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Physiological differences between normobaric and hypobaric hypoxia

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par

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"One may walk over the highest mountain one step at a time."

Barbara Walters

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List of publications

1. Alterations of Neuromuscular Function after the World's Most Challenging Mountain Ultra-Marathon. Saugy J., Place N., Millet G.Y., Degache F., Schena F., Millet G.P. PLoS One. 2013 Jun 26; 8 (6): e65596.
2. Comparison of "Live High-Train Low" in Normobaric versus Hypobaric Hypoxia. Saugy J.J., Schmitt L., Cejuela R., Faiss R., Hauser A., Wehrlin J.P., Rudaz B., Delessert A., Robinson N., Millet G.P. PLoS One. 2014 Dec 17;9(12):e114418.
3. Prooxidant/Antioxidant balance in hypoxia: A crossover study on normobaric vs. hypobaric "Live High-Train Low". Debevec T., Pialoux V., Saugy J.J., Schmitt L., Cejuela R., Mury P., Ehrström S., Faiss R., Millet G.P. PLoS One. 2015 Sep 14;10(9):e0137957.
4. The increase in hydric volume is associated to contractile impairment in the calf after the world's most extreme mountain ultra-marathon. Vitiello D., Degache F., Saugy J.J., Place N., Schena F., Millet G.P. Extrem Physiol Med. 2015 Oct 20; 4:18.
5. Exposure to hypobaric hypoxia results in higher oxidative stress compared to normobaric hypoxia. Ribon A., Pialoux V., Saugy J.J., Rupp T., Faiss R., Debevec T., Millet G.P. Respir Physiol Neurobiol. 2016 Mar; 223:23-7.
6. Similar Hemoglobin Mass Response in Hypobaric and Normobaric Hypoxia in Athletes. Hauser A., Schmitt L., Trösch S., Saugy J.J., Cejuela R., Faiss R., Robinson N., Wehrlin J.P., Millet G.P. Med Sci Sports Exerc. 2016 Apr; 48(4):734-41.
7. Cycling time trial is more altered in hypobaric than in normobaric hypoxia. Saugy J.J., Rupp T., Faiss R., Bourdillon N., Lamon A., Millet G.P. Med Sci Sports Exerc. 2016 Apr; 48(4):680-8.

8. Same performance changes after Live High-Train Low in normobaric versus hypobaric hypoxia. Saugy J.J., Schmitt L., Hauser A., Constantin G., Cejuela R., Faiss R., Wehrlin J.P., Rosset J., Robinson N., Millet G.P. Front Physiol. 2016 Apr 19.
9. Comparison of sleep disorders between real and simulated 3450-m altitude. Heinzer & Saugy J.J., Rupp T., Tobback N., Faiss R., Bourdillon N., Haba Rubio J., Millet G.P. Sleep 2016; Epub Ahead of print.
10. Sleep disordered breathing during "Live High-Train Low" in Normobaric versus Hypobaric Hypoxia. Saugy J.J., Schmitt L., Fallet S., Faiss R., Vesin J.M., Bertschi M., Heinzer R., Millet G.P. HAMB. Epub Ahead of print.
11. Response. Wehrlin JP, Hauser A, Schmitt L, Troesch S, Saugy JJ, Cejula-Anta R, Faiss R, Robinson N & Millet GP. (2016). *Med Sci Sports Exerc* **48,** 1426-1427.
12. Pacing strategies, cerebral and muscle oxygenation during cycling time trial in normobaric versus hypobaric hypoxia. Saugy J.J. and Rupp T., Faiss R., Lamon A., Bourdillon N., Millet G.P. In preparation.

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Abstract

Altitude training is frequently used by elites athletes. Several hypoxic methods have been developed to improve sea-level performances and the Live High–Train Low approach, where athletes live and sleep at altitudes between 2200 and 2500 m and train under 1200 m is recognized as effective. Recent research focused on the potential differences between normobaric (NH) and hypobaric hypoxia (HH), *i.e.* simulated and real altitudes. Slight physiological differences have been found between either acute or chronic hypoxic exposure in NH and HH. Taken together, these differences intimate larger physiological responses to hypoxic exposure in "terrestrial" (*i.e.* HH) versus "simulated" altitudes (*i.e.* NH). However, there is not a sufficient body of knowledge to confirm if one hypoxic condition could induce larger performance enhancement than the other, either during or after acute hypoxic exposure or altitude camps (*i.e.* chronic hypoxic exposure). Many confounding factors and the large inter-individual variability to hypoxic exposure make the comparison very challenging.

Consequently, we first designed a crossover study to assess these potential differences and confirmed for the first time with direct comparison on the same subjects that HH induces different physiological adaptations compared to NH during a 3 weeks LHTL camp. However, physiological differences were not clinically sufficient to induce sea-level performance disparities. Secondly, we showed that acute high altitude hypobaric hypoxia (24h, 3450 m) is more demanding than normobaric hypoxia and induces larger performance impairments on a 250-kJ cycle time trial. Finally, our work demonstrated that sleep is further disturbed in HH compared to NH in both acute and chronic hypoxia.

As previously suggested, HH seems to be a more stressful stimulus than NH at iso-P_iO_2. Consequently, NH and HH should not be used interchangeably and the main factor seems to be the lower peripheral oxygen saturation in HH at rest, as well as during exercise.

Résumé

L'utilisation de l'entrainement en altitude par les athlètes d'élite est de plus en plus courante. Plusieurs méthodes hypoxiques ont été développées pour améliorer les performances au niveau de la mer et l'efficacité de l'approche « Vivre en haut–s'entrainer en bas » (LHTL) où les athlètes vivent et dorment à des altitudes entre 2200 et 2500 m et s'entrainent en-dessous de 1200 m est reconnue. Par ailleurs, les recherches récentes ont mis l'accent sur les différences potentielles entre l'hypoxie normobare (NH) et hypobare (HH), *i.e.* altitude simulée et réelle, et de légères distinctions physiologiques ont été décelées en hypoxie aigue et chronique. Les réponses physiologiques semblent plus marquées en hypoxie réelle que simulée. Par contre, il n'y a pas suffisamment de preuves pour confirmer que l'une des conditions amène de meilleures améliorations de la performance après des camps LHTL ou lors d'expositions hypoxiques aigues. La multitude de facteurs confondant ainsi que la large variabilité interindividuelle à l'hypoxie rendent les comparaisons difficiles. Par conséquent, nous avons mis en place une étude croisée et avons confirmé pour la première fois avec une comparaison directe sur les mêmes sujets que HH est plus sévère que NH lors d'un camp LHTL de 3 semaines. Cependant, les différences physiologiques n'étaient pas cliniquement suffisantes pour engendrer des écarts de performance au niveau de la mer. Nous avons ensuite démontré que HH est plus exigeante que NH en aigue (24 h, 3450m) et impacte la performance de manière plus importante sur un contre la montre de 250 kJ. Finalement, notre travail a attesté que le sommeil est d'avantage perturbé en HH comparé à NH, à la fois lors d'expositions hypoxiques chroniques et aigues. Comme suggéré précédemment, HH semble être un stimulus plus stressant que NH à même P_iO_2. Par conséquent, ces conditions ne devraient pas être utilisés de manière indifférenciées et l'un des principaux facteurs responsable de ces différences s'avère être une saturation périphérique en oxygène plus basse en HH au repos comme pendant l'exercice.

Index of abbreviations

$A\text{-}aDO_2$
Alveolar-to-arterial PO_2 difference

AGE
Alveolar gas equation

AMS
Acute mountain sickness

cDO_2
Cerebral oxygen delivery

CO_2
Carbon dioxide

EAA
Equivalent air altitude

exNO
Exhaled nitric oxide

F_iO_2
Inspired fraction of oxygen

FO_2
Fraction of oxygen

Hbdiff
Mean changes in hemoglobin difference

HHb
Desoxy-hemoglobin

HR
Heart rate

IH
Intermittent hypoxia

IHIT
Interval hypoxic training during IT

IT
Interval training

LHTH
Live high train high

LHTLH
Live high train low and high

MC
Motor cortex

mmHg
Millimetres of mercury

N_2
Nitrogen

NIRS

ADH
Antidiuretic hormone

ALDO
Plasma aldosterone

AOPPs
Advanced oxidation protein products

CHT
Continuous hypoxic training

D01-D18
Day 1 to Day 18

EPO
Erythropoietin

F_iN_2
Inspired fraction of nitrogen

FN_2
Fraction of nitrogen

Hb_{mass}
Haemoglobin mass

HH
Hypobaric hypoxia

HIF-1α
Hypoxia inducible factor 1 alpha

IAE
Intermittent altitude exposure

IHE
Intermittent hypoxic exposure

IHT
Interval hypoxic training

kJ
kilojoules

LHTL
Live high train low

LLTH
Live low train high

MCAv
Mean middle cerebral artery blood flow velocities

mRNA
Messenger ribonucleic acid

NH
Normobaric hypoxia

NN

Near infrared spectroscopy

NO
Nitric oxide

O_2
Oxygen

ODI 3%
Oxygen desaturation index

P_aO_2
Arterial pressure in carbon dioxide

P_B
Barometric pressure

PFC
Pre-frontal cortex

PO_2
Partial pressure of oxygen

Post-21
21 days after training/hypoxic exposure

Post1
1 day after training/hypoxic exposure

Pre-
Before training/hypoxic exposure

Pre2
2 day Before training/hypoxic exposure

REM
Rapid eye-movements

RSH
Repeated sprint training in hypoxia

SOD
Superoxide dismutase

THb
Total hemoglobin changes

VCO_2
Relative carbon dioxide produced

VO_2
Relative oxygen uptake

Normobaric normoxia

NOx
Blood nitric oxide

O_2Hb
Oxy-hemoglobin

P_ACO_2
Alveolar partial pressure in carbon dioxide

P_AO_2
Alveolar partial pressure of oxygen

$P_{ET}CO_2$
End-tidal dioxide pressure

P_iO_2
Inspired pressure of oxygen

Post-
After training/hypoxic exposure

Post-7
7 days after training/hypoxic exposure

Post2
2 day after training/hypoxic exposure

Pre1
1 day Before training/hypoxic exposure

PSG
Polysomnography

RER
Respiratory exchange ratio

S_aO_2
Arterial saturation in oxygen

S_pO_2
Peripheral saturation of oxygen

TT
Time trial

VE
Minute ventilation

VO_{2max}
Maximal oxygen uptake

Chapter One

Introduction

1. Introduction

1.1 General introduction

1.1.1 Hypoxia

Hypoxia is defined as a combination of barometric pressure (P_B) and the inspired fraction of oxygen (F_iO_2) that results in any inspired pressure of oxygen (P_iO_2) under a normoxic value of 150 millimetres of mercury (mmHg) (Conkin & Wessel, 2008).

Oxygen cascade and diffusion.

Before reaching the tissues, oxygen must traverse several steps from the ambient air to the mitochondria. These pathways are called the "oxygen cascade" (Figure 1A) and the partial pressure of oxygen (PO_2) along each step is directly linked to the PO_2 available in the inspired air. At sea level (P_B=760 mmHg, F_iO_2=21%; P_iO_2=149 mmHg), the O_2 in the lungs (alveoli) easily passes to the blood due to a high-pressure gradient, and then passes to the tissues. The overall oxygen cascade from the environment to the mitochondria can be partitioned into the following four major resistances in series: ventilatory resistance, circulatory resistance, tissue resistance, and mitochondrial resistance (Di Prampero, 2003). Each single step of the O_2 cascade can be viewed as a resistance overcome by a specific pressure gradient. In addition, because the resistances are arranged in series, the overall resistance to oxygen flow is given by the accumulation of all the individual resistances, and, likewise, the overall pressure gradient from the environment to the mitochondria is equivalent to the sum of the individual pressure gradients.

In comparison, a decreased P_iO_2 due to a lower P_B with increasing altitude (*e.g.*, the summit of Mt. Everest (8,848 m), P_B=253 mmHg; P_iO_2=50 mmHg) reduces the rate of O_2 diffusion, which results in a decrease in the arterial blood O_2 content (Figure 1B). Thus, less oxygen will

be supplied to the tissue, which leads to a diminished endurance performance at altitude (Sutton *et al.*, 1988; Milledge *et al.*, 2007).

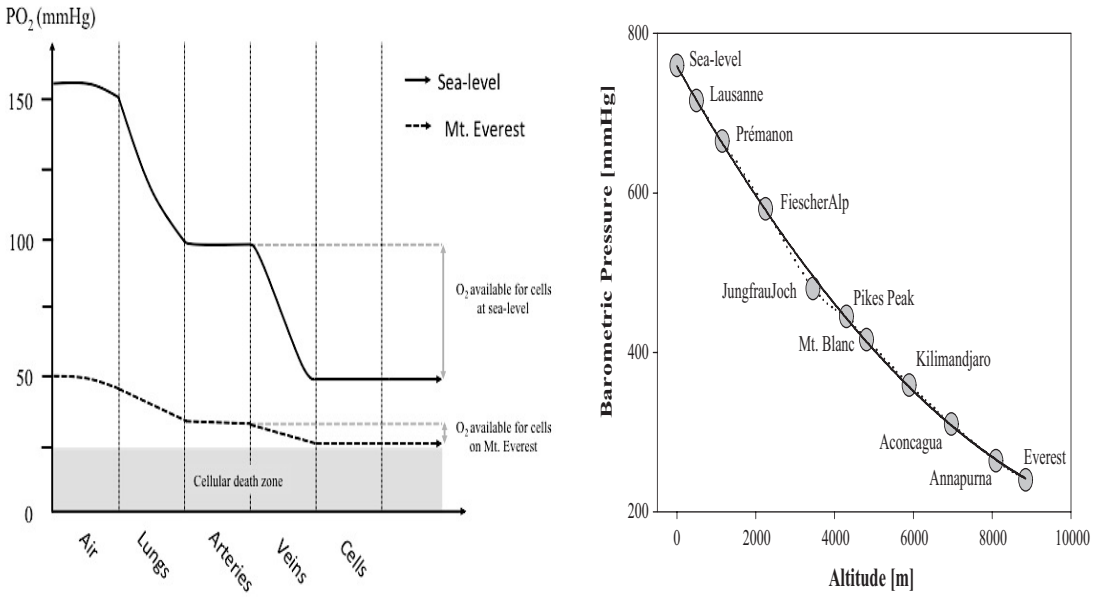

Figure 1 Decrease in oxygen pressure and barometric pressure. (A) Oxygen pressure (PO_2) decrease from ambient air to the cells for sea level (full line) and Mt. Everest (dashed line) depending to the different steps of the oxygen cascade. Adapted from (Richardson *et al.*, 1995). (B) Barometric pressure measured at different altitudes (grey circles and dashed line) and the global theoretical average reported by the International Civil Aviation Organization. Adapted from (West, 1996).

Respiratory responses.

Hypoxia is a potent stimulus, and different physiological systems will change to adapt to this environmental stress. Many of these physiological changes begin immediately upon exposure and are continuously re-adjusted as long as the exposure lasts and even after exposure (Fulco & Cymerman, 1987). The following two successive phases are generally described: an acute adaptation phase to hypoxia, followed by a chronic acclimatization period. The acute adaptation phase lasts eight to ten days and starts as soon as the body is exposed to hypoxia. The increase in minute ventilation (V_E) is the primary defense against low oxygen availability

in the ambient air that begins immediately upon exposure (*i.e.,* 30 minutes) and decreases with time (Fulco *et al.*, 2011; Dempsey & Morgan, 2015). The aim of this increase in V_E is to counteract the reduction in oxygen transport induced by hypoxic environments. The carotid chemoreceptors (which are sensitive to the drop in arterial O_2 pressure (P_aO_2) but also to an increase in arterial CO_2 pressure and decrease in arterial pH) react to arterial hypoxemia within a few seconds by eliciting sympathetic nerve activity and, thus, hyperventilation Therefore, there is a strong relation between peripheral oxygen saturation (S_pO_2) and V_E in hypoxic environments. This increase in ventilation is an indicator of acclimatization as follows: the more acute the ventilation, the better the acclimatization. Thus, the aim of hyperventilation is to limit the above-mentioned decrease in P_aO_2. Moreover, hyperventilation leads to subsequent hypocapnia, which induces a decrease in cerebral perfusion, as well as cardiac and respiratory instability (Lipsitz *et al.*, 1995). In addition, during an exercise in a hypoxic environment, the end-tidal carbon dioxide pressure ($P_{ET}CO_2$) decreases with as a consequence of a respiratory alkalosis due to the reduced plasma concentrations of CO_2 and H^+ (Ursino *et al.*, 2001). Simultaneously, hyperventilation partially counterbalances the decrease in P_AO_2, and therefore P_aO_2 and arterial O_2 saturation (S_aO_2). Thus, the blood pH is elevated and shifts the oxyhaemoglobin dissociation curve to the left (Figure 2) (Calbet *et al.*, 2003).

Haematological responses.

The chronic acclimatization period generally occurs after eight days of exposure to hypoxia. The main adaptations of this second phase concern our haematological properties. A progressive increase in the total haemoglobin mass (Hb_{mass}) driven by an expansion of red cells, which is stimulated by erythropoiesis (Boutellier *et al.*, 1990; Stromme & Ingjer, 1994), occurs during this chronic phase. *Hypoxia Inducible Factor 1 alpha* (HIF-1α) is an O_2-sensitive transcription factor that acts as a master regulator of hypoxia-induced gene

expression. The messenger ribonucleic acid (mRNA) destined to the HIF-1α regulation unit only appears in hypoxia and is undetectable in normoxia (Vogt *et al.*, 2001). One major HIF-mediated adaptation is to stimulate erythropoietin (EPO) production, leading to the above-mentioned red cell production in the bone marrow. However, it is important to keep in mind that the total erythrocyte volume is expressed as a fraction (in %) of the total blood volume according to the haematocrit (Hct) and that red blood cells are diluted in plasma. Altitude exposure triggers a diminution of the plasma volume caused by fluid transfer outside of the vascular compartment. This efflux occurs as a consequence of the greater respiratory loss of water (due to the dryer air at altitude), increased diuresis and augmented exudation at altitude (Hogan *et al.*, 1973; Maher *et al.*, 1974; Butterfield *et al.*, 1992). Subsequently, peripheral resistances are augmented due to the higher blood viscosity, Hct and [Hb] are increased, and blood circulation is altered (Berglund, 1992). On the other hand, the affinity of oxygen for haemoglobin is reduced at high altitudes by 2,3 diphosphoglycerate (2,3 DPG), which facilitates O_2 delivery to the tissues (Mairbaurl, 1994). The compound 2,3 DPG is produced by red blood cell metabolism during prolonged hypoxic exposure (Mines, 1981) to counteract the leftward shift of the oxyhaemoglobin dissociation curve (Figure 2).

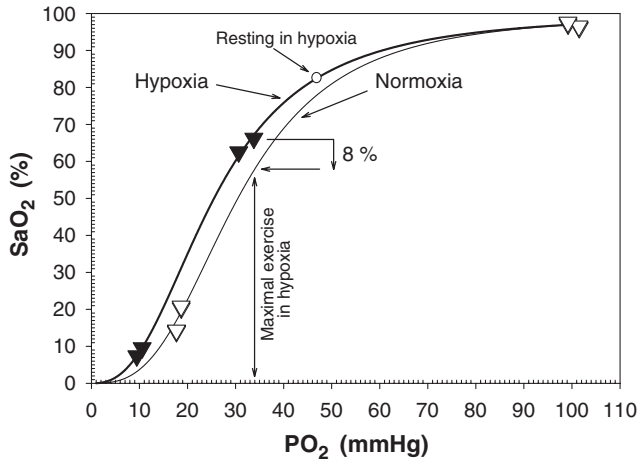

Figure 2. Impact of hypoxia–hyperventilation on the haemoglobin dissociation curve. Effect of acute hypoxia on the O_2 dissociation curve of the haemoglobin during exercise in normoxia (white triangles; fine line) and hypoxia (black triangles; thick line). From (Calbet *et al.*, 2003).

Other mechanisms and adaptations appear after longer exposures to hypoxia (more than 4 weeks), such as weight loss, as a function of both the absolute altitude and the duration of exposure (Kayser, 1994; Westerterp-Plantenga *et al.*, 1999). Indeed, body and muscle mass are significantly reduced after exposure to hypoxia. As a consequence, muscle fibre size is also reduced. The capillary density of muscle tissue is increased because of the reduction in muscle fibre size. The activities of enzymes of the oxidative pathways are also decreased in skeletal muscle tissue (Hoppeler & Desplanches, 1992). However, these long exposures are not the topic of the present thesis, and we will not focus on them.

Cardiovascular responses

In acute hypoxia, the arterial O_2 content (C_aO_2) decreases in parallel with S_aO_2, which requires a higher cardiac output (Q) to preserve the convective O_2 transport. This mechanism, which is assisted by nitric oxide (NO)-dependent local vasodilation with haemoglobin acting as a hypoxic sensor (Crawford *et al.*, 2006), is easy to observe because the heart rate (HR) is increased during submaximal exercise (and at rest). Indeed, the activation of the sympatho-adrenergic axis (with a release of catecholamines) increases the sympathetic tone to increase the HR (Koller *et al.*, 1988; Mazzeo *et al.*, 1994). The increased HR increases the cardiac output and compensates for the decreased stroke volume (mainly due to the reduced plasma volume) (Calbet & Lundby, 2009; Fukuda *et al.*, 2010; Siebenmann *et al.*, 2015). The magnitude of the increase in Q is related to the severity of hypoxia. Although it is usually sufficient to preserve S_aO_2 in moderate hypoxia, it is generally not the case in severe hypoxia, particularly during exercise (Lador et *al.*, 2008). After prolonged exposure (*i.e.*, chronic hypoxia), the increase in C_aO_2 is primarily related to a reduction in PV and a partial recovery of S_aO_2 induced by ventilatory acclimatization. Moreover, a reduction of Q, similar to or below sea level values, is also observed. The primary cause of this reduction is a further reduction in SV, and not a reversion of tachycardia, due to a sustained decrease in plasma

volume caused by elevated diuresis, increased exhaled water, and the loss of fluids from gastrointestinal disturbances at altitude (Wagner, 2000). An overview of cardiac output regulation in both acute and chronic hypoxia is presented in Figure 3 (adapted from Siebenmann *et al.*, 2015).

