### (a) Title:

# Estimation of the neuromuscular fatigue threshold from an incremental cycling test using 1-min exercise periods

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#### **Abstract**

The objectives of this study were: (1) to evaluate the method used for estimating the neuromuscular fatigue threshold from surface electromyographic amplitude (the PWC<sub>FT</sub> test) during a single incremental cycling workout using 1-min exercise periods, and (2) to investigate the possible associations between PWC<sub>FT</sub> and metabolic (onset of blood lactate accumulation, OBLA) and ventilatory (ventilatory threshold, VT, and respiratory compensation point, RCP) variables. Sixteen cyclists performed incremental cycle ergometer rides to exhaustion with bipolar surface sEMG signals recorded from the vastus lateralis. Subsequently, participants performed one constant-workload exercise test at 100 % of their PWC<sub>FT</sub>. During the incremental test, the power output at PWC<sub>FT</sub> was not correlated with that of OBLA (P>0.05), but it was positively correlated with those of VT and RCP (P<0.05). During the constant-workload test, heart rate and blood lactate increased progressively and significantly (P<0.05), whereas sEMG amplitude remained unchanged (P<0.05). The average duration of the constant-workload exercise was 8-9 min. It is concluded that application of the PWC<sub>FT</sub> method using 1-min exercise periods could lead to overestimation of the conformuscular fatigue threshold most likely because this stage duration allows insufficient time for the sEMG response to manifest.

# **Keywords**

Cycling;

Neuromuscular fatigue;

Surface electromyography;

Lactate;

Motor unit recruitment

#### Introduction

Extensive research has been directed towards the identification of the threshold that demarcates fatiguing from non-fatiguing exercise during an incremental workout on a cycle ergometer. <sup>1,2,3</sup> Several fatigue thresholds based on metabolic variables (onset of blood lactate accumulation, OBLA) and ventilatory measurements (ventilatory threshold, VT, and respiratory compensation point, RCP) have been used for this purpose. <sup>4,5</sup> As the exercise intensity increases, fatigue compromises not only the cardiovascular and respiratory systems, but also the neuromuscular system. Specifically, the neuromuscular system counteracts the increasing fatigue by recruiting additional motor units and increasing the discharge rate, <sup>6</sup> as a result of which the amplitude of the surface electromyographic (sEMG) signal increases. <sup>7</sup> Precise identification of the neuromuscular fatigue threshold is of vital importance as it would allow comparison of fatigue states in the cardiovascular and neuromuscular systems.

In a series of studies, deVries and colleagues <sup>1,7</sup> proposed a method for calculating the threshold of fatigue (the Physical Work Capacity at the Fatigue Threshold, PWC<sub>ET</sub>), which consists of examining the sEMG amplitude vs. time relationship obtained during 4 workbours performed at 4 different power outputs. The authors identified the PWC<sub>FT</sub> by determining the highest power output that can be sustained for an exercise period of 2 min without a significant increase in sEMG amplitude. In its original conception, the deVries model was a discontinuous method which requires multiple visits to the laboratory. Subsequently, deVries et al. (1990) performed a further refinement of the method, which allowed the fatigue threshold to be determined from a single (i.e., continuous) incremental test to exhaustion. Recently, the PWC<sub>FT</sub> test has been femamed as "EMG<sub>FT</sub> test" by some authors, <sup>9,10</sup> leading to some confusion

The deVries method (1990) is normally performed using exercise periods of 2 min. This stage duration was chosen on the grounds that "it ensures the linearity of the sEMG vs. time regression lines". Validation of the deVries method with 2-min stage duration was already performed by Briscoe et al. (2014) and, recently, Camic et al. (2010) provided the estimates of PWC<sub>FT</sub> using the deVries test with 2-min exercise periods. However, many researchers interested in assessing the physiological responses of cyclists perform the incremental test using 1-min workbouts. All 11,12,13,14 Therefore, it would be interesting to test the applicability of the deVries method using a shorter (1-min) time period. Moreover, it would be valuable to compare the estimates of PWC<sub>FT</sub> resulting from the 1-min stage duration study with the results reported in Briscoe and Camic studies. Furthermore, in many studies investigating the occurrence of the metabolic and ventilatory thresholds during an incremental test, the

successive exercise periods have 1-min duration. <sup>11,12,13</sup> Therefore, the possibility of extracting, from a single incremental cycling test, both the metabolic/ventilatory and sEMG-based fatigue thresholds is attractive from a practical point of view and, therefore, should be explored.

Recently, Briscoe et al. (2014) demonstrated that application of the deVries method with 2-min exercise periods provides a valid measure of neuromuscular fatigue, i.e., the assessment of PWC<sub>FT</sub> was not overestimated. On the other hand, Evetovich et al. (1996) <sup>15</sup> evaluated the deVries model using stage durations of 2, 3, and 4 min and found that the power output at which the PWC<sub>FT</sub> is reached increased with shorter exercise periods. Collectively, these results prompted us to explore the possibility that application of deVries method with 1-min stages may lead to overestimation of the neuromuscular fatigue threshold.

It is important to understand the relation between the PWC<sub>FT</sub> estimate and other estimates of fatigue threshold (e.g. OBLA, VT, RCP). Previous studies examining these relationships have yielded conflicting results. For example, various authors found significant correlations between lactate accumulation and the occurrence of an inflection point in the sEMC activity, <sup>16,17</sup> whereas others found no significant correlation. <sup>18,19</sup> Similarly, while some authors reported significant correlations between ventilatory thresholds and PWC<sub>F</sub>, <sup>20</sup> others found no connection between these variables. <sup>21</sup> Recently, Camic et al. (2010) <sup>2</sup> found that ventilatory thresholds were not correlated with the sEMG threshold in the time domain (i.e., PWC<sub>FT</sub>), but that these ventilatory indicators were positively associated with a new sEMG-based threshold in the frequency domain (namely, MPF<sub>FT</sub>).

