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Original article (short paper)

Exploratory study of electromyographic behavior of the vastus medialis and vastus lateralis at neuromuscular fatigue onset

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Abstract—This study aimed to determine and analyze the neuromuscular fatigue onset by median frequency (MDF) and the root mean square (RMS) behavior of an electromyographic signal (EMG). Eighteen healthy men with no prior knee problems initially performed three maximum voluntary isometric contractions (MVIC). After two days of MVIC test, participants performed a fatiguing protocol in which they performed submaximal knee-extension contractions at 20% and 70% MVIC held to exhaustion. The MDF and RMS values from the EMG signals were recorded from the vastus medialis (VM) and the vastus lateralis (VL). Analysis of the MDF and RMS behavior enabled identification of neuromuscular fatigue onset for VM and VL muscles in 20% and 70% loads. Alterations between the VM and VL in the neuromuscular fatigue onset, at 20% and 70% MVIC, were not significant. These findings suggest that the methodology proposal was capable of indicating minute differences sensible to alterations in the EMG signals, allowing identification of the moment when the MDF and the RMS showed significant changes in behavior. The methodology used was also a viable one for describing and identifying the neuromuscular fatigue onset by means of the analysis of EMG signals.

Keywords: electromyography, fatigue, isometric contraction, quadriceps muscle

Resumo—"Estudo exploratório do comportamento eletromiográfico do músculo vasto medial e vasto lateral no início da fadiga neuromuscular." Este estudo teve como objetivo determinar e analisar o início da fadiga neuromuscular pelo comportamento do sinal de eletromiográfico (EMG) da frequência mediana (FM) e do *root mean square* (RMS). Dezoito homens saudáveis, sem problemas no joelho, realizaram inicialmente três contrações isométricas voluntárias máximas (CVMs). Após dois dias de CVM os sujeitos realizaram um protocolo de fadiga em que realizaram contrações submáximas de extensão do joelho em 20% e 70% da CVM até a exaustão. Os valores dos sinais de FM e RMS foram registrados a partir do vasto medial (VM) e vasto lateral (VL). A análise do comportamento da FM e RMS permitiu a identificação do início da fadiga neuromuscular para os músculos VM e VL em 20% e 70% da carga máxima. Alterações entre o VM e VL no início da fadiga neuromuscular, com 20% e 70% do MVIC, não foram significativas. Estes resultados sugerem que a metodologia proposta foi capaz de indicar diferenças mínimas sensíveis a alterações nos sinais EMG, permitindo a identificação do momento em que a FM e o RMS apresentaram mudanças significativas no seu comportamento. A metodologia utilizada também foi viável para descrever e identificar o aparecimento da fadiga neuromuscular por meio de análise de sinais de EMG.

Palavras-chave: eletromiografia, fadiga, contração isométrica, músculo quadríceps

Resumén—"Estudio exploratorio de la conducta electromiográfica del vasto medial y vasto lateral en la aparición de fatiga neuromuscular." Este estudio tieve como objetivo determinar y analizar la aparición de fatiga neuromuscular por la frecuencia media (FM) y la media de la raíz cuadrada (RMS) de lo señal electromiográfico (EMG). Dieciocho hombres saludables que no tienen problemas de rodilla previas inicialmente realizaron tres contracciones máximas voluntarias (CVM). Después de dos días de CVM los sujetos realizaron un protocolo de fatiga en la que se presentaron submáximas de extensión de rodilla en 20% y el 70% CVM hasta el agotamiento. Los valores de FM y RMS de las señales EMG se registraron desde el vasto medial (VM) y el vasto lateral (VL). Análisis del comportamiento de FM y RMS activado identificación de inicio fatiga neuromuscular para VM y músculos VL y 20% y el 70 % de la carga maxima. Las alteraciones entre el VM y VL en el inicio de la fatiga neuromuscular, en el 20% y el 70% de la CVM, no fueron significativas. Estos hallazgos sugieren que la propuesta de metodología fue capaz de indicar las diferencias minutos sensibles a las alteraciones en las señales de EMG, que permitan identificar el momento en que el FM y el RMS mostraron cambios significativos en el comportamiento. La metodología utilizada fue también una opción viable para la descripción y la identificación de la aparición de fatiga neuromuscular por medio del análisis de las señales de EMG.

Palabras clave: electromiografía, fatiga, contracción isométrica, músculo cuadríceps

Introduction

Neuromuscular fatigue is known as a decline in the ability to generate muscular force (Montes, Alves, Gomes, Dezan, & Gomes, 2011). The decrease in the force generation caused by neuromuscular fatigue during exercise affects physical performance associated with an increasing in the real and/or perceived difficulty of a task (Boyas & Guével, 2011). The neuromuscular fatigue is generally considered to arise via two main mechanisms: central fatigue and peripheral fatigue. The central factors of fatigue comprise decreases in the voluntary activation of the muscle, which is due to decreases in the number of recruited motor units and their discharge rate. However, the peripheral factors of muscle fatigue include alterations in neuromuscular transmission and muscle action potential propagation and decreases in the contractile strength of the muscle fibers (Babault, Desbrosses, Fabre, Michaut, & Pousson, 2006; Enoka & Duchateau, 2008; Gonzalez-Izal, Malanda, Gorostiaga, & Izquierdo, 2012).

