Respiratory and Muscle Oxygenation Responses to Two Constant-Load Exercise Intensities

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Abstract

Objective: We investigated the respiratory and vastus lateralis oxygenation responses to exhaustive, constant-load cycling at two exercise intensities.

Methods: Eight moderately-trained male randomly cycled to exhaustion at 75% and 85% of maximal work-load (CL75 and CL85, respectively) measured during a maximal incremental test. From continuous recordings of respiratory variables and near-infrared spectroscopy signals of the vastus lateralis muscle, VO2 slow component (SC) and deoxyhemoglobin (HHb) SC were calculated.

Results: At exhaustion, VO2 (+19 ± 25%), VO2 SC (+59 ± 24%) and minute ventilation (+14 ± 14%) were significantly higher during CL85 vs. CL75, whereas oxyhemoglobin (-67 ± 22%) and total hemoglobin (-36 ± 3%) responses were lower (all p<0.05). Additionally, VO2 SC during CL75 and CL85 were strongly correlated (r=0.88, p<0.001). The HHb SC did not differ between CL75 and CL85 (3.10 ± 0.75 vs. 3.44 ± 1.1 A.U, respectively; p=0.60). HHb SC and VO2 SC during CL85 (r=0.94, p<0.001), but not during CL75 (r=0.08, p=0.90), were correlated. Finally, HHb SC during CL75 and CL85 were unrelated (r=0.20, p=0.70).

Conclusion: Our results highlight that only the contribution of locomotor muscles to the VO2 SC magnitude is affected by the exercise intensity and not that of ventilation.

Keywords: Exercise; Ventilation; Deoxyhemoglobin; Cycling; VO2 slow component; Oxygen uptake; Muscle oxygenation

Introduction

During constant-load cycling, when the sustained power output remains below the work rate that stimulates the Gas-exchange Threshold (GET), VO2 initially rises exponentially to reach a steady-state condition within 2-3 min after the onset of exercise [1]. Above this exercise intensity, VO2 continues to rise to eventually reach a delayed steady-state above that predicted from lower power outputs or continues to rise until reaching exercise termination [2,3]. This has been termed the VO2 slow component (SC) phenomenon [1].

Major steps in understanding the VO2 SC origin during constant-load exercise came from the development of the thermodilution technique, which allows muscle VO2 to be measured invasively while controlling for blood flow [4]. Using this technique, Poole et al. [5] have reported that ~86% of the VO2 relates to the work accomplished by the exercising legs. This result suggests a firm link in cycling between the occurrence of the VO2 SC phenomenon and local oxygenation trends of the active musculature (quadriceps muscles).

Despite decades of research, the VO2 SC underpinning mechanisms are not yet fully elucidated. Candidates include an increased muscle temperature [6], lactate and H+ accumulation [7] and/or recruitment of less efficient type II fibres in respiratory and locomotor muscles [8-10]. More recently, measurement of local oxygenation trends by means of Near-infrared Spectroscopy (NIRS) during constant-load cycling paved the way for the suggestion that Deoxyhemoglobin (HHb) kinetics in leg muscles relate to whole-body VO2 kinetics [11-14]. In support, through the deoxyhemoglobin responses, Marles et al. [14] and Oueslati et al. [15] have reported a significant relationship between an HHb SC of the vastus lateralis muscle and VO2 SC during high intensity constant-load cycling exercise.

Recently, Oueslati et al. [15] showed a significant correlation between the VO2 SC and the decline in maximal inspiratory and expiratory pressure during a constant-load exercise at 85% of maximal aerobic power. These results corroborate those of Cross et al. [16,17] showing that the VO2 SC is strongly related to respiratory muscle work during a constant-load exercise at a work rate roughly equal to 25% and 60% of the difference between the GET and VO2peak. Potential limitations of the aforementioned studies include unique exercise intensity being studied and the fact that exercise was not conduct until exhaustion. As VO2 SC is thought to be closely related to development of muscle fatigue (respiratory and locomotor) [2,18], constant-load cycling to exhaustion would represent a more appropriate exercise model to determine the nature of the relationship between VO2 SC, minute ventilation and oxygenation status (HHb SC) in the vastus lateralis muscle. To date, no direct (i.e., same participants) comparison exists examining the effects of different exercise intensities on the nature of these relationships during exhaustive, constant-load cycling.

