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Evaluation of the Electromyography Test for the Analysis of the Aerobic-Anaerobic Transition in Elite Cyclists during Incremental Exercise

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Featured Application: Determination of neuromuscular fatigue threshold in elite cyclists during an incremental test.

Abstract: (1) Background: The aim of this study was to investigate the validity and reliability of surface electromyography (EMG) for automatic detection of the aerobic and anaerobic thresholds during an incremental continuous cycling test using 1 min exercise periods in elite cyclists. (2) Methods: Sixteen well-trained cyclists completed an incremental exercise test (25 W/1 min) to exhaustion. Surface bipolar EMG signals were recorded from the vastus lateralis, vastus medialis, biceps femoris, and gluteus maximus, and the root mean square (RMS) were assessed. The multi-segment linear regression method was used to calculate the first and second EMG thresholds (EMG_{T1} and EMG_{T2}). During the test, gas exchange data were collected to determine the first and second ventilatory thresholds (VT_1 and VT_2). (3) Results: Two breakpoints (thresholds) were identified in the RMS EMG vs. time curve for all muscles in 75% of participants. The two breakpoints, EMG_{T1} and EMG_{T2}, were detected at around 70%–80% and 90%–95% of VO_{2MAX}, respectively. No significant differences were found between the means of VT_1 and EMG_{T1} for the vastii and biceps femoris muscles ($p > 0.05$). There were no significant differences between means of EMG_{T2} and VT_2 ($p > 0.05$). (4) Conclusions: It is concluded that the multi-segment linear regression algorithm is a valid non-invasive method for analyzing the aerobic-anaerobic transition during incremental tests with 1 min stage durations.

Keywords: cycling; neuromuscular fatigue; surface electromyography; Incremental cycling ergometer test

1. Introduction

In recent decades, great effort has aimed at identifying the threshold that delineates the aerobic-anaerobic transition during an incremental workout on a cycle ergometer [1,2]. Such "fatigue threshold" has been calculated on the basis of metabolic, ventilatory, and surface electromyographic (EMG) variables. In particular, EMG-based thresholds are especially attractive because they can be used to characterize neuromuscular fatigue and can be assessed non-invasively [3]. In the time domain, the increase in neuromuscular fatigue during an incremental continuous exercise is manifested by an increase in EMG amplitude as a result of recruitment of additional motor units, increased firing

rates, and/or synchronization [4]. Thus, EMG thresholds can be determined by examining the changes in the profile of the EMG amplitude during the test. EMG thresholds, however, have important limitations since the amplitude of the EMG signal is affected by many confounding factors [4], including (1) electrode location; (2) thickness of the subcutaneous tissues; (3) the detection system used to obtain the recording; (4) changes in the transmembrane action potential; (5) cross-talk from nearby muscles; and (6) signal cancellation. Due to all these confounding factors, caution is needed when using the EMG amplitude as an index of the level of muscle activation.

One of the most common approaches to determine the EMG threshold is based on analyzing the increase in EMG amplitude throughout the whole duration of the incremental test by searching for breakpoints in the linearity of the EMG amplitude vs. time curve. The studies falling in this category vary in the number of breakpoints identified: Zero, one, or two [5]. Among all the factors influencing the identification of EMG breakpoints (for a review of the factors, see Ertl et al. 2016 [5]), the mathematical algorithm utilized is one of the most critical. Remarkably, only the algorithm proposed by Lucía et al. [6] was able to identify two EMG breakpoints in the EMG amplitude vs. time curve: The other automatic methods only identified the first breakpoint [7–9]. However, various studies that analyzed the EMG curve by visual analysis have confirmed the existence of two breakpoints [10–12]. Therefore, it would be interesting to test the algorithm proposed by Lucía et al. [6] using different configurations of the incremental cycling protocol (i.e., pedal cadence, the stage duration, the workload increment, etc.) to investigate the applicability and reliability of this method to find the first and second EMG thresholds.

The multi-segment linear regression method proposed by Lucía et al. [6] was originally implemented in an incremental test where power output was increased by 5 W every 12 s. The short stage duration of this test makes it more similar to a ramp-like protocol compared to the graded steady state tests commonly used for EMG threshold detection. In the literature, there are graded steady tests with stage durations of 1, 2, and 3 min, although those studies that included EMG measures commonly selected 1 min step protocols [11,13,14]. More specifically, of the 27 studies analyzed in the review of Ertl et al. [5], 14 increased their workload every minute, two minute-protocols were used in five studies, while only two studies included a three-minute protocol. Moreover, incremental protocols including one-minute stages are recommended as the most appropriate ones for EMG threshold detection [5]. In addition, metabolic and ventilatory thresholds are commonly determined using incremental tests with one-minute stages [2,15–18]. Therefore, it is of interest to determine whether the multi-segment linear regression method is able to determine two breakpoints in the EMG curve using one-minute stages.

