Wavelet and principal component analysis of electromyographic activity and slow component of oxygen uptake during heavy and severe cycling exercise

<table>
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<tr>
<th>Journal:</th>
<th>Applied Physiology, Nutrition, and Metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID</td>
<td>apnm-2019-0037.R2</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>23-May-2019</td>
</tr>
</tbody>
</table>
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| Novelty bullets: points that summarize the key findings in the work: | □ The expected rise in MPF may be offset by muscle fatigue occurring of the slow component during severe exercise., □ The gradual shift to higher EMG frequencies throughout the slow component phase was reflected in angle θ. |
| Keyword: | Electromyography, Oxygen uptake kinetics, Wavelet analysis, Principal component analysis, Slow component of VO2, exercise intensity < exercise |
| Is the invited manuscript for consideration in a Special Issue? : | Not applicable (regular submission) |
Wavelet and principal component analysis of electromyographic activity and slow component of oxygen uptake during heavy and severe cycling exercise

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Abstract

The aim of the study was to investigate whether the slow component of oxygen uptake (VO$_2$) was concurrent with the recruitment of large alpha-motoneuron muscle fibres by using wavelet and principal component analysis (PCA) of electromyography (EMG) during heavy and severe cycling exercise. 11 male subjects participated in the study. After establishing each subject's maximum value of VO$_2$ through an incremental test on the cycle ergometer, the subjects performed 6-min cycling tests at heavy and severe intensity. EMG signals were collected from **rectus femoris** (RF), **biceps femoris** long head (BF), **tibialis anterior** (TA), **medial gastrocnemius** (MG), and processed by combined use of wavelet and PCA analysis. The time delays to the onset of slow component occurred significantly earlier during severe (105.22 ± 5.45 s) than during heavy (138.78 ± 15.09 s) exercise. ANOVA with repeated measures showed that for all muscles tested, the angle $\theta$ formed by the first and second principal components decreased significantly between time windows during heavy and severe exercise. However significant increases of EMG mean power frequency (MPF) were found only during heavy exercise. Our results show the concurrence of the VO$_2$ slow component with the additional recruitment of muscle fibres, presumably less efficient large alpha-motoneuron fibres.

- The expected rise in MPF may be offset by muscle fatigue occurring in the later time windows of the slow component during severe exercise.
- The gradual shift to higher EMG frequencies throughout the slow component phase was reflected in the progressive and significant decrease of angle $\theta$.

**Key words:** Electromyography, Exercise intensity, Oxygen uptake kinetics, Wavelet analysis, Principal component analysis, Slow component of VO$_2$
Introduction

During heavy constant exercise above the ventilatory threshold (VT), oxygen uptake (VO$_2$) kinetics exhibit a delayed rise in oxygen consumption (Jones and Burnley 2009). This characteristic of heavy exercise is identified as the VO$_2$ slow component. Whipp and colleagues (Whipp and Wasserman 1972) were the first to characterize the presence and behavior of the VO$_2$ slow component and its generation within the exercising muscles (Poole and Gaesser 1985). It is generally accepted that the VO$_2$ slow component is to be attributed largely to the exercising skeletal muscles (Jones and Poole 2005; Poole 1994; Vanhatalo et al. 2011b), particularly the additional recruitment of less efficient large alpha-motoneuron muscle fibres (Bernasconi et al. 2006; Krstrup et al. 2004; Pringle et al. 2003a; Zoladz and Korzeniewski 2001).

One of the non-invasive methods for investigating muscle fibre recruitment patterns is surface electromyography (sEMG). Previous sEMG studies have shown possible changes in muscle fibre recruitment patterns associated with the VO$_2$ slow component. The increase of integrated EMG (iEMG) may reflect the recruitment of additional muscle fibres (Bernasconi et al. 2006; Cleuziou et al. 2004; Garland et al. 2006; Sabapathy et al. 2005; Saunders et al. 2000; Osborne and Schneider 2006; Borrani et al. 2001; Scheuermann et al. 2001). However, changes in mean power frequency (MPF) associated with the VO$_2$ slow component were not consistently noted in scientific literature. Some studies have shown increases in MPF associated with the VO$_2$ slow component (Garland et al. 2006; Sabapathy et al. 2005; Osborne and Schneider 2006;
Borrani et al. 2001), while others reported no detectable increases in MPF during heavy constant exercise (Bernasconi et al. 2006; Tordi et al. 2003; Scheuermann et al. 2001; Cleuziou et al. 2004). This inconsistency can be explained partly by the difficulties in signal processing using the traditional Fourier transform algorithm, in which the signal is assumed to be stationary. However, EMG signals from dynamic contractions contain numerous non-stationary or transitory characteristics. Recently, principal component analysis (PCA) has attracted increasing interest because of its usefulness in spectral classification under various conditions through the use of a large datasets (von Tscharner 2002; Rogers and MacIsaac 2011). In the present study, we proposed a combined wavelet and PCA analysis to detect changes in EMG spectra associated with the VO$_2$ slow component. The aim of the study was to investigate, by using wavelet and PCA analysis of EMG during both heavy and severe cycling exercise, whether the VO$_2$ slow component was concurrent with the recruitment of large alpha-motoneuron muscle fibres.