Based on the experiments of Evetovichand convorkers we hypothesized that application of deVries' model using 1-min stage durations would yield a PWC<sub>FT</sub> estimate close to the severe exercise intensity domain. The objectives of the present study were: (1) to evaluate the applicability of the PWC<sub>FT</sub> test proposed by de Vries using 1-min exercise periods, and (2) to examine the relationships between metabolic/ventilatory and sEMG-based fatigue thresholds during incremental ergometer cycling. Evaluation of the de Vries method with 1-min workbouts was performed by having participants perform a constant workload ride at 100% of the estimated PWC<sub>FT</sub>.

#### 2. Material and Methods

# **Participants**

Sixteen male semi-professional cyclists volunteered to participate in the study. Their anthropometric and physical characteristics are given in Table 1. 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 not to take part in vigorous physical activity for 2 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 performed an average of 20,000 km riding (range 16,000–24,000 km) during the last season. None of the participants reported any injuries or pathologies of limb muscles or joints.

## Screening session

Cyclists underwent a blood test screening prior to participation to check for anemia and possible infections. Blood samples were collected by antecepital reminuncture with Vacutainer system. Red blood cell, white blood cell, platelets, hemoglobin, and bemacerit were determined on a Coulter Counter (model MAX-M). Serum biochemical parameters (glucose, urea, uric acid, creatinine, creatin kinase, lactate dehydrogenase, aspartate transaminase, alanine transaminase, aldolase, total proteins, cholesterol and electrolytes) were measured using coupled enzyme reactions on an automatic autoanalyzer (Hitachi 917, Japan). Subsequently, 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.

# Maximal cycle ergometer test

Participants performed an incremental test to exhaustion on a SRM powermeter (science SRM, SRM GmbH, Germany). The SRM unit consisted of a potentiometer (Powermeter V Science Road Version) connected to a recording system (Powercontrol V). Analysis Software was SRM Training System.

Pedal cadence was maintained at 70 rev·min<sup>-1</sup> during the test, similar to that used in previous studies.<sup>17</sup> For each participant, saddle height and saddle setback were adjusted jointly so that the following criteria were fulfilled: (1) the knee angle in the bottom dead centre position of the pedal was, at most, 150°, and (2) knee joint was positioned above the pedal spindle when the crank arm was in horizontal forward position.<sup>11</sup> 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 minute until the participants could no longer continue to exercise. At this time, the power achieved was referred to as the maximal power. Heart rate was monitored telemetrically using a Polar Heart Watch system (Polar 610 Plus, Polar Electro Oy, Kempele, Finland).

Analysis of expired gas and determination of ventilatory thresholds (VT and RCP)

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 face mask was connected to fast-response differential paramagnetic oxygen and infrared carbon dioxide analyzers. Throughout the incremental test, the software (Oxycon PRO, Carefusion, Germany) averaged, for 5 consecutive seconds, data of oxygen uptake ( $\dot{V}O_2$ ) and carbon dioxide production ( $\dot{V}CO_2$ ) and ventilatory parameters, as well as the ventilatory equivalents for  $O_2$  (EqO<sub>2</sub>)  $\dot{V}E/\dot{V}O_2$ ) and CO<sub>2</sub> (EqCO<sub>2</sub> =  $\dot{V}E/\dot{V}CO_2$ ). The ventilatory threshold (VT) was determined using the criteria of an increase in the ventilatory equivalent for oxygen ( $\dot{V}E/\dot{V}O_2$ ) with no increase in the ventilatory equivalent for carbon dioxide ( $\dot{V}E/\dot{V}CO_2$ ) and the departure from linearity of  $\dot{V}E/\dot{V}$ . The respiratory compensation point (RCP) corresponded to the minimal work rate at which the increase in  $\dot{V}E/\dot{V}O_2$  was accompanied by a parallel increase of  $\dot{V}E/\dot{V}CO_2$ . A test-retest reliability study was conducted for the purpose of determining the stability of the performance of our participants. This method was used to determine the intraclass correlation coefficient (ICC). The repeat study was performed on a second day on six participants (those who lived close to the laboratory where experiments were conducted).

Analysis of blood lactate and determination of lactate threshold (OBLA)

Blood samples (25 µl) were taken from fingertips at rest, every two minutes during the test (i.e, every two workbouts), and 30 s after termination of exercise. Lactate concentration was measured from these blood samples using an automatic analyzer (YSI Model 1500 Sport, Yellow Springs, USA). OBLA was calculated as the power output corresponding to a blood lactate concentration of 4.0 mmol/L.<sup>24</sup>

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 muscle at one-third of the distance between the lateral border of the patella and the anterior superior iliac spine according to the SENIAM (Surface EMG for Non-Invasive Assessment of Muscles) guidelines. 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 sEMG signals were amplified with a bandwidth frequency ranging from 10 to 1000 Hz, and digitized online at a sampling frequency of 1000 Hz using the analog-to-digital conversion system of MP150.

## Determination of $PWC_{FT}$

The PWC<sub>FT</sub> was calculated using the method of deVries et al. (1990). This method is briefly described as follows. During each 1-min workbout of the incremental test, consecutive sEMG epochs (each epoch representing a 128-ms interval chosen during the active period of the vastus lateralis in a single pedaling cycle) were recorded. The first 15s of each 1-min exercise period were not considered since, at the beginning of each period, the cyclist made slight postural adjustments in order to match the target power output and to maintain the required pedal cadence. For each power output of the test, the sEMG amplitude of each of the epochs was calculated and represented vs. time (see Fig. 1 for an example). Then, we identified the lowest power output that resulted in a *significant* positive slope coefficient (P < 0.05 in a single-tailed trest) for the sEMG amplitude vs. time relationship and the highest power output that gave rise to a *non-significant* slope coefficient (P > 0.05). The PWC<sub>FT</sub> was determined by averaging the above-mentioned power outputs (Fig. 1).