The objective of the present study was to investigate the validity and reliability of the multi-segment linear regression method for EMG threshold detection during an incremental cycling test of 60 s stages and compare the results with those obtained by Lucía et al. during the 12 s stage test [6]. The development of muscle fatigue during the incremental tests with 12 s and 60 s stage durations may be slightly different. Indeed, during the 12 s stage test, motor unit recruitment, motor unit discharge rate, and/or the sensation of effort may evolve in a more progressive manner to cope with the quick increases in workload. Based on this, we would expect some differences in the detection of EMG thresholds and in the percentage of VO_{2MAX} at which the EMG thresholds are reached between the 12 s and 60 s stage protocols.

2. Materials and Methods

2.1. Participants

Sixteen well-trained male elite cyclists volunteered to participate in the study. Their mean (±SD) anthropometric characteristics were: Age 21.7 ± 2.9 years, weight 71.3 ± 4.9 kg, and height 1.81 ± 0.06 cm. The study was conducted in accordance with the Declaration of Helsinki, and was approved by the Ethics Committee of the Public University of Navarra. Written informed consent was

obtained from all participants before inclusion. Participants were asked to not take part in vigorous physical activity for two days prior to their test date.

The participants were road cyclists engaged in regular training and amateur road races. On average, all cyclists trained at least four times a week covering a weekly distance ranging between 400 and 600 km, plus competition or Sunday training. Cyclists had a national competitive experience of 4.3 (1.7) mean (SD) years and had ridden for an average of 20,000 km (range 16,000–24,000 km) during the last season. None of the participants reported any injuries or pathologies of limb muscles or joints.

2.2. Screening and Familiarization Session

Cyclists underwent a blood test (biochemical and hematological parameters) prior to participation to check for anemia and possible infections. Participants were asked to attend an orientation session to become familiarized with the testing apparatus and procedures. All tests were performed on a custom-made cycle ergometer. The participants were required to bring in the saddle, pedals, and cycling shoes from their normal road bicycle. The saddle and pedals were installed on the cycle ergometer. A submaximal incremental test was then performed on the cycle ergometer to familiarize the cyclists with the experimental protocol.

2.3. Maximal Cycle Ergometer Test

Participants performed an incremental test to exhaustion on a SRM powermeter (science SRM, SRM GmbH, Germany). Pedal cadence was maintained at 70 rpm during the test, similar to that used in previous studies [7,16,19]. Before the incremental exercise test started, cyclists performed 5 min of unloaded cycling. The test was initiated at a workload of 125 W and the load was increased by 25 W every 1 min [1] until exhaustion or until pedal cadence could not be kept at 70 rpm. For the validity study, each of the 16 subjects performed a single bicycle ergometer test following the above protocol. For the reliability study, 10 subjects performed two exercise tests on a bicycle ergometer on different occasions and separated from each other by a period of no more than five days.

2.4. Analysis of Expired Gas and Determination of Ventilatory Thresholds

During the incremental exercise, breath-by-breath analysis was performed using a turbine flow-meter connected to a face mask (dead space: 30 mL). A side pore of the facemask was connected to fast-response differential paramagnetic oxygen and infrared carbon dioxide analyzers. Throughout the incremental test, the software (Oxycon PRO, Carefusion, Germany) averaged every five consecutive seconds data of oxygen uptake (VO_2) and carbon dioxide production (VCO_2) and ventilatory parameters, as well as the ventilatory equivalents for oxygen (EqO_2 = VE/VO_2) and carbon dioxide ($EqCO_2$ = VE/VCO_2). The first ventilatory threshold (VT_1) corresponded to the power output value at which VE/VO2 exhibited a systematic increase without a concomitant increase in VE/VCO_2 [20]. The second ventilatory threshold (VT_2) corresponded to the minimal power output at which the increase in VE/VO_2 was accompanied by a parallel increase of VE/VCO_2 [20].

2.5. Electromyography

Surface EMG signals were recorded using a pair of circular electrodes Ag/AgCl electrodes (Kendall Meditrace 100, Tyco, Canada) arranged in bipolar configuration. The electrodes had a recording diameter of 10 mm and were separated by a distance of 20 mm (measured from the nearest lateral borders). The electrodes were placed on the dominant leg over the vastus lateralis (VL), vastus medialis (VM), biceps femoris (BF), and gluteus maximus (GLM) according to the SENIAM (Surface EMG for Non-Invasive Assessment of Muscles) guidelines [21]. Before electrode placement, the skin was adequately prepared (light abrasion with sandpaper and cleansing with rubbing alcohol). The electrodes and cables were secured with surgical tape and cloth wrap to avoid movement-induced artifacts. Surface EMG signals were recorded using MP150 equipment (BIOPAC, Goleta, CA, USA).

Raw EMG signals were amplified, filtered (band-pass 10–500 Hz) and digitized (sampling frequency 1000 Hz) using the analog-to-digital conversion system of MP150.

