THE INTERRELATIONSHIP BETWEEN MUSCLE OXYGENATION, MUSCLE ACTIVATION AND PULMONARY VO2 TO INCREMENTAL RAMP EXERCISE: INFLUENCE OF AEROBIC FITNESS

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THE INTERRELATIONSHIP BETWEEN MUSCLE OXYGENATION, MUSCLE ACTIVATION AND PULMONARY VO$_2$ TO INCREMENTAL RAMP EXERCISE: INFLUENCE OF AEROBIC FITNESS

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We investigated whether muscle and ventilatory responses to incremental ramp exercise would be influenced by aerobic fitness status by means of a cross-sectional study with a large subject population. Sixty-four male students (age: 21.2±3.2 years) with a heterogeneous VO\textsubscript{2}peak (51.9±6.3 ml.min\(^{-1}\).kg\(^{-1}\), range 39.7-66.2 ml.min\(^{-1}\).kg\(^{-1}\)) performed an incremental ramp cycle test (20-35 Watt.min\(^{-1}\)) to exhaustion. Breath-by-breath gas exchange was recorded, and muscle activation and oxygenation were measured with surface electromyography (sEMG) and near-infrared spectroscopy (NIRS), respectively. The integrated EMG (iEMG), Mean Power Frequency (MPF), deoxy[Hb+Mb] and total[Hb+Mb] responses were set out as functions of work rate (W) and fitted with a double linear function. The respiratory compensation point (RCP) was compared and correlated to the breakpoints (BPs) (as %VO\textsubscript{2}peak) in muscle activation and oxygenation. The BP in total[Hb+Mb] (83.2±3.0%VO\textsubscript{2}peak) preceded (P<0.001) the BP in iEMG (86.7±4.0%VO\textsubscript{2}peak) and MPF (86.3±4.1%VO\textsubscript{2}peak), which in turn preceded (P<0.01) the BP in deoxy[Hb+Mb] (88.2±4.5%VO\textsubscript{2}peak) and RCP (87.4±4.5%VO\textsubscript{2}peak). Furthermore, the VO\textsubscript{2}peak was significantly (P<0.001) positively correlated to the BPs and RCP, indicating that the BPs in total[Hb+Mb] (r=0.66; P<0.001), deoxy[Hb+Mb] (r=0.76; P<0.001), iEMG (r=0.61; P<0.001), MPF (r=0.63; P<0.001) and RCP (r=0.75; P<0.001) occurred at a higher %VO\textsubscript{2}peak in subjects with a higher VO\textsubscript{2}peak. In this study a close relationship between muscle oxygenation, activation and pulmonary VO\textsubscript{2} was found, occurring in a cascade of events. In subjects with a higher aerobic fitness level this cascade occurred at a higher relative intensity.

**KEYWORDS:** muscle oxygenation; muscle activation; pulmonary VO\textsubscript{2}; respiratory compensation point; VO\textsubscript{2}peak; critical power
INTRODUCTION

In the past decades several physiological thresholds have been determined and used to evaluate exercise tolerance, prescribe training guidelines, monitor training load, etc. Traditionally, thresholds in pulmonary gas exchange (i.e., Gas Exchange Threshold (GET) and Respiratory Compensation Point (RCP)) and blood lactate responses (i.e., Onset of Blood Lactate Accumulation (OBLA) and Maximal Lactate Steady State (MLSS)) were identified. More recently, the application of surface electromyography (EMG) and Near Infrared Spectroscopy (NIRS) allowed the measurements of muscle activation and muscle and cerebral oxygenation responses, respectively. Thresholds in the integrated EMG signal (Hug et al. 2003; Osawa et al. 2011) and deoxygenated [hemoglobin and myoglobin] (deoxy[Hb+Mb]) (Spencer et al. 2011; Racinais et al. 2014) have been found which could help understand the physiological events associated with the respiratory and metabolic responses.

