



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

Oueslati F^{1,2,3*}, Girard O⁴ and Ahmaidi S¹

¹Sport Sciences Department, University of Picardie Jules Verne, Amiens, France

²Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, Québec, Canada

³Faculty of Sciences, University of Carthage, Bizerte, Tunisia

⁴Institute of Sport Sciences, University of Lausanne (ISSUL), Lausanne, Switzerland

*Corresponding author: Ferid Oueslati, Institut universitaire de cardiologie et de pneumologie de Québec, 2725 Ch Ste-Foy, G1V 4G5, Québec, Canada, Tel: (+1) 418-656-8711 #301; E-mail: oueslati.ferid@hotmail.com

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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 VO_{2peak}. Potential limitations of the aforementioned studies include unique exercise intensity being studied and the fact that exercise was not conducted 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% VO_{2max}). 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.

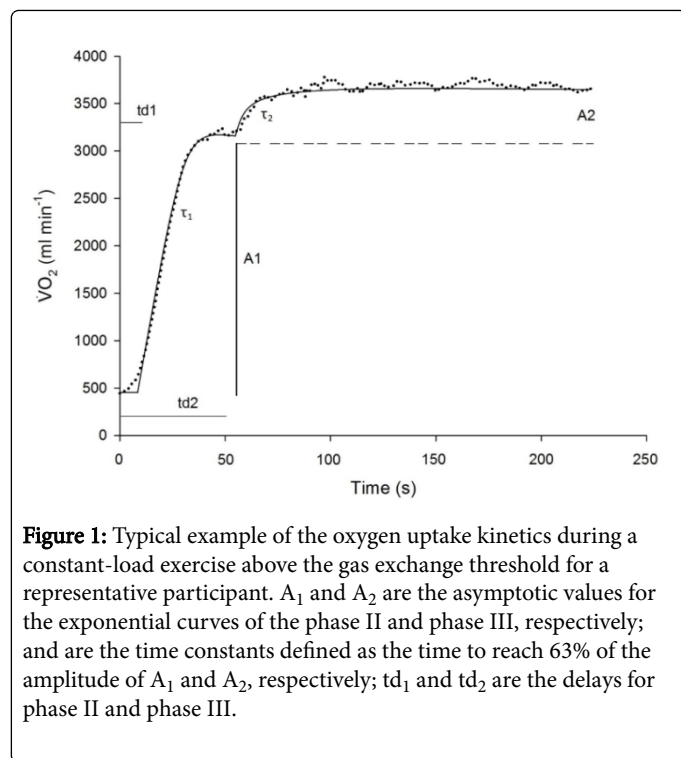


Figure 1: Typical example of the oxygen uptake kinetics during a constant-load exercise above the gas exchange threshold for a representative participant. A₁ and A₂ are the asymptotic values for the exponential curves of the phase II and phase III, respectively; and are the time constants defined as the time to reach 63% of the amplitude of A₁ and A₂, respectively; td₁ and td₂ 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_{2peak}) and maximal power-output (W_{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_{peak} (CL75 and CL85, respectively). All tests were conducted under similar standard environmental conditions (ambient temperature $\sim 22^{\circ}C$ and $\sim 40\%$ 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 $30 W \cdot min^{-1}$ (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_{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 enclosing 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 (PETCO₂). The gas analyzer and the pneumotachograph were calibrated prior to each test following manufacturer's recommendations using a reference gas mixture (16% O₂ and 5% CO₂) 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 (Portamon, 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/VO_2 with no concomitant 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_{2peak} 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):

$$VO_2(t) = VO_{2base} + A1 \left\{ 1 - e^{-\left[\frac{(t-td1)}{\tau1}\right]} \right\} \times U1$$

$$VO_2(t) = VO_{2base} + A2 \left\{ 1 - e^{-\left[\frac{(t-td2)}{\tau2}\right]} \right\} \times U2$$

where (t) is the time; VO_2 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; τ_1 and τ_2 are the time constants defined as the duration of time to reach 63% of the amplitude of A1 and A2, respectively; td_1 and td_2 are the delays for phase II and phase III, respectively. $U_1=0$ when $t \leq td_1$ or $U_1=1$ when $t \geq td_1$ and $U_2=0$ when $t \leq td_2$ or $U_2=1$ when $t \geq td_2$.