2.6. Data Analysis

Data were first analyzed with commercially-available software (AcqKnowledge, BIOPAC Systems, Goleta, CA, USA) to monitor any abnormality in EMG traces. Then, data were exported to Matlab (version R2012b; The Math-Works, Natick, MA, USA) for quantitative analysis using a number of custom scripts. The root mean square voltage (RMS) was used as an index of the global EMG amplitude [15]. For each muscle, the RMS EMG was calculated during the active period of the muscle (EMG burst) of each pedal thrust (64-ms window). Then, the RMS-EMG was averaged every six crank revolutions (corresponding to about 5 s for 70 rpm), as previously described [15].

2.7. Determination of EMG Thresholds

The multi-segment linear regression method was employed to establish whether one or two breakpoints (thresholds) existed in the EMG amplitude response. All muscles were analyzed independently. This method was implemented as described in the following steps:

1. The root mean square (RMS) amplitude of each pedal thrust was calculated and represented vs. time (see the data points in Figure 1A).
2. A single-segment regression curve (a regression line) was fitted to all data points, and then the residual sum of square (RSS) was calculated. The results of this linear regression were used for later statistical analysis (see Figure 1B).
3. The best two-segment regression of the EMG data was computed. To do this, the algorithm calculated all the possible two-segment regressions, and the two-segment regression yielding the least pooled RSS was chosen as representing the best fit. It was observed that the intersection point (breakpoint) between these two segments was very close to the maximum intensities in all subjects. The intersection of both segments defined the instant when EMG_{T2} occurred. An analysis of variance was conducted to determine whether the adjustment using two-segment regression led to a significant ($p < 0.05$) reduction in the total sum of squares when compared to the adjustment using a single-segment regression (see Figure 1C).
4. Finally, the best three-segment regression of the EMG data was computed in order to detect an additional breakpoint in the EMG curve. The third middle segment was obtained by methodically breaking the first (left) segment obtained in the previous step into two segments, and keeping the regression that yielded the lower sum of squares (see Figure 1D). Finally, an analysis of variance was conducted to determine whether the adjustment using three-segment regression led to a significant ($p < 0.05$) reduction in the total sum of squares when compared to the adjustment using two-segment regressions. The two points resulting from the intersection of the above three segments determined the instants when the first (EMG_{T1}) and second (EMG_{T2}) EMG thresholds occurred (see Figure 2).

2.8. Statistics

Kolmogorov-Smirnov tests confirmed that each parameter analyzed in the study (EMG_{T1}, EMG_{T2}, VT_1, and VT_2) was normally distributed. For the validity study (16 subjects), one-way repeated-measures ANOVA was used to determine whether there were significant differences between, on the one hand, EMG_{T1} and VT_1, and on the other hand, between EMG_{T2} and VT_2, when these variables were expressed in W, VO_2 (ml/kg/min), and % VO_{2MAX}. Pearson correlation (r) was used to determine the possible relation between EMG_{T1} and VT_1, as well as between EMG_{T2} and VT_2. To further analyze the validity of the EMG method, the Bland-Altman method was followed [22]. For this analysis, the mean differences (bias) and SD of the differences between the mean values (W) obtained with the two methods (EMG_{T1} vs. VT_1, and EMG_{T2} vs. VT_2) were calculated. The mean

difference plus or minus two SD is shown in Figure 3. In this way, the bias and precision of the EMG technique could be calculated [6]. For the group of six subjects that performed the test on two different days (reliability study), a paired Student's t test was used to compare mean values of EMG_{T1} and EMG_{T2} obtained with both tests. Intraclass Pearson's correlation coefficients were also calculated to determine the degree of correlation between mean values of EMG_{T1} and EMG_{T2} reported with repeated tests. Significance was set at $p < 0.05$.