The reduced ability to generate force has been widely investigated in clinical and sporting areas, in which the understanding of muscular contraction under neuromuscular fatigue conditions is important since it involves a series of significant factors, such as the muscle type involved, contraction duration, overload level, and executed task type (Boyas & Guével, 2011).

Since neuromuscular fatigue development is considered a possible precursor to disorders and muscle pain, (Blangsted, Vedsted, Sjøgaard, & Sjøgaard, 2005), an increasing number of studies have focused on the analysis of it in tasks performed at low levels (Cook, Rosencrance, Zimmermann, Gerleman, & Ludewig, 1998; Roman-Liu & Konarska, 2005) and at high levels of muscle contraction (Kollmitzer, Ebenbichler, & Kopf, 1999, Mathur, Eng, & MacIntyre, 2005; Rainoldi, Falla, Mellor, Bennell, & Hodges, 2008). Merletti, Lo Conte and Orizio (1991) report that the fatigue may be interpreted as an attribute of a specific contraction and can therefore be represented by a number or index associated with the contraction. Thus, the variables related to the amplitude of the electromyographic (EMG) signal (e.g., root mean square [RMS]) and to its frequency content (e.g., median frequency of the power spectrum [MDF]) are commonly used to assess physiologically relevant aspects such as muscle-activation level and development of neuromuscular fatigue (Kallenberg & Hermens, 2008).

There is a demand for studies related to the application of techniques to investigate the moment of transition between the normal muscular work (without neuromuscular fatigue) and the onset of neuromuscular fatigue (De Luca, 1984; De Luca, 1997). Several studies described in the literature evaluated the RMS and MDF behavior only at the beginning and at the end of the tests to verify the neuromuscular fatigue (Callaghan, Mccarthy, & Oldham, 2009; Hedayatpour, Arendt-Nielsen, & Farina, 2008; Mathur et al., 2005; Watanabe & Akima, 2010). However, this concept of the onset of neuromuscular fatigue used by the authors cited above is based on the point at which a contraction can no longer be maintained (the failure point). Many studies have become accustomed to using the force output of a muscle as the index of muscle fatigue. The use of this failure point carries with it some practical disadvantages, for example, fatigue is detected only after it occurs (De Luca, 1984; De Luca, 1997).

Many neurophysiological mechanisms are disturbed before the body feels the effects of neuromuscular fatigue, and these changes sometimes constitute advance warning of neuromuscular fatigue (Boyas & Guevel, 2011), which can be verified by decreasing the force generated during testing (Hedayatpour et al., 2008; Mathur et al., 2005). The neuromuscular fatigue then develops itself progressively until the muscle is no longer able to perform the requested task (Boyas & Guevel, 2011).

Is important to observe that the reduction of force is firstly observed only at the end of testing, at which time the muscle is already working under neuromuscular fatigue. In this context, the aim of this study is to determine and analyze the onset of neuromuscular fatigue using the RMS and MDF behavior as a parameter to define the failure point (not the decrease of the output force), and to verify if the variables extracted from this moment differ from those found at the beginning and at the end of the tests, contributing to a better understanding of the process.

Methods

Participants

This study evaluated 18 male participants (age 21 ± 2 years, weight 79.1 ± 13.0 kg, height 166.4 ± 6.5 cm) who were recreationally active, with no previous history of trauma, surgery, pain, or any other neuromuscular knee disorder. The dominant limb was determined based on the participant preference while performing an act of kicking (Ebersole, O'Connor, & Wier, 2006). All participants provided written informed consent, approved by the local University Ethics Committee, prior to the tests.

Instrumentation

The EMG signal was collected from two pairs of surface electrodes in bipolar configuration on the skin covering the vasti muscles (Meditrace®, Kendall, Mansfield, MA, silver-silver chloride, 10 mm radius disc electrodes, fixed interelectrode distance of 20 mm). The electrode cable circuit had a preamplifier with a gain of 20 times, Common Mode Rejection greater than 80 dB and impedance of 1 KΩ. The areas chosen for electrode placement (electrical stimulation technique in motor points) were prepared by shaving, abraded by fine sandpaper, and cleaned with isopropyl alcohol (Silva et al. 2012); they were positioned parallel to the VM and VL muscles and fixed approximately 2 cm from the motor point in the direction of muscle fibers (De Luca, 1997).

The EMG signals were obtained in a signal conditioner module (BIO EMG 1000 model®, LYNX®, Electronic Technology Ltda, Sao Paulo - SP, Brazil). In this module, two channels were configured for the acquisition of an EMG signal (gain = 1000, sampling frequency = 4000 Hz) and storage of the signals into data files was achieved using the software, Bioinspector 1.8 (LYNX®). During the neuromuscular fatigue test, the force applied by the participant was monitored using a load cell (model MM®, KRATOS®, Cotia - SP, Brazil), synchronized with the EMG signals. A specific channel of the module signal conditioner was set to acquire the signals from the load cell with a second-order Butterworth low pass filter with a cutoff frequency of 100 Hz.

Protocol

The participant sat on a table knee extensor (VITTALY®, model convergent®, São José do Rio Preto - SP, Brazil) with back adjustments and support. The knee-joint position was maintained at 60º (Pincivero, Salfetnikov, Campy, & Coelho, 2004), and the hip was maintained at 90º of flexion. Positions of the hip and the knee were confirmed using a universal goniometer. The trunk, pelvis, and knee were firmly strapped to the table with a seatbelt.

