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ORIGINAL ARTICLE

Acute effects of repeated cycling sprints in hypoxia induced by voluntary hypoventilation

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Abstract
Purpose This study aimed to investigate the acute responses to repeated-sprint exercise (RSE) in hypoxia induced by voluntary hypoventilation at low lung volume (VHL).
Methods Nine well-trained subjects performed two sets of eight 6-s sprints on a cycle ergometer followed by 24 s of inactive recovery. RSE was randomly carried out either with normal breathing (RSN) or with VHL (RSH-VHL). Peak (PPO) and mean power output (MPO) of each sprint were measured. Arterial oxygen saturation, heart rate (HR), gas exchange and muscle concentrations of oxy-([O_2Hb]) and deoxyhaemoglobin/myoglobin ([HHb]) were continuously recorded throughout exercise. Blood lactate concentration ([La]) was measured at the end of the first (S1) and second set (S2).
Results There was no difference in PPO and MPO between conditions in all sprints. Arterial oxygen saturation (87.7 ± 3.6 vs 96.9 ± 1.8% at the last sprint) and HR were lower in RSH-VHL than in RSN during most part of exercise. The changes in [O_2Hb] and [HHb] were greater in RSH-VHL at S2. Oxygen uptake was significantly higher in RSH-VHL than in RSN during the recovery periods following sprints at S2 (3.02 ± 0.4 vs 2.67 ± 0.5 L min^{-1} on average) whereas [La] was lower in RSH-VHL at the end of exercise (10.3 ± 2.9 vs 13.8 ± 3.5 mmol.L^{-1}; $p < 0.01$).
Conclusions This study shows that performing RSE with VHL led to larger arterial and muscle deoxygenation than with normal breathing while maintaining similar power output. This kind of exercise may be worth using for performing repeated sprint training in hypoxia.

Keywords Hypoventilation · Hypoxia · Hypoxemia · Repeated sprints · Exercise

Abbreviations

[HHb]	Muscle concentrations of deoxyhaemoglobin/myoglobin
[La]	Blood lactate concentration
[O_2Hb]	Muscle concentrations of oxyhaemoglobin/myoglobin
[tHb]	Total haemoglobin/myoglobin
ANOVA	Analysis of variance
FRC	Functional residual capacity
HR	Heart rate
MPO	Mean power output
NB	Normal breathing
NIRS	Near-infrared spectroscopy
PPO	Peak power output
RPE	Rating of perceived exertion
RSE	Repeated-sprint exercise
RSH	Repeated sprints in hypoxia
RSH-VHL	Repeated sprints in hypoxia induced by voluntary hypoventilation at low lung volume
RSN	Repeated sprints in normoxia

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SpO_2	Arterial oxygen saturation
$\dot{V}E$	Expired ventilation
$\dot{V}E/\dot{V}CO_2$	Ventilatory equivalent for carbon dioxide
VHL	Voluntary hypoventilation at low lung volume
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2max}$	Maximal oxygen uptake

Introduction

Repeated-sprint exercise (RSE), i.e., short-duration sprints (< 10 s) interspersed with brief recovery periods (< 60 s), is a common practice in most team and racket sports. This kind of exercise is effective to improve the ability to recover and to reproduce performance in subsequent sprints, namely repeated-sprint ability (Bishop et al. 2011). Recently, several studies demonstrated that training by performing repeated sprints in hypoxia (RSH) could be even more effective than the same type of training in normoxia (RSN) for improving peak and/or mean power output during repeated-sprint tests (Brocherie et al. 2015; Faiss et al. 2013a, 2015; Gatterer et al. 2014; Kasai et al. 2015; Millet et al. 2013). A recent meta-analysis confirmed that RSH is more efficient than RSN to improve mean repeated-sprint performance, while additional positive (but non-significant) effects on best repeated-sprint and maximal oxygen uptake ($\dot{V}O_{2max}$) were also reported (Brocherie et al. 2017a). The postulated underlying mechanisms (i.e., compensatory vasodilatation, microvascular oxygen delivery [fast-twitch fibres]) and specific skeletal muscle molecular adaptations mediated by the oxygen sensing pathway (Brocherie et al. 2017b) are likely to be fibre-type and intensity dependant (Faiss et al. 2013b).

So far, the vast majority of the studies that investigated the effects of RSH have used normobaric hypoxia by reducing the ambient fraction of oxygen (Brocherie et al. 2017a). However, it is remarkable that in one study, hypoxia was induced by voluntary hypoventilation at low lung volume (VHL) that is by performing breath holdings near functional residual capacity (Trincat et al. 2017). It is also noticeable that in this study, like in almost all RSH studies, the repeated-sprint performance enhancement was larger than in RSN. Over the last 10 years, it has repeatedly and clearly been shown that exercising with VHL could lead to severe hypoxemia (Woorons et al. 2007, 2008, 2011, 2014) and consequently to muscle tissue deoxygenation (Woorons et al. 2010) and could, therefore, represent a valuable and practical way to train under simulated hypoxic conditions.

Until recently, VHL had always been performed at submaximal exercise intensities (65–80% $\dot{V}O_{2max}$). In addition to the hypoxemic effect, higher carbon dioxide concentrations (i.e., hypercapnic effect), lower level of pH and increased lactate concentration have consistently been reported when exercising with VHL at moderate intensity, compared with the same exercise with normal breathing (NB) (Woorons et al. 2007, 2010, 2011, 2014). Thus, the main feature of this type of exercise is to provoke a combined lactic and respiratory acidosis as a result of both the hypoxic and hypercapnic effects. The latest studies that investigated the effects of VHL training at maximal velocity (Trincat et al. 2017) or at supramaximal intensity (Woorons et al. 2016) showed that it is possible to have a different approach to this kind of training. Nevertheless, little is known on the acute physiological effects of an approach to VHL training involving high exercise intensities. In particular, it is questionable whether, in these conditions, metabolic acidosis is greater than during the same exercise with NB.

