UNIVERSITY OF SÃO PAULO
INSTITUTE OF BIOSCIENCES

ERIC CITO BECMAN

**Effects of increased buffering capacity on electromyogram's
power spectrum density behaviour during muscle fatigue**

**Efeitos do aumento da capacidade tamponante sobre o
comportamento do espectro de potências do eletromiograma
durante a fadiga muscular**

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Thesis submitted to the Institute of Biosciences,
University of São Paulo, in partial fulfilment of the
requirements for the degree of Master of Science

Research field: Physiology

Advisor: José Guilherme de Souza Chaui Mattos
Berlinck

Co-advisor: Bruno Gualano

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APPROVED:

Examination board

Committee member name: _____, PhD

Institution: _____

Signature: _____

Committee member name: _____, PhD

Institution: _____

Signature: _____

Committee member name: _____, PhD

Institution: _____

Signature: _____

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ABSTRACT

Becman, E. C. (2019). *Effects of increased buffering capacity on electromyogram's power spectrum density behaviour during muscle fatigue* (Master of Science thesis). Institute of Biosciences, University of São Paulo, São Paulo.

Surface electromyography (EMG) is a non-invasive technique to measure on-going changes in the electrical potentials of a muscle of interest. The EMG record is known to display alterations when assessed throughout sustained contractions. For instance, a frequency shift and compression of the signal's power spectrum density towards lower-frequencies. It is believed that a reduction of muscle fibre conduction velocity explains at least partially such dynamics. Additionally, the muscle acidosis observed at fatiguing contractions of some exercises seems to alter both conduction velocity and frequency properties. Within this context, the main goal of the present thesis was to explore the effects of muscle buffering capacity manipulation on the EMG power spectrum. More specifically, the mean power frequency (MNF). For manipulating buffering capacity, chronic β -alanine supplementation was used. The randomised and placebo-controlled study measured MNF throughout isometric contractions at submaximal and acidotic intensity (50% of maximal voluntary contraction force) before and after a β -alanine or placebo supplementation period of 28 days. The β -alanine administration was found to increase muscle endurance, expected result according to the literature. Nonetheless, no visible effect was detected for MNF fatigue behaviour. Under theoretical assumptions, the fatigue behaviour of MNF should change when differences at muscle endurance are found. Thus, the lack of a statistically significant result was discussed in the face of the multifaceted nature of myoelectric signals, composed by influences of several biological and non-biological elements. Secondly, investigation of EMG signal complexity via a1ApEn is also presented in the body of the thesis, which makes contextual sense, as it had a crucial contribution on the development of methodological aspects of the protocol employed in the main investigation and the overall Master's project. Additionally, EMG signal complexity is an underexplored topic and with little information known. Hence, a1ApEn provides potential to assess different EMG information not yet provided by better-documented tools, such as MNF.

Keywords: Electromyography. Muscle fatigue. Buffering capacity. Carnosine. Power spectrum density.

RESUMO

Becman, E. C. (2019). *Efeitos do aumento da capacidade tamponante sobre o comportamento do espectro de potências do eletromiograma durante a fadiga muscular* (Dissertação de mestrado). Instituto de Biociências, Universidade de São Paulo, São Paulo.

Eletromiografia de superfície (EMG) se trata de uma técnica não invasiva para medir mudanças de potencial elétrico de membrana em um dado músculo. O registro de EMG é conhecido por apresentar alterações ao longo de contrações até a fadiga. Por exemplo, se observa uma compressão das frequências componentes do sinal em direção a bandas de menor frequência. Acredita-se que tal comportamento esteja relacionado a uma redução de velocidade de propagação de impulsos elétricos nas fibras musculares. A acidose muscular vista durante a fadiga em certos exercícios também parece estar correlacionada com ambos aspectos eletrofisiológicos. Nesse contexto, essa dissertação se propõe explorar os efeitos de alterações na capacidade tamponante muscular sobre o espectrograma do registro de EMG. Mais precisamente, sobre a frequência de potência média (MNF) do mesmo. A manipulação da capacidade tamponante se deu pela administração de suplementação nutricional de β -alanina. O estudo, aleatorizado e controlado por grupo placebo, avaliou o comportamento de MNF durante contrações isométricas antes e após um período de suplementação de 28 dias. A intervenção com β -alanina resultou em um aumento da resistência muscular, resultado esperado de acordo com a literatura. Entretanto, nenhuma diferença significativa foi observada em MNF. Teoricamente, o comportamento de MNF durante a fadiga deve carregar mudanças quando o tempo até a falha muscular é aumentado. Assim sendo, foi discutido que a ausência de significância poderia ser consequência do aspecto multifatorial do sinal eletromiográfico, que incorpora influências de numerosos fatores biológicos a não-biológicos. Secundariamente, o estudo da complexidade de sinal do EMG via a1ApEn também foi apresentado, devido a sua relação contextual com o cerne dessa dissertação. Tal estudo foi fundamental para o desenvolvimento de aspectos metodológicos utilizados ao longo do projeto de mestrado. Adicionalmente, o tópico é pouco explorado pela literatura e, por tanto, com potencial de prover informações diferentes daquelas já fornecidas por ferramentas mais famosas, como a MNF.

Palavras-chave: Eletromiografia. Fadiga muscular. Capacidade tamponante. Carnosina. Espectro de potências.

LIST OF FIGURES

Figure 2.1. Study protocol timeline.....	38
Figure 2.2. Dynamometer system developed for the current investigation.....	39
Figure 2.3. Timeline of exercises within a single session.	40
Figure 2.4. MNF _{initial} by group, session and muscle.....	47
Figure 2.5. MNF _{final} by group, session and muscle.	48
Figure 2.6. MNF _{ratio} by group, session and muscle.	49
Figure 2.7. MNF _{slope} by group, session and muscle.	50
Figure 2.8. Average MNF time-series by group, session and muscle with time relative to T _{lim}	51
Figure 2.9. Average MNF time-series by group, session and muscle.	52
Figure 3.1. Series of EMG variables for all individual records.....	71
Figure 3.2. Average curves of the EMG variables during the contractions.	72
Figure 3.3. a1ApEn intercept values.	73
Figure 3.4. a1ApEn slope values.	74
Figure 3.5. MNF intercept values.....	75
Figure 3.6. MNF slope values.	76
Figure 3.7. RMS intercept values.	77
Figure 3.8. RMS slope values.....	78
Figure 3.9. Pearson correlation coefficient of a1ApEn versus MNF individual curves.....	79
Figure 3.10. Pearson correlation coefficient of a1ApEn versus RMS individual curves.	80
Figure 3.11. Pearson correlation coefficient of MNF versus RMS individual curves.	81
Figure 4.1. a1ApEn _{initial} by group, session and muscle.	90
Figure 4.2. a1ApEn _{final} by group, session and muscle.....	90
Figure 4.3. a1ApEn _{ratio} by group, session and muscle.....	91
Figure 4.4. a1ApEn _{slope} by group, session and muscle.....	91

LIST OF TABLES

Table 2.1. Anthropometric variables by group and session.	45
Table 2.2. Beta-alanine dietary intake by group and session.	45
Table 2.3. Maximal force by group, session and muscle.	46
Table 2.4. Time to fatigue by group, session and muscle.	46
Table 2.5. MNF _{ratio} by group and muscle.	49

LIST OF ACRONYMS

a1ApEn:	Area 1 of Approximate Entropy
AIC:	Akaike information criterion
ANOVA:	Analysis of variance
ApEn:	Approximate Entropy
BI:	Biceps brachii
BMI:	Body mass index
CNS:	Central nervous system
DE:	Lateral deltoid
EMG:	Electromyography
FFT:	Fast Fourier transform
FM:	Familiarisation
HCD:	Histidine-containing dipeptide
LMM:	Linear mixed model
MDF:	Median power frequency
MF:	Muscle fibre
MFAP:	Muscle fibre action potential
MFCV:	Muscle fibre conduction velocity
MNF:	Mean power frequency
MU:	Motor unit
MUAP:	Motor unit action potential
MVC:	Maximum voluntary contraction
PLA:	Placebo
POST:	Post-supplementation
PRE:	Pre-supplementation
PSD:	Power spectrum density
RF:	Rectus femoris
RM:	Repeated measure
RMS:	Root-mean-square
sEMG:	Surface electromyography
SENIAM:	Surface EMG for Non-invasive Assessment of Muscles
STFT:	Short-time Fourier transform

T_{lim}: Time to fatigue ,

WBB: Wii Balance Board

β-ALA: β-alanine

TABLE OF CONTENTS

1. Chapter I: Introduction	15
1.1. Motor units and their muscle fibres	15
1.2. Recruitment and firing rate	17
1.3. Muscle fatigue and acidosis	18
1.4. Conduction velocity and fatigue	20
1.5. Electromyography: an overview	21
1.6. Electromyography analyses	22
1.7. Electromyography during fatiguing contractions	24
1.8. Carnosine and β -alanine.....	25
1.9. Objectives, justification and structure.....	26
1.10. References	28
2. Chapter II: Electromyogram frequency components and buffering capacity relationship during fatiguing contractions.....	34
2.1. Abstract.....	34
2.2. Introduction.....	35
2.3. Materials and methods	37
2.3.1. Ethical approval	37
2.3.2. Subjects	37
2.3.3. Study design.....	37
2.3.4. Exercises	38
2.3.5. Maximal voluntary contractions	40
2.3.6. Submaximal contractions.....	40
2.3.7. Electromyography.....	41
2.3.8. Anthropometric variables	41
2.3.9. Dietary analysis.....	42
2.3.10. Supplementation protocol	42
2.3.11. EMG variables	43
2.3.12. Statistical analysis.....	44
2.4. Results.....	44
2.4.1. Anthropometric variables	44
2.4.2. β -alanine dietary intake.....	45

2.4.3.	Maximal voluntary contraction force	45
2.4.4.	Time to fatigue.....	46
2.4.5.	Mean power frequency	47
2.5.	Discussion.....	52
2.5.1.	Maximal force and time to fatigue.....	52
2.5.2.	Dietary β -alanine intake.....	53
2.5.3.	Anthropometric variables	53
2.5.4.	MNF _{initial}	54
2.5.5.	MNF during fatiguing contractions	54
2.6.	Conclusion	56
2.7.	References.....	57
3.	Chapter III: Characterisation of Areal of Approximate Entropy in electromyographic signals during sustained contractions	65
3.1.	Abstract.....	65
3.2.	Introduction.....	66
3.3.	Materials and methods	67
3.3.1.	Ethical approval	67
3.3.2.	Subjects	68
3.3.3.	Study design.....	68
3.3.4.	Exercises	68
3.3.5.	Electromyography.....	69
3.3.6.	EMG variables	69
3.3.7.	Statistical analysis.....	70
3.4.	Results.....	70
3.4.1.	EMG variables' curves during the sustained contractions.....	70
3.4.2.	a1ApEn linear regression parameters	73
3.4.3.	MNF linear regression parameters.....	74
3.4.4.	RMS linear regression parameters	76
3.4.5.	Correlation between EMG variable curves.....	78
3.5.	Discussion.....	81
3.6.	Conclusion	83
3.7.	References.....	85
4.	Chapter IV: Final points and conclusions	88
4.1.	a1ApEn and β -alanine supplementation	88

4.2.	Discussion.....	92
4.3.	Conclusions.....	93
4.4.	References.....	94

CHAPTER I

INTRODUCTION

1. CHAPTER I: INTRODUCTION

Skeletal muscle contraction is the result of a complex cascade of events with many underlying components (Randall, Burggren, & French, 1997, pp. 351-403). The contraction of a skeletal muscle most frequently happens when it receives a signal from a central controller. This signal travels from the central controller along nerves fibres until it reaches the neuromuscular junction. There, it is transmitted to the muscle fibre (MF) via the release of acetylcholine into the synaptic cleft. An excitatory postsynaptic potential is produced and followed by a propagating wave of depolarisation through the MF membranes in both directions, starting from the innervation zone. These waves of depolarisation, named muscle fibre action potentials, penetrate the fibre through the T-tubules, initiating a series of intracellular biochemical events leading into a mechanical event of MF shortening or tensioning. Countless positive and negative feedback circuits regulate the steps of this cascade, so that the desired muscle contraction, with the desired force, velocity and range, can be achieved.

In this intricate network of events, the myoelectric signal is an important concept that refers to the electrical potential signals transmitted through active muscle fibres, the muscle fibre action potentials (MFAP). Analogously, motor unit action potentials (MUAP) when speaking of the summation of the MFAPs from a single motor unit (MU). The myoelectric signal is a reflex of many of the factors and processes behind the muscle contraction. For one studying these signals, its inherent complexity is at the same time a challenge for interpretation and a window to look inside the properties of both muscle and central controller (Merletti & Parker, 2004, p. XV). For this reason, the understanding of the muscle electrical activity is a major topic in many fields, including medical, life sciences, sport and exercise physiology, rehabilitation and connected areas.

1.1. Motor units and their muscle fibres

A key element in skeletal muscle electrophysiology is the idea of motor units. They consist in the group of a single α -motoneuron in the spinal cord and all the multiple muscle fibres that are innervated by it. MUs are considered a functional and anatomic unit during muscle contraction, since once a depolarisation wave is inducted in the α -motoneuron, this signal is spread to its numerous MFs, inducing them to contract as a group. The total of MFs in

a single MU ranges from about a hundred to over one thousand, relying on factors such as the muscle size where they are located and the kind of task this muscle is associated with (Feinstein, Lindegård, Nyman, & Wohlfart, 1955). Within a motor unit, the muscle fibres tend to be relatively similar and from the same type (Merletti & Parker, 2004, pp. 2-3).

