INSIGHTS GAINED INTO ECCENTRIC EXERCISE-INDUCED MUSCLE DAMAGE FROM HIGH-DENSITY SURFACE ELECTROMYOGRAPHY

Hélio da Veiga Cabral

Tese de Doutorado apresentada ao Programa de Pós-graduação em Engenharia Biomédica, COPPE, da Universidade Federal do Rio de Janeiro, como parte dos requisitos necessários à obtenção do título de Doutor em Engenharia Biomédica.

Orientador: Liliam Fernandes de Oliveira

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Dedication

I would like to dedicate this thesis to my mother Natividade Espirito Veiga, who always loved me unconditionally. Mom, thank you very much for all your efforts to make this thesis and all my dreams possible. I am sure you are still looking through every step mine. I will always love you.

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Resumo da Tese apresentada à COPPE/UFRJ como parte dos requisitos necessários para a obtenção do grau de Doutor em Ciências (D.Sc.)

CONHECIMENTOS ADQUIRIDOS EM DANO MUSCULAR INDUZIDO PELO EXERCÍCIO A PARTIR DA ELETROMIOGRAFIA DE ALTA DENSIDADE

Hélio da Veiga Cabral

Março/2020

Orientador: Liliam Fernandes de Oliveira

Programa: Engenharia Biomédica

O principal objetivo desta tese foi investigar se o dano muscular induzido pelo exercício (DMT) leva a alterações locais na amplitude das ondas-M supramáximas detectadas ao longo do bíceps braquial. Dez homens saudáveis foram submetidos às seguintes medições, realizadas antes e quatro dias consecutivos após 3x10 contrações excêntricas de flexão de cotovelo: avaliação da dor muscular; aquisição de imagens ultrassonográficas (US) do bíceps; aquisição de eletromiogramas de superfície com uma matriz de 64 eletrodos durante estimulação supramáxima do nervo musculocutâneo; 2 contrações isométricas voluntárias máximas (CIVMs) de flexão do cotovelo. A localização longitudinal da zona de inervação (ZI), o número de eletrodos que detectaram as maiores ondas-M (*canais segmentados*) e a coordenada longitudinal do centroide desses eletrodos foram avaliados para caracterizar a distribuição da amplitude das ondas-M. Após a indução do DMT, o pico de torque da CIVM diminuiu, enquanto a dor muscular percebida aumentou em 24, 48, 72 e 96 h ($P<0,004$ para ambos os casos). A intensidade do eco das imagens de US aumentou em 48, 72 e 96 h para as regiões proximal e distal ($P<0,001$), embora nenhuma diferença tenha sido observada entre as regiões ($P=0,136$). A posição da ZI não se alterou ao longo dos dias ($P=0,283$). O número de *canais segmentados* diminuiu significativamente ($P<0,032$) e a coordenada longitudinal do centroide deslocou-se para a região distal em 24, 48 e 72 h ($P<0,032$). O DMT alterou consistentemente as ondas-M supramáximas detectadas principalmente na região proximal do bíceps braquial, sugerindo um efeito local do DMT na excitação muscular.

Abstract of Thesis presented to COPPE/UFRJ as a partial fulfilment of the requirements for the degree of Doctor of Science (D.Sc.)

INSIGHTS GAINED INTO ECCENTRIC EXERCISE-INDUCED MUSCLE DAMAGE FROM HIGH-DENSITY SURFACE ELECTROMYOGRAPHY

Hélio da Veiga Cabral

March/2020

Advisor: Liliam Fernandes de Oliveira

Department: Biomedical Engineering

The purpose of this thesis was to investigate whether exercise-induced muscle damage (EIMD) leads to local changes in the amplitude of supramaximal M waves detected along biceps brachii. Ten healthy male subjects were submitted to the following measurements conducted immediately before and four consecutive days after 3x10 eccentric elbow flexions: evaluation of muscle soreness; acquisition of ultrasound images proximally and distally from biceps; recording of surface electromyograms with a 64-electrodes grid while 10 supramaximal pulses were applied to the musculocutaneous nerve; two isometric, maximal voluntary elbow flexion contractions (MVCs). The innervation zone (IZ) longitudinal location, the number of electrodes detecting the largest M waves (*segmented channels*) and the centroid longitudinal coordinate of these electrodes were assessed in order to characterize the distribution of M-waves amplitude. After EIMD, the MVC torque decreased while the perceived muscle soreness increased at 24, 48, 72 and 96 h ($P<0.004$ for both cases). The echo intensity of ultrasound images increased at 48, 72 and 96 h with respect to baseline for both regions ($P<0.001$) while no differences were observed between regions at any time ($P=0.136$). No time effect was observed for IZ location ($P=0.283$). The number of *segmented channels* significantly decreased ($P<0.032$) and the centroid longitudinal coordinate shifted towards the distal region at 24, 48 and 72 h ($P<0.032$). EIMD consistently changed supramaximal M waves detected mainly proximally from biceps brachii, suggesting a local effect of EIMD on muscle excitation.

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List of abbreviations

ANOVA	Analysis of variance
ARV	Average rectified value
EIMD	Exercise-induced muscle damage
EMGs	Electromyograms
IZ	Innervation zone
MVC	Maximal voluntary contraction
NMES	Neuromuscular electrical stimulation
RMS	Root mean square
US	Ultrasound

Chapter 1: Overview of the Thesis

1.1 General introduction

The accomplishment of eccentric exercises with unaccustomed intensity produces pain, tenderness and swelling, which develop slowly after the exercise, characterizing what is referred to as exercise-induced muscle damage (EIMD) (CLARKSON *et al.*, 1992; HYLDAHL and HUBAL, 2014). The surface electromyography has been used as the standard technique to investigate the neuromuscular adjustments following EIMD and, typically, a pair of electrodes is placed over a single muscle site as representative of the whole muscle (HORTOBÁGYI et al., 1998; BAJAJ et al., 2002; SEEMLER et al., 2007). There are, however, many factors that influence the interpretation of changes in electromyograms (EMGs) amplitude in terms of muscle activation, such as the position and orientation of electrodes (FARINA, 2006). Indeed, previous evidence suggests the alterations of EMGs variables after eccentric EIMD are affected by the proximo-distal location of bipolar electrodes (PIITULAINEN *et al.*, 2009). A possible explanation to this site-dependency is an uneven damage of muscle fibers. Nevertheless, other factors not attributable to EIMD may contribute to the spatially localized activity observed after eccentric contractions, as the prolonged pain that accompanied eccentric exercise (MADELEINE *et al.*, 2006). Additionally, a spatially localized distribution of the amplitude of surface EMGs has been observed even during maximal voluntary contractions and in the absence of muscle damage (MIYAMOTO *et al.*, 2012).

Thus, as can be seen, the question about the EIMD interference on the muscle excitation distribution remains an open issue. If the population of motor units recruited is the same when using surface EMGs to assess the muscle adaptations resulting from EIMD, it would be possible suppress effects other than those resulting from the damage itself on the amplitude distribution of EMGs. During supramaximal electrical stimulation we presumably ensure most, if not all, motor units are recruited in different days (CALDER *et al.*, 2005). Therefore, in this thesis, high-density surface electromyography (HD-sEMG; i.e., muscle activity detection with multiple electrodes) and neuromuscular electrical stimulation (NMES) were combined to investigate the electrophysiological topography of biceps brachii muscle following EIMD. Specifically, the following question is addressed: does EIMD lead to local changes in the amplitude of M waves detected along biceps brachii, from one to four days after eccentric exercise? Chapters 2 provides the essential background on EIMD (*session 2.1*), surface electromyography (*session 2.2*) and neuromuscular electrical stimulation (*session 2.2*) to understand the methods and main results reported in the Chapter 3 of this thesis.

1.2 Objectives

1.2.1 General objective

Investigate the electrophysiological topography of biceps brachii muscle after EIMD.

1.2.2 Specific objectives

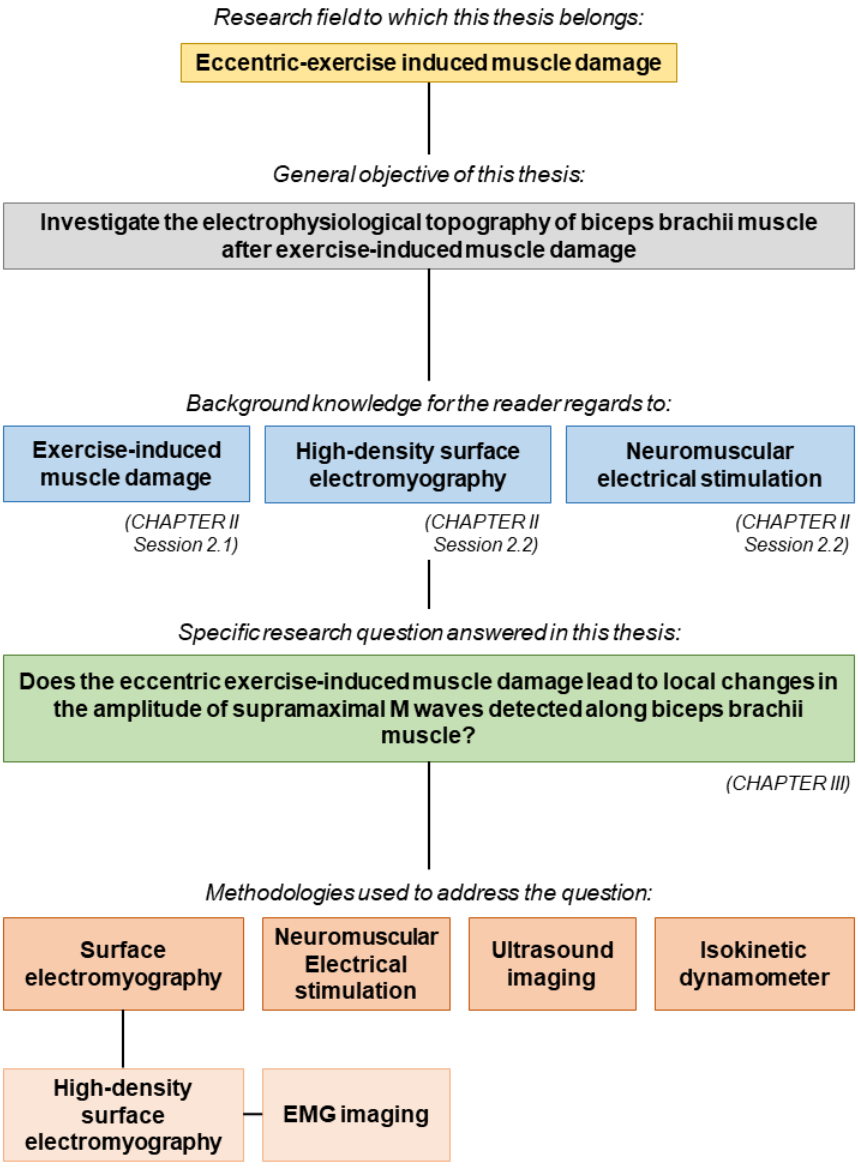
- Provide novel insights into EIMD neuromuscular responses from detection and analysis of HD-sEMG during supramaximal NMES of musculocutaneous nerve.

- Assess the spatial differences in the biceps brachii activity distribution from one to four days after eccentric EIMD.

- Evaluate whether there are differences in the echo intensity of ultrasound images detected from distinct biceps brachii regions from one to four days after eccentric EIMD.

- Examine whether there are similarities in the temporal responses of EIMD indirect markers (i.e., muscle soreness and torque peak) and EIMD neuromuscular responses investigated by HD-sEMG.

1.3 Outline of the Thesis



Chapter 2: State of the Art

2.1 Understanding the exercise-induced muscle damage

2.1.1 What is the exercise-induced muscle damage?

All of those subjected to an exercise session with unaccustomed intensity or duration, mainly when involves eccentric contractions (NEWHAM *et al.*, 1983a; CLARKSON and NEWMAN, 1995), are prone to muscle soreness sensation and force loss, which may last several days after exercise. These symptoms following a novel eccentric exercise are accompanied by others, as muscle swelling, reduced range of motion and efflux of myocellular proteins, and are commonly referred to as exercise-induced muscle damage. The research of HOUGH (1902) was the first to propose that these prolonged force loss and muscle soreness following a novel, intense eccentric exercise would be related to muscle fibers damage. Specifically, in his investigation, subjects contracted a finger against a spring, and he noted that the muscle soreness started to develop some hours after this single exercise session and became more pronounced on the next day. He then established two types of exercise muscle soreness: (i) the muscle soreness during activity (i.e. fatigue); (ii) the delayed-onset muscle soreness. While the first one would be consequence of metabolites accumulation, the other would be caused by structural muscle damage. Over the last years, his findings were subsequently supported and augmented by several studies (ARMSTRONG, 1984; CLARKSON *et al.*, 1992; CLARKSON and SAYERS, 1999; PEAKE *et al.*, 2017). This session briefly describes the etiology and responses to EIMD.