Three Maximal Voluntary Isometric Contractions (MVIC) were performed, and verbal encouragement was provided for a six-second period to induce the participant to reach their highest level in each trial. Visual feedback of the produced force was provided. A five-minute break was given between MVIC trials. The first two seconds and the last two seconds of the MVIC signal were ignored. The overall average value of force recorded over the three attempts was selected as the reference MVIC, allowing targets to be set on a visual-feedback display.

Following two days of the MVIC test, the participants were asked to perform a submaximal knee-extension contraction at 20% (low intensity) and at 70% (high intensity) MVIC held to exhaustion. The choice of the testing order was random, and the exhaustion moment was considered to be the point at which the participant completely ceased exercise, or when the force output appeared to deviate more than 10% of the target (Callaghan et al., 2009; Silva et al., 2012). To assist in the control of load intensity, the force applied was monitored in real time by load cell, representing a visual display of feedback of the force output. A 30-minute rest period was provided between the submaximal contractions.

Data analysis

The off-line processing of the EMG signals was performed in MATLAB (The MathWorks® Inc., Natick, MA). The signal was band-pass filtered (second order Butterworth) at 20-400 Hz. The power spectral density and frequency characteristics were determined using Welch's averaged periodogram method and the fast Fourier transform technique. The stationarity test was not performed because the signal stationarity is assumed in isometric contractions (Bilodeau et al., 1997; De Luca, 1997; De Luca, Foley, & Erim, 1996; Grabiner et al., 1991; Pincivero, Gandhi, Timmons, & Coelho, 2006). Following this processing, the MDF and RMS shifts were computed. To indicate the presence of neuromuscular fatigue, it is important to determine how the final MDF and RMS values relate to the initial MDF and RMS values, respectively (Mathur et al., 2005). Therefore, we chose to normalize the MDF by averages obtained from five initial values of the signal, and the RMS was normalized by the RMS obtained in MVIC tests.

For each testing session, the following were selected from the EMG signal for analysis: (1) beginning of the test; (2) onset of neuromuscular fatigue (NF); and (3) end of the test. At the beginning of the test, the initial MDF (I_{MDF}) and RMS (I_{RMS}) were calculated considering the average of the first five samples (2.5 s) of MDF and RMS from the EMG signal. The final MDF (F_{MDF}) and RMS (F_{RMS}) were calculated considering the average of the last five samples (2.5 s) of MDF and RMS from the EMG signal. In the NF determination, two methodologies were used in this study: (1) the analysis of the MDF slope coefficient; and (2) analysis of the RMS slope coefficient (Figure 1).

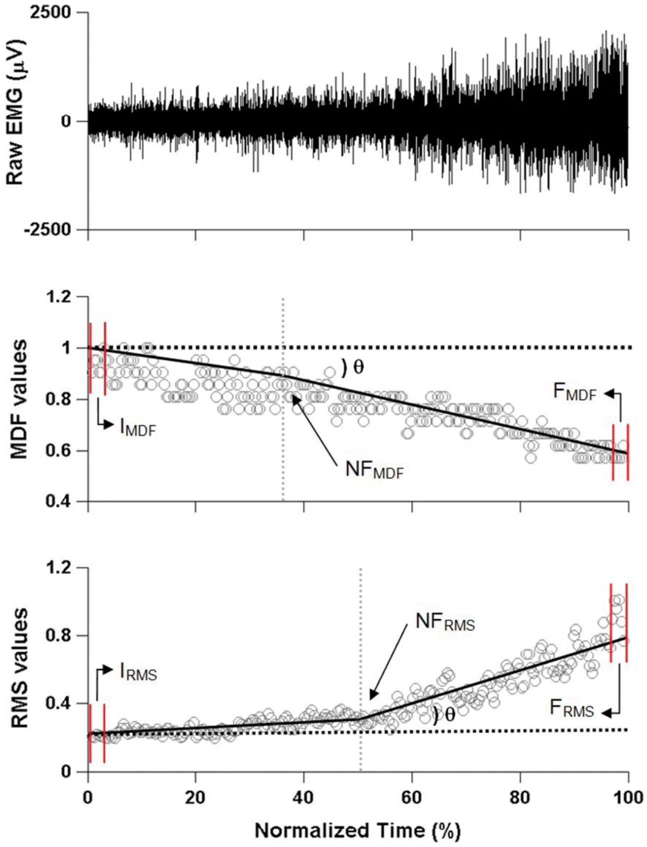

Figure 1. Example of the methodology used in the NF determination during a fatigue test at 20% MVIC.

Table 1. Mean (M), standard deviation (SD) and confidence interval (95% CI) for the normalized time values (%) of MDF slope coefficient (MF_{MDF}) and RMS slope coefficient (MF_{RMS}) at 20% and 70% of maximum voluntary isometric contraction (MVIC) for vastus medialis (VM) and vastus lateralis (VL).