The skeletal muscle fibre types are: (1) slow-twitch oxidative or type I, (2) fast-twitch oxidative or type IIa and (3) fast-twitch glycolytic or type IIb (Randall et al., 1997, pp. 379-381). It should be noted that alternative MF type classifications are present in the literature (Brooke & Kaiser, 1970; Peter, Barnard, Edgerton, Gillespie, & Stempel, 2002). Type I fibres produce relatively low force per cross-sectional area, have long contraction times, small fibre diameters and are found in small MUs. Rich in mitochondria and with abundant blood supply, their predominant energetic pathway is through oxidative phosphorylation. Type IIa presents fast contraction with intermediate force output. Its oxidative capacity is still high, but less pronounced than what is seen in slow oxidative fibres. The third type, type IIb, has the highest force production with fast contraction velocity. Fibre diameter is large and they are found in larger MUs. Few mitochondria are present in the sarcoplasm, making them more dependent on anaerobic glycolysis for ATP synthesis. Due to this dependency, they tend to accumulate the metabolic by-products of that anaerobic pathway during sustained contractions, notably lactate and ions H^+ . The differences go beyond the histological features as shown by Wallinga-De Jonge, Gielen, Wirtz, De Jong, and Broenink (1985). The authors compared type I and type IIb MFs and demonstrated that in the latter the sarcolemma resting potential is more negative, the amplitude of the MFAPs is higher as well as the maximum rates of depolarisation and repolarisation. The three types also differ in terms of fatigability (Edstrom & Kugelberg, 1968). Type IIb is most susceptible to fatigue, while type I is the most resistant.

The distribution of each type of MU fluctuates between various muscles and even between regions of a single muscle (Johnson, Polgar, Weightman, & Appleton, 1973). For instance, the superficial portion of human *biceps brachii* is predominantly composed of fast-twitch fibres (57.7%), whereas in the deep MUs this number goes down to 49.5%. Within the deltoid muscle, the fibre type I predominates in both superficial and deep regions, 53.3% and 61% respectively. In the *rectus femoris* muscle, the average type II amount ranged from 57.2% to 70.5% depending on the region. These discrepancies are reported to be related to the specific muscle functions, like from postural muscles or muscles suitable for faster contractions (Merletti & Parker, 2004, p. 6).

Neuromuscular strategies for regulating power output during a muscle contraction are intimately related to the quality and quantity of MU recruitment. Two primary mechanisms are

the recruitment of new MUs and the firing rate modulation of those currently active (Cormi, McGuigan, & Newton, 2011).

1.2. Recruitment and firing rate

In a ground-breaking work, Henneman, Somjen, and Carpenter (1965) proposed what is called the size principle, unveiling motor unit recruitment strategies until then unknown. The principle states that MUs are recruited in order of the increasing size of their α -motoneuron, with smaller α -motoneuron having fewer innervations of MFs – which likewise have smaller diameters. As the slow-oxidative fibres are those associated with small MUs, that translates into fibres type I being recruited first, followed by fibres type IIa and IIb. Considering that the recruitment of new motor units is one of the core neuromuscular strategies for increasing the power output of a muscle of interest (Cormi et al., 2011), the sequential size recruitment order matches the production with the needs, as progressively stronger and faster fibres are added with increasing force and contraction velocity outputs.

This recruitment is not only applied as a way of increasing power but also to maintain power in fatiguing contractions. During fatigue, the recruitment threshold of inactive MUs drops (Vollestad, Vaage, & Hermansen, 1984) allowing new MUs, when available, to take part of the contraction, possibly as a mechanism of compensating for the impairment of the already fatigued MFs.

Whereas the recruitment of new MUs regulates the amount and type of fibres used for a specific contraction, the control over the amount of electrical activity in each of the recruited MUs is given by the firing rate modulation. The firing rate of a motor unit consists of the frequency of neural impulses transmitted from the α -motoneuron to the muscle fibres and is a product of a complex interaction of descending and reflex signals. By means of two mechanisms, the firing frequency can impact muscle contraction power: increasing the force developed or influencing the rate of force exerted (Cormi et al., 2011). Enoka (1995) discusses that force can vary approximately from 3 to 15 times due to the increase of firing rate from minimum to maximum values. Similarly, Zehr and Sale (1994) present the occurrence of bursts of high-frequency discharges at the beginning of ballistic contractions.

Following the same rationale of the MU recruitment strategy during fatigue, many would suppose that the discharge rate increases during sustained submaximal contraction in order to compensate for the fatigue manifestation. However, both increase (Bigland-Ritchie,

Cafarelli, & Vollestad, 1986) and decrease were reported (Garland, Enoka, Serrano, & Robinson, 1994). The inconsistent results found in the literature are discussed as results of different control strategies between muscles and contraction intensities (Garland et al., 1994; Kuchinad, Ivanova, & Garland, 2004). The reduce of MU firing rate would happen as a reflex of a fall of muscle fibre conduction velocities (MFCV), which is an event frequently seen in high-intensity fatiguing exercises. The slowing of the conduction velocity is discussed further on in this chapter. The most accepted hypothesis is that the accumulation of metabolic by-products during fatigue induces an inhibitory feedback response via group III and IV afferents (Bigland-Ritchie & Woods, 1984; Woods, Furbush, & Bigland-Ritchie, 1987), accordingly matching the firing rate with the MFCV throughout the exercise.

In regard to both recruitment and firing rate modulation, strategies are not uniform across different force levels and muscles. Kukulka and Clamann (1981) found that, in *adductor pollicis*, the discharge rate is the primary force control mechanism and that the majority of the MU recruitment occurs below 30% of maximum voluntary contraction (MVC) force, with no observed recruitment above 50% MVC. The opposite behaviour was present in *biceps brachii*, displaying recruitment up to almost 90% MVC. De Luca, LeFever, McCue, and Xenakis (1982) encountered the same type of difference when comparing deltoid and first *dorsal interosseous* muscles. Deltoid exhibited a predominance of recruitment modulation while in the first *dorsal interosseous* the firing rate was the most influent aspect.

1.3. Muscle fatigue and acidosis

Despite the set of mechanisms our bodies have to regulate the contraction power output, one cannot sustain the desired power indeterminately for most of the intensities. The muscle fatigues after a certain amount of time. This fatigue is a biological phenomenon of high relevance for electrophysiologists and is better defined as the exercise-induced loss of the muscle's ability to maintain an expected force or power. Several are the possible sites that could cause a loss of performance. Based on those sites, fatigue is traditionally categorised into central and peripheral (Phillips, 2015, pp. 5-13). The former refers to events originating within the central nervous system (CNS), while the latter is used for processes outside the CNS. Despite the vast literature available about the topic, its underlying mechanisms are still a matter of debate and it is likely that more than only one factor act together to cause the impairment in the mechanical performance (Phillips, 2015). Furthermore, the impact that some factor has over

fatigue appears to be task dependent, varying with parameters such as the intensity level of the muscle contraction.

One of the most notorious biological events related to the fatigue is the muscle acidosis that occurs more noticeably in high-intensity contractions. For long known, the correlation between the accumulation of ions H^+ in the MF cytosol and fatigue is well accepted in the literature. This fall in the intracellular pH is mostly a result of ATP hydrolysis coupled with anaerobic glycolysis (Vinnakota & Kushmerick, 2011), which is a significant energetic pathway in high-intensity efforts lasting approximately between 60 and 180 seconds (McArdle, Katch, & Katch, 2010, p. 166). As more fatigable MF types present lower aerobic capacity, these MFs end up showing more prominent H^+ concentrations during fatigue (Phillips, 2015). Despite the correlation between muscle acidosis and fatigue during high-intensity exercises being shown by many studies, correlation does not imply in causality and the underlying mechanisms linking both events are far from a consensus (Phillips, 2015).

Supporting the idea that the accumulation of ions H^+ in the MF cytosol during sustained contractions can negatively impact exercise performance, interventions that manipulated the intramuscular pH or related elements have shown to influence fatigue resistance. Studies demonstrated a significant increase on performance when sodium bicarbonate supplementation was administrated (McNaughton, Siegler, & Midgley, 2008; Siegler, Marshall, Bishop, Shaw, & Green, 2016), which has the potential of alkalinising blood pH. A higher blood pH implies greater H^+ concentration differences between intracellular and extracellular environments inside contracting muscles. This new gradient would facilitate H^+ removal from MFs cytosol and then provide resistance to H^+ accumulation. Likewise, the oral administration of β -alanine has a retarding effect over fatigue (for reviews, see: Artioli, Gualano, Smith, Stout, & Lancha Jr., 2010; Sale, Saunders, & Harris, 2010; Saunders et al., 2017). The relationship between β -alanine and acidosis comes from the increase in intramuscular levels of carnosine as a consequence of the supplementation. Carnosine is a dipeptide that acts as an intramuscular buffer and is composed of the amino acids histidine and β -alanine. Furthermore, it was found an adverse effect of induced blood acidosis (due to ammonium chloride ingestion) over muscle resistance (George & MacLaren, 1988).

Numerous mechanisms have been proposed for how muscle acidosis may induce contraction failure. According to Phillips (2015), some of the suggested effects are alterations on membrane excitability, reduced sarcoplasmic reticulum Ca^{2+} release, competition between H^+ and Ca^{2+} to bind to troponin C, the impairment the cross-bridge cycle, inhibition of enzymes

involved in glycolysis (mainly phosphofructokinase and glycogen phosphorylase) and the signalization of a negative feedback via group III and IV afferents.

1.4. Conduction velocity and fatigue

Related with all the above-detailed elements (MF types, MU recruitment, firing rate, muscle fatigue and acidosis) is the concept of muscle fibre conduction velocity, which is the propagation velocity of MFAP along the membrane. The MFCV is a reflex of several factors, comprising MF membrane and cable properties. Fibre diameter affects the velocity of MFAPs directly, with higher velocities associated with larger fibres (Hakansson, 1956). This explains why MFs type II (larger than type I) display faster MFCVs (Methenitis et al., 2016). The conduction velocity is also positively influenced by an increase in MU firing rate under certain conditions (Morimoto & Masuda, 1984). As previously discussed, MU recruitment and firing rates have significant roles in power output control. At low power, primarily slow-twitch MUs are recruited at relatively low discharge rates. With increasing power, larger fast-twitch MUs are recruited and the firing rate of the already-recruited MUs increases. The consequence of these control strategies is reflected as an increasing conduction velocity with progressively higher contraction intensities (Sadoyama & Masuda, 1987). The same changes happen during sustained low-intensity contractions (Lars Arendt-Nielsen, Mills, & Forster, 1989), possibly due to the same recruitment and discharge rate reasons. Propagation velocity is also affected by MF length (Trontelj, 1993). For this reason, isometric contractions are usually preferred for more reliable MFCV measurements. In regards to membrane related factors, changes in the membrane microenvironment due to the accumulation of metabolic by-products seem to decrease the propagation velocity of the MFAPs (Zwarts & Stegeman, 2003).

Several studies pointed out a reduction at muscle fibre conduction velocity concomitantly with fatigue manifestation at moderate to high-power exercises (Dimitrova & Dimitrov, 2003; Merletti & Parker, 2004). A key point behind this behaviour comes from the influence of muscle pH over MFCV, as shown by Brody, Pollock, Roy, De Luca, and Celli (1991). They studied the average MFCV of isolated muscles at different pH baths, proving that lowering environment pH decreases MFCV. Kupa, Roy, Kandarian, and De Luca (1995) demonstrated that the rate of decline in MFCV during sustained contractions is positively correlated to the density of MFs type II in a muscle. The reason for that could be the higher H^+ production associated with fast-twitch fibres. Similarly, it was shown that the decrease in the

propagation velocity is also associated with the contraction intensity. Sustained high-intensity contractions, which are more dependent on anaerobic energetic pathways and tend to display a drop at muscle pH levels, show more significant reductions of MFCV (Lars Arendt-Nielsen & Mills, 1988). Linssen, Stegeman, Merks, Binkhorst, and Notermans (1996) measured the conduction velocity in McArdle's patients, disease that causes an absence of the capacity to use glycogen in the glycolytic pathway. It was observed that no changes occurred in both muscle pH and MFCV during sustained isometric force.

These results show muscle pH as one factor strongly correlated with MFCV changes in fatiguing contractions. Propagation velocity measurements may, therefore, provide pertinent data about muscle acidosis during exercise. However, the biochemical mechanism for how pH changes MFCV is still not clear. One hypothesis is that the accumulation of H^+ could stimulate group III and IV afferents, initiating an inhibitory feedback loop, facilitating mental aspects of fatigue (Phillips, 2015, p. 140) and reducing the firing rate of MUs (Woods et al., 1987).

1.5. Electromyography: an overview

One of the most important methods, if not the most important, for looking at the skeletal muscle electrical signals is the technique called electromyography (EMG). It consists in the use of sensors that are able to measure on-going changes in the electrical potentials in a muscle of interest. Thus, the electromyographic record, known as electromyogram, is a product of the summation of multiple MUAPs within the detection distance of the electrodes. Several technical specifications are possible for the EMG application, such as electrode's shape, size and material. However, it is important to mention that the electrode positioning in relation to a muscle is an essential feature and that EMG is frequently categorised in two types depending on the placement of the electrodes: intramuscular or surface EMG.

Intramuscular EMG is when the electromyogram is recorded with indwelling electrodes (needle or wire) located inside the muscle. One of the main characteristics of this invasive type of EMG is that it can provide very localised information from superficial or deep muscle structures (Merletti & Parker, 2004). The reason is the small detection volume from these intramuscular electrodes that includes only a few groups of motor units around the sensor's tip.

The second type is the surface EMG (sEMG), which consists in the attachment of electrodes on the skin surface above the measured muscle. Common electrodes configurations in sEMG are monopolar and bipolar electrodes (US Department of Health and Human Services,

1992). The first consists of a single electrode positioned over the muscle of interest, whereas in the bipolar arrangement two electrodes are used instead. For both configurations, an additional reference electrode is placed at an electrically neutral site. While the signal recorded from the monopolar EMG is essentially the electrical potentials that reach the single electrode, in the bipolar EMG the record is a function of the difference of the signal detected by each electrode. This method implies a greater ability to reduce interference noise and other biological electric signals produced far from the analysed muscle (US Department of Health and Human Services, 1992).

In contrast with the intramuscular EMG, surface EMG is non-invasive and can detect MUAPs from a much broader volume. Hence, sEMG tends to be seen as a global record of electrical muscle activity, whereas indwelling electrodes bring information from small groups of motor units (Merletti & Parker, 2004). The non-invasiveness and the ease of use make the surface EMG the most vastly applied form of EMG (US Department of Health and Human Services, 1992), however, it requires additional caution when analysing the recorded data.

The reason why is that the signal depicted by the sEMG reflects confounding elements that are not so significant in the intramuscular EMG (Farina, Merletti, & Enoka, 2004). For instance, the distance between the detection system and the active motor units, the inter-electrode distance in a bipolar EMG, thickness of subcutaneous tissues or the shift in the muscle's position relative to the sensors during dynamic contractions. Fortunately, many of these issues are well described in the literature (De Luca, 1997; Dimitrova & Dimitrov, 2003; Farina, 2008; Farina et al., 2004; Merletti & Parker, 2004; US Department of Health and Human Services, 1992; Vigotsky, Halperin, Lehman, Trajano, & Vieira, 2018) and can be easily controlled by following some standardised procedures.

