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Research article

Evaluation of Electromyographic Frequency Domain Changes during a Three-Minute Maximal Effort Cycling Test

Ran Wang, David H. Fukuda ✉, Jeffrey R. Stout, Edward H. Robinson, Amelia A. Miramonti, Maren S. Fragala and Jay R. Hoffman
Institute of Exercise Physiology & Wellness, University of Central Florida, Orlando, FL, USA

Abstract
To evaluate the time course of EMG frequency changes during a three-minute maximal effort cycling test (3MT) session and to examine which parameter between mean (MNF) and median (MDF) frequency is more suitable for evaluation of changes in neuromuscular function throughout a 3MT. Eighteen recreationally-active men volunteered to participate in this study. Maximum voluntary contraction (MVC) was measured using a dynamometer to determine maximal EMG frequency of the vastus lateralis (VL) of the kicking leg during isometric knee extension. A maximal oxygen consumption test (VO_2peak) on a cycle ergometer was performed to establish the appropriate load profile for the 3MT which was completed after a period of at least 48 hours. MNF, MDF and power output (PO) values were measured at 10-second epochs throughout the duration of the 3MT. Repeated measures analysis of variance was used to compare the changes in EMG frequency, relative to maximal values from the MVC, and change in PO during the testing procedure. MNF, Root Mean Square (RMS), and PO significantly decreased during the 3MT, while MDF did not change significantly. Statistically, EMG frequency and PO decreased at first and remained constant in response to the 3MT, which may be reflective of differing patterns of muscle fiber type fatigue throughout the testing session. Due to decreased variability, changes in neuromuscular function during this protocol may be better evaluated using MNF than MDF.

Key words: Critical power, EMG, fatigue, cycling.

Introduction

The estimation of critical power (CP) is a result of the hyperbolic relationship between specific power output levels and the corresponding time that the power output can be sustained. Originally, Monod and Scherrer (1965) described CP based on the results of several (three to five) bouts of repetitive lifting exercises performed using different isolated muscle groups, and they noted that exhaustion did not occur when the dynamic work intensity was inferior or equal to the CP. Theoretically, CP represents the highest power output that can be sustained without exhaustion. Subsequent investigation of this sustainability has shown that exhaustion occurs after about 30 minutes of exercise at CP (Brickley et al., 2002). Moritani and colleagues (1981) extended the CP concept to cycling exercise and found CP to be highly correlated with the ventilatory anaerobic threshold. However, this method still requires a subject to perform exhaustive exercise at different constant work rates on separate days.

Vanhatalo and colleagues (2007) developed a three-minute all-out cycling test (3MT) which yielded a stable power output at the end of the test, thus generalizing the CP concept to all-out exercise. The rationale for the measurement of CP in a single bout of all-out cycling stems from its mathematical definition as the asymptote of the hyperbolic power-time relationship and further outlines a finite and rate-independent capacity for work above the CP (Vanhatalo et al., 2007). The validity and reliability of the 3MT has been demonstrated by comparing the CP assessed from the traditional multi-trial constant load to exhaustion cycling test and the newly developed 3MT, while sensitivity has been shown through similar alterations due to an interval training intervention (Vanhatalo et al., 2008). The 3MT is most often used to evaluate performance through estimation of CP, however, several studies have examined the physiological response to the testing protocol, primarily focused on the metabolic aspects (Bergstrom et al., 2013; McClave et al., 2011; Sperlich et al., 2014) and electromyographic (EMG) amplitude parameters (Bergstrom et al., 2013; Vanhatalo et al. 2011). The 3MT provides an advantageous alternative to the conventional protocol of multiple exhaustive exercise tests to determine CP, and may offer a unique method to examine mechanisms of fatigue.