MVIC		VM			VL		
		M	SD	95% CI	M	SD	95% CI
20%	NF_{MDF}	36.54	21.67	24.99 - 48.09	36.40	25.23	22.95 - 49.84
	NF_{RMS}	26.14	15.14	18.44 - 33.85	25.60	20.86	14.48 - 36.72
70%	NF_{MDF}	43.84	20.36	33.72 - 53.97	39.23	20.10	28.89 - 49.56
	NF_{RMS}	33.77	24.64	20.12 - 47.41	40.06	21.53	28.99 - 51.14

Previous studies have used the slope of the MDF and RMS to describe the shift in these parameters during fatiguing contractions (MacIntyre, Slawnych, Reid, & McKenzie, 1998; Mathur et al., 2005; Petrofsky & Lind, 1980). Therefore, the procedures adopted in this study for the calculation of the MDF through the MDF slope coefficient (NF_{MDF}) and the RMS slope coefficient (NF_{RMS}) were as follows: (1) a linear fit was applied for every five samples of MDF and RMS data during exercise, progressively, and always from the origin until the moment of exhaustion, (2) on each portion of VM e VL, the coefficient slope of the adjusted values MDF and RMS, as well as the value for the t test and their significance ($p < .05$) were analyzed; and (3) the NF was established as the first section where the slope coefficient presented significant difference from zero. The MDF was calculated in the $NF_{MDF,}$ and the RMS was calculated in the NF_{RMS}.

Statistical analyses

Statistical analyses were performed with the Statistical Package for the Social Sciences software program for Windows (version 18.0, SPSS, INC., Chicago, IL). Conducting a normality analysis of the data using the Shapiro-Wilk test, the within and between subject variances was analysed using an analysis of variance (ANOVA). Three-way repeated measures ANOVA with one categorical independent within-subjects variables (moments of the test) and two categorical independent between-subjects variables (loads and muscles) was used to assess the dependency for each EMG variables (MDF and RMS) on contraction force (20% and 70% MVIC) and moments (beginning, NF and the end of the test) for different muscles (VM and VL). Follow-up post-hoc analyses (Tukey honestly significant difference [HSD] test) were used to test for differences among pairs of means when appropriate. Paired *t*-test was applied to compare the NF normalized times for the same muscles (VM at 20% MVIC x VM at 70% MVIC; VL at 20% MVIC x VL at 70% MVIC), and comparisons between muscles the independent *t*-test was applied considering the muscles as different samples groups (VM at 20% MVIC x VL at 20% MVIC; VM at 70% MVIC x VL at 70% MVIC). The statistical significance was set at p < .05. Results are presented in the text and tables as mean (M), standard deviation (SD), and with a 95% confidence interval (95% CI).

Results

$NF_{MDF\ and}$ NF_{RMS} time values

Table 1 shows the time values (normalized by absolute endurance time [%]) of NF_{MDF} and NF_{RMS} for VM and VL at 20% and 70% MVIC. The descriptive analysis of these values indicates the moment at which significant changes occurred in the behavior of the MDF and the RMS. The NF_{RMS} values tended to be lower than the NF_{MDF} values; however the comparison between the intensities of loads did not significantly differ between muscles and methods.

Analysis of MDF: I_{MDF}, NF_{MDF} and F_{MDF}

The analysis of the MDF values found a significant interaction between moments and loads (Wilks Lambda = .88, *F* [2, 63] = 3.99, *p* < .023, partial eta squared = .11), and a significant effect comparing the three moments (Wilks Lambda = .20, *F* [2, 63] = 125.57, *p* < .001, partial eta squared = .79) and two loads (*F* [1, 64] = 11.50, *p* = .001, partial eta squared = .15), showing greater decrease in MDF values at 70% MVIC compared with 20%

Table 2. Median (M), standard deviation (SD) and confidence interval (95% CI) of MDF values obtained at the beginning of the test (I_{MDF}), at the onset of neuromuscular fatigue (NF_{MDF}), and at the end of the test (F_{MDF}) for vastus medialis (VM) and vastus lateralis (VL) muscles.

MVIC		VM			VL		
		M	SD	95% CI	M	SD	95% CI
20%	I_{MDF}	0.99*	0.03	0.97 - 1.00	1.00*	0.06	0.97 - 1.03
	NF_{MDF}	0.92	0.05	0.89 - 0.95	0.95	0.06	0.92 - 0.99
	F_{MDF}	$0.89^{\#}$	0.10	0.84 - 0.95	$0.91^{\#}$	0.09	0.86 - 0.96
70%	I_{MDF}	$0.98^{\#\dagger}$	0.04	0.96 - 1.00	$0.98^{\#\dagger}$	0.03	0.96 - 0.99
	NF_{MDF}	$0.90^{\#}$	0.03	0.88 - 0.92	$0.90^{\#}$	0.04	0.88 - 0.92
	F_{MDF}	0.82	0.08	0.77 - 0.86	0.83	0.08	0.79 - 0.87

[*] Statistical difference of F_{MDF} at 20% MVIC, p < .05.

[#] Statistical difference of F_{MDF} at 70% MVIC, p < .05.

[†] Statistical difference of NF_{MDF} at 70% MVIC, p < .05.

Table 3. Median (M), standard deviation (SD) and confidence interval (95% CI) of RMS values obtained at the beginning of the test (I_{RMS}), at the onset of neuromuscular fatigue (NF_{RMS}), and at the end of the test (F_{RMS}) for vastus medialis (VM) and vastus lateralis (VL) muscles.