Our intention was to investigate the respiratory and vastus lateralis oxygenation responses to constant-load cycling at two exercise intensities above the GET intensity (~60% VO2max). We hypothesized that, the magnitude of VO2 SC, ventilation and vastus lateralis muscle HHb SC would be greater at higher exercise intensities.

Materials and Methods

Participants

Eight young, moderately-trained male cyclists (mean ± SD: age 17 ± 2 years, body weight 65 ± 3 kg, height 1.73 ± 0.07 m) took part in this study. The participants and their parents were provided with the procedures and risks associated with participation in the study. Written informed consent was obtained from all participants (their parents for minors). All participants were non-smokers and free of heart and lung diseases and had normal resting pulmonary function, as assessed by spirometry tests. They declared cycling on average 300 km weekly for
the past 4 years. This study was approved by the Ethics Committee of UHC Farhat-Hached Hospital. Participants were instructed not to engage in any physical activity and not to drink or eat any caffeinated meals on testing days.

Figure 1: Typical example of the oxygen uptake kinetics during a constant-load exercise above the gas exchange threshold for a representative participant. $A_1$ and $A_2$ are the asymptotic values for the exponential curves of the phase II and phase III, respectively; and are the time constants defined as the time to reach 63% of the amplitude of $A_1$ and $A_2$, respectively; $t_d_1$ and $t_d_2$ are the delays for phase II and phase III.

Procedures

The participants visited the laboratory at three occasions, separated by at least 48 h, and at the same time of the day (± 2 h), while wearing shorts and t-shirts. During the first visit (Day 1) a maximal ramp cycling test was completed for the determination of GET, maximal oxygen uptake (VO$_{2\text{peak}}$) and maximal power-output (W$_{\text{peak}}$). During the second and third sessions (Days 2 and 3, respectively, conducted in randomized order) participants cycled at constant-load until exhaustion at exercise intensity corresponding to either 75% or 85% of W$_{\text{peak}}$ (CL75 and CL85, respectively). All tests were conducted under similar standard environmental conditions (ambient temperature ~22°C and ~40% relative humidity). All cycling was conducted on the same electromagnetically braked cycle ergometer (Excalibur Sport, Lode, The Netherlands), which was adjusted to each individual’s specifications and the feet securely strapped on the pedal.

Maximal ramp cycling test

After 2-min resting period (while seated on the bike) followed by 2-min warm up at 60 W (pedaling rate of 60-70 rpm), work rate increased continuously by 30 W.min$^{-1}$ (pedaling rate of 90 ± 5 rpm) until participants reached exhaustion. The test was terminated by volitional cessation of exercise or failure to maintain a pedaling rate of at least 60 rpm for 5 s despite strong verbal encouragement.

Constant-load cycling test

After 2-min resting period (while seating on the bike) followed by 2-min warm up at 60 W (pedaling rate of 60-70 rpm), work rate was set to 75% (242 ± 26 W) or 85% (274 ± 29 W) of W$_{\text{peak}}$ (pedaling rate of 90 ± 5 rpm). Constant-work rate tests were performed until exhaustion using the same criteria as described above. Since our participants were highly familiarized with this type of effort, minimizing the day to day variability, each constant-load test was only performed once [19-22].