Muscle fatigue represents a multifaceted phenomenon with physical and chemical changes in muscle as well as alterations in nervous system efficiency, which are related to different causes, mechanisms and symptoms (Cifrek et al., 2009). The monitoring of local muscle fatigue can be conducted by measuring myoelectric activity via EMG which may represent biochemical and physiological changes during exercise (De Luca, 1984). Traditionally, EMG is used in detecting fatigue through incremental or constant exercise protocols, during which slow twitch and fast twitch fibers are progressively recruited as exercise proceeds (Guffey et al., 2012; Malek et al., 2006; Travis et al., 2011). During maximal all-out exercise, characterized by the attainment of peak power at the start of the 3MT, the majority of available motor units should be recruited along with an alteration of firing rate and a reversal of the progressive muscle activation pattern utilized during incremental or constant exercise may occur (Sargeant et al., 1981; McCartney et al., 1983). Subsequently, fast twitch fibers would be expected to generate a greater proportion of the total external power output (Beelen and Sargeant 1993). Thereafter, these fibers would become progressively fatigued at a rapid rate, potentially leading to a greater reliance on slow twitch fi-

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bers, resulting in decreased power output due to the high fatigue resistance but low contraction speed characteristics of slow twitch fibers (McCartney et al., 1983; Sargeant et al., 1981). Due to this unique muscle recruitment pattern and alteration of firing rate, changes in the EMG signal during the 3MT should be evident. However, the changes in muscle fiber conduction velocity, assessed via surface EMG frequency, associated with this testing protocol have yet to be evaluated.

Mean frequency (MNF) and median frequency (MDF) are two useful frequency-domain parameters of EMG analysis which are frequently used to detect fatigue in the target muscles (De Luca, 1984). According to the definition, both MNF and MDF can represent the shift of the frequency spectrum of the EMG signal. Thus, the general behavior of MNF and MDF should be analogous, however, there are conflicting results regarding which frequency parameter is more suitable for evaluation of fatigue and the results may vary among different muscles or exercise protocols (Stulen and De Luca, 1981). Additionally, some studies have reported differences in variance between MNF and MDF, the MNF had a lower standard deviation (Balestra et al., 1988; Knaflitz et al., 1990). EMG frequency, specifically MNF, has been used to detect fatigue thresholds during graded exercise testing (Camic et al., 2010). To the best of our knowledge, no one has ever compared MNF and MDF in corresponding to the changes in neuromuscular function during the 3MT.

Thus, the purpose of this study was to evaluate the time course of EMG frequency (MNF and MDF) changes during a 3MT cycling session and to examine which frequency parameter (MNF or MDF) is more suitable for evaluation of changes in neuromuscular function throughout the 3MT. The hypotheses were as follows: (1) Both MNF and MDF would decrease over the course of the 3MT, based on the all-out nature of the exercise test; and (2) MNF would be more suitable than MDF to evaluate neuromuscular fatigue during the 3MT due to lower variability.

Methods

Subjects

Eighteen male participants (mean ± SD; age: 23.5 ± 3.1 yrs; height: 1.79 ± 0.06 m; mass: 85.1 ± 9.5 kg) volunteered to participate in this study. The study was approved by New England Institutional Review Board. Testing procedures were fully explained before obtaining written informed consent from each participant. All participants were habitually active (participating in a regular physical activity program or accumulating 150 min per week or more of moderate intensity exercise). In an attempt to eliminate the potential for reduced power output, the participants were asked to refrain from any strenuous physical activity for the previous 72 hours. In addition, participants were instructed to arrive at each testing session 2 hours fasted and in a euhydrated state. Each participant completed a confidential medical and activity questionnaire in order to identify any exclusion criteria, including the inability to perform physical exercise and any chronic illness that required continuous medical care.

Experimental design

All study participants completed two testing sessions on nonconsecutive days separated by a minimum of 48 hours. During the first testing session (T1), participants performed a standardized warm-up consisting of cycling and lower body exercise, the latter included 10 bodyweights squats and 10 alternating lunges. Immediately following the warm up, participants completed a graded cycling exercise protocol to exhaustion. Exhaled gas was analyzed throughout the testing protocol to determine ventilatory threshold. During T2, electromyographic (EMG) electrodes were placed on the vastus lateralis of the participant's kicking leg, and they were instructed to perform a maximal voluntary isometric contraction (MVIC) in order to measure the maximal electrical activity of vastus lateralis for later normalization. After the maximal voluntary isometric contractions, the participants performed a standardized warm-up as described above, followed by five minutes rest, and then a three-minute all-out cycling test. EMG signal data during the three-minute all-out cycling test was collected for further analysis.