To our knowledge, the first and only training study on the effects of RSH induced by VHL (RSH-VHL) was conducted in swimming (Trincat et al. 2017). Yet it could be interesting to define the acute physiological consequences of RSH-VHL in land-based activities (e.g., cycling or running) which are the core component of conditioning in team-sport players. Furthermore, it was recently shown that single-sprint performance is generally not affected by hypoxia whereas larger alterations in repeated-sprint ability (i.e., with earlier and larger performance decrements) occur at altitudes above 3000 m (or inspired fraction of oxygen < 14.5%), when compared to normoxic conditions (Girard et al. 2017). This is unknown for RSH-VHL and is of practical importance. Thus, the main objective of the present study was to analyse the acute effects of RSH-VHL on performance, muscle and blood oxygenation as well as the level of blood lactate concentration. In these conditions, we hypothesized that the decrement in power output could be greater than during RSN. We also assumed that RSH-VHL could lead to severe blood and muscle deoxygenation during most of the exercise. Finally, we postulated that RSH-VHL could provoke an increased blood lactate concentration, compared with RSN.

Methods

Subjects

Nine well-trained subjects, eight men and one woman, were recruited for this study. They were all non-smokers and sea-level residents. None of them reported to have recently been exposed to altitude (i.e., > 500 m above sea level). Four of the subjects (including the woman) were cyclists who trained 4–5 times per week and regularly participated in competition at a regional level. The five other subjects were involved in team sports (four basketball players and one rugby player) with 3–4 training sessions/week. Their physical characteristics (mean ± SD) were age 27.2 ± 9.3 years, height 179.0 ± 8.4 cm, and body mass 74.7 ± 9.6 kg. Subjects were

instructed not to participate in any strenuous exercise other than prescribed by the study protocol from the 48 h before the start of the experiment until the end of the study. They were also instructed to be adequately hydrated and to not have eaten for 3 h prior to each test. The participants gave their written informed consent to participate in this study, which was approved by the local Research Ethics Committee and complied with the Declaration of Helsinki (2008).

Protocol

Before the start of the experiment, the subjects participated in a first session to familiarize with the testing procedures as well as the VHL technique. This breathing technique has already been used in several studies (Trincat et al. 2017; Woorons et al. 2008, 2010, 2014, 2016) and is recognized as one of the various hypoxic training methods (Girard et al. 2017). It consists of repeating short bouts of breath holding while exercising. Unlike a simple apnoea, in which breath is generally held at high lung volume (i.e., total lung capacity) and for as long as possible, VHL requires a rigorous control of breathing to obtain a hypoxic effect while avoiding asphyxia. It stands on four phases: inhalation, exhalation, breath holding and a second exhalation. After inhalation, the first exhalation is carried out naturally, without forcing in order to reach the functional residual capacity (FRC). While FRC represents about one-half of the total lung capacity at rest (Quanjer et al. 1993), it is only about one-third of this lung volume during exercise, in particular when intensities are high. Indeed, FRC has been shown to be reduced with the increase in exercise intensity (Sharratt et al. 1987). In the present study, one can therefore consider that hypoventilation was performed at (relatively) low lung volume. After the first exhalation, a breath holding of a few seconds must be performed and is followed by a second exhalation down to residual volume which aims at the evacuation of the carbon dioxide accumulated within the lungs.

After the familiarization phase, the subjects participated in two testing sessions which consisted of performing two sets of eight "all-out" 6-s sprints on a cycle ergometer (894E, Monark Exercise AB, Vansbro, Sweden). The 6-s sprints were separated by 24 s of inactive recovery (departure every 30 s) and 3 min of passive rest were observed between the two sets. Before starting the first set, subjects performed a 5-min warm-up at low intensity followed by a single 6-s sprint. Exercise began after a 5-min period of rest. At the first sprint of set 1, subjects were required to achieve at least 95% of the mean power output (MPO) reached in the single sprint as recommended (Girard et al. 2011). If not, they had to restart the set again after a 5-min period of rest. Sprints were carried out with NB in one of the two testing sessions (RSN) and with VHL in the other (RSH-VHL). During RSH-VHL, the subjects were required to do a normal exhalation at the start of each sprint, then to hold their breath until the end of the 6-s exertion and finally to perform a second exhalation to empty the remaining air from the lungs. In the pre-experimental phase, we tested whether it could be possible to hold breath at the residual volume to obtain a greater stimulus. It appeared that the manoeuvre could not be conducted that way. In this condition, subjects were not able to hold their breath for more than 4–5 s and made micro-inhalations which prevented SpO_2 to fall down below 90%.

The order of exercise conditions was randomized, but counterbalanced so that five subjects carried out the first testing session in RSH-VHL and the four others in RSN. The two sessions took place at the same time of the day and were separated by 48 h. The mechanical resistance was set at 0.075 kg^{-1} body mass (measured at the first session) and subjects were instructed to stay seated. Computer-generated audio signals provided a 5-s countdown to the start of each sprint. All participants were strongly encouraged during the test to maintain their maximum pedal rate.

Measurements

Performance

For each 6-s sprint, peak power output (PPO) and MPO were measured with the Monark Anaerobic Test software (Monark, Varberg, Sweden). For both sets, we also assessed fatigue by calculating (in absolute value) the percentage decrement score as follows:

$$|(100 \times (\text{total sprint MPO of the set} / \text{ideal sprint MPO of the set}\,\text{ideal sprint MPO of the set})) - 100|,$$

where total sprint MPO is the sum of sprint MPO from all sprints of the set and ideal sprint MPO = number of sprints (i.e., 8) × highest sprint MPO of the set.

This formula has been found to be the most valid and reliable method for quantifying fatigue in tests of multiple-sprint performance (Glaister et al. 2008).

Arterial oxygen saturation and heart rate

Arterial oxygen saturation (SpO_2) and heart rate (HR) were continuously measured during set 1 and set 2 with the pulse oximeter Nellcor N-595 (Pleasanton, CA, USA) and with the adhesive forehead sensor Max-Fast (Nellcor, Pleasanton) which was applied above the right orbital area. This sensor has already been used during intense cycling exercise in hypoxia (Amann et al. 2007; Romer et al. 2007) and has

been shown to provide accurate and reliable estimations of both arterial oxygen saturation and HR (Fernandez et al. 2007; Schallom et al. 2007). An adjustable headband was placed over the forehead sensor to ensure gentle, consistent pressure on the sensor device. Arterial oxygen saturation and HR were recorded in real time every 2 s by the oximeter and collected using a data acquisition system (Score Analysis Software, Nellcor, Pleasanton). Data were then averaged and analyzed over 6 s, which corresponded to the duration of each sprint. It is important to note that in this protocol, SpO_2 began to drop at the end or just after the 6-s sprints. Moreover, the highest values of HR were recorded after each sprint. For these reasons, we chose to present the results for the 6-s periods in which the minimum values of SpO_2 and the maximum values of HR were reached.