# Constant-workload exercise test

Based on the estimated PWC<sub>FT</sub> from the incremental ergometer test, each participant performed one constant-workload exercise test at the power output corresponding to 100 % of their PWC<sub>FT</sub>. During the test, participants were asked to maintain a pedal cadence of 70 rev·min<sup>-1</sup> at the selected power output for as long as they could. Termination of the test was based on the participant's inability to maintain 70 rev·min<sup>-1</sup> despite strong verbal encouragement. The Constant-workload exercise test was performed one (13 participants) or two (3 participants) days after the incremental test. This resting period ensured full recovery in all participants.

## Data analysis

Data were first analysed with commercially-available software (AcqKnowledge, BIOPAC Systems, Goleta, CA, USA) to monitor for any abnormality in sEMG traces. Subsequently, data were exported to Matlab (version R2012b; The Math-Works, Natick, MA, USA) for quantitative analysis using a number of custom scripts. The sEMG signals were filtered using a digital bandpass filter (fourth-order Butterworth) between 15 and 1000 Hz. The root mean square (RMS) was used as an index of the global sEMG amplitude and was calculated for each epoch. The sEMG epoch for each pedal thrust (descending phase of the pedaling cycle) was chosen from the sEMG bursts, i.e., when the vastus lateralis was active. Each sEMG epoch corresponded to a fixed part of a single pedaling cycle, as performed by Dimitrov et al. (2006). Hence, for each exercise period of the test, 52 values of sEMG RMS were calculated (i.e., one for every pedal cycle during 45s at 70 rev min and analyzed as a function of time.

For the constant-workload exercise test, the sEMG amplitude heart rate, and blood lactate data were expressed in relative terms (as a percentage relative to the highest value attained during the incremental test (% max)) in the tables and also in absolute terms in the figures. The time recorded for constant-workload test was normalized as a percentage of total duration. To investigate the behaviour of the muscle activity during the constant-workload exercise, the stope coefficient of the sEMG amplitude vs. percentage of normalized time was calculated.

#### **Statistics**

Statistical analysis was performed using SignaPlot. Kolmogorov-Smirnov tests confirmed that each parameter analysed in the study (RWC<sub>FT</sub>, OBLA, VT, and RCP) was normally distributed. The slope coefficient for each exercise period was calculated from the sEMG amplitude vs. time relationship. To determine whether this stope coefficient was significant, linear regression analysis was performed. Mean and standard deviation values were calculated for the fatigue thresholds studied (PWC<sub>FT</sub>, OBLA, VT, and RCP). One-way repeated-measures ANOVA was used to determine whether there were significant differences in average power output among the PWC<sub>FT</sub>, OBLA, VT, and RCP. When differences were significant, Tukey's post hoc test was used. Pearson correlation coefficients (r) were calculated to determine the relationships among PWC<sub>FT</sub>, OBLA, VT, and RCP. One-way repeated-measures ANOVA was performed to investigate the time-dependent changes (factor = time) of blood lactate, heart rate, and sEMG amplitude within the constant-workload test. When differences were

significant, Tukey's post hoc test was used. Test–retest reliability was calculated using the ICC. Significance was set at P < 0.05.



#### 3. Results

# 3.1 Fatigue thresholds of metabolic/ventilatory and sEMG variables during the incremental test

For all participants, sEMG amplitude increased progressively over the course of the incremental test as a sign of increasing motor unit activation. Figure 1 provides a representative example of the changes in sEMG amplitude during the incremental exercise in one cyclist. As can be seen, sEMG increased slowly at a constant rate from the onset of the protocol until the last three exercise period of the test, when the increase became steep and pronounced. In Fig. 1(b), the regression lines of the sEMG amplitude vs. time relationship are shown separately for each power output of the test. As can be seen, the regression lines corresponding to the low and medium workloads were practically horizontal, and only for the last three power output did the slope of the regression line become significant.

— FIGURE 1 about here —

Table 1 provides the values of the physiological characteristics of the participants calculated from the incremental test. As expected, semi-professional cyclists exhibited high values of  $\dot{\mathbf{V}}O_{2peak}$  and VT and RCP. The maximal power tolerated 406 (36) W was also high. Note that some of the physiological variables shown in Table 1, such as heart rate and blood factate, were measured at the end of the incremental test.

TABLE Tabout here —

The power output corresponding to PWCr Dmean  $\pm$  SD,  $372 \pm 25$ ) was comparable to that of RCP (381  $\pm$  36, P=0.42), but significantly higher than that of VT (337  $\pm$  31, P<0.05) and OBLA (306  $\pm$  32, P<0.05). The power output associated to VT was also significantly higher than that of OBLA (P<0.05).

Table 2 shows the correlations among the power outputs corresponding to OBLA, VT, RCP and PWC<sub>FT</sub>. As can be seen, the lactate threshold was not significantly related to VT, RCP and PWC<sub>FT</sub> (P>0.05). However, PWC<sub>FT</sub> was significantly correlated with VT (r = 0.78, P<0.05) and with RCP (r = 0.85, P<0.05). The two ventilatory thresholds were also significantly associated (r = 0.87, P<0.05).

— TABLE 2 about here —

Test–retest reliability for  $\dot{V}O2_{peak}$  yielded an intraclass correlation coefficient (ICC) of R = 0.95, with no significant differences between test and re-test values.

