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ORIGINAL ARTICLE

Exercise with hypoventilation induces lower muscle oxygenation and higher blood lactate concentration: role of hypoxia and hypercapnia

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Abstract Eight men performed three series of 5-min exercise on a cycle ergometer at 65% of normoxic maximal O_2 consumption in four conditions: (1) voluntary hypoventilation (VH) in normoxia ($VH_{0.21}$), (2) VH in hyperoxia (inducing hypercapnia) (inspired oxygen fraction $[F_IO_2] = 0.29$; $VH_{0.29}$), (3) normal breathing (NB) in hypoxia ($F_IO_2 = 0.157$; $NB_{0.157}$), (4) NB in normoxia ($NB_{0.21}$). Using near-infrared spectroscopy, changes in concentration of oxy-($\Delta[O_2Hb]$) and deoxyhemoglobin ($\Delta[HHb]$) were measured in the vastus lateralis muscle. $\Delta[O_2Hb - HHb]$ and $\Delta[O_2Hb + HHb]$ were calculated and used as oxygenation index and change in regional blood volume, respectively. Earlobe blood samples were taken throughout the exercise. Both $VH_{0.21}$ and $NB_{0.157}$ induced a severe and similar hypoxemia (arterial oxygen saturation $[SaO_2] < 88\%$) whereas SaO_2 remained above 94% and was not different between $VH_{0.29}$ and $NB_{0.21}$. Arterialized O_2 and CO_2 pressures as well as P50 were higher and pH lower in $VH_{0.21}$ than in $NB_{0.157}$, and in $VH_{0.29}$ than in $NB_{0.21}$. $\Delta[O_2Hb]$ and $\Delta[O_2Hb - HHb]$ were lower and $\Delta[HHb]$ higher at the end of each series in both $VH_{0.21}$ and $NB_{0.157}$ than in $NB_{0.21}$ and $VH_{0.29}$. There was no difference in $\Delta[O_2Hb + HHb]$ between testing conditions. [La] in $VH_{0.21}$ was greater than both in $NB_{0.21}$ and $VH_{0.29}$ but not different from $NB_{0.157}$. This study demonstrated that exercise with VH induced a lower tissue oxygenation and a higher [La] than exercise with NB. This was caused by a severe arterial O_2 desaturation induced by both hypoxic and hypercapnic effects.

Keywords Hypoventilation · Hypoxemia · Hypercapnia · Breath holding · NIRS · Tissue oxygenation

Introduction

In two recent studies, it has been suggested that training with voluntary hypoventilation (VH) could be an interesting method for athletes who want to benefit from hypoxia without going to altitude or using expensive devices that simulate hypoxic environment (Woorons et al. 2007, 2008). According to these studies, the key point when using VH is to perform this technique at low pulmonary volumes, near functional residual capacity (FRC) or residual volume (RV). In those conditions, rather than with a simple VH, there is a decrease in O_2 alveolar stores and a greater inequality in the ventilation–perfusion ratio leading to a widened alveolar–arterial difference for O_2 (Woorons et al. 2007). Thus, a significant arterial desaturation occurs, unlike a VH performed at high pulmonary volumes (Yamamoto et al. 1987). In addition to severe hypoxemia (arterial oxygen saturation $[SaO_2] = 87\%$), submaximal exercise with VH also induces an increased arterial carbon dioxide pressure ($PaCO_2$) leading to respiratory acidosis (Woorons et al. 2007). After a 4-week training with VH

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carried out at FRC, both pH and bicarbonate concentration were found increased at a high submaximal intensity but not at maximal exercise (Woorons et al. 2008). Furthermore, the athletes tended to increase their velocity at exhaustion of 0.4 km/h on average. It was also found that maximal lactate concentration was maintained after training whereas the training intensity was low. On the other hand, there was no change in maximal oxygen uptake ($\dot{V}O_{2\,max}$), lactate threshold or time to exhaustion.

Very few studies have focused on this topic so far and we do not know much about what happens during and after training with VH. In particular, no study has ever been undertaken to assess the effects of exercise with this respiratory technique on the working muscle. One could hypothesize that the severe hypoxemia that occurs during exercise with VH, when carried out at low pulmonary volumes, may impact on muscle oxygenation ($MuscO_2$) as reported during exercise in hypoxia (Costes et al. 1996; Maehara et al. 1997; Subudhi et al. 2007) or in athletes presenting exercise-induced hypoxemia (Legrand et al. 2005). However, this is not obvious because even at a high degree of hypoxia, $MuscO_2$ may not be different from normoxia during exercise (DeLorey et al. 2004; Peltonen et al. 2009; Rupp and Perrey 2009). Furthermore, unlike a classical exercise in hypoxic conditions, the arterial desaturation is discontinuous during exercise with VH since it is alternated with short periods with normal breathing (Woorons et al. 2007). Thus, it would be interesting to assess whether exercise with VH could lead to a low $MuscO_2$. This phenomenon may indeed induce specific adaptations when regularly undergone during training, as reported with the "living low training high" method (LLTH) which is somewhat similar to VH training (Geiser et al. 2001; Terrados et al. 1990; Vogt et al. 2001; Zoll et al. 2006). $MuscO_2$ can be non-invasively assessed with near-infrared spectroscopy (NIRS), a recent technique based on the principle of differential absorption properties of oxygenated and deoxygenated forms of haemoglobin and myoglobin. This method has been widely used to estimate changes in O_2 saturation in human muscle during dynamic exercise (Belardinelli et al. 1995a, b; Costes et al. 1996; Esaki et al. 2005; Grassi et al. 1999, 2003; Legrand et al. 2005; MacDonald et al. 1999; Maehara et al. 1997; Mancini et al. 1994; Subudhi et al. 2007).