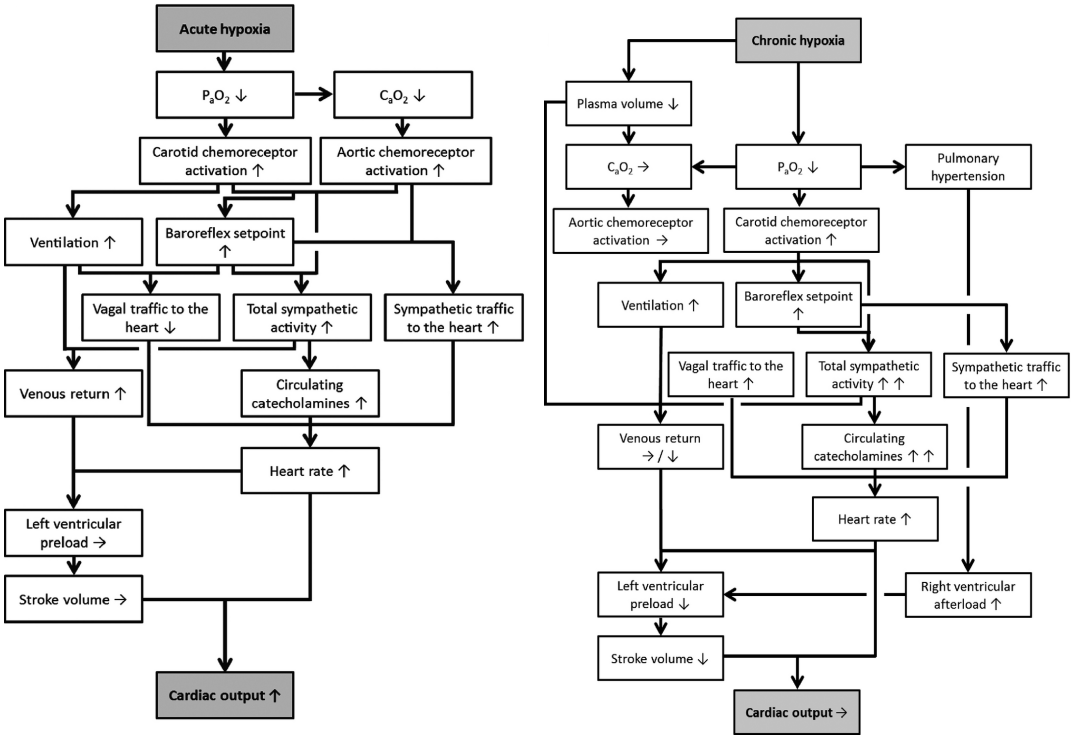

Figure 3. Regulation of cardiac output in acute and chronic hypoxia. Adapted from (Siebenmann *et al.*, 2015)

1.1.2 Exercise and training in hypoxia

The incapacitating effect of hypoxia has been recognized since humans started to ascend to altitude, but the underlying mechanisms were not really understood. From the beginning of the 20th century, the reduced partial pressure of oxygen (PO_2) has been identified as the perpetrator of the incapacitating effect of hypoxia instead of the barometric pressure per se (Douglas *et al.*, 1913); then, research focused on how the aerobic capacity is impaired by these environmental conditions. The key event for altitude training and exercise is certainly

the 1968 Olympic Games in Mexico City at ~2,300 m. Since then, research and knowledge about altitude training have increased continuously. Prior to these competitions, the impact of altitude on aerobic performance was proposed, but the significant below-records performances in endurance disciplines during these Olympiads confirmed the hypothesis. Moreover, numerous Olympic medals and world records in endurance sports were beaten by athletes residing above sea level or in high altitude environments. Thus, it seems rational to assume that living and/or training at altitude is beneficial, in one-way or another, at improving aerobic performance. Aerobic performance is related to the following three components: i) maximal oxygen uptake (VO_{2max}), which represents the upper limit for aerobic performance, ii) the fraction of VO_{2max}, and iii) the exercise efficiency. The first component (VO_{2max}) is the one that is most impacted by the decrease in PO_2 induced by altitude. Numerous studies have reported decreases in aerobic performance and maximal oxygen uptake in hypoxia (Squires & Buskirk, 1982; Chapman *et al.*, 1999; Peltonen *et al.*, 1999; Friedmann *et al.*, 2005; Mollard *et al.*, 2007; Peltonen *et al.*, 2007; Chapman *et al.*, 2011). This decrease has been described as a linear relation by Wehrlin & Hallen (Wehrlin & Hallen, 2006) (Figure 4). However, other authors described the relationship between VO_{2max} and P_iO_2 as a faithful reflection of the oxygen equilibrium curve (Ferretti *et al.*, 1997); thus, this relation would be curvilinear. Nevertheless, it is now generally assumed that each 1,000 m step is associated with an approximately 7.7% decrease in performance. It should be noted that altitude adaptations and performance decreases are under the influence of a great inter-individual variability (Schouweiler & Stray-Gundersen, 2002; Chapman, 2013).

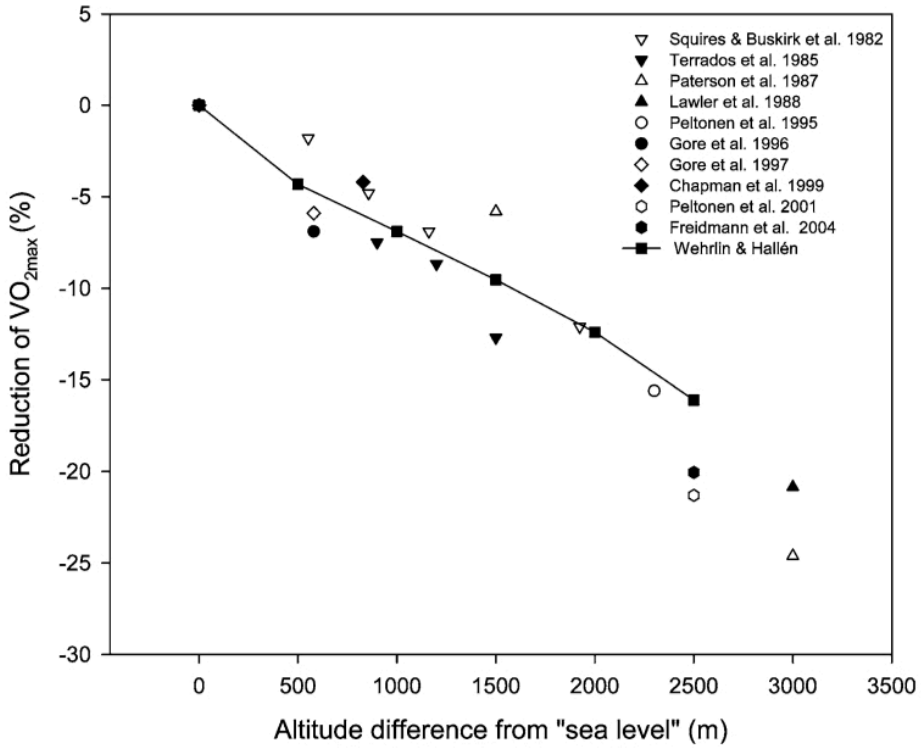

Figure 4. Percent decrease in maximal oxygen uptake (VO_{2max}) with altitude compared to sea level values (0-363 m). From (Wehrlin & Hallen, 2006).

Currently, athletes commonly use altitude training with the aim of enhancing their ability to compete successfully at a high level, and a variety of hypoxic training and/or exposure approaches have been applied and investigated within the last few decades (Figure 3). Before the 1990s, the most widely used method was the Live High-Train High (LHTH) method, which was aimed at spending the maximum time at altitude. Nevertheless, the decrease in aerobic capacity with altitude (Figure 4) limits the training intensities during LHTH. Scientists and the endurance training community then moved to another altitude training alternative, which allows athletes to maintain optimal training intensities for enhanced sea-level performance. This new mixed altitude training modality appeared in the 1990s and is called "Live High-Train Low", where athletes live at high altitudes but train at lower altitudes (LHTL) (Levine & Stray-Gundersen, 1997; Stray-Gundersen *et al.*, 2001; Stray-Gundersen & Levine, 2008). Since then, more than 15 years of research have revealed that LHTL is an effective training method to enhance sea level performance in endurance athletes, and it

provided 1-3% of additional benefit compared to similar normoxic training. However, in a recent study using a simulated altitude set-up (Siebenmann *et al.*, 2012), neither endurance performance nor the associated physiological responses were modified after 4 weeks of LHTL. However, LHTL is still recognized as an effective method that can improve performance in athletes, despite the large inter-subject variability in responses (Lundby *et al.*, 2012).

During the past few years, more than 70 studies have analysed diverse modalities of LHTL (Bonetti & Hopkins, 2009). Beyond the classical parameters of these types of altitude training camps (*i.e.,* duration, ratio between training and living, or altitude level), the development of several altitude simulation methods (nitrogen house, hypoxic chamber, and portable devices, such as the AltiTrainer®) allowed researchers to design many new combinations of training strategies (Figure 5, from (Millet *et al.*, 2013)) using either "simulated" (*i.e.,* normobaric hypoxia, NH) or "real" (*i.e.,* hypobaric hypoxia, HH) hypoxia. The purpose of the present thesis was to assess the physiological differences between these two types of hypoxia and their implications on sea level or altitude performances. The next chapter introduces the state of the actual literature about the potential differences between these two modalities, which underpins the rationale for this thesis.

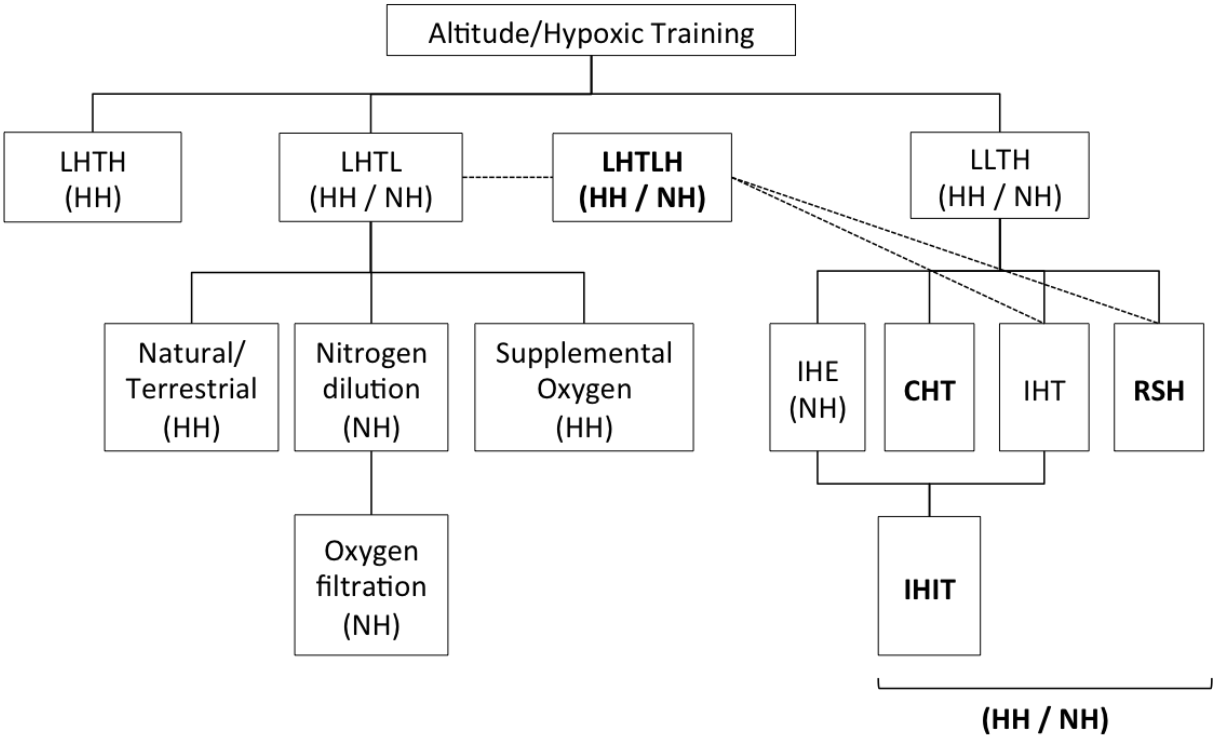

Figure 5. Actual altitude training panorama. LHTH, live high train high; LHTL, live high train low; LHTLH, live high train low and high; LLTH live low train high; IHE, intermittent hypoxic exposure; CHT, continuous hypoxic training; IHT, interval hypoxic training; RSH, repeated sprint training in hypoxia; IHIT, IHE during interval-training; NH, normobaric hypoxia; HH, hypobaric hypoxia. From (Millet *et al.*, 2013).

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1.2 Normobaric versus hypobaric hypoxia

1.2.1 General considerations

Since Paul Bert published his monograph on barometric pressure in 1878 (Bert, 1878), it has been admitted that the combination of the inspired fraction of oxygen (F_iO_2) and ambient pressure (P_B) that produces the same inspired partial pressure of oxygen (P_iO_2) will produce the same physiological responses. This model is called the equivalent air altitude model (EAA), which is an isoP_iO_2 model where $P_iO_2 = (P_B - 47) * F_iO_2$, where 47 mmHg is the partial pressure of water vapour at 37°C. The EAA model has been used to describe isohypoxia, which is *"the same distribution of hypoxia signs and symptoms under any circumstances of equivalent hypoxic dose"* (Conkin & Wessel, 2008). However, these authors concluded that this EAA model was inaccurate (Conkin & Wessel, 2008). The symptoms of

AMS (acute mountain sickness) and physiological measures diverge for a same hypoxic P_iO_2, depending on the barometric pressure. However, at that time, the underlying mechanisms remained elusive.

Nevertheless, in a very recent a short communication, Conkin reminded us of the historical conception concerning the alveolar gas composition (Conkin, 2016). This paper is based on the work of Fenn *et al.* and Rahn & Otis (Fenn *et al.*, 1946; Rahn & Otis, 1949), who derived the alveolar gas equation (AGE) and named it the alveolar air equation:

$$P_AO_2 = (P_B - 47) \times F_iO_2 - P_ACO_2 \times [F_iO_2 + ((1-F_iO_2) / RER)]$$

In this equation, in addition to the previously explained terms, RER is respiratory exchange ratio (VCO_2/VO_2 $ml.min^{-1}$ in STPD) and P_AO_2 and P_ACO_2 are the alveolar partial pressures of oxygen and carbon dioxide, respectively. In addition, "1- F_iO_2" is the dry-gas fraction of nitrogen (F_iN_2), and thus F_iN_2 is a contributor to P_AO_2. Through the study of five different conditions resulting in the same P_iO_2 (including NH and HH), the authors discovered different P_AO_2 values that were attributed to a N_2 dilution effect or the respiratory exchange effect. By noting these forgotten facts, Johnny Conkin made an appreciable step in the comprehension of the underlying mechanisms of different physiological adaptations under NH and HH and confirmed that these conditions cannot be used interchangeably (Conkin, 2016).

Nevertheless, one should be aware of the different ways to induce hypoxia itself (Figure 6), all of which lead to a decrease in arterial pressure (P_aO_2) and oxygen saturation (S_aO_2), to better interpret the potential differences between normobaric and hypobaric hypoxia.

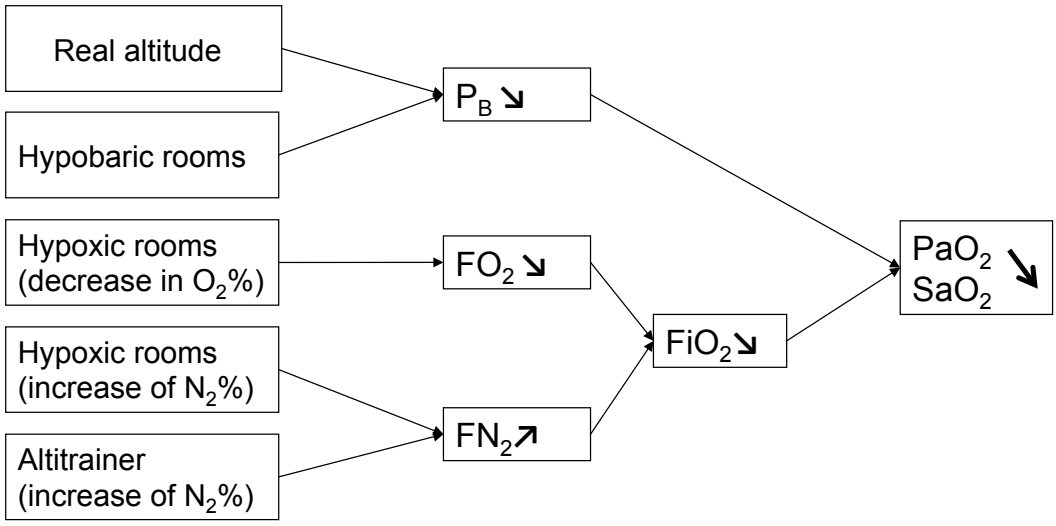

Figure 6. Different methods inducing hypoxia, and thus decreased arterial pressure in oxygen (P_aO_2). O_2, oxygen; N_2, nitrogen; P_B, barometric pressure; FO_2, oxygen fraction; FN_2, nitrogen fraction; F_iO_2, inspired oxygen fraction; S_aO_2, arterial oxygen saturation. Adapted from (Millet & Schmitt, 2011).

As stated above, hypoxia can result from a decreased partial pressure of O_2 in the inspired air that is either caused by reduced barometric pressure (hypobaric hypoxia, HH) or a decreased fraction of inspired O_2 (normobaric hypoxia, NH). Normobaric hypoxia has been developed within the last 20 years because of the lack of sufficiently high mountains in many countries (*e.g.*, Australia), and thus the impossibility of remaining or staying at natural high altitudes (hypobaric hypoxia). Therefore, natural altitude environments were simulated via either O_2 filtration, inspiration of low-O_2 gas mixtures, or the addition of nitrogen to ambient air (Figures 5 and 6, (Millet *et al.*, 2013)). Oxygen filtration was used throughout the different studies conducted in the present thesis. It is really fundamentally necessary to understand that the inspired pressure of oxygen (P_iO_2) can be perfectly matched between natural and simulated altitudes, despite the differences in the oxygen fraction and barometric pressure (Figure 7).

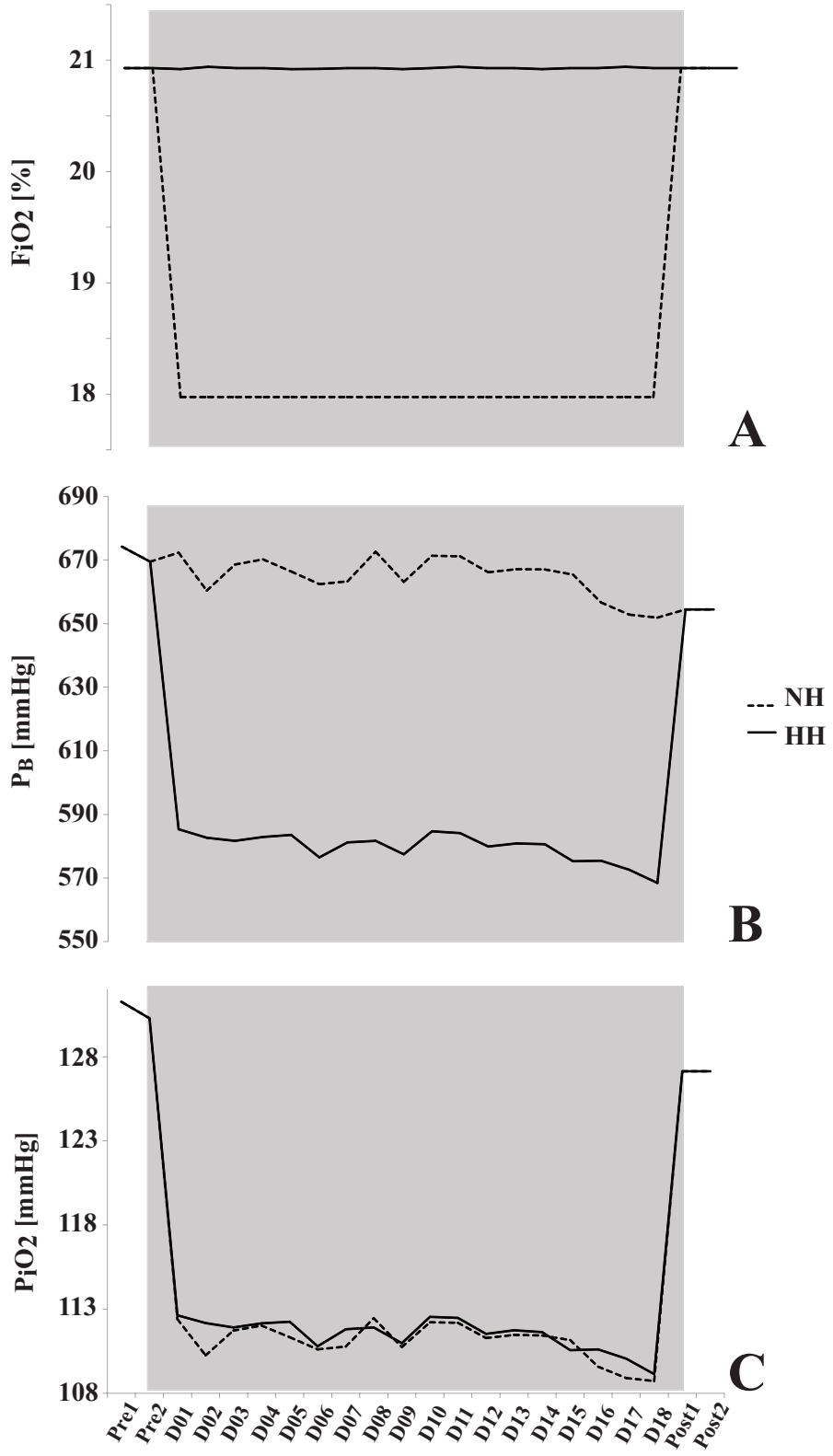

Figure 7. Fraction of inspired oxygen (F_iO_2, %) (**A**), barometric pressure (P_B, mmHg) (**B**) and corresponding pressure of inspired oxygen (P_iO_2, mmHg) (**C**) before (Pre1-Pre2), during (D01-D18, grey part) and after (Post1-Post2) a Live High–Train Low altitude training camp in either normobaric (NH) or hypobaric hypoxia (HH). Adapted from (Saugy *et al.*, 2014).

1.2.2 NH versus HH: State of the actual literature

A growing body of literature has reported physiological differences between NH and HH in various scientific areas over the past few years. A recent point-counterpoint (Millet *et al.*, 2012) brought the debate to the top of the scientific reflexion in the associated fields. Since then, altitude studies have considered that their results could be different based on the way in which hypoxia is induced. Recently, Coppel *et al.* reviewed the physiological effects of hypobaric and normobaric hypoxia in crossover trials and confirmed that these two hypoxic conditions cannot be used interchangeably (Coppel *et al.*, 2015). This conclusion is shared by other recent studies (Fulco *et al.*, 2011; DiPasquale *et al.*, 2015b; Conkin, 2016). The aim of this sub-chapter is to present the actual state of the scientific literature about the above-mentioned differences between the two hypoxic conditions.

Ventilation responses.

The main perceived consequence of a decrease in P_iO_2 following exposure to hypoxia is an increase in pulmonary ventilation to maintain oxygen delivery to the tissues (Wagner *et al.*, 1986). The peripheral chemoreceptors in the carotid bodies, which are sensitive to the drop in arterial oxygen pressure (P_aO_2), are stimulated by hypoxia to centrally regulate ventilation through the cortical respiratory centres (Teppema & Dahan, 2010). However, this ventilatory adaptation to hypoxia is not applied to the same extent when individuals are exposed to either normobaric or hypobaric hypoxia. Tucker *et al.* already highlighted the potential effect of hypobaria on ventilation more than 30 years ago (Tucker *et al.*, 1983). Subjects were exposed to either terrestrial, 4,570 m, or normobaric hypoxia with an oxygen fraction of 14%. Although the arterial desaturation levels were similar between conditions, breathing 14% O_2 resulted in a greater hyperventilatory response compared to altitude exposures. These conclusions were later confirmed by Loeppky *et al.* (Loeppky *et al.*, 1997), who observed

higher ventilation in NH (F_iO_2 = 14.2%) compared to HH (P_B = 432 mmHg) after a 3 h exposure that was still higher after 6 and even 9 hours. A few years later, Savourey *et al.* compared the potential physiological differences between these two types of hypoxia after chronic exposure to high altitudes (40 min, 4,500 m) and found that compared to NH, HH leads to a greater hypoxemia, hypocapnia, and blood alkalosis, but lower oxygen arterial saturation and ventilation (Savourey *et al.*, 2003). Recently, our team also observed lower ventilatory responses both at rest and during exercise in HH versus NH at 3,000 m that lasted for 24 h (Faiss *et al.*, 2013). Finally, in their review of the crossover trials comparing NH and HH (Coppel *et al.*, 2015), Coppel *et al.* highlighted the decrease in minute ventilation and alveolar ventilation in HH compared to NH.