## 3.2 Metabolic/ventilatory and sEMG variables during the constant-workload test

Individual analysis of the sEMG amplitude - time relationship revealed that, for most cyclists (12 of 16), sEMG amplitude remained approximately stable during most part of the constant-workload test and underwent a slight increase towards the end of the test. One representative example of this behaviour is provided in Fig. 2. In 2 cyclists a negative trend in sEMG amplitude vs. time was observed and, in another 2 cyclists, sEMG amplitude increased progressively over the course of the test. Group analysis of the sEMG - time relationship indicated that sEMG amplitude remained unchanged during the constant-workload test [Fig. 3(a)]. The slight increase in sEMG towards the end of the test did not reach statistical significance and, in fact, the slope coefficient of the sEMG amplitude vs. percentage of normalized time was nearly zero.

The mean time to exhaustion during the constant-workload test is shown in Table 3. Group analysis of the heart rate - time relationship showed that heart rate increased progressively throughout the constant-workload test [Fig. 3(b)], this increase being significant during the second half of the test (P<0.05). It is noteworthy that the heart rate at the end of the constant-workload test was roughly the same as the one reached during the incremental test (Table 3). Blood lactate also increased rapidly and significantly during the constant-workload exercise [Pig. 3(c)]. Lactate levels at the end of the constant test were slightly lower than those reached in the incremental test (Table 3).

FIGURE 2 about here —

FIGURE 3 about here —

— TABLE 3 about here —

#### 4. Discussion

4.1 Comparison between sEMG-based fatigue thresholds and metabolic/ventilatory thresholds

One of the purposes of the present study was to determine whether the PWC<sub>FT</sub> derived from the incremental test with 1-min stages was associated with fatigue thresholds determined via blood and gas exchange analysis. The main findings connected with this purpose were: (1) the power output at  $PWC_{FT}$  was similar to that of RCP, but significantly higher than those of VT and OBLA, and (2) The power output at  $PWC_{FT}$  was not correlated with that of OBLA, but it was positively correlated with those of VT and RCP.

During the course of an incremental workload test, a gradual rise in sEMG amplitude is observed, mainly as a result of two strategies adopted by the neuromuscular system to counteract the development of fatigue: a progressive recruitment of additional motor units and a rise of discharge rate. Many authors conjectured that fatigue-induced increases in sEMG activity might be due to the accumulation of metabolic byproducts of muscular contraction (i.e., lactate fordrogen ions, potassium, and inorganic phosphate). The reason is that these metabolites have been proposed to be responsible for manifestations of local muscle fatigue, such as loss of membrane excitability <sup>27</sup> and impaired excitation—contraction coupling. However, the specific metabolites that underlie fatigue-induced changes in sEMG amplitude have not been clearly identified. Some investigators postulate that the decrease in intracellular pH caused by lactate accumulation generates the physiological signal that triggers the recruitment of additional motor units. Indeed, the accumulation of lactate has been suggested as the main contributor to the fatigue-related increases in sEMG amplitude. The accumulation of lactate has been suggested as the main contributor to the fatigue-related increases in sEMG amplitude.

We showed that PWC<sub>FT</sub> was significantly digher than OBLA, indicating that blood lactate concentration had already begun to increase in a nonlinear fashion before the sEMG breakpoint had been surpassed. More importantly, we found no significant correlation between the power outputs corresponding to PWC<sub>FT</sub> and OBLA. Therefore, collectively, our data indicate that, during a single continuous incremental test, blood lactate accumulation may not have a direct effect on the recruitment of additional motor units and/or on the onset of neuromuscular fatigue. Our results support the study of Viitasalo and colleagues <sup>32</sup> who reported no associations between the sEMG vs. time curves and the anaerobic threshold (OBLA in our study). Moreover, the lactate hypothesis was also challenged by the finding that, during incremental treadmill running, the lactate threshold was not related with the occurrence of an inflection point in the sEMG activity. <sup>19</sup>

The possible associations between ventilatory thresholds (VT and RCP) and sEMG-based fatigue thresholds have also been the subject of research. Our results indicated that the power output corresponding to PWC<sub>FT</sub> was similar to RCP, but significantly higher than VT (Tables 2 and 3). These results are not in line with the findings of Glass et al. (1998) <sup>33</sup> and Lucia et al. (1999), <sup>17</sup> who reported that, for the vastus lateralis and rectus femoris, the power output at sEMG threshold was similar to that corresponding to VT. Consistent with this, Camic et al. (2010) <sup>2</sup> found that, when the deVries method was applied using 2-min stages, the power outputs at PWC<sub>FT</sub> and VT had comparable values.

Therefore, one plausible explanation for the high values of PWC<sub>FT</sub> reported in the present study could be the short duration of the exercise periods. Indeed, it might be that, with 1-min periods, higher workload would be necessary to induce a significant increase in sEMG amplitude, as compared to longer exercise periods.

We found significant positive correlations between PWC<sub>FT</sub> and both ventilatory indicators (VT and RCP). This positive association might suggest that PWC<sub>FT</sub> and ventilatory thresholds are linked to a common physiological mechanism of fatigue. One possible candidate could be interstitial and/or arterial [K<sup>+</sup>], as this metabolite has been shown to change concurrently and in the same direction as ventilatory variables.<sup>34</sup> In addition, animal studies indicate that group III-IV muscle afferents, which activation leads to the stimulation of the respiratory neurons, may detect potassium outflow from muscle fibres.<sup>29</sup> Furthermore, elevated levels of interestitial [K+] has been linked to signs of peripheral muscle fatigue <sup>35</sup> and, therefore, an increase in [K+] might result in an increase in descending drive onto the motoneuron pool, necessary for the recrutment of additional motor units.

4.2 Evaluation of the sEMG-based model of devries using 1-min exercise periods

We found that, during the constant-workload test performed at 100% of PWC<sub>FT</sub>, heart rate and blood lactate increased progressively and significantly, whereas sEMG amplitude remained unchanged. In addition, we found that the average duration of the constant-workload exercise was 8-9 min.