MVIC		VM			VL		
		M	SD	95% CI	M	SD	95% CI
20%	I_{RMS}	0.29* #	0.09	0.25 – 0.34	0.27* #	0.09	0.22 – 0.32
	NF_{RMS}	0.32†	0.08	0.28 – 0.36	0.31†	0.11	0.25 – 0.37
	F_{RMS}	0.46♦	0.17	0.38 – 0.55	0.45♦	0.19	0.35 – 0.54
70%	I_{RMS}	0.73	0.16	0.65 – 0.81	0.66	0.18	0.56 – 0.75
	NF_{RMS}	0.78	0.16	0.68 – 0.87	0.71	0.20	0.60 – 0.81
	F_{RMS}	0.90	0.22	0.78 – 1.01	0.80	0.26	0.66 – 0.92

* Statistical difference of F_{RMS} at 20% MVIC, $p < .05$.
\# Statistical difference of I_{RMS} at 70% MVIC, $p < .05$.
† Statistical difference of NF_{RMS} at 70% MVIC, $p < .05$.
♦ Statistical difference of F_{RMS} at 70% MVIC, $p < .05$.

MVIC, always decreasing during the fatigue tests for the two muscles (Table 2). Subsequent follow-up post-hoc comparisons (Tukey HSD) indicated that, in both loads and muscles, there was a significant increase in MDF values obtained in the end of the test (F_{MDF}) compared with the beginning of the test (I_{MDF}). In fatigue tests performed at 20% MVIC, no statistical differences were found when comparing the MDF values obtained at the onset of neuromuscular fatigue (NF_{MDF}) compared with I_{MDF} and F_{MDF}. However at 70% MVIC, the NF_{MDF} was significantly lower than the F_{MDF}. For comparison between sustained contraction at 20% and 70% MVIC, the only statistical difference found was between F_{MDF} for all muscles (Table 2).

Analysis of RMS: I_{RMS}, NF_{RMS} and F_{RMS}

The analysis of the RMS values found a significant interaction between moments and loads (Wilks Lambda = .90, *F* [2, 63] = 3.40, *p* = .04, partial eta squared = .90), and a significant effect comparing the three moments (Wilks Lambda = .40, *F* [2, 63] = 46.40, *p* < .001, partial eta squared = .60) and two loads (*F* [1, 64] = 123.58, *p* < .001, partial eta squared = .66), showing greater increase in RMS values at 70% MVIC compared with 20% MVIC, always increasing during the fatigue tests for the two muscles (Table 3). Follow-up *post-hoc* comparisons (Tukey HSD) indicated a significant increase in RMS values obtained in the end of the test (F_{RMS}) compared with RMS values in beginning of the test (I_{RMS}) for all muscles at 20% MVIC, and no differences were observed at 70% MVIC. In addition, to all muscles and both loads the RMS values obtained at onset muscle fatigue (NF_{RMS}) were not significantly different compared with I_{RMS} and F_{RMS}. Comparisons between loads intensities revealed that, for all muscles, the values of I_{RMS}, NF_{RMS}, and F_{RMS} at 70% MVIC were statistically higher compared to the values of I_{RMS}, NF_{RMS}, and F_{RMS} at 20% MVIC, respectively, for both days (Table 3).

Discussion

NF_{MDF} and NF_{RMS}

The values of normalized time obtained at the time of neuromuscular fatigue onset demonstrated patterns in which the NF_{MDF} generally occurred after the NF_{RMS}. There were no significant differences between muscles and between loads, probably due to the high variability present in time values found in NF_{MDF} and NF_{RMS}. This demonstrates that muscles evaluated for each participant entered a state of localized neuromuscular fatigue at different times. The explanation for this variability is not clear. Merletti et al. (1991) report that when an index or EMG variable under fatigue conditions is estimated for a specific experimental condition by repeating the same experiment on a number of participants, its value is affected by two types of errors: the error due to inter-subject variability and the estimation error due to experimental noise for each experiment. These errors may be influenced by the activity level of the participants (Hodgson et al., 2005), gender (Clark, Manini, Thé, Doldo, & Ploutz-Snyder, 2003; Pincivero et al., 2004; Pincivero, Green, Mark, & Campy, 2000) and other factors, in which these changes in the EMG signal during fatigue tests is a function of both physiological and psychological factors, and it is difficult to know accurately the causal relationship of each to the failure point (De Luca, 1997). It is therefore the first study that addresses the analysis of these moments while providing an opportunity to check for differences in the determination of this index and to verify its behavior under the influence of exercises performed with different loads of both low and high intensities.

MDF and RMS

In this study, F_{MDF} was significantly lower than the I_{MDF} for both muscles and loads. The same behavior was found for the RMS at 20% MVIC, wherein the I_{RMS} was significantly lower than the F_{RMS}. These behaviors were consistent with previous findings that observed an increase of the RMS and a decrease in MDF values in submaximal knee-extension contractions. Moreover, this pattern was consistent with that described in many previous studies (Clark, Collier, Manini, & Ploutz-Snyder, 2005; Hedayatpour et al., 2008; Kallenberg & Hermens, 2008; Watanabe & Akima, 2010).