These lower ventilation levels in HH compared to NH are in accordance with smaller tidal volumes (see Figure 8) and most often, but not always, with higher respiratory frequencies (Coppel *et al.*, 2015). Overall, HH induced greater respiratory frequencies, lower tidal volumes, and minute ventilation values over short time periods, suggesting a higher alveolar physiologic dead space, which is associated with ventilatory alkalosis and hypocapnia (Savourey *et al.*, 2003; Millet *et al.*, 2012). Similar conclusions have also been reported by Richard and Koehle (Richard & Koehle, 2012) and Faiss *et al.* (Faiss *et al.*, 2013). The N_2 and oxygen concentrations in the cerebrospinal fluid could be influenced by the barometric pressure, which could change the central regulation of ventilation (Conkin & Wessel, 2008). Moreover, fluid circulation and trans-alveoli-capillary flux are also known to be modified by the barometric pressure (Levine *et al.*, 1988). This modification could induce greater pulmonary vasoconstriction in hypobaric hypoxia and thus alter the oxygen diffusion by decreasing the pressure gradient. Finally, the $P_{ET}CO_2$ values observed in several studies did not change significantly between conditions, despite the decreased ventilation (Coppel *et al.*, 2015), but the $P_{ET}CO_2$ values were lower for NH than for HH in other cases (Loeppky *et al.*,

1997; Loeppky *et al.*, 2005; Faiss *et al.*, 2013). Twenty years ago, Reeves *et al.* described $P_{ET}CO_2$ as 'a good indicator of acclimatization' (Reeves *et al.*, 1993). This ventilatory acclimatization, characterized by an increased hypoxic ventilatory response, is obviously of great importance for altitude acclimatization and results in a decreased $P_{ET}CO_2$ associated with an increased S_aO_2 (Fulco *et al.*, 2013).

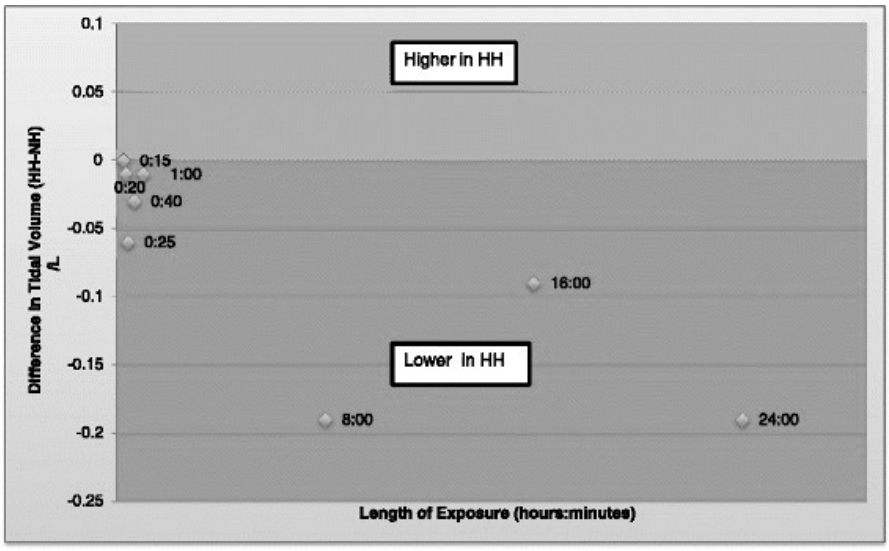

Figure 8. Differences in tidal volume between normobaric (NH) and hypobaric hypoxia (HH) over time. Each point represents data obtained from a study in the systematic review of Coppel *et al.* From (Coppel *et al.*, 2015).

Fluid balance.

Levine *et al.* first showed that ascent to a hypobaric hypoxic environment induced significant changes in both pulmonary fluid balance and O_2 transport and provided some theoretical basis for the clinical maxim that descent is the treatment of choice for high-altitude illness (Levine *et al.,* 1988). Later, Loeppky *et al.* confirmed that the fluid balance is not influenced in the same way by normobaric and hypobaric hypoxia (Loeppky *et al.*, 2005). The effects of barometric pressure and reduced PO_2 seem to be combined with reduced antidiuretic hormone (ADH) and plasma aldosterone (ALDO) levels, which induce fluid retention (*i.e.,* lower urine volume in HH). However, the significant decrease in plasma volume, higher diuresis and lower ADH level in NH compared with HH results in a negative fluid balance (*i.e.,* greater urine volume in NH).

Acute mountain sickness.

Most studies have shown that the severity of acute mountain sickness symptoms was higher in HH compared to NH at high altitudes of approximately 4,500 m (Roach *et al.*, 1996; Loeppky *et al.*, 2005; Conkin & Wessel, 2008). In 2004, Beidleman *et al.*, designed a study to assess the effect of intermittent altitude exposure (IAE) on AMS (a 3-week period of IAE for 4 h/day, 5 days/week, at 4,300 m). The authors concluded that intermittent altitude exposure was more effective for altitude acclimatization and the reduction of the severity of AMS than normobaric hypoxia (Beidleman *et al.*, 2004). In addition, Coppel *et al.* also concluded that AMS symptoms increased to a larger extent in HH compared to NH (Coppel *et al.*, 2015). These results were later confirmed by DiPasquale *et al.* in their latest study showing that AMS severity was not equivalent when individuals were exposed to either NH or HH (DiPasquale *et al.*, 2015b). Furthermore, a recent review evaluated six altitudes and hypoxia preacclimatization strategies to determine their effectiveness at reducing AMS. These strategies used either NH or HH and the differences in the main physiological response parameters to acclimatization (*e.g.*, $P_{ET}CO_2$ and S_aO_2) were between the hypoxic conditions. Thus, it seems that pre-acclimatization with HH is far more effective at reducing AMS symptoms (Fulco *et al.*, 2013). The potential mechanisms that induce differences in AMS severity or the efficiency of pre-acclimatization strategies that prevent AMS in NH and HH are still unclear. The roles of the density of the breathing gas, nitrogen movement, and the influence of the central nervous system have been discussed (Conkin & Wessel, 2008; Conkin, 2016). Moreover, the higher peripheral oxygen saturation level in NH compared to HH may be involved in these differences in AMS.

Nitric oxide metabolism and oxidative stress.

Nitric oxide (NO) is of great importance in the physiological responses to hypoxia. A decreased partial pressure of exhaled NO (exNO) at high altitude (Donnelly *et al.*, 2011) has been observed and could influence pulmonary vasoconstriction. On the other hand, exNO was reported to be higher in NH compared to HH after an acute exposure to high altitude (up to 10 minutes at 5,000 m) (Dweik *et al.*, 1998; Hemmingsson & Linnarsson, 2009; Hemmingsson *et al.*, 2012), and the barometric pressure plays a role in this difference (Kerckx *et al.*, 2010). Recently, Faiss *et al.* also reported a higher amount of exNO in NH than HH after 24 h of exposure at 3,000 m (Faiss *et al.*, 2013). The potential underlying mechanisms for these reductions in exhaled NO in HH could be a higher back-diffusion to the alveoli and then to the haemoglobin in HH, suggesting that more NO is recaptured by the blood compartment in HH (Millet *et al.*, 2012). Moreover, nitrate and nitrite (*i.e.*, precursors of plasma NO and NOx) concentrations are also higher in NH than in HH (Faiss *et al.*, 2013), suggesting decreased systemic NO bioavailability in HH. A potential explanation for this decrease in the NOx concentrations in HH compared to NH could be a larger increase in oxidative stress in HH, as revealed by higher plasma concentrations of advanced oxidation protein products (AOPPs) and superoxide dismutase (SOD). Taken together, these results suggest that the lower minute ventilation in HH described above could be due to a decrease in NO bioavailability as an indirect consequence of the decreased barometric pressure in this condition (Millet *et al.*, 2012; Faiss *et al.*, 2013).

Performance.

As mentioned before, aerobic performance is decreased at altitude, whereas hypoxic exposure can increase sea-level performances. Nevertheless, it is important to be aware that it is very difficult to compare the training benefits between different hypoxic training methods because

of the multitude of parameters that induce changes in performance. Moreover, training content and the hypoxic dose are key factors for sea-level performance enhancement (Millet *et al.*, 2010). In addition, responses to training and competition at high altitudes and the timing of the return to competition after altitude training is specific to each individual (Chapman, 2013; Chapman *et al.*, 2014b). However, differences between NH and HH have been shown in both of these performance fields. On one hand, a recent meta-analysis highlighted that the mean performance improvements (represented by power output increases) following LHTL were lower when the altitude camps were accomplished under NH compared to HH conditions (0.6% and 1.4% vs. 4.0% and 4.2% for elite and non-elite athletes, respectively) (Bonetti & Hopkins, 2009). In addition, most of the LHTL studies with NH conditions did not produce any performance enhancement, although some induced positive erythropoietic responses (Clark *et al.*, 2009; Robertson *et al.*, 2010). However, most studies reporting both erythropoietic and performance enhancements were completed under HH conditions (Wehrlin *et al.*, 2006; Chapman *et al.*, 2014a). Nevertheless, at this time, no study has directly compared LHTL camps in NH vs. HH to confirm whether the benefits would really be greater following training in HH compared to NH. Thus, we designed a complete crossover study over a period of two years with the aim of assessing this interesting point (see studies 1 and 3 in the summary of results, discussion, and in the annexes).

On the other hand, and as mentioned above, the decrease in VO_{2max} (Figure 4, (Wehrlin & Hallen, 2006)) is the main mechanism for endurance performance impairments in hypoxia, which is strongly linked to S_pO_2 levels that are also decreased by altitude (Millet *et al.*, 2012; Chapman, 2013). These performance impairments with altitude have already been reported either in NH or HH by several authors. These decreases do not seem as large when the exercises are conducted under normobaric hypoxia compared to hypobaric hypoxia. At simulated altitudes of approximately 3,000 m, performance impairments of 10% (Chapman *et*

al., 1998), 18% (Faiss *et al.*, 2014) or 20% (Ventura *et al.*, 2003) were reported versus 10% at a lower real altitude of 2,100 m (Chapman *et al.*, 2011). When considering higher altitudes, HH seems also to induce larger performance impairments (41% (Fulco *et al.*, 2011) and 44% (Richalet, 2010)) in HH versus 30% (Bourdillon *et al.*, 2014) in NH. Regarding all these studies, we can conclude that some differences in the decreased performance might arise from the type of hypoxic stimulus (*e.g.*, NH or HH). However, considering the large inter-individual variability in hypoxia sensitivity (Chapman *et al.*, 1998; Chapman, 2013) and the considerable number of variables affecting these results, one can only speculate that these differences are effective without a direct comparison. To our knowledge, only one study has compared aerobic performances in both hypoxic conditions to date, but they did not use a direct comparison with the same subjects (Beidleman *et al.*, 2014). In this study, two different groups of subjects were exposed to either NH or HH and performed a 720-kJ time trial (TT). Compared to the control condition (*i.e.*, sea-level, normobaric normoxia = NN) the TT performance was decreased to a larger extent in HH (-65%) compared to NH (-36%). Accordingly, we designed an experimental crossover protocol to directly compare the decreases in aerobic performance in the same subjects at the same acute exposure durations to both NH and HH, with a normoxic control condition (see studies 2 and 6 in the summary of results, discussion, and in the annexes).

Sleep.

Sleep quality in hypoxia is impaired at both high (Sargent *et al.*, 2013) and moderate altitudes (Latshang *et al.*, 2013), which could compromise aerobic performance (Davenne, 2008; Oliver *et al.*, 2009). Until now, sleep and its disturbances have been studied in either NH or HH. On one hand, oxygen transport has been shown to decrease during sleep both in continuous (Berssenbrugge *et al.*, 1983) and intermittent normobaric hypoxia (Tamisier *et al.*,

2009), as well as in high altitude hypobaric hypoxia (Bloch *et al.*, 2010). Periodic breathing has been observed in NH at a simulated altitude of 2,650 m, and the rate of rapid eye-movement during sleep (%REM) was increased compared to normoxia (Kinsman *et al.*, 2003; Kinsman *et al.*, 2005a; Kinsman *et al.*, 2005b). In addition, ventilation has been shown closely related to O_2 and CO_2 chemosensitivity during sleep in NH (Nespoulet *et al.*, 2012).

On the other hand, several authors have reported that sleep is disordered when an individual is exposed to hypobaric hypoxia. Reductions in REM sleep and periodic breathing during the night have been observed at a terrestrial altitude of 3,600 m (Roach *et al.*, 2013; Sargent *et al.*, 2013). These periodic breathing events lead to nocturnal intermittent hypoxia (IH), which implies intermittent arterial hypoxemia and its maladaptive consequences (Berssenbrugge *et al.*, 1983; Kinsman *et al.*, 2002; Dempsey *et al.*, 2010). Moreover, these maladaptive effects could persevere after waking and become deleterious to the athlete's training and performances (Dempsey & Morgan, 2015). Consequently, sleeping in hypobaric hypoxia has been reported to induce maladaptive effects that directly alter aerobic performance, even at sea level, through alterations in night ventilatory physiology.

In contrast, recent studies considered that periodic breathing in either normobaric or hypobaric hypoxia was a protective mechanism. Indeed, average nocturnal peripheral oxygen saturation seems to be decreased to a lower level in subjects experiencing more hypopneas/apneas compared to others who do not experience these conditions (Nespoulet *et al.*, 2012). Based on these results, it is obvious that hypoxia has an influence on sleep patterns and recovery in response to NH or HH. In light of all the differences exposed above, it could be supposed that barometric pressure might also have an influence on sleep. Nevertheless, these hypoxic conditions have never been directly compared in the field of nocturnal and sleep pattern disturbances. Therefore, in this context, we designed two different studies to compare sleep and breathing disorders during either acute or chronic normobaric versus

hypobaric hypoxia (see studies 4 and 5 in the summary of results, discussion, and in the annexes).

The aim of the present thesis work was then to further comprehend the mechanisms underlying the potential differences between normobaric and hypobaric hypoxia. As presented above, several disparities have been identified in many physiological areas between these two hypoxic conditions. We have tried to provide new insights by designing carefully controlled protocols that allow us to directly compare NH and HH. The main results of our work are presented in the next chapter.

Chapter Two

Summary of experimental results

2. Summary of experimental results

2.1 Performance changes after Live High–Train Low in normobaric versus hypobaric hypoxia.

With a study design replicating the "real" practices of endurance athletes, we investigated both performance and physiological parameters resulting of Live High–Train Low (LHTL) altitude training camps under either normobaric (NH) or hypobaric hypoxia (HH). Breathing frequency was lower (13.9 ± 2.1 vs. 15.5 ± 1.5 breath.min^{-1}, P<0.05) and night S_pO_2 was higher (92.1 ± 0.3 vs. 90.9 ± 0.3%, P<0.001) for NH compared HH during the whole LHTL camps. Similar increases in Hb_{mass} (2.6 ± 1.9 vs. 3.4 ± 2.1%) and VO_{2max} (6.1 ± 6.8 vs. 5.2 ± 4.8%) were measured in NH and HH groups, respectively, immediately after the camps, where no 3-km running performance was observed. However, this aerobic performance was improved to a larger extent for HH compared to NH 21 days following the LHTL (3.3 ± 3.6 vs. 1.2 ± 2.9%, P<0.05, Figure 9).

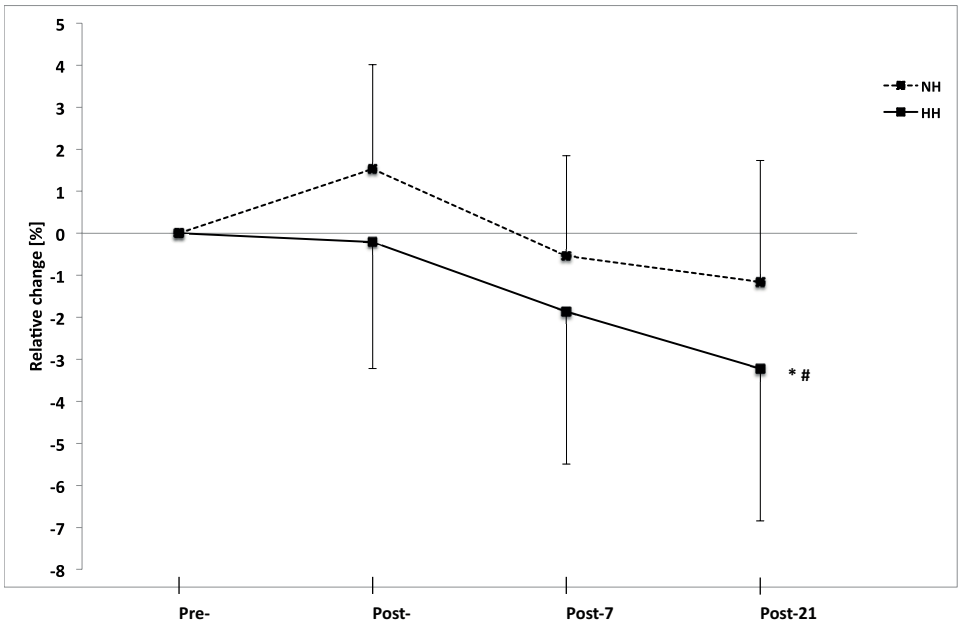

Figure 9. Relative change in 3-km run time from Pre- to Post-, Post-7, and Post-21 as determined on a running track near sea level for the normobaric hypoxia (NH) and hypobaric hypoxia (HH) groups (in %). Data are mean ± standard error *P<0.05 for differences with Pre- and #P<0.05 for differences between groups.

2.2 Performance changes during acute exposure to normobaric versus hypobaric hypoxia.

The aim of this study was to assess the aerobic performance changes during a cycle time trial in acute exposure to normobaric (NH) versus hypobaric hypoxia (HH) (24 h at 3450 m) with a control condition in normobaric normoxia (NN).

The mean time trial (TT) performance in NN was significantly faster than in the two hypoxic conditions ($P<0.001$, Figure 10) and the time trial performance was also faster in NH compared to HH ($P<0.01$, Figure 10). Mean peripheral oxygen saturation (S_pO_2) during the TT was significantly higher in NN compared to hypoxic conditions ($P<0.001$, Figure 11) and significantly lower for HH than NH ($P<0.05$, Figure 11).

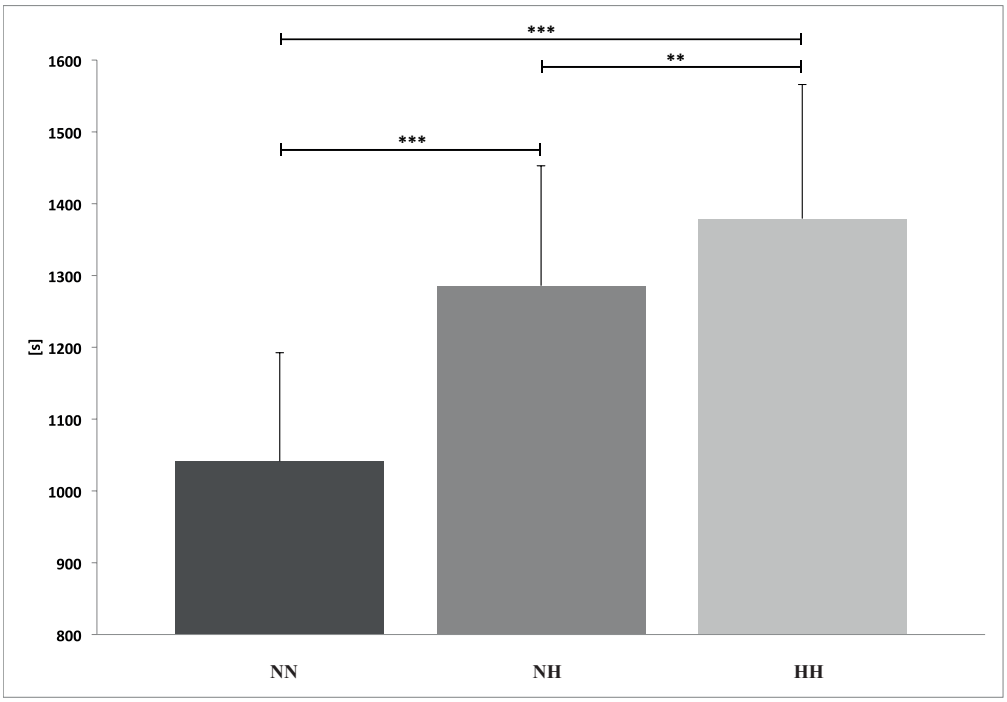

Figure 10. Mean time trial performance in normobaric normoxia (NN), normobaric hypoxia (NH) and hypobaric hypoxia (HH). Data, expressed in seconds (s) are mean ± standard errors. **P<0.01 and ***P<0.001 for differences between conditions.

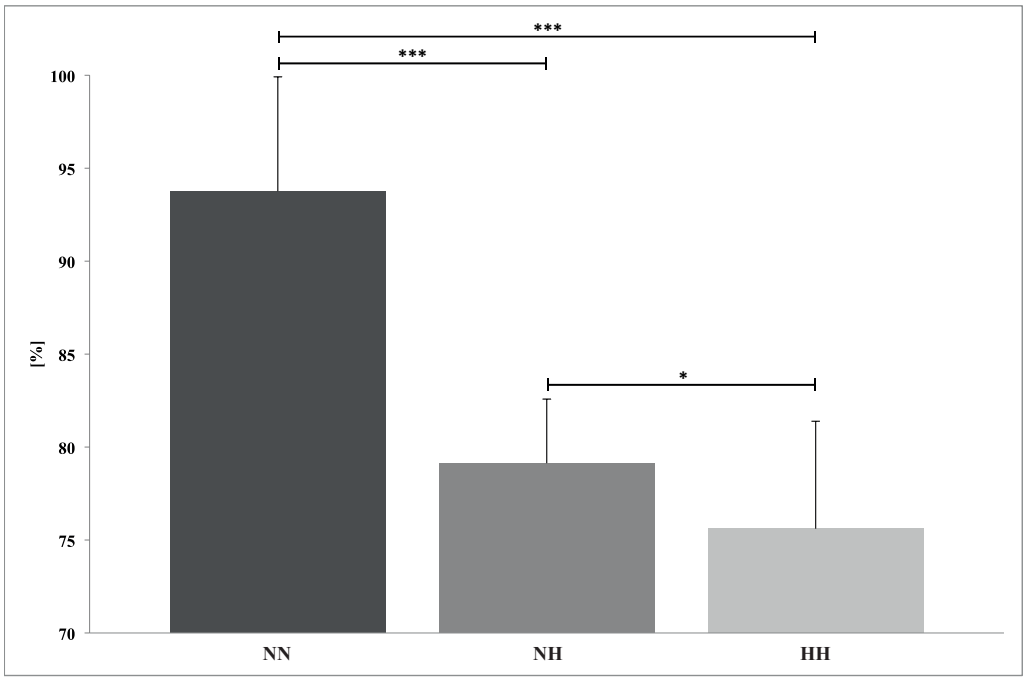

Figure 11. Mean pulse oxygen saturation (S_pO_2) during the time trial in normobaric normoxia (NN), normobaric hypoxia (NH) and hypobaric hypoxia (HH). Data are mean ± standard errors. *P<0.05 and ***P<0.001 for differences between conditions.