In the present study, the PWC<sub>FT</sub> was calculated by applying the deVries method using 1-min exercise periods (instead of the 2-min periods used in the original work of deVries and co-workers). Our results showed that sEMG amplitude did not significantly increase during the constant-workload exercise at 100% of PWC<sub>FT</sub>. Moreover, for 2 participants, sEMG amplitude exhibited a decreasing trend over the test period. It is noteworthy that, in our cyclists, the 100% of PWC<sub>FT</sub> corresponded to 91% of the maximal power output calculated from the incremental test. This high exercise intensity was most likely in the heavy exercise intensity domain, as evidenced by the fact that the constant-workload

exercise could be maintained for a short period of time (between 8 and 9 minutes for most participants). This short endurance times suggests that application of deVries' model using 1-min workbout durations might result in an overestimation of the fatigue threshold.

In the current study, heart rate and blood lactate concentration were also monitored during the constant-workload test. Interestingly, we found that the heart rate at the end of the constant test was about 94% of the maximal heart rate observed during the incremental test, which might explain why cyclists could not sustain the 100%-PWC<sub>FT</sub> workload for a prolonged period of time (less than 10 min). This result is consistent with the study of Wagner and Housh (1993) <sup>36</sup> who paposed a heart rate fatigue threshold of approximately 110 beats min<sup>-1</sup>, which should be maintained (theoretically) indefinitely. Furthermore, several authors agree that exercise tasks during which heart rate exceeds 130 beats min<sup>-1</sup> would result in the onset of fatigue.<sup>37</sup> Based on the aforementioned studies, we conclude that the elevated heart rates found at the end of the constant-intensity test (between 178 and 195) reflect a high level of fatigue in our cyclists. Whereas the heart rate data suggest that the cardiovascular system was seriously compromised during the constant-workload task, the lack of a significant increase in sEMG amplitude may indicate that the neuromuscular fatigue of the vastus lateralis (as assessed by sEMG) was not a major limiting factor in performance. This means that participants reached volitional exhaustion before the vastus lateralis reached a critical level of fatigue, as witnessed by the relatively constant sEMC amplitude. This lack of correspondence between the heart and leg muscles for the same exercise intensity may underlie dissociation between the cardiovascular (heart rate modulation) and neuromuscular (muscle activity) factors involved in fatigue. In this respect, our results are in agreement with previous studies which showed that the fatigue threshold estimated from the heart rate response was significantly lower than that estimated from the sEMG response. 10,38

Similar to heart rate, blood lactate increased significantly over the course of the constant-workload test at 100% of PWC<sub>F</sub>, and, at the end of this test, lactate was only slightly lower (93%) than the maximum value reached during the incremental exercise. Despite these high values of lactate concentration, sEMG amplitude did not increase significantly towards the end of the constant test. Moreover, the lack of correlation between the lactate and sEMG curves suggests that either group III–IV muscle afferents are not as sensitive to changes in intracellular pH as some conjectured (Kaufman and Rybicki 1987) or their influence on alpha motoneurons is counteracted or masked by other processes (see below). In the light of these results, the widespread hypothesis that fatigue-induced

increases in sEMG activity might be due to the accumulation of metabolic byproducts in the muscle may need to be revisited.<sup>17</sup>

An unexpected finding of the current work was the absence of a significant increase in sEMG amplitude during the constant test, although this test was performed in the severe intensity exercise domain. This finding, however, should not be considered unusual <sup>39</sup> and is probably related to the fact that sEMG amplitude is influenced by factors other than motor unit recruitment, such as firing rate and/or the level of synchronization between motor units. Indeed, it is well known that firing rate decreases over the course of a fatiguing task. <sup>6</sup> Then, the expected increase in sEMG amplitude throughout the constant test caused by additional motor unit recruitment could have been offset by the decrease in firing rate.

The above reasoning could also explain the high estimate of PWCFT obtained from the incremental test with 1-min stage durations. It is certainly possible that, at the beginning of each new power output during the incremental test, discharge rate undergoes a further decline within the first 30 s (as suggested by Bigland-Ritchie and Woods 1984)<sup>40</sup>, thereby neutralizing the fatigue-induced increases in sEMG during the initial part of the workbout. With longer exercise periods (2 min and longer), firing rate might remain approximately stable after the first 30 s of each period, thereby allowing the increases in sEMG amplitude to manifest clearly. Consequently, exercise periods of 1-min duration would be too short to detect clear sEMO signs of neuromuseular fatigue.

One possible reason for the different responses of physiological (heart rate and blood lactate) and neuromuscular (sEMG) variables during the constant-workload task is that, for a population of highly trained cyclists, the onset of quantifiable local muscle fatigue might occur at exercise intensities close to the maximal power output, as suggested by a recent study. Indeed, it is plausible that trained cyclists might have developed a tolerance to muscle fatigue, for example by adopting specific muscle activation patterns. This explanation is consistent with the work of Takaishi et al. (1998), who showed that the activation of the vastus lateralis, vastus medialis and biceps femoris was significantly different between trained and untrained participants. Consequently, it might well be that, in highly trained cyclists, termination of the exercise is due to cardiovascular limitations (for instance, inability to provide oxygen to the working muscles) which occur before neuromuscular fatigue becomes evident and measurable.

#### 5. Conclusions

In summary, the present study indicates that application of the PWC<sub>FT</sub> method proposed by deVries using 1-min exercise periods could lead to overestimation of the neuromuscular fatigue threshold (PWC<sub>FT</sub>). This conclusion is inferred from the observation that during the constant-workload test performed at 100% of PWC<sub>FT</sub> heart rate and lactate concentration increased progressively and significantly and also from the fact that this constant-workload test was maintained for only 8-9 min. It is suggested that the neuromuscular fatigue threshold cannot be estimated from an incremental test with 1-min workbouts because this stage duration allows insufficient time for the sEMG response to manifest.