An assessment of whether exercise with VH could increase muscle lactate concentration and therefore metabolic acidosis represents another interesting issue, even though the role of lactic acid on muscle fatigue has been challenged (Westerblad et al. 2002). Although muscle and blood lactate concentrations are dependent on a number of factors, a higher lactate concentration could reflect both an enhanced glycolysis and pyruvate production. Therefore, athletes could take advantage of this training method to improve or at least stimulate this energetic pathway. Although not reflecting muscle concentration, blood lactate concentration ([La]) is a good indicator of metabolic acidosis especially when associated with a decrease in blood bicarbonate concentration ($[HCO_3^-]$). So far, all studies dealing with exercise with VH failed to find an increase in [La] (Holmer and Gullstrand 1980; Hsieh and Hermiston 1983; Sharp et al. 1991; Town and Vanness 1990; Woorons et al. 2007; Yamamoto et al. 1987, 1988). Several hypotheses could explain this lack of increase. First, it has been reported that hypercapnia, which is another main effect of VH exercise, could reduce lactate release from muscle to blood (Ehrsam et al. 1982; Graham et al. 1980, 1986). Second, an increased [La] is probably more likely to occur if exercise is carried out in acute hypoxia. Hitherto, only two studies have reported a significant decrease in SaO_2 during exercise with VH (Woorons et al. 2007; Yamamoto et al. 1987). Third, exercise duration should probably be longer and, unlike previous studies, organized in series separated by periods with normal breathing. These relative recovery periods between series should decrease $PaCO_2$ and thus facilitate lactate release in the blood compartment. In studies that reported a severe arterial hypoxemia during VH, exercise had a short duration (<5 min) and the periods with normal breathing did not exceed 30 s (Woorons et al. 2007; Yamamoto et al. 1987), which probably prevented an increase in [La].

One could consider that the hypoxic effect is predominant during exercise with VH and may contribute to the changes in $MuscO_2$ and/or [La] that might occur. However, the hypercapnic effect cannot be neglected. Indeed, hypercapnia induces a respiratory acidosis and consequently a right shift of the oxygen dissociation curve (ODC). This phenomenon accentuates the decrease in both SaO_2 and venous oxygen saturation (SvO_2) and may therefore play a role in the change of $MuscO_2$ and [La].

The goal of the present study was to assess the effects of a prolonged exercise with an intermittent VH carried out at FRC on [La] and $MuscO_2$ and to distinguish the respective role played by hypoxia and hypercapnia. We therefore compared exercise with VH to exercise in hypoxia on the one hand and to exercise inducing hypercapnia on the other hand. We first hypothesized that exercise with VH would lead to a lower $MuscO_2$ than exercise with normal breathing under the effect of both hypoxia and hypercapnia. We also made the assumption that $MuscO_2$ should be even lower in VH than in hypoxic exercise. As we used an exercise organized in series separated by periods with normal breathing in normoxia, we finally expected, for the reasons mentioned above, a greater increase in [La] during the prolonged exercise with VH than with normal breathing.

Method

Subjects

Eight men, with no sign or history of cardiovascular or respiratory disease, volunteered to participate in this study. All men were physically active with a recreational sport practice of 3–4 h a week. Subject characteristics (mean ± SD) were: age 29.3 ± 6.4 years, height 178.1 ± 6.4 cm, weight 74.8 ± 9.8 kg and $\dot{V}O_{2max}$ 53.2 ± 3.7 mL kg^{-1} min^{-1}. They were informed about the nature, the conditions and the risks of the experiment and gave their written informed consent. All the procedures were approved by the ethical committee Ile de France II, Paris, France.

Protocol

Preliminary procedures were conducted and included: (1) clinical interviews and examinations; (2) a maximal exercise test on an electrically braked cycle ergometer (Jaeger ER 900, Wuerzburg, Germany) aiming to assess $\dot{V}O_{2max}$ and maximal power output. After a 3-min rest, the test began at an intensity of 30 W. The workload was then increased by 30 W every 2 min until exhaustion. Subjects were verbally encouraged to continue the exercise as long as possible; (3) a test to familiarize with hypoventilation technique. After a resting period of about 1 h following maximal test, the subjects performed a cycle exercise at 50% $\dot{V}O_{2max}$ with VH carried out at or near FRC. To perform this respiratory technique, the subjects were asked to inspire every 4 s instead of about 1 s in normal conditions (Fig. 1). After each inspiration, the subjects had to make a normal expiration in order to target FRC and then had to hold their breath until the next inspiration.

To precisely control the respiratory technique, we used the pedalling frequency which was established at 75 rpm and permanently monitored. Thus, the subjects had to inspire every 5 rev (4 s). The 5 rev were organized as follows: a normal expiration over 1 or 1.5 rev (0.8–1.2 s) and then a breath holding over 3.5–4 rev (2.8–3.2 s). The inspiration was made over ½ or 1 rev (0.4–0.8 s). During the familiarizations test, the transcutaneous arterial O_2 saturation was measured by an ear pulse oximeter (Ohmeda Biox 3740, Louisville, CO, USA).

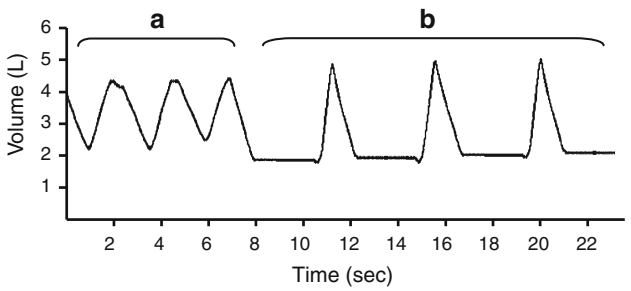

Fig. 1 Pulmonary volume kinetics over three respiratory cycles of normal breathing (**a**) and voluntary hypoventilation carried out at functional residual capacity (**b**)

One week after the preliminary tests, each subject had to perform a cycle exercise (same ergometer as previously) including three 5-min series at 65% of normoxic $\dot{V}O_{2max}$ under the following conditions: (1) normal breathing (NB) in normoxia ($NB_{0.21}$), (2) VH in normoxia ($VH_{0.21}$), (3) VH in hyperoxia ($VH_{0.29}$) (inspired oxygen fraction $[F_IO_2] = 0.29$). This intervention was undertaken to induce hypercapnia without undergoing arterial O_2 desaturation, (4) NB in hypoxia ($NB_{0.157}$). For each subject, the level of hypoxia was individually determined to induce a same level of arterial desaturation as during the familiarization test with VH. Thus, the parameters in these two interventions were compared at a same SaO_2. The average FIO_2 (mean ± SD) was 0.157 ± 0.01.