Data measurements

Pulmonary responses: Expired gases were collected by a mask encircling both the mouth and nose and recorded by a breath by breath portable analyzer (Cosmed K4b2, Rome, Italy) to measure VO$_2$, ventilation (VE), carbon dioxide production (VCO$_2$), tidal volume, respiratory rate and partial pressure of end tidal carbon dioxide production (PETCO$_2$). The gas analyzer and the pneumotachograph were calibrated prior to each test following manufacturer’s recommendations using a reference gas mixture (16% O$_2$ and 5% CO$_2$) and a 3 l syringe (Cosmed, Rome, Italy), respectively. During the ramp test, heart rate was continuously measured with a chest strap polar device (RS. 300, Polar Electro, Kempele, Finland).

Near-infrared spectroscopy responses (NIRS): On days 2 and 3, uninterrupted measurements of vastus lateralis muscle oxygenation trends were obtained during constant-load cycling via NIRS (Portamont, Artinis, Medical System, Zetten, The Netherlands). An emitter-detector pair was placed in the same position between visits, on the distal part of the right vastus lateralis (approximately 15 cm above the proximal border of the patella). The probe was fixed with a black, plastic spacer held in place via double-sided tape to reduce the intrusion of extraneous light. Skinfolds were measured at the location of the probe using a Harpenden caliper (Baty International, west Sussex, United kingdom) to ensure that skin fold was lower than 1.5 cm, as recommended to avoid signal alterations [23,24].

Data analysis

Maximal ramp cycling test: Data from the maximal ramp cycling test were averaged into 30-s intervals for further analysis. The GET was determined using the criteria of a disproportionate increase in VCO$_2$ in reference to VO$_2$ [25]; i.e., a first departure from the linear increase in V$_{E}$ and an increase in V$_{E}$/VCO$_2$. Each VCO$_2$/VO$_2$ relationship was printed and analyzed by three experienced exercise physiologists (random-order and blind conditions). Values were retained when at least two of them agreed on GET assessment (8 values were retained). The VO$_{2\text{peak}}$ was calculated as the highest averaged VO$_2$ value over 30 s.

Constant-load cycling test: VO$_2$ responses. For the two constant-load tests, time to exhaustion (TTE) was determined (in s) and assigned the value 100% for the ease of between-tests comparisons. Breath-by-breath VO$_2$ values were averaged into a sliding interval of 30 s. All respiratory variables were expressed at rest, 20%, 40%, 60%, 80% and 100% of TTE (averaged values over a period of 30 s prior to each time point of interest).

In line with previous studies, data corresponding to the first 20 s after the onset of exercise were excluded from analysis [14,26,27]. A bi-exponential model was used to characterize changes in VO$_2$ over time during constant-load cycling allowing the VO$_2$ SC amplitude to be quantified (Figure 1):
VO₂(t) = VO₂base + A₁ \left(1 - e^{-\frac{t-t₁}{τ₁}}\right) \times U₁
VO₂(t) = VO₂base + A₂ \left(1 - e^{-\frac{t-t₂}{τ₂}}\right) \times U₂

where (t) is the time; VO₂ base is the unloaded cycling baseline value; A₁ and A₂ are the asymptotic values for the exponential curves of the phase II and phase III, respectively; t₁ and t₂ are the delays for phase II and phase III, respectively; U₁=0 when t ≤ t₁ or U₁=1 when t ≥ t₁ and U₂=0 when t ≤ t₂ or U₂=1 when t ≥ t₂.

The amplitude of the VO₂ SC represents the difference in VO₂ at the end of phases II and at exhaustion.

Near-infrared spectroscopy

A modified form of the Beer-Lambert Law was used to calculate micromolar changes in tissue deoxyhemoglobin (HHb), oxyhemoglobin (O₂Hb) and total hemoglobin (tHb) across time using received optical densities from two continuous wavelengths of NIRS light (760 and 850 nm). The oxygen tissue saturation index (TSI, expressed in %), which reflects the dynamic balance between O₂ supply and O₂ consumption, was calculated as $[\text{HHb}] / ([\text{HHb}] + [\text{O₂Hb}]) \times 100$ [28]. NIRS data were acquired at 1 Hz.