#### References

- 1. deVries HA, Moritani T, Nagata A, Magnussen K. The relation between critical power and neuromuscular fatigue as estimated from electromyographic data. Ergonomics 1982;25:783–791.
- 2. Camic CL, Housh TJ, Johnson GO, Hendrix CR, Zuniga JM, Mielke M, Schmidt RJ. An EMG frequency-based test for estimating the neuromuscular fatigue threshold during cycle ergometry. Eur J Appl Physiol 2010;108:337–345.
- 3. Malek MH, Coburn JW, Tedjasaputra V. Comparison of electromyographic responses for the superficial quadriceps muscles: cycle versus knee-extensor ergometry. Muscle Nerve 2009;39:810–818.
- 4. Jansen R, Ament W, Verkerke GJ, Hof AL. Median power frequency of the surface electromyogram and blood lactate concentration in incremental cycle ergometry. Eur J Appl Physiol 1997;75:102–8.
- 5. Lucía A, Sánchez O, Carvajal A, Chicharro JL. Analysis of the aerobic anaerobic transition in elite cyclists during incremental exercise with the use of electromyography. Br J Sports Med 1999;3:178-85.
- 6. Bigland-Ritchie B, Johansson R, Lippold QCJ, Smith & Woods JJ. Changes in motoneurone firing rates during sustained maximal contractions. J Physiol Dond 1983;340:335–346.
- 7. deVries HA, Tichy MW, Housh TJ, Smyth KD, Tichy AM, Housh DJ. A method for estimating physical working capacity at the fatigue threshold Ergonomics 1987;30:1195–1204.
- 8. deVries HA, Housh TJ, Johnson GO, Evans SA, Tharp GD, Housh DJ, Hughes RA. Factors affecting the estimation of physical working capacity at the fatigue threshold. Ergonomics 1990;33:25–33.
- 9. Briscoe MJ, Forgach MS, Trifan E, Malek MH. Validating the EMGFT from a Single Incremental Cycling Test. Int J Sports Med 2014;35(7):566-70.
- 10. Guffey DR, Gervasi BJ, Maes AA, Malek MH. Estimating electromygraphic and heart rate fatigue threshold from a single treadmill test. Muscle Nerve 2012;46:577–581.
- 11. Hug F, Faucher M, Kipson N, Jammes Y. EMG signs of neuromuscular fatigue related to the ventilatory threshold during cycling exercise. Clin Physiol Funct Imaging 2003;23:208-14.

- 12. Gravier G, Steinberg JG, Lejeune PJ, Delliaux S, Guieu R, Jammes Y. Exercise-induced oxidative stress influences the motor control during maximal incremental cycling exercise in healthy humans. Respir Physiol Neurobiol 2013;186(3):265-72.
- 13. Lenti M1, De Vito G, Sbriccoli P, Scotto di Palumbo A, Sacchetti M. Muscle fibre conduction velocity and cardiorespiratory response during incremental cycling exercise in young and older individuals with different training status. J Electromyogr Kinesiol 2010;20:566-71.

- 14. Sbriccoli P, Sacchetti M, Felici F, Gizzi L, Lenti M, Scotto A, De Vito G. Non-invasive assessment of muscle fiber conduction velocity during an incremental maximal cycling test. J Electromyogr Kinesiol 2009;19(6):e380-6.
- 15. Evetovich TK, Housh TJ, Johnson GO, Evans SA, Stout JR, Bull AJ Smith DB, Evetovich MM. Effect of workbout duration on the physical working capacity at fatigue threshold (PWCFT) test. Ergonomics 1996;39(2):314-21.
- 16. Helal JN, Guezennec CY, Goubel F. The aerobic-anaerobic transition re-examination of the threshold concept including an electromyographic approach. Eur J Appl Physiol 1987;56:643–649.
- 17. Lucía A, Sánchez O, Carvajal A, Chicharro JL. Analysis of the aerobic-anaerobic transition in elite cyclists during incremental exercise with the use of electromyography. Br J Sports Med 1999;3:178-85.
- 18. Bouissou P, Estrade PY, Goubel F, Guezennee CV, Serrurier B. Surface EMG power spectrum and intramuscular pH in human vastus lateralis muscle during dynamic exercise. J Appl Physiol 1989;67:1245-9.
- 19. Taylor AD, Bronks R Electromyographic correlates of the transition from aerobic to anaerobic metabolism in treadmill running. Eur J Appl Physiol 1994;69:508–515.
- 20. Bergstrom HC, Housh TJ, Cochrane KC, Jenkins ND, Lewis RW, Traylor DA, Zuniga JM, Schmidt RJ, Johnson GO, Cramer JT. An examination of neuromuscular and metabolic fatigue thresholds. Physiol Meas 2013;34(10):1253-67.
- 21. Zuniga JM, Housh TJ, Camic CL, Hendrix CR, Schmidt RJ, Mielke M, Johnson GO. A mechanomyographic fatigue threshold test for cycling. Int J Sports Med 2010;31(9):636-43.
- 22. Davis JA. Anaerobic threshold: a review of the concept and directions for future research. Med Sci Sports Exerc 1985;17:6–18.

- 23. Wassermann K, McIlroy MB. Detecting the threshold of anaerobic metabolism in cardiac patients during exercise. Am J Cardiol 1964,14:844-852.
- 24. Heck H, Mader A, Hess G, Mücke S, Müller R, Hollmann W. Justification of the 4-mmol/l lactate threshold Int J Sports Med 1985;6:117–30.