Each series was divided into five periods of 1 min including 15 s in $NB_{0.21}$ followed by 45 s in one of the previous conditions. Furthermore, between series, we included transition periods in $NB_{0.21}$ where subjects kept on cycling for 1 min at the same exercise intensity. This was done to minimize excessive hypoventilation-induced hypercapnia which could increase cerebral vasodilatation and blood flow, and cause discomfort due to headaches (Woorons et al. 2007). Before the start of the series, the subjects remained seated for 5 min at rest on the cycle ergometer and then warmed up for 3 min at an exercise intensity corresponding to 40% of $\dot{V}O_{2max}$. The first 2 min of the warming up were performed in $NB_{0.21}$ and the third one in the same condition as in the series to come, in order to prepare their organism and adjust their breathing to the pedalling frequency if they had to hypoventilate. Figure 2 provides a description of a test in $VH_{0.21}$. The four tests were carried out in 2 days and randomized. Both days were separated by 72 h while there was a 4-h rest between tests performed the same day. The randomization excluded two tests performed with VH the same day. During a test where VH had to be carried out, the subjects were coached all the time in order to carry out the exercise as adequately as possible. The subjects were asked to focus on the pedalling frequency (75 rpm) while they were given the number of rates at each breathe so that they knew when to inspire, expire or hold their breath.

To create conditions of normobaric hypoxia or hyperoxia, we used a gas mixing device (AltiTrainer$^{®}_{200}$, S.M. TEC, Geneva, Switzerland) which was connected to a N_2 or an O_2 gas bottle. Throughout the test, each gas was manually added to ambient air with a three-way valve with a small dead space. Thus, the transitions in gas concentration took only a few seconds to obtain the desired F_IO_2. The gas mixture was stocked in a buffer tank (30 L) before

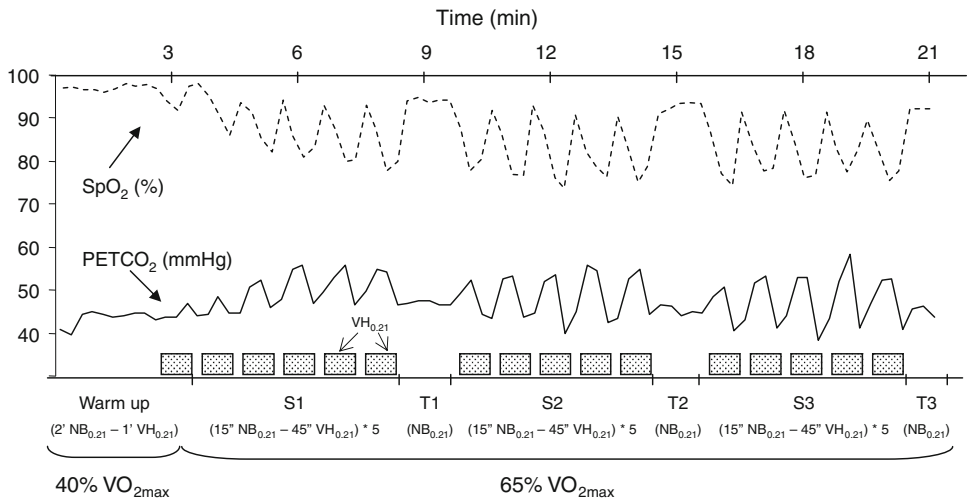

Fig. 2 Description of the exercise protocol. Example for exercise with voluntary hypoventilation in normoxia ($VH_{0.21}$); SpO_2 transcutaneous arterial oxygen saturation; $PETCO_2$ end-tidal carbon dioxide pressure; *S1*, *S2*, *S3* series 1, 2 and 3; *T1*, *T2*, *T3* transition 1, 2 and 3; $NB_{0.21}$ normal breathing in normoxia; $\dot{V}O_{2max}$ maximal oxygen consumption

being inhaled by the subjects. Inspired O_2 pressure was continuously monitored throughout the tests by an oxygen probe, located in the buffer tank (electrochemical O_2 probe MOX3, City Technology, Portsmouth, UK). According to the manufacturer, the maximal difference between the PO_2 measured by the AltiTrainer$^{®}_{200}$ O_2 probe and the PO_2 calculated from the O_2 fraction measured by an external probe (Servomex 720A, Geneva, Switzerland) is less than 1 mmHg over the whole range of PO_2 (150–69 mmHg). The device is reliable for ventilation less than 200 L/min.

Measurements

[La] and blood gases

Four arterialized blood samples (95 μL) were drawn from the left earlobe of the subjects at rest and in the last 15 s of S1, T2 and S3. The earlobe was prewarmed with a vasodilating capsaicin cream and the arterialized measurements were performed by a well-trained technician in order to improve their accuracy. The earlobe samples were collected with a capillary tube. [La] was immediately determined enzymatically in hemolyzed blood thanks to amperometric electrodes with enzymatic membranes (Radiometer ABL 700, Copenhagen, Denmark). We also analyzed blood gases with the same device for arterialized oxygen pressure (PaO_2), $PaCO_2$, SaO_2, pH, P50, [HCO_3^-] and hemoglobin concentration ([Hb]).