In the past years, the relationship between these physiological thresholds has been studied. Murias et al. (2013a) observed an association between the breakpoint in deoxy[Hb+Mb] and RCP in a small sample of male and female subjects. This association was also observed by Fontana et al. (2014), in which a large sample of 118 subjects between 18 and 80 years old was studied. Additionally, Racinais et al. (2014) and Osawa et al. (2011) found that the EMG threshold was related to both the RCP and breakpoint in deoxy[Hb+Mb] but occurred at a slightly higher intensity. In contrast to these studies, in a recent study from our laboratory, a cascade of events was detected at an intensity between 75% and 90%VO\textsubscript{2}peak, starting with a levelling-off in total[Hb+Mb], followed by the EMG threshold, which in turn preceded the breakpoint in deoxy[Hb+Mb] and RCP (Boone et al. 2015). In a recent study, Keir et al. (2015) observed that the Critical Power (i.e., the boundary between the heavy and very heavy exercise domain (as outlined by Whipp et al. 2005)), MLSS, RCP and breakpoint in deoxy[Hb+Mb] occurred at a similar oxygen uptake (VO\textsubscript{2}) suggesting that they originate from
a similar physiological phenomenon. Although the above studies clearly indicate the association between muscle oxygenation, muscle activation and pulmonary VO\(_2\) responses, the contrasting results, especially on the order in which the breakpoints occur, shows that the underlying mechanisms for the association are not completely clear.

Since exercise training has been shown to induce both central and peripheral adaptations (Saltin et al. 1968; Ekblom 1969; Roca et al. 1992; Hawley 2002; Gibala et al. 2012) affecting both the QO\(_2\) (i.e., \(O_2\) delivery to the tissues) and microvascular \(O_2\) extraction, it can be predicted that aerobic fitness status affects the dynamic balance between \(O_2\) supply and \(O_2\) demand and might therefore, not only alter muscle oxygenation and activation to ramp exercise, but also the pulmonary VO\(_2\) response (Jones and Carter 2000). Therefore, in order to better understand the possible association between muscle activation, muscle oxygen utilization and ventilatory responses, it would be valuable to assess the impact of aerobic fitness status on the interrelationship of these physiological variables. To address these questions, a large study population with a heterogeneous aerobic fitness level performed an incremental ramp exercise to exhaustion. During the exercise the general metabolic responses (pulmonary VO\(_2\)), muscle activation (by means of EMG) and muscle oxygenation (by means of NIRS) were determined. It was hypothesized first, that the breakpoints in muscle oxygenation responses, muscle activation and ventilatory responses would occur in a cascade of events (i.e., in a specific order) within each subject, independent of the individual VO\(_2\)\(_{\text{peak}}\). Second, it was hypothesized that the \%VO\(_2\)\(_{\text{peak}}\) at which the breakpoints occurred would be a function of fitness (as VO\(_2\)\(_{\text{peak}}\), i.e., the higher the peak VO\(_2\), the greater the \%VO\(_2\)\(_{\text{peak}}\) at which they occur.

**MATERIALS AND METHODS**
Subjects

64 male physically active students (PA students), mean ± SD age 21.2 ± 3.2 years volunteered to take part in this study. The subjects had a mean body mass of 71.8 ± 4.8 kg and a mean height of 1.80 ± 0.08 m and were all active in a wide variety of non-competitive sport activities. The subjects were informed about the protocol and the aim of the study, and they signed an informed consent approved by the ethical committee of the Ghent University Hospital. A medical history questionnaire and an examination including rest and exercise ECG were performed prior to the start of the study. All subjects were declared to be in good health and none of them presented medical contraindications for participation in the study.

Experimental procedure and protocol

The experimental protocol consisted of an incremental ramp protocol to exhaustion with an increase in work rate of 20-35 Watt·min\(^{-1}\), based on the subjects’ anthropometry and self-reported physical fitness level. The actual ramp increase in work rate was preceded by three minutes of baseline cycling at 50 Watt on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands). Throughout the test, subjects were asked to cycle at a pedal rate of 70-80 rpm and the test was terminated when the pedal rate dropped below 65 rpm despite strong verbal encouragement. Ten minutes prior to the start of the ramp exercise the subjects performed three repetitions of maximal voluntary contractions (MVC) while seated upright on a bench with their legs hanging above the floor (90° flexion). The subjects were asked to attempt to extend the right leg using maximal force (with the surface EMG-electrodes attached to the M. Vastus Lateralis) against an immovable bar in order to obtain a maximal isometric contraction. The MVC-trials were held for at least 5s and
subjects were allowed to rest for 3 min in between trials. The subjects were asked to abstain from strenuous exercise and training for at least 24 hours prior to their visit to the laboratory.

**Measurements**

During the exercise tests, VO$_2$ was measured continuously on a breath-by-breath basis by means of a computerized O$_2$-CO$_2$ analyzer-flowmeter combination (Jaeger Oxycon Pro, Germany). Prior to the start of each test, the gas analyzers and volume transducer were calibrated as per manufacturer’s recommendations.