NIRS data were averaged into a 30 s sliding interval and normalized to express the magnitude of changes from the resting period (arbitrarily defined as 0 µM) [21]. Additionally, the HHb data were fitted from 20 s after the onset of the exercise with a bi-exponential model of the form [14]:

$$\text{HHb}(t) = \text{HHb}(b) + \text{HHb amplitude} \left(1 - e^{-\frac{(t-tdd)}{τHHb}}\right)$$

where HHb(b) is the baseline measured at rest, HHb amplitude is the asymptotic amplitude for the exponential curve, tdd HHb is the time delay. Also, the HHb SC was defined and at 20%, 40%, 60%, 80% and 100% of TTE (averaged values over 30 s prior each time point of interest). To study the relationship between $V_{E}$ and tHb, the difference in $V_{E}$ (Δ $V_{E}$) and tHb (ΔtHb) between CL85 and CL75 was assessed.

Finally, to further investigate limiting factors of constant-load cycling, respiratory and NIRS data were also determined during CL75 for an exercise duration corresponding to TTE during CL85 (iso-time CL85).

Figure 2: Typical example of deoxyhemoglobin responses during the constant-load exercise above the gas exchange threshold for a representative participant. HHb amplitude is the asymptotic amplitude for the exponential curve, τHHb is the time constant and td HHb is the time delay.

Statistical analysis

Descriptive statistics (mean, standard deviation) were calculated for all variables. Data were processed using SigmaPlot (version 11, Systat software, Germany). A non-parametric ANOVA of Friedman was used to compare physiological data. Relationships between different parameters were investigated using the Spearman correlation coefficient. The threshold for statistical significance was set at p<0.05.

Results

Maximal ramp cycling test

The mean VO₂peak and Wₚeak were 3.64 ± 0.35 4 ml.min⁻¹ and 322 ± 35 W, respectively. The GET was observed at 191 ± 10 W (59 ± 5% of Wₚeak) and corresponded to a VO₂ of 2.25 ± 0.38 l.min⁻¹ (73 ± 6% of VO₂peak). Maximal heart rate and respiratory exchange ratio were 189 ± 5 beats.min⁻¹ and 1.30 ± 0.10, respectively.

Constant-load cycling exercise

Compared to CL75 (567 ± 177 s), TTE was shorter during CL85 (303 ± 87 s) (p<0.05).

Pulmonary responses: The kinetics of the main pulmonary data are given in Figure 3: tidal volume, $V_{E}$ and VO₂ but not respiratory rate, were significantly higher during CL85 compared to CL75 (p<0.05). At exhaustion, PETCO₂ was higher during CL85 vs. CL75 (38 ± 4 vs. 33 ± 3 mmHg, p<0.05).

Whereas baseline VO₂ and VO₂ of phase II did not differ, VO₂ at exhaustion (end phase III) was ~9% higher in CL85 vs. CL75 (Table 1). Greater VO₂ SC amplitudes occurred during CL85 (Table 1). Amplitudes of VO₂ SC during CL75 and CL85 were positively correlated (r=0.88, p<0.001).
Near-infrared spectroscopy

Mean values of the NIRS parameters during CL75 and CL85 are shown in Figure 4. Compared to CL75, O₂Hb and tHb values were lower (-67 ± 22% and -36 ± 3%, respectively, p<0.05) during CL85, whereas no difference between conditions occurred for HHb and TSI values.

The HHb responses fitted with a bi-exponential curve are given in Table 2. No differences for HHb related-parameters were observed between the two conditions. The HHb SC during CL75 and CL85 did not correlate significantly (r=-0.20, p=0.7).

Relationship between pulmonary and local oxygenation responses

The VO₂ SC was correlated significantly with the HHb SC during CL85 (r=0.94, p<0.001) but not during CL75 (r=-0.08, p=0.9). No significant correlation occurred between the ΔVE and ΔHb at exhaustion (r=-0.60, p=0.1).