The concerted action on “Surface EMG for Non-invasive Assessment of Muscles” (SENIAM) was an initiative by the European Commission in order to develop such standardised guidelines. The results (Hermens et al., 1999) of the action consist in bipolar sEMG recommendations for features including sensor placement, electrodes shape, size and material, skin preparation, inter-electrode distance and testing procedures.

1.6. Electromyography analyses

Once the muscle electrical signal is collected, mathematical methods to extract information from the sEMG are often needed. Here, the researcher has several possibilities

available. The most widely used techniques are traditionally classified into time domain analysis or frequency domain analysis. Important to notice that both types can be used together to examine changes in frequency parameters through time. In this case, the method is said as a time-frequency domain analysis. Lastly, another group of mathematical methods has gained importance more recently, the non-linear analysis.

Maybe the most common information extracted from the EMG is the signal amplitude through a specific time window. Using the pre-processed signal (i.e., noise and interference treated), this time domain data is obtained in 3 steps (Merletti & Parker, 2004): (1) demodulation, (2) smoothing and (3) re-linearization. For a better explanation, let us take the root-mean-square (RMS) indication as an example. The RMS is an extensively used time domain parameter that consists of “the square root of the arithmetic mean of the squares of a set of values” (Oxford Dictionaries, 2019). The first step is obtained by squaring the set of values, transforming all data into positive values. The second stage is smoothing, represented by the arithmetic mean of the demodulated values. The final step is the re-linearization, achieved by calculating the square root of the smoothed and demodulated data.

The second listed set of methods, frequency domain analysis, extracts information about the underlying frequency components and their energy rather than changes of a parameter over time. The relationship of frequency components of a time series and their power is known as the power spectral density (PSD) and can easily be found with a fast Fourier transform (FFT). From the PSD, one can estimate several parameters such as mean power frequency (MNF) or median power frequency (MDF), both broadly found in EMG fatigue studies.

Changes in the PSD over time are calculated by time-frequency domain analysis. Used mathematical methods include the short-time Fourier transform (STFT) and wavelets. Despite wavelets have some theoretical advantages over STFT, especially in regards to signal resolution, physiologists have been using STFT preferentially, as the latter has a more straightforward computational implementation and shows similar results in practical grounds (Merletti & Parker, 2004).

Finally, the last type of is non-linear analysis. Compared to the previously explained analyses, non-linear methods are relatively new in the muscle electrophysiology literature and have been presenting increasing importance (Merletti & Parker, 2004). Some of the findings include pattern changes during fatiguing contractions and the ability to differentiate healthy and pathological EMG signals (Acharya, Ng, Swapna, & Michelle, 2011; Istenic, Kaplanis, Pattichis, & Zazula, 2010; Xie, Guo, & Zheng, 2010). One example of a non-linear method is recurrence quantification analysis, which measures recurring patterns and non-stationarities in

experimental data sets. Another approach is to quantify the signal complexity over time. For that, algorithms like Approximate Entropy (ApEn) and its derivatives can be used.

1.7. Electromyography during fatiguing contractions

During fatiguing contractions, electromyographic changes are seen in time, time-frequency and nonlinear analysis. These changes have multiple causes and different characteristics depending on the contraction force, type, muscle used and others. Understanding how sEMG behaves and how it is a reflection of physiological structures and processes is fundamental for one to draw proper conclusions from electromyographic results.

EMG amplitude can increase or decrease during fatigue depending on the force used. Consistently, studies report increased RMS values through sustained submaximal contractions and the opposite behaviour at maximal force (Farina et al., 2004). The two core mechanisms explain those reports are the MU recruitment and firing rate modulation (Vigotsky et al., 2018). Both have a significant and similar positive influence over RMS values. At maximal intensity, when nearly no new MU is recruited and discharge rate falls, RMS follows the same decrease. At submaximal fatiguing contractions, the occurrence of recruitment of new MUs has a positive influence over RMS. The same is valid for firing rate. However, as a fall at firing rate is seen in high-intensity submaximal contractions and muscle control strategies vary considerably across different muscles and tasks, the sEMG amplitude can be influenced in unintuitive ways. The existence of other physiological elements beyond recruitment and rate coding that can potentially be reflected in the RMS, such as MU synchronisation and volume conductor alteration, adds another layer of complexity (Vigotsky et al., 2018).

For both maximal and submaximal fatiguing contractions, frequency components are shifted and compressed towards low frequencies (Farina et al., 2004). The EMG power spectrum density is explained at least partially by the muscle's average MUAP (Brody et al., 1991), including the propagation velocity and MUAP shape. The reduction of MFCV seen during fatigue results in a widening of MUAP, shifting frequency components of the MUAP towards lower frequencies. However, Brody et al. (1991) showed that reduction in MDF values during fatigue could not be explained by the MFCV behaviour alone. Their result evidenced changes not only in velocity but also in shape. In Farina et al. (2004) review, the authors present other factors that could be reflected in the frequency shift, among them MU recruitment and

firing rate. However, these factors' impact may be limited and hard to fully understand, restricting our ability to extract neural strategies from the sEMG.

Non-linear analyses are underused in electromyographic researches in general and information about the effects of fatigue over non-linear variables is relatively scarce. Studying the dynamics of signal complexity and fatigue manifestation, Xie et al. (2010) observed progressively reduced Fuzzy Approximate Entropy (function based on ApEn developed by the researchers) during sustained contractions. The reported decrease in complexity was followed by similar changes in MNF, which is discussed to be mathematically related to signal complexity.

1.8. Carnosine and β -alanine

Many organic reactions and enzymes require a specific environment pH for optimal performance. Thus, it is natural that our bodies have ways of defending themselves against pH changes such as those related to high-intensity exercises. The presence of the molecule carnosine in skeletal muscles is believed to be one of those mechanisms, due to its buffering properties.

Carnosine is a histidine-containing dipeptide (HCD) and is composed of the combination of the amino acids β -alanine and histidine (Saunders et al., 2017). This HCD is mainly found in skeletal muscles, where it is synthesised through a reaction catalysed by the carnosine synthetase (Artioli et al., 2010). Nonetheless, MFs are not able to produce themselves either β -alanine or histidine, which implies dependency of blood supply for both amino-acids (Painelli, Freitas, Gualano, & Artioli, 2015). Humans have the carnosine as the unique HCD present in their muscles (Boldyrev & Severin, 1990). Nevertheless, carnosine is not the only HCD found in skeletal muscles among different animals. Anserine and balenine (also known as ophidine) are other HCDs commonly found in other groups (Boldyrev & Severin, 1990). Furthermore, not only in terms of quality are the differences between animal groups, but also in quantity. Some animals involved in hypoxic dives or sprint-like efforts present high intramuscular HCD contents, while endurance profiles were related to lower HCD concentrations (Artioli et al., 2010).

The most prominent role for carnosine and the other HCDs so far is their intracellular buffering ability, due to their imidazole ring (Artioli et al., 2010). The phylogenetic concentration distribution is one of several pieces of evidence supporting this main property.

Another set of indications comes from the differences in carnosine concentration between different people. Athletes of modalities that use anaerobic energetic pathways tend to have greater intramuscular carnosine content when compared to athletes of more aerobic modalities (Artioli et al., 2010).

Given the lower blood availability of β -alanine and its higher affinity to the carnosine synthetase, it was hypothesised that this amino acid would be a limiting factor for carnosine synthesis (Painelli et al., 2015). This idea was proven correct with the demonstration that the nutritional supplementation with β -alanine improves intramuscular content of carnosine more prominently than histidine supplementation (Sale et al., 2010). The discovery that β -alanine supplementation has a positive effect over the carnosine content in the skeletal muscle opened a new field of research: the application of β -alanine supplementation in studies about muscle fatigue. The rationale comes from the correlation between fatigue and pH and the increasing effect of β -alanine supplementation and the intracellular buffer carnosine. Since then, several authors pointed out a retarding effect over time to fatigue induced by chronic β -alanine ingestion, when highly anaerobic glycolytic efforts were investigated (Saunders et al., 2017).

1.9. Objectives, justification and structure

Looking back at the available literature, the underlying events linking the sEMG record PSD changes during fatigue and muscle fibre acidosis are still uncovered. Similarly, even the correlation of pH and fatigue itself is still a matter of debate. Interesting progress was made with the studies like the one of Brody et al. (1991), pointing out how pH changes caused alterations on the EMG power spectrum. However, the strictly controlled *in vitro* environment often used is unlikely to be perfectly transposable to *in vivo* situations.

To bring more clarity to this convoluted topic, we investigated the relationship between EMG and pH *in vivo*. More specifically, the effects of changes on muscle buffering capacity over EMG changes due to fatigue. The study had an exploratory nature where our concern was to determine if increasing MF carnosine content affects the power spectrum density, measured by MNF, of the single-channel surface electromyogram. For obtaining such an increase in intramuscular carnosine, chronic β -alanine supplementation was used. We hypothesised that a higher buffering capacity would not alter the initial values of MNF, while the MNF fatigue dynamic would be affected. This investigation is the core of the current thesis and is presented in the next chapter in more details as an article.

Additionally, as a secondary objective, we investigated the EMG data collected during the master's project period using a non-linear variable developed by our group: Area 1 of Approximate Entropy (a1ApEn). The a1ApEn is an improvement, developed by our group, to the ApEn algorithm (Natali & Chaui-Berlinck, 2016). This tool is based on the construction of the area under the curve of ApEn *versus* a normalised tolerance vector for the probing window size $m = 1$, which was demonstrated to be more reliable and objective compared to the ApEn. Two goals were set: (1) characterise EMG signal complexity by a1ApEn, not yet studied in myoelectrical data, and (2) examine whether alterations on buffering capacity are reflected in EMG a1ApEn. Reported in chapter 3, following the article format, the characterisation objective used electromyograms recorded during a pilot experiment prior to our core study (chapter 2). This preliminary experiment was essential to evaluate the feasibility of the system developed to measure muscle force employed and explained in the next chapter. The relationship between a1ApEn and buffering capacity was assessed through the same data of chapter 2. Nevertheless, these results are just briefly presented in chapter 4, which consists of a general discussion of the elements presented in the current thesis. The reason for this choice is to avoid excessive text repetition (as most of the related discussion is shared with chapters 2 and 3) and due to the results of both chapter 2 and chapter 3, which raised the discussion about the relevance of the producing a third article exclusively for the effects of β -alanine supplementation over a1ApEn (see chapter 4).

1.10.References

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CHAPTER II

ELECTROMYOGRAM FREQUENCY COMPONENTS AND BUFFERING CAPACITY RELATIONSHIP DURING FATIGUING CONTRACTIONS

2. CHAPTER II: ELECTROMYOGRAM FREQUENCY COMPONENTS AND BUFFERING CAPACITY RELATIONSHIP DURING FATIGUING CONTRACTIONS

2.1. Abstract

Studies demonstrate that the surface electromyogram (EMG) shows a frequency shift towards lower frequencies during fatiguing contractions, which is often associated at least partially to lower muscle fibre conduction velocity and muscle acidosis. The goal of the present work was to determine whether an increase in muscle buffering capacity would result in different EMG frequency patterns during fatiguing contractions. For manipulating buffering capacity, chronic β -alanine supplementation was used. Fourteen healthy men (mean $\pm$ SD: age, 23.36 ± 4.92 years; weight, 72.54 ± 10.79 kg; height, 177.53 ± 7.87 cm) participated in the investigation. Subjects were randomly allocated between a group that received β -alanine supplementation (β -ALA) or a group placebo (PLA). EMG was recorded at an experimental session before (PRE) and after supplementation (POST) while volunteers performed isometric contractions at 50% of maximal voluntary isometric contraction force (MVC) until fatigue. From the EMG, the mean power frequency (MNF) was obtained. Time to fatigue (T_{lim}) was also analysed. The β -alanine supplementation had a significant effect on T_{lim} , increased by 9.54% at POST for group β -ALA ($p = 0.02$). Conversely, no differences were found in the MNF dynamics during fatigue. It is discussed that MNF fatigue curve differences were theoretically expected given greater T_{lim} and that the lack of significance may be connected to the complex, multifaceted nature of surface EMG signal.

Keywords: mean power frequency; β -alanine; electromyography; submaximal force; fatigue.

2.2. Introduction

Several studies have observed changes in surface electromyography (EMG) during fatiguing muscle contractions. When performing time-frequency analyses within EMG records, a shift in the spectral components happens towards lower frequencies simultaneously with an increase in low-frequency energy. The use of central parameters of the power spectrum density (PSD) is broadly employed to evaluate this behaviour. For instance, Viitasalo and Komi (1977) observed an approximately linear decrease in the EMG mean power frequency (MNF) during isometric contractions sustained at 60% of the maximal voluntary contraction force (MVC). Similar findings were reported for other submaximal and maximal intensities (Bilodeau, Schindler-Ivens, Williams, Chandran, & Sharma, 2003; Broman, Bilotto, & De Luca, 1985; Moritani, Nagata, & Muro, 1982).

A reduction of muscle fibre conduction velocity (MFCV) is one of the factors considered to have a role in the frequency shift. Lower MFCV values imply in a widening of muscle fibre action potentials (MFAP) recorded, enhancing the low-frequency components. Numerous authors reported a simultaneous drop of MNF and MFCV in the course of a fatiguing effort (Arendt-Nielsen & Mills, 1985, 1988; Brody, Pollock, Roy, De Luca, & Celli, 1991; Broman et al., 1985; Sadoyama, Masuda, & Miyano, 1983). Nevertheless, differential rates of decline between the two variables (Brody et al., 1991; Broman et al., 1985) and even the occurrence of the frequency shift in the absence of alteration on the conduction velocity (Arendt-Nielsen & Mills, 1985; Eberstein & Beattie, 1985; Sadoyama et al., 1983) suggest that MNF behaviour is explained only partially by MFCV. For example, Brody et al. (1991) observed that median power frequency (MDF) initial values of contractions varied proportionally to changes on initial MFCV, whereas during sustained contractions the same proportionality was not held, with MDF displaying stronger declines than MFCV.