Peak oxygen consumption test

A continuous graded exercise test was performed on an electronically-braked cycle ergometer (Excalibur Sport, Lode, Groningen, Netherlands) to determine the power output at gas exchange threshold (GET) and the peak power output in watts (W) following the procedures described by Bergstrom et al. (2013). The final workload was recorded as the peak power output (PPO) for each participant. Open-circuit spirometry was used to estimate gas exchange threshold using the V-slope method with a metabolic cart (True One 2400, Parvo Medics, Inc., Sandy, UT) by sampling and analyzing the breath-by-breath expired gases.

Maximum Voluntary Isometric Contraction (MVIC) strength test

To obtain the MVIC strength, participants began by warming up for five minutes on a cycle ergometer. After the warm up, individuals were positioned in a Biodex S4 isokinetic dynamometer (Biodex Medical System, Inc., New York, NY, USA) in a seated position with the hip at an angle of 110° and strapped to the machine at the waist and shoulders. Next, the evaluators positioned the individual's knee at an angle of 110° of extension (180° representing full extension). Familiarization with the isokinetic dynamometer began with a warm-up of several submaximal isometric muscle actions of the leg extensors. Five minutes after the warm-up, the participants were instructed to exert their maximum strength when trying to extend the knee and to produce the contraction as fast and as hard as possible. Participants had three attempts at obtaining the MVIC, each lasting six seconds; there was a three-minute interval between each attempt to ensure recovery of ATP-PC energy system.

Three-minute cycling test

Following a standardized warm-up, which included 10 bodyweight squats and 10 alternating lunges, the participant completed 60 seconds of unloaded cycling at 90 rpm,

followed by an all-out three-minute effort with resistance being set as a function of pedaling rate (Vanhatalo et al., 2007). Participants were asked to accelerate to approximately 110 rpm over the last 5 s of the baseline period. The resistance was adjusted during the all-out effort using the pedaling rate dependent linear mode on the cycle ergometer published by Jeukendrup et al. (1996), which used a scaling factor based on the power output at a given pedaling rate (70 rpm) being equal to 50% of the difference between the power output at GET and peak power output assessed during the graded exercise test.

EMG assessment

To assess EMG activity during the MVIC and the three-minute all-out cycling test, a bipolar (4.6 cm center-to-center) surface electrode (Quinton Quick-Prep silver-silver chloride) arrangement was placed over the vastus lateralis (at 2/3 on the line from the anterior spina iliaca superior to the lateral side of the patella) and in the estimated direction of the muscle fibers, the reference electrode was placed on the surface of lateral tibia condyle (Hermens et al., 1999). Inter-electrode impedance was kept below 5,000 ohms with shaving of hair and cleaning of skin beneath the electrodes. The raw EMG signals were pre-amplified using a differential amplifier (MP150 BIOPAC Systems, Inc., Santa Barbara, CA), sampled at 1,000 Hz, and stored on a personal computer (Dell Latitude E6530, Dell Inc., Round Rock, TX) for subsequent analysis.

Signal processing

All signal processing was performed using AcqKnowledge software (Version 4.2, BIOPAC Systems, Inc., Santa Barbara, CA). The EMG signals were band-pass filtered (zero-lag, fourth-order Butterworth) at 10 Hz to 500 Hz with a hamming window, first, and then processed via a fast Fourier transformation algorithm with an epoch of 10 seconds. MNF, MDF and RMS during the 3MT were calculated and expressed every 10 seconds. During the three MVICs, the time point which had the maximum EMG amplitude was detected, then a one-second window consisting of 0.5 second before and after that time point was selected and analyzed. For each participant, MNF and MDF values from each epoch of data during the cycling test were normalized relative to the maximum value derived from the three MVICs.