NIRS measurements

We estimated the oxygenation of the left vastus lateralis muscle throughout each exercise using NIRS (Tissue Spectrometer Model 325, Hutchinson Technology, MN, USA). NIRS theory has been fully described previously (Belardinelli et al. 1995a; Subudhi et al. 2007) and its validity well established (Mancini et al. 1994). However, it is probably more relevant to use NIRS technique during dynamic exercise involving large muscle mass since it has been reported that muscle desaturation was related to whole body oxygen consumption ($\dot{V}O_2$) in these conditions (Im et al. 2001). Briefly, the technique is based on the modified Beer–Lambert law to quantify light attenuation, accounting for scattering through biological tissues. The light source of the device we used was provided by four different wavelength laser diodes at 680, 720, 760 and 800 nm for a better detection of chromophores as well as a good sensitivity of the instrument (Belardinelli et al. 1995b; Mancini et al. 1994). Thus, we could reliably assess concentration changes of oxyhemoglobin ($\Delta[O_2Hb]$) and deoxyhemoglobin (Δ[HHb]). We used the longest probe furnished by the manufacturer which was positioned longitudinally at mid-thigh level of the vastus lateralis muscle of the left leg ($\sim$15 cm above the patella) and at about 5 cm lateral to the midline of the thigh. The distance between the light emitters and the detectors was 2.5 cm. After a careful shave of the skin, the probe was secured around the thigh with Velcro® straps and adhesive bands. No movement of the probe was observed in any exercise test. After each test, the probe was removed. It was replaced at the same spot at the following test thanks to pen marks made over the skin to indicate the position of the probe.

The main limit of the method is the impossibility to obtain accurate quantitative measurements. For each exercise intervention, we normalized muscle measurements to reflect changes from the beginning of the warm up (arbitrarily defined as 0 μM) to express the magnitude oxygenation throughout exercise. The normalization was made in the same conditions whatever the intervention, since the subjects breathed normally and were in normoxia during the first 2 min of exercise. Measurements were not expressed relatively to resting values since NIRS signal can

be influenced by skin blood flow at rest (Maehara et al. 1997) whereas this is minored at exercise (Mancini et al. 1994). Furthermore, during the resting period, while sitting on the ergocycle, there is a venous blood pooling phenomenon in the vastus lateralis. Besides, NIRS data at rest were not stable in four among the eight subjects of our study. On the other hand, at the very beginning of exercise, the muscle pump expels the blood from the venous territory and provokes a well described deep fall in NIRS signal (Grassi et al. 2003; MacDonald et al. 1999). The latter then stabilizes during the warm-up period. A recent study also calculated the NIRS data from the warming up (Boone et al. 2009) because the measurements are more stable in these conditions. The sum of $\Delta[O_2Hb]$ and $\Delta[HHb]$ was used as an index of change in total haemoglobin volume ($\Delta[HbT]$) whereas the difference between these variables ($\Delta[O_2Hb - HHb]$) was taken as an "oxygenation index" as suggested in previous studies (Belardinelli et al. 1995b; Grassi et al. 1999, 2003; MacDonald et al. 1999; Maehara et al. 1997). In the present study, we will define $MuscO_2$ as the oxygen available in muscle depending on arterial, capillary and venous O_2 saturation, as reflected by the NIRS signal (Mancini et al. 1994), on the one hand, and the regional blood volume on the other hand.

NIRS values were obtained every second. However, we averaged values over 15 s to attenuate the noise in the signal. Data were analyzed in the last 15 s of each series and transition period.

Statistics

All the results are expressed as mean ± SD. To determine whether there was a difference between exercise interventions at the end of each series and recovery period, we performed a two-way analysis of variance (ANOVA). For each intervention, we also performed a one-way ANOVA for repeated measures to assess whether there was a time effect. This was done to determine whether there were differences in the NIRS variables between the baseline values (0 μM) and the three series, and between each series and the following transition period. This was also carried out to analyse the lactate kinetics from S1 to S3. In both statistical testing, when a significant main effect was found, the Bonferroni post hoc test was used. The level of significance was set at $P < 0.05$.

Results

Blood gases and acid–base variables

The results are presented in Table 1 and Fig. 3 and can be summarized as follows:

As expected, there were differences in PaO_2 between interventions at S1 and S3. On the other hand, PaO_2 was not different between interventions at T2. While SaO_2 dropped to the same level during exercise in $VH_{0.21}$ and $NB_{0.157}$, PaO_2 was lower in $NB_{0.157}$ than in $VH_{0.21}$ at S3. At S1 and S3, $PaCO_2$ and P50 were higher and arterial pH lower in both $VH_{0.29}$ and $VH_{0.21}$ than in $NB_{0.157}$ and $NB_{0.21}$. Throughout exercise, [La] in both $VH_{0.21}$ and $NB_{0.157}$ were not different but were greater than in $NB_{0.21}$ and $VH_{0.29}$. Moreover, [La] was higher at T2 and S3 than at S1 in $VH_{0.21}$, $NB_{0.157}$ and $NB_{0.21}$ but not in $VH_{0.29}$ (Fig. 3). At T2 and S3, $[HCO_3^-]$ in $VH_{0.21}$ and $NB_{0.157}$ was lower than in $VH_{0.29}$ and $NB_{0.21}$.

NIRS measurements

The results are presented in Figs. 4 and 5. In summary, $\Delta[O_2Hb]$, $\Delta[HHb]$ and $\Delta[HbT]$ increased at S1 in the four exercise interventions as compared with the beginning of the warm up, but did not vary afterwards. $\Delta[O_2Hb - HHb]$ was lower in $VH_{0.21}$ and in $NB_{0.157}$ at the end of each series than at the beginning of the warm up whereas it was not different in both $NB_{0.21}$ and $VH_{0.29}$.

At S1, S2 and S3, $\Delta[O_2Hb]$ in $VH_{0.21}$, $VH_{0.29}$ and $NB_{0.157}$ were not different but were lower than $\Delta[O_2Hb]$ in $NB_{0.21}$. Furthermore, $\Delta[HHb]$ in $VH_{0.21}$ and in $NB_{0.157}$ were not different whereas they were higher than $\Delta[HHb]$ in both $NB_{0.21}$ and $VH_{0.29}$ during most part of exercise. $\Delta[O_2Hb - HHb]$ was lower in both $VH_{0.21}$ and $NB_{0.157}$ than in $NB_{0.21}$ and $VH_{0.29}$. Finally, ANOVA revealed no intervention effect for $\Delta[HbT]$.