The study conducted by Brody et al. (1991) not only addressed the relationship between conduction velocity and EMG frequency components, but also the connection between both and muscle pH. Their intervention consisted of supramaximal stimulations of *in vitro* muscle preparations repeated at different pH baths, which caused the variations in the initial values of MDF and MFCV. Although the exact mechanism by which muscle pH changes correlates with the myoelectrical signal is not yet understood, many studies support the existence of such association. For instance, Mortimer, Magnusson, and Petersen (1970) analysed the conduction velocity of stimulated *gastrocnemius* and *soleus* muscles under ischemia and concluded that

the ability to preserve normal propagation velocity depends on metabolic by-products removal via blood flow. Ions H^+ are a well-known metabolic by-product, which tends to accumulate in the sarcoplasm during anaerobic efforts as a result of high rates of ATP hydrolysis coupled with anaerobic glycolysis (Vinnakota & Kushmerick, 2011). Furthermore, Mortimer et al. (1970) observed MFCV differences between the studied muscles, with velocity decreases less prominent in the *soleus*. Compared to the *soleus*, the *gastrocnemius* displays a larger proportion of muscle fibres (MF) type II (Edgerton, Smith, & Simpson, 1975), which have low aerobic potential (Essen, Jansson, Henriksson, Taylor, & Saltin, 1975) and high fatiguability (Edstrom & Kugelberg, 1968). Additionally, Tesch, Komi, Jacobs, Karlsson, and Viitasalo (1983) show a correlation between fibre type II, lactate accumulation and MNF decrease during a fatiguing exercise. The same association with MF type was found for MFCV and MDF (Kupa, Roy, Kandarian, & De Luca, 1995). Moreover, more significant propagation velocity declines are observed at high-intensity sustained contractions (Arendt-Nielsen & Mills, 1988), which are more dependent on anaerobic pathways and are frequently correlated with muscle acidosis. Lastly, Linssen, Stegeman, Merks, Binkhorst, and Notermans (1996) found that no changes occurred in both muscle pH and MFCV during sustained isometric force in McArdle's patients, unable to use glycogen in the glycolytic pathway.

The correlation between sarcoplasmic H^+ accumulation and fatigue is mostly a result of ATP hydrolysis coupled with anaerobic glycolysis (Vinnakota & Kushmerick, 2011). Interestingly, some interventions that manipulated the muscle pH related elements have shown an impact on fatigue resistance. This is the case of studies reporting a significant increase on performance due to sodium bicarbonate supplementation (McNaughton, Siegler, & Midgley, 2008; Siegler, Marshall, Bishop, Shaw, & Green, 2016), which acts alkalinising the blood pH. Similarly, it was found an adverse effect of induced blood acidosis (due to ammonium chloride ingestion) over muscle resistance (George & MacLaren, 1988). Furthermore, the oral administration of the amino acid β -alanine has a fatigue retarding effect, especially in scenarios when strong muscle acidosis is expected (for reviews, see: Artioli, Gualano, Smith, Stout, & Lancha Jr., 2010; Craig Sale, Saunders, & Harris, 2010; Saunders et al., 2017). The relationship between β -alanine and acidosis comes from the positive effect of β -alanine supplementation on the concentration of the intramuscular buffer carnosine.

Heretofore, several connections between muscle acidosis, fatigue, conduction velocity and EMG power spectrum were presented. In this context, the question of whether the muscle resistance to acidosis could or not have a distinguishable effect on the myoelectrical signal remains unexplored. Thus, the present paper aimed to investigate this issue. Our goal was to

determine if an increase in muscle buffering capacity would result in different EMG patterns during fatiguing contractions. More specifically, EMG power spectrum changes. For manipulating muscle buffering capacity, chronic β -alanine supplementation was used.

2.3. Materials and methods

2.3.1. Ethical approval

All procedures were approved by the ethical committee of the Biosciences Institute of the University of São Paulo (CAAE 67648617.0.0000.5464). Each participant was informed of any possible risk and benefits regarding the procedures and provided written informed consent prior to enrolment.

2.3.2. Subjects

Fourteen healthy men (mean $\pm$ SD: age, 23.36 ± 4.92 years; weight, 72.54 ± 10.79 kg; height, 177.53 ± 7.87 cm) volunteered to participate in this investigation and completed all the procedures. All subjects were physically active, currently enrolled in resistance training programs (2-4 sessions/week; 2-6 hours/week). Health history was assessed through a questionnaire. No participant declared to have any disease or health condition that could preclude the participation in the study. Likewise, none of them had previous use of β -alanine supplementation or anabolic steroids. Preceding the study, the individuals were instructed to maintain their regular diet and physical activity routine during the course of the investigation period and not to exercise in the previous 48 hours before the procedures.

2.3.3. Study design

A randomised, double-blind, placebo-controlled, repeated measures design was used. Subjects were randomly allocated into two experimental groups: (1) one that received β -alanine supplementation (β -ALA; $n = 7$; mean $\pm$ SD: age, 24.14 ± 6.2 years; weight, 71.33 ± 11.5 kg; height, 175.79 ± 9.4 cm); and (2) other which placebo supplementation was given to (PLA; $n = 7$; mean $\pm$ SD: age, 22.57 ± 3.55 years; weight, 73.75 ± 10.78 kg; height, 179.27 ± 6.23 cm).

The study consisted of one familiarisation session (FM) and two experimental sessions, one before (PRE) and other after (POST) the supplementation period (Figure 2.1). The interval between FM and PRE was one-week long. The supplementation protocol lasted 28 days, which was shown to be sufficient to increase intramuscular carnosine (Saunders et al., 2017). At the FM session, the participants were familiarised with the procedures to be used in the experimental sessions. At PRE and POST, isometric submaximal contractions until failure were requested for three different muscles: *biceps brachii* (BI), *rectus femoris* (RF) and lateral deltoid (DE). The submaximal force was equal to half of the maximal voluntary isometric contraction force (MVC) measured at each session before the submaximal contractions. This 50% of MVC was chosen due to its potential to promote muscle acidosis (Ahlborg et al., 1972; Beliveau et al., 1992; Bendahan et al., 1996; Laurent, Portero, Goubel, & Rossi, 1993). Surface electromyograms were recorded for both contractions' forces and three muscles. Time to fatigue (T_{lim}) was also analysed. In order to test either a priori dietary β -alanine intake is similar between groups and that no significant changes in anthropometric variables happened during the investigation, subjects' data about body composition and habitual meat consumption were collected before the start of PRE and POST exercises. Day time was held constant across the sessions for each volunteer.

Figure 2.1. Study protocol timeline. FM: familiarisation session; PRE: pre-supplementation session; POST: post-supplementation session.

2.3.4. Exercises

All exercises consisted of isometric contractions, in standardised and stabilised positions, using the muscles of the dominant side, against a dynamometer system developed for this study, which was composed of a Wii Balance Board (WBB; Nintendo, Kyoto, Japan) supporting weights with an adjustable-size strap and a gym single handle attached (Figure 2.2).

The WBB was connected to the computer software LabChart v.8.1.13 (ADInstruments, Sydney, Australia), which continuously registered real-time force output. The scale was zeroed and force values multiplied by -1 to correctly represent muscle output. Force direction was always against gravity.

Figure 2.2. Dynamometer system developed for the current investigation. An adjustable-size strap, with a gym handle, was attached to the weights on the Wii Balance Board.

Biceps brachii exercise consisted of an elbow flexion against the dynamometer. Volunteers were positioned in a standing position, with the elbow in the body side and flexed at 45° and the forearm supinated. For *rectus femoris*, knee extensions were employed. Subjects sat in a Leg Extension Machine (Extensor/Flexor Sentado Conj. Classic, Lion Fitness®, Valentim Gentil, Brazil) with the dynamometer strap attached to the ankle pad. The knee was flexed at 45° . Lateral abduction was used for lateral deltoid exercises. The shoulder was set laterally abducted at an angle of 45° and the forearm pronated.

Participants' exercise sequences were randomly assigned from one of two possibilities ($n = 7$ for each): BI→RF→DE at FM, DE→RF→BI at PRE and BI→RF→DE again at POST; or DE→RF→BI at FM, BI→RF→DE at PRE and DE→RF→BI at POST. All MVCs were performed first in the assigned sequence. Then, after a 5-minute rest interval, all submaximal contractions were attained following the same order (Figure 2.3).

Figure 2.3. Timeline of exercises within a single session. Maximal voluntary contractions (MVC) are performed first for all muscles. Then, after a 5-minute interval, submaximal contractions until muscle fatigue are executed in the same order.

Few cases of discomfort were reported during the exercises. Muscle soreness from a heavy resistance training session in the previous day was reported as the reason, despite the fact that the volunteers were instructed not to train within the 48 hours preceding the sessions. In these cases, exercises for the affected muscles were interrupted and muscle data discarded. In total, analysed data consisted of registers of 13 volunteers for BI (7 β -ALA and 6 PLA), 10 for RF (4 β -ALA and 6 PLA) and 12 for DE (5 β -ALA and 7 PLA).

2.3.5. Maximal voluntary contractions

The subjects performed three 5-second MVCs for each muscle, while force and EMG were recorded. Between each MVC attempt, there was a 1-minute interval. Volunteers were verbally encouraged to exert the maximal possible force. Peak force value was extracted from each of the three contractions and MVC force (100% MVC) was calculated as the average of those peaks.

2.3.6. Submaximal contractions

One submaximal isometric contraction per muscle was required, until failure, with force equal to 50% of maximal force (50% MVC). Visual feedback of the force output was provided throughout the experimental session. Participants were requested to hold the force as long as possible, using the visual feedback as reference. Muscle failure was considered as the point

when the subject could not sustain the force output within the 50 ± 2.5 % MVC force zone. Volunteers were verbally motivated during the fatigue protocol.

2.3.7. Electromyography

The surface EMG activity was recorded employing two pre-gelled, silver chloride surface electrodes (Eletrodo 2223, 3M, Sumaré, Brazil). The interelectrode distance was 20 mm. A single ground electrode was placed over the ankle for RF and the wrist for BI and DE. Prior to electrode attachment, the skin was prepared by abrading and cleaning it with alcohol. *Biceps brachii* electrodes were placed on and aligned with the line connecting the medial acromion border and the cubital fossa, at one third from the fossa cubit. *Rectus femoris* was measured with electrodes placed halfway through the line connecting the anterior superior iliac spine to the top of the patella, following the same orientation. Lateral deltoid electrodes were attached to the region of the most prominent bulge of the muscle on the line between the acromion and the lateral epicondyle of the elbow, oriented according to the same line. The raw EMG signal was obtained with an Octal Bio Amp (ADInstruments, Sydney, Australia) and converted into digital using a PowerLab 16/35 (ADInstruments, Sydney, Australia). Data were sampled at 1 kHz, bandpass filtered from 10 to 500 Hz and additionally filtered for electrical noise with an adaptive mains filter. Recordings were done with the software LabChart v8.1.13 (ADInstruments, Sydney, Australia).

2.3.8. Anthropometric variables

Weight, height, body mass index (BMI) and body fat percentage were obtained for each volunteer at the beginning of sessions PRE and POST, before the exercises and after a dietary recall interview. The first was collected with a digital scale (Tecsilver, Tech Line, São Paulo, Brazil) and height with a stadiometer (Personal Caprice, Sanny, São Bernardo do Campo, Brazil). BMI was calculated by dividing the weight by the squared height. Body fat was taken with the use of a calliper Neo II Comfort Plus P (Prime Med, São Paulo, Brazil) to measure seven skinfold sites: chest, axilla, triceps, subscapular, abdominal, suprailiac and thigh. The measurements were inserted into the Jackson and Pollock (1978, eq. 1) equation to calculate body density, from which the percentage of body fat was obtained with the Siri equation (Siri, 1961).

2.3.9. Dietary analysis

With the use of dietary recall interview, information regarding the habitual food consumption of the meat types most frequently consumed in the Brazilian diet (Instituto Brasileiro de Geografia e Estatística. Coordenação de Trabalho e Rendimento, 2011) were retrieved. These types were beef, pork, poultry and fish. The interviews were applied at the beginning of sessions PRE and POST. The PRE interview consisted of a 6-month dietary recall while POST interview was a 4-week recall (time difference between PRE and POST). All subjects were asked about which meat-contained meals that they ingested during the period relative to the interview. Consumption frequency and the number of servings were then obtained. The latter was asked in natural or household units, with the help of the illustrated serving sizes in Monego et al. (2013). Household units were converted into weight with the use of their tables. Average serving sizes were calculated for each meat type and average daily consumption was obtained by combining them with consumption frequency.

Estimated daily HCD intake was retrieved from the daily consumption of the meats of interest. The HCDs concentrations for each type were extracted from the average of the values reported by several different studies (Abe, Dobson, Hoeger, & Parkhouse, 1985; Boldyrev & Severin, 1990; Carnegie, Ilic, Etheridge, & Collins, 1983; Jones, Smith, & Harris, 2011; Plowman & Close, 1988; Wołos, Jabłonowska, Faruga, & Jankowski, 1982; Yeum et al., 2010). Concentrations are expressed as $\mu\text{mol/g}$ of wet weight. As the β -alanine and the HCDs are in a 1:1 proportion, estimated daily β -alanine intake is equal to total HCD consumption.

2.3.10. Supplementation protocol

The β -ALA group received 6.4 g/day of β -alanine supplementation (Carnosyn[®], Natural Alternatives International, San Marcos, USA) for 28 days. This represents a total of 8 sustained-release tablets per day, containing 800 mg of β -alanine. The PLA group received maltodextrin tablets (Natural Alternatives International, San Marcos, USA) instead. The volunteers were asked not to take more than two tablets at once and to give at least 3 hours between tablet ingestion.

2.3.11. EMG variables

Short-time Fourier Transform was applied to all EMG records. Non-overlapping, rectangular, 256-ms windows were used and mean power frequency (MNF) obtained for each one. For the three maximal contractions, MNF was found at the time of peak force. MNF associated with maximal force (MNF_{MVC}) was calculated by averaging these three values. Next, the whole MNF array was normalised by dividing it by MNF_{MVC}. From the first and the last 10% of this array, the normalised MNF values of the submaximal contractions were extracted and averaged, resulting in the variables MNF_{initial} and MNF_{final}, respectively. In order to obtain the degree of MNF changes due to fatigue, two other variables were calculated:

1. MNF_{ratio}, which can be understood as the MNF_{final} value expressed in relative terms to MNF_{initial}. Calculated as follow:

$$MNF_{ratio} = \frac{MNF_{final}}{MNF_{initial}} \quad (2.1)$$

2. MNF_{slope}, representing the rate of MNF change during sustained contraction and also uses MNF_{initial} as reference. It is obtained with the following expression:

$$MNF_{slope} = \left(\frac{MNF_{final} - MNF_{initial}}{MNF_{initial}} \right) / T_{lim} \quad (2.2)$$

Also expressed as:

$$MNF_{slope} = \frac{(MNF_{ratio} - 1)}{T_{lim}} \quad (2.3)$$

All data processing was done in MATLAB v.2018b (The MathWorks Inc, Natick, United States of America).

