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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.

Keywords:

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

Abbreviations:

ANOVA: Analysis of variance

EIMD: exercise-induced muscle damage

EMGs: electromyograms

ICC: Intraclass Correlation Coefficient

MVC: maximal voluntary contraction

SD: standard deviation

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)

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

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

Methods

Participants

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

Eccentric exercise and experimental protocols

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

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

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

Figure 1

Positioning of stimulation and detection electrodes

The musculocutaneous nerve was stimulated in monopolar derivation (Botter et al. 2009). First, the nerve was identified through palpation of the skin region near the right clavicle. During this procedure, participants were asked to rotate their head to the left to facilitate the nerve trunk 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

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

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

Ultrasound image and EMG processing

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

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

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

Figure 2

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

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

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

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

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

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

Statistical analysis

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

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

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

Results

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

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

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

Figure 3

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

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

Figure 4

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

Figure 5

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

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

Figure 6

Discussion

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

Are changes in M-wave amplitude associated with EIMD?

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

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

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

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

EIMD leads to regional changes in muscle excitation

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

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

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

Limitations and future, practical considerations

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

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

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

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

Compliance with Ethical Standards

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

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

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Figure captions

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

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

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

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

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.

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 ($P<0.05$).