Discussion

This study reports several original findings concerning the physiological effects of exercise with VH. It first shows that even though inducing a discontinuous hypoxemia, a prolonged exercise with VH, when carried out at FRC, leads to a lower $MuscO_2$ than exercise with normal breathing. It also demonstrates that this type of exercise can increase blood lactate concentration, unlike what was reported so far. Finally, this study shows that both the lower $MuscO_2$ and the higher [La] were not only the consequence of the hypoxic effect of exercise with VH but were also induced by the hypercapnic effect.

To assess the effects of VH on $MuscO_2$, we utilized NIRS, a technique widely employed during exercise in humans (Belardinelli et al. 1995a, 1995b; Costes et al. 1996; Esaki et al. 2005; Grassi et al. 1999, 2003; Legrand et al. 2005; MacDonald et al. 1999; Maehara et al. 1997; Mancini et al. 1994; Subudhi et al. 2007) and which has been shown to be reliable (Mancini et al. 1994). The device

Table 1 Blood gases and acid–base variables

	Rest	S1	T2	S3
PaO_2 (mmHg)				
$NB_{0.21}$	85.4 ± 5.9	84.8 ± 5.0	81.8 ± 6.2	81.0 ± 6.7
$VH_{0.21}$	83.6 ± 6.0	64.1 ± 5.6*‡	81.5 ± 3.4	66.4 ± 8.1*†‡
$VH_{0.29}$	89.1 ± 6.3	114.2 ± 9.7*†	83.5 ± 4.2	104.1 ± 8.6*†
$NB_{0.157}$	85.0 ± 5.9	60.8 ± 4.3*	82.0 ± 4.4	55.9 ± 6.6*
$PaCO_2$ (mmHg)				
$NB_{0.21}$	39.3 ± 2.8	40.4 ± 2.5	38.4 ± 4.0	37.3 ± 3.8
$VH_{0.21}$	40.2 ± 2.6	49.1 ± 3.1*†‡	39.7 ± 3.9	43.1 ± 5.3*†‡
$VH_{0.29}$	40.0 ± 3.8	54.4 ± 6.0*†	40.1 ± 5.3	53.6 ± 4.4*†
$NB_{0.157}$	40.2 ± 2.6	36.7 ± 3.1*	36.8 ± 4.4	33.8 ± 4.2*
pH				
$NB_{0.21}$	7.42 ± 0.02	7.36 ± 0.03	7.36 ± 0.03	7.37 ± 0.03
$VH_{0.21}$	7.42 ± 0.02	7.29 ± 0.03*†	7.32 ± 0.02*	7.28 ± 0.04*†
$VH_{0.29}$	7.42 ± 0.02	7.26 ± 0.05*†	7.31 ± 0.04*	7.26 ± 0.04*†
$NB_{0.157}$	7.42 ± 0.02	7.37 ± 0.03	7.34 ± 0.03*	7.36 ± 0.04
SaO_2 (%)				
$NB_{0.21}$	96.1 ± 0.9	94.5 ± 0.5	94.5 ± 0.7	94.2 ± 0.7
$VH_{0.21}$	96.4 ± 1.2	87.0 ± 2.9*‡	93.4 ± 0.8*‡	86.9 ± 3.3*‡
$VH_{0.29}$	96.0 ± 0.6	95.7 ± 0.8	94.8 ± 1.4	95.0 ± 1.2
$NB_{0.157}$	96.2 ± 1.0	87.7 ± 1.4*‡	93.7 ± 0.9‡	85.7 ± 1.8*‡
[Hb] (g/dl)				
$NB_{0.21}$	15.5 ± 1.0	16.3 ± 1.1	16.2 ± 1.1	16.1 ± 1.0
$VH_{0.21}$	15.4 ± 1.0	16.2 ± 1.3	16.4 ± 1.3	16.4 ± 1.1
$VH_{0.29}$	15.5 ± 1.0	16.2 ± 0.9	16.3 ± 1.0	16.2 ± 1.0
$NB_{0.157}$	15.3 ± 0.8	15.9 ± 1.4	16.0 ± 1.3	16.1 ± 1.0
$[HCO_3^-]$ (mmol/L)				
$NB_{0.21}$	25.2 ± 0.7	22.4 ± 2.2	21.1 ± 2.5	20.9 ± 2.9
$VH_{0.21}$	25.8 ± 1.8	22.8 ± 2.1†	19.7 ± 2.9*‡	19.3 ± 2.8*‡
$VH_{0.29}$	25.6 ± 1.9	23.6 ± 2.3*	21.2 ± 2.7	22.5 ± 3.0*
$NB_{0.157}$	25.6 ± 1.4	20.9 ± 1.8*‡	19.2 ± 2.8*‡	18.6 ± 3.0*‡
P50 (mmHg)				
$NB_{0.21}$	25.3 ± 1.3	26.7 ± 1.0	26.6 ± 0.8	26.5 ± 0.9
$VH_{0.21}$	25.6 ± 1.1	30.6 ± 1.3*†	29.2 ± 1.6*	31.2 ± 2.0*†
$VH_{0.29}$	25.0 ± 1.3	30.8 ± 1.7*†	28.9 ± 1.8*	30.6 ± 1.6*†
$NB_{0.157}$	24.4 ± 1.4	27.1 ± 2.3	27.7 ± 1.7	27.3 ± 1.2

Values are mean ± SD

S1, *S3* series 1 and 3; *T2* second transition; *PaO_2* arterial oxygen pressure; *$PaCO_2$* arterial carbon dioxide pressure; *SaO_2* arterial oxygen saturation; [*Hb*] haemoglobin concentration; *$[HCO_3^-]$* blood bicarbonate concentration; *P50* PO_2 for $SaO_2 = 50\%$; *$NB_{0.21}$* normal breathing in normoxia; *$VH_{0.21}$* voluntary hypoventilation in normoxia; *$VH_{0.29}$* voluntary hypoventilation in hyperoxia; *$NB_{0.157}$* normal breathing in hypoxia; different from *$NB_{0.21}$, ‡$VH_{0.29}$, †$NB_{0.157}$