2.3.12. Statistical analysis

Between-group differences were assessed through the group by session interaction effect (group*session) analysed with two-way repeated measures (RM) analyses of variance (ANOVA) or with linear mixed models (LMM). Two-way RM ANOVAs were used for anthropometric measurements and β -alanine dietary intake, taking into account group, session and group*session effects, with the session set as RM. LMMs were applied to MVC, T_{lim} and MNF data, including group, session, muscle and all possible interactions as fixed effects. Both muscle and session factors were considered RM. Covariance matrix structures were those with the lowest Akaike information criterion (AIC) values via restricted maximum likelihood. Three-way interactions were further analysed by re-applying the LMM separately for each muscle. In case of a significant group by session interaction for either ANOVA or LMM, PRE *versus* POST pairwise comparisons were performed using paired t-tests and Cohen's d for significance and effect size measurements, respectively. Statistics were performed using SPSS v.23 (IBM® SPSS® Statistics Version 23, SPSS Inc, Chicago, United States of America). Statistical significance level was set at $p < 0.05$. Values are expressed as means $\pm$ standard deviations (SD).

2.4. Results

2.4.1. Anthropometric variables

No differences were found for body composition measurements between experimental groups and sessions. Two-way RM ANOVAs reported no significant group by session interaction effects [weight: $F(1, 12) = 0.08$, $p = 0.79$; BMI: $F(1, 12) = 3.4 \times 10^{-3}$, $p = 0.95$; body fat: $F(1, 12) = 0.97$, $p = 0.34$]. Likewise, neither group [weight: $F(1, 12) = 0.17$, $p = 0.69$; BMI: $F(1, 12) = 0.03$, $p = 0.87$; body fat: $F(1, 12) = 0.26$, $p = 0.62$] nor session effects [weight: $F(1, 12) = 0.18$, $p = 0.68$; BMI: $F(1, 12) = 0.01$, $p = 0.93$; body fat: $F(1, 12) = 0.19$, $p = 0.67$] were found. The weight, BMI and body fat means and SDs values are presented in Table 2.1.

Table 2.1. Anthropometric variables by group and session. Data are expressed as mean $\pm$ SD. BMI = body mass index; PRE = pre-supplementation session; POST = post-supplementation session.

Group	Session	Weight (kg)	BMI (kg/m ²)	Body fat (%)
β -alanine	PRE	71.16 $\pm$ 11.52	23.13 $\pm$ 3.47	10.02 $\pm$ 3.82
	POST	71.50 $\pm$ 11.52	23.11 $\pm$ 3.49	9.62 $\pm$ 3.63
Placebo	PRE	73.71 $\pm$ 10.24	22.86 $\pm$ 2.07	10.91 $\pm$ 5.19
	POST	73.79 $\pm$ 11.43	22.86 $\pm$ 2.48	11.07 $\pm$ 4.56

2.4.2. β -alanine dietary intake

Average daily dietary β -alanine consumption by group and session are reported in Table 2.2. Analysis of variance reported no significant differences [group: $F(1, 12) = 2.3$, $p = 0.15$; session: $F(1, 12) = 0.09$, $p = 0.77$; group*session: $F(1, 12) = 2.53$, $p = 0.14$].

Table 2.2. Beta-alanine dietary intake by group and session. Data are expressed as mean $\pm$ SD. PRE = pre-supplementation session; POST = post-supplementation session.

Group	Session	β -alanine dietary intake ($\mu\text{mol/kg.day}$)
β -alanine	PRE	153.60 $\pm$ 67.34
	POST	113.25 $\pm$ 67.32
Placebo	PRE	86.04 $\pm$ 41.34
	POST	113.37 $\pm$ 49.48

2.4.3. Maximal voluntary contraction force

Maximal force values per group, muscle and session are presented in Table 2.3. The unstructured covariance matrix had the lowest AIC values (348.93) and, therefore, was employed in LMM. Type III tests of fixed effects pointed out no significant group*session or group*session*muscle interaction effects, implying that β -alanine supplementation did not result in any significant changes in maximal voluntary contraction force, as expected [group*session: $F(1, 6.66) = 0.38$, $p = 0.56$; group*session*muscle: $F(2, 10.31) = 0.22$, $p =$

0.81]. Also as expected, muscle was the only effect found with $p < 0.05$ [$F(2, 9.68) = 382.98$, $p < 0.01$].

Table 2.3. Maximal force by group, session and muscle. Data are expressed as mean $\pm$ SD. MVC = maximal voluntary contraction force; PRE = pre-supplementation session; POST = post-supplementation session; BI = biceps brachii; RF = rectus femoris; DE = lateral deltoid.

Group	Session	MVC (kgf)		
		BI	RF	DE
β -alanine	PRE	21.47 $\pm$ 4.49	63.17 $\pm$ 2.63	13.83 $\pm$ 1.52
	POST	21.73 $\pm$ 3.92	62.36 $\pm$ 6.64	13.52 $\pm$ 2.06
Placebo	PRE	20.18 $\pm$ 5.52	54.13 $\pm$ 9.86	15.34 $\pm$ 3.18
	POST	20.94 $\pm$ 4.97	56.80 $\pm$ 6.15	14.69 $\pm$ 3.80

2.4.4. Time to fatigue

The β -alanine supplementation had an effect on time to fatigue [group*session: $F(1, 11.9) = 12.18$, $p = 5 \times 10^{-3}$]. Tests of fixed effects reported significance only in group by session interaction. PRE vs POST paired t-test within PLA shows that data cannot be distinguished from differences by chance [$t(18) = 1.57$, $p = 0.13$; means $\pm$ SD: PRE, 81.43 $\pm$ 18.91 s; POST, 77.73 $\pm$ 21.31 s]. However, T_{lim} was found significantly increased by 9.54% (7.44 s increased; $d = 0.32$) in POST for group β -ALA [$t(16) = -2.52$, $p = 0.02$; means $\pm$ SD: PRE, 77.96 $\pm$ 23.09 s; POST, 85.4 $\pm$ 24.17 s]. Toeplitz covariance type was used (AIC = 520.18). T_{lim} values per group, muscle and session are presented in Table 2.4.

Table 2.4. Time to fatigue by group, session and muscle. Data are expressed as means $\pm$ SD. T_{lim} = time to fatigue; PRE = pre-supplementation session; POST = post-supplementation session; BI = biceps brachii; RF = rectus femoris; DE = lateral deltoid.

Group	Session	T_{lim} (s)		
		BI	RF	DE
β -alanine	PRE	74.90 $\pm$ 19.62	87.40 $\pm$ 22.6	72.79 $\pm$ 29.72
	POST	81.80 $\pm$ 19.61	98.91 $\pm$ 22.34	76.92 $\pm$ 30.32
Placebo	PRE	75.52 $\pm$ 17.27	90.21 $\pm$ 26.86	78.97 $\pm$ 10.20
	POST	79.80 $\pm$ 10.35	88.88 $\pm$ 30.43	66.40 $\pm$ 14.91

2.4.5. Mean power frequency

The intervention had no significant effect on $MNF_{initial}$ [group*session: $F(1, 11.1) = 1.07$, $p = 0.32$; group*session*muscle: $F(2, 10.48) = 0.41$, $p = 0.67$]. Similarly, none the other main or interaction effects were found. Unstructured covariance matrix was used, which had the lowest AIC (418.63). Data per group, session and muscle are presented in Figure 2.4 as mean $\pm$ SD.

Figure 2.4. $MNF_{initial}$ by group, session and muscle. Data are expressed as mean $\pm$ SD. MNF = mean power frequency; MNF_{MVC} = MNF at maximal force; $MNF_{initial}$ = average MNF of the first 10% MNF values of the submaximal contraction; PRE = pre-supplementation session; POST = post-supplementation session; BI = biceps brachii; RF = rectus femoris; DE = lateral deltoid.

The use of β -alanine supplementation showed no distinguishable effect on MNF_{final} [group*session: $F(1, 58) = 0.05$, $p = 0.82$; group*session*muscle: $F(2, 58) = 0.07$, $p = 0.93$]. Group and muscle main effects were significant [group: $F(1, 58) = 4.11$, $p = 0.047$; muscle: $F(2, 58) = 26.79$, $p < 0.01$]. With AIC equal to 418.44, identity covariance structure was applied. Overall MNF_{final} for β -ALA and PLA was 73.11 ± 0.9 % MNF_{MVC} and 77.18 ± 0.27 % MNF_{MVC} , respectively. *Rectus femoris* presented the highest values for MNF_{final} , 85.12 ± 6.72

% MNF_{MVC} . The lowest average MNF_{final} was found in the lateral deltoid, 68.27 ± 5.187 % MNF_{MVC} . Finally, the *biceps brachii* average was 74.29 ± 7.13 % MNF_{MVC} . MNF_{final} results are displayed in Figure 2.5.

Figure 2.5. MNF_{final} by group, session and muscle. Data are expressed as mean $\pm$ SD. MNF = mean power frequency; MNF_{MVC} = MNF at maximal force; MNF_{final} = average MNF of the last 10% MNF values of the submaximal contraction; PRE = pre-supplementation session; POST = post-supplementation session; BI = biceps brachii; RF = rectus femoris; DE = lateral deltoid.

No effect of the intervention was present in the MNF_{ratio} data [group*session: $F(1, 58) = 0.02$, $p = 0.89$; group*session*muscle: $F(2, 58) = 0.45$, $p = 0.64$], illustrated in the Figure 2.6. Significance was found in the LMM (covariance type = identity; AIC = 378.33) for group [$F(1, 58) = 7.17$, $p = 0.01$], muscle [$F(2, 58) = 44.51$, $p < 0.01$] and group*muscle effects [$F(2, 58) = 4.89$, $p = 0.01$]. Results by group and muscle are presented in Table 2.5. The main source of variation comparing group by muscles MNF_{ratio} comes from RF, with PLA values 9.06% $MNF_{initial}$ higher than in β -ALA ($d = 2.79$). Furthermore, muscles reflect the pattern found in MNF_{final} . RF had the highest average among muscles and DE the lowest (mean $\pm$ SD: BI, 77.72 ± 6.18 % $MNF_{initial}$; RF, 87.71 ± 5.56 % $MNF_{initial}$; DE, 71.73 ± 4.12 % $MNF_{initial}$).

Figure 2.6. MNF_{ratio} by group, session and muscle. Data are expressed as mean $\pm$ SD. MNF = mean power frequency; $MNF_{initial}$ = average MNF of the first 10% MNF values of the submaximal contraction; MNF_{ratio} = MNF_{final} divided by $MNF_{initial}$; MNF_{final} = average MNF of the last 10% MNF values of the submaximal contraction; PRE = pre-supplementation session; POST = post-supplementation session; BI = biceps brachii; RF = rectus femoris; DE = lateral deltoid.

Table 2.5. MNF_{ratio} by group and muscle. Results from both experimental sessions are included. Data are expressed as mean $\pm$ SD. MNF = mean power frequency; MNF_{ratio} = MNF_{final} divided by $MNF_{initial}$; MNF_{final} = average MNF of the last 10% MNF values of the submaximal contraction; $MNF_{initial}$ = average MNF of the first 10% MNF values of the submaximal contraction; BI = biceps brachii; RF = rectus femoris; DE = lateral deltoid.

Group	Muscle	MNF_{ratio} (% $MNF_{initial}$)
β-alanine	BI	76.95 ± 5.55
	RF	82.27 ± 2.84
	DE	72.06 ± 5.06
Placebo	BI	78.61 ± 7.23
	RF	91.33 ± 3.92
	DE	71.48 ± 3.67

MNF_{slope} results are shown in Figure 2.7. The three-way interaction was found significant [covariance type = unstructured; AIC = -101.34; $F(2, 9.66) = 4.6$, $p = 0.04$]. Analysing the group and session factors by each muscle, the group*session interaction presented $p < 0.05$ for DE [covariance type = compound symmetry; AIC = -36.83; $F(1, 10) = 6.01$, $p = 0.03$]. Pairwise comparisons between PRE and POST by group for DE data reported a decrease in MNF_{slope} in group placebo [$t(6) = 2.91$, $p = 0.03$, $d = 0.941$; means $\pm$ SD: PRE, -0.38 ± 0.05 % MNF_{initial}/s; POST, -0.43 ± 0.06 % MNF_{initial}/s]. This difference seems to be a consequence of reduced T_{lim} (Table 2.4) values in POST rather than changes in MNF_{initial} (Figure 2.4) or MNF_{final} (Figure 2.5) in this set of data. Again, this group*muscle interaction was not significant for none of the three variables when analysed individually.

Figure 2.7. MNF_{slope} by group, session and muscle. Data are expressed as mean $\pm$ SD. MNF = mean power frequency; MNF_{slope} = division of the difference between MNF_{final} and MNF_{initial} by MNF_{initial} and then by T_{lim} ; MNF_{final} = average MNF of the last 10% MNF values of the submaximal contraction; MNF_{initial} = average MNF of the first 10% MNF values of the submaximal contraction; T_{lim} = time to fatigue; PRE = pre-supplementation session; POST = post-supplementation session; BI = biceps brachii; RF = rectus femoris; DE = lateral deltoid.

Average MNF time-series are presented in Figure 2.8, with time expressed relative to T_{lim} . Data are categorised by group, session and muscle. Considering the y-intercepts, the values at T_{lim} and the results above detailed, little or no influence is seen from β-alanine

supplementation in the $MNF_{initial}$ and MNF_{final} during fatiguing contractions. Figure 2.9 shows the same average MNF curves but expressed as absolute values in seconds. The impact of increased time to fatigue in β -ALA POST on MNF_{slope} can be visualised in the biceps MNF time-series. However, the influence on the other muscle results is not distinguishable.