Gas exchange

Gas exchange was continuously recorded during the whole exercise through a breath-by-breath portable system ($K4b^2$, Cosmed, Rome, Italy). Before the beginning of exercise, we performed the standardized calibration procedures as recommended by the manufacturer. These included air calibration, turbine calibration with a standard 3000-mL syringe, gas calibration with a certified commercial gas preparation (oxygen: 16%, carbon dioxide: 5%) and delay calibration to ensure accurate readings during the testing and to check the alignment between the gas flow and gas concentrations. The breath-by-breath measurements were performed for tidal volume, breathing frequency, expired ventilation ($\dot{V}E$), oxygen uptake ($\dot{V}O_2$), carbon dioxide production, end-tidal oxygen and carbon dioxide end-tidal pressures. The ventilatory equivalent for oxygen and carbon dioxide were calculated. Since gas exchange could obviously not be measured during sprints with breath holdings, data were analysed in the last 20 s of the recovery periods.

Near-infrared spectroscopy

Muscle oxygenation was assessed using a near-infrared spectroscopy (NIRS) technique which was well described elsewhere (Boushel and Piantadosi 2000). The NIRS device (Portamon Artinis, Zetten, The Netherlands) was used to measure changes in muscle oxygenation by placing a triple optode sensor on the left-leg vastus lateralis muscle (at mid-thigh) with an interoptode spacing of 40 mm. The probe was attached to the skin with double-sided tape and firmly fastened with an opaque cotton elastic band wrapped around subjects' thigh. Position of the probe was marked at the first testing session with a permanent pen for accurate repositioning at the second testing session. A standard differential pathlength factor (DPF) of 4.0 was used in lack of any clear standard value for human quadriceps muscle during cycling sprints (Racinais et al. 2007). All signals were recorded with a sampling frequency of 20 Hz. They were then down sampled at 1 Hz to remove possible artefacts and smooth the pedalling-induced perturbations. Muscle concentrations of deoxyhaemoglobin/myoglobin ([HHb]) and total haemoglobin/myoglobin ([tHb]) were recorded. We also chose to analyse the muscle concentrations of oxyhaemoglobin/myoglobin ($[O_2Hb]$) even though it has been reported that this factor might be confounded by rapid volume changes during sprints (Grassi et al. 2003). For the two sets, the change (Δ) in [HHb], [tHb] and $[O_2Hb]$ were calculated from the resting values recorded over the 2 min following the warm-up. Muscle measurements were, therefore, normalized from these recordings (arbitrarily defined as 0 µm). For all variables, data were averaged over 6 s and analysed for the greater changes obtained over a 6-s period during or just after each sprint.

Rating of perceived exertion (RPE) and blood lactate concentration [La]

In both exercise conditions, just after the end of each set, RPE was obtained using the Borg scale (range 0–10). In addition, a blood sample was taken from the earlobe of the subjects 90 s after the end of the first (S1) and the second set (S2) as well as 180 s after the end of S2 to obtain [La] (Lactate Pro, Arkray, Japan). The highest value of the two samples collected at the end of S2 was kept for the analyses.

Statistics

All the results are expressed as mean ± SD. To determine whether there was a difference in PPO, MPO, SpO_2, HR, $\dot{V}O_2$ and NIRS data between exercise conditions for each sprint and within each set, we performed two-way analyses of variance (ANOVA) for repeated measures. We performed one-way ANOVA for repeated measures to determine whether there were differences in the same data between sprints of S1 and S2 within each condition. To compare the percentage decrement score, RPE, [La] and gas exchange data (except $\dot{V}O_2$) between and within conditions at the end of each set (i.e., over the last 20 s of the recovery period of the 8th repetition for gas exchange), we also used two-way ANOVA for repeated measures. When a significant effect was found, the Bonferroni (two-way ANOVA) or the Tukey post hoc test (one-way ANOVA) was carried out to localize the differences. We used the Student *t* test to compare the single sprint MPO of the first sprint between the two exercise conditions. The relationships between the differences (Δ) in $\dot{V}O_2$, HR, SpO_2, [La], Δ[HHb] and $\Delta[O_2Hb]$ between RSH-VHL and RSN at the end of S2 were analysed by performing Person linear regressions. In particular we tested the following relationships: $\Delta\dot{V}O_2/\Delta$[La], $\Delta SpO_2/\Delta(\Delta$[HHb]),

$\Delta SpO_2/\Delta(\Delta[O_2Hb])$, $\Delta SpO_2/\Delta HR$, $\Delta SpO_2/\Delta \dot{V}O_2$. We also analysed the relationships between [La] and $\dot{V}E/\dot{V}CO_2$ within each exercise condition. All analyses were made using Sigmastat 3.5 software (Systat Software, CA, USA). Null hypothesis was rejected at $p < 0.05$.

Results

Performance

Single-sprint MPO was not different between RSH-VHL (858 ± 138 W) and RSN (879 ± 154 W) ($p = 0.20$). Mean power output of the first sprint of S1 relative to single-sprint MPO was 96.5 ± 1.1% in RSH-VHL and 98.3 ± 2.6% in RSN. There was no difference between conditions in both PPO and MPO either at S1 or S2 (Fig. 1a, b). There was also no difference between sprints of same number at S1 and S2 in the two exercise conditions. The percentage decrement score was not different between RSH-VHL and RSN at S1 (6.2 ± 2.2% vs 7.7 ± 4.1%; $p = 0.26$) and was lower in RSH-VHL than in RSN at S2 (9.5 ± 3.8% vs 12.1 ± 4.8%; $p < 0.01$). Finally, the percentage decrement score was higher at S2 than at S1 in both conditions (RSN: $p < 0.01$; RSH-VHL: $p = 0.03$).