Structural alterations. FRIDÉN *et al.* (1981) were the first to report morphological damage in human muscles following eccentric exercise. In their study, electron microscopy images from biopsies taken from subjects who repeatedly came down stairs revealed disturbance and spreading of Z lines, overstretched sarcomeres and disorganization of contractile proteins (Figure 1). They also showed that these structural alterations were spread along the damaged muscle and could be limited to a single myofibril or involve many adjacent myofibrils. These acute structural changes were further supported by studies of NEWHAM and colleagues (1983a;1983b), where subjects were instructed to continuously step up with one leg and down with the other during a box stepping exercise. Muscle biopsies taken 24-72 h after exercise revealed morphological changes involving broadening and loss of Z lines and appearance of the intracellular enzyme creatine kinase in the plasma. Additionally, they showed that these disruptions resulted primarily from the eccentrically exercised muscle, which was further confirmed by other studies (CLARKSON and SAYERS, 1999).

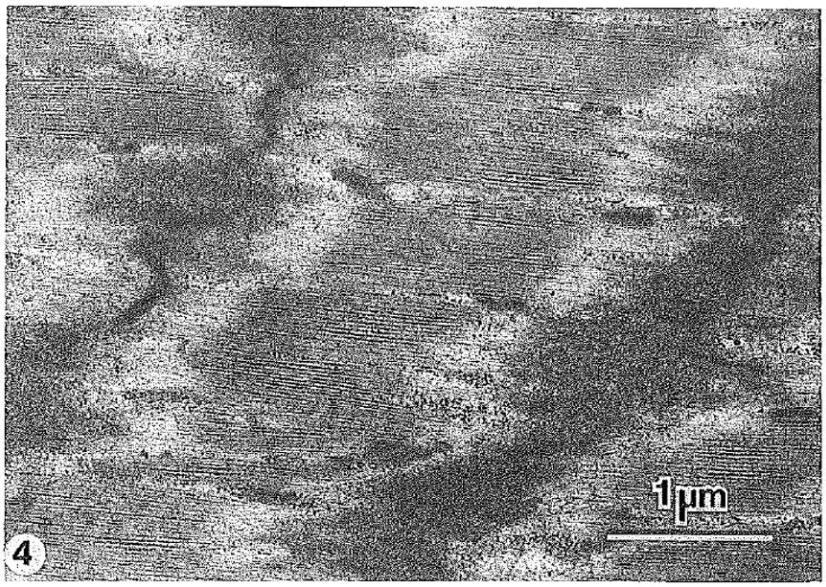


Figure 1: Examples of structural muscle damage in humans after novel eccentric contractions. The electron micrograph shows disorganized Z-band material extending into the I-band as consequence of eccentric exercise. Figure obtained from FRIDÉN et al. (1983) with permission approved by Georg Thieme Verlag KG (License number 4777160298365).

Posteriorly, other morphological research with eccentric EIMD supported these first findings and, over last decades, several investigations demonstrated that Z line is the most susceptible structure to alterations (FRIDÉN *et al.*, 1983; GIBALA *et al.*, 1995; HORTOBÁGYI *et al.*, 1998; FRIDÉN and LIEBER, 2001). In contrast to prevalent evidence, few studies proposed that minor or no damage could be observed after voluntary eccentric contractions in humans (CRAMERI *et al.*, 2007) and that ultrastructural changes would be just signs of muscle adaptation and regeneration (YU *et al.*, 2004). However, more recently, LAURITZEN *et al.* (2009) counteracted these ideas, demonstrating an extensive ultrastructural change in biceps brachii muscle after 5 subjects performed 70 voluntary maximal eccentric actions. They specifically reported widespread Z-line disturbances and significant myofibrillar disruption with a few infiltrated macrophages. Collectively with these myofibrillar changes, the damage following a novel eccentric exercise also impairs the extracellular matrix and sarcomeres' structural proteins (STAUBER *et al.*, 1990; LIEBER *et al.*, 1996). For instance, both animal (LIEBER *et al.*, 1994) and human (BEATON *et al.*, 2002) studies demonstrated loss of desmin protein following eccentric exercise, although some studies did not observe the same behavior (YU *et al.*, 2002).

In conclusion, the EIMD unleash acute structural changes in the muscle tissue as myofibrillar and sarcoplasmic reticulum disruptions, Z-line streaming, breakdown of some cytoskeleton proteins, sarcomere overstretching/disruption, and muscle fiber damage (FRIDÉN *et al.*, 1981; FRIDÉN *et al.*, 1983; NEWHAM *et al.*, 1983b; FRIDÉN and LIEBER, 2001). However, these alterations on its own do not explain the very prolonged symptoms following a novel eccentric exercise and jointly with them, a calcium overload phase and inflammatory alterations would play an important role.

Calcium homeostasis disturbance and inflammatory alterations. The structural damage to membrane (MCNEIL and KHAKEE, 1992) combined with the opening of stretch-activated ion channels (MCBRIDE *et al.*, 2000) after lengthening contractions result in disturbance of ion concentration over the sarcolemma (i.e. there is a persistent increase in Na^+ and Ca^{2+} intracellular over time; ALLEN, 2004). This increase of intracellular calcium, for instance, triggers calcium-dependent degradation pathways (LIEBER and FRIDÉN, 1999) through the activation of calcium-dependent proteases (e.g. calpain; BELCASTRO, 1993), which hydrolyze contractile proteins, further contributing to the sarcomere disorder.

Furthermore, the structural alterations due to EIMD also stimulate satellite and inflammatory cells to interact with others within the extracellular matrix. For instance, HUBAL *et al.* (2008) have shown that eccentric contraction results in upregulation of MCP-1, a protein involved in activation/attraction of inflammatory cells. The muscle inflammatory response following eccentric exercise is related with more tissue damage, fibrosis, soreness and delayed recovery (CANNON *et al.*, 1990; HYLDAHL and HUBAL, 2014; CHAZAUD, 2016). However, in contrast to what was initially thought, the inflammation is not solely related with that and encompasses tissue repair and return to homeostasis (HYLDAHL and HUBAL, 2014; DAMAS *et al.*, 2016; PEAKE *et al.*, 2017).

Leukocytes are mobilized into the circulation immediately after eccentric exercise (PEAKE *et al.*, 2005). Specifically, they accumulate in the muscle extracellular space at 24-48h after exercise (PAULSEN *et al.*, 2010), with an early accretion of neutrophils in the first 24h (RAASTAD *et al.*, 2003). Monocytes/macrophages are lately observed in the muscle, from 48h to 7 days after exercise (PAULSEN *et al.*, 2010). Subsequently to this mobilization in the muscle tissue, leukocytes cells transmigrate into the muscle where

they breakdown damaged tissue (PAULSEN *et al.*, 2012; PEAKE *et al.*, 2017). For instance, the neutrophils produce tissue degradation through the release of oxygen-reactive radicals and cytotoxic proteins (PEAKE *et al.*, 2017; TOUMI and BEST, 2003). They are also the major producers of cytokines (i.e. IL-1, IL-6 and TNF), which are immunomodulatory polypeptides with a proinflammatory effect. The monocytes, in turn, migrates with neutrophils and change their immunophenotypic characterization when infiltrate in the muscle tissue, becoming macrophages. The macrophages produce oxygen-reactive radicals, cytotoxic proteins and enzymes capable to degrade and remodel the cells of extracellular matrix. Therefore, the excessive production of reactive species by these inflammatory cells may worsen the muscle damage (MACINTYRE *et al.*, 1995).

2.1.2 Responses to eccentric exercise-induced muscle damage

A series of novel eccentric contractions produce, among other things, prolonged reduction in force, delayed-onset muscle soreness, muscle swelling, range of motion decrease and muscle-specific proteins appearance in the blood. These consequences are commonly referred as indirect muscle damage markers and have been extensively used to quantify the eccentric EIMD. Although their characteristics are not yet fully understood, this section will shortly discuss two eccentric EIMD responses.

Force loss. Declines in force production following repeated eccentric contractions range from 15-60% with respect to the pre-exercise value and may last up to 2 weeks (Figure 2; CLARKSON *et al.*, 1992). Conversely, the force is completely restored in 2 h after concentric contractions (WALSH *et al.*, 2004) and, therefore, force decrements measured at 2h or later after eccentric contractions are likely associated just to muscle damage and not to metabolic fatigue (PROSKE and ALLEN, 2005). Force loss is also well-correlated

with direct, histological evidence of muscle damage (PAULSEN *et al.*, 2012; CLARKSON and HUBAL, 2002) and it is thus considered one of the most reliable indirect markers of EIMD (WARREN *et al.*, 1999; PAULSEN *et al.*, 2012; DAMAS *et al.*, 2016). The degree of force reduction is dependent on the nature and intensity of eccentric stimulus, explaining why some discrepancies in force loss among individuals are reported in the literature (PROSKE and ALLEN, 2005).

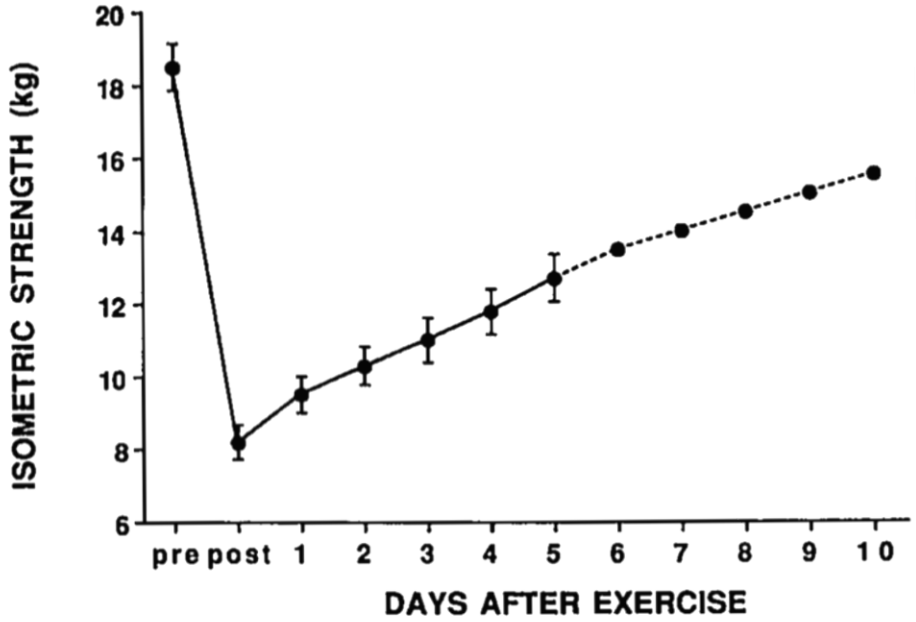


Figure 2: Isometric strength (mean ± S.D.) for 109 participants measured before and 10 days after eccentric exercise-induced muscle damage. It is possible to observe a clear decrease in force immediately after eccentric exercise which extends up to 10 days. Figure obtained from CLARKSON et al. (1992) with permission approved by Wolters Kluwer Health, Inc. (License number 4777700609949).

Many hypotheses have been argued to explain the prolonged force decline after eccentric exercise (ALLEN, 2001; HYLDAHL and HUBAL, 2014). In 1990, MORGAN early proposed a possible explanation known as *popping sarcomere theory* (for review see PROSKE and MORGAN, 2001). According to MORGAN's hypothesis, when contracting muscles are lengthened, some sarcomeres receive stretch more than others

(i.e. weaker sarcomeres; see Figure 2 of ALLEN, 2001). If this stretch occurs on the descending limb of the length-force curve, the weaker sarcomeres start to quickly elongate, becoming gradually weaker until there is no myofilament overlap. After a single eccentric contraction, the muscle relaxes and the overstretched sarcomeres re-interdigitate (TALBOT and MORGAN, 1996). However, with repeated eccentric actions, it is likely that in some sarcomeres the myofilaments fail to correctly re-interdigitate, producing myofibrillar damage and disruption. The disrupted sarcomeres are located randomly along the myofibrils length and their disruptions could spread the damage longitudinally and transversally to adjacent myofibrils, leading to membrane damage. The key point of *popping sarcomere theory* is that the sarcomere instability would happen only on the descending limb of the length-force relation, explaining why shortening contractions would not result in overextended sarcomeres. Eccentric contractions therefore lead to a shift of the muscle length-force relation in the direction of longer muscle lengths. This shift is also correlated with the amount of damage (JONES *et al.*, 1997) and thus considered a reliable measure of EIMD.