2.3 Same performance changes after Live High–Train Low in normobaric versus hypobaric hypoxia.

The aim of the study was to investigate the changes in physiological and performance (3-km run) parameters after a Live High–Train Low (LHTL) altitude camp in either normobaric hypoxia (NH) or hypobaric hypoxia (HH) using a crossover designed protocol reproducing the actual training practices of endurance athletes. This study complete the first study presented above.

Nocturnal peripheral oxygen saturation was higher for NH than HH during the whole LHTL (92.3 ± 1.2 vs. 91.3 ± 1.0%, P<0.001, Figure 12). VO_{2max} increased to the same extent in both conditions (4.9 ± 5.6 vs. 3.2 ± 5.1%, for NH and HH) while no difference was found in haematological parameters. No difference was found between conditions at any time for the 3-km running time. However, the performance was significantly improved 21 days after the camps compared to before LHTL (4.5 ± 5.0 vs. 6.2 ± 6.4% for NH and HH, Figure 13).

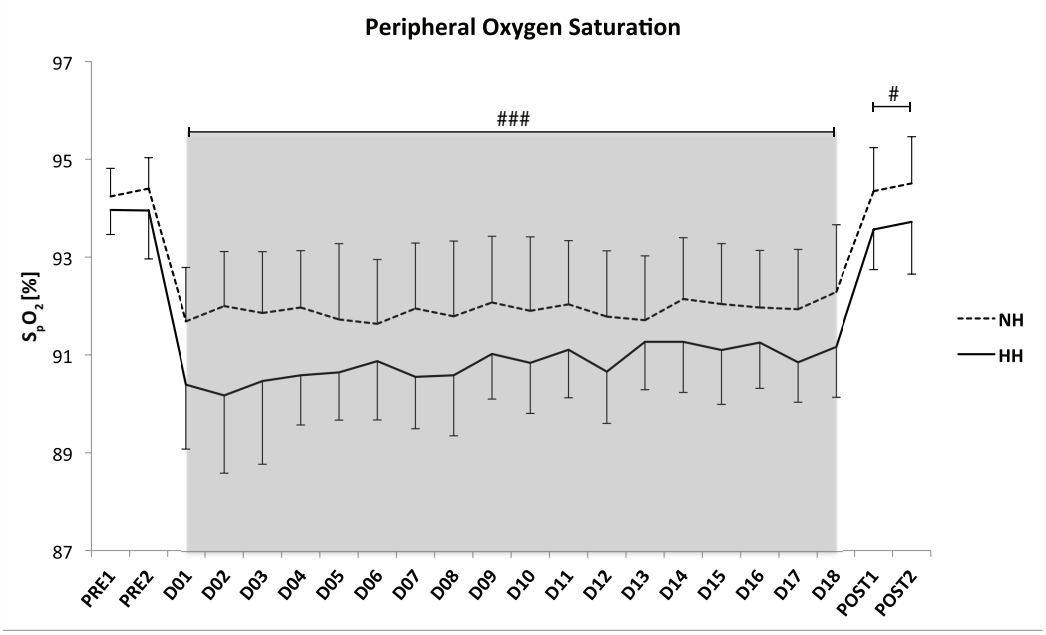

Figure 12. Mean values of night oxygen pulse saturation (S_pO_2) for the crossover data. Data are presented as mean ± standard error. Pre1-Pre2: measurements before the camps (1150 m, Prémanon, France); D01-D18: measurement during the camps (NH: hypoxic room in Prémanon, France; HH: Fiescheralp, Switzerland); Post1-Post2: measurements after the camps (1150 m, Prémanon, France). $^{\#}P<0.05$, $^{\#\#\#}P<0.001$ for differences between conditions.

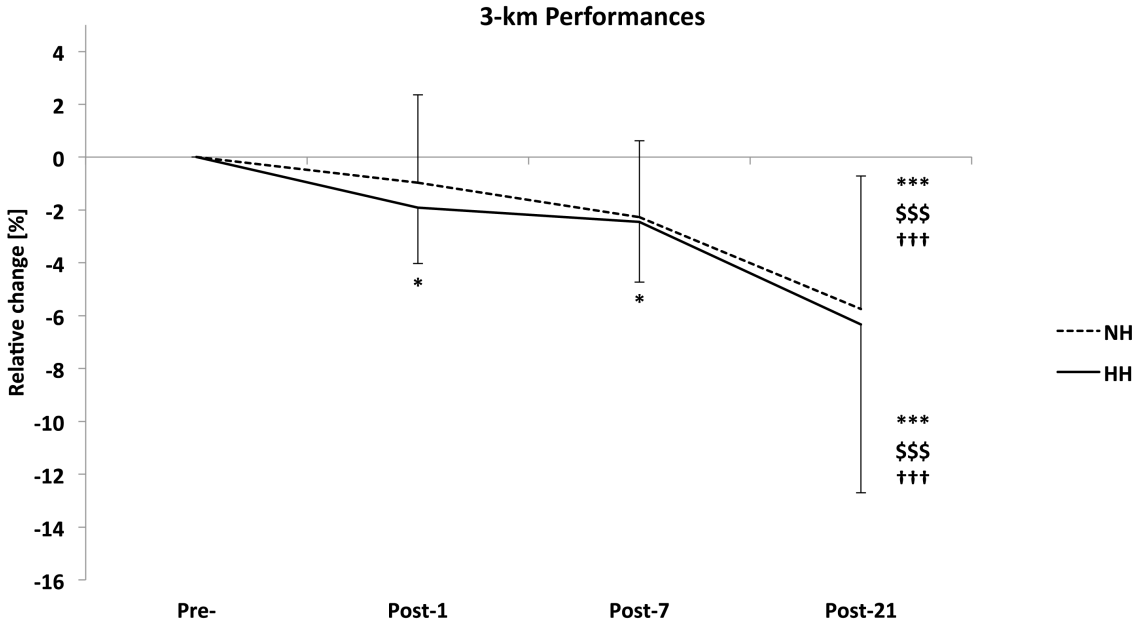

Figure 13. Relative change in 3-km run time from Pre- to Post-1, Post-7, and Post-21 as determined on a running track near sea level for the normobaric hypoxia (NH) and hypobaric hypoxia (HH) conditions (in %) for the crossover (n = 16). Data are mean ± standard error *P<0.05, **P<0.01 and ***P<0.001 for differences with Pre-; $^{\$\$\$}P<0.001$ for differences with Post-1; $^{†††}P<0.001$ for differences with Post-7.

2.4 Sleep assessment in acute normobaric versus hypobaric hypoxia.

The aim of the study was to assess the sleep disorders induced by acute high altitude exposure (3450 m) in either normobaric (NH) or hypobaric hypoxia (HH), compared to a control condition in normobaric normoxia (NN) using the gold standard nocturnal sleep polysomnography (PSG).

Heart rate was higher in HH than in NH (61±10 vs. 55±6 bpm; $P<0.05$) and NN (48±5 bpm; $P<0.001$). Breathing frequency did not differ between conditions, while mean nocturnal oxygen saturation was further decreased during HH than in NH (81.2±3.1 vs. 83.6±1.9%; $P<0.01$) when compared to NN (95.5±0.9%; $P<0.001$). Total sleep time was longer in HH than in NH (351±63 vs. 317±65 min, $P<0.05$), and both were shorter compared to NN (388±50 min, $P<0.05$). Apnea-hypopnea index was higher in HH than in NH (20.5 [15.8-57.4] vs. 11.4 [5.0-65.4]; $P<0.01$) and NN (8.2 [3.9-8.8]; $P<0.001$). Oxygen desaturation index 3% (ODI 3%) was higher in HH (47.6 [22.1-82.2]) compared to NH (22.7 [13.1-73.8]) and NN (4.4 [2.2-4.8]). Subjective sleep-quality was similar between hypoxic conditions but lower than in NN.

2.5 Disordered sleep during Live High–Train Low in normobaric versus hypobaric hypoxia.

We investigated for the first time sleep quality and disordered breathing during a 18-days LHTL altitude camp using either NH or HH in a controlled crossover designed study.

The night breathing frequency was lower for NH than HH during the whole camps (14.6 ± 3.1 vs. 17.2 ± 3.4 breath.min^{-1}, $P<0.001$, Figure 14). The nocturnal peripheral oxygen saturation was lower for HH compared to NH for all LHTL nights (90.8 ± 0.3 vs. 91.9 ± 0.2, $P<0.001$)

and the number of oxygen desaturation index 3% (ODI 3%) was higher for HH compared to NH (15.1 ± 3.5 vs. 9.9 ± 1.6, P<0.001, Figure 15).

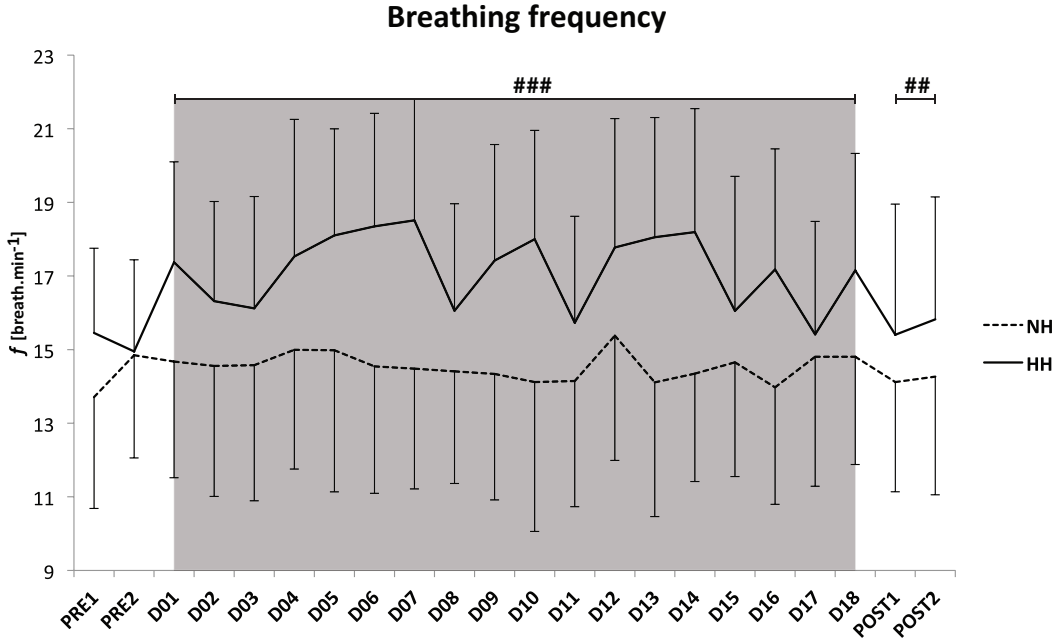

Figure 14. Values (mean ± standard error) of breathing frequency (*f*) measured during the night with instrumented t-shirts for NH and HH groups. Pre1-Pre2: measurements before the camps (1150 m); D01-D18: measurement during the camps (NH: hypoxic room; HH: ski resort). #P<0.05, ###P<0.001 for differences between conditions.

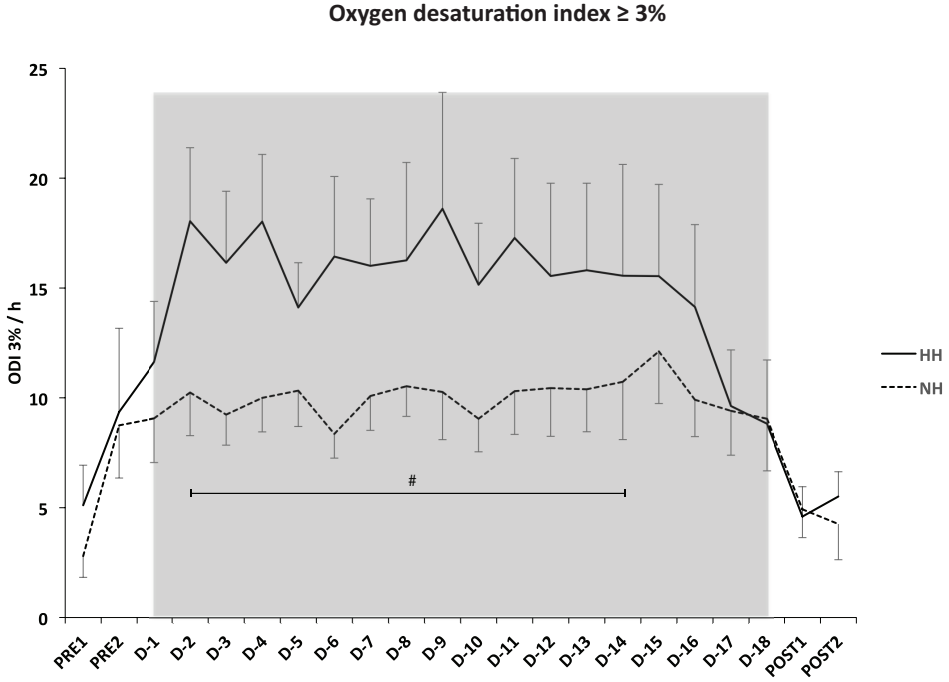

Figure 15. Values (mean ± standard error) of night oxygen desaturation index larger than 3% ≥per hour (ODI > 3%). Pre1-Pre2: measurements before the camps (1150 m); D01-D18: measurement during the camps (NH: hypoxic room; HH: ski resort). #P<0.05, ###P<0.001 for differences between conditions.

2.6 Pacing strategies during time trial performances in normobaric versus hypobaric hypoxia.

The aim of the present study was to assess the pacing strategies and exercise regulation during a self-paced cycle time trial of 250 kilojoules (kJ) in normobaric normoxia (NN), normobaric hypoxia (NH) and hypobaric hypoxia (HH). For this purpose, the exercises were divided in 25 slices of 10 kJ. We demonstrated that compared to NN the whole time trial pace was reduced for both hypoxic conditions and that HH power output was significantly further reduced compared to NH from 140 to 220 kJ (Figure 16, $P<0.05$). The near infrared spectroscopy (NIRS) measurements showed lower oxygenation values in HH compared to NH in pre-frontal cortex (PFC) HbDiff at the beginning of the time trial (20-120 kJ, $P<0.05$, Figure 17A), while higher values were observed in HH compared to NH in motor cortex (MC) HbDiff in the second part of the time trial (160-240 kJ, $P<0.05$, Figure 17B). The S_pO_2 was lower for both hypoxic conditions during the whole time trial and it was further decreased in HH compared to NH at rest and during the first half of the time trial (from 0 to 140 kJ Figure 18A; $P<0.05$). Mean middle cerebral artery blood flow velocities (MCAv) at rest was not different between conditions while it was increased in the first quarter of the time trial in all conditions, the increase being lower in NH compared to HH (from 10 to 150 kJ, $P<0.05$) and NN (from 10 to 100 kJ, $P<0.05$) while no difference was found along time trial between HH and NN (Figure 18B). Muscle HbDiff was lower for NN compared to NH from 20 to 210 kJ ($P<0.05$) and lower for HH compared to NN from 190 to 240 kJ ($P<0.05$, Figure 19A). HbDiff was also lower in NH compared to HH from 50 kJ onward ($P<0.05$). Muscle THb were higher in HH compared to both NN and NH conditions from 90 kJ onward ($P<0.05$) with no difference between NN and NH (Figure 19B). No difference was found between hypoxic conditions in any subjective feeling assessments (*i.e.* RPE, VAS for legs and

breathing discomfort) while the subjective breathing discomfort was lower in NN compared to hypoxic conditions.

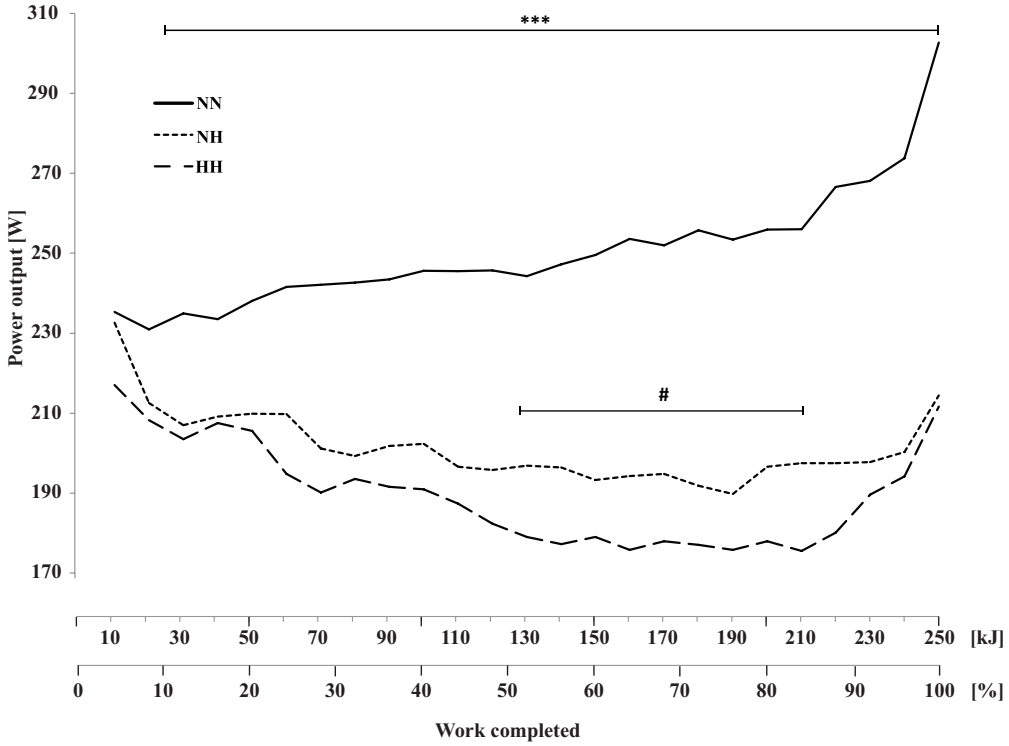

Figure 16. Power output during the self-paced 250-kJ time trial in normobaric normoxia (NN), normobaric hypoxia (NH) and hypobaric hypoxia (HH). ***P<0.001 for difference between NN and both hypoxic conditions; #P<0.05 for difference between NH and HH.

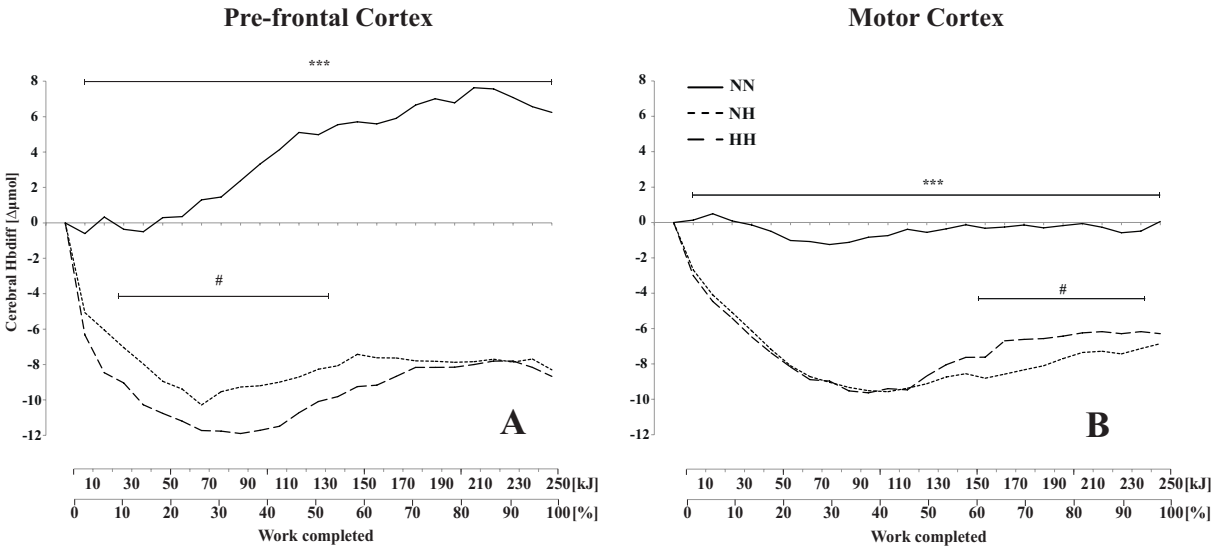

Figure 17 Mean changes in cerebral haemoglobin difference (HbDiff = O_2Hb-HHb) for the pre-frontal cortex **(A)** and the motor cortex **(B)** during the self-paced 250-kJ time trial in normobaric normoxia (NN), normobaric hypoxia (NH) and hypobaric hypoxia (HH). ***P<0.001 for difference between NN and both hypoxic conditions; #P<0.05 for difference between NH and HH.

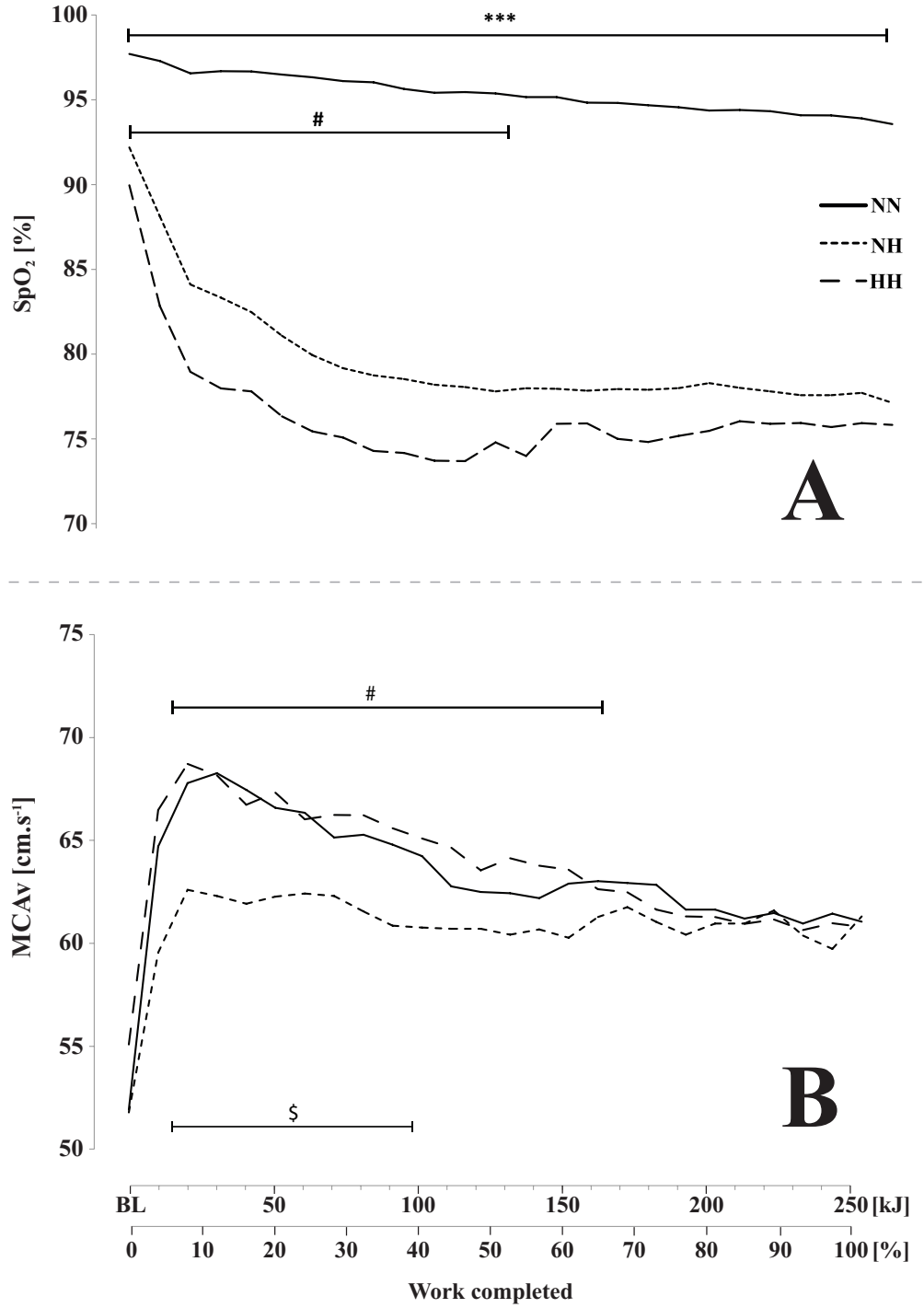

Figure 18 (A) Peripheral oxygen saturation (S_pO_2) and **(B)** middle cerebral artery blood flow mean velocity (MCAv, panel B) during the self-paced 250-kJ time trial in normobaric normoxia (NN), normobaric hypoxia (NH) and hypobaric hypoxia (HH). ***P<0.001 for difference between NN and both hypoxic conditions; $P<0.05 for difference between NN and NH; #P<0.05 for difference between NH and HH.