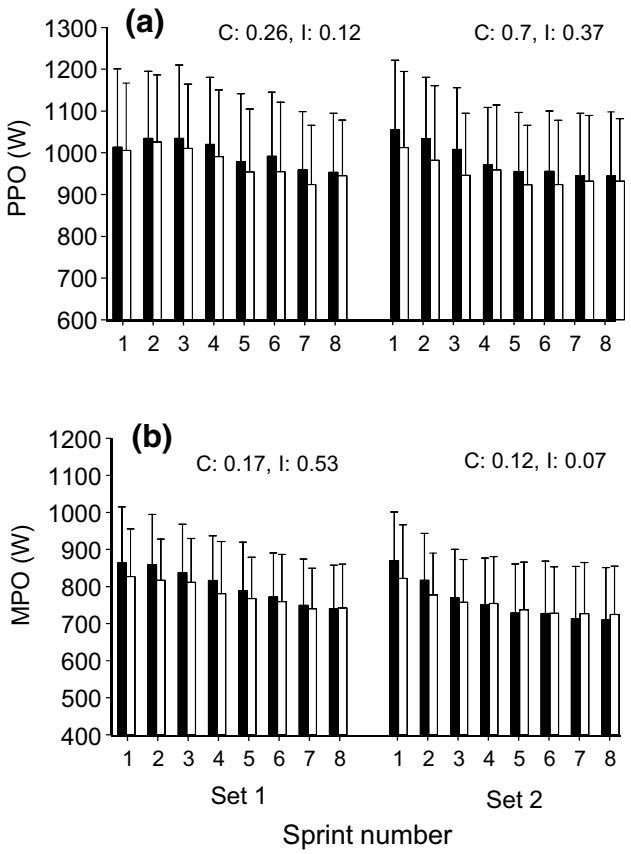

Fig. 1 Peak power output (PPO) (**a**) and mean power output (MPO) (**b**) achieved at each sprint of the first and the second set of the repeated-sprint exercise performed with voluntary hypoventilation at low lung volume (RSH-VHL) and with normal breathing (RSN). RSH-VHL: white bars; RSN: black bars

Arterial oxygen saturation and HR

Arterial oxygen saturation was significantly lower in RSH-VHL than in RSN from the 2nd sprint of both S1 and S2 (Fig. 2a). Heart rate was lower in RSH-VHL than in RSN from the 4th sprint of S1 and from the 2nd sprint of S2 (Fig. 2b). The one-way ANOVA for repeated measures showed a significant effect for SpO_2 and HR ($p < 0.01$), but there was no difference in SpO_2 between sprints of same number at S1 and S2 in both conditions. On the other hand, HR was higher at all sprints of S2 compared with sprints of same number at S1 during RSN and higher from the 1st to the 6th sprint of S2 during RSH-VHL. There was a significant correlation between ΔSpO_2 and ΔHR at the end of S2 ($r = 0.84$, $p < 0.01$). The absolute and relative times spent at different levels of SpO_2 are presented in Table 1.

Gas exchange

Oxygen uptake was not different between conditions at S1 and higher in RSH-VHL than in RSN from the 2nd sprint to the 8th sprint of S2 (Fig. 2c). Furthermore, there was no difference in $\dot{V}O_2$ between sprints of same number at S1 and S2 in both conditions except for the 1st sprint of RSH-VHL in which $\dot{V}O_2$ was higher at S2. Breathing frequency, the ventilatory equivalent for oxygen and end-tidal oxygen pressure were lower whereas tidal volume and end-tidal carbon dioxide pressure were higher in RSH-VHL than in RSN at the 8th repetition of both S1 and S2 (Table 2). Carbon dioxide production was higher and $\dot{V}E/\dot{V}CO_2$ lower in RSH-VHL at the end of S2 only. Minute ventilation was not different between conditions in both sets. End-tidal carbon dioxide pressure was lower and $\dot{V}E/\dot{V}CO_2$ higher at S2 than at S1 in both conditions whereas carbon dioxide production was lower at S2 than at S1 in RSN only. There was no difference between S1 and S2 for all the other ventilatory variables in the two exercise conditions.

Muscle oxygenation

There was no difference in $\Delta[O_2Hb]$ and $\Delta[HHb]$ between exercise conditions at S1 (Fig. 3a, b) and no difference in $\Delta[tHb]$ both at S1 and S2 (Fig. 3c). On the other hand, compared to normalized resting values, the decrease in $[O_2Hb]$ was greater in RSH-VHL than in RSN at sprints 1, 5, 6, 7 and 8 of S2 whereas [HHb] was higher in RSH-VHL than in RSN from the 5th to the 8th sprints of S2.