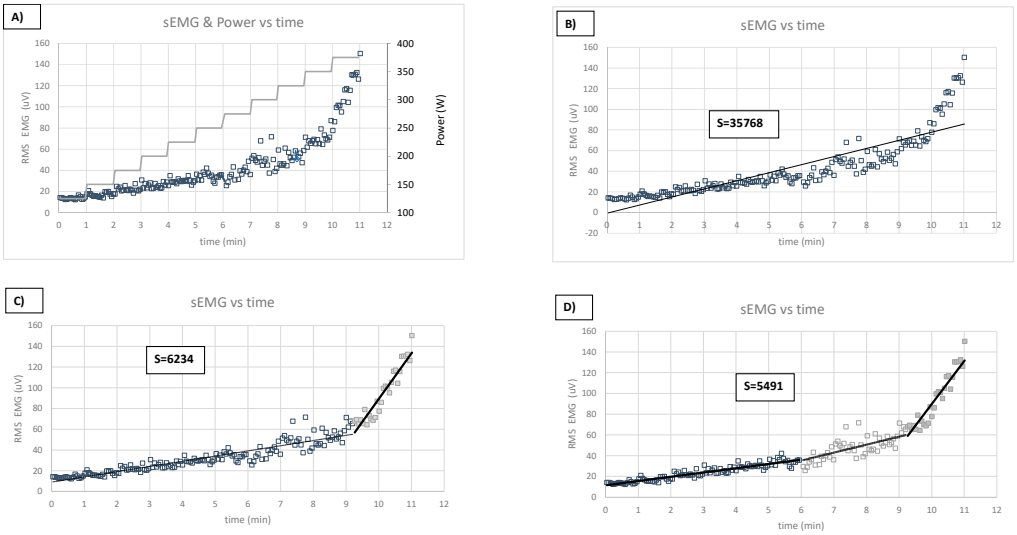


Figure 1. Multi-regression method step by step, S represents the residual sum of squares (RSS). (**A**) Original data, workload (gray line), and EMG amplitude for each epoch. (**B**) Best single-segment regression. (**C**) Better two-segment regression. (**D**) Better 3-segment regression.

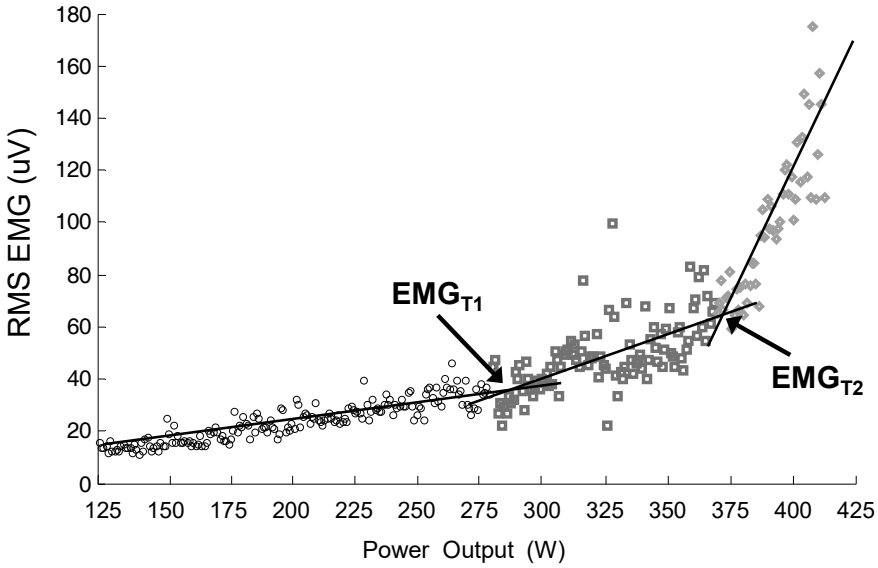


Figure 2. A representative example of a surface electromyographic (EMG) recording of the vastus lateralis obtained during the incremental test of one cyclist. Each data point represents the root mean square of the EMG (RMS EMG), obtained at two-second intervals. The RMS EMG data were fitted with three regression lines using the multi-segment linear regression method. The intersections between the regression lines mark the breakpoints in the linearity of the curve, thus yielding the first (EMG_{T1}) and second (EMG_{T2}) thresholds.

3. Results

3.1. Physiological Values

Table 1 provides the physiological characteristics of the participants calculated from the incremental test. As expected, well-trained cyclists reached high values of VO_2 at the end of exercise: 71.6 ± 4.1 mL/kg/min (range 62.3–81.0). The maximal power output of 406 ± 36 W (range 325–450) was also high. The duration of the test was 11.1 ± 1.3 min (range 8–13 min).

Table 1. Physiological (mean ± SD) characteristics of the participants (N = 16) calculated from the incremental test.

Variables	Mean (SD)	Range
VT_1 (L min^{-1})	3.3 ± 0.6	1.9–4.4
VT_2 (L min^{-1})	4.6 ± 0.5	3.4–5.6
VO_{2MAX} (ml Kg^{-1} min^{-1})	4.9 ± 0.7	4.5–5.7
VO_{2MAX} (L min^{-1})	71.6 ± 4.1	62.3–81.0
Power at end of exercise (W)	406 ± 36	325–450

3.2. Time Course of EMG Amplitude during the Incremental Test

Figure 2 provides an example of an EMG response during the incremental exercise of one cyclist. As can be seen, three regression lines with different slopes were fitted to the data points, which allows for identification of two breakpoints in the linearity of the RMS EMG curve. This two-EMG-threshold pattern was observed in 75% of subjects for all muscles investigated, with EMG_{T1} and EMG_{T2} occurring, respectively, around 70%–80% and 90%–95% of $VO2_{MAX}$.

The second threshold, EMG_{T2}, was detected in all 16 subjects for all muscles. However, EMG_{T1} could not be detected in the VL, VM, and BF in 20%–25% of the cases, whereas it was identified in the GLM for all subjects. It should be noted that EMG_{T1} was always detected in at least two of the muscles tested.