The MDF is an EMG signal variable that is influenced by the action potential conduction velocity of the muscle fiber contraction, affecting the muscle behavior throughout the exercise, but it is also sensitive to many other physiological processes and methodological factors (Farina, Merletti, & Enoka, 2004). The RMS amplitude is a variable that reflects the number and/or size of motor units present in the muscle and its firing rate (Basmajian & De Luca, 1985; Moritani, Stegeman, & Merletti, 2004). The increase of the RMS and decrease in the MDF during submaximal contraction, such as was observed in the present study, may be attributed to an increase in the recruitment of new motor units and/or a change in the firing rate of these recruited units and a possible decrease in action potential speed conduction in the muscle (Enoka, 2012; Enoka et al., 2011; Watanabe & Akima, 2010).

Isometric contraction over 15–30% MVIC results in increased intramuscular pressure and, subsequently, resulting in reduced blood flow in the contracted muscle. Consequently, energy depletion, metabolite accumulation, decreased membrane potential, and intramuscular acidosis take place (Akima & Watanabe, 2010). Fitts and Holloszy, (1976) and Moritani et al. (2004) stated that these chemical changes interfere with the excitation-contraction coupling process, resulting in a decrease in the force developed. In this physiological situation, neural compensation is necessary to control the loss of contractility of motor units activated in order to maintain the target force level during a sustained contraction. These compensations are performed through an additional recruitment of motor units and/or an increase in the firing rate of these motor units. Moreover, the accumulation of metabolites or a decrease in membrane potential has been reported as being responsible for the decrease in action potential conduction velocity of the muscle fiber, which is the main cause of a decrease in MDF values from an EMG signal (Boyas & Guével, 2011).

Regarding to the above mentioned physiological processes during muscle contraction, it is important to note that the VM and VL behaviors had similar tests results at 20% and 70% MVIC. The initial and final values of RMS were different at 20% MVIC and similar at 70% MVIC. The fact that the variable is closely linked with the rate of motor units recruitment during the exercise (Dideriksen, Farina, & Enoka, 2010; Watanabe & Akima, 2010), suggests that in tasks of high intensity, muscle activity starts with a high level of muscle contraction and, during the neuromuscular fatigue onset, these RMS values tend to increase, however discreetly. In low-intensity exercises, this increase occurs more effectively, differentiating statistically the behavior of this variable between the beginning and the end of the test.

NF_{RMS} values were not different from the I_{RMS} and F_{RMS} for both muscles and loads, but it is noted that the behavior of the RMS at 20% MVIC was statistically different compared to 70% MVIC. The RMS from the three analyses (beginning, MF, and final), at 20% MVIC, were significantly lower than the RMS values at 70% MVIC. This shows that the behavior of the muscle is strongly influenced by the intensity of the load during testing, which determines the pattern of motor-units recruitment present in the active muscle (Enoka et al., 2011).

NF_{MDF} values were similar from the I_{MDF} and F_{MDF} for both muscles at 20% MVIC, but at 70% MVIC the MF_{MDF} and I_{MDF} were different compared to F_{MDF}. In submaximal contractions at 70%, the MVIC values decreased more sharply than 20% MVIC, demonstrating that the variable is also influenced by the intensity level of the contraction. It is suggested that these differences between loads applied in the test can be explained by physiological responses produced during the sustained contraction. During a muscle contraction, there is a significant decrease in the action potential conduction velocity of the muscle fiber, resulting in an increase in intramuscular pressure, reduced blood flow, and/or chemical changes during contraction (Watanabe & Akima, 2010). Depending on the degree of contraction, these changes can occur in different ways, which explains the MDF behavior during the 20% and 70% MVIC.

Even if there is a strong relationship between intracellular metabolism and membrane potential with blood flow in the muscle (Enoka et al., 2011; Moritani, Sherman, Shibata, Matsumoto, & Shinohara, 1992), influencing the behavior of the EMG signal, it is important to report that the physiological responses in the muscle that occurred in the present study are unclear, because no data were collected to directly assess the blood flow, metabolism, and intracellular pressure. We believe that further studies are needed to deepen the understanding of the mechanisms involved in sustained submaximal contractions. Other important factors may have contributed to the behaviors observed in EMG signals, which could be related to the positioning of the electrodes and the surface composition of muscle-fiber type present in the VM and VL (Watanabe & Akima, 2010).

Hedayatpour et al. (2008) reported that the amplitude and frequency components behavior of the EMG signals are influenced by the location of the surface electrodes in the muscles VM and VL. However, previous studies, such as Roy, De Luca, and Schneider, (1986), and Hogrel, Duchene, and Marini (1998) showed that the local coupling of the electrodes did not affect the temporal pattern and frequency of EMG signals. Watanabe and Akima (2010) reported that the influence of the location of surface electrodes on the behavior of EMG signals are not clear, and that this is a methodological limitation that requires further study to investigate the actual interference promoted by surface electrodes in EMG signals of the quadriceps femoris muscle.

The results suggest that VM and VL were recruited in a similar manner in sustained submaximal contractions. However, there is no consistency in relation to the composition of muscle fibers from the quadriceps femoris muscle (Edgerton, Smith, & Simpson, 1975; Watanabe & Akima, 2010). The VM and VL work together to perform common actions and have some differences in physiology, function, and mainly, anatomy. It is generally accepted that the principal function of the VM is to control tracking of the patella by overcoming the lateral forces imposed by other vasti muscles, and the main function of VL is to extend the knee (Rainoldi et al., 2008).