Data analysis

Data are presented as mean ± *95% confidence intervals*. Shapiro-Wilk test was performed for normality analyses of normalized MNF, MDF and RMS as well as the power output throughout the duration of the three-minute cycling test. One-way repeated measures analysis of variance (ANOVA) comparisons with Bonferroni *post-hoc* tests were used if the data was normally distributed, otherwise the Friedman ANOVA by ranks was used. The partial eta squared (η^2) statistic was used to evaluate effect size for each ANOVA according to classifications set forth by Green et al. (1996) with η^2= 0.01 corresponding to a small effect size, η^2= 0.06 to a medium effect size, and η^2= 0.14 to a large effect size. An alpha level of 0.05 (or 0.05/18 for *post-hoc* comparisons) was used to determine statistical significance. In order to further illustrate changes over the course of the 3MT, change scores (Δ) with 95% confidence intervals for each time period compared to the initial 10s epoch for MNF and MDF or from the previous epoch for RMS and PO were calculated. When the 95% confidence interval includes zero, the mean change score is not different from zero, which can be interpreted as no statistical change ($p > 0.05$). However, if the 95% confidence interval does not include zero, the mean change for that variable may be considered statistically significant ($p \leq 0.05$). Linear regression was used to assess the relationship between MNF and MDF across each set of epochs for all testing sessions. The correlation coefficient (r) of this relationship was evaluated as low (0.5 - 0.7), moderate (0.7 - 0.8), or high (0.9 - 1.0) using the criteria set forth by Vincent and Weir (1994).

Results

Shapiro-Wilk tests showed that all time points from MNF and MDF were normally distributed, while time points from PO and RMS were not normally distributed. Similar to the values for power output ($\chi^2 = 266.402$, $p \leq 0.01$) (as shown in Figure 1), both MNF ($F = 8.123$, $p \leq 0.01$, $\eta^2 = 0.323$) (as shown in Figure 2) and RMS ($\chi^2 = 200.140$, $p \leq 0.01$) (as shown in Figure 4) significantly decreased throughout the 3MT, while MDF showed a non-significant decline ($F = 2.410$, $p = 0.088$, $\eta^2 = 0.124$) (as shown in Figure 3) throughout the testing procedure.

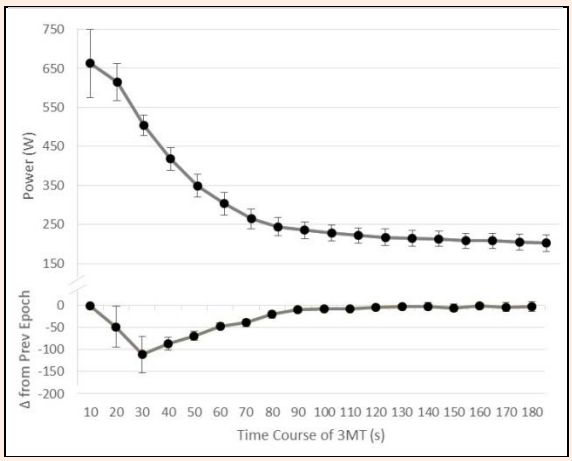

Figure 1. Power output and change (Δ) in power output compared to the previous 10s epoch throughout the three-minute maximal cycling test (3MT). Data are reported as mean values ± 95% confidence intervals.

The results from post-hoc testing showed that MNF during the first two 10-second epochs of the 3MT was 71 ± 9 % and 70 ±11 % of the MVIC value, respectively. MNF in the 1st epoch was significantly higher than MNF in the 5th-7th, 9th-12th, and 14th- 18th epochs ($p < 0.05$), MNF in the 2nd epoch was significantly higher than MNF in the 6th, 7th, 9th and 10th epoch ($p < 0.05$). There were no significant differences in MNF between any of the last sixteen epochs ($p > 0.05$).

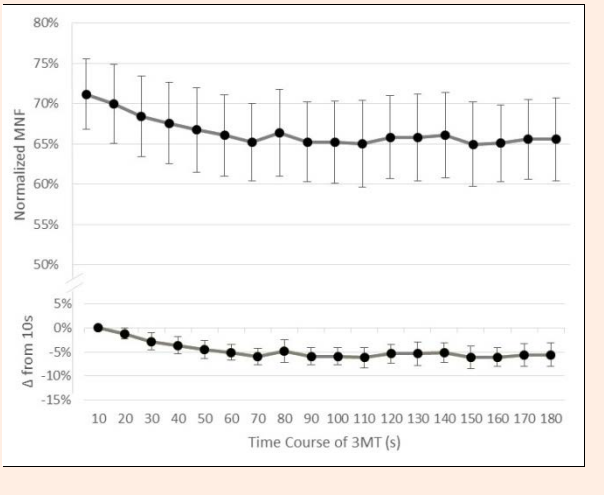

Figure 2. Mean frequency (MNF) normalized to maximal MNF and change (Δ) in MNF compared to the initial 10s epoch throughout the three-minute maximal cycling test (3MT). Data are reported as mean values ± 95% confidence intervals.