3.3. Validity: Comparison between Ventilatory and Electromyography Thresholds

Table 2 summarizes the average values of VT_1 and EMG_{T1} expressed in W, VO_2 (ml/kg/min), and $\%VO_{2MAX}$ for all muscles. There were no significant differences between the means of EMG_{T1} and VT_1 for the VL, VM, and BF muscles. However, for the GLM muscle, the means of EMG_{T1} were significantly greater than those of VT_1 ($p < 0.05$).

Table 2. Mean values of the power output (W), VO_2, and $\%VO_{2MAX}$ obtained at the first electromyographic threshold (EMG_{T1}) and at the first ventilatory threshold (VT_1). All values are expressed as mean ± SD.

		EMG_{T1}			
Variable	**VT_1**	**Vastus Lateralis**	**Vastus Medialis**	**Biceps Femoris**	**Gluteus Maximus**
W	278.2 ± 34.5	291.1 ± 44.3	294.0 ± 46.9	295.6 ± 47.3	300.8 ± 37.9 *
VO_2 (ml/kg/min)	51.5 ± 6.8	54.5 ± 6.9	55.7 ± 5.9	56.5 ± 8.0	57.8 ± 5.7 *
$\%VO_{2MAX}$	71.9 ± 9.4	76.1 ± 8.9	77.8 ± 9.5	78.9 ± 11.5	80.7 ± 6.1 *

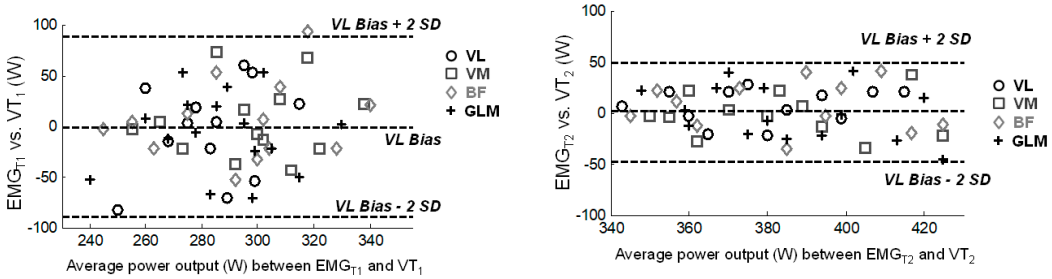
* Significant difference between the mean values at EMG_{T1} and at VT_1 ($p < 0.05$).

Table 3 summarizes the average values of VT_2 and EMG_{T2} expressed in W, VO2 (ml/kg/min), and $\%VO2_{MAX}$ for all muscles. There was no significant differences between means of EMG_{T2} and VT_2 ($p > 0.05$) for four muscles.

Table 3. Mean values of the power output (W), VO2, and %V obtained at the second electromyographic threshold (EMG_{T2}) and at the second ventilatory threshold (VT_2). All values are expressed as mean ± SD.

		EMG_{T2}			
Variable	**VT_2**	**Vastus Lateralis**	**Vastus Medialis**	**Biceps Femoris**	**Gluteus Maximus**
W	383.5 ± 44.4	378.6 ± 24.5	372.2 ± 27.4	374.4 ± 31.6	379.8 ± 30.2
VO2 (mL/kg/min)	65.6 ± 4.5	66.9 ± 3.5	65.8 ± 3.8	66.9 ± 4.8	67.2 ± 4.7
$\%VO2_{MAX}$	91.6 ± 8.3	93.5 ± 6.0	91.9 ± 6.0	93.4 ± 5.2	93.8 ± 4.9

To further examine the validity of the EMG method, the Bland-Altman method was applied to assess the degree of agreement between the EMG thresholds and ventilatory thresholds (see Figure 3). This figure shows that, for all muscles, the bias and error of the multi-segment regression method was relatively low. Note that the levels of agreement were especially low for the comparison between VT2 and EMG_{T2} (Figure 3b). Moreover, for all comparisons, at least 90% of the individual values were within the limits of agreement.



(**a**) (**b**)

Figure 3. Graphic analysis of power output (W) data corresponding to (**a**) the first electromyographic threshold (EMG_{T1}) and ventilatory threshold (VT_1), and (**b**) the second electromyographic threshold (EMG_{T2}) and ventilatory threshold (VT_2), for the vastus lateralis (VL), vastus medialis (VM), biceps femoris (BF), and gluteus maximus (GLM).

3.4. Correlation between Ventilatory and Electromyography Thresholds

In all muscles, a significant correlation was found between the power outputs (W) at VT_1 and EMG_{T1} (Table 4). Likewise, for all muscles, there was a significant correlation between the power outputs at VT_2 and EMG_{T2} (Table 4).

Table 4. Correlations between the power output (W) obtained at the first electromyographic threshold (EMG_{T1}) and at the first ventilatory threshold (VT_1), and between the power output obtained at the second electromyographic threshold (EMG_{T2}) and at the second ventilatory threshold (VT_2).