**Materials and methods**

*Subjects*

11 male athletic subjects (age 20±1.1 years; height 179±4.8 cm; weight 68±5.9 kg) participated in the study. The study was approved by the Institution's Ethics Committee. Subjects all gave their informed consent to participate, in compliance with the procedures set out by a local Ethics Committee. The subjects were fully familiar with laboratory exercise testing procedures.
Test procedure

The tests were performed on an electrically braked cycle ergometer (PowerMax VII, Combi Wellness, Japan), with seat height adjusted and reproduced in subsequent tests for each subject. Subjects were asked to begin each session with a 5 min warm-up cycling at a speed of 80 rpm and 1.5kp workload. They then completed an incremental test to volitional exhaustion in order to determine their individual ventilatory threshold (VT) and maximum oxygen uptake (VO\textsubscript{2max}). The pedal speed was maintained at 80±5rpm in the incremental test. VT was calculated by the V-slope method (Beaver et al. 1986). The VO\textsubscript{2max} was determined as the point where the heart rate was greater than 180, the oxygen consumption had plateaued at <150 ml/min, and the respiration exchange ratio (RER) was greater than 1.1. The results of the VO\textsubscript{2max} tests are shown in Table 1. Based on the results of incremental test, individual work rates were determined that elicited the VO\textsubscript{2} responses corresponding to approximately 50% (heavy exercise) and 70% (severe exercise) between VT and VO\textsubscript{2max} for each subject (Lansley et al. 2011).

\[
\Delta 50\% = \text{VT} + (V O_{2\text{max}} - \text{VT}) \times 0.5 \quad (1)
\]

\[
\Delta 70\% = \text{VT} + (V O_{2\text{max}} - \text{VT}) \times 0.7 \quad (2)
\]

On the second visit to the laboratory, subjects first performed a 5 min cycling warm-up. They then completed two exercise bouts at heavy (3-min seated rest; 6-min cycling at \Delta 50\% work rate; and a 3-min recovery) and severe (3-min seated rest; 6-min cycling at \Delta 70\% work rate; and a 3-min recovery) exercise intensity. Subjects were given 20-
30 min rest time between bouts.

**Instrumentation**

For all tests, pulmonary gas exchange (VO\textsubscript{2} and VCO\textsubscript{2}) and minute ventilation were continuously measured using a metabolic measurement system (MetaLyzer 3B, Cortex Biophysik, Leipzig, Germany) that was calibrated before each test using known-composition gases and a 3-liter syringe. Respiratory gas exchange variables were recorded every 5 seconds. Heart rate was obtained telemetrically.

Surface EMG (sEMG) activity of right lower limb muscles was recorded from the muscle bellies of *rectus femoris* (RF), *biceps femoris* long head (BF), *tibialis anterior* (TA), and *medial gastrocnemius* (MG) using parallel-bar EMG sensors (Trigno standard EMG sensor, Delsys Inc., Boston, MA, USA) at 2000Hz sampling rate, after removal of hair and cleaning with alcohol swipes. Cycle ergometer, Cortex 3B MetaLyzer, and EMG sensors were synchronized.

**Data Analysis**

**VO\textsubscript{2} kinetics**

The VO\textsubscript{2} data were visually inspected to remove non-physiological data points resulting from aberrant breaths (i.e. swallow, cough, etc.). Then the VO\textsubscript{2} data were interpolated to give second-by-second values using the spline method. Nonlinear regression techniques were used to fit VO\textsubscript{2} with a bi-exponential function:
\[ V_{O_2}(t) = V_{O_2}(b) + A_1 \times (1 - e^{-(t - TD_1)/\tau_1}) \quad \text{Primary component (3)} \]
\[ + A_2 \times (1 - e^{-(t - TD_2)/\tau_2}) \quad \text{Slow component (4)} \]

Where the \( V_{O_2}(t) \) is the \( VO_2 \) at any given time point; \( V_{O_2}(b) \) is the average value of 3 min rest baseline; \( A_1 \) and \( A_2 \) are the asymptotic amplitudes for the exponential terms; \( \tau_1 \) and \( \tau_2 \) are the time constants; \( TD_1 \) and \( TD_2 \) represent the time delays.