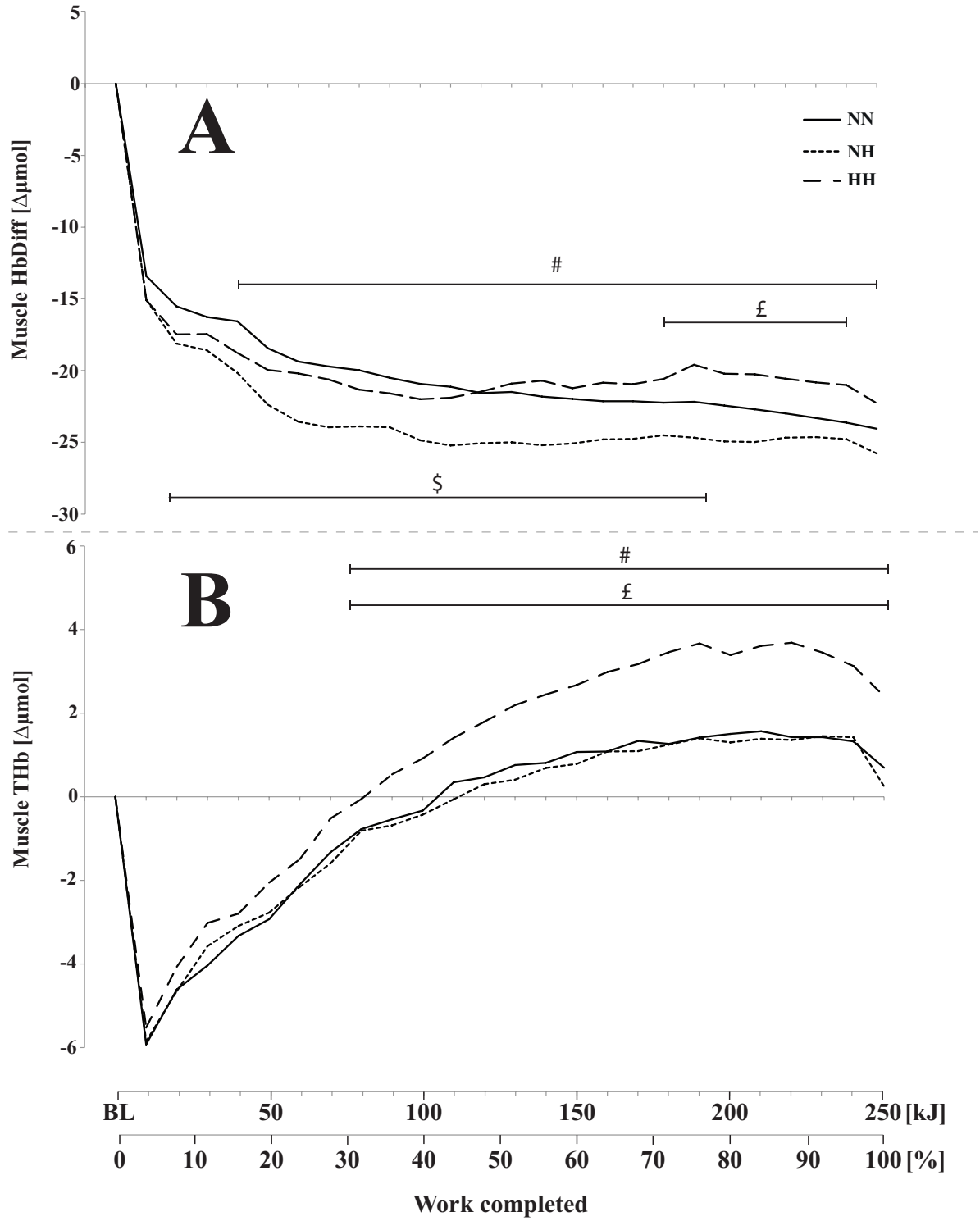

Figure 19. Mean changes in muscle haemoglobin difference (HbDiff = O_2Hb-HHb, panel A), deoxy-(HHb, panel B) during the self-paced 250-kJ time trial in normobaric normoxia (NN), normobaric hypoxia (NH) and hypobaric hypoxia (HH) for the *vastus lateralis*. $^{\$}P<0.05$ for difference between NN and NH; $^{£}P<0.05$ for difference between NN and HH; $^{\#}P<0.05$ for difference between NH and HH.

Chapter Three

Discussion and perspectives

3. Discussion and perspectives

3.1 General Discussion

Spending time at altitude to pursue several recreational mountain sports, such as ski touring, trekking or alpinism, has become increasingly widespread over the past few years. In addition, altitude/hypoxic training is similarly more and more frequently adopted by athletes to enhance their sea level or altitude performances through an improved oxygen transport capacity as a result of a hypoxia-induced erythropoietic response (Millet *et al.*, 2010) or better altitude acclimatization (Fulco *et al.*, 2013).

During the last decade, there has been a growing interest in the following two different types of hypoxia: normobaric and hypobaric hypoxia. It is still highly debated whether both hypoxic conditions bring equivalent patho-physiological responses, despite the recent increasing body of evidence presented above. Therefore, our work focused on direct comparisons of the same subjects under both chronic and acute hypoxic exposures.

First, we investigated both physiological and performance parameters after an 18-day LHTL altitude camp under either normobaric or hypobaric hypoxia, based on the current real practices of endurance athletes (*i.e.,* elite triathletes). We had hypothesized that an LHTL camp conducted under HH would be more demanding regarding the physiological adaptations (*e.g.,* peripheral oxygen levels, haematological parameters, Hb_{mass}, etc.) compared to NH and therefore result in a larger post-hypoxic increase in performance. This project was conducted over two years to complete the first real crossover-designed study on this topic. The crossover design indisputably increased the statistical power of our analysis compared to the first part of the project (Saugy *et al.*, 2014). Our results partially confirmed our hypothesis because larger night desaturation levels and (in our ecological design) longer hypoxic exposures were

observed in the HH condition compared to the NH condition. However, the 3-km running performances were not different between conditions at any measurement point (Figure 13), which was unlike the first phase of the project. Nevertheless, both conditions induced sea-level performance enhancements at Post-21 (*i.e.,* 21 days after returning to sea level) compared to Pre- (*i.e.,* before the LHTL camps). This result is consistent with the highest plateau in performance observed after the altitude training camps (beginning on day 18) described by Sinex & Chapman (2015) and Millet *et al.* (2010). These enhanced performances could be partially explained by the VO_{2max} increases, which were equivalent between the two conditions (4.9 ± 5.6 vs. 3.2 ± 5.1% for NH and HH, respectively). Running economy and efficiency upon return from altitude camp are highly debated (Schmitt *et al.*, 2006; Lundby *et al.*, 2007; Chapman, 2013), but were not involved in the subsequent 3-km running performance in our case, as VO_2, ventilation and the HR modifications were not different between conditions after the LHTL intervention during a maximal aerobic test (*i.e.,* conducted both Pre- and Post-LHTL).

Different sea-level performance enhancements have been observed between the two phases of the project (*i.e.,* larger improvements for HH in the first phase, but no difference in the second phase), but our crossover analysis reduced this trend and highlighted the substantial importance of inter-individual variations in response to altitude training (Friedmann *et al.*, 2005; Garvican *et al.*, 2010; Chapman, 2013; Sinex & Chapman, 2015).

Daily hypoxic exposures were consistent with the common recommended exposures in the literature (8-12 $h.d^{-1}$ (Roberts *et al.*, 2003; Saunders *et al.*, 2004) and 18 $h.d^{-1}$ (Levine & Stray-Gundersen, 1997; Wehrlin & Marti, 2006) in NH and HH, respectively) and induced higher total hypoxic exposures for HH compared to NH (230 vs. 310 h). However, the aim of our work was to construct two LHTL camps with real conditions in an ecological manner to obtain recommendations for athletes and coaches. Moreover, this exposure disparity is not the

central factor for the S_pO_2 difference between NH and HH because it was lower for HH from the first night of exposure and remained stable (*i.e.,* lower) throughout the camps (Figure 12). Moreover, these peripheral oxygen saturations recorded throughout the protocol are central parameters of great importance. To date, no study has directly compared LHTL using both normobaric and hypobaric hypoxia with a crossover design. This result is consistent with previous studies using short exposures and higher altitudes (Savourey *et al.*, 2003; Savourey *et al.*, 2007; Self *et al.*, 2011), but not studies using longer exposures (Roach *et al.*, 1996; Loeppky *et al.*, 2005; Miyagawa *et al.*, 2011; Faiss *et al.*, 2013). The underlying mechanisms inducing this lower S_pO_2 in HH compared to NH were not directly measured, but could be postulated based on previous findings. Greater respiratory frequencies in HH were first reported by Savourey *et al.* (Savourey *et al.*, 2003) and then confirmed in our work (Saugy *et al.*, 2014; Saugy *et al.*, 2016b). Lower tidal volume and minute ventilation values have also been highlighted, implying higher amounts of alveolar physiologic dead space, which is associated with ventilatory alkalosis and hypocapnia in hypobaric hypoxia (Millet *et al.*, 2012; Richard & Koehle, 2012; Faiss *et al.*, 2013). In addition, changes in fluid balance have also been shown, with a significant decrease in plasma volume and higher diuresis for NH (*i.e.,* greater urine volume in NH) (Loeppky *et al.,* 2005). Furthermore, barometric pressure modifies fluid circulation and trans-alveoli-capillary membrane flux (Levine *et al.*, 1988). Taken together, these results might induce a stronger pulmonary vasoconstriction in hypobaric hypoxia and modify oxygen diffusion by decreasing the pressure gradient (Millet *et al.*, 2012). The N_2 and O_2 concentrations in the cerebrospinal fluid could also be influenced by P_B, as well as the central regulation of ventilation (Conkin & Wessel, 2008). Overall, these alterations could be the trigger for the lower mean nocturnal S_pO_2 values reported in the HH group.

Our findings also led us to highlight the differences in the prooxidant/antioxidant balance under LHTL in NH and HH. Lower oxidative stress levels following LHTL in NH were observed compared to HH, which were explained by the lower hypoxic dose and different physiological responses between conditions (Debevec *et al.*, 2015). In addition, the influence of the LHTL paradigm in HH vs. NH on Hb_{mass} was also analysed by our group and resulted in similar values for the same hypoxic dose, with large individual variability in the responses (Hauser *et al.*, 2016).

Our work on chronic hypoxic exposure during both normobaric and hypobaric LHTL hypoxic camps then allows us to conclude that these conditions are definitely not interchangeable (Fulco *et al.*, 2011; Millet *et al.*, 2012; Saugy *et al.*, 2014; Coppel *et al.*, 2015). Slight physiological differences have been highlighted but were apparently not clinically sufficient to induce sea level performance differences. However, regarding the large individual variability in the responses to both hypoxia types, athletes and coaches should definitely consider NH as a surrogate to HH when including altitude training in their complex programmes.

The second major purpose of the present work was to directly compare the physiological parameters and aerobic performances during acute high altitude exposures in either normobaric or hypobaric hypoxia. Regarding the slight physiological differences observed between these hypoxic conditions and the apparently greater severity of HH, we hypothesized that performance at real altitude would be more impaired compared to performance at simulated altitude. With the growing interest in recreational altitude sports and the increasing use of altitude training in national teams and elite athletes, several authors have already compared performance between normoxia and either real or simulated altitude. Nevertheless, it was astounding that only one study directly compared endurance performance after acute

exposure to NH versus HH (Beidleman *et al.*, 2014). Although the latter study was neither a direct comparison with the same subjects nor with the same protocol, it provides some clues to strengthen our hypothesis of a more severe hypoxic stimulus in HH compared to NH. However, as the main cardiovascular, ventilatory and haematologic responses did not differ between conditions during rest or exercise, the mechanisms underpinning the greater performance impairments in HH were not clear and require further investigations. One plausible reason for the lack of physiological differences between the hypoxic conditions in this study could be that the hypoxic exposure was too short (2 hours), particularly when we consider that most of the differences in the physiological adaptations between NH and HH were observed after longer durations. Nonetheless, our results confirmed this greater performance impairment in HH compared to NH, and, for the first time, directly compared identical subjects using the same protocol (Saugy *et al.*, 2016a). Compared to the control condition (*i.e.,* 25 and 33% longer than NN for NH and HH, respectively), the decreases were consistent with the existing literature, which describes an approximately 30% decrease in NH (Bourdillon *et al.*, 2014) versus 40% in HH (Richalet, 2010; Fulco *et al.*, 2011). The main cause of the greater decrease in endurance performance in HH compared to NH (*i.e.,* 8%, Figure 10) seems to be the lower S_pO_2 measured at rest and during exercise in HH (Figure 11), which was already explained above as the modified oxygen diffusion induced by the decreasing pressure gradient between alveoli and capillaries in HH (Levine *et al.*, 1988; Loeppky *et al.*, 1997; Conkin & Wessel, 2008). Furthermore, the amplified oxidative stress reported in HH by Faiss et al. (Faiss *et al.*, 2013) and confirmed by our work (Ribon *et al.*, 2016) might affect NO bioavailability and thus weaken oxygen unloading to the tissues.

Furthermore, the sensitivity to hypoxia determined by cerebral oxygenation (Bourdillon *et al.*, 2014), the changes in muscle activity (Amann *et al.*, 2006a), and the cerebral perturbations that deeply affect motor output and performance in hypoxia (Verges *et al.*, 2012) are all

significant clues that prompted us to further analyse cerebral blood flow and oxygenation, as well as muscular oxygenation.

Thus, we assessed the physiological regulations implying different pacing strategies of these exercises (*i.e.,* distribution of work or energy expenditure through a given exercise duration or distance) to better understand the performance differences between NH and HH. Prolonged self-paced exercise is enjoying a growing success in sport sciences due to its relevance to real competitions (Racinais *et al.*, 2015). Different models have been proposed to explain the regulation of such exercises. Most models involve central and peripheral regulations through different sensitive feedbacks selected by the central nervous system (St Clair Gibson *et al.*, 2006; Edwards & Polman, 2013; Roelands *et al.*, 2013; Renfree *et al.*, 2014). One of the most frequently used pacing concepts was proposed by Tucker (Tucker, 2009), called *the anticipatory feedback rate of perceived exertion (RPE) model.* The aim is to maintain the homeostasis of the athlete so that he/she can finish the exercise using the brain as a central governor (Figure 20).

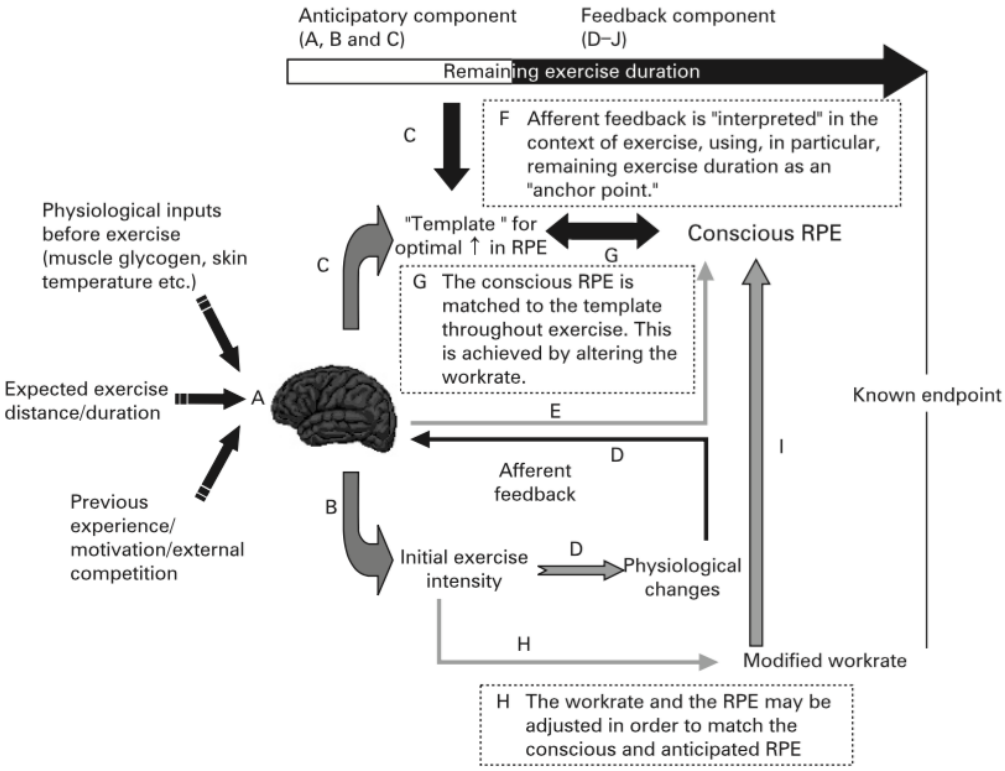

Figure 20. Schematic Figure proposed by R. Tucker to explain the anticipatory regulation of exercise performance during self-paced exercise (*e.g.* time trials). From (Tucker, 2009).

Hypoxia is a major environmental factor that is thought to mediate central motor command, as well as afferent feedbacks at rest and during exercise (Verges *et al.*, 2012; Goodall *et al.*, 2014). Numerous aspects of the supraspinal function, such as cortical oxygenation and cerebral blood flow, seems to have a predominant role in the aerobic performance impairment with hypoxia either with incremental (Subudhi *et al.*, 2009) or constant (Amann *et al.*, 2007) workload. In addition, motor output is likely influenced by an effect on peripheral locomotor muscles through decreased arterial oxygenation (Amann *et al.*, 2006b; Amann *et al.*, 2007; Amann & Calbet, 2008).

Currently, there are only a few studies that have investigated self-paced exercise in hypoxia versus normoxia, and they have suggested minor differences (Clark *et al.*, 2007; Beidleman *et al.*, 2014; Bourdillon *et al.*, 2014) or similar trends (Amann *et al.*, 2006b; Periard & Racinais, 2016). Our results highlighted the different pacing strategies used between NN and the

hypoxic conditions, with reduced power output until an end spurt in hypoxia, whereas a slightly positive trend was observed in normoxia over the same period (Figure 16). In addition, variations in power outputs (as a function of mean power during the trial) from the start to 85% of time trial were increased two-fold in hypoxia compared to normoxia (~22% and ~11%, respectively). These differences in pacing regulation between normoxia and hypoxia agree with some studies on the acute exposure to higher altitude (~5,000 m, NH; (Fan & Kayser, 2013; Bourdillon *et al.*, 2014)) but not with most of the studies that were conducted on acute exposure to lower altitudes for shorter (5-min or 5-km cycling, ~3,200 m in HH or ~2,700 m in NH; (Clark *et al.*, 2007) and (Amann *et al.*, 2006b), respectively), similar (30-min cycling, 3,800 m, HH;(Van Cutsem *et al.*, 2015)) or longer periods (750-kJ cycling, ~3,000 m, NH (Periard & Racinais, 2016)).

Moreover, NIRS is now widely used as an appropriate tool for studying muscle and cerebral haemodynamic and oxygenation responses to exercise with challenging environmental conditions, such as altitude (Verges *et al.*, 2012). However, previous studies almost exclusively considered the pre-frontal cortex (PFC), which synthesizes information from several different brain systems and exerts control over both executive and cognitive behaviours. The PFC is indeed of particular interest in our work, but the central motor drive is ultimately regulated by the premotor and primary motor areas. These cortical regions have never been investigated during self-paced exercise and could give us substantive clues to understand not only pacing and regulation in hypoxia but also the potential differences between self-paced aerobic exercises under both hypoxic conditions (*i.e.,* NH and HH). In addition, Rupp *et al.* recently showed that the PFC and motor cortex (MC) oxygenation profiles could differ during submaximal fatiguing exercise (Rupp *et al.*, 2013). For the first time, we simultaneously studied macro-circulation (*i.e.,* cerebral blood flow) and both PFC and MC micro-haemodynamics and oxygenation during a self-paced exercise. These

innovative assessments give us an exclusive opportunity to further comprehend the underlying mechanisms driving the performance differences between NH and HH, as previously described (Saugy *et al.*, 2016a).

As observed by Beidleman *et al.* (Beidleman *et al.*, 2014), the power outputs followed the same trend under both hypoxic conditions. However, where they only found a trend towards a lower power in HH compared to NH (P=0.08), in our case, the difference was great enough to become significant in the second part of the time trial (Figure 16). The reason why it was only a trend for Beidleman *et al.* could be the lower pre-exposure duration (approximately 2 hours) in their study.

A closer look at the different physiological parameters measured during the exercise allows us to better understand their regulation. First, the initial power output was the same under both hypoxic conditions (Figure 16), although a lower S_pO_2 was observed for HH at rest and during the first part of the TT (Figure 18A). In addition, cerebral oxygen delivery (cDO_2) remained matched between NH and HH, due to higher vasodilatory compensation in HH, where hypoxemia is significantly enhanced (Figure 18B). Moreover, the prefrontal cortex oxygenation was further diminished compared to NH (Figure 17A); thus, subjects must decrease their power production to prevent a larger deoxygenation of the brain when the cerebral blood flow is substantially decreased in HH. In addition, a lower work rate was achieved by the alteration in the degree of skeletal muscle recruitment (*i.e.,* lower iEMG activity for HH) in accordance with a previous result (Peltonen *et al.*, 1997), corresponding to the lower MC activity (Figure 17B) and lower muscle deoxygenation (Figure 19, *i.e.,* lower mechanical and energetic demands).

Based on all these results, it seems that aerobic exercises in NH and HH are not regulated in the same way. Several physiological mechanisms are involved to allow the maintenance of

acceptable values and prevent the brain from disturbances or damage. The system seems to be regulated in a protective way, according to the central governor model proposed by Tucker (Tucker, 2009). Nevertheless, considering the higher adaptive cost of hypobaric hypoxia induced by a more severe environment, the strategies adopted need to be more protective.