The amplitude of the VO_2 SC represents the difference in VO_2 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 (O_2Hb) 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 O_2 supply and O_2 consumption, was calculated as $[HbO_2]/([HbO_2] + [HHb]) \times 100$ [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]:

$$HHb(t) = HHb(b) + HHb \text{ amplitude} \left\{ 1 - e^{-\left[\frac{(t-tdHHb)}{\tauHHb}\right]} \right\}$$

where HHb(b) is the baseline measured at rest, HHb amplitude is the asymptotic amplitude for the exponential curve, τ_{HHb} is the time constant and td_{HHb} is the time delay. Also, the HHb SC was expressed as the difference between HHb maximum value and HHb amplitude (Figure 2) [14]. Similar to respiratory variables, NIRS-related parameters (HHb, O_2Hb , tHb and TSI) were calculated at rest 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 (ΔV_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).

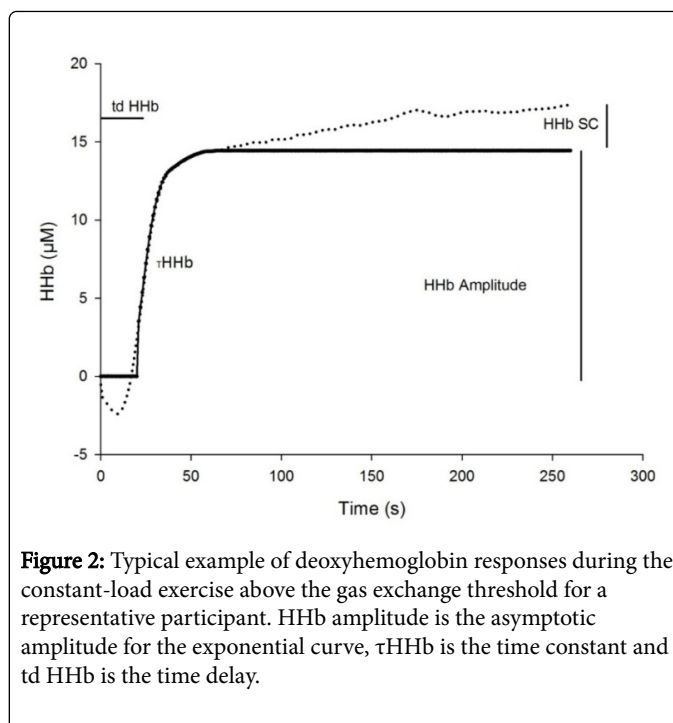


Figure 2: Typical example of deoxyhemoglobin responses during the constant-load exercise above the gas exchange threshold for a representative participant. HHb amplitude is the asymptotic amplitude for the exponential curve, τ_{HHb} is the time constant and td_{HHb} is the time delay.

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 VO_{2peak} and W_{peak} were 3.64 ± 0.35 l.min⁻¹ and 322 ± 35 W, respectively. The GET was observed at 191 ± 10 W ($59 \pm 5\%$ of W_{peak}) and corresponded to a VO_2 of 2.25 ± 0.38 l.min⁻¹ ($73 \pm 6\%$ of VO_{2peak}). 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 VO_2 but not respiratory rate, were significantly higher during CL85 compared to CL75 ($p < 0.05$). At exhaustion, $PETCO_2$ was higher during CL85 vs. CL75 (38 ± 4 vs. 33 ± 3 mmHg, $p < 0.05$).

Whereas baseline VO_2 and VO_2 of phase II did not differ, VO_2 at exhaustion (end phase III) was ~9% higher in CL85 vs. CL75 (Table 1). Greater VO_2 SC amplitudes occurred during CL85 (Table 1). Amplitudes of VO_2 SC during CL75 and CL85 were positively correlated ($r=0.88$, $p < 0.001$).