**sEMG analysis**

Stationary signals are constant in their statistical parameters over time. However, EMG signals from dynamic contractions contain numerous non-stationary or transitory characteristics stemming from abrupt changes, beginnings and ends of events, etc. Thus, the spectral estimation technique used to describe them must be carefully chosen, taking into account the specific type of non-stationarity exertion affecting the signal of interest.

Wavelet analysis with well-defined time and frequency resolution, used in the present study, has proven to be a sensitive method of assessing non-stationary EMG (von Tscharner 2000; Qi et al. 2011). The method has been described in detail in previous papers (Wakeling and Rozitis 2004). In short, the method uses a filter bank of a series of non-linearly scaled wavelets (10-350 Hz, \( k = 1-9 \)) (Beck et al. 2008; von Tscharner 2000; 2002; Qi et al. 2011). In this study the first wavelet covered a frequency band of 0-10 Hz, which is associated with movement artefacts (De Luca 1979). So the first wavelet, \( k = 0 \), was excluded. The mean power frequency (MPF) was calculated by:

\[
\text{MPF} = \frac{\sum f_c(k) \times j_k}{\sum j_k} \quad (5)
\]

Where \( f_c(k) \) represents the centre frequency for wavelet \( k \), and the mean frequency was...
calculated as the mean of the MPF values taken from each cycling cycle. The start of each cycling cycle was determined by a potentiometer.

We used principal component analysis (PCA) to identify the major features of the spectra from 11 subjects. To bring the EMG changes in line with the time windows of the VO$_2$ slow component, cycles were selected with respect to the primary component (-30%), the beginning of the slow component (0% slow component), 30%, 60%, and 90% slow component. Therefore, the matrix is $p \times N$, where $p = 9$ wavelet domains and $N = 1100$ cycles (subjects $\times$ 10 cycles of each time window $\times$ exercise intensities) for each muscle. The principal components (PCs) were calculated from the covariance matrix of spectra (Wakeling and Rozitis 2004). The principal component weighting is given by the eigenvector, and the principal component loading score is given by the eigenvalue, a scalar value that describes the amount of each eigenvector in each measured spectrum (Hodson-Tole and Wakeling 2007). The first two principal components account for more than 80% of the signals. A quantitative measure of the contribution of high and low frequency content within the signal is given by the angle $\theta$ formed between the PCI and PCII loading scores (Hodson-Tole and Wakeling 2007; Wakeling 2004; Wakeling and Rozitis 2004). A higher value of $\theta$ represents relatively more low frequency signal content and a smaller $\theta$ value is associated with relatively more high frequency content in the EMG signal.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics (version 22, IBM, Inc.,
New York, USA). Shapiro-Wilk test was used to assess data normality. Paired sample T-test was used to examine the effect of exercise intensity (heavy and severe) on VO₂ kinetics parameters. One-way ANOVA with repeated measures with was performed to compare MPF and θ values across the slow component time windows. Bonferroni post-hoc multiple comparisons were then performed to assess significant differences determined by repeated-measures ANOVA. All data are reported in the text as mean (standard deviation (SD)), while the results are graphed in each figure as mean (standard error of mean (SEM)). Significant level was set at $P < 0.05$ for all statistical procedures.

**Results**

*VO₂ kinetics*

The physical and physiological characteristics and results of the incremental tests are shown in Table 1. The mean value of VO₂ max was $3.2 ± 0.4 \text{ l min}^{-1}$, the highest heart rate reached $183 ± 6 \text{ beat/min}$, while the mean RER was $1.13 ± 0.05$ at the end of the incremental test.

Figure 1 illustrates differences in VO₂ responses at heavy and severe exercise intensity by the curve fit method of the bi-exponential model in a representative subject. Kinetic parameters for the exponential curve-fitting of the individual VO₂ responses at heavy and severe exercise intensity was shown in Table 2. The paired-sample T-test showed that the exercise intensity had a significant effect on TD₂. The onset of slow component,
TD₂, is significantly earlier at severe exercise than at heavy exercise.