The third intent of the present work was to directly compare sleep-disordered breathing parameters during the night in either normobaric or hypobaric hypoxia both at chronic and acute altitudes. The addition of altitude to their training programmes implies that athletes will spend time sleeping in hypoxic environments, which seems to have an impact on respiratory physiology in both NH (Berssenbrugge *et al.*, 1983; Tamisier *et al.*, 2009) and HH (Bloch *et al.*, 2010). Our work confirmed that both hypoxic conditions induced a decreased sleep quality and an increase nocturnal breathing disturbances. The main finding is that the addition of hypobaria to hypoxia (*i.e.,* HH) amends sleep quality and nocturnal breathing to a larger extent than hypoxia alone (*i.e.,* NH). This result was observed after several hours spent at high altitude (Heinzer & Saugy, 2016) and during LHTL camps using chronic, moderate altitude exposures (Saugy *et al.*, 2016b).

The larger nocturnal S_pO_2 decrease observed in HH compared to NH after one night of acute hypoxia (3,450 m) agreed with previous studies (Saugy *et al.*, 2014; DiPasquale *et al.*, 2015a, b). The potential underlying mechanisms for this lower peripheral oxygen saturation in HH have already been explained in detail in this discussion, but it is important to consider that the mechanisms remain speculative. The breathing frequency was not different between conditions during our study conducted in acute hypoxia, but differences in breathing pattern have already been observed (Saugy *et al.*, 2014; Coppel *et al.*, 2015; Saugy *et al.*, 2016b), with a higher breathing frequency in HH, even if this point is still debated (Coppel *et al.*, 2015).

Both hypoxic conditions induced night respiratory events (*i.e.,* central apneas and hypopneas), and they might be associated with a hypoxia-induced increase in higher loop gain with greater chemoreceptor sensitivity (Edwards *et al.*, 2014). However, the breathing and respiratory disorders seem more severe in HH compared to NH, with a higher apnea-hypopnea index (AHI) and more hypopnea in acute hypoxia. Moreover, the oxygen desaturation index 3% (*i.e.,* number of times per hour of sleep that S_pO_2 level drops by 3 percent or more, ODI 3%) was increased in both acute (Heinzer & Saugy, 2016) and chronic hypobaric hypoxia during LHTL (Saugy *et al.*, 2016b; Saugy *et al.*, 2016c) compared to an equivalent P_iO_2 in normobaric hypoxia. This oxygen desaturation index is considered a consistent marker of sleep apnea (Hang *et al.*, 2015) and significantly decreases the sleep quality, leading to disordered sleep.

Our present work on sleep-disordered breathing in either acute or chronic NH versus HH showed that differences also exist in the field of sleep patterns between the tested hypoxic conditions. Real altitude seems to have a greater impact on nocturnal breathing and sleep structure than simulated altitude induced by reducing the inspired oxygen fraction (*i.e.,* NH). The main trigger for these differences seems the same as the trigger for the previous performance disparities, namely alterations in peripheral oxygenation. One cannot exclude the possibility that NO metabolism has also an important role in these differences. Hence, our results add significant elements to the growing body of evidence stating that NH and HH cannot be used interchangeably (Fulco *et al.*, 2011; Millet *et al.*, 2012; Saugy *et al.*, 2014; Coppel *et al.*, 2015; Ribon *et al.*, 2016; Saugy *et al.*, 2016a).

3.2 Potential mechanisms

One cannot rule out the possibility that the potential underlying mechanisms that were not measured in the present work could induce the above-described differences between normobaric and hypobaric hypoxia. Therefore, it is important to consider them in further studies in this field. Some of the main conceivable clues that are yet to be explored are presented here. However, these parameters have not yet been compared between NH and HH, and future studies are required to assess their hypothetical responsibilities in the differences between hypoxic conditions.

3.2.1 Ventilation-Perfusion inequality

Inhomogeneous pulmonary vasoconstriction could lead to greater ventilation-perfusion inequality, due to the changes in NO metabolism described above. The relative contribution of each factor to the efficiency of gas exchange in humans during exercise in hypoxic environments has been studied for a long time (Gale *et al.,* 1985). It is now well settled that pulmonary gas exchange efficiency, which is determined by the alveolar-to-arterial PO_2 difference (A-aDO_2) and thus incorporates contributions from pulmonary (*i.e.,* alveolar ventilation-to-perfusion inequality, diffusion limitation, and the intrapulmonary right-to-left shunt) and non-pulmonary factors (*i.e.*, the extrapulmonary right-to-left shunt), is progressively exacerbated (*i.e.*, the A-aDO_2 increases) during exercise at sea level in a workload-dependent manner (Dempsey & Wagner, 1999). This impairment in pulmonary gas exchange proficiency during exercise is exacerbated in acute hypoxia, such that for any given VO_2, the A-aDO_2 is greater than the value observed during exercise while breathing room air at sea level (Wagner *et al.,* 1986). Although the overall topographical distributions of perfusion and ventilation in the lungs become more uniform with exposure to high altitude

(Dawson, 1972), there is evidence suggesting that the functional ventilation-perfusion relationships are actually reduced at high altitude (Haab *et al.,* 1969).

3.2.2 Patent foramen ovale

The patent foramen ovale (PFO) is interatrial communication that allows the blood flow to bypass the pulmonary circulation during foetal life. The initiation of breathing room air causes a reduction in pulmonary vascular pressure, which reverses the atrial pressure gradients such that the foramen ovale closes in the majority of people. However, PFO prevalence has been suggested to be as high as 38% in the general population (Woods *et al.,* 2010). It has been suggested that a shunt through a PFO can be as high as 21% of the cardiac output (Devuyst *et al.,* 2004). Recently, Lovering *et al.* explored the consequences of an intra-cardiac right-to-left shunt via the PFO in healthy humans during exercise while breathing room air and in acute normobaric hypoxia at sea level (Lovering *et al.,* 2015). During these investigations, it became obvious that the presence of a PFO could be critical to the interpretation of studies where pulmonary gas exchange efficiency is a key parameter. Moreover, the contribution of this right-to-left shunt has been shown to be reduced at altitude and may only partially explain the lack of improvement in pulmonary gas exchange efficiency at 5,260 m (Elliott *et al.*, 2015). In this study, subjects with a PFO exhibited a less pronounced degree of ventilatory acclimatization to 5,260 m, as determined by the significant increase in P_aCO_2 and concomitant increase in A-aDO_2 and decrease in P_aO_2 and S_aO_2.

3.3 Conclusion

Our work intended to provide new insights into the potential differences between normobaric and hypobaric hypoxia and their underlying physiological mechanisms. We first highlighted the conclusive differences between both hypoxic conditions during chronic moderate altitude

exposure using a complete and well-controlled crossover study of Live High-Train Low camps. A greater decrease in the peripheral oxygen saturation level was observed in HH, and one of the underlying mechanisms seems to be NO metabolism (Debevec *et al.*, 2015). However, the physiological differences between NH and HH do not appear to be clinically sufficient to induce sea level performance distinctions. Nevertheless, the type of hypoxia may now systematically be reported and considered by athletes and coaches when they are contemplating including altitude training in their programmes.

Second, our work on direct comparisons of acute exposure to high altitude exposure also highlighted the physiological differences between the two hypoxic conditions, which were substantial enough to induce greater performance impairments when the subjects were exposed to real altitude. Once again, peripheral oxygen saturation seems to be a main factor, in addition to muscle oxygenation, cerebral blood flow and oxygenation. NO metabolism and oxidative stress are also involved in these differences (Ribon *et al.*, 2016). Finally, the present work shows that hypobaric hypoxia has a greater impact on sleep structure and nocturnal breathing.

Regarding the strong reliability of our results, which used direct comparisons with crossover studies for the first time, we added substantial insights into the growing body of evidence claiming that normobaric and hypobaric hypoxia cannot be used interchangeably.

3.4 Perspectives

We did not state that one hypoxic condition is better than the other, instead each condition is different and has advantages and drawbacks for coaches and athletes from a practical point of view. HH leads to longer hypoxic doses for a given training period, which could be easier for

coaches to include in the complex training plans for elite athletes, but the costs and logistics could be harmful. In contrast, NH requires longer time periods to obtain sufficient hypoxic doses, but it provides individualization opportunities. An interesting perspective would be to conduct altitude-training camps where the hypoxic dose could be adjusted depending on the athlete's physiological responses and training levels.

In addition, we showed that acute exposure to hypoxia in NH versus HH does not result in the same physiological adaptations. However, it is still not clear whether the combination of hypoxic stimulus and lowered barometric pressure induced such differences or if the modified pressure itself could induce significant adaptations. It would be interesting to make assessments using every possible combination (*i.e.,* normobaric hypoxia, normobaric normoxia, hypobaric hypoxia and hypobaric normoxia) under the same experimental conditions (*e.g.,* using a hypobaric chamber and hypoxic/hyperoxic devices) to further understand the underlying physiological mechanisms. These considerations could have important implications for aerospace physiology.

Finally, anti-doping is a constant battle, and the Athlete Biological Passport (ABP) is actually the best way to detect cheaters. However, the ABP principally monitors an athlete's biological variables over time to identify abnormal biases on a longitudinal basis, and several factors are known to influence the results of these markers. Altitude is one of these factors, and the method by which it is evaluated still needs to be standardized. This standardization is even more crucial in the current era, due to the wide combination of hypoxic methods being developed. Causal relationships between the haematological variables and the use of hypoxia should be properly integrated into ABP analyses. In particular, modifications of

haematological parameters during and after exposure to different altitudes/hypoxia protocols (*i.e.,* using either NH or HH) must be properly considered in detection models.

Chapter Four

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4. References

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Chapter Five

Article 1 - Comparison of "Live High-Train Low" in Normobaric versus Hypobaric Hypoxia.

5. Article 1- Comparison of "Live High-Train Low" in Normobaric versus Hypobaric Hypoxia.

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Abstract

We investigated changes in both performance and selected physiological parameters following a Live High-Train Low (LHTL) altitude camp in either normobaric hypoxia (NH) or hypobaric hypoxia (HH) replicating current 'real' endurance athletes' practices. Well-trained triathletes were split into two groups (NH, n=14 and HH, n=13) and completed an 18-d LHTL camp during which they trained at 1100-1200 m and resided at 2250 m (P_iO_2 =111.1 ± 1.1 vs. 111.5 ± 1.0 mmHg) under either NH (hypoxic chamber; F_iO_2 17.9 ± 0.2%) or HH (real altitude; barometric pressure 580 ± 23 mmHg) conditions. Oxygen saturations (S_pO_2) were recorded continuously daily overnight. P_iO_2 and training loads were matched daily. Before (Pre-) and 1 day after (Post-) LHTL, blood samples, VO_{2max}, and total haemoglobin mass (Hb_{mass}) were measured. A 3-km running-test was performed near sea level twice before and 1, 7, and 21 days following LHTL. During LHTL, hypoxic exposure was lower for NH than HH group (220 vs. 300 h; P<0.001). Night S_pO_2 was higher (92.1 ± 0.3 vs. 90.9 ± 0.3%, P<0.001), and breathing frequency was lower in NH than HH group (13.9 ± 2.1 vs. 15.5 ± 1.5 breath.min^{-1}, P<0.05). Immediately following LHTL, similar increases in VO_{2max} (6.1 ± 6.8 vs. 5.2 ± 4.8%) and Hb_{mass} (2.6 ± 1.9 vs. 3.4 ± 2.1%) were observed in NH and HH groups, respectively, whilst 3-km performance was not improved. However, 21 days following LHTL, 3-km run time was significantly faster in HH (3.3 ± 3.6%; P<0.05) *versus* NH (1.2 ± 2.9%; ns) group. In conclusion, the greater degree of race performance enhancement by day 21 after an 18-d LHTL camp in HH group was likely induced by a larger hypoxic dose. However, one cannot exclude other factors including differences in sleeping desaturations and breathing patterns, thus suggesting higher hypoxic stimuli in HH.

Introduction

Live High - Train Low (LHTL) training camps are commonly used by athletes under either normobaric hypoxia (NH) [1-5] or hypobaric hypoxia (HH) [6-10] conditions. These two types of hypoxia are obtained by the combination of a lowered value of barometric pressure (PB) and/or a reduced inspired fraction of oxygen (F_IO_2) (NH: F_IO_2 < 20.9%; PB = 760 mmHg vs. HH: F_IO_2 = 20.9%; PB < 760 mmHg) resulting in an inspired partial pressure of oxygen (P_IO_2) less than 150 mmHg. NH and HH were, until recently, thought to be interchangeable since P_IO_2 was assumed as the only factor influencing the physiological responses to hypoxia [11]. This "*equivalent air altitude model*" [12] has now been criticized and a growing body of literature has reported physiological differences between acute exposures to NH and HH [13,14]. Specifically, acute mountain sickness (AMS) symptoms are less severe under NH than HH conditions [15]. Pre-acclimatisation at real altitudes (HH) resulted in a significant decrease in the severity of AMS under HH conditions [16], which was not the case in individuals subjected to pre-acclimatisation under NH conditions [16]. Furthermore, according to Fulco *et al.*, 2011, NH and HH could not "*be used interchangeably*" and do not exhibit the same levels of effectiveness relative to pre-acclimatisation strategies for the prevention of AMS (*e.g.*, significant decrease in the severity of AMS under HH conditions following pre-acclimatization under HH but not NH) and for the improvement of exercise performance at higher altitudes [16]. In addition, minute ventilation was lower under HH than NH conditions and was associated with the combination of lower tidal volumes and higher respiratory frequencies [17]. Interestingly, oxidative stress markers were also elevated when individuals were continuously exposed to HH conditions in reference to NH for 24 h, whereas nitric oxide (NO) in exhaled air and plasma was lower under HH *versus* NH [18]. Moreover, exhaled NO and NO end-products (NO_x) decreased in HH but remained stable in NH [18]. Finally, differences in fluid balance were highlighted by

larger volumes of urine excretion and significant decreases in plasma volume under NH conditions [15]. While the afore-mentioned studies support our recent suggestion that "*HH is a more severe environmental condition*" [14], this would also make the assumption that larger physiological adaptations would occur after prolonged hypoxic exposure under HH compared to NH conditions realistic. This still needs to be demonstrated in research.

Of interest is that the training practices of athletes are dependent upon hypoxic conditions, which reflect protocols described in the literature. It has indeed been reported that: 1) daily hypoxic exposure is shorter in NH (*i.e.* 8-12 $h.d^{-1}$; [4,5]) compared to HH (*i.e.* 16-18 $h.d^{-1}$ in HH; [7,19]) during LHTL protocols; 2) total hypoxic dose is reduced accordingly in NH (*i.e.* ~150-300 h; [1,4,5,20,21]) compared to HH (*i.e.* 300-600 h; [9,22-25]); 3) total camp duration varies between NH (0 to 23 days [2,3,26]; and HH (13 and 28 days; [9,22-25]). Moreover, the mean performance improvements (*i.e.* power output increases) following LHTL were lower when the intervention was completed under NH compared to HH conditions (0.6% vs. 4.0%) [27]. Finally, most of the LHTL studies conducted under NH conditions did not elicit any performance improvement, although some induced positive erythropoietic responses [1,28], and most of the studies reporting both performance and erythropoietic enhancements were performed under HH conditions [19,29]. However, these findings of the differences in the physiological responses to NH vs. HH, which suggest larger adaptations in the HH condition, are based only on short-term hypoxic exposure. To the best of our knowledge, no study to date has directly compared altitude-induced adaptations (*i.e.* haematological, peripheral oxygen saturation, etc.) and performance changes following LHTL training camps under both NH and HH conditions. This is an important issue as the development of NH facilities (*e.g.,* nitrogen houses; hypoxic rooms, etc.) worldwide is increasing, and since coaches and athletes often consider NH and HH conditions to provide the same hypoxic stimulus. The issue of

whether additional benefits occur by LTHL using HH conditions rather than NH conditions has never been directly investigated. Therefore, the present study aimed to compare the physiological responses and the performance gains in trained triathletes during and after LHTL camps with matched P_iO_2 in NH versus HH conditions. Of importance is that we replicated common or 'real' altitude training practices of endurance athletes (*e.g.*, daily exposure, total hypoxic doses under NH and HH conditions, respectively). We hypothesised that a LHTL intervention conducted under HH compared to NH was more detrimental to the physiological adaptations (*e.g.,* desaturation level, Hb_{mass}, haematological parameters, breathing pattern, etc.) and the post-hypoxic increases in performance.

Methods

Experimental Design. Our experimental design (Figure 1) consisted of a 33-week period divided into the following four phases: 1) 24 weeks (January to May) were completed at sea level where training loads were quantified; followed by 2) a 3-wk lead-in period at sea level during which all training sessions were supervised and loads were quantified; 3) an 18-d LHTL training camp under either NH or HH conditions; and 4) a 3-wk post-altitude period at sea level during which all training sessions were once again supervised and loads were quantified.

Two groups (NH, n=14 and HH, n=13) were matched based on the VO_2 max values prior to the training camp and completed an 18-d LHTL camp during which all athletes trained at 1100-1200 m and resided at an altitude of 2250 m (P_IO_2=111.1 ± 1.1 vs. 111.5 ± 1.0 mmHg) under either NH (hypoxic house; exposure 12.2 ± 0.3 $h.d^{-1}$; F_iO_2 17.9 ± 0.2%, Prémanon, France) or HH conditions (real altitude; 16.8 ± 3.1 $h.d^{-1}$; barometric pressure 580 ± 23 mmHg, Fiescheralp, Switzerland). Normobaric hypoxia was obtained by extracting oxygen from ambient air (OBS, Husøysund, Norway). Calculations of F_IO_2 values corresponding to the

required altitude took into account the altitude of Prémanon. As gas compositions were continuously monitored, O_2 fractions were permanently adjusted during sessions in order to maintain stability. Moreover, it was determined that opening the door several times for periods of a few seconds did not change F_IO_2 values. For safety reasons, O_2 and CO_2 compositions were monitored. Each room was connected to a central monitoring station under the control of an independent investigator.

The NH group was split into smaller groups of seven athletes and alternated using the hypoxic chamber, whereas the HH athletes went into the valley twice daily via cable car to perform the training.

Before (Pre-) and 24 h after (Post-) the LHTL period, several physiological tests were performed on both groups in the same location (Prémanon, France, 1150 m). These sessions were conducted in a well-ventilated laboratory (temperature 22 ± 1 °C) in the same order and at the same time of the day in the Pre- and Post- testing condition. Measurements included blood samples, anthropometric measurements, maximal incremental tests on an cycle ergometer (VO_{2max}), and total haemoglobin mass (Hb_{mass}) assessments. Subjects performed five 3-km running tests at the following times: prior to lead-in, before LHTL, after LHTL, seven days after LHTL (Post-7), and twenty one days following LHTL (Post-21). All 3-km running tests were performed near sea level (100-390 m).

Subjects. Twenty-seven well-trained male triathletes living at or near sea level (age 23 ± 4 years, body height 179 ± 5 cm, body weight 71 ± 7 kg, fat mass 10.1 ± 1.6%, and VO_{2max} 66.9 ± 8.4 $mL \cdot kg^{-1} \cdot min^{-1}$) participated in this study. Three subjects were excluded following the lead-in period due to insufficient training loads and fitness. All subjects were non-smokers who had not been acclimatised or recently exposed to testing altitudes. Volunteers provided their written, voluntary, informed consent before participation. The experiment was approved

by a Medical Ethics Committee (Commission Cantonale Valaisanne d'Ethique Médicale, CCVEM; Agreement 051/09) and performed in accordance with the Declaration of Helsinki.

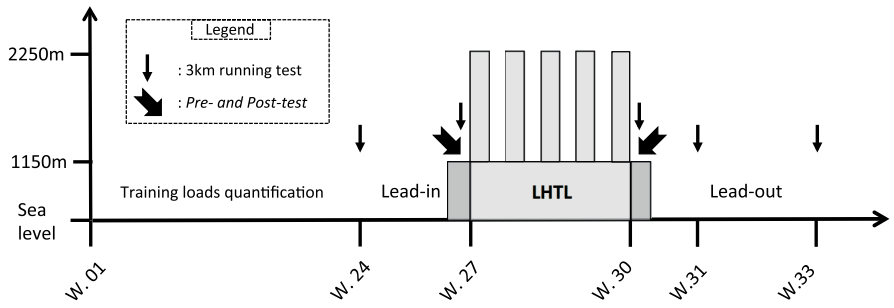

Figure 1. Overview of the study design separated by weeks (W.) and in order of the testing altitude, including the six months before the lead-in period where the training loads were assessed, the lead-in, the LHTL camp of 18 days, and the lead-out period. Testing included 3-km test = the 3-km running tests on the track near sea level; Pre-, Post-, Post-7 and Post-21 = testing sessions; LHTL= Live High-Train Low training camp for normobaric hypoxia (NH) and hypobaric hypoxia (HH), where athletes were exposed to both higher altitude for living and sleeping and lower altitude for training.

Measurements

Training content and training loads. Two experienced certified coaches supervised and advised the athletes during all training sessions of the lead-in period and matched training content and loads for both groups during the LHTL period. Additionally, each subject's daily training loads were quantified individually by both subjective and objective means to evaluate their effects on physiological adaptation and each subject's subsequent performance. Training load quantification was performed using the 'Objective Load Scale' (ECOs) [30]. The training loads for the triathletes included in our study were similar to those described in other studies involving endurance athletes. The Objective Load Scale allowed for the quantification of all training loads in each sport of the triathlon (swim, bike, run, and transitions). The daily and weekly training loads (ECOs) of each subject were quantified based on each subject's physical characteristics and training program intensity. Volume was quantified by time and allowed for better comparisons of different performance levels and conditions (*e.g.,* ground

surface, environmental temperature) [30].

3-km run test. The 3-km running tests were completed on a 400 m outdoor synthetic track near sea level. To avoid any group or pacing influences, starts were given in time-trial form (*e.g.,* 30 s between each start and randomisation of the order in which each athlete competed).

Incremental cycling test. VO_{2max} was tested before and after LHTL using subjects' own bicycles, which were linked to a computerised ergometer system (Cyclus 2, RBM elektronik-automation GmbH, Leipzig, Germany). The exercise protocol began with a warm-up period of 5 min at a workload of 90 W. The workload was subsequently increased by 30 $W \cdot min^{-1}$ until voluntary exhaustion. During the final minutes of the test, subjects were strongly encouraged to perform until they reached maximal exhaustion and had achieved VO_{2max} based on the standard criteria for all tests. Each subject wore a mouthpiece and nose clip for breath collection. O_2 and CO_2 levels in expired gas were continuously measured and monitored as breath-by-breath values (Ultima Cardio 2 gas exchange analysis system, MGC Diagnostics with Breezesuite software, Saint Paul, MN, USA). Both the gas analyser and the flowmeter of the gas analyser were calibrated prior to each test. The highest 30 s average value served as the VO_{2max}. Maximal heart rates (HR_{max}) and the lowest S_pO_2 values were each recorded during the same time period. The maximal power output (P_{max}) was the load of the last stage completed.

Anthropometrics values. Athletes' body weights and heights were measured in the morning before breakfast.

Haemoglobin mass. Hb_{mass} was measured in duplicate by using a slightly modified version [31] of the optimised carbon monoxide (CO)-rebreathing method described by Schmidt and Prommer [32]. The subjects inhaled a bolus of 100 mL of pure CO (Multigas SA, Domdidier, Switzerland), followed by 3.5 L of oxygen. Each Hb_{mass} measurement was performed in duplicate on two consecutive days (12- to 24-h time lag between measurements). In average

over all measurement time points, the typical error for duplicate Hb_{mass} measurement was 2.1% in our mobile laboratory. Hb_{mass} data are expressed as the mean values of the duplicate measurements.