Myoelectric activity was detected by means of surface electromyography (surface EMG) and recorded with bipolar 34 mm diameter Ag-AgCl electrodes (Blue Sensor) at a sampling frequency of 1000 Hz. The electrodes were placed longitudinally on the distal part of the M. Vastus Lateralis of the right leg. Each electrode site was prepared by shaving, abrading and swabbing the site with diluted ethanol. The reference electrode was placed over the spiny process of a prominent cervical vertebrae. The EMG-signal was checked for movement artefacts and the wires connected to the electrodes were taped to the thigh of the subjects. Myoelectric signals were relayed from the bipolar electrodes to a Telemetry device (Noraxon Inc., Scottsdale, USA). During the ramp protocol EMG was recorded continuously. The highest averaged iEMG from the MVC trial was used as the value for MVC to which the iEMG values during ramp exercise were normalized.

Muscle oxygenation (oxy[Hb+Mb] and deoxy[Hb+Mb]) was measured by means of a frequency-domain multidistance near-infrared spectroscopy system (Oxiplex TS, ISS, Champaign, Illinois, USA). The NIRS probe consisted of eight light-emitting diodes, operating at wavelengths of 690 and 830 nm and one detector fibre bundle (source-detector distance = 2.0-3.5cm). The probe was positioned longitudinally on the distal section of the left
M. Vastus Lateralis and secured with Velcro® straps around the thigh. Prior to the placement of the NIRS probe, the NIRS system was calibrated and the skin was carefully shaved. Pen marks were made on the skin to indicate the margins of the probe to check for any downward sliding during the cycling exercise (none occurred). The NIRS signals were recorded at 25 Hz and afterwards digitally averaged into 1s-values.

Data analysis

The breath-by-breath data from the gas exchange responses were filtered upon exportation based on the following criteria: tidal volume <0.2 and >10 l·min\(^{-1}\); fraction of expired CO\(_2\) <1 and >10% (Fontana et al. 2014). The obtained breath-by-breath values were averaged into 10s intervals and these values were used for further analysis. The VO\(_2\)peak was calculated as the highest 30s average VO\(_2\) throughout the test. The gas exchange threshold (GET) and respiratory compensation point (RCP) of each ramp exercise test were determined by two independent experienced physiologists. The GET was determined using the criteria of a disproportionate increase in VCO\(_2\) to VO\(_2\) (Beaver et al. 1986), a first departure from the linear increase in V\(_E\) and an increase in V\(_E\)/VO\(_2\) with no increase in V\(_E\)/VCO\(_2\). The RCP corresponded to a second departure from linearity in V\(_E\) and an increase in both V\(_E\)/VO\(_2\) and V\(_E\)/VCO\(_2\) (Wasserman and McIlroy 1964). In case the physiologists found divergent results, the data were reevaluated together until a consensus was reached. The baseline VO\(_2\) was calculated as the mean VO\(_2\) of the final 90 s of the baseline cycling at 50 Watt.

The raw EMG signals were rectified, band-pass filtered (5-1000Hz) and integrated using commercially available software (MyoResearch2.10, Noraxon Inc., Scottsdale, USA). The iEMG and MPF values of each ramp exercise test were averaged over 1s-intervals. The iEMG-values were normalized to the maximum iEMG obtained during the MVC trials.
whereas MPF was normalized to the MPF of baseline cycling (100%). The iEMG- and MPF-values, and deoxy[Hb+Mb]- and total[Hb+Mb]- values were plotted as functions of work rate (W). The pattern of iEMG, MPF, deoxy[Hb+Mb] and total[Hb+Mb] (as functions of W and %VO₂peak) was analyzed by means of a double linear model (Osawa et al. 2011; Spencer et al. 2011; Boone et al. 2015) (SigmaPlot 11.0, Systat Software, CA, USA), yielding

\[ y = m_1 \cdot x + b_1 \] for the first linear function (low-to-moderate work rates)

\[ y = m_2 \cdot x + b_2 \] for the second linear function (high to maximal work rates)

where \( m \) represents the slope and \( b \) is the y-intercept value. From this double linear model the breakpoint (BP) in linearity of iEMG, MPF, deoxy[Hb+Mb] and total[Hb+Mb] could be determined.