Electromyographic responses

Figure 2 displays the EMG mean power frequency (MPF) and theta (θ) at -30% (primary component), 0%, 30%, 60%, and 90% of the slow component phase (x-axis) for heavy and severe exercise. Repeated measures ANOVA showed that MPF values of the 4 tested muscles increased significantly from the 0% to the 90% slow component time windows during heavy exercise (RF: from 77.4 ± 7.2 Hz to 80.9 ± 8.1 Hz; BF: 84.0 ± 13.2 Hz to 86.1 ± 12.5 Hz; TA: 110.3 ± 9.1 Hz to 114.2 ± 11.0 Hz; MG: 136.0 ± 15.9 Hz to 140.1 ± 17.8 Hz) (p < 0.05), however, a significant increase in MPF was found only for RF (from 84.5 ± 10.2 Hz to 86.7 ± 11.6 Hz) during severe exercise (Figure 2). On the other hand, θ decreased significantly from 0% to 90% of the slow component phase at both heavy (RF: from 1.61 ± 0.13° to 1.57 ± 0.13°; BF: 1.77 ± 0.21° to 1.71 ± 0.20°; TA: 1.69 ± 0.13° to 1.65 ± 0.12°; MG: 1.49 ± 0.14° to 1.43 ± 0.15°) and severe (RF: from 1.66 ± 0.20° to 1.61 ± 0.19°; BF: 1.78 ± 0.24° to 1.74 ± 0.25°; TA: 1.69 ± 0.18° to 1.64 ± 0.18°; MG: 1.55 ± 0.20° to 1.49 ± 0.20°) exercise intensity for the 4 tested muscles. Post hoc tests using the Bonferroni correction revealed that VO₂ slow component elicited a significant decrease of θ between the 0% slow component time window and other % slow component time windows for RF, TA, and MG during both heavy and severe exercise (Figure 2).

Discussion

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The findings of the VO$_2$ kinetics analysis of the present study demonstrate that the bi-exponential parameters of the VO$_2$ slow component are associated with the intensity of the exercise, which is consistent with previous studies (Carter et al. 2000; Carter et al. 2002; Wilkerson et al. 2004). It has been reported that the increase in MPF was associated with the VO$_2$ slow component (Burnley et al. 2002; Borrani et al. 2001; Bernasconi et al. 2006; Sabapathy et al. 2005). However, some other studies observed decreased or unchanged MPF during the VO$_2$ slow component phase (Garland et al. 2006; Pringle and Jones 2002). In the present study, the EMG MPF increased significantly during the slow component phase at heavy exercise intensity in all tested muscles, whereas MPF increased significantly only in RF during severe exercise. We did not find any significant increase in MPF during the slow component phase at severe exercise intensity for BF, TA, and MG.

It has been suggested that the appearance of the VO$_2$ slow component is a precursor of muscle fatigue during exercise (Grassi et al. 2015). A decreased MPF of the power spectrum is widely used to examine muscle fatigue (Orizio et al. 2003; Gonzalez-Izal et al. 2010). As additional muscle fibres, particularly the fatigable large alpha-motoneuron muscle fibres, are recruited during the slow component phase, the expected rise in MPF may be offset by muscle fatigue occurring in the later time windows of the slow component during severe exercise. The reduction of high frequency content from neuromuscular fatigue might conceal the MPF rise.

The combined wavelet and PCA analyses allow us to analyze the non-stationary EMG
The spectral properties of the EMG signals were processed by a wavelet analysis that was well-defined in time and frequency resolution, and with non-linear scaling adjusted to the physiological response time of the muscle (20, 23). Then a quantitative method, PCA, was used to describe the contribution of high- and low-frequency contents within the signal. PCA extracts the important features in the signal, so some variables, such as movement artifacts, were given lower order components and were excluded. In the present study the major source of variation in the EMG signal can be expected to be associated with the slow component of VO$_2$, and the first two principal components (PCI and PCII), which account for more than 80% of the EMG signal.

EMG $\theta$ decreased progressively and significantly during the slow component phase at both heavy and severe exercise intensity. The decreases in $\theta$ were associated with increases in the higher frequency components of the EMG spectral during the VO$_2$ slow component phase. There was a significant difference between the 0% and later time windows of the slow component for all the tested muscles during both heavy and severe exercise. This indicated that relatively more high frequency components in the EMG power spectra concur with the VO$_2$ slow component. What has been shown is that the EMG spectrum shifts to higher frequencies as more muscle fibres, presumably larger alpha-motoneuron muscle fibres, are recruited (Beck et al. 2004; Croce et al. 2012; Moritani et al. 1992). The consistent increases in activity of the muscle fibres toward higher frequency content could be interpreted as a reflection of additional recruitment.
of muscle fibres, particularly large alpha-motoneuron muscle fibres.