In 1993, WARREN and colleagues proposed an alternative hypothesis for the fall of force following eccentric contractions. The *excitation-contraction (E-C) uncoupling theory* (for review see WARREN *et al.*, 2001) proposes that the force-capability decline is triggered by interference with E-C coupling. They showed in mouse soleus muscle that the force produced during caffeine-induced contraction was not significantly different between normal and damaged muscles. Caffeine directly releases Ca^{2+} from sarcoplasmic reticulum, bypassing part of normal excitation-contraction coupling. Their results therefore suggest that changes in Ca^{2+} release are the main cause of the reduced force after eccentric contractions. According to them, approximately 75% of force loss during the 3 days after eccentric activity could be attributed to E-C uncoupling and the remaining

25% by damage to the structures related with force production (including "popped" sarcomeres). Subsequent works supported the E-C uncoupling hypothesis (BALNAVE and ALLEN, 1995; CORONA *et al.*, 2010; MURPHY *et al.*, 2013).

There is discussion on which hypothesis would be the best explanation for the prolonged force decline after eccentric exercise. However, it has been proposed the loss of force after eccentric exercise is result from a combination of various mechanisms (MORGAN and PROSKE, 2004; HYLDAHL and HUBAL, 2014). Mechanical strain during lengthening contractions causes uneven overstretching of sarcomeres, resulting in greater forces placed on sarcolemma and t-tubules. These alterations produce membrane disruption, opening of stretch-activated channels and E-C dysfunction. The Ca^{2+} release in the cytosol resulted from sarcolemma permeable sections or stretch-activated channels promote contractile proteins degradation by calpain enzymes, leading to prolonged loss of muscle force.

Delayed-onset muscle soreness. The soreness usually peaks between 24-48h after EIMD and subsides within 5-9 days (Figure 3). The soreness rise is preceded by a pain-free period, explaining why the term delayed-onset muscle soreness is adopted to describe this response (GRAVEN-NIELSEN and ARENDT-NIELSEN, 2003). As well as force loss, the delayed-onset muscle soreness presents variability among subjects submitted to the same eccentric exercise. In 1984, ARMSTRONG proposed that different processes work together to produce muscle soreness: (i) structural damage to muscle; (ii) disturbance in Ca^{2+} homeostasis; (iii) type IV nerve ends sensitization as consequence of products invading inflammatory cells.

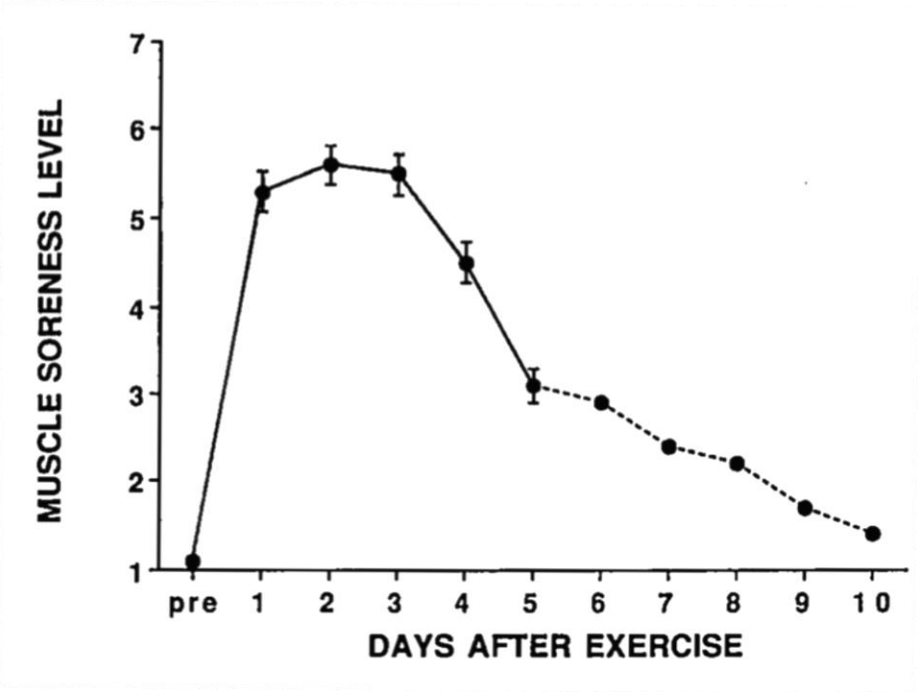


Figure 3: Muscle soreness level (mean ± S.D.) for 109 participants measured before and 10 days after eccentric exercise-induced muscle damage. It is possible to note an increase in the perceived muscle soreness after eccentric exercise which extends up to 9 days. Figure obtained from CLARKSON et al. (1992) with permission approved by Wolters Kluwer Health, Inc. (License number 4777700609949).

Although the delayed-onset muscle soreness is mainly associated with myofibers damage, it was recently observed in rats that hyperalgesia (i.e. abnormally increased sensitivity to pain) may occur 1-3 days after eccentric contractions without damage symptoms (HAYASHI *et al.*, 2017). MIZUMURA and TAGUCHI (2016) concluded in their review that neurotrophic factors (e.g. activation of receptor-nerve growth factor B2-bradykinin; activation of neurotrophic factor COX-2-glial cell) would be then the main cause to the hyperalgesia induced by eccentric contractions. Even though there are several hypotheses to explain the delayed-onset muscle soreness, their causes are not fully understood by literature.

2.2 Combining NMES with HD-sEMG to investigate the electrophysiological topography of human muscles

2.2.1 Surface electromyography

The functional unit of the neuromuscular system comprises the motor neuron and the muscles fibers innervated by the axon of this neuron, composing the motor unit (HECKMAN and ENOKA, 2012; Figure 4). When the motor unit receives an electrical stimulus that overcomes its recruitment threshold (e.g. during voluntary muscle contractions), the motor neuron discharges, and action potentials are generated in the muscle fibers. The action potentials initiate in the neuromuscular junction and propagate towards to the myotendinous junctions.

Electromyography is the technique used to record the sum of action potentials (electromyograms) produced during muscle contractions. With electrodes positioned over the skin surface or inserted in the muscle it is possible to extract relevant physiological information, as the degree and timing of muscle activation, the discharge rate of active motor units and myoelectric manifestation of muscle fatigue (MERLETTI *et al.*, 1990; FARINA *et al.*, 2004a; MERLETTI and FARINA, 2009). The detection selectivity is the main difference between surface and intramuscular electromyography, with the last one being able to record individual motor unit action potentials (MERLETTI and FARINA, 2009). Conversely, the surface EMGs reflect a more general information about muscle activity, since the activation of single motor units is not equally evident (GARCIA and VIEIRA, 2011). Due to its practicality and non-invasiveness characteristic, the surface electromyography is typically used by the literature of sports and rehabilitation sciences (VIGOTSKY *et al.*, 2018).

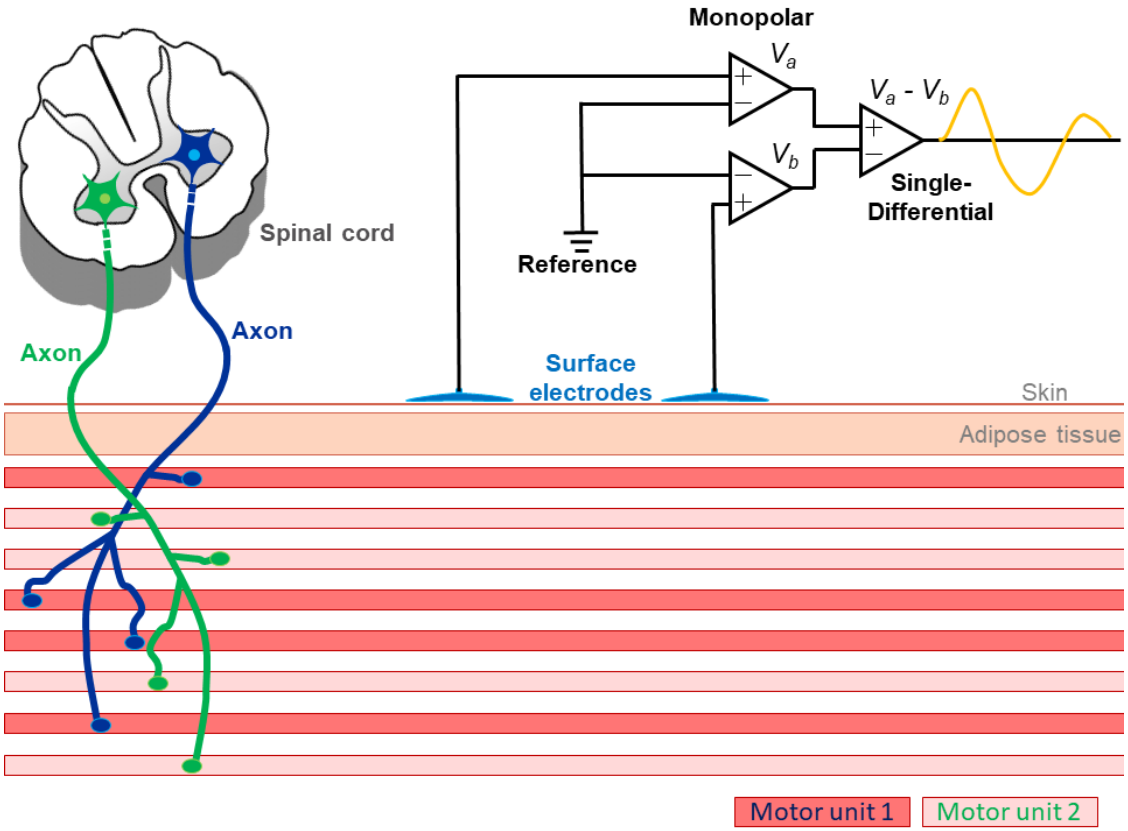


Figure 4: Figure shows examples of two motor units (i.e., the motor neuron and the muscles fibers innervated by the axon of this neuron) and the two main modes of surface electromyography acquisition, the monopolar and single-differential configurations.

There are two types of electrodes configuration, the monopolar and bipolar derivations (GARCIA and VIEIRA, 2011). Monopolar EMGs correspond to the difference between the voltage measured by the electrode and that measured by the reference electrode, which is usually positioned over the skin in a bone region (i.e. voltage presumably equal to zero). In contrast, the bipolar or single-differential EMGs are obtained from the difference between two monopolar EMGs (Figure 4). In this type of EMGs recording, the power line interference and the common-mode voltage, for instance due to crosstalk are considerable attenuated compared with the monopolar derivation (DE LUCA and MERLETTI, 1988). Moreover, the detection volume is reduced, and the myoelectric activity of deep motor units are also attenuated in the bipolar configuration (ROELEVELD at al., 1997). The single-differential EMGs reflect therefore a local activity of the muscle.

A single pair of electrodes positioned over the skin is standardly applied to investigate the relation between muscle force demands and motor unit activity. There are two ways of increase the muscle force during a specific motor task: increasing the discharge rate of the motor units already activated (temporal summation) and recruiting new motor units (spatial summation; HECKMAN and ENOKA, 2012). Regardless of the type of motor unit modulation, the higher the muscle force, the more motor unit potentials are summed and the higher EMGs amplitude recorded by the surface electrodes pair. The main physiological information that can be obtained using a couple of surfaces electrodes is thus an indication of the muscle contraction degree. For this purpose, indexes have often been used by the literature to quantify variations in the intensity of muscle activation. The average rectified value (ARV), the peak-to-peak (PP) value and the root mean square value (RMS) are the most common amplitude descriptors and they are calculated as follows:

$$ARV = \frac{1}{N}\sum_{n=1}^{N}|x_n|$$

$$PP = x_{max} - x_{min}$$

$$RMS = \sqrt{\frac{1}{N}\sum_{n=1}^{N}x_n^2}$$

where N is the total number of samples, x_n is the value of the sample n, x_{max} is the value of the sample with higher value and x_{min} of the sample with lower value.

Notwithstanding the effectiveness of all descriptors in quantify EMGs amplitude variation, the information provided by each one is slightly distinct (GARCIA and

VIEIRA, 2011). For instance, due to its square operator, the RMS descriptor emphasizes periods of high EMGs amplitude with respect to periods of low myoelectric activity. On the other hand, the ARV descriptor quantifies temporal variations in the EMGs amplitude directly related to the degree of muscle activity. The PP value, in turn, is not sensible to the window size used to quantify the signal amplitude and has been frequently used with electrically-evoked potentials.

What physiological information can be obtained from the high-density surface electromyography in fusiform muscles? The term high-density surface electromyography or multichannel surface electromyography is used to denote the detection of the myoelectric activity using groups of electrodes organized in linear arrays (MERLETTI *et al.*, 2003) or grids (STEGEMAN *et al.*, 2012). This possibility of acquiring EMGs from a muscle with higher spatial resolution has progressively gained attention in the clinical and applied research (DROST *et al.*, 2006). The main reason for that is the large number of anatomical and physiological information that can be obtained from the HD-sEMG. In this section, we will briefly describe some information that can be extracted from HD-sEMG in fusiform muscles.