The VO₂ at the work rate corresponding to the breakpoints in iEMG, MPF, deoxy[Hb+Mb] and total[Hb+Mb] was calculated based on the VO₂ response to the ramp exercise by first quantifying the Mean Response Time (MRT) in each individual (Fontana et al. 2014). The MRT was determined as the time interval between the onset of the incremental test and the intersection of the forward extrapolation of the baseline VO₂ and the backwards extrapolation of the linear VO₂-time relationship below the GET (Boone and Bourgois 2012). The individual MRT was then used to align the deoxy[Hb+Mb], total[Hb+Mb], iEMG and MPF data with the VO₂ data (Fontana et al. 2014), thus accounting for the kinetics of VO₂ and the transit time between muscles and lungs. In this way the VO₂ at which the breakpoints occurred was determined.

Statistical analysis
The descriptive statistics were calculated as mean values ± SD (ranges). RCP and the breakpoints (in VO2 and %VO2peak) in the iEMG, MPF, deoxy[Hb+Mb] and total[Hb+Mb] were compared by means of Repeated Measures ANOVA (SPSS 19.0). In case of a significant main effect, the breakpoints were compared pairwise by means of Paired Sample T-tests (with Bonferroni correction). To determine the influence of aerobic fitness status on the physiological responses to ramp exercise, VO2peak (as indicator of aerobic fitness status) was correlated with the breakpoints (%VO2peak) obtained from the double linear model fit to iEMG, MPF, deoxy[Hb+Mb] and total[Hb+Mb]. Statistical significance was set at P<0.05.

RESULTS

The ramp test lasted on average 14’53s (range 12’48s – 17’21s) with the three minute baseline cycling included.

General metabolic responses

The indicators of aerobic fitness status (Wpeak, VO2peak, GET, RCP) are presented in Table 1 for the entire subject group. The mean baseline VO2 for cycling at 50 Watt was 761 ± 89 ml·min\(^{-1}\) (range 629 – 891 ml·min\(^{-1}\)). RCP (%VO2peak) was significantly correlated to VO2peak (r=0.75; P<0.001).

Muscle activity

The mean iEMG for the MVC trials was 76 ± 10 mV (range 55 – 98 mV). The breakpoints in the iEMG and MPF response (VO2 and %VO2peak) could be consistently determined in 93% of the subjects and are presented in Table 2. In Figure 1 (upper panels) the iEMG and MPF of
three representative subjects with a similar Wpeak but different VO\textsubscript{2}peak are presented. The breakpoints (%VO\textsubscript{2}peak) in the iEMG ($r=0.61$; $P<0.001$) and MPF response ($r=0.63$; $P<0.001$) were significantly correlated to the VO\textsubscript{2}peak (Figure 2).

Muscle oxygenation

In Figure 1 (lower panels) the deoxy[Hb+Mb] and total[Hb+Mb] responses for the same three representative subjects with a similar Wpeak but different VO\textsubscript{2}peak are presented. The breakpoints of the deoxy[Hb+Mb] and total[Hb+Mb] response (VO\textsubscript{2} and %VO\textsubscript{2}peak) could be identified in 96% of the subjects and are presented in Table 2. The breakpoints (%VO\textsubscript{2}peak) in the deoxy[Hb+Mb] ($r=0.76$ respectively; $P<0.001$) and total[Hb+Mb] response ($r=0.66$ respectively; $P<0.01$) were significantly correlated to the VO\textsubscript{2}peak (Figure 2).

Muscle activation vs. muscle oxygenation vs. general metabolic responses

ANOVA revealed significant differences between the RCP and the BP (%VO\textsubscript{2}peak) of the iEMG, MPF, deoxy[Hb+Mb] and total[Hb+Mb] responses ($P<0.001$). Pairwise comparison showed that the BP in the deoxy[Hb+Mb] response was significantly correlated to ($P<0.001$) (Figure 3) and significantly higher than ($P<0.01$) the BP in the iEMG ($r=0.91$; 2.2 ± 2.0%VO\textsubscript{2}peak difference) and MPF response ($r=0.90$; 1.9 ± 2.1%VO\textsubscript{2}peak difference), and significantly correlated ($P<0.001$) but not significantly different ($P=0.13$) from the RCP ($r=0.96$; 0.8 ± 1.3%VO\textsubscript{2}peak difference). The BP in total[Hb+Mb] was significantly correlated to ($P<0.001$) (Figure 3) and significantly lower than ($P<0.001$) the BP in the iEMG ($r=0.87$; -2.8 ± 2.1%VO\textsubscript{2}peak difference), MPF response ($r=0.85$; -3.1 ± 2.2%VO\textsubscript{2}peak difference).
difference), and the RCP (r=0.85; -6.5 ± 2.1%VO\textsubscript{2}peak difference). The BPs in iEMG and MPF were significantly correlated (P<0.001) and significantly lower (P=0.047) than the RCP (r=0.88; -1.4 ± 2.1%VO\textsubscript{2}peak difference and r=0.87; -1.1 ± 2.2%VO\textsubscript{2}peak difference for iEMG and MPF, respectively).