Figure 2.8. Average MNF time-series by group, session and muscle with time relative to T_{lim} . MNF = mean power frequency; $MNF_{initial}$ = average MNF of the first 10% MNF values of the submaximal contraction; T_{lim} = time to fatigue; PRE = pre-supplementation session; POST = post-supplementation session; BI = biceps brachii; RF = rectus femoris; DE = lateral deltoid.

Figure 2.9. Average MNF time-series by group, session and muscle. MNF = mean power frequency; $MNF_{initial}$ = average MNF of the first 10% MNF values of the submaximal contraction; PRE = pre-supplementation session; POST = post-supplementation session; BI = biceps brachii; RF = rectus femoris; DE = lateral deltoid.

2.5. Discussion

2.5.1. Maximal force and time to fatigue

In our study, muscle endurance increased whereas the maximal force remained unchanged with the ingestion of β -alanine supplementation. Similar findings were reported by

C. Sale, Hill, Ponte, and Harris (2012) for both variables in isometric knee extensions at 45% MVC. Additionally, the maximal force seems not to be influenced by the supplementation not only in isometric but also in dynamic contractions (Hoffman et al., 2006; Kendrick et al., 2008), which seems logical given that maximal force output is not limited by buffering capacity. On the other hand, contrasting results are available when analysing muscle endurance in different protocols, as reviewed by Artioli et al. (2010), possibly due to inconsistencies in the exercise acidotic potential between studies. For intensities around the 50% MVC here used, muscle acidosis and accumulation of anaerobic glycolysis by-products have been reported for isometric contractions (Ahlborg et al., 1972; Beliveau et al., 1992; Bendahan et al., 1996; Laurent et al., 1993). These data support that our intervention may have successfully enhanced buffering capacity in β -ALA.

2.5.2. Dietary β -alanine intake

Considering β -alanine molecular weight as 89.094 g/mol (National Center for Biotechnology Information, 2019) and the results from both weight and daily consumption of the amino acid, average intake preceding the study was around 559.52 ± 243.09 mg/day in PLA and 952.32 ± 387.52 mg/day in β -ALA. Dietary recalls from placebo group pointed out to an increase of daily ingestion of β -alanine about 190.55 ± 473.01 mg/day, when a decrease of 245.03 ± 462.76 mg/day was calculated for the β -alanine supplemented group. Subtracting this decrease from the given 6.4 g/day, the effective intake increase during the investigation was 5.96 g/day in β -ALA. Higher intramuscular carnosine concentrations have been reported for supplementation doses equal to or greater than 3.2 g/day (Artioli et al., 2010; Craig Sale et al., 2010). Thus, it is reasonable to assume that discrepancies in dietary β -alanine intake did not interfere with our results, especially considering that no significant differences in such intakes were found.

2.5.3. Anthropometric variables

Any extreme variation on a volunteer's anthropometric variables between PRE and POST would imply in alterations on the relative position of motor units in regards to the EMG detection system. It is accepted that anatomic and geometrical properties, such as the thickness of subcutaneous tissue layers, impact how the MUAPs are perceived by the electrodes (Farina,

Merletti, & Enoka, 2004). However, none of the anthropometric measures was found altered between groups and sessions; hence, their effects are not relevant in our results.

2.5.4. $MNF_{initial}$

Fluctuations in muscle pH are a critical element behind the EMG spectral parameters since spectrum density reflects variations on fibre conduction velocity, which is responsive to muscle acidosis (Brody et al., 1991). In the current study, volunteers in group β -ALA consumed β -alanine supplementation for 28 days, which is demonstrated to improve the intramuscular carnosine content, mostly known as an intramuscular buffer (Artoli et al., 2010; Craig Sale et al., 2010). In other words, the extra amino acid ingestion would enhance intramuscular buffering capacity without any projected outcome on proton concentration in a resting muscle. Therefore, the lack of significance in $MNF_{initial}$ brings no surprise.

2.5.5. MNF during fatiguing contractions

Our main concern was to determine whether a modification on the fatigue behaviour of the EMG power spectral density could be distinguished when buffering capacity is manipulated. All MNF variables were found not significantly altered by β -alanine supplementation. Therefore, the answer to our main question is no. On the other hand, the absence of statistical significance does not specify the exact meaning of this negation. The possible interpretations are that β -alanine supplementation does not affect EMG frequency components or that an effect exists, but it was too small to be significant given data variance.

Considering exclusively the eq. 2.3 and the T_{lim} results, it is not possible to hold constant both MNF_{ratio} and MNF_{slope} while T_{lim} varies. By definition, one of the two variables should change, so the other remains the same. This reasoning suggests that, theoretically, an effect of β -alanine should be present in one or more of the four MNF variables. In other words, the best interpretation for the non-significance of the presented results is that any existent effect was not sufficiently strong to be distinguished. Although possible, $MNF_{initial}$ is not likely to be affected, as already discussed, and at least one of the other three variables are better candidates. EMG records are a complex biological signal, a function of numerous factors (Farina et al., 2004). Such complexity may be the key behind a small effect size and the inability to detect significant intervention changes.

Firstly, the MNF exhibited the anticipated and well-known frequency compression towards lower frequencies during fatigue (Merletti & Parker, 2004). As already cited, the fall in fibre conduction velocity is considered to be a key component behind this compression (Bigland-Ritchie, Donovan, & Roussos, 1981; Brody et al., 1991; Zwarts, Van Weerden, & Haenen, 1987) and muscle acidosis has been associated with the reduction of both the propagation velocity and MNF values (Brody et al., 1991; Mortimer et al., 1970; Vestergaardpoulsen et al., 1992). Still, contrasting results were reported in the literature. For instance, a dissociation of the post-exercise recovery of myoelectric variables and pH (Miller et al., 1987; Vestergaardpoulsen, Thomsen, Sinkjaer, & Henriksen, 1995) and occurrences of PSD compression with no decrease in MFCV (Arendt-Nielsen & Mills, 1985; Eberstein & Beattie, 1985; Sadoyama et al., 1983). Brody et al. (1991) elucidated such inconsistencies by demonstrating modifications in both MFCV and fundamental shape of the M-wave shape during sustained electrically stimulated contractions from *in vitro* muscle preparations. Consequently, conduction velocity behaviour explains the MNF dynamics during fatigue only partially. The changes in the M-wave shape were considered to be coupled with differential rates of depolarisation and repolarisation of the sarcolemma. These rates may be shaped by modifications of ions Na^+ and K^+ gradients around the fibre membrane, alterations which are observed during fatiguing contractions (Clausen, 2008; Clausen, Nielsen, Harrison, Flatman, & Overgaard, 1998).

Furthermore, *in vivo*, other elements might modulate the frequency shift, such as the recruitment of new motor units and firing rate modulation. Through the course of the fatigue, a lowering of recruitment threshold is reported with consequential recruitment of new MUs with progressively larger fibres (Vollestad, Vaage, & Hermansen, 1984), following the size principle (Henneman, Somjen, & Carpenter, 1965). These changes have a positive effect on the average MFCV, as greater fibre diameters are correlated with faster conduction velocities (Hakansson, 1956). The conduction velocity is also positively influenced by an increase in MU firing rate under certain conditions (Morimoto & Masuda, 1984). Nonetheless, both increase (Bigland-Ritchie, Cafarelli, & Vollestad, 1986) and a decrease in discharge rates (Garland, Enoka, Serrano, & Robinson, 1994) were reported during sustained contractions. The contradictory findings are discussed as a product of distinct control strategies between muscles and exerted powers (Garland et al., 1994; Kuchinad, Ivanova, & Garland, 2004). Rises in firing rate may be a mechanism to compensate for mechanical loss during fatigue under certain circumstances. On the other hand, the drop in those rates is hypothesised to be induced by an accumulation of metabolic by-products during fatigue, which would cause an inhibitory feedback response via

group III and IV afferents (Bigland-Ritchie & Woods, 1984; Woods, Furbush, & Bigland-Ritchie, 1987).

Hence, the rate of MNF reduction (MNF_{slope}) and MNF value at failure (MNF_{final} and MNF_{ratio}) are a complex interaction of those factors and possibly others. Unfortunately, without the concomitant access to metabolical variables (for instance, muscle pH or lactate concentration) or MFCV, the ability to draw physiological precise and valid conclusions from bipolar surface EMG is limited (for reviews, see: Dimitrova & Dimitrov, 2003; Farina et al., 2004; Vigotsky, Halperin, Lehman, Trajano, & Vieira, 2018) and beyond the scope of the current investigation.

2.6. Conclusion

The β -alanine supplemented group showed an elevation in the time to fatigue, as expected. Also, according to the expectations, MNF displayed the well-documented movement towards lower frequencies during fatigue and $MNF_{initial}$ seems to be not affected by β -alanine supplementation. It was discussed that within a context of increased muscle endurance, changes on the MNF fatigue curve could be theoretically presumed. However, our data reported non-significance for all $MNF_{initial}$, MNF_{final} , MNF_{ratio} and MNF_{slope} variables. Thus, it is concluded that increased buffering capacity does not imply in a practical and distinguishable effect on the EMG power spectrum, regardless of the presumed behaviour. The lack of significance is discussed as that any present effect is too subtle to be distinguished, possibly due to the EMG multifaceted nature.

2.7. References

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CHAPTER III

CHARACTERISATION OF AREA1 OF APPROXIMATE ENTROPY IN ELECTROMYOGRAPHIC SIGNALS DURING SUSTAINED CONTRACTIONS

3. CHAPTER III: CHARACTERISATION OF AREA1 OF APPROXIMATE ENTROPY IN ELECTROMYOGRAPHIC SIGNALS DURING SUSTAINED CONTRACTIONS

3.1. Abstract

The purpose of the present study was to investigate the behaviour of electromyogram's (EMG) complexity via Area1 of Approximate Entropy (a1ApEn) during sustained isometric contractions at submaximal forces. Fourteen healthy volunteers participated in this project. Sustained isometric contractions of triceps brachii were performed at three different intensities (30, 50 and 70 % of maximum voluntary contraction - MVC) for 25.6 s, while surface EMG was recorded. This protocol was repeated on two different days. From the EMG records, the a1ApEn estimator was calculated with non-overlapping sequential windows of 256 ms. Also, root mean square (RMS) and mean power frequency (MNF) estimators were obtained for comparison. Linear regressions of the estimators against time were fitted. Lastly, correlations between a1ApEn, RMS and MPF were calculated. During the sustained contraction, a1ApEn demonstrated a consistent pattern in both days. EMG complexity showed a linear decrease at all intensities. The more intense the contraction, the more pronounced the decrease. No significant effect of intensity was found among the y-intercepts of a1ApEn regressions. The a1ApEn showed similarities with MNF results and an increasing correlation between both is seen with greater contraction forces. The findings here reported suggest that a1ApEn is responsive to electrophysiological alterations of muscles during sustained contractions. However, its ability to bring new information about the myoelectrical signal is questioned.

Keywords: a1ApEn; complexity; electromyography; fatigue; submaximal force.

3.2. Introduction

Electromyography (EMG) is a method that measures the electrical activity of a particular muscle and is a crucial tool in investigations in a wide range of areas, from physiology to sport and clinical sciences. Generally speaking, the EMG results are a reflex of several physiologic properties, including motor unit (MU) recruitment, firing rate, conduction velocity, shape of the muscle fibre action potentials and MU synchronisation (for review, see: Farina, Merletti, & Enoka, 2004; Merletti & Parker, 2004; Vigotsky, Halperin, Lehman, Trajano, & Vieira, 2018). Traditionally, the EMG signal is approached by time and time-frequency analyses. For instance, both EMG amplitude and power spectrum density (PSD) vary with contraction force requirement and with time during sustained efforts. Signal amplitude measured by root mean square (RMS) was found increased with fatigue and higher submaximal contraction intensities (Vigotsky et al., 2018). MU recruitment and firing rate dynamics seem to play a major role in those scenarios (Merletti & Parker, 2004). Another example is the EMG frequency compression observed during fatigue (Merletti & Parker, 2004). Alterations in muscle fibre conduction velocity (MFCV) and action potential shape are related to this compression (Brody, Pollock, Roy, De Luca, & Celli, 1991). However, relatively few studies investigated non-linear tools in the myoelectrical records. Acharya, Ng, Swapna, and Michelle (2011) reported high-accuracy when using classification algorithms to distinguish between healthy *versus* pathologic EMG using non-linear features such as Approximate Entropy (ApEn). Xie, Guo, and Zheng (2010) found that Fuzzy Approximate Entropy (*f*ApEn), another entropy estimator based on ApEn, decreases throughout sustained contractions, whereas ApEn remained unchanged.

Information entropies (IE) have been established since Shannon's studies in communication systems and his proposition of a measure (broad sense) to estimate the uncertainty degree in a message (Shannon, 1948). The basic idea behind these entropies is to give a value to the occupancy of a vectorial space (the so-called phase-space) of a given arbitrary dimension by the elements of a time-series. The vaster such an occupancy is, the higher the entropy value associated with the series, and, thus, more uncertainty one has in respect to the data. Eventually, a substantial number of entropies were developed on theoretical grounds, and some became applied in several types of analysis. For instance, Approximate Entropy developed by S. M. Pincus (1991) is one of the established tools and has been used in a wide spectrum of scenarios to measure the uncertainty of a signal. In a few words, ApEn

evaluates the complexity of an array by counting the occurrences of repeated events of a sub-array along the original one, given a chosen tolerance. The ApEn estimator was found useful to provide information about biological data, such as body temperature (Cuesta et al., 2007), heart rate (Shin et al., 2006) and muscle electrical activity (Zhou, Barkhaus, Zhang, & Rymer, 2011).

Recently, our group developed a new IE estimator (Natali & Chaui-Berlinck, 2016). This estimator is derived from the Approximate Entropy and seeks to overcome the subjectivity problems in the choice of parameters to calculate ApEn. Namely, the choice of the tolerance of equality in and of the dimension of the phase-space (Castiglioni & Di Rienzo, 2008). In other words, one might classify as more or less uncertain a given data set depending on an arbitrary choice of these two parameters. Sample Entropy, broadly used IE estimator derived from the ApEn (Richman & Moorman, 2000), presents the same arbitrariness problem. In general terms, our estimator computes ApEn for all possible tolerances and fixes the dimension to 1, and, then, computes the (numerical) area under the curve of these ApEn values. Therefore, we named the estimator as a1ApEn (for the area of dimension 1 of ApEn), and we proved that this estimator has consistency without subjectivity (Natali & Chaui-Berlinck, 2016) and is a valid tool to assess heart rate data (Natali, 2015). Hence, the applicability of the a1ApEn in other biological contexts is promising and not yet explored, especially in EMG records.