Figure 5 shows an example of single-differential EMGs detected from the fusiform, biceps brachii muscle using a linear array of 16 electrodes. When we sample the EMGs using a one-dimensional array of electrodes aligned in parallel with the muscle fibers, it is possible to track the movement of surface potentials along them (MERLETTI and MUCELI, 2019). Indeed, as represented in Figure 5, the motor unit action potentials generated at the innervation zone (IZ), i.e. region where the neuromuscular junctions of the muscle are located, were continuously detected by the electrodes until the extremities

of the fibers. The capability of HD-sEMG to provide information in two domains (time and space) allows the extraction of valuable physiological knowledge.

It is possible to note in Figure 5 that some single-differential EMGs have small amplitude with respect to others (e.g. differential channels 7 and 15). Since the surface electrodes 7 and 8 are located symmetrically at both sides of the IZ, as the action potentials propagate in opposite direction from the IZ toward both proximal and distal tendons, they record the same monopolar potential at the same time. Thus, the difference between these monopolar EMGs have almost zero amplitude (cf. grey rectangle box in Figure 5). Therefore, when the bipolar derivation is used, the amplitude of action potentials acquired over the IZ is very low (MERLETTI *et al.*, 2003; RAINOLDI *et al.*, 2004). This fact has implications for the positioning of bipolar surface electrodes. If we are interested in knowing whether a muscle is active or not, positioning a couple of electrodes above the IZ provide a disguised information. This issue is especially critical in dynamic contractions or when comparing the amplitude of EMGs collected for different joint angles, given the relative position between IZ and electrodes changes with changes in joint angle (FARINA *et al.*, 2006; MANCEBO *et al.*, 2019a).

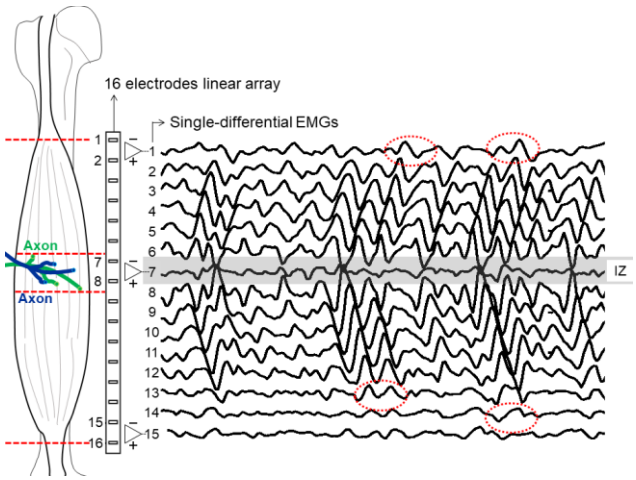


Figure 5: Example of 15 single-differential (SD) electromyograms obtained from the biceps brachii muscle with a linear array of 16 electrodes during an isometric elbow flexion. Anatomical information, as innervation zone (IZ; SD channel 7) and tendon regions (e.g. SD channels 1 and 14), may be easily extracted from these signals as fully explained along the session 2.2.1.

The common mode signals cancellation in the single-differential detection has also implications for the EMGs detected over tendon regions (e.g. differential channels 1, 14 and 15). The end-fiber-effect is characterized by a more uniform distribution of the surface potential over the skin caused by the extinction of the action potentials at the tendon regions (MERLETTI *et al.*, 2003). Consequently, the surface electrodes in these regions acquire similar monopolar EMGs at the same time, which leads to differential EMGs with small amplitude. The end-fiber-effect is denoted in Figure 5 with the dashed red circles. In conclusion, the ideal solution to study the activation of a fusiform muscle as the biceps brachii using a pair of electrodes would be to position them somewhere between differential channels 2 and 6 or channels 8 and 12.

The conduction velocity of motor unit action potentials also can be estimated using HD-sEMG (FARINA *et al.*, 2000; FARINA *et al.*, 2001). Since successive surface electrodes provide the same action potentials delayed in time, it is possible to estimate the conduction velocity as the ratio between the interelectrode distance and the time delay. The techniques to calculate the delay between EMGs encompass the time delay between peaks of the same potential detected by consecutive electrodes (Figure 6) as well as more robust methods in the time and frequency domains.

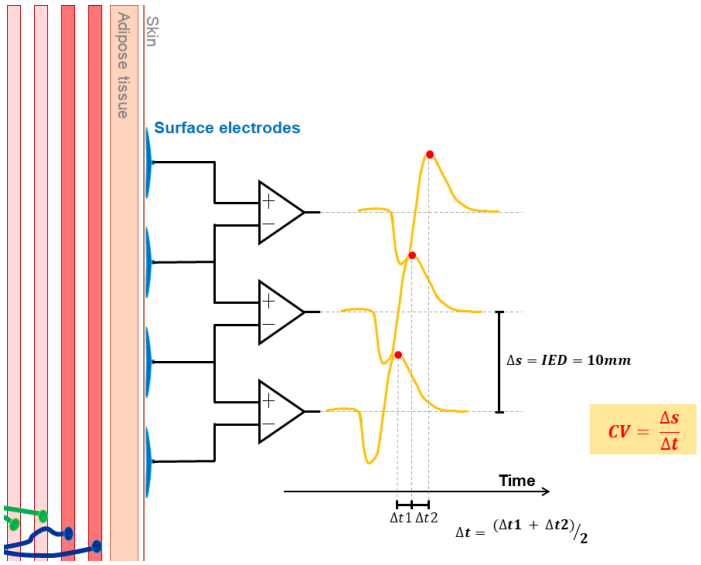


Figure 6: An example of how to estimate the motor unit conduction velocity (CV) using high-density surface electromyography. The CV was estimated as the ratio between the interelectrode distance (IED) and the action potential time delay (Δt; measured trough the delay between peaks of the same potential detected by consecutive electrodes).

The uneven spatial distribution of human muscles activity. The possibility to collect EMGs from multiple skin regions covering the target muscle also revealed that EMGs detected from a single muscle region do not really reflects the neural changes within the whole muscle volume (GALLINA *et al.*, 2013). Indeed, inhomogeneities in the spatial distribution of EMGs amplitude have been consistently reported for individual muscles in different circumstances, among which during isometric contractions (GALLINA and BOTTER, 2013), fatiguing contractions (HOLTERMANN *et al.*, 2010), muscle pain (MADELEINE *et al.*, 2006) and dynamic condition (MANCEBO *et al.*, 2019b). For instance, using a 16-electrodes linear array transversally positioned to pectoralis major muscle (Figure 7A), MANCEBO and colleagues (2019b) showed that the distribution of EMGs activity is localized rather than diffused, along muscle longitudinal axis during the inclined bench press. Specifically, they showed that the coordinate of the barycenter (i.e. the region detecting relatively large EMGs) was identified around the channels located near to clavicular region (Figure 7B), suggesting the trunk inclination play a role in the spatial distribution of pectoralis major.

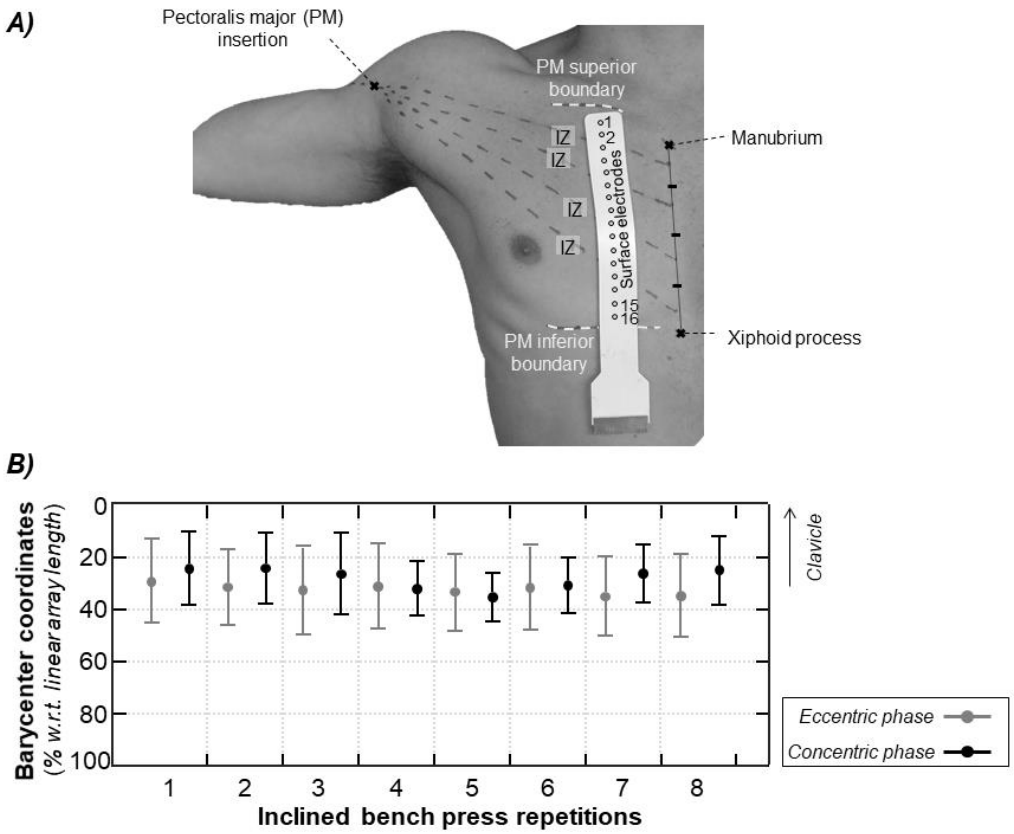


*Figure 7: **A**, shows the linear array of electrodes position. The innervation zone (IZ, gray rectangles) is marked for the four reference lines (dashed black lines). The limits of the pectoralis major muscle (PM) identified with the ultrasound are marked with dashed white lines. The adhesive vector of 16 electrodes was positioned parallel to the sternum, 1 cm below the upper limit of the PM and between the identified IZ and the sternum. **B**, mean and standard deviation of the normalized barycenter coordinates of PM activation with respect to linear array length. The barycenter coordinate is presented separately for each phase of the exercise along the 8 repetitions. Figure adapted from MANCEBO et al. (2019b) with permission.*

Inferences on regional variations in muscle activity detected from surface EMGs require, therefore, electrodes positioned at muscle regions covering different muscle fibers. In this regard, both linear arrays (DE SOUZA *et al.*, 2017; MANCEBO *et al.*, 2019b) and grids of electrodes (WATANABE *et al.*, 2012; VIEIRA *et al.*, 2017a; BORZELLI *et al.*, 2020) have been used by the literature. However, as displayed in Figure 8A, the information provided by raw EMGs acquired with a matrix may be somewhat redundant and difficult to interpret. A possible solution, when electrodes grids are applied, is to generate topographic maps (or EMG imaging) indicating the distribution

of electrical potentials (MERLETTI *et al.*, 2016). In that case, the EMGs intensity of each channel (calculated using ARV or RMS) is represented with a false color within a scale (Figure 8B) and thus valuable, physiological information can be easily extracted. In Figure 8B, it is possible to clearly identify the innervation zone (the central region in blue) and the location where the muscle activity is higher (distal region in red). When the EMG imaging is interpolated as shown in Figure 8C, such obtainable information become even easier to visually detect.

