3A; $P < 0.001$ for all
329 cases). Conversely, the perceived muscle soreness significantly increased at 24 h, 48 h, 72 h
330 and 96 h after eccentric exercise ($P < 0.005$ for all cases), with significant differences shown also
331 at 48 h and 72 h compared with 24 h (Figure 3B; $P < 0.005$ for both cases).

332

333

Figure 3

334

335 As shown for a representative participant in Figure 4A, the EIMD altered the US grayscale
336 image average intensity for both proximal and distal regions. Close inspection of Figure 4A
337 suggests that the change in grayscale intensity was most evident from 48 h to 96 h after EIMD.
338 Also, differences in echo intensity between days appear to span across a large cross-sectional
339 area of the biceps brachii and thus were well included in the region of interest (cf. rectangles in
340 Figure 4A). When considering all participants, a significant effect of time on echo intensity
341 was observed for both detection sites (ANOVA main effect; $P < 0.001$). Specifically, echo
342 intensity significantly increased at 48 h (mean \pm S.D. proximal: 41.6 ± 12.8 ; distal: 16.3 ± 12.3),
343 72 h (proximal: 44.4 ± 13.4 ; distal: 22.6 ± 18.5) and 96 h (proximal: 47.1 ± 19.7 ; distal: $24.9 \pm$
344 19.6) with respect to baseline (proximal: 26.5 ± 11.6 ; distal: 10.3 ± 7.7) for both regions (Figure

345 4B; Bonferroni's post-hoc; $P < 0.005$ for all cases). No significant differences were observed
346 among the proximal and distal regions at any time (Figure 4B; ANOVA interaction effect;
347 $P = 0.136$).

348

349 **Figure 4**

350

351 The effect of EIMD on supramaximal M-waves elicited from the biceps brachii muscle can be
352 well appreciated from results of a representative participant. As shown in the bottom panel of
353 Figure 5, the IZ longitudinal position was roughly the same across experimental sessions; from
354 baseline to 96 h the IZ was located within channels 6 and 7. In contrast, local differences in M-
355 wave amplitude distribution were observed from 24 to 72 h after EIMD. The number of
356 *segmented channels* decreased, mainly in the proximal region (cf. black circles on the top panel
357 of Figure 5), and the longitudinal coordinate of the centroid shifted from the IZ towards the
358 distal region of the biceps brachii at 24, 48 and 72 h after EIMD (bottom panel of Figure 5).

359

360 **Figure 5**

361

362 Group data revealed that the EIMD significantly affected the spatial distribution of M-wave
363 peak-to-peak amplitude, although it did not affect the IZ longitudinal position. No significant
364 change in IZ location was observed across time for all subjects and sessions (Figure 6A;
365 ANOVA; $P = 0.283$). Conversely, the size (i.e., number of *segmented channels*) and center of
366 M-wave amplitude distribution (i.e., centroid longitudinal coordinate) were affected by EIMD
367 (ANOVA; $P < 0.011$ for both cases). With respect to baseline, the number of *segmented*
368 *channels* significantly decreased (Figure 6B; Bonferroni's post-hoc; $P < 0.027$) and the centroid
369 longitudinal coordinate shifted towards the distal region at 24 h, 48 h, and 72 h (Figure 6C;

370 Bonferroni's post-hoc; $P < 0.041$ for all cases). Changes in both the number of *segmented*
371 *channels* and in the centroid of M-waves were relatively consistent across all subjects (cf. grey
372 lines in Figures 6B and 6C). Collectively, these results indicate that relatively larger peak-to-
373 peak values tended to be detected over a smaller and more distal biceps brachii region up to 72
374 h from EIMD.

375

376 **Figure 6**

377

378 **Discussion**

379 In this study, we hypothesized that any local change along the biceps fibers resulting from
380 EIMD would lead to a reduction in the amplitude of supramaximal M-waves detected from the
381 damaged site. Our results revealed the amplitude distribution of M-waves changed consistently
382 in the proximal biceps brachii region up to four days after the initial exercise. As discussed
383 below, these results suggest: i) regional changes in M-wave amplitude may reflect local effects
384 of EIMD on muscle excitation; ii) EMG and US appear to be sensitive to different processes
385 taking place within the muscle after damage; iii) EMGs may be used to assess both temporal
386 and spatial effects of exercise-induced damage on muscle function.

