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(Article begins on next page)

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Title:

Changes in supramaximal M-wave amplitude at different regions of biceps brachii following eccentric exercise of the elbow flexors.

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Abstract:

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

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

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

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

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

Introduction

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

Imaging and electrophysiological evidence suggests the EIMD responses may manifest unevenly within the muscle (Hedayatpour et al. 2008; Piitulainen et al. 2009; Guilhem et al. 2013; Maeo et al. 2017; Matta et al. 2019). For example, Maeo et al. (2017) reported greater variations in T2-MRI after EIMD proximo-centrally within the quadriceps, suggesting that the muscle distal site would be less susceptible to damage. Studies using multichannel surface 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 45º 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 30º/s (Chan et al.

140   2012) and from 110º to 0º, with 0º 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 45º and 90º (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

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

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

Stimulation protocol and surface EMG recordings

Ten biphasic, rectangular current pulses (100 μs per phase; 1 pps) were applied to evoke supramaximal M-waves from the biceps brachii (Rehastim Science Mode, Hasomed, Germany). For all experimental sessions, the stimulation intensity was set at 20% over the maximal current level, which was identified at the baseline session with a staircase current 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 ± S.D.: 41.4 ± 8.2 mA). For each current intensity, two biphasic, rectangular
223 current pulses (100 μs 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 cm^2) 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
$$X = \frac{1}{A} \sum_{n=1}^{N} a_n x_n$$

276

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

278

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

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

14

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

16

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.

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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

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

731

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