Regression analyses for MNF and MDF indicated that the both the slope parameter ($p < 0.01$) and the intercept ($p < 0.01$) were significant different from zero. Although low, there was a positive correlation between MNF and MDF ($n = 324$, $r = 0.577$, $r^2 = 0.333$, $p < 0.001$). The standard error of the estimate was 0.0883 or 7.5% of average MNF value.

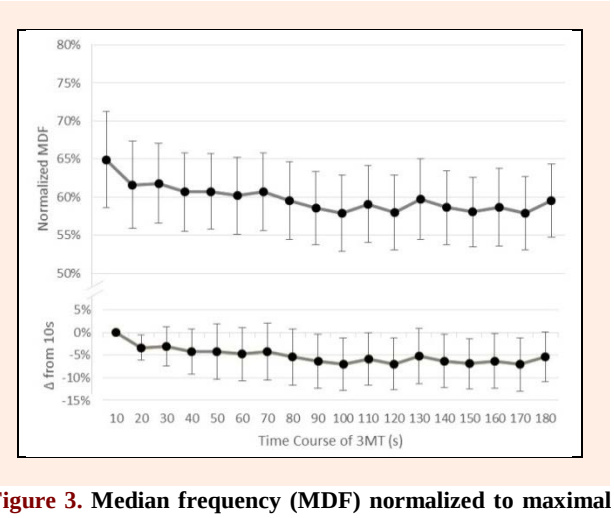

Figure 3. Median frequency (MDF) normalized to maximal MDF and change (Δ) in MDF compared to the initial 10s epoch throughout the three-minute maximal cycling test (3MT). Data are reported as mean values ± 95% confidence intervals.

Furthermore, pairwise comparison of the Friedman test showed that the power output values decreased significantly from the 1st epoch to the 8th epoch ($p = 0.009$). Power output values from the 9th to 18th epoch did not significantly differ from one another ($p > 0.05$).

Discussion

The results showed that MNF significantly decreased from the initial epoch to the 5th epoch and remained constant throughout the remaining course of the 3MT. The most important finding of this study was that both EMG amplitude (presented as RMS) and frequency (presented as MNF) decreased in parallel with power output during the 3MT, indicating that there was a decline in motor unit recruitment and muscle fiber conduction velocity (Stewart et al., 2011). The power output value was decreasing at a very low constant rate after 70 seconds and plateaued during the last 60 seconds during the 3MT (Figure 1), which was similar to that found previously (Vanhatalo et al., 2011). The current reduction in EMG amplitude is consistent with published data (Vandewalle et al. 1991) examining the EMG amplitude response during a 45-second Wingate test, which reported a parallel decline in power output and integrated EMG of the VL. The researchers suggested a progressive attenuation of spatial and/or temporal recruitment of motor units during the test, which indicated that both central and peripheral fatigue occurred during the bout of all-out, maximal exercise. Greer et al. (2006) reported a significant decline in integrated EMG and a reduction in MDF of the plantar flexors and the knee extensors during a 30-second Wingate test. In addition, Hunter et al. (2003) observed no change in the EMG amplitude of VL during a 30-second cycling sprint, but the authors reported a significant shift of the MNF towards lower values similar to the current study. The reduction in EMG frequency may be attributed to increased intramuscular acidosis and a concomitant slowing of muscle fiber conduction velocity (Hunter et al., 2003). However, interpretation of spectral frequencies should be made with caution, as these measures are indirect physiological variables (Farina et al. 2004).