Isotime comparisons

Compared to data measured at exhaustion during CL85 (100% of TTE), VO₂ and VE values were significantly lower (-12 ± 13% and -13 ± 11%, all p<0.05) at isotime CL75, whereas the Hb and O₂-Hb values were significantly higher (Table 3).

Discussion

Our intention was to investigate the effect of exercising at two different intensities (75% vs. 85% of W_peak) during constant-load cycling to exhaustion on the relationships between vastus lateralis oxygenation, ventilation and VO₂ SC. As expected, higher respiratory and metabolic loads occurred for CL85 vs. CL75 when comparisons were made at absolute relative (% of TTE) time points. Our principal original findings were that i) vastus lateralis HHb SC did not differ between conditions, despite larger amplitude of the VO₂ SC when exercising at a higher exercise intensity; and ii) HHb SC and VO₂ SC were correlated during CL85 but not CL75.

VO₂ slow component

The magnitude of VO₂ SC amplitude values during CL75 and CL85 (0.44 ± 0.14 and 0.73 ± 0.22 L.min⁻¹, respectively) corroborates previous findings obtained at similar relative exercise intensities (i.e., 0.22 L.min⁻¹ at 75% of W_peak [29]; 0.65-0.67 L.min⁻¹ at 80% of VO₂max [20,30]; 0.88 L.min⁻¹ at 85% of W_peak). Also in line with previous studies, higher exercise intensity led to greater values for ventilation, VO₂ and therefore the amplitude of the VO₂ SC [31-33]. An important finding of our study was also that amplitude of VO₂ SC during CL75 and CL85 were significantly correlated (r=0.88, p<0.001). At exercise intensities above GET, the VO₂ SC can be traced to common fatigue manifestations associated with an increase in active muscles work: increase in muscle temperature, lactate and hydrogen accumulation, preferential recruitment of type Ia fibers as well as decreased ATP-synthese and/or of mechanical efficiency [8,10,32,33]. Despite the same mechanistic basis the respective contribution of the aforementioned mechanisms, in turn influencing VO₂ SC amplitude, likely differs with

### Table 1: Respiratory responses fitted with a bi-exponential curve during the constant-load cycling at 75% (CL75) and 85% (CL85) of maximal work-load. Values are means ± SD. *, p<0.05, different between conditions.

<table>
<thead>
<tr>
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<th>CL75</th>
<th>CL85</th>
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<tbody>
<tr>
<td>Baseline VO₂ (l.min⁻¹)</td>
<td>0.41 ± 0.21</td>
<td>0.46 ± 0.09</td>
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<tr>
<td>VO₂ of phase II (l.min⁻¹)</td>
<td>2.94 ± 0.35</td>
<td>2.93 ± 0.28</td>
</tr>
<tr>
<td>VO₂ of phase III (l.min⁻¹)</td>
<td>3.39 ± 0.43</td>
<td>3.63 ± 0.33</td>
</tr>
<tr>
<td>VO₂ slow component (l.min⁻¹)</td>
<td>0.44 ± 0.14</td>
<td>0.70 ± 0.22</td>
</tr>
<tr>
<td>VE of phase II (l.min⁻¹)</td>
<td>98.7 ± 18.7</td>
<td>89.0 ± 17.9</td>
</tr>
<tr>
<td>VE of phase III (l.min⁻¹)</td>
<td>123.1 ± 7.1</td>
<td>140.8 ± 10.9</td>
</tr>
<tr>
<td>VE between phases II and III (l.min⁻¹)</td>
<td>25.6 ± 17.2</td>
<td>51.8 ± 18.0</td>
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<tr>
<td>VO₂: oxygen uptake, VE: minute ventilation.</td>
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### Table 2: Deoxyhemoglobin (HHb) responses fitted with a bi-exponential curve during the constant-load cycling at 75% (CL75) and 85% (CL85) of maximal work-load. Values are means ± SD.