we used provides $\Delta[O_2Hb]$ and $\Delta[HHb]$ data. However, it is important to take into account other parameters such as $\Delta[O_2Hb - HHb]$, which represents an oxygenation index (Belardinelli et al. 1995b; Grassi et al. 1999, 2003; MacDonald et al. 1999; Maehara et al. 1997) or $\Delta[HbT]$ to compare $MuscO_2$ in different exercise conditions. Our results showed that both $\Delta[O_2Hb]$ and $\Delta[O_2Hb - HHb]$ were always lower and $\Delta[HHb]$ higher in $VH_{0.21}$ than in $NB_{0.21}$ at any moment of exercise. If we consider that $\Delta[HbT]$ was not different between interventions, we can therefore conclude that $MuscO_2$ was lower in $VH_{0.21}$ than in $NB_{0.21}$. While previous studies especially focused on the effect of VH on SaO_2 (Dicker et al. 1980; Holmer and Gullstrand 1980; Woorons et al. 2007; Yamamoto et al. 1987), no study had ever investigated the effects of this respiratory technique on $MuscO_2$ so far.

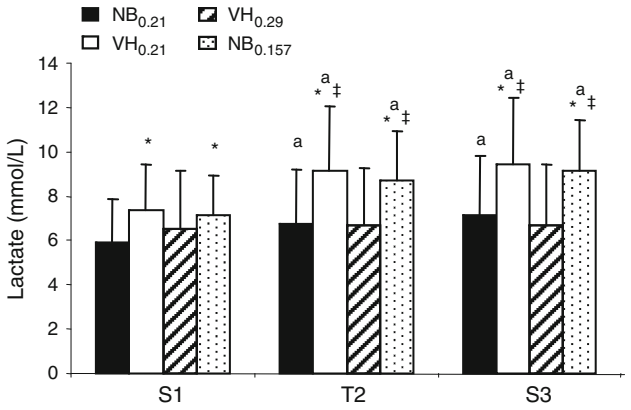

Fig. 3 Blood lactate concentration at series 1 (*S1*), transition 2 (*T2*) and series 3 (*S3*). *$NB_{0.21}$* normal breathing in normoxia, *$VH_{0.21}$* voluntary hypoventilation in normoxia, *$VH_{0.29}$* voluntary hypoventilation in hyperoxia, *$NB_{0.157}$* normal breathing in hypoxia. Significantly different from *$NB_{0.21}$, ‡$VH_{0.29}$, †$NB_{0.157}$, (*a*) S1, (*b*) S2, (*c*) S3; Values are mean ± SD; $P < 0.05$

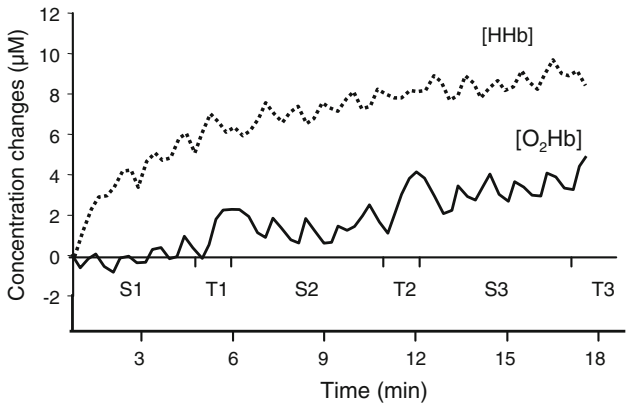

Fig. 5 Time course of near-infrared spectroscopy concentration changes of oxyhemoglobin ($\Delta[O_2Hb]$) and deoxyhemoglobin ($\Delta[HHb]$) during exercise with voluntary hypoventilation in normoxia ($VH_{0.21}$) in one representative subject. *S1*, *S2*, *S3* series 1, 2 and 3; *T1*, *T2*, *T3* transition 1, 2 and 3

Two main reasons could explain the lower $MuscO_2$ in $VH_{0.21}$. First, the drop in SaO_2 certainly played a role since arterial blood participates in the NIRS signal. Some authors put forward this hypothesis to partly explain a greater deoxygenation during hypoxic than during normoxic exercise (Costes et al. 1996). In the present study, this hypothesis can be reinforced by the fact that during $NB_{0.157}$, where SaO_2 was not different from $VH_{0.21}$, $MuscO_2$ was also lower than in $NB_{0.21}$ during the whole exercise. Second, it is likely that a lower SvO_2 in $VH_{0.21}$ than in $NB_{0.21}$ has contributed to the lower $MuscO_2$. Some studies reported a correlation between NIRS measurements and SvO_2 (Esaki et al. 2005; Wilson et al. 1989). Furthermore, NIRS signal seems to parallel femoral SvO_2 during steady-state exercise in hypoxia, unlike in normoxia (Costes et al. 1996). During hypoxic exercise or in hypoxemic athletes, a low PaO_2 and SaO_2 may be partially compensated by a lower SvO_2, and probably a greater O_2 extraction. Costes et al. (1996) reported both a lower femoral venous oxygen pressure (PvO_2) and SvO_2 during steady-state exercise at 80% of $\dot{V}O_{2max}$ in hypoxia ($F_IO_2 = 0.105$) than during the same exercise in normoxia. Furthermore, when compared with sea level, PvO_2 seems to be lower from altitude as low as 1,000 m during maximal exercise (Bourdillon et al. 2009a). Albeit the exercise