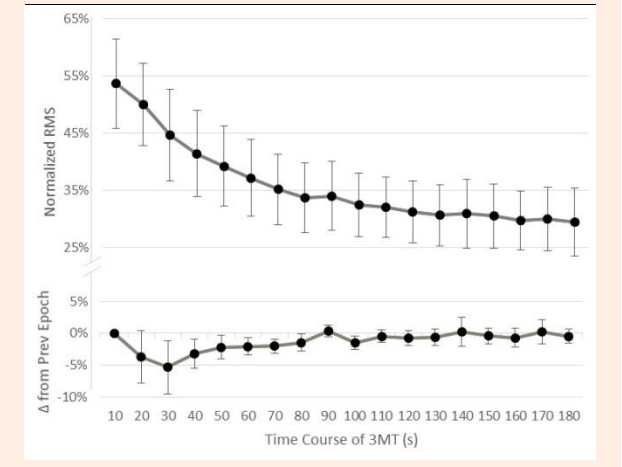

Figure 4. Electromyography amplitude (RMS) normalized to maximal RMS and change (Δ) in RMS compared to the previous 10s epoch throughout the three-minute maximal cycling test (3MT). Data are reported as mean values ± 95% confidence intervals.

An additional observation from this study is that although there was a parallel reduction of both MNF and MDF with power output. However, MNF may better reflect neuromuscular fatigue during the 3MT than MDF due to the reduced variability of results, which is in accordance with previous findings (Balestra et al., 1988; Knaflitz et al., 1990). Although both features represent

measures of central tendency, the performance of MNF in the evaluation of frequency change is quite different compared to that of MDF. Generally speaking, MNF is always slightly higher than MDF because of the skewed shape of the EMG power spectrum; whereas the variance of MNF is typically lower than that of MDF (Knaflitz et al., 1990). In theory, the standard deviation of MDF is higher than that of MNF by a factor 1.253 (Balestra et al., 1988). However, the estimation of MDF is more affected by muscle fatigue than random noise, especially the noise located in the high frequency band of the EMG power spectrum (Stulen and De Luca, 1981). Further, regression analysis revealed that even with data extrapolated from the same EMG signal and similarly reflective of central tendency, MNF and MDF were not well correlated. Moreover, the ability to predict MNF from MDF was very limited with only 33% of the variability in MNF explained by MDF, which may indicate these measures reflect different properties of the change of central tendency in EMG signal. This discrepancy may be important in future studies examining changes in EMG spectral parameters during high-intensity cycling or during the determination of EMG-related fatigue thresholds.

Typical neuromuscular responses during shorter duration (< 1 min) all-out cycling exercise are well documented (Inbar et al., 1996). Peak power output is reached in the initial stage, and from this point, power output declines until the end of the test. The decreased power output is accompanied by a decline in muscle pH, which is commonly related with fatigue and inhibition of anaerobic metabolism (Stewart et al., 2011). Anaerobic metabolism is well-developed in type II muscle fibers, and the reduction in power output during all-out exercise is due to declining contribution from type II fibers (Inbar et al., 1996). The reduction in fiber type contribution also suggests altered motor unit recruitment and therefore may affect neuromuscular activity during this type of exercise, which was confirmed by this study. Our finding is supported by McCartney et al. (1983) and Sargeant et al. (1981), who conclude that the vast majority of available motor units are recruited at the onset of all-out exercise. Given the rapid pedaling rate at the start of the 3MT, type II fibers would be expected to preferentially contribute to power production (Beelen et al., 1993). Thereafter, these fibers (along with some type I fibers) would become progressively fatigued, such that, later in the exercise bout, the power output may be predominantly produced by type I fibers (McCartney et al., 1983; Sargeant et al., 1981; Vanhatalo et al., 2008).