387

388 *Are changes in M-wave amplitude associated with EIMD?*

389 Ensuring muscle damage was induced by the exercise protocol we applied was necessary to test
390 for our hypothesis. As for other eccentric exercises such as downhill running (Maeo et al.
391 2017), the protocol applied in this study has been shown to successfully induce muscle damage
392 (Chan et al. 2012). The effectiveness of eccentric-exercise protocols in inducing damage is
393 usually quantified by changes in indirect variables related to muscle function, such as the
394 maximal force-generation capability and the perceived muscle soreness (Warren et al. 1999).
395 The prolonged decrease in peak torque following novel eccentric contractions, for instance, is

396 well-correlated with direct, histological evidence of muscle damage and is thus considered one
397 of the most reliable markers of EIMD (Warren et al. 1999). Similarly, given that soreness has
398 been documented to last up to seven days after eccentric exercise (Hyldahl and Hubal 2014),
399 the term ‘delayed-onset muscle soreness’ is frequently adopted to describe EIMD. Here we
400 observed a respectively significant decrease and increase in biceps brachii force and perceived
401 soreness after exercise (Figure 3). These results are well in agreement with the decrease of
402 MVC force and the increased muscle pain (Matta et al. 2019; Chan et al. 2012; Chen 2003)
403 reported in the literature. Based on these considerations, it seems therefore that the exercise
404 protocol we applied here effectively resulted in biceps brachii EIMD.

405

406 Considering that we successfully induced biceps brachii damage, the remaining issue is whether
407 the changes in M-wave amplitude we observed are associated with EIMD or not. Addressing
408 this issue urges a few considerations. First, as typically reported for biceps brachii (Piitulainen
409 et al. 2009), we observed only one IZ. Corroborating previous studies (Piitulainen et al. 2009),
410 the IZ position did not change between days for all subjects (Figure 6A) and, additionally, the
411 average amplitude value of all channels of the grid showed an excellent between-day reliability,
412 suggesting an accurate repositioning of the electrodes’ grid across experimental sessions.
413 Second, repositioning is supposedly not an issue for stimulation electrodes as well. In addition
414 to positioning stimulation electrodes at marked skin regions, the musculocutaneous nerve was
415 stimulated with current intensities 20% over that which led to maximal M-waves (cf. Methods).
416 Seemingly most, if not all, biceps brachii motor units were elicited in all experimental sessions
417 (Calder et al. 2005). Third, the analysis of single-differential EMGs detected with 8 mm inter-
418 electrode distance likely suppressed crosstalk from other elbow flexors (Vieira et al. 2017) that
419 were possibly elicited during stimulation of the musculocutaneous nerve (Pinto et al. 2018).
420 Far-field potentials would indeed be expected to appear with equal amplitude across the grid

421 (Roeleveld et al. 1997) and thus would hardly account for the proximo-distal variations we
422 observed in M-wave amplitude (Figure 5). Finally, even though subcutaneous thickness has
423 been shown to affect EMG amplitude (Cescon et al. 2008), it is unlikely that region anatomical
424 changes would take place between consecutive days. Collectively, these arguments seem to
425 suggest that the regional changes in M-wave amplitude reported here primarily arise from
426 EIMD.

427

428 *EIMD leads to regional changes in muscle excitation*

429 Here we raise the question if EIMD could affect excitation of the biceps brachii locally.
430 Different from previous studies that were focused on regional changes in EMG amplitude
431 during voluntary contractions after EIMD (Hedayatpour et al. 2008; Piitulainen et al. 2009;
432 Guilhem et al. 2013), here we assessed regional differences in biceps brachii excitation through
433 supramaximal stimulation. During supramaximal stimulation we presumably ensured most, if
434 not all, motor units were recruited in different days, suppressing effects other than those
435 resulting from the damage itself on the amplitude distribution of EMGs (e.g. pain, recruitment
436 patterns; (Madeleine et al. 2006; Miyamoto et al. 2012)). Our results indicate clear and
437 consistent alterations in the amplitude distribution of M-waves; up to 72 h from EIMD,
438 supramaximal M-waves with the largest amplitude were detected from a smaller, distal biceps
439 brachii region for all subjects (Figures 5 and 6). It seems tempting to suggest these EIMD-
440 induced changes could result from the impairment of gross sarcolemmal function. Structural
441 damage to the sarcolemma and the opening of stretch-activated ion channels, reported for
442 example after lengthening contractions (McBride et al. 2000; McNeil and Khakee 1992), lead
443 to increased intracellular Na^+ and Ca^{2+} concentrations. The increased permeability of the
444 sarcolemma, which may last until 4 days after eccentric exercise (McNeil and Khakee 1992),
445 could inhibit propagation or reduce propagation speed of action potentials beyond the damaged