The definition of a precise time when occurs neuromuscular fatigue onset is an issue addressed in the literature (De Luca, 1997; Merletti et al., 1991), however this determination is unknown in experimental studies through specific methodologies applied to the EMG signal. The methodology proposed in this study is suitable for MDF and RMS behavior, showing the moment where significant changes were observed in a period of exercise. We believe that this method should not be definitive, because the success of its applicability does not exclude the need to evaluate other variables extracted in the time and frequency domain for similar applications that address the same goal. Therefore, we believe that further studies are needed to understand the changes that occur in the EMG signals prompted by a neuromuscular fatigue onset.

The determination of neuromuscular fatigue onset by means of observing the MDF and RMS behavior over time proved to be viable, but showed no differences between both muscles and loads. The evaluation of neuromuscular fatigue through noninvasive techniques, such as surface electromyography, provides an understanding of how changes occur in muscles and is an important tool to be used in future studies that seek to investigate issues that address the same topic.

References

Babault, N., Desbrosses, K., Fabre, M-S., Michaut, A., & Pousson, M. (2006). Neuromuscular fatigue development during maximal concentric and isometric knee extensions. *Journal of Applied Physiology*, *100*, 780-785.

Basmajian, J.V., & De Luca, C.J. (1985). *Muscles alive: Their functions revealed by electromyography*. Baltimore, Maryland: Williams and Wilkins.

Bilodeau, M., Cincera, M., Arsenault, A.B., & Gravel, D. (1997). Normality and stationarity of EMG signals of elbow flexor muscles during ramp and step isometric contractions. *Journal of Electromyography and Kinesiology*, *7*, 87-96.

Blangsted, A.K., Vedsted, P., Sjøgaard, G., & Sjøgaard, K. (2005). Intramuscular pressure and tissue oxygenation during low-force static contraction do not underline muscle fatigue. *Acta Physiologica Scandinavica, 183*, 379-88.

Boyas, S., & Guével, A. (2011). Neuromuscular fatigue in healthy muscle: Underlying factors and adaptation mechanisms. *Annals of Physical and Rehabilitation Medicine, 54*, 88-108.

Callaghan, M.J., Mccarthy, C.J., & Oldham, J. A. (2009). The reliability of surface electromyography to assess quadriceps fatigue during multi joint tasks in healthy and painful knees. *Journal of Electromyography and Kinesiology*, *19*, 172-180.

Clark, B.C., Manini, T.M., Thé, D.J., Doldo, N.A., & Ploutz-Snyder, L.L. (2003). Gender differences in skeletal muscle fatigability are related to contraction type and EMG spectral compression. *Journal of Applied Physiology*, *94*, 2263-72.

Clark, B.C., Collier, S.R., Manini, T.M., & Ploutz-Snyder, L. L. (2005). Sex differences in muscle fatigability and activation patterns of the human quadriceps femoris. *European Journal of Applied Physiology*, *94*, 196-206.

Cohen, J.W. (1988). *Statistical power analysis for the behavioral sciences*. Hillsdale, NJ: Lawrence Erlbaum Associates.

Cook, T., Rosencrance, J., Zimmermann, C., Gerleman, D., & Ludewig, P. (1998). Electromyographic analysis of a repetitive hand gripping task. *International journal of occupational safety and ergonomics*, *4*, 185-200.

De Luca, C.J. (1984). Myoelectrical manifestations of localized muscular fatigue in humans. *Critical Review in Biomedical Engineering*, *11*, 251-79.

De Luca, C.J., Foley, P.J., & Erim, Z. (1996). Motor unit control properties in constant-force isometric contractions. *Journal of Neurophysiology*, *76*, 1503-16.

De Luca, C.J. (1997). The use of surface electromyography in biomechanics. *Journal of Applied Biomechanics*, *13*, 135-163.

Dideriksen, J.L., Farina, D., & Enoka, R.M. (2010). Influence of fatigue on the simulated relation between the amplitude of the surface electromyogram and muscle force. *Philosophical Transactions of the Royal Society a-Mathematical Physical and Engineering Science*s, *368*, 2765-2781.

Ebersole, K.T., O'Connor, K.M., & Wier, A.P. (2006). Mechanomyographic and electromyographic responses to repeated concentric muscle actions of the quadriceps femoris. *Journal of Electromyography and Kinesiology*, *16*, 149-157.

Edgerton, V., Smith, J., & Simpson, D. (1975). Muscle fibre type populations of human leg muscles. *The Histochemical Journal, 7*, 259-266.

Enoka, R. M., & Duchateau, J. (2008). Muscle fatigue: what, why and how it influences muscle function. *The Journal of Physiology*, *586*, 11-23.

Enoka, R.M., Baudry, S., Rudroff, T., Farina, D., Klass, M., & Duchateau, J. (2011). Unraveling the neurophysiology of muscle fatigue. *Journal of Electromyography and Kinesiology*, *21*, 208-219.

Enoka, R.M. (2012). Muscle fatigue – from motor units to clinical symptoms. *Journal of Biomechanics*, *3*, 427-433.

Farina, D., Merletti, R., & Enoka, R.M. (2004). The extraction of neural strategies from the surface EMG. *Journal of Applied Physiology*, *96*, 1486-1495.