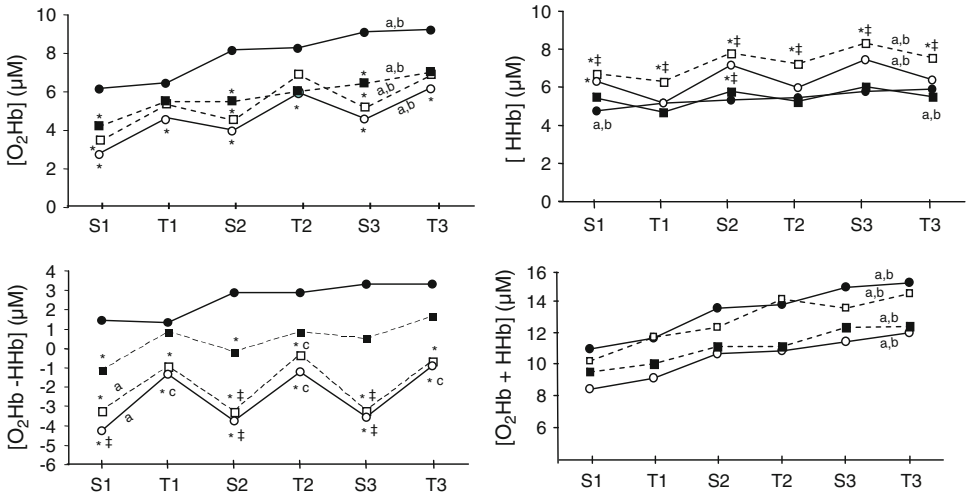

Fig. 4 Near-infrared spectroscopy concentration changes during exercise with normal breathing in normoxia ($NB_{0.21}$, *filled circle*), voluntary hypoventilation in hyperoxia ($VH_{0.29}$, *filled square*), voluntary hypoventilation in normoxia ($VH_{0.21}$, *open square*), normal breathing in hypoxia ($NB_{0.157}$, *open circle*); $\Delta[O_2Hb]$ concentration changes of oxyhemoglobin; $\Delta[HHb]$ concentration changes of deoxyhemoglobin; *S1*, *S2*, *S3* series 1, 2 and 3; *T1*, *T2*, *T3*, transition 1, 2 and 3. Significantly different from *$NB_{0.21}$, ‡$VH_{0.29}$, †$NB_{0.157}$, (*a*) beginning of warm up for all series, (*b*) beginning of warm up for all transitions, (*c*) previous series; ($P < 0.05$). Standard deviation is not included for more clarity

intensity was lower in the present study, the same phenomenon may have occurred anyway and impacted on NIRS results.

The increase in both $\Delta[O_2Hb]$ and $\Delta[HHb]$ during $VH_{0.21}$ may appear surprising since an increase in one of these parameters should lead to a decrease in the other. However, this would be the case if $\Delta[HbT]$ remained constant. In the present study, we also reported an increase in $\Delta[HbT]$. Yet it is likely that exercise with VH led to muscle deoxygenation. First, it is noticeable that $\Delta[HHb]$ increased more than $\Delta[O_2Hb]$. This is the reason why the oxygenation index, represented by $\Delta[O_2Hb - HHb]$, was negative and decreased significantly in $VH_{0.21}$ as compared with the beginning of exercise. Furthermore, [HHb] is closely associated with changes in venous oxygen content and is essentially blood-volume insensitive during exercise (De Blasi et al. 1993; Grassi et al. 2003). Thus, it could be a reliable estimator of tissue deoxygenation caused by changes in fractional oxygen extraction (De Blasi et al. 1994; DeLorey et al. 2004; Grassi et al. 2003). The hypothesis of a greater tissue deoxygenation in $VH_{0.21}$ is reinforced by the fact that several studies reported a higher muscle deoxygenation during exercise in hypoxia than in normoxia (Costes et al. 1996; Maehara et al. 1997; Subudhi et al. 2007). On the other hand, unlike a classical exercise in hypoxia, it is remarkable that $VH_{0.21}$ led to strong oscillations in $\Delta[O_2Hb]$ and $\Delta[HHb]$ within each 5-min series. This particular kinetics matches with SaO_2 kinetics and is the consequence of the intermittent pattern of exercise with VH. Although not presented in this study, we already noted in our previous study (Woorons et al. 2007) that $\dot{V}O_2$ kinetics was more stable during a single 5-min series carried out with VH. Thus, muscle deoxygenation and reoxygenation probably compensated the decrease and increase in SaO_2 in order to match the metabolic demand. Finally, the increase in $\Delta[O_2Hb]$ was not reported in several studies (Peltonen et al. 2009; Subudhi et al. 2007, 2008). However, in these studies, the exercise intensity was maximal. On the opposite, an increase in $\Delta[O_2Hb]$ during light and moderate exercise in normoxia or hypoxia has also been reported (Bourdillon et al. 2009b; Rupp and Perrey 2009). This was probably caused by a higher regional blood volume induced by muscular vasodilation. The fact that the NIRS measurements were normalized from the warming up, rather than from rest, is probably another reason explaining the increase we found in $\Delta[O_2Hb]$ since the normalization was made after the initial fall of NIRS signal at the beginning of exercise. Besides, some studies have reported an increase in $\Delta[O_2Hb]$ after its early drop (DeLorey et al. 2004; MacDonald et al. 1999).

The second main finding of this study was that exercise with VH induced a higher [La] than exercise with normal breathing. This is the first study reporting such a phenomenon. Previously, all the studies focusing on the glycolytic effects of exercise with VH failed to find an increase in [La] (Holmer and Gullstrand 1980; Hsieh and Hermiston 1983; Sharp et al. 1991; Town and Vanness 1990; Woorons et al. 2007; Yamamoto et al. 1987, 1988). We also found that [La] in $NB_{0.157}$ was higher than in $NB_{0.21}$ while it was not different from $VH_{0.21}$. On the other hand, $VH_{0.29}$ did not induce a greater [La] than $NB_{0.21}$. According to these results, the hypoxic effect of VH seems to be responsible for the higher [La]. Thus, a lack of arterial hypoxemia during VH exercise could explain why [La] did not change in some previous studies. In studies where VH led to a large decrease in SaO_2 (Woorons et al. 2007; Yamamoto et al. 1987), the exercise was probably not long enough (<5 min) and not separated by recovery periods with normal breathing, as in the present study, to increase [La]. In the presence of hypercapnia, [La] release from the muscle to the blood is lower (Ehrsam et al. 1982; Graham et al. 1980, 1986) whereas it seems to increase more during recovery following exercise with VH than following exercise with normal breathing (Yamamoto et al. 1988). This could explain why [La] was greater in $VH_{0.21}$ than in $NB_{0.21}$ from the transition of the first series to the end of exercise. A slightly greater hypoxemia in the present study as compared with the previous ones may partly explain that [La] was also higher at the end of S1 in $VH_{0.21}$ than in $NB_{0.21}$.