In such a scenario, this work investigated the behaviour of a1ApEn of EMG data collected during submaximal isometric contractions of triceps brachii. Three submaximal intensities were used, 30, 50 and 70% of maximal voluntary contraction force (MVC), and procedures were repeated at two different days. The main objectives were to characterise a1ApEn behaviour during sustained submaximal contractions, investigate its consistency between different days, and to assess possible influences of contraction intensity. Additionally, we compared the findings with other well-known EMG variables patterns: RMS and mean power frequency (MNF) of the PSD changes through time.

3.3. Materials and methods

3.3.1. Ethical approval

The study was approved by the ethical committee of the Biosciences Institute of the University of São Paulo (CAAE 67648617.0.0000.5464). Before the start of the procedures, all

participants were aware of possible risks or benefits related to their enrolment in the study and conferred written informed consent.

3.3.2. Subjects

A total of fourteen healthy adults (mean $\pm$ SD: age, 26.71 ± 10.06 years; weight, 63.32 ± 18.45 kg; height, 165.86 ± 11.73 cm) volunteered to participate in this investigation. The group was composed of eight women (mean $\pm$ SD: age, 22.38 ± 1.6 years; weight, 50.13 ± 7.7 kg; height, 157.75 ± 4.68 cm) and six men (mean $\pm$ SD: age, 32.5 ± 13.75 years; weight, 80.92 ± 12.31 kg; height, 176.67 ± 9.05 cm). Health history was evaluated through an interview and no case of disease or health condition was declared. The physical activity level of the participants ranged between sedentary and light physical activities practice (0 to 4 hours of light/medium intensity physical activities per week).

3.3.3. Study design

The protocol consisted of two identical experimental sessions 10 to 15-day distant apart from each other. At the beginning of both first (D1) and last (D2) sessions, the maximal voluntary contraction force (MVC) was obtained from triceps brachii. Then, surface electromyography was recorded for the muscle during three sustained isometric elbow extensions at different submaximal forces: 30% MVC (i30), 50% MVC (i50) and 70% MVC (i70). The order of submaximal intensities which each subject performed the contractions was random. From the EMG records, a1ApEn, MNF and RMS were calculated using a windowed analysis with a 256-ms non-overlapping window. The contractions were sustained for 25.6 seconds, corresponding to 100 windows. The individuals were instructed not to exercise in the previous 48 hours before the experimental sessions.

3.3.4. Exercises

Both maximal and submaximal contractions of triceps brachii were performed by elbow extensions of the dominant arm, in a standardised and stabilised position, against the digital scale Wii Balance Board (WBB; Nintendo, Kyoto, Japan). The position consisted of volunteers

sitting in a chair, stabilising their backs against the backrest, with the elbow alongside the body flexed at 90° and forearm in a neutral position, with the wrist against the scale, which was positioned over a wooden board on top of their legs. The WBB was connected to the computer software LabChart v.8.1.13 (ADInstruments, Sydney, Australia), which continuously registered real-time force output. The maximal force was obtained as the highest force value within three maximal elbow extensions of 4 seconds, intertwined with 1-minute rest intervals. Submaximal contractions were executed in the i30, i50 and i70 intensities, relative to the maximal force previously measured. Visual feedback of the force output was provided during the exercises and the subjects were requested to hold the desired force using the visual feedback as a reference, remaining within a zone of $\pm 2.5\%$ MVC around the target intensity. Between the three 25.6-second contractions, it was given a rest interval of 5 minutes. The interval between the end of the MVC assessment and the first submaximal intensity was of 10 minutes.

3.3.5. Electromyography

The surface EMG activity was recorded employing two pre-gelled, silver chloride surface electrodes (Eletrodo 2223, 3M, Sumaré, Brazil). The interelectrode distance was 20 mm. A single ground electrode was placed over the wrist. Prior to electrode attachment, the skin was prepared by abrading and cleaning it with alcohol. Triceps brachii electrodes were placed over the long head, on the midpoint of the line connecting the posterior crista of the acromion and the olecranon at two finger widths medial to the line. The raw EMG signal was obtained with an Octal Bio Amp (ADInstruments, Sydney, Australia) and converted into digital using a PowerLab 16/35 (ADInstruments, Sydney, Australia). Data were sampled at 1 kHz, bandpass filtered from 10 to 500 Hz and additionally filtered for electrical noise with an adaptative mains filter. Recordings were done with the software LabChart v8.1.13 (ADInstruments, Sydney, Australia).

3.3.6. EMG variables

Short-time Fourier Transform was applied to all EMG records. Non-overlapping, rectangular, 256-ms windows were used and a1ApEn, MNF and RMS obtained for each one. For each volunteer at each session, the EMG variables were normalized by dividing them by their maximum observed value. Data showed a linear behaviour, therefore, we proceeded with

linear regressions for each submaximal contraction and y-intercept and the slope was obtained. All data processing was done in MATLAB v.2018b (The MathWorks Inc, Natick, United States of America).

3.3.7. Statistical analysis

Correlations between a1ApEn, MNF and RMS and their linear regression parameters were analysed. Correlations were assessed with Pearson's correlation coefficients between the normalised time-series of the three EMG variables. The intercept and slopes of a1ApEn, MNF and RMS were utilised. From these data, the influence of different sessions and intensities were accessed through two-way repeated measures analysis of variance (ANOVA) using session, intensity and the interaction session*intensity as factors. Post-hoc comparisons were performed with Tukey's test. Statistics were performed using SPSS v.23 (IBM® SPSS® Statistics Version 23, SPSS Inc, Chicago, United States of America). Statistical significance level was set at $p < 0.05$. All numbers are rounded to two decimal places and values are expressed as means $\pm$ standard deviations (SD).

3.4. Results

3.4.1. EMG variables' curves during the sustained contractions

Figure 3.1 shows the a1ApEn, MNF and RMS values during the course of contraction for all individual EMG records. Complexity presented an approximately linear decline in all scenarios. The same pattern was observed in MNF. EMG amplitude increased through time, with the RMS curve resembling a logarithmic growth.

Figure 3.1. Series of EMG variables for all individual records. Data are expressed by EMG variable, session and intensity.

Average curves for the three variables are presented in Figure 3.2. Overall, no clear visual distinction exists between sessions D1 and D2. Average RMS curve at D2 and i50 is slightly shifted towards lower values. However, similar observations are absent in all other scenarios.

Figure 3.2. Average curves of the EMG variables during the contractions. Data are expressed by EMG variables, sessions and intensities.

As both a1ApEn and MNF displayed a visual linear curve behaviour and the logarithmic aspect of RMS was not so extreme, the three EMG variables were processed with linear regressions for the sake of simplicity and greater compatibility. The overall linear regression R^2 for a1ApEn, MNF and RMS were 0.12 ± 0.10 , 0.30 ± 0.22 and 0.34 ± 0.22 , respectively.

3.4.2. a1ApEn linear regression parameters

No significant differences were found on the a1ApEn y-intercept due to effects of intensity [$F(2, 26) = 3.11, p = 0.06$], session [$F(1, 13) = 1.65, p = 0.22$] and the interaction intensity*session [$F(2, 26) = 1.51, p = 0.24$]. Results are shown in Figure 3.3.

Figure 3.3. a1ApEn intercept values. Data are expressed as means $\pm$ SD and grouped by session and intensity.

On the other hand, the intensity effect was significant for the slope in a1ApEn [$F(2, 26) = 23.02, p < 0.01$], whereas neither session [$F(1, 13) = 0.25, p = 0.63$] nor intensity*session [$F(2, 26) = 1.06, p = 0.36$] influence was present. Post-hoc Tukey's multicomparison test expressed that all intensities were significantly different between each other (i30 vs. i50, $p = 0.02$; i30 vs. i70, $p < 0.01$; i50 vs. i70, $p < 0.01$), with progressively steeper declines with greater force levels (Figure 3.4).

Figure 3.4. a1ApEn slope values. Data are expressed as means $\pm$ SD and grouped by session and intensity.

3.4.3. MNF linear regression parameters

The intensity was found to have an influence over MNF intercept [$F(2, 26) = 4.44$, $p = 0.02$]. None of the other effects had $p < 0.05$ in the ANOVA [session: $F(1, 13) = 0.24$, $p = 0.64$; intensity*section: $F(2, 26) = 1.08$, $p = 0.35$]. The Tukey's test pointed out that the MNF intercept was higher in i30 than in i50 (i30 vs. i50, $p = 0.03$; i30 vs. i70, $p = 0.06$; i50 vs. i70, $p = 0.94$). Data are presented in Figure 3.5.

Figure 3.5. MNF intercept values. Data are expressed as means $\pm$ SD and grouped by session and intensity.

The slopes of MNF (Figure 3.6) demonstrated a similar pattern to those reported for a1ApEn, with effects of intensity [$F(2, 26) = 33.41, p < 0.01$] and no influence of session main effect [$F(1, 13) = 1.46, p = 0.25$]. However, the interaction intensity*session presented $p < 0.05$ [$F(2, 26) = 4.17, p = 0.03$]. Post-hoc comparisons pointed differences between i30 and i70 at D1 ($p < 0.01$) and between all intensities at D2 (i30 vs. i50, $p = 0.02$; i30 vs. i70, $p < 0.01$; i50 vs. i70, $p < 0.01$). No significance was found between sessions within intensity levels, except by i70*D1 *versus* i70*D2 ($p = 0.03$).

Figure 3.6. MNF slope values. Data are expressed as means $\pm$ SD and grouped by session and intensity.

3.4.4. RMS linear regression parameters

The EMG amplitude results showed that the intercept value of their curves is sensitive to the contraction force [$F(2, 26) = 34.27$, $p < 0.01$], but not the other factors [session: $F(1, 13) < 0.01$, $p = 0.97$; intensity*session: $F(2, 26) = 0.18$, $p = 0.84$]. Progressively higher forces implied in greater initial amplitudes in the EMG (i30 vs. i50, $p < 0.01$; i30 vs. i70, $p < 0.01$; i50 vs. i70, $p < 0.01$), as seen in Figure 3.7.

Figure 3.7. RMS intercept values. Data are expressed as means $\pm$ SD and grouped by session and intensity.

The last linear regression parameter left is the RMS slope. Again, the exclusive influence of the intensity main effect was obtained [$F(2, 26) = 34.27$, $p < 0.01$]. Similarly, to the y-intercept, higher contraction forces implied in steeper amplitude curves (i30 vs. i50, $p = 0.01$; i30 vs. i70, $p < 0.01$; i50 vs. i70, $p < 0.01$). Data are reported in Figure 3.8.

Figure 3.8. RMS slope values. Data are expressed as means $\pm$ SD and grouped by session and intensity.

3.4.5. Correlation between EMG variable curves

The Pearson correlation coefficients between a1ApEn and MNF values ranged from 0.31 ± 0.15 at D1*i30 to 0.48 ± 0.09 at D2*i70, as displayed in Figure 3.9. These values showed to be affected by intensity [$F(2, 26) = 9.96$, $p < 0.01$], with i70 presenting greater correlations between the EMG variables than the other two contraction forces (i30 vs. i50, $p = 0.44$; i30 vs. i70, $p < 0.01$; i50 vs. i70, $p = 0.01$).

Figure 3.9. Pearson correlation coefficient of a1ApEn versus MNF individual curves. Data are expressed as means $\pm$ SD and grouped by session and intensity.

Conversely, the correlations between the complexity values and EMG amplitude seemed to remain about the same independently of the force elicited and the experimental session [intensity: $F(2, 26) = 0.47$, $p = 0.63$; session: $F(1, 13) = 1.38$, $p = 0.26$; intensity*session: $F(2, 26) = 0.2$, $p = 0.82$]. The correlation results varied between -0.42 ± 0.12 at D1*i70 and -0.36 ± 0.14 at D2*i30 (Figure 3.10).

Figure 3.10. Pearson correlation coefficient of a1ApEn versus RMS individual curves. Data are expressed as means $\pm$ SD and grouped by session and intensity.

The analysis of the correlation between the MNF and RMS values resulted in coefficients from -0.58 ± 0.17 to -0.44 ± 0.16 at D2 i70 and i30, respectively. These results, presented in Figure 3.11, were influenced by intensity level only [$F(2, 26) = 5.21$, $p = 0.01$], with stronger negative correlations observed in the most intense contractions (i30 vs. i70, $p = 0.03$; i50 vs. i70, $p = 0.02$), while i30 and i50 were not distinguishable ($p = 0.96$).

Figure 3.11. Pearson correlation coefficient of MNF versus RMS individual curves. Data are expressed as means $\pm$ SD and grouped by session and intensity.

3.5. Discussion

The current study aimed to characterise any possible changes of EMG complexity measured by a1ApEn during sustained isometric contractions at three different submaximal intensities. Our results provided that complexity drops linearly with the exercise. These findings are in accord with Xie et al. (2010), who observed similar complexity reduction at 80% MVC by utilising Fuzzy Approximate Entropy. Complementarily, we provided new information about how complexity may vary across submaximal forces. First, complexity at the beginning of the muscle contraction, represented by the y-intercept values, is unaffected by modifications of muscle force output. The opposite observation comes from the rate of complexity reduction, which gets more prominent with increased intensities.

The explanation for these results may rely at some degree on the spectral components of the electromyogram. MNF presented this same reduction at all submaximal forces here

analysed. Furthermore, y-intercepts and slopes from MNF curve presented similar dynamics to those seen in a1ApEn, with no influence overall of intensity over the intercept and a positive effect of it over the rate of decline during the contractions. A signal's power spectrum density and complexity changes seem to be related (Steven M. Pincus, 2006), with power spectrum curve compression towards an arbitrary frequency implying in a higher data organisation state and a decrease of complexity (Steven M. Pincus, 1994). For the EMG during fatigue, both an MNF fall and an increase of low-frequency energy are seen concomitantly (Merletti & Parker, 2004). However, the relationship between myoelectrical spectrum and complexity is likely due to the enhanced band energy rather than the overall frequency shift or the low-frequency aspect associated with the shift and compression.