DISCUSSION

The present study assessed the effect of aerobic fitness status on the physiological responses to ramp exercise. In a large subject group with heterogeneous aerobic fitness levels it was found first, that breakpoints (BP) in muscle oxygenation (deoxy[Hb+Mb] and total[Hb+Mb]), muscle activation (iEMG and MPF) and ventilatory responses (RCP) were associated and occurred in a specific order, independent of the individual VO\textsubscript{2}peak. More specifically it was observed that the BP in total[Hb+Mb] preceded the BP in iEMG and MPF which in turn preceded the BP in deoxy[Hb+Mb] and RCP. Second, it was found that the %VO\textsubscript{2}peak at which the BPs occurred was a function of VO\textsubscript{2}peak. In subjects with higher aerobic fitness status the BPs in muscle oxygenation and activation occurred at a higher %VO\textsubscript{2}peak compared to subjects with lower aerobic fitness status.

This is not the first study relating thresholds in NIRS-parameters to landmarks of exercise intensity (Bhambani et al. 1997; Miura et al. 1998; Grassi et al. 1999; Terakado et al. 1999; Wang et al. 2006; Soller et al. 2008; Bellotti et al. 2013). However, these studies differ strongly in methodology, especially in the marker of change in the different NIRS signals studied and the thresholds in respiration and/or lactate concentration to which these NIRS thresholds are related. Murias et al. (2013a, 2013b) were the first to observe, in a small subject sample, that the VO\textsubscript{2} at the breakpoint in deoxy[Hb+Mb] coincided with the RCP. Recently, this association was confirmed in a large subjects sample (n=118, age=18-80 years).
old) (Fontana et al. 2014). Additionally, Bellotti et al. (2013) showed a similar association between the BP in deoxy[Hb+Mb] and the maximal lactate steady state. Osawa et al. (2011) and Racinais et al. (2014) also added measurements of muscle activity to the experimental procedure to be able to relate muscle oxygenation to neuromuscular activation. In both studies, it was shown that the BP in deoxy[Hb+Mb] preceded the EMG threshold. A recent study from our laboratory (Boone et al. 2015) showed contrasting results and a cascade of events (BP total[Hb+Mb] < BP iEMG and MPF < BP deoxy[Hb+Mb] and RCP) was found. In the present study a similar cascade of events was found in a large subject population with a heterogeneous level of aerobic fitness level. It should be noted that the intensity at which the BP in deoxy[Hb+Mb] has been detected strongly differs between the above studies. In the studies of Osawa et al. (2011), Murias et al. (2013a) and Racinais et al. (2014) the BP occurred at 78 and 80%VO\textsubscript{2}peak, and 80%Wpeak, respectively, which is lower than the 88%VO\textsubscript{2}peak in the present study. However, these latter studies did not account for the kinetics of the VO\textsubscript{2} response, inherent to incremental ramp exercise. In the present study the leftward shift of the VO\textsubscript{2} response by the individual Mean Response Time (MRT) (44 ± 10s) induced an upward shift of 5.4 ± 1.4%VO\textsubscript{2}peak (i.e., BP in deoxy[Hb+Mb] from 82.7 to 88.2%VO\textsubscript{2}peak). It should be noted that this cannot explain the lower BP in deoxy[Hb+Mb] in the studies of Fontana et al. (2014) (74%VO\textsubscript{2}peak) and Belotti et al. (2013) (76%VO\textsubscript{2}peak), in which a similar data analysis procedure was used compared to the present study. However, in these latter two studies the mean aerobic fitness level was lower (40 ml·min\textsuperscript{-1}·kg\textsuperscript{-1}) compared to the present study and also to the studies of Osawa et al. (2011), Murias et al. (2013a) and Racinais et al. (2014)(50 ml·min\textsuperscript{-1}·kg\textsuperscript{-1}). In the present study, in which the impact of aerobic fitness level was addressed, the subject with the lowest VO\textsubscript{2}peak (39.7 ml·min\textsuperscript{-1}·kg\textsuperscript{-1}) had a BP in deoxy[Hb+Mb] at 77.7%VO\textsubscript{2}peak, which is more in line with the study of Fontana et al. (2014).