The higher [La] in $VH_{0.21}$ than in $NB_{0.21}$, which was accompanied most of the time by a lower $[HCO_3^-]$, argues in favour of a greater metabolic acidosis during exercise with VH. An imbalance between O_2 supply and O_2 demand in the working muscles, under the effect of the severe hypoxemia, may be at the origin of this phenomenon. However, this hypothesis is controversial (Brooks 1991; Katz and Sahlin 1988). A more relevant hypothesis could be that the relative exercise intensity was greater during $VH_{0.21}$ than $NB_{0.21}$ since $\dot{V}O_{2max}$decreases in the presence of arterial O_2 desaturation, especially in trained subjects (Bourdillon et al. 2009a; Mollard et al. 2007; Woorons et al. 2005). Anyway, irrespective of the reasons leading to a greater metabolic acidosis during exercise with VH, this phenomenon is interesting since it reflects a higher solicitation of anaerobic glycolysis.

So far, this study tends to demonstrate that the greater muscle deoxygenation as well as the higher [La] during prolonged exercise with VH is mainly due to a hypoxic effect. There was no difference in all NIRS data between $VH_{0.21}$ and $NB_{0.157}$, and [La] increased at the same extent in both interventions. However, we cannot disregard the role played by hypoventilation-induced hypercapnia. Indeed, this phenomenon generally induces a respiratory acidosis and consequently a right shift of ODC. This was the case in this study as shown by the higher P50 we found

in both $VH_{0.21}$ and $VH_{0.29}$ as compared with $NB_{0.21}$. On the other hand, a greater metabolic acidosis, as suggested by the higher [La], did not increase P50 since this variable was not different between $NB_{0.157}$ and $NB_{0.21}$. A right shift of ODC probably had an effect on $MuscO_2$ by accentuating the decrease in both SaO_2 and SvO_2. A high P50 has been shown to contribute importantly to the arterial desaturation during VH exercise (Woorons et al. 2007). Furthermore, physiologically, a right shift of ODC leads to a lower SvO_2 for a given PaO_2. According to these considerations, it is surprising that, in contrary to the hypothesis we made, $MuscO_2$ was not lower in $VH_{0.21}$ than in $NB_{0.157}$. However, it is noteworthy that, for a same arterial desaturation, PaO_2 was higher in $VH_{0.21}$ than in $NB_{0.157}$. A similar PaO_2 in both interventions would have probably led to a lower $MuscO_2$ in $VH_{0.21}$. The greater [La] during $VH_{0.21}$, as discussed above, was probably due to the severe hypoxemia. Therefore, hypercapnia also played a role in this phenomenon since the high P50 accentuated the drop in SaO_2. But more interestingly, it is likely that hypercapnia also enhances muscle acidosis. The increase in both hydrogen ions and $PaCO_2$ in blood during VH exercise decreases the diffusion gradient and probably the release of H+ and CO_2 from the muscle to the blood. The consecutive accumulation of H+ and CO_2 within the muscle should result in a drop of intracellular pH. This phenomenon could be at the origin of the improvement in muscular buffering capacity that probably occurred after VH training (Woorons et al. 2008).

The higher lactate concentration and the pronounced muscle deoxygenation we found during prolonged exercise with VH represent an interesting finding for this new training method. A higher lactate concentration reflects an increased contribution of anaerobic glycolysis. Thus, training with VH could help to improve, or at least maintain the power of glycolytic metabolism, as previously suggested (Woorons et al. 2008). Muscle deoxygenation, when repeatedly undergone, may induce interesting cellular (greater activity of oxidative enzymes, higher mitochondrial or capillary densities) and molecular (increase in mRNA contents of HIF-1, myoglobin and vascular endothelial growth factor) adaptations, as reported in the LLTH method (Geiser et al. 2001; Terrados et al. 1990; Vogt et al. 2001; Zoll et al. 2006). If it was so after VH training, tissue O_2 utilization may be improved which might have a positive impact on aerobic performance like in LLTH studies (Dufour et al. 2006; Meeuwsen et al. 2001), even though this remains controversial. Nevertheless, the only study carried out on VH training did not find any change in aerobic parameters or performance (Woorons et al. 2008). The present study shows that arterial desaturation was the same as an exercise carried out at $F_IO_2 = 0.157$, equivalent to an altitude of about 2,400 m. This constitutes a sufficient hypoxic stimulus with regard to the altitudes used in LLTH studies (Melissa et al. 1997; Meeuwsen et al. 2001; Terrados et al. 1988, 1990). On the other hand, since arterial desaturation is not continuous during VH, the altitude dose may be too low as compared with the one required to improve aerobic performance (Millet et al. 2010). In addition, it is probable that exercise intensity should be higher than 65% of $\dot{V}O_{2max}$, like in the present study, or even 70% like in the previous ones to have positive effects on the aerobic metabolism. A recent review on hypoxic training reported that LLTH is more likely to be efficient when performed at high intensity exercise, i.e. at or above the ventilatory threshold (Millet et al. 2010). Anyway, regarding the hardness of training with VH, it is probably wiser to limit the exercise intensity under the ventilatory threshold. Altogether, these findings show that the benefits of this training method remain questionable for endurance activities. On the other hand, athletes involved in short time duration activities or even in intermittent sports, where anaerobic glycolysis and metabolic acidosis play a major role, may find interest to perform VH training. However, longitudinal VH training studies in these sports are required for validation.

In conclusion, this study demonstrated that exercise with VH can increase muscle deoxygenation as well as blood lactate concentration as compared to exercise with normal breathing. It also suggests that for a same PaO_2, exercise with VH could induce a lower $MuscO_2$ than the same exercise in hypoxia. These results were the consequence of both hypoxic and hypercapnic effects. Thus, training with VH should be of particular interest since it could increase the contribution of anaerobic glycolysis and may lead to adaptations at the muscular level inducing a better O_2 utilization and probably a greater buffer capacity.

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Conflict of interest statement The authors declare that they have no conflict of interest.

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