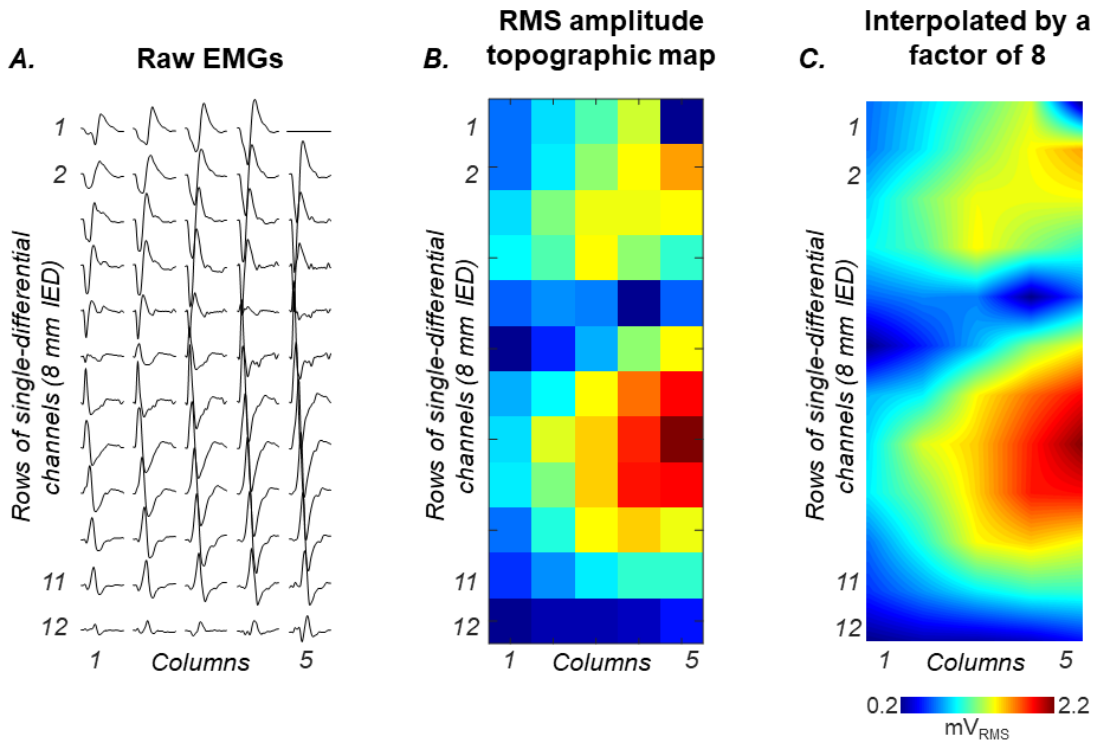


*Figure 8: **A**, shows an example of raw single-differential M-waves obtained with a 64 electrodes grid during a supramaximal neuromuscular stimulation protocol. **B**, displays the topographical map (or EMG imaging) obtained from the root mean square computed for each channel of the panel A. **C**, the topographical map is interpolated by a factor of 8 allowing to identify more easily physiological information, as the innervation zone location (central region in blue).*

The spatial distribution of these EMG amplitude topographic maps may be also assessed quantitatively. For instance, the region with higher muscle activity can be identified through a process known as EMGs segmentation (VIEIRA *et al.*, 2010). A segmentation commonly applied in literature is to consider the channels detecting EMGs

with an activity equal or higher than 70% of the maximum activity of the map (VIEIRA *et al.*, 2010; VIEIRA *et al.*, 2017a; MANCEBO *et al.*, 2019b). These channels are referred as *segmented channels* (or *active channels*) and, from those, it is possible to identify where the muscle activity is most strongly represented in the matrix (i.e., the longitudinal and transverse coordinates of *segmented channels*' centroid).

2.2.2 Electrical stimulation of the peripheral nervous system

When the electrical stimuli received by the motor units is evoked rather than voluntary, the factors of variability are decreased (MAFFIULETTI, 2010). The NMES is the technique that involves the application of electrical stimuli directly to the muscle nerve or to the muscle surface (HULTMAN and SJÖHOLM, 1983; MERLETTI *et al.*, 1992). The NMES has been extensively used for in vivo assessment of neuromuscular function (MAFFIULETTI, 2010). In this session, some concepts involving NMES will be discussed.

NMES techniques. The muscle contractions could be electrically elicited with the stimulation electrodes superficially positioned in the nerve trunk (i.e. nerve stimulation) or over the muscle belly (i.e. muscle stimulation). Regardless of the type of stimulation (nerve or muscle), when electrodes with different dimensions are used, the stimulation technique is called monopolar configuration. In that case, one electrode has smaller dimensions (known as "negative" or "cathode" electrode) and is located near to the nerve or to the motor point of the muscle. The other stimulation electrode has larger dimensions (known as "positive" or "anode" electrode) and is located on the opposite site of the active electrode. In this type of configuration, the electrical stimulation takes place in the proximity of the cathode electrode and therefore the current density is more localized.

Conversely, when two electrodes with similar dimensions are placed over the muscle, the stimulation technique is called bipolar configuration. The current distribution is confined in the area above to the stimulation electrodes in this case and therefore the current density is more uniform with respect the monopolar stimulation (MERLETTI *et al.*, 1992). Figure 9 illustrates the NMES techniques commonly used.

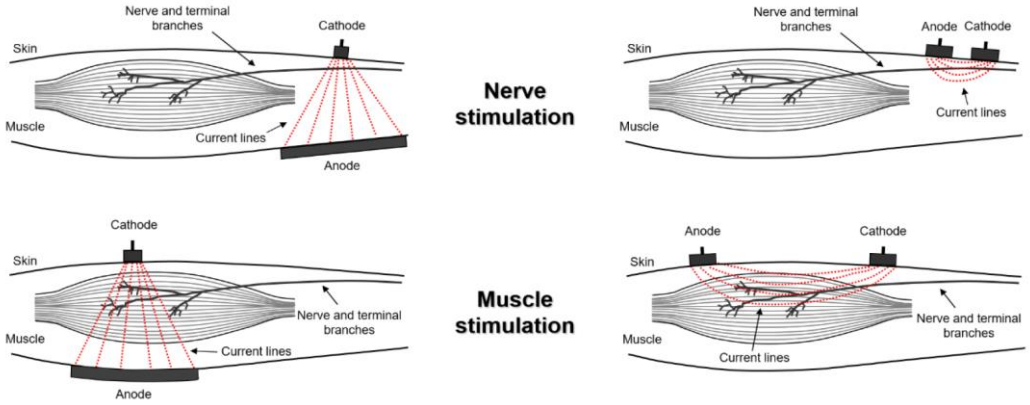


Figure 9: Neuromuscular electrical stimulation techniques.

M-waves. During the NMES, the surface electromyography detected over the muscle is a compound of motor unit action potentials. This sum of the potentials of concurrently activated motor units is called M-wave. A change in the M-wave size is therefore related to a change in the number of motor units activated (FARINA *et al.*, 2004b). Figure 10 shows an example of M-wave detected in the single-differential configuration by a pair of electrodes.

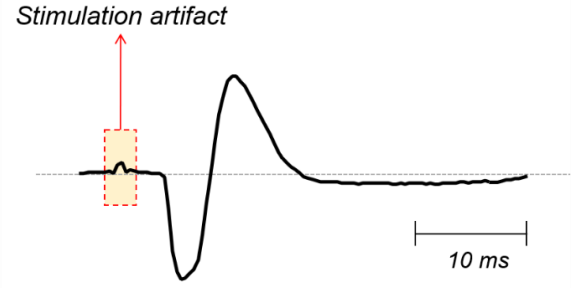


Figure 10: An example of M-wave detected with a pair of electrodes in bipolar mode. The yellow rectangle indicates the stimulation artifact.

During the stimuli is common that the current field extends to a volume including the detection volume of the surface electrodes (MANDRILE *et al.*, 2003). When this occurs, a stimulation artifact is recorded in the EMGs. The stimulation artifact is usually spike-shaped (Figure 10) and has influence the EMGs temporal and spectral characteristics since is constituted by a high-frequency component. There are available on the literature some hardware and software methods to remove or reduce the stimulation artifact. In this regard, a common and simple technique used is the offline blanking, where the samples of the artifact stimulation are digitally removed (MANDRILE *et al.*, 2003).

How could we identify the maximal level of NMES? Since the motor neurons have different recruitment thresholds, the stimulus intensity enhancement leads to the recruitment of new motor units. This progressive increment in the stimulus intensity produces therefore progressively greater amplitudes of M-waves (FARINA *et al.*, 2004b; KEENAN *et al.*, 2006). When no variations in the M-wave amplitude are observed even with the increase of stimulation intensity, the maximal M-wave response was generated in the skeletal muscle. This means that possibly all motor neurons innervated by the stimulated nerve are recruited. The literature typically uses the incremental stimulation technique to identify the maximal level of NMES (BOTTER *et al.*, 2009; PIITULAINEN *et al.*, 2011). The Figure 11 illustrates the identification of the maximal M-wave response using an incremental stimulation protocol.

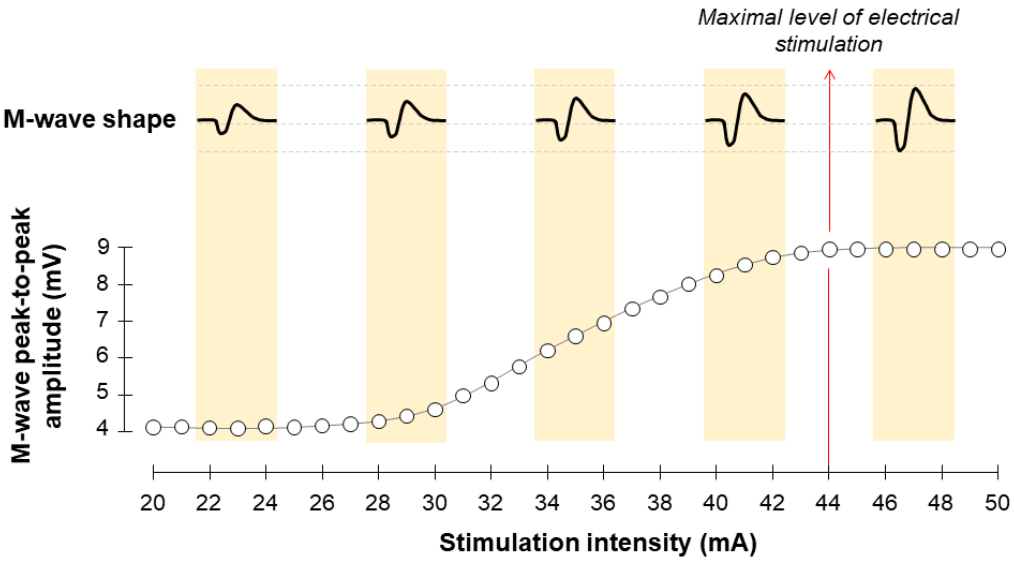


Figure 11: Example of how the maximal stimulation intensity is identified. The current intensity is gradually increased (bottom panel) until no clear increment of M-wave peak-to-peak value may be visually evident (top panel). This current intensity is, therefore, defined as the maximal (44 mA in this case).

Chapter 3: Exercise-induced muscle damage leads to local changes in biceps brachii excitation

3.1 Introduction

It is well-established that eccentric contractions may lead to EIMD, in particular when contraction intensity and duration are novel to the subject (CLARKSON *et al.*, 1992; HYLDAHL and HUBAL, 2014). Although EIMD can be assessed directly by histological analyses (NEWHAM *et al.*, 1983b; LAURITZEN *et al.*, 2009), changes in peak torque, muscle soreness, ultrasound (US) image echo intensity and T_2-MRI have been often used to evaluate the damage following novel, eccentric exercises (WARREN *et al.*, 1999; RADAELLI *et al.*, 2012; MATTA *et al.*, 2018). The indirect, non-invasive assessment of EIMD is motivated by documented observations of, for example, reduced capacity of generating maximal force, muscle soreness sensation, muscle swelling, decreased range of motion, an increase in B-mode ultrasound gray scale echo intensity of the muscle, and appearance of muscle-specific proteins in the blood, lasting up to several days after exercise (CLARKSON *et al.*, 1992; HYLDAHL and HUBAL, 2014). Notwithstanding the overt advantage of using indirect, non-invasive assessments of EIMD, attention has been typically focused on a single muscle site.

Imaging and electrophysiological evidence suggests, however, the EIMD responses may manifest unevenly within the muscle (HEDAYATPOUR *et al.*, 2008; PIITULAINEN *et al.*, 2009; MAEO *et al.*, 2017; MAEO *et al.*, 2018; MATTA *et al.*, 2019). For example, MAEO *et al.* (2017) reported greater variations in T2-MRI after EIMD proximo-centrally within quadriceps, suggesting the muscle distal site would be less susceptible to damage. Studies using multichannel surface electromyography have also reported site-dependent changes in the EMGs following EIMD during voluntary contractions (HEDAYATPOUR *et al.*, 2008; PIITULAINEN *et al.*, 2009). For instance, PIITULAINEN and colleagues (2009) observed a proximo-distal dependent decrease in the amplitude of biceps brachii EMGs after eccentric contractions. However, during voluntary contractions, other factors may contribute to the spatially localized activity following EIMD. First, the prolonged pain that accompanied eccentric exercise may induce a differential distribution of muscle activity (MADELEINE *et al.*, 2006; HEDAYATPOUR *et al.*, 2008). Second, spatially localized distribution of the amplitude of surface EMGs has been observed even during maximal voluntary contractions and in the absence of muscle damage (MIYAMOTO *et al.*, 2012). It seems therefore advisable to check for the population of motor units recruited when using surface EMGs to assess the local muscle adaptations resulting from EIMD. Otherwise, spatial changes in muscle activity following eccentric exercise may be not attributable to 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 ask whether EIMD leads to local changes in the amplitude of M waves detected along biceps brachii, from one to four days after eccentric exercise. We hypothesize 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 on. Otherwise, eccentric exercise is expected to elicit variations in M-waves' amplitude equally distributed over biceps brachii muscle. By assessing supramaximal M waves, we ensured most, if not all, biceps brachii motor units were elicited (BOTTER and MERLETTI, 2016), presumably suppressing effects other than those resulting from the induced muscle damage on the surface EMGs.