The present study however, is the first in which a cascade of events is found relating muscle activation (iEMG and MPF) and oxygenation (total[Hb+Mb], deoxy[Hb+Mb]) to functional indexes in subjects with different levels of aerobic fitness status. A specific order at which the BPs occur has recently been found in another study from our laboratory and this order was unaffected by pedal rate (Boone et al. 2015). In this latter study the BP in deoxy[Hb+Mb] occurred at a significantly lower %VO\textsubscript{2}peak compared to the RCP, whereas this is not the case in the present study. The lower BP in deoxy[Hb+Mb] compared to RCP in this study (Boone et al. 2015) is probably related to the fact the VO\textsubscript{2} data were only shifted by 20s instead of the individual MRT. It should be noted however, that the differences in the breakpoints, especially in iEMG and deoxy[Hb+Mb] are very small (2.2%VO\textsubscript{2}peak) and it can be questioned whether the difference is meaningful, given the variability in the recording of the pulmonary gas exchange, muscle activity (surface EMG) and oxygenation (NIRS). In this concern it should be argued that almost all subject in the present study showed this typical order in the appearance of the breakpoint as can be assessed from Figure 3, in which the correlation between the breakpoints is presented. To obtain insight into the individual day-to-day variability of RCP and the BPs in muscle activation and oxygenation, four subjects performed four incremental ramp exercises, following the same protocol as the present study, within a period of 2 weeks, with a least 48 hours of rest in between the trials. The RCP and BPs were determined using the exact same procedure as described above. It was found that the coefficient of variation in each individual for each BP was within the narrow range of 0.48-2.59%. These results indicate that even a small difference of 2.2%VO\textsubscript{2}peak is probably a meaningful difference supporting the cascade of events (BP in total[Hb+Mb] < iEMG, MPF < deoxy[Hb+Mb], RCP) occurring at the level of muscle oxygenation and activation. In our opinion the greatest source of error in the determination of the %VO\textsubscript{2}peak at which the breakpoints occur originates from the leftward shift of the VO\textsubscript{2} data by the individual MRT.
Although this should be considered as the most accurate method available, it has been shown that there is a high test-retest variability in MRT (Hughson and Inman 1986; Markovitz et al. 2003) and the calculation of the MRT is influenced by several methodological issues, such as baseline work rate, ramp slope, etc. (for review see Boone and Bourgois 2012). Further research is needed in this concern to increase the accuracy in the determination of the VO$_2$ at a given work rate during ramp exercise.

The deoxy[Hb+Mb] response showed a leveling-off at 89%VO$_2$peak, which might be the reflection of microvascular O$_2$ extraction reaching a ceiling. This implies that above this work/metabolic rate a further increase in pulmonary VO$_2$ originates from an increased aerobic energy turnover in other leg muscles or muscle groups not observed with NIRS (Harms et al. 1998). It cannot be excluded however, that a local increase in blood flow at the location of the NIRS probe induces the levelling-off in deoxy[Hb+Mb], implying that, in this case, muscle VO$_2$ can continue to increase (Murias et al. 2013b). The observation of the stable total[Hb+Mb] at this intensity however, does not support this hypothesis. While the current observations relate to incremental ramp exercise, it remains to be confirmed if these observations and relationships are also valid for constant work rate exercise and might be related to the critical power. Critical power has been shown to occur at a similar intensity (80-90%VO$_2$peak) (Poole et al. 1988; Hill et al. 2002) and has been associated with changes in neuromuscular activity (Burnley et al. 2012). Additionally, Keir et al. (2015) observed that the maximal lactate steady state, critical power and RCP occurred at the same oxygen uptake (VO$_2$) during steady state exercise. It remains to be elucidated however, whether the specific changes in NIRS, EMG and gas exchange that take place during incremental exercise in the vicinity of the critical power work rate and/or metabolic rate (~80-90%VO$_2$max) also describe the changes in muscle oxygenation and recruitment when constant work rate exercise is performed above vs below critical power. It should be noted that the NIRS and EMG analyses
were performed on one site of the M. Vastus Lateralis. Given the existing heterogeneity in blood flow and $O_2$ extraction responses not only between muscle fiber types but also between muscle regions and entire muscles (Koga et al. 2014), it is not clear whether the responses in NIRS and EMG are representative enough to establish the link with the systemic responses. In the present study the BP in iEMG preceded the BP in deoxy[Hb+Mb]. This is in contrast to the studies of Osawa et al. (2011) and Racinais et al. (2014) in which the BP in deoxy[Hb+Mb] occurred at a lower intensity compared to the BP in iEMG. Given the small differences between the BPs and the contrasting results additional research (e.g., a longitudinal training study) is needed to clarify the interrelationship between muscle activation, muscle oxygenation and pulmonary gas exchange.