The observed MNF reduction is a well-documented phenomenon and has been reported for both maximal and submaximal contractions (Farina et al., 2004). The EMG power spectrum density is explained at least partially by the MFCV and the fundamental shape of MU action potentials (Brody et al., 1991). Although the exact mechanism is still unknown, the propagation velocity reduction is associated with changes in muscle pH, which gets more acidotic at high-intensity sustained contractions (McArdle, Katch, & Katch, 2010), due to greater contributions of the anaerobic glycolysis pathway (Vinnakota & Kushmerick, 2011). The changes in the MU shape were considered to be coupled with differential rates of depolarisation and repolarisation of the sarcolemma. These rates may be shaped by modifications of ions Na^+ and K^+ gradients around the fibre membrane, alterations those are observed during fatiguing contractions (Clausen, 2008; Clausen, Nielsen, Harrison, Flatman, & Overgaard, 1998), particularly at more intense efforts.

Interestingly, we found an increasing correlation between a1ApEn and MNF values at higher intensities. Being the EMG record a complex interaction of several physiological (e.g., MFCV, MU recruitment, firing rate and MU synchronisation) and non-physiological (e.g., interelectrode distance, volume conductor shape and subcutaneous tissue layers thickness) elements (Farina et al., 2004), it is reasonable to assume a correspondent multifactorial nature also for a1ApEn. The better correlation between both EMG variables at higher intensities may be hypothesised as a differential relative contribution of the many factors underlying EMG signal a1ApEn, with a greater influence of common elements affecting both MNF and a1ApEn on signal complexity.

Notwithstanding the relationship between the frequency shift and loss of complexity, it is important to notice the magnitude of each dynamics. Although MNF and a1ApEn intercept values were similar (Figure 3.3 and Figure 3.5), the MNF slope was about the double of a1ApEn

slopes at all intensities (Figure 3.4 and Figure 3.6), showing that in EMG the a1ApEn measurement is less sensitive to fatigue-related events than the MNF. Conversely, these findings raise the interpretation that, despite the similarities, both EMG variables have unique properties and that a1ApEn may be relatively more influenced by elements independent to fatigue manifestation.

In regards to the RMS, the EMG amplitude gain observed in the current investigation was expected, since many studies report increased RMS values through sustained submaximal contractions and the opposite behaviour at maximal force (Farina et al., 2004). The two core mechanisms to explain those reports are the MU recruitment and firing rate modulation (Vigotsky et al., 2018). Both have a significant and similar positive influence over RMS values. At maximal intensity, when nearly no new MU is recruited and discharge rate falls, RMS follows the same decrease. At submaximal fatiguing contractions, the occurrence of recruitment of new MUs has a positive influence over RMS. The same is valid for firing rate. However, as a fall at firing rate is seen in high-intensity submaximal contractions and muscle control strategies vary considerably across different muscles and tasks, the sEMG amplitude can be influenced in unintuitive ways.

Although both RMS and a1ApEn slopes are responsive to changes in contraction force requirements, it is more likely that the similarities between both are due to the common element of fatigue rather than RMS values reflecting directly on signal complexity, especially considering that y-intercept displayed different patterns. The relatively constant correlations across the three submaximal forces may support that idea. Following the same rationale used for a1ApEn vs MNF correlations, it would remain constant the proportion between factors present in both complexity and amplitude *versus* factors that influence only one of the EMG variables or noise.

3.6. Conclusion

Our study demonstrates a reduction of EMG complexity during sustained isometric contractions, which is more pronounced with increased muscle force outputs. The a1ApEn data showed high resemblance with the observed results of MNF. However, differences in the magnitude of the fatigue behaviour between both variables shows that a1ApEn has unique aspects and has potential to provide novel information about muscle electrophysiology. On the

other hands, in regard to muscle fatigue, no evidence was found showing a1ApEn ability to provide new or better EMG information when compared to other broadly employed analyses.

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CHAPTER IV

FINAL POINTS AND CONCLUSIONS

4. CHAPTER IV: FINAL POINTS AND CONCLUSIONS

4.1. a1ApEn and β -alanine supplementation

In the previous chapters, it was discussed the core aspects of muscle electrophysiology along with concepts related to energetic pathways and fatigue. The main concern of the current thesis was to explore if changes in muscle buffering capacity could be tracked down along a myoelectrical record. This question was raised due to some key elements. The first is the occurrence of changes in EMG variables during sustained contractions, including time, time-frequency and non-linear ones (Farina, Merletti, & Enoka, 2004; Merletti & Parker, 2004; Vigotsky, Halperin, Lehman, Trajano, & Vieira, 2018). Second, the association between muscle acidosis and fatigue (Phillips, 2015). Third, the pH impact on muscle fibre conduction velocity (Brody, Pollock, Roy, De Luca, & Celli, 1991). Fourth, the relationship between MFCV and EMG frequency compression towards low frequencies (Merletti & Parker, 2004). Consequentially, the time-frequency analysis was the most obvious candidate to display possibly some influence of changes in buffering capacity. Therefore, the fundamental objective of the current thesis was to study the effects of β -alanine supplementation on the MNF, and the results were presented in chapter 2.

Additionally, we aimed to look at the non-linear variable a1ApEn developed by our group (Natali & Chaui-Berlinck, 2016) as a secondary objective. In the EMG field, few studies have approached non-linear analysis, but impressive results were reported. For instance, the ability to categorise healthily *versus* pathological EMG (Zhou, Barkhaus, Zhang, & Rymer, 2011) and evidence of loss of complexity during sustained contractions (Xie, Guo, & Zheng, 2010). As the a1ApEn has some unique aspects, namely the lack of arbitrariness at choosing a tolerance value, it was a natural path to question whether buffering capacity could also be detected with the use of a1ApEn. Nonetheless, one step was missing. Prior to assessing the relationship between a1ApEn and increased acidosis resistance during fatiguing contractions, the behaviour of a1ApEn itself during sustained efforts needed to be unveiled. This task was achieved in chapter 3.

The next step would have been analysing the a1ApEn patterns with β -alanine supplementation. However, given the findings reported in both chapter 2 and chapter 3, this subsequent investigation seemed less appropriate.

The characterisation of EMG complexity by a1ApEn presented a loss of complexity during the exercises. The higher the required force output, the steeper the slope was. Also, the y-intercept of a1ApEn remained the same between 30 and 70% MVC. A similar dynamic was found for MNF in the same experiments, but with greater responsiveness. It was discussed that the a1ApEn behaviour might be a function of the frequency compression observed and supporting literature was presented (Pincus, 1994, 2006). Important to notice that the power spectrum does not explain the signal complexity completely, especially at the lower forces, where the correlation between a1ApEn and MNF was weaker. However, the results did not provide strong reasons to expect that a1ApEn could bring new information on EMG fatigue behaviour or to be better and differentially related to muscle acidosis than MNF.

In chapter 2, it was demonstrated the inability to detect MNF changes due to the increased buffering capacity. As previously discussed, the observed higher time to fatigue means, by definition, that at least one parameter of MNF fatigue curve must change. However, significance was not found, implying that any possible MNF pattern change is not evident enough to be detected given EMG natural variability and sample size. If the MNF showed to be unable to portrait the expected changes, a1ApEn, which was found less responsive to fatigue and with greater variance, does not seem to be more appropriate for the objective of capturing EMG changes due to alterations in the buffering capacity.

For these reasons and to avoid excessive text repetition, the a1ApEn changes with β -alanine supplementation are not presented in an individual chapter following the article structure of the other two investigations. Still, the data was assessed and is presented briefly in the following paragraph.

Using the same data and methodology applied in chapter 2, a1ApEn_{initial}, a1ApEn_{final}, a1ApEn_{ratio} and a1ApEn_{slope} were calculated from the EMG a1ApEn time series and results are presented in Figure 4.1, Figure 4.2, Figure 4.3 and Figure 4.4, respectively. The β -alanine supplementation was found not to affect significantly none of the four a1ApEn variables, neither by the group*session effect [a1ApEn_{initial}: $F(1, 12) = 1.88$, $p = 0.2$; a1ApEn_{final}: $F(1, 12) = 0.5$, $p = 0.5$; a1ApEn_{ratio}: $F(1, 12) = 1.89$, $p = 0.19$; a1ApEn_{slope}: $F(1, 12) = 0.03$, $p = 0.87$] nor by the group*session*muscle interaction [a1ApEn_{initial}: $F(2, 12) = 0.45$, $p = 0.65$; a1ApEn_{final}: $F(2, 12) = 0.01$, $p = 0.99$; a1ApEn_{ratio}: $F(2, 12) = 1.03$, $p = 0.39$; a1ApEn_{slope}: $F(2, 12) = 1.42$, $p = 0.28$].

Figure 4.1. a1ApEn_{initial} by group, session and muscle. Data are expressed as mean $\pm$ SD. a1ApEn_{MVC} = a1ApEn at maximal force; a1ApEn_{initial} = average a1ApEn of the first 10% a1ApEn values of the submaximal contraction; PRE = pre-supplementation session; POST = post-supplementation session; BI = biceps brachii; RF = rectus femoris; DE = lateral deltoid.

Figure 4.2. a1ApEn_{final} by group, session and muscle. Data are expressed as mean $\pm$ SD.; a1ApEn_{MVC} = a1ApEn at maximal force; a1ApEn_{final} = average a1ApEn of the last 10% a1ApEn values of the submaximal contraction; PRE = pre-supplementation session; POST = post-supplementation session; BI = biceps brachii; RF = rectus femoris; DE = lateral deltoid.

Figure 4.3. a1ApEn_{ratio} by group, session and muscle. Data are expressed as mean ± SD. a1ApEn_{initial} = average a1ApEn of the first 10% a1ApEn values of the submaximal contraction; a1ApEn_{ratio} = a1ApEn_{final} divided by a1ApEn_{initial}; a1ApEn_{final} = average a1ApEn of the last 10% a1ApEn values of the submaximal contraction; PRE = pre-supplementation session; POST = post-supplementation session; BI = biceps brachii; RF = rectus femoris; DE = lateral deltoid.

Figure 4.4. a1ApEn_{slope} by group, session and muscle. Data are expressed as mean ± SD.; a1ApEn_{slope} = division of the difference between a1ApEn_{final} and a1ApEn_{initial} by a1ApEn_{initial} and then by T_{lim}; a1ApEn_{final} = average a1ApEn of the last 10% a1ApEn values of the submaximal contraction; a1ApEn_{initial} = average a1ApEn of the first 10% a1ApEn values of the submaximal contraction; T_{lim} = time to fatigue; PRE = pre-supplementation session; POST = post-supplementation session; BI = biceps brachii; RF = rectus femoris; DE = lateral deltoid.

4.2. Discussion

As stated before, $\alpha 1ApEn$ followed the same pattern than MNF. It becomes evident that EMG is not an appropriate tool to access muscle buffering capacity changes during fatigue. It was discussed in chapter 2 that, on theoretical grounds, EMG fatigue curves should change with alterations in time to fatigue. Absence of significance implies in lack of statistical power to detect such changes. It could be due to either a small sample size or small effect size.

The sample size is relative to the field of research. In some fields, 50 subjects may be insufficient, while 10 can be enough in others. Important discoveries in the muscle electrophysiology field were achieved with studies with similar sample sizes than ours. For instance, Arendt-Nielsen, Mills, and Forster (1989) used only five volunteers to study EMG amplitude, MNF and MFCV at isometric knee extensions at 10, 20, 30 and 40% MVC and observed a rise in the variables for the two first intensities and declines in MNF and MFCV for 30 and 40% MVC. It was concluded that at lower force outputs the recruitment of new MUs (following the size principle) caused the increase of MFCV and consequently MNF at 10 and 20% MVC (as larger MFs tend to have faster MFCVs), whereas at progressively higher intensities a negative effect from fatigue on MFCV and MNF dominates the behaviour of both variables. Eberstein and Beattie (1985) recorded EMG MNF and MFCV in *biceps brachii* of nine subjects at 60 and 70% MVC, observing declines of both variables with MFCV varying linearly with MNF. Xie et al. (2010) reported a loss of signal complexity measured by $fApEn$ from *biceps brachii* EMG data of 12 volunteers performing 80% MVC sustained isometric contractions. Hence, the sample size used chapter 2 was within the expected.

The effect size concept relates to the magnitude of a particular effect and several methods are available to estimate it (Fritz, Morris, & Richler, 2012). Cohen's d (Cohen, 1962) is a popular statistics to approach this magnitude when two conditions are compared. It is the difference between the means of the conditions expressed relatively to the standard deviation. In this scenario, a small effect size is another relative idea and depends on both the magnitude of changes and data variance, with the former being small compared to the latter. Considering the context of chapter 2, relatively high data dispersion may be explained by EMG multifaceted nature and the applied protocol.

Farina et al. (2004) list several physiological and non-physiological components of the myoelectrical signal, which could increase data variance considerably. Some of the non-physiological included features are the shape of the volume conductor, the thickness of

subcutaneous tissue layers, endplates and tendon regions position relative to the EMG detection system, interelectrode distance and muscle fibre shortening. These elements were controlled within our protocol, following the European recommendations for surface EMG (Hermens et al., 1999), which was developed in order to standardise EMG procedures and address the effects of the mentioned features over the electrical records. The physiological factors comprise of fibre membrane or motor unit properties. The last one englobes MU recruitment and firing rate modulation, discussed in chapter 1. Individual differences in MU control strategies throughout sustained contraction may influence directly global MFCV and MNF values, given the positive influence of faster discharge frequencies and larger newly recruited MUs over MFCV (Hakansson, 1956; Methenitis et al., 2016; Morimoto & Masuda, 1984).

Another possible source of high variance could be the inherent subjectivity of maximal voluntary efforts. To sustain a muscle contraction until muscle failure is a task dependent on the motivation levels of the volunteers and some variance due to psychological factors is expected in the maximal voluntary contraction time, the time to fatigue (T_{lim}). The variables MNF_{slope} and $a1ApEn_{slope}$ are particularly sensitive to T_{lim} alterations as both are a function of the time to fatigue.

4.3. Conclusions

Without extending any further the discussion, as more in-depth discussion and conclusions were presented in chapter 2 and chapter 3, we concluded that muscle buffering capacity changes are not detectable in EMG fatigue curves by the employed protocol. Small effect size is believed to be the cause of the lack of significance. Possibly, in much more controlled environments, such as *in vitro* experiments changes could be detected. However, these setups are distant from real situations. For those situations, electromyography is not an appropriate tool to evaluate muscle buffering capacity changes in the context of fatiguing contractions.

4.4. References

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