3.2 Methods

3.2.1 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. They were not engaged in systematic exercise within the preceding 12 months and were not taking any medication or nutritional supplements during the experimental period. After being informed about the experimental procedures and possible risks and before participating in the study, all subjects provided written informed consent. 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, CAAE number 20819219.7.0000.5257).

3.2.2 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^{o} 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 30º/s (CHAN *et al.*, 2012) and from 110º to 0º, with 0º 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.

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

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

Below follows a detailed description of the experimental procedures applied for the evaluation of muscle soreness, US imaging and for M-wave stimulation and detection. Each of these procedures were applied separately for each experimental session.

3.2.3 Muscle soreness and ultrasound imaging

Subjective perceived muscle soreness of the right elbow flexors was assessed using a visual analogue scale, ranging from 0 (no pain) to 10 cm (worst possible pain) (CHAN *et al.*, 2012; MATTA *et al.*, 2019). Subjects were asked to rate the level of perceived soreness immediately after having their elbow passively extended from 110° to 0° (MATTA *et al.*, 2018).

Ultrasound B-mode images (GE Logic, USA; 8 MHz excitation 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 US probe was positioned. Three *reference lines* were drawn on the skin, perpendicularly to the muscle longitudinal axis (Figure 12A). 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 from *proximal* and *distal reference lines,* each at a time, with the probe aligned parallel to them and with the muscle at rest. A water-based gel was used for acoustic coupling and the US acquisition configuration was kept constant during all sessions.

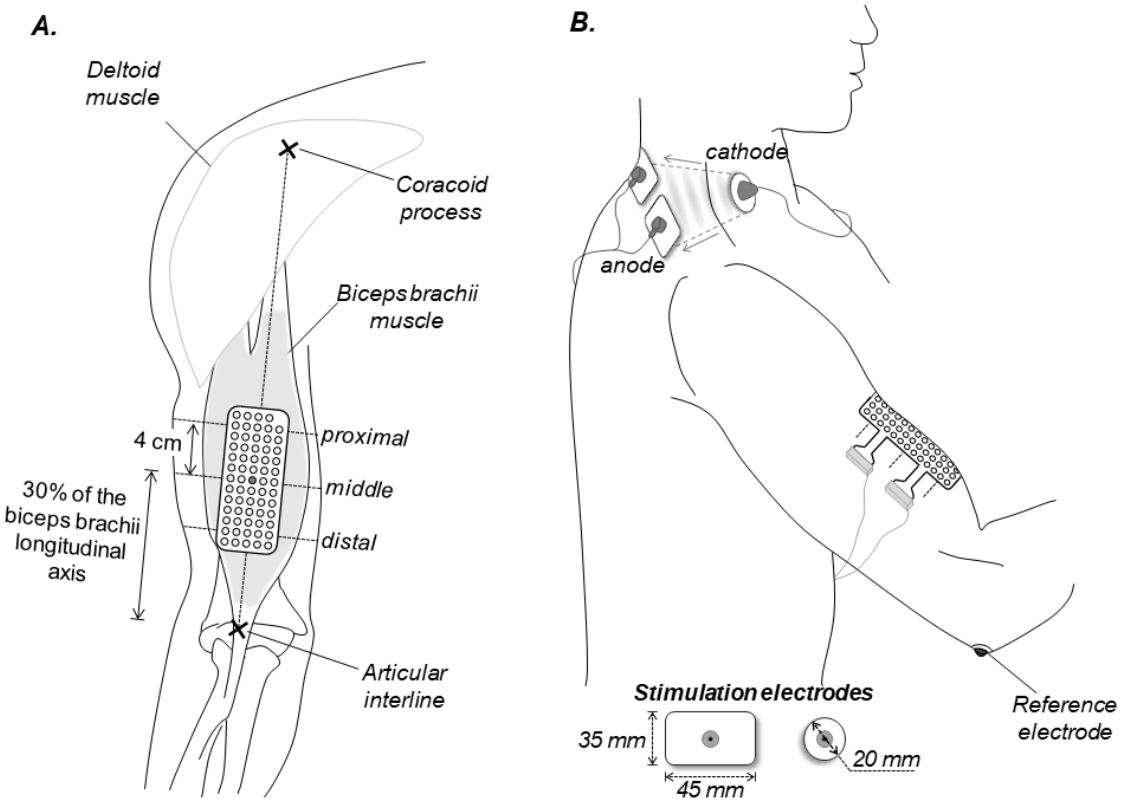


Figure 12: A, shows a schematic representation of where grid of 64 surface electrodes was positioning in the biceps brachii muscle. The ultrasound images were obtained from the proximal and distal reference lines. B, illustrates the position of electrodes used to stimulate the musculocutaneous nerve.

3.2.4 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 nearby 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 of short-circuited rectangular anode electrodes (size 35 x 45 mm each) were positioned on the opposite side (Figure 12B). 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 (Figure 1A; WATANABE *et al.*, 2015). In this way, the 2nd, 7th and 12th rows of electrodes were respectively aligned with the *proximal*, *middle* and *distal reference lines* (Figure 12A). 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 abrasive paste.

3.2.5 Stimulation protocol and surface EMGs recordings

Ten biphasic, rectangular current pulses (duration of 100 μs per phase; frequency of 1 pulse per second) were applied to evoke supramaximal M waves from biceps brachii (Rehastim Science Mode, Hasomed, Germany). The stimulation intensity was set at 20% over the maximal current level, identified with a staircase current profile (PIITULAINEN *et al.*, 2011). Specifically, the current intensity was gradually increased (steps of 2 mA) until no clear increment of the M-wave peak-to-peak value could be visually appreciated; this level was defined as the maximal stimulation intensity (PIITULAINEN *et al.*, 2011). For each current intensity, two biphasic, rectangular current pulses (duration of 100 μs per phase; frequency of 1 pulse per second) were applied.

Monopolar surface EMGs were amplified (200 gain, 10-500 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 EMGs.

3.2.6 Ultrasound images and EMGs processing

The echo intensity was considered for the texture analysis of 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 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, proximal and distal, was considered for further analyses.

The spatial distribution of M-waves peak-to-peak value 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 across all participants (total of 97 out of 3200 signals; 10 subjects x 64 electrodes x 5 sessions) and were replaced with the linear interpolation of neighbor 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 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 13A). Finally, the innervation zone was identified visually for each column using a Matlab script, where phase opposition between consecutive action potentials, followed by propagation, could be well appreciated (Figure 13A; MANCEBO *et al.*, 2019a). This procedure provides half a channel resolution for IZ identification (GALLINA *et al.*, 2013). Action potential propagation and phase inversion were clearly identified for all cases and the median IZ position across columns was considered to define the IZ position for each experimental session.

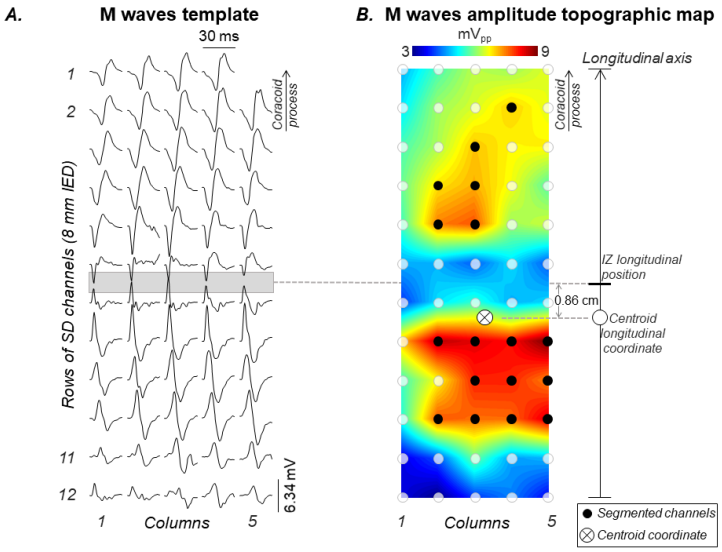


Figure 13: Raw, single-differential M waves collected at baseline are show in panel A. The innervation zone (IZ; shaded, grey rectangles) is clearly seen in the region where there is phase opposition between consecutive action potentials, followed by propagation. B, shows the topographic map obtained from peak-to-peak value of M waves displayed in A. Black circles denote electrodes for which the M waves peak-to-peak value 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.

M-wave peak-to-peak value was computed for each of the 59 single-differential channels, providing topographic maps for the biceps brachii muscle (Figure 13B). 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 M waves with peak-to-peak value 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 13B):

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

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

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

where N is the total number of *segmented channels* for each subject, A is the sum of all peak-to-peak value values of *segmented channels* in the map and a_n is the peak-to-peak value of *segmented channel* with coordinates x_n and y_n. The centroid longitudinal coordinate (Y), indicating where the EMGs amplitude was most strongly represented along the muscle, was retained for analysis (Figure 13B).

3.2.7 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, with the exception of eccentric exercise. The Intraclass Correlation Coefficient (ICC) was considered to assess between-day consistency of (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 experimental session). ICC values were 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).

Based on the effect size (0.55) estimated from our data, high-density EMGs measures collected from 10 subjects during five experimental sessions ensured high (91.98%) statistical power (post-hoc power analysis; FAUL *et al.*, 2007). After ensuring data normality (Shapiro-Wilk normality test; $P>0.06$) and homoscedasticity (Bartlett's test; $P>0.08$ for all cases), parametric analysis was considered for inferential statistics. The one-way repeated measures analysis of variance (ANOVA) was applied to compare 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 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 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%.

3.3 Results

3.3.1 Reliability analysis

For all variables used to examine the difference between days, the average ICC was always higher than 0.974, indicating excellent between-day reliability. Specifically, the average ICC values (95% confidence interval) were 0.976 (0.880-0.990) for MVC peak torque, 0.989 (0.944-0.998) for echo intensity at proximal region, 0.974 (0.869-0.999) for echo intensity at distal region, and 0.995 (0.973-0.998) for average amplitude value of the grid.

3.3.2 Indirect markers of biceps brachii EIMD

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 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 14A; $P<0.001$). Conversely, the perceived muscle soreness significantly increased at 24, 48, 72 and 96 h after eccentric exercise ($P<0.004$), with still significant difference at 48 and 72 h compared with 24 h (Figure 14B; $P<0.004$).

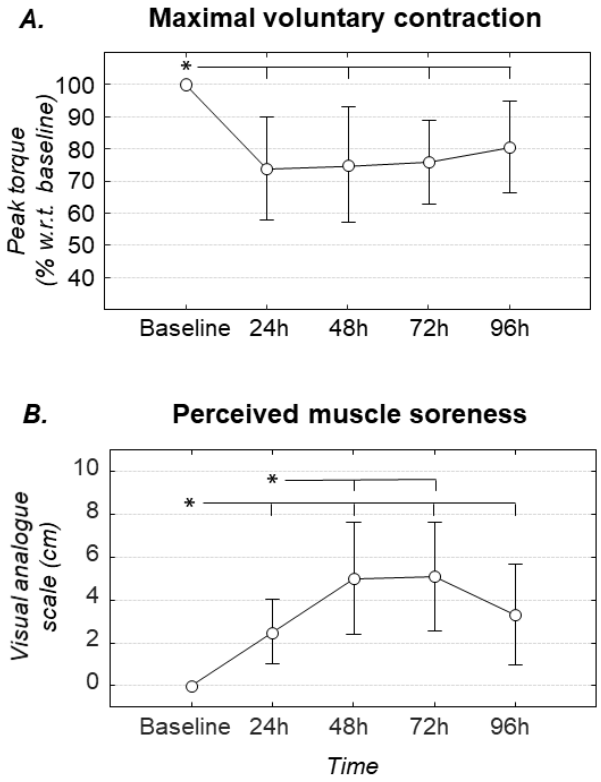


Figure 14: Mean (circles) and standard deviation (whiskers; N = 10 subjects) are shown for the maximal voluntary contraction peak torque (A) and perceived muscle soreness (B), separately for each experimental session. Asterisk denotes statistical significance (P<0.05).

3.3.3 Ultrasound image echo intensity of biceps brachii EIMD

As shown for a representative participant in Figure 15A, the EIMD altered the US grayscale image intensity for both proximal and distal regions. Close inspection of Figure 15A suggests the change in grayscale intensity was most evident from 48 to 96 h after EIMD. Also, differences in echo intensity between days appear to span a large cross-sectional area of biceps brachii and thus were well included in the region of interest (cf. rectangles in Figure 15A). 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, 72 and 96 h with respect to baseline for both regions (Figure 15B; Bonferroni's post-hoc; P<0.001 for all cases). No significant differences were observed among regions at any time (Figure 15B; ANOVA interaction effect; P=0.136).