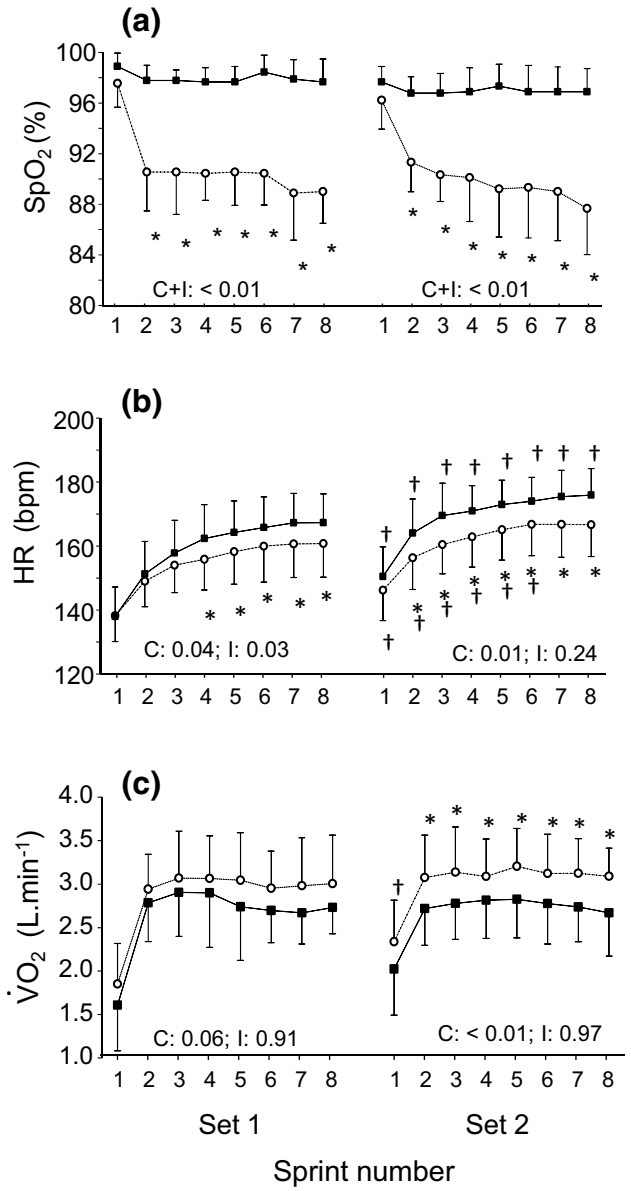

Fig. 2 Minimum arterial oxygen saturation (SpO_2) (**a**) and maximum heart rate (HR) (**b**) measured at each sprint of set 1 (S1) and set 2 (S2) of the repeated-sprint exercise performed with voluntary hypoventilation at low lung volume (RSH-VHL) and with normal breathing (RSN). **c** Oxygen uptake ($\dot{V}O_2$) measured over the last 20 s of the recovery periods of each sprint during RSH-VHL and RSN. Open circles: RSH-VHL; filled squares: RSN. *Significant difference with RSN; †significant difference with sprint of same number at S1 ($p < 0.05$)

The one-way ANOVA for repeated measures showed a significant effect for $\Delta[O_2Hb]$, Δ[HHb] and Δ[tHb] in the two exercise conditions ($p < 0.01$). However, there were no significant differences for these variables between sprints of same number at S1 and S2 both in RSH-VHL and in RSN. Figure 3d–f show the typical NIRS signals of $[O_2Hb]$, [HHb] and [tHb] recorded during one set of RSH-VHL and RSN. Finally, we found a positive correlation between $\Delta(\Delta[O_2Hb])$ and ΔSpO_2 ($r = 0.89$, $p < 0.01$) and an inverse relationship between $\Delta(\Delta$[HHb]) and ΔSpO_2 ($r = -0.71$, $p = 0.03$) at the end of S2.

Rating of perceived exertion and [La]

Blood lactate concentration was not different between exercise conditions after S1 ($p = 0.45$) and was lower in RSH-VHL than in RSN after S2 ($p < 0.01$) (Fig. 4). There was no difference in RPE between RSH-VHL and RSN both at S1 (8.9 ± 0.3 vs 8.3 ± 0.7) and S2 (9.4 ± 0.6 vs 9.3 ± 0.6). Blood lactate concentration and RPE were both significantly higher at S2 than at S1 in the two exercise conditions ($p < 0.05$). There was no relationship between Δ[La] and $\Delta\dot{V}O_2$ at the end of S2 ($r = -0.12$, $p = 0.76$). On the other hand, [La] was positively correlated with $\dot{V}E/\dot{V}CO_2$ at the end of S2 in RSN ($r = 0.83$, $p < 0.01$) but not in RSH-VHL ($r = 0.41$, $p = 0.27$).

Discussion

This study was the first to investigate the acute effects of repeated sprints in hypoxia induced by VHL. It brought out several major findings. First, performing RSH-VHL in cycling did not provoke a greater fatigue (i.e., decrease in power output) than the same exercise performed under normal breathing conditions. Second, RSH-VHL induced a greater muscle deoxygenation than RSN in the second part of exercise. Third, lactate concentration was lower in RSH-VHL than in RSN after the end of exercise. Finally, oxygen uptake was significantly higher in RSH-VHL during the recovery periods following sprints in the second half of exercise.

The first and unexpected result of the present study was that PPO and MPO were not different between the two exercise conditions throughout the whole RSE. Yet we postulated a greater decrement in performance in RSH-VHL due to the combined respiratory and metabolic acidosis that was consistently reported during exercise with VHL (Woorons et al. 2007, 2010, 2014). Unlike our hypothesis, the percentage decrement score was similar in both exercise conditions at S1 and was surprisingly lower in RSH-VHL at S2. Noticeably, during RSE performed in moderate normobaric hypoxia (i.e., below 3000 m or inspired fraction of oxygen > 14.5%), fatigue development was not greater than in normoxic conditions (Girard et al. 2017). On the other hand, in severe hypoxia (i.e., altitude > 3000 m; inspired fraction of oxygen < 14.5%), the decrease in performance has repeatedly been found larger than in normoxia (Billaut et al. 2013; Brocherie et al. 2016; Morrison et al. 2015). Our results

Table 1 Time spent at different levels of SpO_2 during repeated sprint exercise

Range of SpO_2 (%)	Time (s)		Time (%)	
	RSH-VHL	RSN	RSH-VHL	RSN
[98–100]	167.5 ± 99.4*	373.5 ± 139.6	33.9 ± 20.2*	73.7 ± 27.6
[95–97]	175.0 ± 61.4	119.0 ± 121.4	35.3 ± 12.2	23.4 ± 23.9
[92–94]	88.5 ± 40.0*	14.5 ± 22.2	17.9 ± 8.1*	2.9 ± 4.4
[89–91]	39.0 ± 19.8*	0.0 ± 0.0	7.9 ± 4.0*	0.0 ± 0.0
≤ 88	25.5 ± 19.9*	0.0 ± 0.0	5.1 ± 3.9*	0.0 ± 0.0

SpO_2 arterial oxygen saturation, *RSH-VHL* repeated-sprint exercise with voluntary hypoventilation at low lung volume, *RSN* repeated-sprint exercise with normal breathing