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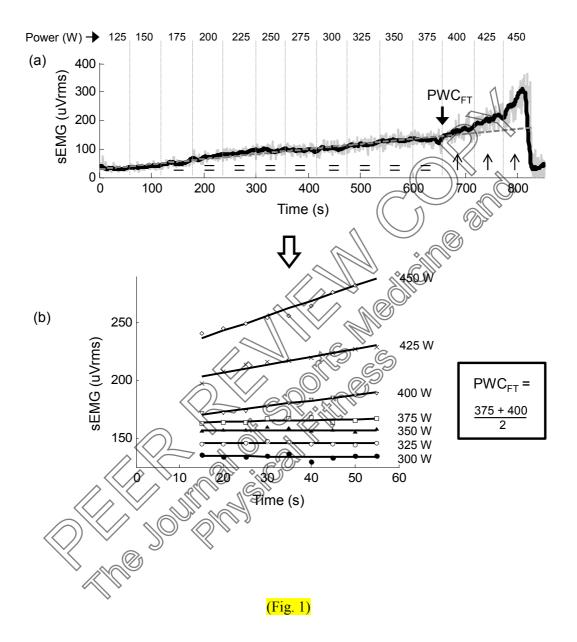
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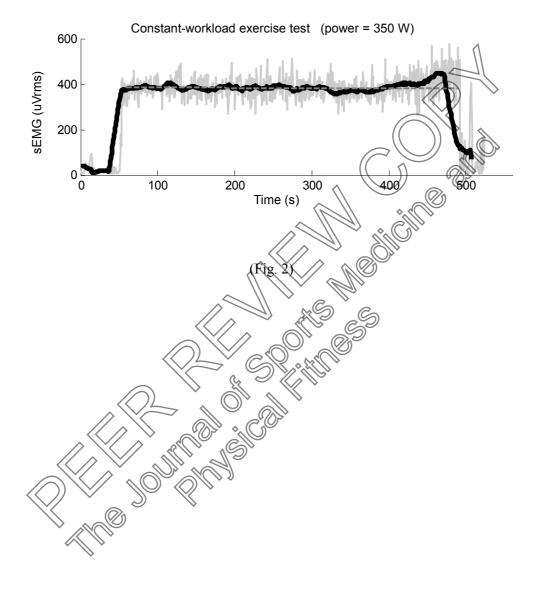
- 25. Hermens HJ, Freriks B, Merletti R, Stegeman D, Blok J, Rau G, Disselhorst-Klug C, Hägg GM. SENIAM European recommendations for surface electromyography: results of the SENIAM project Roessingh Research and Development, Enschede. 1999.
- 26. Dimitrov GV, Arabadzhiev TI, Mileva KN, Bowtell JL, Crichton N, Dimitrova NA. Muscle fatigue during dynamic contractions assessed by new spectral indices. Med Sci Sports Exerc 2006;38(11): 1971-9.
- 27. Enoka RM, Stuart DG. Neurobiology of muscle fatigue. J.Appl. Physiol 1992;72:1631–1648.
- 28. MacLaren DP, Gibson H, Parry-Billings M, Edwards RH, A review of metabolic and physiological factors in fatigue. Exerc Sport Sci Rev 1989;17:29–66
- 29. Kaufman MP, Rybicki KJ. Discharge properties of group III and IV muscle afferents: their responses to mechanical and metabolic stimuli. Circ Res 1987:601-60-I-65.
- 30. Woods JJ, Furbush F, Bigland-Ritchie B. Evidence for a fatigue induced reflex inhibition of motoneuron firing rates. J Neurophysiol 1987;58:125–137.
- 31. Moritani T, Nagata A, Muro M, Electromographic manifestations of muscular fatigue. Med Sci Sport Exerc 1982;14:198-202.
- 32. Viitasalo JT, Luhtanen P, Rahkila P, Rusko H. Electromyographic activity related to aerobic and anaerobic threshold in ergometer bicycling. Acta Physiol Scand 1985;124:287-93.
- 33. Glass SC, Knoltown RG, Sanjabi PB, Sullivan JJ. Identifying the integrated electromyographic threshold using different muscles during incremental cycling exercise. J Sports Med Phys Fitness. 1998;38: 47–52.
- 34. McLoughlin P, Popham P, Linton RA, Bruce RC, Band DM. Exercise-induced changes in plasma potassium and the ventilator threshold in man. J Physiol 1994;479:139–147.

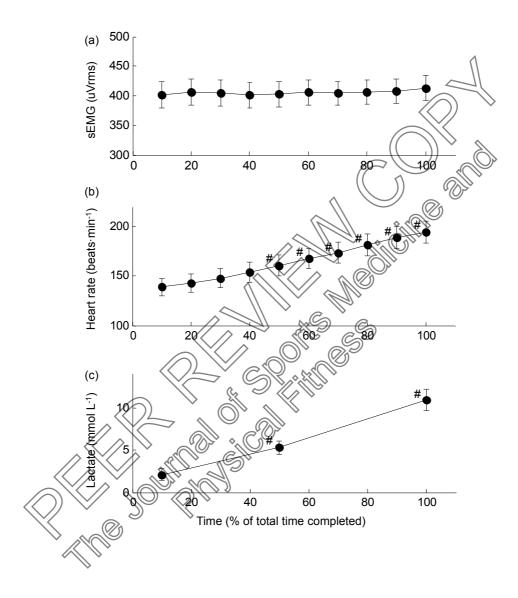
35. Fortune E, Lowery MM. The effect of extracellular potassium concentration on muscle fiber conduction velocity examined using model simulation. Conf Proc IEEE Eng Med Biol Soc 2007;2726–2729.