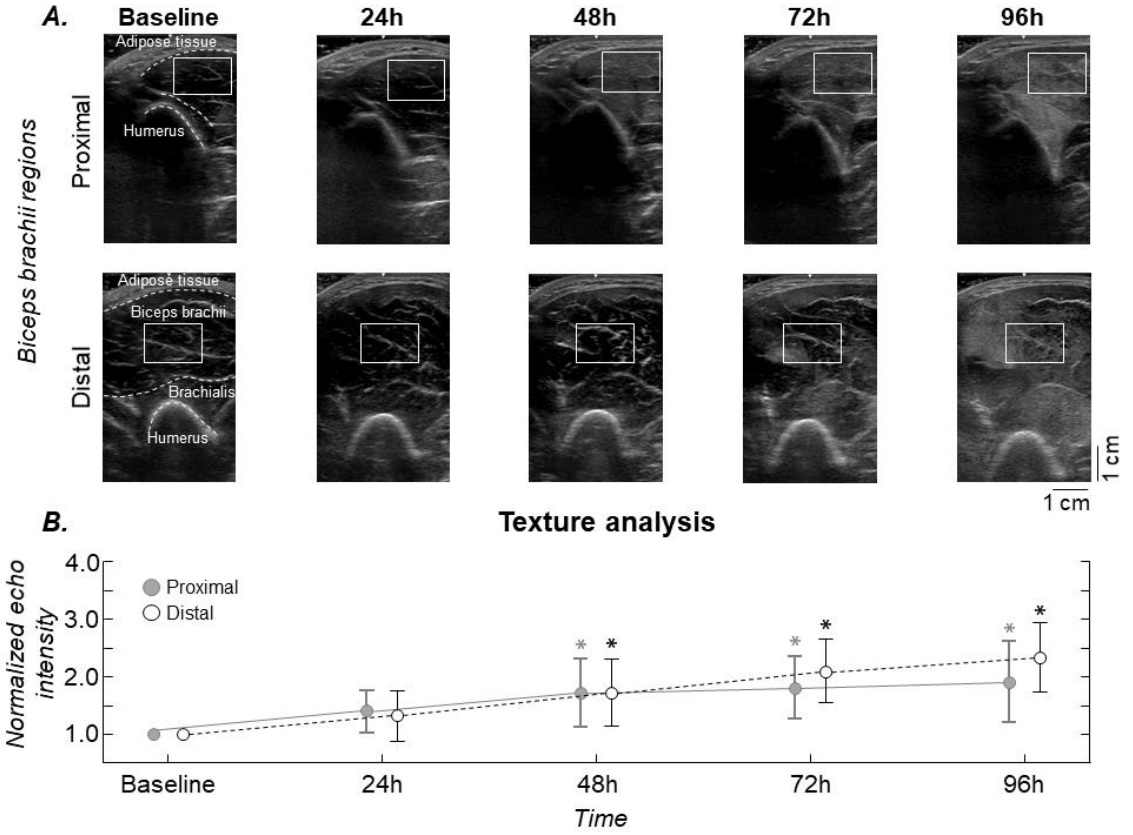


Figure 15: A, shows ultrasound images collected from the biceps brachii proximal (top) and distal (bottom) regions of a single participant. The rectangles with white lines illustrate the region of interest used to calculate the echo intensity. B, shows the mean (circles) and standard deviation (whiskers) for the echo intensity, separately for each region and experimental session. Asterisk denotes statistical significance (P<0.05) with respect to baseline values.

3.3.4 Electrophysiological topography of biceps brachii EIMD

The effect of EIMD on supramaximal M waves elicited from the biceps brachii muscle could be well appreciated from results of a representative participant. As shown in the bottom panel of Figure 16, 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*, mainly in the proximal region, decreased (cf. black circles on the top panel of Figure 16) and the longitudinal coordinate

of the centroid shifted from IZ towards the distal region of biceps brachii at 24, 48 and 72 h after EIMD (bottom panel of Figure 16).

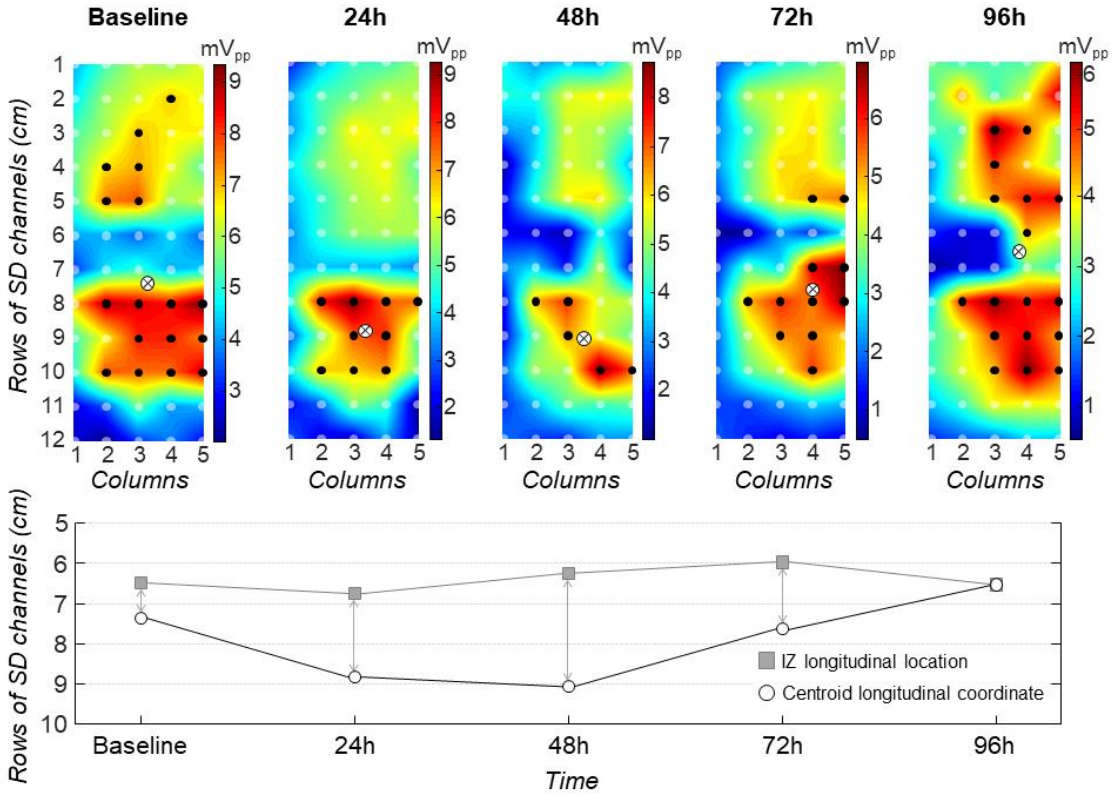


Figure 16: The top panel shows the peak-to-peak value maps of M waves for a representative participant, separately for each experimental session. Black circles indicate segmented channels and the crossed, white circles denote the centroid location of these channels. The centroid longitudinal coordinates with respect to the innervation zone (IZ) longitudinal locations are displayed on the bottom panel, separately for each experimental session.

Group data revealed the EIMD affected significantly the spatial distribution of M-wave peak-to-peak value though not the IZ longitudinal position. No significant change in IZ location was observed across time for all subjects and sessions (Figure 17A; 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 17B; Bonferroni's post-hoc; P<0.032) and the centroid longitudinal coordinate shifted towards the distal region at 24,

48 and 72 h (Figure 17C; Bonferroni's post-hoc; P<0.032 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 17B and 17C). 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.

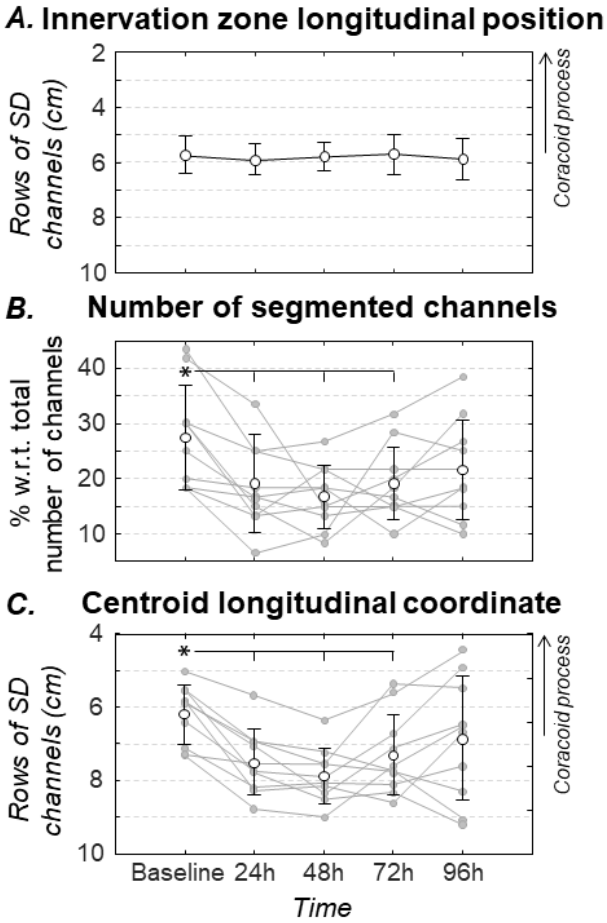


Figure 17: Mean (circles) and standard deviation (whiskers; N = 10 subjects) are shown for the innervation zone longitudinal position (A), number of segmented channels (B) and centroid longitudinal coordinate (C) within the grid, separately for each experimental session. Grey circles and lines in the panels B and C indicate individual results. Asterisk denotes statistical significance (P<0.05).

3.4 Discussion

In this study we hypothesized any local damage of biceps brachii would lead to local changes in the amplitude of supramaximal M waves. Our results revealed the

amplitude distribution of M waves changed consistently in the proximal biceps brachii region up to four days after exercise. Changes in M wave amplitude were well in agreement with changes in muscle soreness and force though not with US echo intensity. 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 seem 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.

3.4.1 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 (e.g. downhill running (MAEO *et al.*, 2017); maximal vertical jumps (TWIST and ESTON, 2005); resistance training exercise (KANDA *et al.*, 2013)), 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, 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; DAMAS *et al.*, 2016). Similarly, given soreness has been documented to last up to seven days after eccentric exercise (CLARKSON *et al.*, 1992; 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 14). These results are well in agreement with the decrease of MVC force and the increased muscle pain (MATTA *et al.*, 2019; CHAPMAN *et al.*, 2008) reported in the literature. Based on these considerations, it seems therefore the exercise protocol we applied here effectively resulted in biceps brachii EIMD.

Considering 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 (NISHIHARA *et al.*, 2013), we observed only one IZ. Corroborating previous studies (PIITULAINEN *et al.*, 2009), the IZ position did not change between days for all subjects (Figure 17A) 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 presumably 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 leading to maximal M waves (cf. Methods). Presumably, 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.*, 2017b), 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 16). Finally, even though subcutaneous thickness and muscle architecture have been shown to affect EMG amplitude (CESCON *et al.*, 2008; VIEIRA *et al.*, 2017a), it is unlikely that regional, anatomical changes would take place between

consecutive days. Collectively, these arguments seem to suggest the regional changes in M-wave amplitude reported here primarily arise from EIMD.

3.4.2 EIMD leads to regional changes in muscle excitation

Here we raise the question as per whether EIMD could affect excitation of biceps brachii locally. Different from previous studies focused on regional changes in EMG amplitude during voluntary contractions after EIMD (PIITULAINEN *et al.*, 2009; HEDAYATPOUR *et al.*, 2008), 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 largest amplitude were detected from a smaller, distal biceps brachii region for all subjects (Figures 16 and 17). 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 (ALLEN, 2004). 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 site in damaged fibers (MCNEIL and KHAKEE, 1992; PIITULAINEN *et al.*, 2010). Local inhibition of propagation would result in a smaller number of single fiber action potentials elicited by stimulation while local

reduction of propagation would result in a greater temporal spread of single fiber action potentials. While both factors would be expected to decrease the peak-to-peak value of compound surface potentials (FARINA *et al.*, 2004a), inhibition though not reduced propagation velocity would most likely explain the decrease in muscle maximal force after EIMD (Figure 14A; see also PIITULAINEN *et al.*, 2008; 2010). On the other hand, maximal force was measured during voluntary contractions and therefore not all fibers may have been recruited during MVCs after EIMD. Although it is currently unviable to ascertain the occurrence of local, structural damage of human muscles in vivo and its consequences on muscle excitation, our results suggest EIMD affects muscle excitation locally.

