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Research article

Non-Uniformity of Elbow Flexors Damage Induced by an Eccentric Protocol in Untrained Men

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Abstract
Muscle structure disorganization is a consequence of intense eccentric contractions, with symptoms that characterize exercise-induced muscle damage (EIMD). To date, few studies have described EIMD parameters at different muscle sites. The aim of the present study was to analyse indirect markers of EIMD at two elbow flexors sites over three days. Eleven healthy untrained men were submitted to a session of three sets of 10 eccentric elbow flexion repetitions on an isokinetic dynamometer. The isometric peak torque (PT), muscle soreness, elbow flexors oedema, (normalized muscle thickness [MT]) and echo-intensity (EI) were measured. There was a significant decrease in PT immediately after (Post) and 10 min, 24 h, 48 h and 72 h after intervention compared to that at baseline ($p < 0$ .05). MT% increased after 72 h compared with that immediately, 10 min and 24 h after intervention ($p < 0.05$). No statistical changes were observed in muscle soreness and oedema between the two muscle sites. With respect to EI%, significant differences were observed for the 24 h, 48 h and 72 h measures compared with those of the Post, 10 min and 24 h measures for both muscle sites; at the distal site, EI% was significantly higher than at the proximal site for measures after 24 h ($p < 0.05$). The presence of differences in EI% 24 h after eccentric training on distal sites of elbow flexors indicates non-uniform EIMD in this region.

Key words: Eccentric contractions, resistance training, ultrasound, echo intensity, muscle soreness.

Introduction

Muscle damage can be induced either by impact (IIMD) (Naughton et al., 2018) or by exercise (EIMD); even a single session of unaccustomed heavy eccentric training is able to trigger this physiological event in muscle tissue (Clarkson and Hubal, 2002; Friden and Lieber, 2001). Acute EIMD results in delayed-onset muscle soreness (DOMS), maximal voluntary force decrease, and local oedema (Butterfield, 2010; Warren et al., 1999). The hypothesis for the EIMD physiological mechanism is sarcomere rupture and structure disorganization, which is based on the non-uniform lengthening of sarcomeres in activated muscle as they are stretched beyond the optimum length (Friden and Lieber, 2001).

Indirect markers have often been used in EIMD investigation as global or local analyses. Global parameters can be associated with biochemical markers, force production and range of motion (Warren et al., 1999), and muscle soreness, oedema and muscle texture of echo-intensity (EI) from ultrasound images are considered local ones (Chen and Nosaka, 2006). In general, local EIMD parameters are described for a single muscle site (Chen and Nosaka, 2006; Radaelli et al., 2012), assuming it is homogeneous along the entire muscle. In this sense, ultrasound image EI has been commonly used as a local and non-invasive method to evaluate EIMD based on the histogram of the grey-scale pixels of the region-of-interest (ROI), which reflects the inflammatory process (Nosaka and Sakamoto, 2001; Pillen and van Alfen, 2011).

EIMD levels at different muscle sites are not well understood, and to date, few studies have investigated this issue (Chen, 2003; Chen and Nosaka, 2006; Dierking et al., 2000; Hedayatpour et al., 2008). Some studies have presented non-significant differences between arm sites of increasing circumference one to five days after eccentric intervention for elbow flexors (Chen, 2003; Chen and Nosaka, 2006; Jubeau et al., 2012). On the other hand, delayed-onset soreness was reported primarily in the distal region of the elbow flexors and knee extensors with EIMD (Dierking et al., 2000; Hedayatpour et al., 2008). In contrast, greater sensitivity to comprehensive soreness in the tibialis anterior muscle belly compared with the region near the myotendinous junction were observed (Andersen et al., 2006), demonstrating that this variable can be muscle dependent. Nevertheless, the claim of more robust indirect EIMD markers based on ultrasonography image processing, such as EI analyses, on different muscle sites could elucidate this question. In this sense, local recovery techniques such as massage or cryotherapy (Dupuy et al., 2018) could optimize tissue regeneration. To fill this gap in the literature, the aim of the present study was to compare EIMD at two sites of the elbow flexors after an eccentric training session.

Methods

Participants

Eleven men participated in the study (age 26.5 ± 5.7 years; body mass 74.9 ± 10.8 kg; height 176.4 ± 8.2 cm). The subjects were informed about the general procedures, completed the PAR-Q questionnaire on physical fitness on their own, and signed a consent form that was approved by the Ethics in Research Committee at Clamenteino Fraga Filho Hospital (number 44100215.6.0000.5257). The subjects were not engaged in strength training of their upper limbs for at least 12 months. The exclusion criteria were previous orthopaedic lesions and daily medication for chronic diseases.