Values of red cell volume (RCV), blood volume (BV), and plasma volume (PV) were estimated using the following formulas: RCV = Hb_{mass}/MCHC x 100, BV = RCV x (100/Hct) and PV = BV – RCV, where MCHC is the mean corpuscular haemoglobin concentration, and Hct is the haematocrit corrected to whole-body haematocrit by the cell factor of 0.91. For the calculation of RCV, BV, and PV, venous haemoglobin concentrations [Hb] and venous Hct were used. Blood gas analyses were conducted using an ABL 800flex (Radiometer A/S, Copenhagen, Denmark).

Blood samples. Antecubital vein blood samples (4.9 mL EDTA tube, Sarstedt, Nümbrecht, Germany) were taken during three time periods including either before breakfast or immediately after waking up, before LHTL, and after LHTL. Blood was subsequently analysed via fluorescent flow cytometry and hydrodynamic focusing (XT-2000i, Sysmex Europe, Norderstedt, Germany), and the following primary haematological parameters were quantified: red blood cells (RBC), haemoglobin (Hb), haematocrit (Hct), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), reticulocyte percentage (RET%), absolute number of reticulocytes (RET#), and immature reticulocyte fraction (IRF). The Sysmex XT-2000i underwent regular internal quality control procedures as required by the standards of laboratory medicine. During the period of our study, the coefficient of variations (CV), which was determined using internal quality controls, was far below 1.5% for Hb and 1.5% for RET% (within CV limits accepted by the manufacturer of the instrument). Plasma EPO was quantified using a standard procedure with an ELISA kit (Stemcell Technologies, Grenoble, France). Plasma EPO concentrations below the limit of quantification (1.6 mU/mL) were excluded from the analyses. CVs determined by

three internal quality controls (levels: low, medium and high) were below 15% in our WADA (World Anti-Doping Agency) accredited laboratory [33]. All plasma samples were analysed in duplicate, where the mean values of the duplicate were used for this study. Additionally, baseline ferritin was quantified using standard laboratory procedures (Dimension EXL, Siemens Healthcare Diagnostics SA, Zürich, Switzerland) to evaluate the subject's iron stores. It is important to note that all athletes were tested for doping by the accredited laboratory according to the standards of the biological passport. This was done to avoid performance enhancement via doping.

Questionnaires. Subjects completed three different questionnaires on a daily basis immediately after waking up (hypoxic rooms for NH and normal rooms for HH, but all were hypoxic) and during three phases before, during, and after the LHTL training camp. The three questionnaires were as follows: 1) *The Lake Louise score questionnaire, 2) The Daily Analysis of Life Demands for Athletes (DALDA), and 3) Sleep assessment questionnaire.*

The Lake Louise score questionnaire is scoring system developed by the 1991 International Hypoxia Symposium consensus committee, which met at Lake Louise in Alberta, Canada. It is widely used today to assess the severity of AMS. The DALDA is a self-reported sport-specific tool describing the stress sources and characteristics of each person, which allows for the differentiation of the individuality of stress responses. This questionnaire is divided into two parts; Part A describes the general stress sources that occur in everyday life for an athlete (diet, home life, school, work, friends, training, climate, sleep, recreation, and health); and Part B determines which symptoms of any existence in stress reactions of the athlete. The sleep assessment questionnaire was the Groningen Sleep Quality Scale (GSQS), which was used to evaluate for high altitude sleep (HAS) disturbances. It consists of a sleep quality score (GSQSS) and two visual analogue scales (VAS), which yield a score between 0 and 10 for sleep quality and waking state.

Sleep assessment. S_pO_2 and HR were recorded each evening at 0.25 Hz with a wrist oximeter connected to a finger sensor (Wristox 3150 with 8000SM-WO Sensor, Nonin, Plymouth, MN). Subjects wore an instrumented t-shirt (model SEW, CSEM, Neuchâtel, Switzerland) each night (including the 2 nights before and the 2 nights after LHTL), a device made of comfortable fabric that was used to measure breathing frequency via an elastic sensor included in the textile, as well as each subject's sleeping position via accelerometers.

Data Analysis and Statistics. Data are reported as the means and standard deviations. Data were tested for equality of variance (Fisher-Snedecor *F-test*) and for normality (Shapiro-Wilk test). When both conditions were met, a two-way ANOVA was performed for repeated measures for each condition (NH and HH) to determine time effects for variables measured on several occasions during the camps with pairwise multiple comparison procedures (Holm-Sidak method). Differences between results obtained before and after LHTL for both the NH and HH groups were subsequently also compared using a two-way ANOVA. Differences in percentage changes between the groups were tested with a Wilcoxon signed rank sum test. When either equality of variance or normality were not satisfied, variables were analysed for each condition using a Friedman test for repeated measures to determine time effects using pairwise multiple comparison procedures (Bonferroni test). In this case, differences between the NH and HH groups at baseline (Pre-) were tested using a Mann-Whitney rank sum test. The correlation between values of Hb_{mass} initial (in g) or pre-to-post change (in %) and VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$) as well as correlations between all haematological and physiological parameters were calculated via the Pearson product moment correlation. Null hypotheses were rejected at $P<0.05$. All analyses were completed using Sigmaplot 11.0 software (Systat Software, San Jose, CA).

Results

Training loads. No differences were found in daily or average training loads between the two groups during the 18-d LHTL camp (232 ± 159 vs. 217 ± 129 ECOs for the NH and HH groups, respectively; Figure 2A). Additionally, no differences were found in weekly training loads monitored during the 6 months prior to the study (1161 ± 130 vs. 1208 ± 168 ECOs per week for the NH and HH groups, respectively; Figure 2B), nor were any differences noted during the lead-in period or the post-hypoxic period.

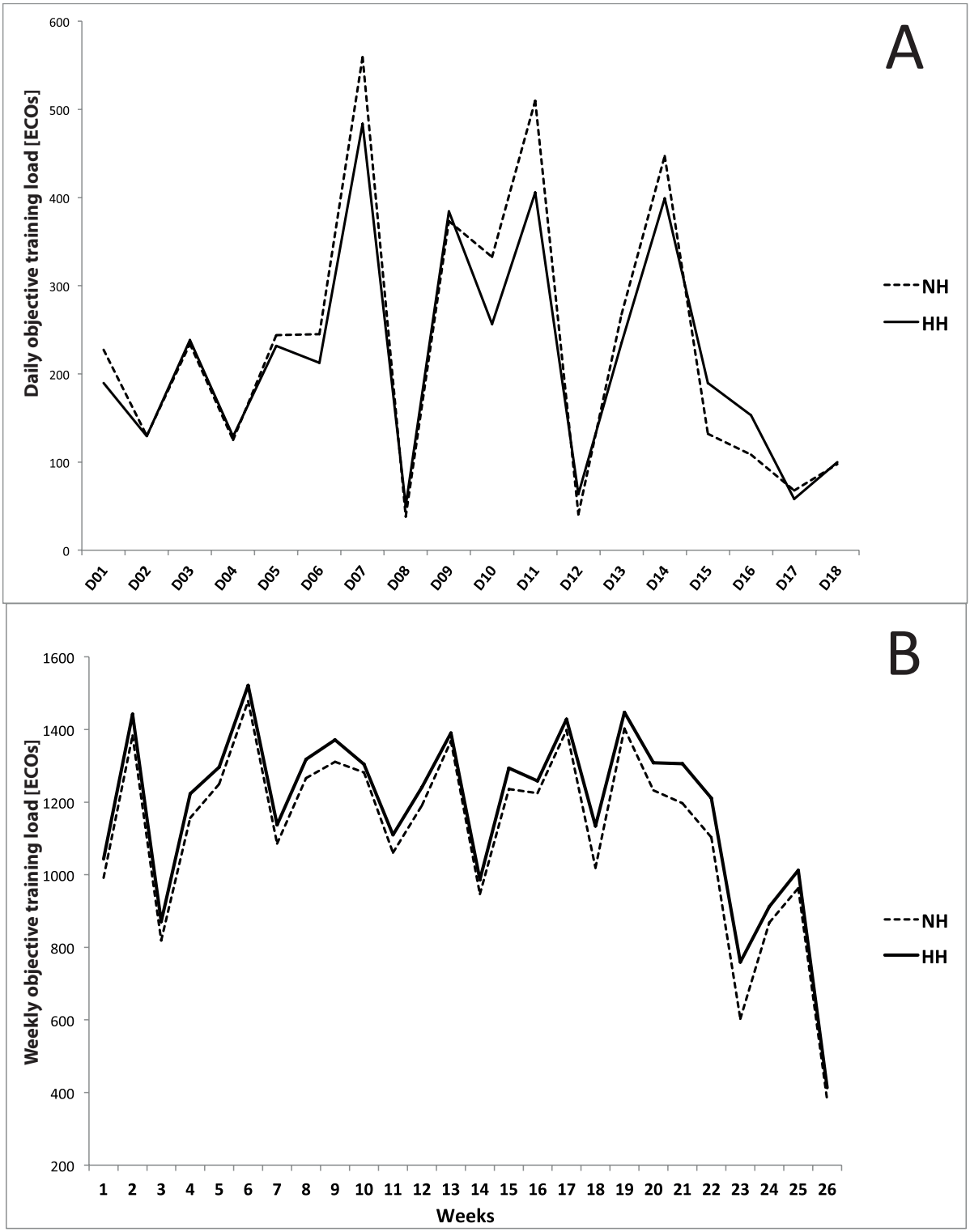

Figure 2. A. Daily objective training (D01-D18: day 01 to day 18) loads during the Live High-Train Low (LHTL) camp for normobaric hypoxia (NH) and hypobaric hypoxia (HH) groups. **B**. Weekly objective training loads during the six months before the intervention for NH and HH groups.

3-km performance test. Compared to Pre-, 3-km performance remained unchanged at Post- and Post-7 in both groups. Whereas run performance did not improve significantly in the NH

group (630.1 ± 64.8 vs. 621.8 ± 54.8 s, P>0.05) from Pre- to Post-21, however, faster 3-km run times occurred in the HH group (611.1 ± 48.5 vs. 588.3 ± 32.2 s; -3.3 ± 3.6%, P<0.05, Figure 3). No differences were found between the groups during the lead-in period, before LHTL, after LHTL, or 7 days post-LHTL. In addition, no differences were found between Lead-in and Pre- for both groups (626.3 ± 63.8 vs. 630.1 ± 64.8 s and 602.4 ± 44.3 vs. 611.1 ± 48.5 s, for NH and HH at Lead-in vs. Pre-, respectively).

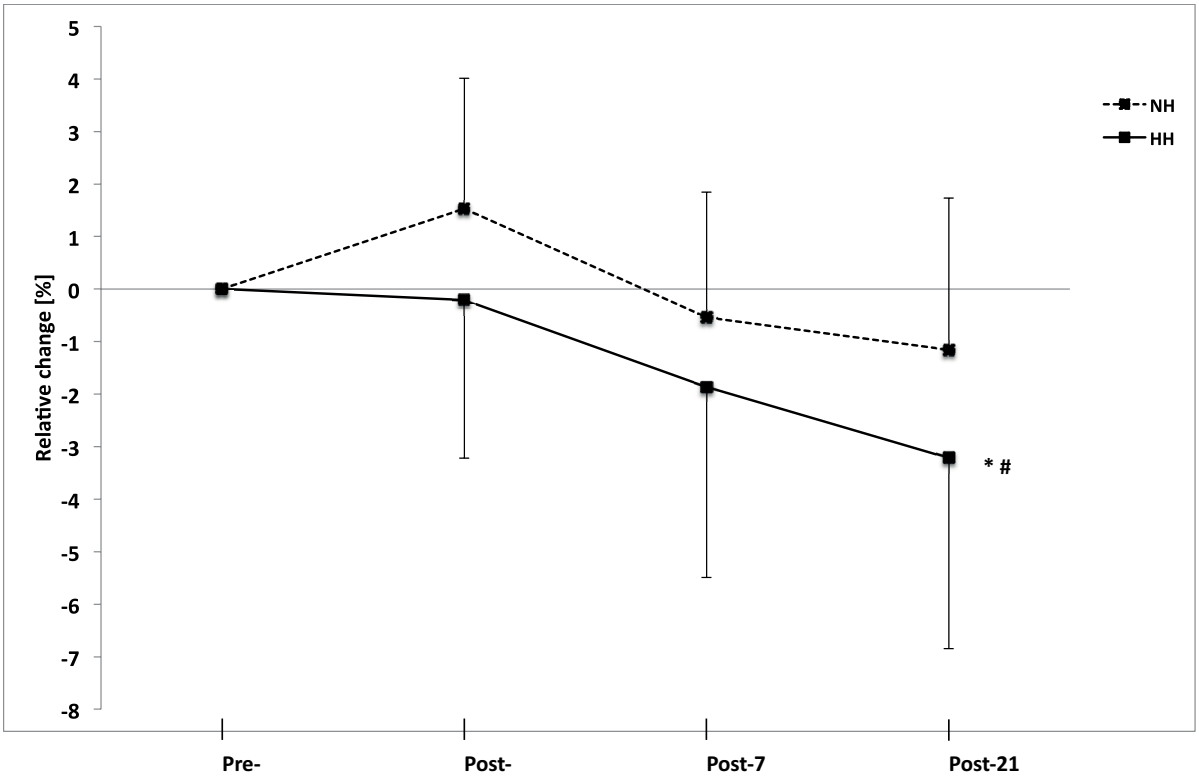

Figure 3. Relative change in 3-km run time from Pre- to Post-, Post-7, and Post-21 as determined on a running track near sea level for the normobaric hypoxia (NH) and hypobaric hypoxia (HH) groups (in %). Data are mean ± standard error *P<0.05 for differences with Pre- and #P<0.05 for differences between groups.

Maximal test on cycle ergometer. These results are presented in Table 1. Both groups increased their maximal oxygen uptake values and power output values by the same amount immediately after the LHTL training camp period (+6.1 ± 6.8 vs. +5.2 ± 4.8% VO_{2max} and +9.6 ± 5.2 vs. +6.6 ± 4.7% P_{max}, for the NH and HH groups, respectively).

Table 1 Physiological parameters before (Pre-) and after (Post-) the Live High-Train Low (LHTL) camps for the normobaric hypoxia (NH) and hypobaric hypoxia (HH) groups.

		Pre-	Post-	Delta %
VO_{2max}	NH	65.4 ± 7.8	69.1 ± 5.6 **	6.1 ± 6.8
[mL.·kg^{-1}·.min^{-1}]	HH	69.2 ± 8.9	73.2 ± 7.1 **	5.2 ± 4.8
HR_{max}	NH	187 ± 7	188 ± 5	0.6 ± 2.6
[b·.min^{-1}]	HH	185 ± 9	185 ± 8	0.1 ± 1.9
P_{max}	NH	353 ± 43	385 ± 38 ***	9.6 ± 5.2
[W]	HH	378 ± 24	403 ± 32 ***	6.6 ± 4.7
VE_{max}	NH	178.9 ± 17.8	184.3 ± 14.6	3.7 ± 10.2
[l·.min^{-1}]	HH	182.6 ± 34	188.1 ± 19.3	4.5 ± 9.3

VO_{2max} maximal oxygen uptake; HR_{max} maximal heart rate; P_{max} maximal power output; VE_{max} maximal ventilation. Data are mean ± SD; **P<0.01 and ***P<0.001 for differences between Pre- and Post-.

Body fat mass and weight. Body weight (69.5 ± 5.9 vs. 69.6 ± 5.6 kg for the NH group and 69.9 ± 6.4 vs. 69.1 ± 6.2 kg for the HH group) and fat mass percentage (9.9 ± 1.8 vs. 9.1 ± 1.3% for the NH group and 10.3 ± 1.4 vs. 8.4 ± 0.7% for the HH group) did not differ between groups.

Night S_pO_2 and heart rate. No differences in average values of night HR were found between the groups or between different days (51 ± 1 and 50 ± 2 bpm, for the NH and HH groups, respectively). Conversely, although mean S_pO_2 values (Figure 4B) were similar during the control nights (before the camps, Pre1 and Pre2), they were higher in the NH group than in the HH group between day 1 and day 18 (D1 to D18) (92.1 ± 0.3 vs. 90.9 ± 0.3, for the NH and HH groups, respectively; P<0.001) and remained higher (P<0.05) during each of the two

nights following the camps (Post 1: 94.7 ± 0.5 vs. 93.5 ± 0.9% and Post2: 94.8 ± 0.6 vs. 93.7 ± 1.3%).

Breathing frequency. The average values of night breathing frequency were similar between the groups during the two evenings prior to the camp (14.0 ± 1.8 and 13.9 ± 1.5 breath.min^{-1} for NH and HH, respectively). However, breathing frequencies were lower in the NH group than in the HH group (13.9 ± 2.1 vs. 15.5 ± 1.5 breath·min^{-1}, $P<0.05$) during the LHTL camp (D1 to D18) and remained lower upon the camp's completion (13.7 ± 1.9 vs. 15.1 ± 1.3 breath·min^{-1}, $P<0.05$).

***Total Haemoglobin Mass*.** All results are presented in Table 2. Both groups increased their total haemoglobin masses during the study period (912 ± 96 vs. 936 ± 103 g and 950 ± 115 vs. 967 ± 122 g for the NH and HH groups, respectively, $P<0.001$).

***Blood Parameters*.** All blood parameters are presented in Table 2. The RBC number, [Hb] and Hct were each lower in the NH group than in the HH group following camp. Additionally, the initial ferritin values were not different between groups and were within reference ranges (98.7 ± 75.9 *vs.* 105.3 ± 51.9 ng/mL for NH and HH, respectively). A larger decrease in [EPO] was noted in the HH group compared with the NH group with return to 1150m (table 2).

Hypoxic doses and P_iO_2. The daily (12.2 ± 0.3 vs. 16.8 ± 3.1 h, $P<0.001$) and total (220.1 ± 0.9 *vs.* 302.9 ± 5.5 h, $P<0.001$) hypoxic doses were lower in the NH group than in the HH group. No differences were found in either daily or average P_iO_2 values between the two training camps (111.1 ± 1.1 vs. 111.5 ± 1.0 mmHg for the NH and HH groups, respectively, Figure 4A).

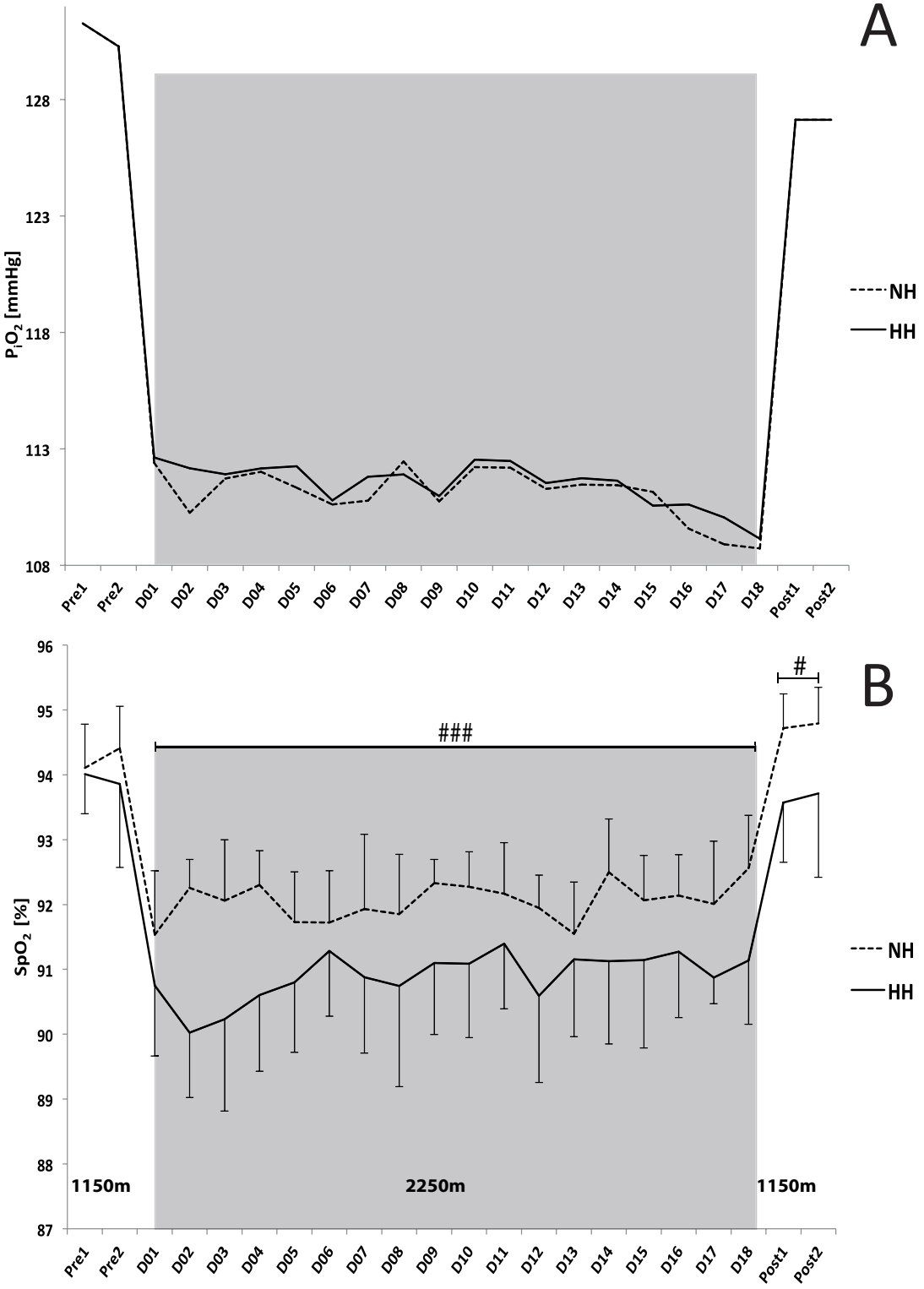

Figure 1. A. Daily values of inspired pressure of oxygen (P_iO_2 in mmHg) during the Live High-Train Low (LHTL) camps for the normobaric hypoxia (NH) and hypobaric hypoxia (HH) groups. **B.** Mean values of night oxygen pulse saturation (S_pO_2). Data are presented in mean ± standard error. Pre1-Pre2: measurements before the camps (1150 m, Prémanon, France); D01-D18: measurement during the camps (NH: hypoxic room in Prémanon, France; HH: Fiescheralp, Switzerland). $^{\#}P<0.05$, $^{\#\#\#}P<0.001$ for differences between groups.

***Questionnaires*.** The mean Lake Louise Score was 1.2 ± 0.4 for the NH group and 1.1 ± 0.4 for the HH group. No differences were found between the groups. DALDA Part B results were not different between the groups, and scores did not change across days (2.3 ± 0.7 vs. 2.3 ± 0.6 for the NH and HH groups, respectively). DALDA Part A results included a significantly higher score for the NH group than for the HH group from D07 to D11 and D15 to D16 ($P<0.05$). The average VAS value for the sleep quality of the entire camp was lower in the NH group (6.0 ± 0.4 vs. 6.4 ± 0.4 for the NH and HH groups, respectively; $P<0.001$). The GSQSS was significantly higher for the NH group (4.7 ± 1.1 vs. 3.6 ± 0.8 for the NH and HH groups, respectively; $P<0.001$), indicating poorer sleep quality for the NH group than for the HH group. However, waking state VAS scores were not different between the groups (5.9 ± 0.5 vs. 5.7 ± 0.5 for the NH and HH groups, respectively).

Correlations. A positive correlation was found between the mean Hb_{mass} (in g) and VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$) values of both groups following camp ($r=0.68$, $P<0.01$; $r=0.86$, $P<0.001$ for the NH and HH groups, respectively). We did not find any correlations between changes in Hb_{mass} and VO_{2max} or between initial value of Hb_{mass} and any other parameter.