446 site in damaged fibers (McNeil and Khakee 1992; Piitulainen et al. 2010). Local inhibition of
447 propagation would result in a smaller number of single fiber action potentials elicited by
448 stimulation while local reduction of propagation would result in a greater temporal spread of
449 single fiber action potentials. While both factors would be expected to decrease the peak-to-
450 peak amplitude of compound surface potentials (Farina et al. 2004), inhibition though not
451 reduced propagation velocity would most likely explain the decrease in muscle maximal force
452 after EIMD (Figure 3A; see also Piitulainen et al. 2010). On the other hand, maximal force was
453 measured during voluntary contractions and therefore not all fibers may have been recruited
454 during MVCs after EIMD. Our considerations therefore solely explain the peripheral, but not
455 the central mechanisms as the corticospinal excitability, which may contribute to MVC torque
456 reduction after damaging eccentric exercise (Doguet et al. 2019). Although it is currently
457 unviable to ascertain the occurrence of local, structural damage of human muscles in vivo and
458 its consequences on muscle excitation, our results do suggest EIMD affects muscle excitation
459 locally.

460

461 Interestingly, our results revealed that the eccentric protocol used in this study consistently
462 affected the proximal region of the biceps brachii. As shown in Figure 5, a significant decrease
463 in the M-waves' amplitude was observed at the proximal muscle site, suggesting that this region
464 would be more susceptible to EIMD or to its effect on muscle excitation. Architectural
465 differences along the muscle could possibly explain the presumable, greater vulnerability of the
466 biceps brachii proximal region to EIMD in eccentric contractions. The distal tendon of the
467 biceps brachii flattens into an internal aponeurosis, located in the centerline of the muscle and
468 extending over the distal third of the muscle longitudinal axis (~34% of the muscle length on
469 average; Asakawa et al. 2002). The internal aponeurosis would likely impact the amount of
470 movement along the centerline fascicles during the elbow flexion, with the proximal and middle

471 regions undergoing greater shortening-lengthening movements than the distal region (cf. Figure
472 6 in Pappas et al. 2002). Thus, the degree of muscle strain during lengthening contractions
473 would be higher at more proximal biceps brachii sites. The fact that the magnitude of EIMD is
474 a function of muscle strain (Lieber and Friden 1993; Guilhem et al. 2016), combined with
475 greater displacements at the muscle proximal region, could potentially explain a preference for
476 damage induced by eccentric exercise to take place proximally in the biceps brachii muscle.

477

478 Contrarily to M-waves, variations in echo intensity across days did not depend on whether US
479 images were collected proximally or distally from biceps brachii (Figure 4). The increased
480 grayscale intensity of US images following EIMD is possibly due to edema and intracellular
481 material leakage and production of connective tissue (Wong et al. 2020). The suggested
482 association between echo intensity and inflammatory responses following EIMD (Radaelli et
483 al. 2012) would explain why increased echo intensity persisted some days after the eccentric
484 exercise, with highest intensities occurring at ~3-4 days from baseline (Figure 4B; (Radaelli et
485 al. 2012; Matta et al. 2018)). Moreover, the lack of proximo-distal differences in echo intensity
486 of US images confining exclusively the biceps brachii muscle (Figure 4) is in agreement with
487 the view that edema arises more diffusely within the damaged muscle (Chen 2003). Taken
488 together, the local changes in M-wave amplitude and the similar changes in the echo intensity
489 of US images collected from different biceps brachii regions indicate US images and EMGs
490 may reflect different processes coalescing from EIMD.

491

492 *Limitations and future, practical considerations*

493 Notes on three potential limitations are made here. First, due to methodological issues, we were
494 unable to collect data during and immediately after the exercise protocol. These data could
495 have revealed immediate changes in EMG amplitude following exercise, possibly revealing a

496 greater proximal difference in EMG amplitude when compared to those observed for
497 consecutive days after the exercise protocol. Second, although the a-posteriori analysis
498 revealed high statistical power, it seems advisable to extend our study to a larger sample of
499 subjects unaccustomed with eccentric exercises. Third, the biceps brachii is one of the three
500 elbow flexors prime movers and therefore the effect of maximal eccentric exercise reported
501 here may not apply to the other elbow flexors. However, as per our research question, we do
502 not believe these limitations discredit the localised change in the amplitude of surface EMGs
503 after eccentric exercise (Figures 5-6).