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General procedures

The study consisted of five visits at the same time of day for each subject. During the first visit, the subjects were familiarized with the single maximal voluntary isometric contraction (MVIC) test, which was performed over five seconds with the elbow at 90 degrees. Measurements were performed for reliability to establish baseline measurements over two days. After one week, the subjects returned for the eccentric intervention and were instructed to maintain the same level of hydration and avoid any physical activity. Elbow flexion MVIC was tested, as well as muscle soreness at two muscle sites. Ultrasound images were acquired at the same sites for posterior oedema and EI measures. The measurements were conducted before the exercise intervention (Baseline), immediately after (Post), 10 min later (10 min) and repeated after 24, 48 and 72 h by the same evaluator.

Peak torque measurement

For the MVIC tests, the subjects were seated (Biodex 4 System Pro - *Biodex Medical Systems Inc.*, New York, USA) with the hip flexed at 85º and the pelvis firmly immobilized by straps. The right arm was placed with the shoulder flexed at 45º and the elbow flexed at 90º with the arm supinated (Chapman et al., 2005). The subjects were verbally encouraged to perform two repetitions of five-second MVIC with a one-min rest interval. The peak torque (PT) was considered the highest value between the two trials.

Muscle soreness

Muscle soreness was assessed by a 10-cm visual analogic scale (VAS), where zero (0 mm) represented “no pain” and 10 “extreme pain” (Jubeau et al., 2012). A manual algometer (I7 Wooley Lane, NY, USA) was used to apply 60 N/cm^2 pressure over one second on two muscles sites at 60% (proximal site) and 80% (distal site) of the arm length from the acromial process scapula to the lateral epicondyle of the humerus (Matta et al., 2011). The subjects were asked to rate their apparent soreness on the VAS when the elbow was extended, and an evaluator applied the algometer on the respective arm site.

Oedema

Two US images (GE Logic, USA; 40 mm linear probe; 8 MHz excitation frequency; 6 cm depth) were acquired at the same sites with the probe positioned transverse to the arm for complete visualization of the biceps brachii and brachialis and the humerus interfaces at the proximal and distal sites. A water-soluble gel was used for acoustic coupling. The acquisition configuration was kept constant during the protocol. The arm was supinated and relaxed at the same position for soreness measurements.

Muscle oedema for the two sites was measured as the thickness of the elbow flexors, defined as the vertical distance between the humerus and the superficial aponeurosis of the biceps brachii (Figure 1). Public domain software, ImageJ (National Institutes of Health, USA, v.1.43), was used to measure the MT and EI.

Echo-intensity

The EI was calculated over the same images for the two sites and was defined as the mean value from the greyscale histogram (0=black and 255=white). The selected larger ROI was a rectangle from the humerus to the first contact with the superficial aponeuroses of each image (Figure 1), based on other studies (Chen and Nosaka, 2006; Radaelli et al., 2012). EI and MT were normalized by baseline measurements.

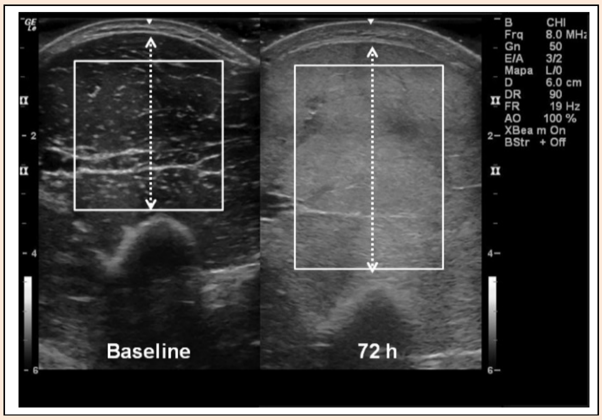

Figure 1. A transversal ultrasound image at the Baseline (left) and 72 hours (right) after eccentric intervention. Muscle Thickness of elbow flexors and the region of interest for Echo Intensity are represented in each image.

Eccentric intervention

The exercise intervention was performed on the dynamometer in eccentric mode, in the same seated position as described for the MVIC tests. The subjects performed three sets of 10 repetitions of elbow flexion eccentric contractions (Chan et al., 2012) at 90°/s angular velocity (Newton et al., 2013) with a two-min rest interval between sets. After each eccentric phase, the elbow joint was passively returned to the initial position. The range of motion adopted was from 90° elbow flexion to full extension of the elbow joint. Volunteers were instructed to perform all repetitions with maximum effort through verbal encouragement.