*Significantly different from RSN, values are mean ± SD, $p < 0.05$

Table 2 Gas exchange at the end of S1 and S2

%	RSH-VHL		RSN		ANOVA *P* value		
	S1	S2	S1	S2	T	C	T × C
Vt (L)	2.50 ± 0.5*	2.52 ± 0.5*	2.34 ± 0.7	2.15 ± 0.6	0.11	< 0.01	0.02
Bf (breaths.min^{-1})	44.5 ± 5.8*	47.1 ± 9.7*	58.4 ± 17.0	62.9 ± 13.5	0.02	< 0.01	0.68
$\dot{V}$E (L.min^{-1})	108.5 ± 16.8	115.8 ± 19.8	120.0 ± 20.5	123.1 ± 21.6	0.09	0.13	0.46
$\dot{V}CO_2$ (L.min^{-1})	3.15 ± 0.4	3.01 ± 0.3*	3.06 ± 0.4	2.56 ± 0.4†	0.01	0.01	< 0.01
$\dot{V}E/\dot{V}O_2$ (L.min^{-1})	35.6 ± 5.1*	37.1 ± 5.1*	43.9 ± 7.6	46.0 ± 8.9	0.14	0.01	0.88
$\dot{V}E/\dot{V}CO_2$ (L.min^{-1})	33.6 ± 3.6	37.7 ± 5.8*,†	38.7 ± 4.5	47.2 ± 7.9†	< 0.01	< 0.01	0.15
$PETO_2$ (mmHg)	111.5 ± 4.8*	112.3 ± 4.6*	116.9 ± 3.4	118.9 ± 3.2	0.12	< 0.01	0.54
$PETCO_2$ (mmHg)	37.1 ± 3.7*	33.8 ± 4.2*,†	34.1 ± 2.9	28.9 ± 4.5†	< 0.01	< 0.01	0.18

Values are mean ± SD

RSH-VHL repeated sprints in hypoxia induced by voluntary hypoventilation at low lung volume, *RSN* repeated sprints with normal breathing, *S1* first set, *S2* second set, *T* time effect, *C* condition effect, *T* × *C* interaction effect (time × condition), *Vt* tidal volume, *Bf* breathing frequency, $\dot{V}$E expired ventilation, $\dot{V}CO_2$ carbon dioxide production, $\dot{V}E/\dot{V}O_2$ ventilatory equivalent for oxygen, $\dot{V}E/\dot{V}CO_2$ ventilatory equivalent for carbon dioxide, *$PETCO_2$* end-tidal carbon dioxide pressure, *$PETO_2$* end-tidal oxygen pressure

*Significantly different from the set in RSN, †significantly different from S1 within condition, $p < 0.05$

may, however, be taken with care. Although MPO of the first sprint of S1 was not different between conditions and reached at least 95% of MPO of the single sprint, a slight pacing may have occurred in some subjects at the beginning of RSH-VHL. The fact that during RSH-VHL the subjects could maintain a power output as high as in RSN is a key result since the efficiency of RSH relies on the repetition of maximal intensity efforts in order to recruit and induce physiological adaptation in fast-twitch fibres (Faiss et al. 2013b).

Our second hypothesis that performing a cycling RSE with VHL could provoke a larger blood and muscle deoxygenation than the same exercise with normal breathing is partly validated. Arterial oxygen saturation decreased to 87% at the end of exercise which represents a severe hypoxemia (Dempsey and Wagner 1999). This level of blood oxygenation is very similar to what has been reported in VHL studies in different exercise modalities (Woorons et al. 2008, 2011, 2014). Noteworthy, SpO_2 values of about 90% have been reported during RSE performed at simulated altitude of 1800–2000 m (Brocherie et al. 2016; Goods et al. 2014) whereas in severe hypoxia (i.e., 3000 m, inspired fraction of oxygen = 14%), SpO_2 values of 82–85% have been recorded (Billaut et al. 2013; Goods et al. 2014). Arterial oxygen saturation values as low as 75–78% have even been reported during RSH at 3000 m (Bowtell et al. 2014; Morrison et al. 2015). The greater increase in [HHb] and decrease in [O_2Hb] recorded in the second part of exercise also support the fact that VHL has an impact on muscle oxygenation and leads to tissue hypoxia (Woorons et al. 2010). This is of particular importance since muscle deoxygenation has been shown to be a paramount physiological response for inducing specific skeletal muscle adaptations leading to greater resistance to fatigue and increased performance during repeated sprint tests after RSH training (Faiss et al. 2013b). The present results must, however, be pondered. First, the minimum SpO_2 reached at each sprint remained in the range of 88–90% during most part of RSH-VHL. Furthermore, regarding the total time spent at low levels of SpO_2 (Table 1), it is clear