The present study showed that aerobic fitness level has a strong impact on the muscle oxygenation and activation responses to ramp exercise. The BPs in iEMG, MPF, deoxy[Hb+Mb] and total[Hb+Mb] occurred at a higher relative intensity in subjects with a higher $VO_2$ peak. This rightward shift in the muscle oxygenation and muscle activation responses in more aerobically trained subjects coincides with a shift in the RCP to a higher intensity, which is a known effect of endurance training (Jones and Carter 2000) and this indicates that the responses are probably mechanistically linked. Additionally, this confirms the hypothesis of Fontana et al. (2014) that NIRS can help to determine the RCP, especially since also the responses of muscle oxygenation seem to be sensitive to changes in aerobic fitness. A longitudinal study addressing the effects of an endurance training program could shed a more comprehensive light on this issue.

The observation that the BPs of muscle oxygenation (total[Hb+Mb] and deoxy[Hb+Mb]) were closely related to the BPs of muscle activation (iEMG and MPF) suggests that muscle fiber characteristics and recruitment patterns might be at least in part attributable to the responses in muscle oxygenation. First, it can be assumed that muscle fiber distribution will
differ among the subjects in the present study and perhaps, similar to the study of Bergh et al. (1978) that subjects with the highest VO$_2$peak had the highest proportion of slow-twitch fibers. Second, it has been shown in rats that the relationship between O$_2$ delivery and O$_2$ extraction is fiber type dependent such that fast-twitch muscles are characterized by a greater fractional O$_2$ extraction compared to slow-twitch muscles (Behnke et al. 2003; McDonough et al. 2005; Ferreira et al. 2006). Third, aerobic training programs have been shown to induce adaptations at the central and peripheral level that might affect DO$_2$ and microvascular O$_2$ extraction. Nonetheless, it can be assumed that the initial trigger in the cascade of events (described above), i.e., a leveling-off in DO$_2$ (BP in total[Hb+Mb]) occurs at a higher relative intensity in trained subjects possibly due to a higher capillary-to-myocyte ratio and a higher proportion of slow-twitch fibers. This will in turn delay the recruitment of easy fatiguable fast-twitch fibers. Additionally, the ceiling of microvascular O$_2$ extraction is delayed due to a better matching of QO$_2$ to VO$_{2m}$.

In conclusion, the present study showed that aerobic fitness status has an impact on the muscle activation and oxygenation to incremental ramp exercise. A higher aerobic fitness status resulted in a coinciding shift of ventilatory responses (RCP) and the breakpoints in muscle oxygenation and muscle activation responses to higher relative intensities. This indicates that peripheral and central adaptations to (endurance) training positively impact the mechanisms which govern the QO$_2$ to VO$_{2m}$-relationship so as to better adjust to changes in metabolic demand in subjects with higher aerobic fitness levels. Additionally, it was observed that, independent of aerobic fitness level, muscle oxygenation, muscle activation and ventilatory response are closely interrelated with the breakpoint in total[Hb+Mb] preceding the breakpoint in iEMG and MPF, in turn preceding the breakpoint in deoxy[Hb+Mb] and the respiratory compensation point. These observations support the notion that NIRS and EMG parameters can aid in the determination of landmarks of exercise intensity.
ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

There are no conflicts of interest to be reported.
REFERENCES


Table 1

Indicators of aerobic fitness (Wpeak, VO$_2$peak, GET and RCP) and VO$_2$ response (S1$_{VO2}$, S2$_{VO2}$, ΔS$_{VO2}$) to ramp exercise (mean ± SD and range) for the subject group (n=64). * indicates significantly different from S1$_{VO2}$ (P<0.05).