Statistical analysis

The normal distribution was confirmed with a Shapiro-Wilk test for all parameters. For PT, the differences were tested by repeated measures ANOVA. ANOVA with two factors (2 [arm site] x 5 [time measures]) was performed to compare the relative differences in soreness, EI and MT between the two sites (60% and 80% of the arm length) at different time points. If interactions between the factors were detected, a Fischer LSD post hoc test was applied to identify significant differences. The significance level was set at 5% ($p < 0.05$) and the Statistica package (Statsoft, Inc., Tulsa, OK, USA) was used for the analyses.

Measurements of the first and second visits were used for inter-day reliability calculations. The inter-class correlation coefficient (ICCr) and the 95% confidence intervals (95% CIs) were as follows: for MT at 80% (ICCr = 0.961 and 95% CI= [0.854 to 0.989]) and at 60% (ICCr = 0.981 and 95% CI= [0.930 to 0.995]); for EI at 80% (ICCr = 0.926 and 95% CI = [0.727 to 0.980]) and at 60% (ICCr = 0.914 and 95% CI= [0.681 to 0.977]); and for peak torque (ICCr = 0.986 and 95% CI = [0.948 to 0.996]), considered good reliability.

Results

Significant reductions in PT were found between baseline and all other time points (Figure 2).

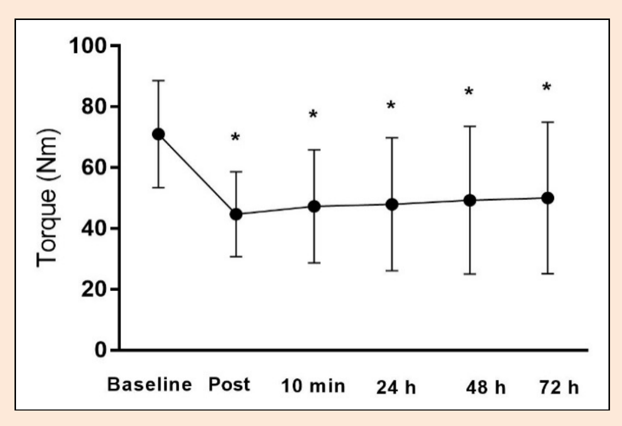

Figure 2. Torque values (Nm) expressed on average and standard deviation. * - Significant differences for the baseline measure (p < 0.05).

For DOMS parameters, a significant interaction was observed (F = 32.12; p < 0.001) at both arm sites. Additionally, significant variations were found from baseline, Post and 10 min to the measures at 24 h (p = 0.009), 48 h (p < 0.001) and 72 h (p < 0.001) after exercise. Additionally, significant differences were found between 24 h and 48 h (p=0.021) for both arm sites, and only for the distal site between 24 h and 72 h (p=0.009) (Figure 3).

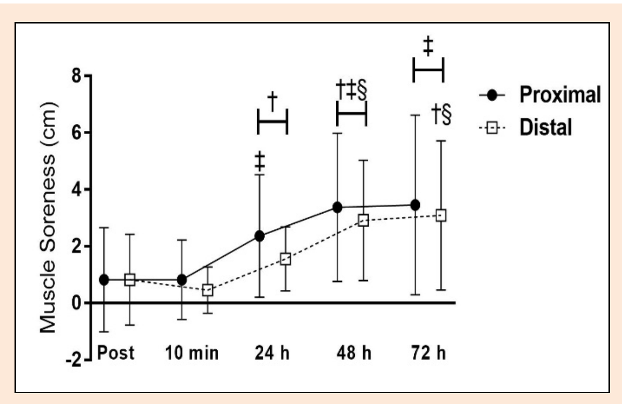

Figure 3. Muscle soreness expressed on average and standard deviation for proximal and distal sites. † - Significant differences for Post; ‡ - Significant differences for 10 min (p < 0.05); § - Significant differences for 24h (p < 0.05).

For the MT parameter, significant interactions between factors were observed (F = 36.34; p < 0.001). The MT% at 72 h was significantly higher than Post, 10 min and 24 h (p < 0.05) (Figure 4) for both arm sites. For the proximal site, significant increases were observed at 48 h (p = 0.020) when compared with Post and 24 h (p=0.039).