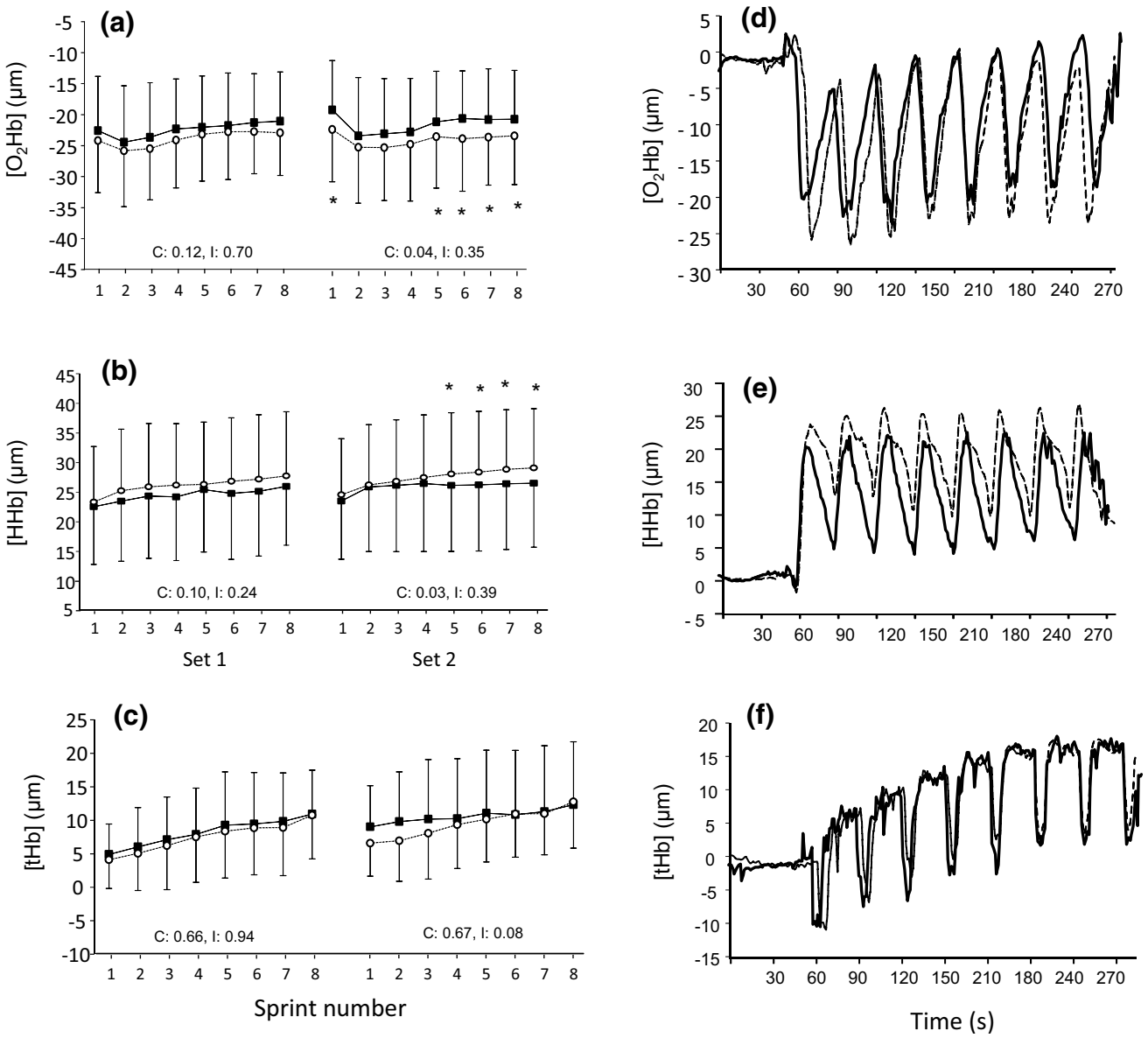

Fig. 3 Changes in concentrations of oxyhaemoglobin/myoglobin ([O_2Hb]) (**a**), deoxyhaemoglobin/myoglobin ([HHb]) (**b**) and total haemoglobin/myoglobin ([tHb]) (**c**) measured by near-infrared spectroscopy (NIRS) at each sprint of set 1 and set 2 of the repeated-sprint exercise performed with voluntary hypoventilation at low lung volume (RSH-VHL, open circles) and with normal breathing (RSN, filled squares). *Significant difference with RSN ($p < 0.05$). Typical NIRS signals (after treatment) of [O_2Hb] (**d**), [HHb] (**e**) and [tHb] (**f**) during one set in RSH-VHL (dashed line) and RSN (solid line) expressed as changes in µM from resting baseline set to 0 µM

that the hypoxic dose was low when performing RSH-VHL, particularly if we use the new metrics based on the time spent at low saturation level (Millet et al. 2016). It is also important to note that there was a large interindividual variability for arterial desaturation. While SpO_2 dropped down below 85% in some subjects (lowest SpO_2 recorded = 80%), it remained above 90% in others. Finally, it is noticeable that muscle tissue deoxygenation did not occur during the entire exercise. Altogether, it is therefore questionable whether or not RSH-VHL could lead to the same physiological adaptations as RSH performed in ambient hypoxia.

The lower [La] in RSH-VHL at the end of exercise represents another surprising result. So far, the majority of the studies on VHL exercise have reported an increased glycolytic activity and higher [La] compared with exercise with NB, mainly because of the hypoxemic state (Woorons et al. 2010, 2014). However, in these studies, exercise was carried out at submaximal intensity. One may argue that the room for increased anaerobic glycolysis is greater when exercise is

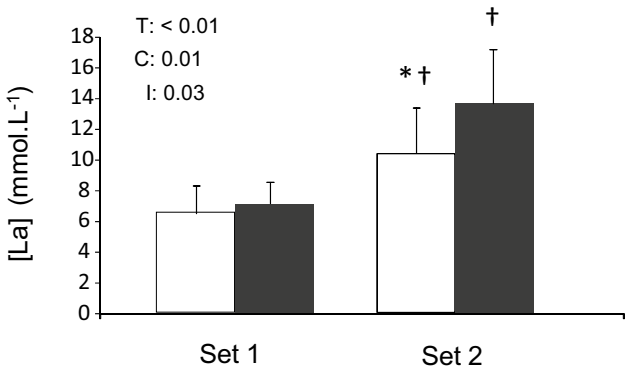

Fig. 4 Blood lactate concentrations at the end of the first and the second set of the repeated-sprint exercise performed with voluntary hypoventilation at low lung volume (white bars) and with normal breathing (black bars). *Significant difference with RSN; †significant difference with S1 ($p < 0.05$)

carried out at submaximal rather than at maximal intensity. Still, it has been found that RSE performed at different levels of normobaric hypoxia could lead to higher [La] than the same exercise in normoxia (Bowtell et al. 2014). Thus, in the present study, we could expect at least a maintenance of [La] in RSH-VHL compared with RSN. Considering that power output was not different between exercise conditions and that [La], and maybe the contribution of anaerobic glycolysis was lower in RSH-VHL, our findings raise the question of how energy was supplied during this specific exercise.

During RSE, energy supply is mainly ensured by phosphocreatine (Bogdanis et al. 1996). However, both anaerobic and aerobic glycolysis have been reported to play a role, in particular in the first part of RSE for the former and in the second part for the latter (Girard et al. 2011). The reduced [La] after RSH-VHL may not reflect a lower contribution from anaerobic glycolysis. In this regard, the measurements of gas exchange were of a great interest since they revealed a markedly greater $\dot{V}O_2$ in RSH-VHL during the recovery periods following sprints at S2. This is an important finding because an enhanced oxidative pathway is essential for the maintenance of high exercise intensities and for reducing fatigue during RSE (Bishop et al. 2011). Thus, the lower [La] in RSH-VHL could be explained either by an enhanced lactate oxidation or a greater phosphocreatine resynthesis during the resting periods between sprints.