- 36. Wagner LL, Housh TJ. A proposed test for determining physical working capacity at the heart rate threshold. Res Q Exerc Sport 1993;64:361–364.
- 37. Leger L, Tokmakidis S. Use of the heart rate defl ection point to assess the anaerobic threshold J Appl Physiol 1988;64:1758–1760.
- 38. Miller JM , Housh TJ , Coburn JW , Cramer JT , Johnson GO. A proposed test for determining physical working capacity at the oxygen consumption threshold (PWCVO2). J Strength Cond Res 2004;18:618–624.
- 39. Scheuermann BW, Hoelting BD, Noble ML, Barstow TJ. The slow component of O(2) uptake is not accompanied by changes in muscle EMG during repeated bouts of neavy exercise in humans. J Physiol 2001;531:245-56.
- 40. Bigland-Ritchie B, Woods JJ (1984) Changes in muscle contractile properties and neural control during human muscular fatigue Muscle Nerve 7(9):691-9.
- 41. Tenan MS, McMurray RG, Blackburn BT, McGrath M, Leppert K (2011) The relationship between blood potassium, blood lactate and electromyography signals related to fatigue in a progressive cycling exercise test J Electromyogr Kinesiol 21(1):25-32.
- 42. Takaishi T, Yamamoto T, Ono T, Ito T, Moutani T (1998) Neuromuscular, metabolic, and kinetic adaptations for skilled pedaling performance in cyclists Med Sci Sports Exerc 30:442–449.

# **FIGURES**



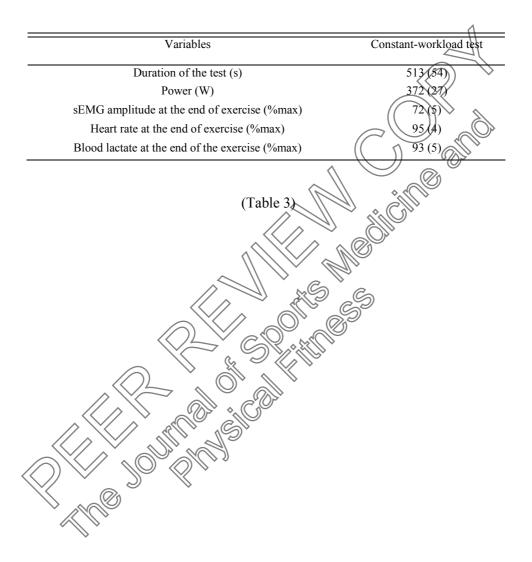




(Fig. 3)

Variables	Mean (SD)	Range
Age (years)	21.7 (2.9)	183-28.5
Body weight (Kg)	71.3 (4.9)	62.4 79.4
Height (m)	1.81 (0.06)	1.71 -2.92
VT (L min <sup>-1</sup> )	3.3 (0.6)	1.9 - 4.4
RCP (L min <sup>-1</sup> )	4.6 (0(5)	3.4 – 5.6
VO₂peak (L min⁻¹)	5.1 (0.4)	4.5 – 5.9
Heart rate at end of exercise (beats min <sup>-1</sup> )	198 (8)	212
Blood lactate at end of exercise (mmol L <sup>-1</sup> )	12.1 (5.0)	7.7 – 14.8
sEMG amplitude at end of exercise (μVrms)	572 (59)	369 - 843
Power at end of exercise (W)	406 (36)	325 - 450
(Fable 1)		





## **LEGENDS**

Fig. 1 Representative example of the method for estimating the neuromuscular fatigue threshold using the surface EMG amplitude (PWC<sub>FT</sub>) from a single incremental cycle ergometry test in one cyclist. (a) Time plot of the sEMG recording obtained in the cyclist during the incremental test. The black solid line represents the sEMG amplitude averaged every 15 bursts (sEMG signal resulting from the active period of the vastus lateralis in each pedal thrust). (b) Regression lines corresponding to the sEMG amplitude vs. time relationship for each power output. The highest power output with a non-significant slope was 375 W (P>0.05), whereas the lowest power output with a significant slope was 400 W (P<0.05). The PWC<sub>FT</sub> (387.5 W) is calculated as the average of these two power outputs.

Fig. 2 Time plot of a surface electromyographic (sEMG) recording obtained in one cyclist from the vastus lateralis during the constant-workload test. Note that sEMG amplitude remained approximately constant except during the last minute of the test. The black solid line represent the sEMG amplitude averaged every 15 bursts. The grey dashed line is the regression line of the sEMG vs. time relationship.

Fig. 3 Group data (mean  $\pm$  SEM) of sEMG amplitude (a) heater rate (b) and blood lactate (c) as a function of the percentage of total time completed during a constant-workload test performed at the power output corresponding to 100% PWC<sub>FT</sub>, # Significantly higher than the value at 10% of the total time completed at P < 0.05.

**Table 1**. Anthropometric (mean  $\pm$  SD) and physiological characteristics of the participants (N = 16) calculated from the incremental test.

**Table 2**. Correlation matrix for the power outputs of the fatigue thresholds (N = 16). \* indicates that the correlation was significant (P < 0.05).

**Table 3**. Results (mean  $\geq$  SD) of the constant-workload test (N = 16). Blood lactate, heart rate and sEMG amplitude are expressed as relative percent of the maximal values obtained during the incremental-workload test (max).

Variables	Mean (SD)	Range
	21.7.(2.0)	1000005
Age (years)	21.7 (2.9)	183 – 28.5
Body weight (Kg)	71.3 (4.9)	62.4 / 79.4
Height (m)	1.81 (0.06)	1.71 - 2.92
VT (L min <sup>-1</sup> )	3.3 (0.6)	1.9 – 4.4
RCP (L min <sup>-1</sup> )	4.6 (0(5)	3.4 – 5.6
VO₂peak (L min <sup>-1</sup> )	5.1 (0.4)	4.5 - 8.9
Heart rate at end of exercise (beats min <sup>-1</sup> )	198 (8)	212
Blood lactate at end of exercise (mmol L <sup>-1</sup> )	12.1 (5.0)	7.7 – 14.8
sEMG amplitude at end of exercise (μVrms)	572 (59)	369 - 843
Power at end of exercise (W)	406 (36)	325 - 450
(Fable 1)		