For the EI parameter, significant interactions between factors were observed (F = 111.24; p < 0.001). For both sites, a significant EI% increase was observed at 48 h and 72 h compared with Post, 10 min and 24 h (p < 0.05). Significant differences were observed between Post and 24 h (p < 0.001) as well as between 10 min and 24 h (p < 0.001) h only for the distal site (p = 0.014). At the distal site, the EI% was significantly higher than the proximal site for measurements at 24 h (p < 0.001), 48 h (p < 0.001) and 72 h (p < 0.001) (Figure 5).

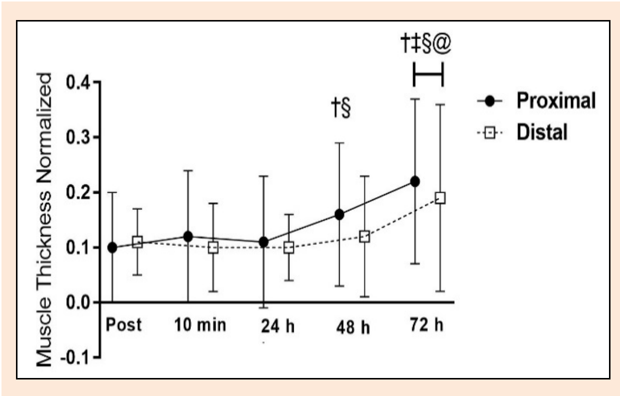

Figure 4. Muscle Thickness expressed on average and standard deviation for proximal and distal sites. † - Significant differences for Post; ‡ - Significant differences for 10 min (p<0.05); § - Significant differences for 24h (p < 0.05). @ - Significant differences for the measure for 48 h (p < 0.05).

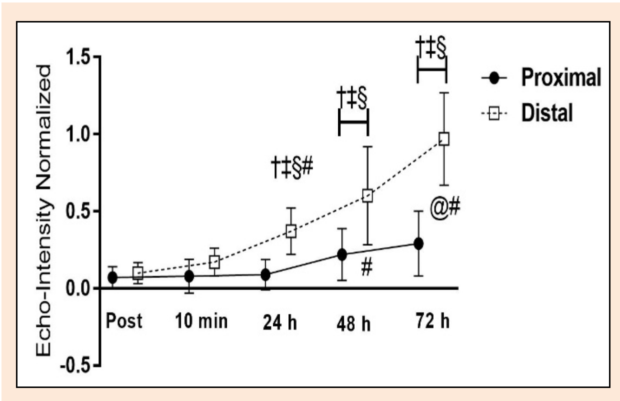

Figure 5. Echo Intensity normalized expressed on average and standard deviation for proximal and distal sites. † - Significant differences for Post; ‡ - 10 min (p < 0.05); § - Significant differences for 24h (p < 0.05). @ - Significant differences for 48 h (p < 0.05). # - Significant differences for distal site (p < 0. 05).

Discussion

The purpose of this study was to investigate the effect of an eccentric training session on EIMD at different arm sites immediately before and time points ranging from 10 min after to three days after the intervention. Our initial hypothesis of non-homogeneity along the muscles was supported only for the variable EI% at 24 h, 48 h and 72 h. The other variables changed similarly between the two arm sites after eccentric exercise.

The disorganization of the muscle tissue structures with possible intracellular material extravasation may be the principal structural reason for the changes in the ultrasound image brightness observed by EI (Pillen and van Alfen, 2011). Healthy skeletal muscle is a hypoechoic tissue that appears darker on ultrasound images than bone, fat or fascia (connective) tissues, which are hyperechoic and reflective. Muscle exudates from inflammatory processes due to the eccentric training also appear lighter on US images. In this sense, EI is thought to be a promising approach to quantify EIMD at local muscle sites (Chen and Nosaka, 2006; Radaelli et al., 2012; Nosaka and Sakamoto, 2001). The mean of the histogram of grey levels on the US image provides a unidimensional image analysis and classifies the ROI of the tissue as hyperechoic or hypoechoic (Matta et al., 2018).

In general, the EI% values at proximal (60%) and distal (80%) arm lengths increased significantly from 24 h to 72 h after the Post and 10 min measurements for both arm sites. These results confirmed previous findings seen at 48 h after the training session (Chen and Nosaka, 2006; Nosaka and Sakamoto, 2001; Pillen and van Alfen, 2011). Chen and Nosaka (2006) reported a significant EI increase (approximately 20%) three days after an eccentric exercise for elbow flexors among untrained men, corroborating our findings for the proximal site at 48 h (22.4%). For the distal site, our findings revealed higher EI% changes after 24 h (37%), increasing at 48 h (60%) and 72 h (97%).