	CL75	CL85
Baseline $\dot{V}O_2$ (l.min ⁻¹)	0.41 ± 0.21	0.46 ± 0.09
$\dot{V}O_2$ of phase II (l.min ⁻¹)	2.94 ± 0.35	2.93 ± 0.28
$\dot{V}O_2$ of phase III (l.min ⁻¹)	3.39 ± 0.43	3.63 ± 0.33*
$\dot{V}O_2$ slow component (l.min ⁻¹)	0.44 ± 0.14	0.70 ± 0.22*
\dot{V}_E of phase II (l.min ⁻¹)	98.7 ± 18.7	89.0 ± 17.9
\dot{V}_E of phase III (l.min ⁻¹)	123.1 ± 7.1	140.8 ± 10.9*
\dot{V}_E between phases II and III (l.min ⁻¹)	25.6 ± 17.2	51.8 ± 18.0*
$\dot{V}O_2$; oxygen uptake, \dot{V}_E ; minute ventilation.		

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.

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 $\dot{V}O_2$ 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 ΔV_E and ΔtHb at exhaustion (r=-0.60, p=0.1).

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

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.

Isotime comparisons

Compared to data measured at exhaustion during CL85 (100% of TTE), $\dot{V}O_2$ and \dot{V}_E values were significantly lower (-12 ± 13% and -13 ± 11%; all p<0.05) at isotime CL75, whereas the tHb and O₂Hb values were significantly higher (Table 3).

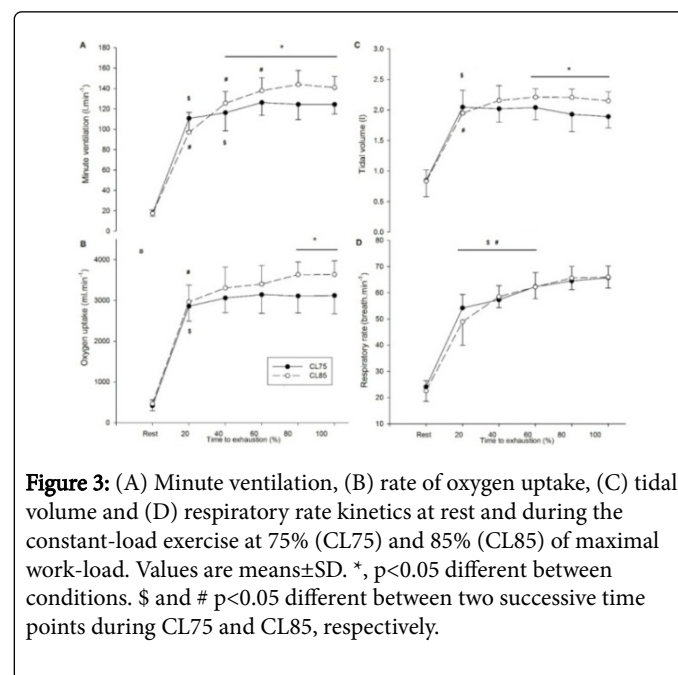


Figure 3: (A) Minute ventilation, (B) rate of oxygen uptake, (C) tidal volume and (D) respiratory rate kinetics at rest and during the constant-load exercise at 75% (CL75) and 85% (CL85) of maximal work-load. Values are means±SD. *, p<0.05 different between conditions. \$ and # p<0.05 different between two successive time points during CL75 and CL85, respectively.

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 $\dot{V}O_2$ 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 $\dot{V}O_2$ SC when exercising at a higher exercise intensity; and ii) HHb SC and $\dot{V}O_2$ SC were correlated during CL85 but not CL75.

$\dot{V}O_2$ slow component

The magnitude of $\dot{V}O_2$ 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 $\dot{V}O_{2max}$ [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, $\dot{V}O_2$ and therefore the amplitude of the $\dot{V}O_2$ SC [31-33]. An important finding of our study was also that amplitude of $\dot{V}O_2$ SC during CL75 and CL85 were significantly correlated (r= 0.88, p<0.001). At exercise intensities above GET, the $\dot{V}O_2$ 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 IIa fibers as well as decreased ATP-synthase and/or of mechanical efficiency [8,10,32,33]. Despite the same mechanistic basis the respective contribution of the aforementioned mechanisms, in turn influencing $\dot{V}O_2$ SC amplitude, likely differs with

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 O₂ as a consequence of an increase in muscle VO₂ 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 VO₂ 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 VO₂ 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 VO₂ SC occurrence is likely affected by the exercise intensity. Additionally, the origin of VO₂ SC can be localized in the respiratory muscles especially when the exercise intensity is above 85% of VO_{2max} [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.