	EMG_{T1}			
	Vastus Lateralis	**Vastus Medialis**	**Biceps Femoris**	**Gluteus Maximus**
VT_1	0.75 *	0.69 *	0.72 *	0.88 *
	EMG_{T2}			
	Vastus Lateralis	**Vastus Medialis**	**Biceps Femoris**	**Gluteus Maximus**
VT_2	0.90 *	0.82 *	0.82 *	0.83 *

* Significant correlation ($p < 0.05$).

3.5. Reliability

No significant differences were found between mean values of either EMG_{T1} or EMG_{T2} obtained in the first and second tests ($p < 0.05$). Intraclass correlation coefficients (ICC) between repeated measurements were significant ($p < 0.05$) and high (Table 5).

Table 5. Reliability of measurements of the electromyographic thresholds EMGT1 and EMGT2.

Muscle	Threshold	First Test	Second Test	ICC
Vastus lateralis	EMGT1 (W)	289.2 ± 50.4	294.3 ± 47.8	0.85
	EMGT2 (W)	375.2 ± 27.2	380.4 ± 31.6	0.87
Vastus medialis	EMGT1 (W)	291.2 ± 27.0	295.4 ± 31.5	0.93
	EMGT2 (W)	370.2 ± 25.9	374.4 ± 30.1	0.89
Biceps femoris	EMGT1 (W)	293.2 ± 27.7	297.4 ± 31.5	0.93
	EMGT2 (W)	371.2 ± 29.9	376.4 ± 28.1	0.86
Gluteus maximus	EMGT1 (W)	298.2 ± 27.9	302.4 ± 31.2	0.96
	EMGT2 (W)	377.2 ± 28.3	381.4 ± 27.7	0.91

4. Discussion

The main findings of our study were the following: (1) the multi-segment linear regression method was able to detect two breakpoints (thresholds) in the RMS EMG vs. time curve from all muscles in 75% of participants during an incremental cycling test of 60 s stages; (2) the two breakpoints, EMG_{T1} and EMG_{T2}, were detected at around 70%–80% and 90%–95% of VO_{2MAX}, respectively; and (3) the two EMG breakpoints, EMG_{T1} and EMG_{T2}, occurred at similar power outputs than VT_1 and VT_2, respectively.

4.1. Possible Mechanisms for the Non-Linear Increases in EMG Amplitude

Most studies on cycling performance attribute the occurrence of breakpoints in the linearity of the EMG curve to the recruitment of additional motor units and/or to the change in the type of motor units recruited [5]. However, to date, the mechanisms underlying the abrupt increases in EMG amplitude during an incremental test are not entirely clear, and could involve both local and central (global) factors.

At the local (muscle) level, it has been shown that as fatigue develops, there is a progressive accumulation of metabolic byproducts of muscular contraction (i.e., lactate, hydrogen ions, potassium, and inorganic phosphate), which could result in impairment of excitation–contraction coupling [23,24]. To counteract this deficit in the contractile apparatus, muscle power output during an incremental test might be increased by (1) recruiting more motor units; and (2) recruiting type IIa and type IIb motor units to obtain energy through anaerobic glycolysis. Based on this reasoning, it has been hypothesized that the first breakpoint would correspond to the moment when a large number of type IIa (fast twitch oxidative-glycolytic) motor units come into play, whereas the second threshold would occur when a large population of type IIb (fast twitch glycolytic) motor units are recruited [6]. These hypotheses, however, have not been experimentally verified, and thus remain speculative. Indeed, EMG amplitude could increase due to factors other than the number and type of active motor units, such as broadening of the trans-membrane action potential [25], crosstalk [4], and even signal cancellation [4]. Thus, motor unit recruitment is not the only peripheral factor that can account for the non-linear increases in EMG amplitude.

Central (and not only local) factors could also explain the occurrence of abrupt increases in EMG amplitude. Indeed, impairment of the contractile machinery during the course of an incremental test could be compensated by an enhancement of motor unit rate coding. Specifically, it might be speculated that one or two of the EMG thresholds could be related to increases in motor unit discharge rate and/or synchronization [4]. The possible involvement of neural mechanisms in the EMG breakpoints is further supported by the experiments of Airaksinen et al. [26], who demonstrated that, during an incremental cycling test, both working and non-working muscles showed a shift in EMG at the same load. Airaksinen's observations are suggestive of a global change in the neural activation of muscles. It is therefore plausible that the abrupt increases in EMG activity may be initiated not only by impairment of excitation–contraction coupling, but also by increases/adjustments in the

neural activity [26]. We conclude by stating that, because multiple factors influence EMG amplitude, the assumption that EMG breakpoints are caused by changes in motor unit recruitment should be treated with caution, as it remains empirically unsupported.