<table>
<thead>
<tr>
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<th>CL75</th>
<th>CL85</th>
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<tbody>
<tr>
<td>Baseline HHb (μM)</td>
<td>-0.58 ± 1.55</td>
<td>-0.08 ± 1.37</td>
</tr>
<tr>
<td>HHb of phase II (A.U)</td>
<td>10.54 ± 3.76</td>
<td>10.54 ± 5.09</td>
</tr>
<tr>
<td>HHb of phase III (A.U)</td>
<td>13.81 ± 4.26</td>
<td>13.97 ± 4.89</td>
</tr>
<tr>
<td>HHb slow component (A.U)</td>
<td>3.10 ± 0.75</td>
<td>3.44 ± 1.10</td>
</tr>
<tr>
<td>HHb slow component (%)</td>
<td>21 ± 5</td>
<td>24 ± 12</td>
</tr>
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<td>HHb, deoxyhemoglobin</td>
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</table>

**Figure 3:** (A) Minute ventilation, (B) rate of oxygen uptake, (C) tidal volume and (D) respiratory rate kinetics at rest and during the constant-load exercise at 75% (CL75) and 85% (CL85) of maximal work-load. Values are mean±SD. *, p<0.05 different between conditions. $ and # p<0.05 different between two successive time points during CL75 and CL85, respectively.
exercise intensity, but could not be fully ascertained with present methodology.

**HHb slow component**

In the first time, vastus lateralis muscle oxygenation was recorded by means of NIRS to investigate the effect of exercise intensity on local oxygenation during two different cycling intensities. Our results show that HHb increased exponentially (from the onset to 20% TTE), reflecting an increased extraction of O$_2$ as a consequence of an increase in muscle VO$_2$ muscle [13]. HHb then continued to rise slowly yet progressively (the HHb SC) but similarly in both conditions (75 vs. 85% W$_{peak}$) until exhaustion. Marles et al. [14] observed an amplitude of 14 ± 5 A.U for HHb SC during a constant-load cycling exercise at an intensity corresponding to a power output half-way between the GET and VO$_{2max}$. Additionally, we report that amplitudes of HHb and VO$_2$ SC during CL85 were correlated ($r=0.94$, $p<0.05$). This observation is in accordance with the literature supporting a locomotor muscle origin of the VO$_2$ SC [5,32,34]. However, the lack of significant difference for the HHb SC between CL75 and CL85 would indicate that the contribution of locomotor muscles in VO$_2$ SC occurrence is likely affected by the exercise intensity. Additionally, the origin of VO$_2$ SC can be localized in the respiratory muscles especially when the exercise intensity is above 85% of VO$_2$max [16,17,20,35]. That said, as respiratory muscles are skeletal muscles, all the aforementioned potential ‘peripheral’ could also play a role in the respiratory hypothesis.

<table>
<thead>
<tr>
<th></th>
<th>CL85 at exhaustion</th>
<th>CL75 at isotime CL85</th>
<th>CL75 at exhaustion</th>
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<tbody>
<tr>
<td>VO$_2$ (l.min$^{-1}$)</td>
<td>3.63 ± 0.33</td>
<td>3.23 ± 0.20</td>
<td>3.12 ± 0.45</td>
</tr>
<tr>
<td>VE (l.min$^{-1}$)</td>
<td>140 ± 10$^*$</td>
<td>124 ± 13</td>
<td>123 ± 7</td>
</tr>
<tr>
<td>HHb (A.U)</td>
<td>14.0 ± 3.9</td>
<td>13.4 ± 5.3</td>
<td>14.0 ± 4.8</td>
</tr>
<tr>
<td>O$_2$Hb (A.U)</td>
<td>-20.2 ± 3.6$^*$</td>
<td>-13.3 ± 3.2</td>
<td>-13.0 ± 4.3</td>
</tr>
<tr>
<td>IHB (A.U)</td>
<td>-5.7 ± 3.5$^*$</td>
<td>0.1 ± 5.1</td>
<td>0.9 ± 4.7</td>
</tr>
<tr>
<td>TSI (%)</td>
<td>63 ± 11</td>
<td>60 ± 5</td>
<td>60 ± 4</td>
</tr>
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</table>