	CL85 at exhaustion	CL75 at isotime CL85	CL75 at exhaustion
VO ₂ (l.min ⁻¹)	3.63 ± 0.33*	3.23 ± 0.20	3.12 ± 0.45
Ṡ _E (l.min ⁻¹)	140 ± 10*	124 ± 13	123 ± 7
HHb (A.U)	14.0 ± 3.9	13.4 ± 5.3	14.0 ± 4.8
O ₂ Hb (A.U)	-20.2 ± 3.6*	-13.3 ± 3.2	-13.0 ± 4.3
tHb (A.U)	-5.7 ± 3.5*	0.1 ± 5.1	0.9 ± 4.7
TSI (%)	63 ± 11	60 ± 5	60 ± 4

Ṡ_{O₂}: Oxygen Uptake; Ṡ_E: Minute Ventilation; HHb: Deoxyhemoglobin; O₂Hb: Oxyhemoglobin; tHb: Total Hemoglobin; TSI: Tissue Saturation Index. Note that iso-time CL85 corresponded to 55 ± 12% of CL75 time to exhaustion

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 V_E 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 CO₂ production increases the cerebral oxygenation and activity [36] and in turn raises V_E. 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}CO₂ (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 V_E increase enhances sympathetic vasoconstrictor 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 ΔV_E and ΔtHb (r=-0.60, p=0.1).

Isotime comparisons

Previous studies have proposed that the exercise-induced increase in V_E 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

V_E and VO₂ 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 O₂Hb and tHb as measured with NIRS only reflect the balance between O₂ delivery and O₂ extraction and not the actual muscle VO₂ 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.

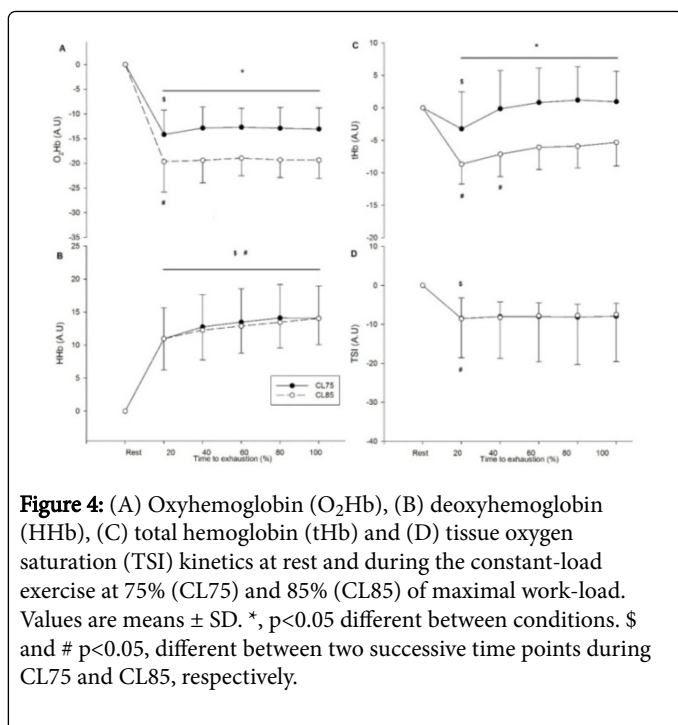


Figure 4: (A) Oxyhemoglobin (O₂Hb), (B) deoxyhemoglobin (HHb), (C) total hemoglobin (tHb) and (D) tissue oxygen saturation (TSI) kinetics at rest and during the constant-load exercise at 75% (CL75) and 85% (CL85) of maximal work-load. Values are means ± SD. *, p<0.05 different between conditions. \$ and # p<0.05, different between two successive time points during CL75 and CL85, respectively.

Conclusion

In conclusion, sustained 85% of W_{peak} compared to 75% W_{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.

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