4.2. Comparison of the Multi-Segment Linear Regression Method with Stage Durations of 12 s and 60 s

To date, the multi-segment linear regression method had only been tested during an incremental test with 12 s stage durations [6], and the applicability of this algorithm to incremental protocols with longer stage durations (one or two minutes) remained to be verified. Indeed, the 12 s stage test employed by Lucía et al. resembles a ramp-like protocol, in which muscular effort is continuously increased to cope with the quick increases in workload. Such test differs noticeably from the graded steady state protocol employed here, where subjects were asked to maintain a fixed power output for 1 min. Based on this, it was predicted that the development of fatigue, the pattern of motor unit recruitment, and/or the sensation of muscular effort may be different in protocols with 12 s and 60 s stage durations, and that this may result in differences in the detection of EMG thresholds and in the % of VO_{2MAX} at which the EMG thresholds are reached. Interestingly, we found that the two breakpoints encountered, EMG_{T1} and EMG_{T2}, occurred at higher percentages of VO_{2MAX} (around 70%–80% and 90%–95% of VO_{2MAX}, respectively), as compared to those found during the 12-s stage protocol (around 60%–70% and 80–90% of VO_{2MAX}, respectively). One possible explanation for these differences may be that, during exercise periods of longer duration, adjustments in motor unit rate coding may "delay" the recruitment of a significant number of motor units to higher relative intensities of exercise. Specifically, it may be that, with longer stage durations, exercise intensity is maintained at the expense of changes in motor unit discharge rate and/or synchronization and less so due to recruitment of additional motor units [27]. Due to this, great recruitment of additional motor units would be "delayed/postponed" until higher relative intensities of effort are reached, which explains why EMG thresholds occur at higher percentages of VO_{2MAX}. Alternatively, it might be hypothesized that, with longer stage durations, the sensation of muscular effort appears at higher relative intensities, and thus the signal that triggers the increases in neural activity occurs at a higher percentage of VO_{2MAX} [26].

It is also worth noting that, with the 60 s stage duration protocol, we detected the first EMG breakpoint in 75% of participants, whereas, with the 12-s stage duration protocol, the first breakpoint was identified in 90% of subjects by Lucía et al. [6]. This small discrepancy may be attributed to slight differences in the development of fatigue in these protocols. Indeed, it is possible that, with the 60 s stage test, the sensation of muscular effort/fatigue increased gradually at the beginning and middle of the exercise, but increased more steeply towards the end of the test. Such progression of fatigue would lead to a EMG time course in which the first breakpoint is not so well marked (for an example, see Figure 1 of Latasa et al. [28]). In contrast, it is likely that, with the 12 s stage test, fatigue increased more progressively, thus resulting in an EMG time course in which two breakpoints are more easily recognized.

4.3. Comparison with Previous Studies

The mathematical method utilized here has clearly identified two breakpoints in the linearity of the EMG recordings in most subjects. However, in reviewing the literature there is clearly a disparity of results regarding the number of EMG breakpoints encountered. The existence of a non-linear increase in EMG amplitude at around the aerobic-to-anaerobic transition intensity was reported by some authors since the 1980s [1,12], but it was not until 1999 that Lucia et al. noted the existence of a second EMG breakpoint occurring at near to maximum intensities [6]. Studies prior to Lucía's findings overlooked and missed this breakpoint, which was most likely due to the great interest in determining the transition between the aerobic and anaerobic metabolism, known to be at lower intensities. For this reason, investigations prior to Lucía's work only detected one non-linear increase in EMG variables at about 60%–70% of VO_{2MAX} [1,29]. After the key study of Lucía et al., there continues to be a

discrepancy in the number and interpretation of EMG breakpoints identified during incremental tests [30,31]. Remarkably, automatic methods used by most authors for EMG-threshold detection had the limitation that only one EMG breakpoint could be possibly identified [8,9,32,33]. Essentially, these methods are based on calculating all the two-segment regressions for the data, and then choosing the two-segment regression with the least residual sum of squares, thus yielding a single intersection point. In contrast, the multi-segment linear regression method presented here offers the possibility to fit the data with three linear segments, which allows better adjustment of data and, most importantly, determination of two EMG breakpoints. It might be argued that the two EMG thresholds do not really exist and that they are artificially determined by the algorithm. However, various authors have confirmed the existence of two EMG thresholds by visual analysis [10,11].