In order to further clarify spectral EMG data, such as those presented in the current investigation, Cifrek et al. (2009) outlined four possible interpretations as follows: (1) increased muscle force coinciding with increased EMG amplitude and a rightward shift in the EMG spectrum (as denoted by increased MNF or MDF) , (2) decreased muscle force coinciding with decreased EMG amplitude and a leftward shift in the EMG spectrum (as denoted by decreased MNF or MDF), (3) muscle fatigue coinciding with increased EMG amplitude and a leftward shift in the EMG spectrum, or (4) recovery from previous muscle fatigue coinciding with decreased EMG amplitude and a rightward shift in the EMG spectrum. The results of the current study fall into the second category, which indicates decreased muscle force due to the failure of neural excitation and the gradual reduction of power output during the 3MT. While not directly measured in this study, muscle fiber conduction velocity (MFCV) is hypothesized to have decreased throughout the fatiguing exercise bout, directly changing the shape of the motor unit action potential (MUAP) waveform, and the resulting recruitment pattern of the surface EMG signal. In support of the previous discussion with regard to declined power output and altered muscle fiber type utilization, decreased muscle pH has been shown to result in decreased MFCV and, subsequently, MDF *in vitro* (Brody et al., 1991). Furthermore, Hunter et al. (2009) demonstrated the same effect *in vivo*, they reported that induced metabolic alkalosis can increase muscle fiber conduction velocity following prolonged submaximal cycling.

It was speculated that the vast majority of available motor units would be activated at the beginning of the 3MT, however, the highest normalized RMS achieved at the onset of 3MT was only 55%. This value may have been affected by the examination of 10-second epochs and the portion of the cycling movement in which the VL was not contracting. Many factors may affect EMG amplitude, including the fatigue status and recruitment condition of motor units. Decreased MFCV results in both a power spectrum shift toward lower frequencies and increased EMG amplitude during exercise because of a spatial low-pass filtering effect due to biological tissue acting as a volume conductor (De Luca, 1984). Therefore, decreased EMG amplitude may indicate the human body cannot maintain the recruitment of motor units or recruit new motor units in response to fatigue during the 3MT. The spatial low-pass filtering effect of tissue may result in an increase of electrical activity that can be detected from the surface electrodes which would lead to increased EMG amplitude. However, during the 3MT this effect may not have been offset by the influence of decreased motor unit recruitment, especially the loss of fast twitch muscle fibers, and ultimately resulted in the reported decrease in EMG amplitude.

Conclusion

EMG frequency, an indicator of muscle fiber conduction velocity, and EMG amplitude, a sign of neural excitation, initially decreased in response to all-out exercise followed by an eventual stabilization period during the 3MT. This result and the similar time course exhibited for PO may be reflective of differing patterns of muscle fiber type fatigue throughout the testing procedure. Finally, MNF may be a more sensitive measure than MDF when evaluating changes in neuromuscular function during this protocol.

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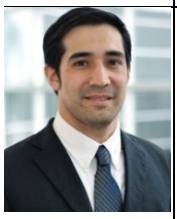
Key points

- EMG frequency decreased initially and remained constant in response to all-out cycling test.
- The change in EMG frequency and power output were similar during all-out cycling test.
- MNF may be better than MDF for neuromuscular function evaluation during all-out cycling test due to decreased variability.

AUTHOR BIOGRAPHY

Ran WANG
Employment
University of Central Florida
Degree
MEd
Research interests
Sport Science
E-mail: Ran.Wang@ucf.edu

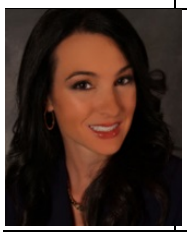


David H. FUKUDA
Employment
University of Central Florida
Degree
PhD
Research interests
Performance Testing
E-mail: David.Fukuda@ucf.edu

Jeffrey R. STOUT
Employment
University of Central Florida
Degree
PhD
Research interests
Sports Nutrition
E-mail: Jeffrey.Stout@ucf.edu

Edward H. ROBINSON
Employment
University of Central Florida
Degree
PhD
Research interests
Sports Nutrition
E-mail: Ned.Robinson@ucf.edu

Amelia A. MIRAMONTI
Employment
University of Central Florida
Degree
BSc
Research interests
Neuromuscular Fatigue
E-mail: Amelia.Miramonti@ucf.edu



Maren S. FRAGALA
Employment
University of Central Florida
Degree
PhD
Research interests
Resistance Training
E-mail: Maren.Fragala@ucf.edu

Jay R. HOFFMAN
Employment
University of Central Florida
Degree
PhD
Research interests
Sport Science
E-mail: Jay.Hoffman@ucf.edu

✉ **David Fukuda**
Institute of Exercise Physiology & Wellness, University of Central Florida, 4000 Central Florida Blvd, Orlando, FL 32816, USA