Fitts, R H., & Holloszy, J.O. (1976). Lactate and contractile force in frog muscle during development of fatigue and recovery. *American Journal of Physiology*, *231*, 430-433.

Gonzalez-Izal, M., Malanda, A., Gorostiaga, E., & Izquierdo, M. (2012). Electromyographic models to assess muscle fatigue. *Journal of Electromyography and Kinesiology*, *22*, 501-12.

Grabiner, M.D., Koh, T.J., & Miller, G.F. (1991). Fatigue rates of vastus medialis oblique and vastus lateralis during static and dynamic knee extension. *Journal of Orthopedic Research*, *9*, 391-397.

Hedayatpour, N., Arendt-Nielsen, L., & Farina, D. (2008). Non-uniform electromyographic activity during of the vastus medialis and lateralis fatigue and recovery muscles. *Journal of Electromyography and Kinesiology*, *18*, 390-396.

Hodgson, J.A., Roy, R.R., Higuchi, N., Monti, R.J., Zhong, H., Grossman, E. (2005). Does daily activity level determine muscle phenotype? *Journal of Experimental Biology*, *208*, 3761-70.

Hogrel, J-Y., Duchene, J., & Marini, J-F. (1998). Variability of some SEMG parameter estimates with electrode location. *Journal of Electromyography and Kinesiology*, *8*, 305-315.

Kallenberg, L.A.C., & Hermens, H.J. (2008). Behaviour of a surface EMG based measure for motor control: Motor unit action potential rate in relation to force and muscle fatigue. *Journal of Electromyography and Kinesiology*, *18*, 780-788.

Kollmitzer, J., Ebenbichler, G.R., & Kopf, A. (1999). Reliability of surface electromyographic measurements. *Clinical Neurophysiology*, 110, 725-734.

MacIntyre, D.L., Slawnych, M.P., Reid, W.D., & McKenzie, D.C. (1998). Fatigue of the knee extensor muscles following eccentric exercise. *Journal of Electromyography and Kinesiology*, *38*, 3-9.

Mathur, S., Eng, J.J., & MacIntyre, D.L. (2005). Reliability of surface EMG during sustained contractions of the quadriceps. *Journal of Electromyography and Kinesiology*, *15*, 102-110.

Merletti, R., Lo Conte, L.R., & Orizio, C. (1991). Indices of muscle fatigue. *Journal of Electromyography and Kinesiology*, *1*, 20-33.

Montes, F.A., Alves, F.J.B.F., Gomes, S. C., Dezan, D., & Gomes, D. C. (2011). Concepçöes de fadiga. *EFDeportes*, 16, 161.

Moritani, T., Sherman, W.M., Shibata, M., Matsumoto, T., & Shinohara, M. (1992). Oxygen availability and motor unit activity in humans. *European Journal of Applied Physiology and Occupational Physiology, 64*, 552-556.

Moritani, T., Stegeman, D., Merletti, R. (2004). Basic physiology and biophysics of EMG signal generation. In: R. Merletti & P. Parker (Eds.), *Electromyography physiology, engineering, and noninvasive application*. Hoboken (pp. 1-20). New Jersey: John Wiley & Sons, Inc.

Petrofsky, J.S., & Lind, A.R. (1980). Frequency analysis of the surface electromyogram during sustained isometric contractions. *European Journal of Applied Physiology*, *43*, 173-182.

Pincivero, D.M., Green, R.C., Mark, J.D., & Campy, R.M. (2000). Gender and muscle differences in EMG amplitude and median frequency, and variability during maximal voluntary contractions of the quadriceps femoris. *Journal of Electromyography and* Kinesiology, *10*, 189-96.

Pincivero, D.M., Salfetnikov, Y., Campy, R.M., & Coelho, A.J. (2004). Angle and gender-specific quadriceps femoris muscle recruitment and knee extensor torque. *Journal of Biomechanics*, *37*, 1689-1697.

Pincivero, D.M., Gandhi, V., Timmons, M.K., & Coelho, A.J. (2006). Quadriceps femoris electromyogram during concentric, isometric and eccentric phases of fatiguing dynamic knee extensions. *Journal of Biomechanics*, *39*, 246-54.

Rainoldi, A., Falla, D., Mellor, R., Bennell, K., & Hodges, P. (2008). Myoelectric manifestations of fatigue in vastus lateralis, medialis obliquus and medialis longus muscles. *Journal of Electromyography and Kinesiology*, *18*, 1032-1037.

Roman-Liu, D., Konarska, M. (2009). Characteristics of power spectrum density function of EMG during muscle contraction below 30%MVC. *Journal of Electromyography and Kinesiology*, *19*, 864-74.

Roy, S.H., De Luca, C.J., & Schneider, J. (1986). Effects of electrode location on myoelectric conduction velocity and median frequency estimates. *Journal of Applied Physiology*, *61*, 1510-1517.

Silva, C.R., Geres, B.S., Kuriki, H.U., Negrao-Filho, R.F., Alves, N., & Azevedo, F.M. (2012). Analysis of reliability of EMG signal frequency domain parameters used in the characterization of localized muscle fatigue. *Motriz*, *18*, 456-464.

Watanabe, K., & Akima, H. (2010). Neuromuscular activation of vastus intermedius muscle during fatiguing exercise. *Journal of Electromyography and Kinesiology*, *20*, 661-666.

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