Interestingly, our results revealed that the eccentric protocol used in this study consistently affected the proximal region of biceps brachii. As shown in Figure 16, a significant decrease in the M-waves' amplitude was observed at the proximal muscle site, suggesting that this region would be more susceptible to EIMD or to its effect on muscle excitation. Architectural differences along the muscle could possibly explain the presumable, greater vulnerability of the biceps brachii proximal region to EIMD in eccentric contractions. The distal tendon of biceps brachii flattens into an internal aponeurosis, located in the centerline of the muscle and extending over the distal third of the muscle longitudinal axis (~34% of the muscle length on average; ASAWAKA *et al.*, 2002). The internal aponeurosis would likely impact on the amount of 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 FRIDÉN, 1993),

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 15). The increased grayscale intensity of US images following EIMD is possibly due to edema, intracellular material leakage and production of connective tissue (RADAELLI *et al.*, 2012; MATTA *et al.*, 2018). The suggested association between echo intensity and inflammatory responses following EIMD (RADAELLI *et al.*, 2012; MATTA *et al.*, 2018) would explain why increased echo intensity persisted some days after eccentric exercise, with highest intensities occurring at ~3-4 days from baseline (Figure 4B; RADAELLI *et al.*, 2012; MATTA *et al.*, 2018; MATTA *et al.*, 2019). Moreover, the lack of proximo-distal differences in echo intensity of US images confining exclusively the biceps brachii muscle (Figure 15) 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.

3.4.3 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. Even though these data could have revealed immediate changes in EMG amplitude following exercise, possibly revealing 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 localized change in the amplitude of surface EMGs after eccentric exercise (Figures 16-17).

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 14), 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 17B and 17C). This result indicates 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).

Chapter 4: General conclusion

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

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Appendix: Publications resulted from this Thesis.

Changes in supramaximal M wave induced by eccentric exercise are site-dependent in the biceps brachii muscle.

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INTRODUCTION:
The accomplishment of eccentric exercises with unaccustomed intensity induces muscle damage. Regardless of the method used to quantify this exercise-induced muscle damage (EIMD), attention is typically focused on a single muscle site [1]. Previous evidence suggests however the EIMD may manifest in different muscle regions [2]. Here we combine nerve stimulation with high-density surface EMG to investigate the electrophysiological topography of biceps brachii EIMD.

METHODS:
Ten male subjects were submitted to following measures during five consecutive days (before and four days after EIMD): (i) perceived soreness of right elbow flexors during passive stretching; (ii) monopolar surface EMGs from biceps brachii with a 64 grid of electrodes while 10 supramaximal current pulses were applied transcutaneously to the musculocutaneous nerve; (iii) two isometric, elbow flexion maximal voluntary contractions (MVC) on a dynamometer. After measurements have been taken at the end of the first day, participants performed 3x10 eccentric, maximal elbow flexions. Single-differential EMGs were computed and for each of the resulting 59 signals, M waves were averaged and their peak-to-peak value was computed. Innervation zone (IZ) was identified visually and, separately for proximal and distal regions from the IZ, the number of active channels (EMGs with greatest amplitude), their relative longitudinal position and their averaged EMG amplitude [3] were assessed to characterize EIMD induced changes on M waves. The one-way repeated measures ANOVA and Tukey post-hoc tests were applied to compare the variables across days.

RESULTS:
The MVC significantly decreased at 24, 48, 72 and 96 h after EIMD ($P<0.001$), while the perceived muscle soreness increased progressively from 24 to 96 h after EIMD ($P<0.001$). The number of proximal active channels significantly decreased and the longitudinal coordinate of the proximal centroid shifted towards the IZ at 24, 48 and 72 h after EIMD ($P<0.007$ in both cases). No time effect was observed for the distal number of active channels, the distal centroid location and the average amplitude of EMGs ($P>0.146$ for all cases).

CONCLUSION:
EIMD consistently changed supramaximal M waves elicited from the biceps brachii muscle. With respect to baseline, M waves with largest amplitude were detected from a smaller biceps brachii region up to 72 h from EIMD, possibly because EIMD took place predominantly proximally. While this possibility urges further testing, our results suggest muscle tissue damage manifests locally within biceps brachii, within three days from EIMD.

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Eccentric exercise-induced muscle damage leads to local changes in the amplitude of supramaximal M waves detected along biceps brachii muscle.

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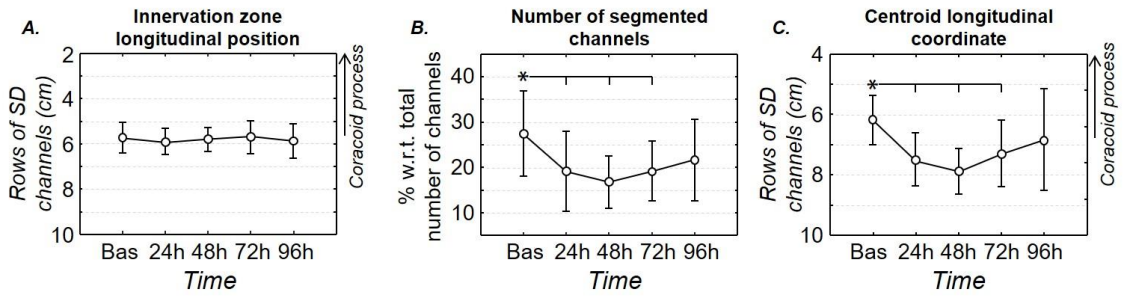
BACKGROUND AND AIM: Previous evidence suggests site-dependent changes in the electromyograms (EMGs) may be triggered following eccentric exercise-induced muscle damage (EIMD) [1,2]. However, other factors not attributable to EIMD may contribute to this spatially localized activity, as the prolonged pain that accompanied eccentric exercise [3]. It seems therefore advisable to make sure the population of motor units recruited is the same when using surface EMGs to assess the local muscle adaptations resulting from EIMD. In this study, we combined supramaximal electrical stimulation of the musculocutaneous nerve with high-density surface electromyography (HD-sEMG) to ask whether EIMD leads to local changes in the amplitude of M waves detected along biceps brachii. **METHODS:** Ten healthy, male subjects were submitted to following measures conducted immediately before and four consecutive days after 3x10 eccentric elbow flexions: (i) perceived soreness of right elbow flexors during passive stretching; (ii) acquisition of ultrasound images proximally and distally from biceps; (iii) recording of monopolar HD-sEMG (64 electrodes) from biceps while 10 supramaximal pulses were applied transcutaneously to the musculocutaneous nerve; (iv) two isometric, elbow flexion maximal voluntary contractions (MVC) on a dynamometer. For each of 59 single-differential signals, M waves were averaged and their peak-to-peak value was computed. The innervation zone (IZ) longitudinal location, the number of electrodes detecting the largest M waves (segmented channels; [4]) and their relative longitudinal position were assessed to characterize EIMD induced changes on M waves. **RESULTS:** The perceived muscle soreness increased with respect to baseline at 24, 48, 72 and 96 h after EIMD ($P<0.004$), while the MVC peak torque significantly decreased at 24, 48, 72 and 96 h after EIMD ($P<0.001$). The echo intensity of ultrasound images increased from 48 to 96 h with respect to baseline for both proximal and distal regions ($P<0.001$), while no differences were observed among regions at any time ($P=0.136$). No time effect was observed for the IZ location (panel A; $P=0.283$). The number of segmented channels significantly decreased (panel B) and the longitudinal coordinate of the centroid shifted towards the distal region of the muscle at 24, 48 and 72 h after EIMD (panel C; $P<0.032$ in both cases). **CONCLUSIONS:** The amplitude distribution of M waves changed consistently in the proximal biceps brachii region up to four days after EIMD. Our results therefore suggest a local effect of EIMD on biceps brachii muscle excitation and demonstrate the potential of HDs-EMG to assess both spatial and temporal effects of EIMD on muscle function. **REFERENCES:** [1] Hedayatpour *et al.* 2008; Med Sci Sports Exerc 40(2):326–34. [2] Piitulainen *et al.* 2009; Muscle Nerve 40(4):617–25. [3] Madeleine *et al.* 2006; Clinical Neurophysiology 117(11):2436–45. [4] Vieira *et al.*, 2010; J Biomech 43(11):2149–58.



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Innervation zone locations distribute medially within the pectoralis major muscle during bench press exercise

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ABSTRACT

Changes in innervation zone (IZ) position may affect the amplitude of surface electromyograms (EMGs). If not accounted for, these changes may lead to equivocal interpretation on the degree of muscle activity from EMG amplitude. In this study we ask how much the IZ position changes within different regions of the pectoralis major (PM) during the bench press exercise. If expressive, changes in IZ position may explain the conflictual results reported on PM activation during bench press. Single-differential surface EMGs were collected from 15 regions along the PM cranial, centro-cranial, centro-caudal and caudal fibres, while 11 healthy participants gently, isometrically contracted their muscle. IZs were identified visually, from EMGs collected with the glenohumeral joint at extreme bench press positions; 20° and 110° of abduction in the horizontal plane. Except for 3 out of 88 acquisitions (4 detection sites × 2 glenohumeral angles × 11 participants), for which no phase opposition and action potential propagation were observed, IZs could be well identified. Group results revealed the IZ moved medially from 110° to 20° of glenohumeral joint abduction in the horizontal plane, regardless of the PM region from where EMGs were detected ($P < 0.01$). IZs were confined medially within PM, from ~20% to ~40% of the muscle-tendon unit length, and their position changed up to 13.3%. These results suggest that changes in the amplitude of EMGs detected mainly medially from PM may be not associated with changes in the degree of PM activity during bench press.

1. Introduction

Surface electromyography has been extensively used to investigate the pattern of pectoralis major (PM) activation in resistance training studies. Attention is often focused on the effect of exercise variants on PM activation, such as trunk inclination, hand grip distance on the barbell and different training methods (Barnett et al., 1995; Glass and Armstrong, 1997; Gomo and Van Den Tillaar, 2016; Keogh et al., 1999; Lauver et al., 2016; Lehman, 2005; Mookerjee and Ratamess, 1999; Sakamoto and Sinclair, 2012; Snyder and Fry, 2012; Trebs et al., 2010). Notwithstanding such well-conducted research, contradictory findings on the pattern of PM activation have been reported. For example, while Glass and Armstrong (1997) did not observe a significant effect of bench press inclination on the degree of activation of the PM clavicular region, Trebs et al. (2010) observed greater activation of this region for more inclined bench press positions. Whether such discrepancies indicate different patterns of PM activation between individuals or are possibly attributable to a broad spectrum of well-described limitations in surface electromyography remains an open issue.

Inferences on the pattern of PM activation are usually drawn from variations in the amplitude of surface electromyograms (EMGs). There is however a number of factors limiting the interpretation of changes in EMG amplitude in terms of variations in the degree and timing of muscle activation (Farina, 2006; Vigotsky et al., 2018). Muscle fibre orientation, thickness of subcutaneous tissues and the proximity of electrodes to tendon regions and to the innervation zone (IZ) location are some examples of non-physiological sources affecting the amplitude of EMGs (Farina et al., 2004). In particular, studies using arrays of electrodes have consistently reported spurious decreases in the amplitude of bipolar EMGs when detected nearby the muscle IZ (Nishihara

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Exercise-induced muscle damage leads to local changes in biceps brachii excitation.

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Abstract:	Purpose: Investigate whether exercise-induced muscle damage (EIMD) leads to local changes in the amplitude of supramaximal M waves detected along biceps brachii, from one to four days after eccentric exercise. Methods: Ten healthy male subjects were submitted to the following measures conducted immediately before and four consecutive days after 3x10 eccentric elbow flexions: evaluation of muscle soreness; acquisition of ultrasound images proximally and distally from biceps; recording of surface EMGs from biceps with a 64-electrodes grid while 10 supramaximal pulses were applied to the musculocutaneous nerve; two isometric, maximal voluntary elbow flexion contractions (MVCs). The innervation zone (IZ) longitudinal location, the number of electrodes detecting the largest M waves (segmented channels) and the centroid longitudinal coordinate of these electrodes were assessed in order to characterize the spatial distribution of M-waves amplitude. Results: The MVC torque decreased at 24, 48, 72 and 96 h after EIMD ($P<0.001$), while the perceived muscle soreness increased at 24, 48, 72 and 96 h after EIMD ($P<0.004$). The echo intensity of ultrasound images increased at 48, 72 and 96 h with respect to baseline for both regions ($P<0.001$) while no differences were observed among regions at any time ($P=0.136$). No time effect was observed for IZ location ($P=0.283$). The number of segmented channels significantly decreased ($P<0.032$) and	