Although the changes in MPF and $\theta$ changes observed in the present study may well point to the recruitment of additional muscle fibres during heavy exercise, there may be other factors causing changes in EMG spectra, such as muscle temperature and/or PH. Analyzing sEMG spectral properties as indicators of motor unit recruitment and muscle fibre type remains controversial as a method (von Tscharner and Nigg 2008a; Farina 2008b; Pincivero 2008; Dimitrov 2008; Farina 2008a; Wakeling 2008; von Tscharner and Nigg 2008b). We do agree that sEMG as a quantitative tool for assessing muscle fibre recruitment has to be used with caution. We hope nevertheless that by examining EMG spectra in conjunction with the VO$_2$ slow component during heavy and severe exercise, we are on the way to determining with more certainty if and how advanced sEMG analyses can become a viable tool for monitoring muscle activity.

Study limitation

We have opted for the delta ($\Delta$) method in the present study because it has been used widely in exercise physiology and sports communities for the classification and prescription of exercise intensity (Osborne and Schneider 2006; Pringle et al. 2003b; Barstow et al. 1996; Sabapathy et al. 2005; Saunders et al. 2000; Berger and Jones 2007) . However, critical power have been used to designate the demarcation between heavy and severe exercise intensity (Vanhatalo et al. 2011a; Poole et al. 2016) in recent years. The notion of critical power might be generally preferable.
Conclusion

Our results based on the analysis of VO$_2$ kinetics and EMG wavelet and principal component during heavy and severe cycling show the concurrence of the VO$_2$ slow component with the additional recruitment of muscle fibres, in particular less efficient large alpha-motoneuron fibres. The gradual shift to higher EMG frequencies throughout the slow-component phase was reflected in the progressive and significant decrease of EMG 0.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (grant award number: 31600797) and Fundamental Research Funds for the Central Universities (grant award number: DUT16QY25).

Conflict of interest statement: The authors have no conflicts of interest to report.

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Table 1. Individual physical and physiological characteristics and results of incremental test.

<table>
<thead>
<tr>
<th>Subject code</th>
<th>Age (year)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>VO₂max (l min⁻¹)</th>
<th>HRmax (beat min⁻¹)</th>
<th>RER</th>
<th>Time to exhaustion (second)</th>
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<tr>
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<td>575</td>
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<td>2.51</td>
<td>184</td>
<td>1.12</td>
<td>500</td>
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Mean±SD  
VO₂max: highest VO₂ achieved at the end of exercise.
HRmax: highest heart rate achieved at the end of exercise.
RER: respiration exchange ratio.
Table 2. Parameters of the VO$_2$ kinetic response to heavy and severe exercise (mean ± SD).

<table>
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<tr>
<th>Parameters</th>
<th>Heavy exercise</th>
<th>Severe exercise</th>
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<tr>
<td>$A_1$ (ml/min)</td>
<td>$1289.78±305.13$</td>
<td>$1527.00±268.44$</td>
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<td>$\tau_1$ (s)</td>
<td>$33.33±9.08$</td>
<td>$24.00±5.50$</td>
</tr>
<tr>
<td>TD$_1$ (s)</td>
<td>$18.22±5.80$</td>
<td>$15.89±2.93$</td>
</tr>
<tr>
<td>$A_2$ (ml/min)</td>
<td>$295.11±84.93$</td>
<td>$420.56±132.69$</td>
</tr>
<tr>
<td>$\tau_2$ (s)</td>
<td>$221.22±15.09$</td>
<td>$218.67±57.44$</td>
</tr>
<tr>
<td>TD$_2$ (s)</td>
<td>$138.78±15.09^*$</td>
<td>$105.22±5.45^*$</td>
</tr>
<tr>
<td>$r$ Model</td>
<td>$0.98±0.01$</td>
<td>$0.98±0.01$</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD

$A_1$, and $A_2$, amplitudes for the primary and slow component VO$_2$;

$\tau_1$, and $\tau_2$, the time constants of primary and slow component;

TD$_1$ and TD$_2$, time delays to onset of primary and slow component.

Significant level $^* p < 0.05$
**Figure 1.** Oxygen uptake response to heavy (black circle) and severe (black dot) intensity cycling in a representative subject. The data show breath-by-breath $O_2$ uptake values with the regression fit. The time 0 indicates when the predetermined work rate was applied.

**Figure 2.** EMG mean power frequency (MPF) and theta ($\theta$) change with VO$_2$ slow component at heavy (black diamond line) and severe (grey square line). Time base was normalized with respect to the value of MPF at the onset of the slow component (0% slow component) and with the duration of slow component. * Significant differences between 0 % slow component and other % slow component windows ($P < 0.05$).
Figure 1. Oxygen uptake response to heavy (black circle) and severe (black dot) intensity cycling in a representative subject. The data show breath-by-breath O2 uptake values with the regression fit. The time 0 indicates when the predetermined work rate was applied.
RF

BF

TA

MG

slow component (%)