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wpeak</td>
<td>Watt</td>
<td>362 ± 39</td>
</tr>
<tr>
<td></td>
<td>Watt.kg$^{-1}$</td>
<td>5.05 ± 0.64</td>
</tr>
<tr>
<td>VO$_2$peak</td>
<td>ml.min$^{-1}$</td>
<td>3726 ± 377</td>
</tr>
<tr>
<td></td>
<td>ml.min$^{-1}$.kg$^{-1}$</td>
<td>51.9 ± 6.3</td>
</tr>
<tr>
<td>GET</td>
<td>Watt</td>
<td>189 ± 31</td>
</tr>
<tr>
<td></td>
<td>ml.min$^{-1}$</td>
<td>2221 ± 247</td>
</tr>
<tr>
<td></td>
<td>ml.min$^{-1}$.kg$^{-1}$</td>
<td>30.6 ± 3.9</td>
</tr>
<tr>
<td>RCP</td>
<td>Watt</td>
<td>302 ± 42</td>
</tr>
<tr>
<td></td>
<td>ml.min$^{-1}$</td>
<td>3255 ± 446</td>
</tr>
<tr>
<td></td>
<td>ml.min$^{-1}$.kg$^{-1}$</td>
<td>45.6 ± 7.5</td>
</tr>
<tr>
<td>S1$_{VO2}$</td>
<td>ml.min$^{-1}$.Watt$^{-1}$</td>
<td>9.59 ± 0.24</td>
</tr>
<tr>
<td>S2$_{VO2}$</td>
<td>ml.min$^{-1}$.Watt$^{-1}$</td>
<td>9.98 ± 0.28 *</td>
</tr>
<tr>
<td>ΔS$_{VO2}$</td>
<td>ml.min$^{-1}$.Watt$^{-1}$</td>
<td>0.38 ± 0.26</td>
</tr>
</tbody>
</table>
Table 2

Respiratory compensation point (RCP) and breakpoints (VO₂, %VO₂peak) in the responses of muscle activation (iEMG and MPF) and muscle oxygenation (deoxy[Hb+Mb] and total[Hb+Mb]) to the ramp exercise. Equal superscripts (a, b, c) indicate that the breakpoints did not differ significantly, whereas different superscripts point at significant differences (P<0.05).

<table>
<thead>
<tr>
<th></th>
<th>VO₂ (ml·min⁻¹·kg⁻¹)</th>
<th>%VO₂peak (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
</tr>
<tr>
<td>Total[Hb+Mb]</td>
<td>3096 ± 384 ³</td>
<td>2431-4022</td>
</tr>
<tr>
<td>iEMG</td>
<td>3202 ± 425 ²</td>
<td>2440-4288</td>
</tr>
<tr>
<td>MPF</td>
<td>3211 ± 428 ²</td>
<td>2458-4311</td>
</tr>
<tr>
<td>RCP</td>
<td>3254 ± 447 ²</td>
<td>2403-4318</td>
</tr>
<tr>
<td>Deoxy[Hb+Mb]</td>
<td>3285 ± 457 ²</td>
<td>2480-4352</td>
</tr>
</tbody>
</table>
FIGURE CAPTIONS

Figure 1

The iEMG, MPF, deoxy[Hb+Mb] and total[Hb+Mb] responses of three representative subjects with a similar Wpeak (range 365-374 Watt) but a different VO$_2$peak (44.1 ml.min$^{-1}$.kg$^{-1}$, 51.4 ml.min$^{-1}$.kg$^{-1}$ and 58.0 ml.min$^{-1}$.kg$^{-1}$). In the present figure the rightward shift of the breakpoints in deoxy[Hb+Mb] and total[Hb+Mb] in subjects with a higher aerobic fitness level is shown. Dashed, gray and black vertical lines represent the breakpoints in the responses of the subject with the low, average and high VO$_2$peak, respectively.

Figure 2

Correlation between VO$_2$peak and breakpoints (BP) (in %VO$_2$peak) of the iEMG and MPF responses (upper panels), and deoxy[Hb+Mb] and total[Hb+Mb] (lower panels) in the entire population.

Figure 3

Correlation of the breakpoint (BP) in deoxy[Hb+Mb] and total[Hb+Mb] with the breakpoint in iEMG and Respiratory compensation point (RCP). In the fifth panel the correlation between the BP in deoxy[Hb+Mb] and total[Hb+Mb] is presented.