The EI% significantly increased at 24 h, 48 h and 72 h. In particular, after 24 h, 48 h and 72 h, this difference reached 37%, 60% and 97%, respectively, from baseline for the distal site and was much lower for the proximal site, suggesting a non-uniform damage response along the elbow flexors. At the distal site, close to the muscle-tendon junction, the brachialis muscle can be visualized on US images, contributing to higher EI values, as has also been documented by others (Nosaka and Sakamoto, 2001). At the proximal arm site, only the biceps brachii is visualized. Using image processing on magnetic resonance images of contracting biceps, Pappas et al. (2002) demonstrated non-uniform shortening along the anterior surface of the biceps brachii during isometric elbow flexion contraction that was more pronounced at the proximal relative to the distal site of the muscle. Additionally, the authors demonstrated a specific level of stretching of the biceps brachii fibres on the distal site.

Oedema is a commonly observed consequence of EIMD, as described by Hydahl and Hubal (2014) on an invited review. In this sense, our study demonstrated a significant increase in elbow flexors oedema after eccentric intervention on both arms sites. The MT% of the distal site increased significant only after 72 h (18%) for the Post measurement; for the proximal site, the MT% exhibited higher values at 48 h (16%) and 72 h (22%). Our results corroborate the findings of Chapman et al. (2008) in experiments performed on untrained men with an increase of up to 15-20% after eccentric training in only one muscle length from 24 h to 72 h. However, to date, no study has assessed MT at different arm sites. Some studies evaluating oedema at different muscle lengths used arm circumference measurements; their results did not demonstrate significant differences between arm sites (Chen, 2003; Chen and Nosaka, 2006; Jubeau et al., 2012).

The elbow flexion PT was significantly reduced immediately after the intervention compared with baseline (approximately 50%), exhibiting a 37% deficit at 72 h. Corroborating our findings, Chen and Nosaka (2006) reported a PT decrease of approximately 40% volume for elbow flexors in untrained men 72 h after EIMD was induced. Furthermore, a decrease in PT after eccentric interventions among untrained subjects was reported by others with a similar exercise protocol (Chan et al., 2012), decreasing the peak torque by nearly 50% 24 h after EIMD. The failure in the coupling of cross-bridges is believed to be the main contributor to the force depression together with other structural disorganization after tissue injury (Warren et al., 2002). These mechanisms can account for approximately 57-75% of the strength loss in the first five days after injury, with the remaining 25% attributed to structural damage in the cytoskeleton (Warren et al., 2002).

A similar increase in DOMS was observed for both sites, with higher values occurring between 24 h and 48 h after eccentric intervention, as has been reported by others (Chen et al., 2012; Pillen and van Alfen, 2011). In the present study, no significant difference in muscle soreness was found between the two sites, differing from the results of Dierking et al. (2000), who demonstrated higher soreness perception in the distal region of the elbow flexors using a modified non-controlled pressure plumber apparatus (Dierking et al., 2000). DOMS is accepted to result from physical damage to muscle tissue that triggers subsequent inflammatory processes (Chan et al., 2012). Lau et al. (2015) have recently related muscle soreness to inflammation primarily in the muscle fascia using an electrical threshold (Lau et al., 2015). These authors described a pattern that differs from the one described in our study; their results indicated lower DOMS in the central line of the elbow flexors and distal regions following six sets of 10 eccentric repetitions of the elbow flexors.

The present data should be considered restricted to untrained subjects performing a single eccentric training session applied with an isokinetic dynamometer. Nevertheless, conventional training should be tested to ensure a practical field response in the EIMD parameters for the same behaviour. Furthermore, acute EIMD results should not be generalized for chronic effects, for example, selective elbow flexors hypertrophy.

Conclusion

Present results indicate a non-uniformity of elbow flexors damage after one bout of heavy eccentric training based on the echo-intensity (EI) parameter, from muscles ultrasound images. Higher EI values at the distal site after 24 h from intervention, suggest a pronounced inflammatory process on this specific site. These results indicate that the measurement of EIMD at a single site may not be an appropriate strategy for assessing muscle damage as a whole. Additionally, accordingly to all parameter, the elbow flexors are not completely recovered after 72h from a heavy eccentric training session.

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Key points

- The Echo-intensity indicates prominent EIMD on distal site of elbow flexors.
- Eccentric intervention had negative effects on muscle soreness and performance.
- EIMD parameters do not return to the baseline within 72 hours of recovery.

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