504

505 Practical and methodological observations may be cautiously made from our results. First, the
506 high-density surface electromyography may provide relevant information about EIMD
507 recovery. While MVC force and muscle soreness significantly differed from baseline values
508 during the four days following EIMD (Figure 3), both the number of *segmented channels* and
509 the centroid longitudinal coordinate returned to baseline values at 96 h after eccentric exercise
510 (cf. grey traces in Figure 6B and 6C). This result indicates that any peripheral alterations to
511 biceps brachii excitation may restore within 96 h from EIMD and are unlikely to explain
512 persistent experiences of force decline and soreness. Future studies are necessary to verify the
513 latter possibility, measuring elbow force elicited by nerve stimulation and assessing perceived
514 soreness from different muscle sites with less subjective metrics (Matta et al. 2019). Although
515 the eccentric exercise has numerous benefits for rehabilitation, sports and pathological
516 conditions, its ensuing clinical symptoms (e.g. the delayed-onset muscle soreness and decreased
517 muscle function) may disturb training and rehabilitation programs (Hody et al. 2019).
518 Considering that the risk of further injuries increases during a delayed-onset muscle soreness
519 episode or during the subsequent days, following the temporal recovery of the EIMD has an
520 applied, clinical relevance (Hedayatpour and Falla 2002). Moreover, information about the

521 EIMD recovery may be mistakenly conceived when obtained from a single muscle site. Our
522 results indeed suggest that the EIMD effects on muscle excitation should not be assessed with
523 EMGs collected from a single muscle region. At least for the biceps brachii, EMGs collected
524 distally and proximally would appear to provide contrasting information on the temporal
525 evolution of EIMD. In conclusion, here we demonstrated that the high-density surface
526 electromyography technique may be therefore used as a promising, diagnostic tool to assess
527 both spatial and temporal effects of EIMD on muscle function.

528 **Compliance with Ethical Standards**

529 *Conflict of Interest:* The authors declare that the research was conducted in the absence of any
530 commercial or financial relationships that could be construed as a potential conflict of interest.

531 *Ethical approval:* All procedures performed in this study were in accordance with the ethical
532 standards of the committee of our university hospital (HUCFF/UFRJ) and with the 1964
533 Helsinki declaration and its later amendments or comparable ethical standards.

534

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700 **Figure captions**

701 **Fig 1** *A*, shows a schematic representation of where grid of 64 surface electrodes was
702 positioning in the biceps brachii muscle. The ultrasound images were obtained from the
703 proximal and distal reference lines. *B*, illustrates the position of electrodes used to stimulate
704 the musculocutaneous nerve.

705

706 **Fig 2** Raw, single-differential M-waves collected at baseline are show in panel *A*. The
707 innervation zone (IZ; shaded, grey rectangles) is clearly seen in the region where there is phase
708 opposition between consecutive action potentials, followed by propagation. *B*, shows the
709 topographic map obtained from peak-to-peak amplitude of M-waves displayed in *A*. Black
710 circles denote electrodes for which the M-waves peak-to-peak amplitude exceeded 70% of the
711 maximal peak-to-peak, termed as *segmented channels*. Crossed, white circle indicates
712 *segmented channels*' centroid location. Note the centroid longitudinal location is very close to
713 IZ longitudinal position at baseline day.

714

715 **Fig 3** Mean (circles) and standard deviation (whiskers; N = 10 subjects) are shown for the
716 maximal voluntary contraction peak torque (*A*) and perceived muscle soreness (*B*), separately
717 for each experimental session. Asterisk denotes statistical significance ($P < 0.05$). w.r.t, with
718 respect to.

719

720 **Fig 4** *A*, shows ultrasound images collected from the biceps brachii proximal (top) and distal
721 (bottom) regions of a single participant. The rectangles with white lines illustrate the region of
722 interest used to calculate the echo intensity. *B*, shows the mean (circles) and standard deviation
723 (whiskers) for the echo intensity, separately for each region and experimental session. Asterisk
724 denotes statistical significance ($P < 0.05$) with respect to baseline values. EI, echo intensity.

725

726 **Fig 5** The top panel shows the peak-to-peak amplitude maps of M-waves for a representative
727 participant, separately for each experimental session. Black circles indicate *segmented*
728 *channels* and the crossed, white circles denote the centroid location of these channels. The
729 centroid longitudinal coordinates with respect to the innervation zone (IZ) longitudinal
730 locations are displayed on the bottom panel, separately for each experimental session.

731

732 **Fig 6** Mean (circles) and standard deviation (whiskers; N = 10 subjects) are shown for the
733 innervation zone longitudinal position (*A*), number of *segmented channels* (*B*) and centroid
734 longitudinal coordinate (*C*) within the grid, separately for each experimental session. Grey
735 circles and lines in the panels *B* and *C* indicate individual results. Asterisk denotes statistical
736 significance ($P < 0.05$).