The question remains of why the majority of studies have reported only one EMG threshold instead of two. Several factors may contribute to the conflicting results. First, it has been suggested that the second EMG breakpoint can only be detected when subjects are capable of reaching high intensities during the incremental tests, possibly higher than VT_2. This would explain why EMG_{T2} is more easily found in professional cyclists: They have the capability of recruiting a sufficient number of motor units at high intensities so as to induce an abrupt detectable increase in EMG activity [6,11,33]. The two-threshold response reported here was obtained for well-trained cyclists, which lends further support to this theory. A second factor may be the choice of the fixed pedaling cadence. Indeed, with a fixed cadence, the increase in exercise intensity is reached at the expense of increasing muscular effort, which favors the recruitment of fast motor units and possibly facilitates threshold detection at EMG_{T2} [6,34]. Finally, it might be possible that previous studies had problems in finding the both EMG breakpoint because they only assessed one muscle, typically the vastus lateralis. In the present study, we have shown that EMG_{T2} is more frequently detected in the gluteus maximus than in the vastus, and that EMG_{T2} is more easily detected when various leg muscles are examined. It is known that the contribution of gluteus maximus to total force is higher at highest workloads [35], and that hip extensors muscles, like gluteus maximus, compensate the loss of force production of knee extensors [36,37]. As a consequence, the onset of fatigue would be delayed in the gluteus maximus compared to the knee extensors; this would also imply a wider range of EMG amplitudes at the highest workloads, thereby explaining the easier detection of both thresholds in the gluteus maximus.

The present findings suggest that the aerobic-anaerobic transition may take place in two stages, thus reinforcing the view that such transition may be more complex than expected. The occurrence of two breakpoints in the EMG vs. time curve would be in accordance with the existence of three exercise intensity domains (moderate, heavy, and severe), and may signify that, during a continuous incremental ergometer test, the increase in fatigue takes place in various phases.

4.4. Comparison between EMG Fatigue Thresholds and Ventilatory Thresholds

Our results showed a correspondence between the occurrence of EMG_{T1} and VT_1, on the one hand, and between EMG_{T2} and VT_2 on the other. This correlation between EMG and ventilatory thresholds could be due to the enhanced activation of respiratory centers triggered by intramuscular accumulation of potassium and hydrogen ions, although this possibility is not based on the present results and thus is speculative. Indeed, it has been suggested that group III–IV muscle afferents are sensitive to changes in muscle acidosis and extracellular potassium concentration [38,39]. The chemo-sensitive activation of these afferents could elicit a marked stimulation of the respiratory neurones [40,41], thus leading to an abrupt increase in minute ventilation. Based on this sensorimotor reflex loop, it might be hypothesized that ventilation exhibits a first breakpoint (VT_1) when an abrupt increase in the number of active motor units (EMG_{T1}) occurs, and a second breakpoint (VT_2), coinciding with the change in motor unit recruitment from predominantly slow twitch motor units to fast twitch motor units (EMG_{T2}). In agreement with this theory, there are many reports showing a concomitant parallel increase in ventilation and EMG activity during an incremental cycling test [2,6,12–14,28,33].

Alternatively, there are studies suggesting that changes in ventilation observed during incremental exercise are not triggered by accumulation of potassium and hydrogen ions, but that such changes are mediated by an increase in neural activity originating from the subthalamic motor region or indirectly via α-γ coactivation of motor neurons innervating exercising limbs [42]. Such increase in neural activity may occur in response to the need to progressively recruit fast twitch muscle fibers as exercise intensity is increased. Finally, there are reports suggesting that ventilation variables are not influenced by motor unit recruitment, as evidenced by the dissociation between the occurrence of EMG and ventilation thresholds [2,43].

4.5. Methodological Limitations

The present experiments had various limitations that deserve comment. First, we did not assess the isometric maximal voluntary contraction and, as a consequence, we had no means to quantify the level of muscle activation and the possibility to normalize EMG data. Moreover, apart from the multi-segment linear regression method, no other techniques have been employed to examine neuromuscular fatigue during the incremental test, such as a decline in maximal voluntary force, and measures of conduction velocity or spectral EMG analysis. As a result, it was impossible to relate the occurrence of the two thresholds encountered (EMG_{T1} and EMG_{T2}) in the EMG RMS response with other indexes of neuromuscular fatigue. A third limitation is that EMG-based thresholds are not fully reliable indexes of the level of muscle activation because such EMG measures are affected by numerous uncontrollable factors (for a review, see Reference [4]). Finally, many factors influence the relation between EMG amplitude and force, including the thickness of subcutaneous tissue, the recruitment strategy, and the peak discharge rates of the different MUs [4]. Due to many factors that can influence this relation, the correlation between surface EMG amplitude the energy system used during continuous cycling may be lower than expected.

5. Conclusions

In conclusion, it has been shown that, during an incremental cycling test with 60 s stage durations, the multi-segment linear regression method is able to detect a two-threshold response in the RMS EMG curve from four lower limb muscles in 75% of participants. The first and second EMG thresholds occurred at exercise intensities of 70%–80% and 90%–95% of VO_{2MAX}, respectively. The correspondence between, on the one hand, the first EMG breakpoint and the first ventilatory threshold, and on the other hand, between the second EMG breakpoint and the second ventilatory threshold, indicates that the multi-segment linear regression algorithm is a valid non-invasive method for analyzing the aerobic-anaerobic transition during incremental tests with 1 min stage durations.

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