Even though the higher $\dot{V}O_2$ during the recovery periods of RSH-VHL is an interesting finding, it was not so surprising since this phenomenon had already been reported in a previous VHL study (Woorons et al. 2011). The authors hypothesized that it could have been caused by a greater work and consequently a larger oxygen cost of respiratory muscles during the hyperventilation phase following the periods with VHL. They also postulated that the elevated $\dot{V}O_2$ could be the consequence of the oxygen debt contracted during the breath holding periods. Since $\dot{V}E$ was not different between exercise conditions in the present study, the first assumption can be rejected whereas the "oxygen debt" hypothesis represents a likely explanation for the excess oxygen consumption recorded after the 6-s sprints at S2 (Gaesser and Brooks 1984).

The mechanism by which $\dot{V}O_2$ was increased during RSH-VHL cannot be clearly established with the present data. An improved blood perfusion is unlikely since Δ[tHb] was not different between exercise conditions. On the other hand, the greater Δ[HHb] in the second part of S2 suggests that a better oxygen extraction may have occurred in RSH-VHL. [HHb] is closely associated with changes in venous oxygen content and has been shown to be a reliable estimator of changes in fractional oxygen extraction (Grassi et al. 2003). However, one could argue that the greater Δ[HHb] in RSH-VHL was a compensation for the lower SpO_2, as suggested by the significant inverse correlation between Δ[HHb] and ΔSpO_2. Therefore, we may assume that the increase in $\dot{V}O_2$ was partly the consequence of a greater cardiac output as already reported (Woorons et al. 2011). Considering that HR was lower in RSH-VHL than in RSN during most part of exercise, a marked increase in stroke volume may have occurred in the former condition like what happens during moderate exercise with VHL (Woorons et al. 2011).

The lower HR in RSH-VHL was not really expected either. So far, HR has been found unchanged or even increased either during or after moderate VHL exercise not exceeding 5 min (Woorons et al. 2007, 2011). The author attributed this augmentation to the hyperventilation phase that follows the period with VHL. The same phenomenon did not occur in this study since $\dot{V}E$ reached high levels in both exercise conditions and was not different between RSH-VHL and RSN. Noteworthy a decrease in HR has already been found during a short-lasting supramaximal cycling exercise performed with apnea (Ahn et al. 1989). These authors postulated that this decrease could be due to an arterial chemoreceptor mechanism under the effect of the fall in arterial oxygen saturation and consequently hypoxia. Based on the significant positive correlation between ΔSpO_2 and ΔHR, it is possible that the same physiological mechanism occurred in the present study.

While $\dot{V}E$ was not different between exercise conditions after the last sprint of each set, two observations are worth mentioning. First the ventilatory pattern was not the same in both conditions. RSN was characterized by a high breathing frequency whereas tidal volume was greater during RSH-VHL. This finding may not be trivial since one could speculate that large inspirations might lead to an increased stroke volume through a "pump effect" and might therefore partly explain the higher $\dot{V}O_2$ in RSH-VHL (Woorons et al. 2010). Second, when pertained to the metabolic oxygen demand, $\dot{V}E$ was much higher in RSN than in RSH-VHL as indicated

by the greater ventilatory equivalents for oxygen and carbon dioxide in the former condition. This phenomenon is in accordance with the fact that RSN generated a greater lactic acidosis than RSH-VHL at the end of exercise. Hyperventilation is a well-known physiological response to eliminate the excess H^+. The metabolic acidosis-induced hyperventilation in RSN is reinforced by the strong correlation between [La] and $\dot{V}E/\dot{V}CO_2$ for this exercise condition and that was absent in RSH-VHL.

The fact that the physiological parameters were not measured at the same time during exercise constitutes the main limitation of the present study. Consequently, some of the results may appear difficult to reconcile and in contradiction with what has been reported so far. For instance, RSH-VHL elicited hypoxaemia and tissue deoxygenation whereas the results show normocapnic conditions and lower [La]. However, unlike blood and muscle oxygenation, gas exchange could not be measured during sprints because of the breath holding. Data, therefore, show what happened during the recovery periods. Yet it is remarkable that the partial pressure of carbon dioxide rapidly returns to normal conditions after VHL exercise (Woorons et al. 2011). It is likely that the 6-s sprints with VHL elicited hypercapnia since hypoventilation systematically leads to elevated carbon dioxide partial pressure (West 1997). The lower [La] recorded after RSH-VHL probably also reflects what occurred during the recovery periods with duration four times longer than the sprinting bouts (ratio 1:4) and with $\dot{V}O_2$ that was much higher than in RSN. To overcome these limitations, it would be interesting to take a blood sample just after the end of the sprints through catheterization, to rapidly obtain blood gases and lactate concentration. Another limitation of this protocol was that RPE was indistinctly measured. The assessment of both perceived dyspnoea and pain in the legs would have been useful, in particular because subjects especially complained from the former after RSH-VHL and from the latter after RSN, probably due to the greater lactic acidosis.

This study provides interesting information for the implementation of RSH-VHL. First it shows that this kind of training could be managed like a "classical" RSH considering that RSH-VHL did not induce greater fatigue nor was perceived harder than RSN. Second, it is recommended to perform at least two sets including six sprints or more to expect a benefit from this method since the main physiological effects occurred in the second part of RSH-VHL. Third it is not advised to exceed a 6-s time for the sprints with VHL. Some of the subjects were not always capable of holding their breath for the whole duration and had to take an inhalation between the 5th and 6th s. One could however consider using sprint durations beyond 6 s since significant performance gains have been reported after RSH with 10-s sprints followed by 20 s of recovery (Faiss et al. 2013a, b, 2015; Gatterer et al. 2014). The feasibility of using such ratio with RSH-VHL remains, however, to be tested.

In conclusion, this study demonstrated that VHL represents an interesting way to induce significant arterial and muscle deoxygenation during RSE whilst maintaining high intensity level. Oxygen uptake was surprisingly higher in RSH-VHL than in RSN during the recovery periods following sprints at S2. This increase was accompanied by an even more unexpected reduction in [La] at the end of exercise. On the basis of the present findings, further studies are required to investigate the use of VHL as an effective training strategy to improve repeated sprint ability.

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