Table 3: Respiratory and NIRS data at exhaustion during the constant-load cycling at 75% (CL75 at exhaustion) and 85% of maximal work-load (CL85 at exhaustion) and during CL75 at an exercise duration corresponding to exhaustion during CL85 (CL75 at isotime CL85). Values are means ± SD. *p<0.05, CL85 at exhaustion different from the other two conditions.

**Ventilatory and total hemoglobin responses**

In our study, differences in VE between the two constant-load exercise intensities were primarily related to changes in tidal volume (from 60% to 100% of TTE, p<0.05), as the respiratory rate did not differ (p>0.05). It has been reported that CO$_2$ production increases the cerebral oxygenation and activity [36] and in turn raises VE. In support, it can be suggested that the larger increase in tidal volume during CL85 compared to CL75 is closely related to the cerebral stimulation due to the higher P$_{ET}$CO$_2$ (at 80% and 100% of TTE, p<0.05). Future studies measuring brain oxygenation status are needed to verify this assumption. Compared to CL75, we further demonstrated that CL85 was associated with lower tHb values throughout the exercise. It is well documented that VE increase enhances sympathetic vasconstrictor activity via respiratory muscle metaboreflex and therefore decreases regional blood volume (i.e., tHb) [37]. In our study frame, however, the effect of VE on blood flow distribution cannot be ascertained due to a lack of significant correlation observed between ΔVE and ΔHb ($r$=-0.60, p=0.1).

**Isotime comparisons**

Previous studies have proposed that the exercise-induced increase in VE with exercise intensity could induce respiratory muscle fatigue, influencing exercise tolerance via activation of the metaboreflex [38,39]. Specifically, it has been demonstrated that the decreased blood flow in exercising legs is caused by an increase in ventilatory work [40,41]. With this in mind, we made the interesting observations that VE and VO$_2$ were higher and tHb lower at the end of CL85 compared to the same absolute exercise time during CL75 (after ~300 s of pedaling or 55% of TTE). Pending confirmatory research, it seems plausible that the increase in VE (the difference between the end of CL85 and the same absolute exercise time during CL75) could limit exercise tolerance via a reduction of legs blood flow (i.e., tHb).

**Limitations**

A limitation of the current study is that the concentration changes in O$_2$Hb and tHb as measured with NIRS only reflect the balance between O$_2$ delivery and O$_2$ extraction and not the actual muscle VO$_2$ that cannot be quantified using the present methodology. Furthermore, blood volume measurement was not performed in respiratory muscles to confirm the occurrence of respiratory muscle metaboreflex activation and blood flow redistribution between respiratory and locomotor muscles [42]. Another limitation was our small sample size. Finally, the reader should be cognizant to the fact that present experiments were conducted with young moderately-trained cyclists, who notably presented specific cardiopulmonary characteristics (i.e., greater respiratory work at exhaustion) compared to middle-aged cyclists [43,44]. Our results may not be extrapolated to other athletic populations.
Conclusion

In conclusion, sustained 85% of $W_{\text{peak}}$ compared to 75% $W_{\text{peak}}$ is associated with higher respiratory responses and larger VO$_2$ SC amplitudes. Unique to this study was that HHb SC amplitudes were similar between the two exercise intensities. Furthermore, HHb SC and VO$_2$ SC were correlated during CL85 but not CL75. Altogether, our results highlight that the contribution of locomotor muscles to the VO$_2$ SC magnitude is affected by the exercise intensity.

Acknowledgments

We thank our cyclists for their enthusiastic participation. We dedicate this paper to the Tunisian Cycling Federation.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

References