Table 2 Haematological parameters before (Pre-) and after (Post-) the Live High-Train Low (LHTL) camps for the normobaric hypoxia (NH) and hypobaric hypoxia (HH) groups.

		Pre-	Post-	Delta %
EPO [mU/mL]	NH	3.85 ± 1.42	3.37 ± 1.59	-14.1 ± 18.3
	HH	4.96 ± 3.73	3.92 ± 3.94*	-34.5 ± 27.5$^{\#}$
RBC [u/µl]	NH	5.26 ± 0.39	5.08 ± 0.46	-3.5 ± 4.8
	HH	5.15 ± 0.36	5.18 ± 0.43 $^{\#\#}$	0.6 ± 3.8 $^{\#}$
HGB [g/dl]	NH	15.75 ± 1.07	15.75 ± 1.14	0.1 ± 5.1
	HH	15.53 ± 0.88	16.18 ± 1.08 *$^{\#}$	4.2 ± 3.9 $^{\#}$
Hct [%]	NH	46.25 ± 2.89	45.19 ± 2.97	-2.2 ± 5.6
	HH	45.24 ± 2.43	46.33 ± 2.62 *$^{\#}$	2.5 ± 3.8 $^{\#}$
MCV [fl]	NH	88.06 ± 3.71	89.22 ± 3.16 **	1.4 ± 1.3
	HH	88.06 ± 4.74	89.69 ± 4.51 ***	1.9 ± 1.4
MCH [pg]	NH	29.96 ± 1.13	31.09 ± 1.27 ***	3.8 ± 0.9
	HH	30.22 ± 1.27	31.32 ± 1.34 ***	3.6 ± 1.2
MCHC [g/dl]	NH	34.03 ± 0.72	34.85 ± 0.58 ***	2.4 ± 1.2
	HH	34.33 ± 1.01	34.92 ± 0.79 ***	1.7 ± 2.0
RET [%]	NH	0.89 ± 0.31	1.03 ± 0.28 **	21.6 ± 30.0
	HH	0.98 ± 0.24	1.18 ± 0.38**$^{\#}$	23.2 ± 34.1$^{\#}$
IRF [%]	NH	6.72 ± 3.61	5.37 ± 2.17	-9.02 ± 33.95
	HH	6.06 ± 1.77	4.76 ± 2.75	-19.09 ± 36.46
Hb_{mass} [g]	NH	912.4 ± 96.6	935.9 ± 102.6 ***	2.6 ± 1.9
	HH	946.8 ± 126.7	978.6 ± 131.6 ***	3.4 ± 2.1
RCV [ml]	NH	2675.8 ± 295.4	2692.1 ± 289.9	0.7 ± 2.8
	HH	2734.1 ± 306.5	2778.7 ± 324.1	1.64 ± 3.1
BV [ml]	NH	6358.3 ± 583.8	6553.6 ± 664.1	3.1 ± 4.8
	HH	6617.1 ± 744.1	6557.5 ± 821.4	-1.0 ± 4.2 $^{\#\#}$
PV [ml]	NH	3682.6 ± 384.6	3861.6 ± 460.9	5.1 ± 8.6
	HH	3883.1 ± 505.3	3778.8 ± 551.8	-2.7 ± 6.1 $^{\#\#}$

EPO erythropoietin; RBC red blood cells; HGB haemoglobin; Htc haematocrit; MCV mean cell volume; MCH mean cell haemoglobin; MCHC mean cell haemoglobin concentration; RET reticulocytes; IRF immature reticulocyte fraction; Hb_{mass} haemoglobin mass; RCV red cell volume; BV blood volume; PV plasma volume. Data are mean ± SD; *P<0.05, **P<0.01 and ***P<0.001 for differences between Pre- and Post-; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 for differences between NH and HH.

Discussion

The present study demonstrated that an 18-d LHTL altitude camp performed under either NH or HH conditions induced different physiological and systemic responses: (i) during LHTL, longer hypoxic exposure, larger night desaturation levels, and higher breathing frequencies were noted in the HH group; (ii) immediately after LHTL, larger haematological changes occurred in the HH group, but similar increases were observed in Hb_{mass} and VO_{2max}; (iii) finally, larger performance enhancements were measured in the HH group after 3 weeks at sea level.

Differences in daily responses were found between our experimental groups. The hypoxic dose was higher for the HH group than for the NH group (300 vs. 220 h). The present study aimed to compare two LHTL altitude training camps (simulated versus real altitude) in "real conditions", corresponding to those encountered by elite athletes in their training practices. In this context, the daily exposures reported in the present study (12 vs. 17 $h \cdot d^{-1}$ in the NH and HH groups, respectively) are consistent with those reported previously with hypoxic exposures of 8-12 $h \cdot d^{-1}$ [4,5] and 18 $h \cdot d^{-1}$ [7,19] in NH and HH conditions, respectively. Meta-analysis results would suggest that optimal durations are of 11 and 18 $h \cdot d^{-1}$ [27]. There is a clear dose-response effect between the hypoxic dose and the haematological responses, as highlighted by Levine and Stray-Gundersen's study [23]. However, differences in hypoxic doses between the NH and HH groups cannot be easily reduced, as long duration stays in confinement for individuals of the NH group may cause other complications, including

detrimental reductions in plasma volume [34]. Aside from the difference in total hypoxic exposure, the HH group also experienced fewer transitions between "high" (2250 m) and "low" altitudes. LHTL under NH conditions implies numerous daily exposures to normoxia and many transitions to and from hypoxic conditions. Therefore, the exposure is more intermittent than it would be for LHTL under HH conditions (*e.g.*, the number of daily shifts between altitudes was 7 vs. 2 in the NH and HH groups, respectively). Taken together, we assume that these intermittent characteristics cannot be ruled out as explanations for noted altitude training adaptations. In addition, Navarette-Opazo & Mitchell [35] demonstrated in a recent meta-analysis, that the number of cycles per day was one of the most relevant variable inversely correlated with the qualitative effect of intermittent exposures to hypoxia. Interestingly, in a similar way, Garvican *et al.* [22] described the "*oscillating nature of LHTL,*" the daily descents to sea level and the associated normoxic exposures as an explanation for the less dramatic fall in [EPO] compared to changes associated with continuous exposure to high altitudes. This observation may also explain the differences (almost twice as much in the HH group) in [EPO] decreases noted between the two groups.

Night measurements also indicated that the two conditions were not similar. Night arterial oxygen saturations were higher in the NH group than in the HH group, while breathing frequencies were lower (Figure 4B). Such differences in ventilatory patterns may be explained by differences in the magnitude of alteration of pulmonary diffusion. Similarly, Savourey *et al.* [17] first demonstrated that HH induced greater respiratory frequencies, lower tidal volumes, and minute ventilation values over short time periods; thus, suggesting higher amounts of alveolar physiologic dead space, which is associated with ventilatory alkalosis and hypocapnia [14]. Later, similar conclusions have also been reported by Richard and Koehle [13] and Faiss *et al.* [18]. Changes in fluid balance have been shown as well with differences between the HH and NH conditions [15]. Additionally, barometric pressure (PB) modifies

fluid circulation and trans-alveoli-capillary membrane flux [36]. This may induce a stronger pulmonary vasoconstriction in the HH group and modify oxygen diffusion by decreasing the pressure gradient [14]. PB may also influence N_2 and O_2 concentrations in cerebrospinal fluid, as well as central regulation of ventilation [12]. Taken together, these alterations are the likely inducers of the lower mean sleeping S_pO_2 values reported in the HH group. It is interesting to note that these lower S_pO_2 values were maintained after subjects returned to 1150 m during the two nights period immediately following the camp. Several studies [17,37] have reported a more rapid blood desaturation under HH conditions, leading to a longer duration of hypoxemia. This is in line with the present results as we observed a larger decrease in S_pO_2 from the first night under HH conditions and the maintenance of these lower values during the whole LHTL camp. Interestingly, the higher breathing frequencies while sleeping in HH conditions at 2250 m were also maintained during the two nights spent at 1150 m following the camp. This larger desaturation, which was most likely influenced by the higher breathing frequencies, potentially induced more severe hypoxemia in the HH group and delayed performance enhancement.

Specific short-term post-hypoxic responses were observed immediately following the camps. These responses were noted primarily among haematological parameters and revealed differences between the groups that were clearly influenced by differences in both daily and total hypoxic exposures. To date, there is no consensus on neither the dose-response relationship between hypoxic stimulus and Hb_{mass} increase nor on the recommendation in terms of total hypoxic exposure duration. For instance, Richalet and Gore have recommended an exposure of 216 h [20], Garvican *et al.* of 300 h [22], and Wilber at al. a minimum of 4 weeks with at least 22 $h.d^{-1}$ [25]. In the present study, the difference of 80 h (220 vs. 300 h for the NH and HH groups, respectively) is likely the primary cause of the larger increases in Hct, [Hb], and RET in the HH group. However, although the increase in Hb_{mass} immediately

following camp was significant, the magnitude of the increase was the same in both groups (2.6 vs. 3.4% for the NH and HH groups, respectively). Our results are consistent with those of previous studies in which Hb_{mass} was increased by 3-4% following several LHTL protocols [7,19,22,28,38] and illustrate an enhanced oxygen transport capacity as a result of an erythropoietic response. These findings are also consistent with the dose-response relationship and correspond to an average rise of 1% per 100 h of exposure [1,38]. Gore *et al.* [38] also suggested that the amount of the hypoxic dose or the level of altitude should be higher in cases involving the use of simulated altitudes to produce equivalent results at real altitudes (*i.e.* 3000m in NH with LHTL corresponding to 2320 m in HH with classical altitude training). Further, our results showed a 5-6% increase in VO_{2max} in both groups following LHTL, an increase that is commonly observed under HH conditions [19] as well as NH conditions [26,39], although the phenomenon is more common in the former [40,41].

We also reported differences in plasma and blood volume changes during the study period between the NH and HH groups. Of interest, is that diuresis and changes in fluid balance have been shown to be different between HH and NH (*i.e.* larger diuresis for NH and larger fluid retention for HH) [15,42]. The influence of PV changes (*e.g.*, expansion and reduction) on performance enhancement are well-known [43]. It is known that plasma volume may increase until at least 16 days following an altitude training camp [44]. One may speculate that the non-significant difference in plasma volume observed at Post- between the two groups would not occur anymore at Post-21, suggesting a potential larger increase in PV for the HH group during these 3 weeks. This potential hemodynamic enhancement would partially explain the longer delay in performance enhancement compared to the NH group. However, based on the existing contradictory literature, it is difficult to speculate on the maintenance of the Hb_{mass} gains at Post-21. Garvican-Lewis *et al.* [45] reported a 4% increase two weeks after 11 days of LHTL under NH conditions at 3000 m (14 $h.d^{-1}$). However, a persistent increase in Hb_{mass}

post-exposure does not mean that it did not decrease from the initial elevation during the days following the exposure. Several studies show a relatively linear decrease in Hb and Hct starting as soon as hypoxic stimulus is removed. For example, Gough *et al.* [46], reported a drop from post to post-14; and Garvican *et al.* [22], demonstrated that Hb_{mass} had started to drop off by Post-4 and was no different from control after 10 days at sea level. Similarly, elite altitude-native Kenyan runners showed a significant 20 g decrease at 21 days at sea level (Figure 2A in [47]).

One of the most important findings of the present study is the performance enhancement noted in the HH group three weeks after camp. Our results are consistent with those of the meta-analysis by Bonetti and Hopkins [27], which described a "terrestrial" LHTL protocol that induced additional benefits relative to the performances of elite athletes as estimated by power output increases of 4.0% under HH conditions and 0.6% under NH conditions.

Delayed (in our case, three weeks after LHTL) performance enhancement has been observed in several [7,26] but not all studies [39]. The following mechanisms have been proposed: enhanced stroke volume compensating for the reduction in heart rate [48], enhanced efficiency [42], and increased VO_2 and power output at the lactic threshold [26]. Recently, Chapman *et al.* [49] described the following three components, which may influence performance changes following altitude training: the timing of the decay of red cell mass, the consequences of ventilatory acclimatisation, and the alterations in the biomechanical and neuromuscular factors associated with force production. Regarding the first component, we cannot determine if the unknown decay of Hb_{mass} (see above) in the present study influenced the difference in performance enhancement between groups at Post-21. The observed differences in the ventilatory pattern, as evidenced by the higher night breathing frequency in the HH group, could have influenced the delayed performance difference between groups.

Katayama *et al.* reported that the ventilatory adaptations upon returning to sea level were maintained for at least 4 weeks, after prior high altitude acclimatization, as these acclimatization benefits were observed during a new subsequent altitude exposition [50]. Finally, it is unlikely that there was any biomechanical alteration in either group who trained "low" at 1100-1200 m, as suggested by the preliminary results of Laymon *et al.* [51]. Therefore, a hypoxic-induced alteration in running style was probably not involved in the observed difference at Post-21.

Strengths and limitations. Our primary aim was to compare LHTL training camps under real and simulated altitude in an ecological setting (*e.g.*, by reproducing "real life" conditions of daily exposures and camp durations as described in previous LHTL studies under NH and HH conditions, respectively) rather than investigating the efficiency of LHTL, which has previously been documented. For this reason, we did not include a sea level control group. To the best of our knowledge, our study is the first to report differences in performance enhancement following direct comparisons of prolonged altitude training under NH and HH conditions. The athletes were well trained as shown by their training loads, VO_{2max}, and performance levels. The groups were matched according to the VO_{2max} values. Additionally, the athletes' training load and content were quantified and matched during the 6-month period before the study, which included a suitable lead-in period. To our knowledge, this study is the first where training loads and altitude levels were entirely matched on a daily basis during the entire LHTL period. The current study emphasizes the importance of well-controlled studies to achieve a better understanding of the mechanisms and potential benefits of altitude training [52].

The primary limitation of this study was that no measurements of total haemoglobin mass or other haematological parameters were completed at one or three weeks following LHTL due to logistical constraints. In addition, since our aim was to compare two typical 18-d LHTL

camps in “real” conditions, the hypoxic doses were different. One cannot rule out that the physiological and performance responses would be less dissimilar between NH and HH with a close matching of hypoxic doses.

Perspectives. This study questions the relationship between modes of prolonged hypoxic exposure and subsequent performance improvement. Real altitude conditions (HH) were more demanding than the simulated altitude (NH) utilised in training camps of the same duration. However, in general, hypoxic chambers make adjustments possible and continue to attract interest because of their practicality. Chapman [53] emphasises that the response to training and competition at high altitudes is individual, and that timing the return to competition after altitude training must also be individualised to obtain optimal sea level performance [49]. Normobaric hypoxia devices offer these individualisation possibilities in terms of hypoxic doses and altitude adjustments. Finally, further studies are necessary to assess the physiological responses of these hypoxic training methods to equivalent hypoxic doses.

This study highlights the different physiological adaptations noted in the HH and NH LHTL camps. Our results suggest that future investigations should increase the altitude of the normobaric hypoxia group to reach the same level of desaturation as that experienced under hypobaric hypoxic conditions and lengthen the durations of the camps to obtain hypoxic doses similar to those experienced under hypobaric hypoxic conditions.

Conclusion. The primary finding of the study is that there were significant differences in the responses to a LHTL training camp in NH compared to HH. Specifically, our results included greater performance enhancements in the HH group three weeks after LHTL, greater significance in haematological changes within the HH group following camp, greater night desaturation levels, and higher breathing frequencies in the HH group, with similar increases in Hb_{mass} and VO_{2max} following LHTL in both NH and HH. Additionally, one cannot rule out other factors, including differences in sleep quality, desaturation level, breathing patterns,

fewer transitions between high and low altitudes (*e.g.*, intermittence) or different responses relative to plasma volumes and [EPO] following camp.

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Chapter Six

Article 2 - Cycling time trial is more altered in hypobaric than normobaric hypoxia.

6. Article 2 - Cycling time trial is more altered in hypobaric than normobaric hypoxia.

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Abstract

Purpose: Slight physiological differences between acute exposure in normobaric hypoxia (NH) and hypobaric hypoxia (HH) have been reported. Taken together, these differences suggest different physiological responses to hypoxic exposure to a simulated altitude (NH) versus a terrestrial altitude (HH). For this purpose, in the present study, we aimed to directly compare the time-trial performance after acute hypoxia exposure (26 h, 3450 m) by the same subjects under three different conditions: normobaric hypoxia (NH), hypobaric hypoxia (HH) and normobaric normoxia (NN). Based on all of the preceding studies examining the differences among these hypoxic conditions, we hypothesized greater performance impairment in HH than in NH. **Methods:** The experimental design consisted of three sessions: NN (Sion, F_iO_2 20.93); NH (Sion, hypoxic room, F_iO_2 13.6%, PB 716 mm Hg); and HH (Jungfraujoch, F_iO_2 20.93, PB 481 mm Hg). The performance was evaluated at the end of each session with a cycle time-trial of 250 kilojoules. **Results:** The mean TT duration in NN was significantly shorter than under the two hypoxic conditions ($P<0.001$). In addition, the mean duration in NH was significantly shorter than in HH ($P<0.01$). The mean SpO_2 during the TT was significantly lower for HH than for NH ($P<0.05$), and it was significantly higher in NN than for the two other sessions ($P<0.001$). **Conclusion:** As previously suggested, HH seems to be a more stressful stimulus, and NH and HH should not be used interchangeability when endurance performance is the main objective. The principal factor in this performance difference between hypoxic conditions seemed to be the lower peripheral oxygen saturation in HH at rest, as well as during exercise. **Key words:** normobaric hypoxia, hypobaric hypoxia, performance, exercise.

Introduction

The use of altitude training is now very common among elite athletes, and several altitude-training methods have been developed to optimize training in both endurance (30) and team sports (28). Recent research has focused on the potential differences between normobaric (NH) and hypobaric hypoxia (HH), *i.e.*, simulated and terrestrial altitudes (13). Slight physiological differences between acute exposure to NH and HH have been reported (29, 39): minute ventilation was higher in NH than in HH, with a combination of a higher tidal volume and lower respiratory frequency (12). AMS symptoms have been shown to be less severe in NH than in HH (27), and pre-acclimatization at a 'terrestrial' altitude (HH) resulted in a significant decrease in the severity of AMS when traveling to HH conditions, while this change did not occur with NH pre-acclimatization (18). Differences in fluid balance have also been observed, with higher diuresis and a larger decrease in plasma volume for NH, while HH induced greater fluid retention (12, 27). The nitric oxide (NO) in exhaled air or in plasma and oxidative stress markers were also shown to be lower in NH than in HH (16). Taken together, these differences suggest larger physiological responses to hypoxic exposure in 'terrestrial' (HH) versus 'simulated' altitudes (NH) (35). These results suggest the hypothesis that the type of hypoxia (*e.g.*, normobaric or hypobaric hypoxia) could influence performance at different altitudes, even with exposure durations or ambient oxygen pressures strictly matched between the two conditions.

Currently, many athletes or teams playing different sports integrate altitude or hypoxic training into their programs to improve performance through several physiological adaptations. Moreover, hypoxia could be applied for many therapeutic uses in cardiovascular diseases or in obese populations. These uses suggest that hypoxia could be of prime importance to understanding better the extent to which NH and HH differ from each other. However, surprisingly, only one study to our knowledge compared endurance performance

after short exposure to HH versus NH (5). In this study, two different groups were exposed to either NH or HH and then performed a 720-kJ time trial (TT). Compared to sea level, the TT performance decreased by less (-36 ± 14%) in NH (simulated altitude of 4300 m) than in HH (-65 ± 24%); however, we cannot exclude the possibility that the hypoxia sensitivity might have been different between the two groups. Accordingly, we designed an experimental protocol to compare for the first time TT endurance performance in NH versus HH versus control (normobaric normoxia, NN) conditions with the same subjects and the same acute exposure durations. We tested the hypothesis that greater performance impairment would occur in HH than in NH, compared to NN.

Methods

Subjects

Sixteen trained male subjects volunteered for this study, but three of them did not finish all of the experiments. Therefore, thirteen trained males subjects were included in the analyses (mean ± SD; age 34.7 ± 9.5 years old, body weight 75.2 ± 7.2 kg, height 179.7 ± 5.7 cm, fat mass 13.2 ± 5.9%, peak oxygen consumption [VO_{2max}] 60.2 ± 9.9 $mL.kg^{-1}.min^{-1}$ [range: 43-75 $mL.kg^{-1}.min^{-1}$]. All of the subjects provided written, voluntary informed consent before participation. The subjects were all non-smokers and were neither acclimatized nor recently exposed to significant altitudes for at least the month before the beginning of the experiment. In addition, all of the subjects were born and lived at altitudes of less than 700 m. The experiment was conducted according to the Declaration of Helsinki, and the study was approved by the local Ethical Committee (Commission Cantonale Valaisanne d'Ethique Médicale, CCVEM; Agreement 051/09).

Experimental Design

The experimental design consisted of three testing sessions preceded by a preliminary visit. For this preliminary visit, the subjects came to the laboratory for baseline anthropological

measurements, including body composition analysis by air plethysmography (BodPod®, Life Measurement, Concord, CA, USA). They completed a consent form and preliminary questionnaires, and they performed: (a) a maximal incremental exercise test (F_iO_2: 20.93%; 60 W + 30 $W.min^{-1}$) on a magnetic braked cycle ergometer equipped with a power meter (Cycleops IC 400 Pro, Madison, WI, USA) for the determination of maximal aerobic power output and VO_{2max}; and (b) a preliminary 250-kJ TT on the same cycle ergocycle.

The study then consisted of three sessions separated by at least 12 days and a maximum of 20 days in a randomized order. The subjects were asked to maintain their usual training and physical activities during the whole experimental protocol to avoid fitness changes between sessions. Two sessions were completed in a hypoxic chamber (ATS Altitude, Sydney, Australia) built in our laboratory at an altitude of 485 m (Sion, Switzerland), and a third session was performed at a terrestrial altitude. The hypoxic chamber is a well-ventilated 30 cu. m room (2.4 × 5.0 × 2.5 m) with transparent glass panels. The system consists of a compressor storing air in pressurized tanks with serial connection to air filters, allowing for oxygen reduction in the air input flow to the chamber (altitude simulation by lowering the inspired fraction of O_2, F_iO_2). The temperature inside the chamber was maintained constant at 23°C on average using an internal air conditioning system. The NN session was performed in the hypoxic chamber with a F_iO_2 of 20.93%, a measured barometric pressure (P_B) of 718.1 ± 3.3 mm Hg, an inspired pressure of oxygen (P_iO_2) of 140.5 ± 0.6 mmHg, a temperature of 23 ± 1°C and a humidity of 42.8 ± 4.4%. The NH session, corresponding to a simulated altitude of 3450 m, was performed with a F_iO_2 of 13.6%, P_B of 715.8 ± 3.8 mmHg, P_iO_2 of 91.0 ± 0.6 mm Hg, a temperature of 22.7 ± 0.8°C and a humidity of 41.0 ± 4.8%. F_iO_2, P_B and the temperature were measured regularly with an electronic oximeter (GOX 100 oximeter, Greisinger, Regenstauf, Germany) and a barometer (GPB 2300, Greisinger).

For blinding, the system also ran normoxic airflow into the chamber during the NN sessions.

The HH session was performed at the Altitude Research Station, Jungfraujoch, Switzerland (3450 m, F_iO_2 of 20.93%, P_B of 481.8 ± 4.7 mm Hg, P_iO_2 of 90.9 ± 1.0 mm Hg, temperature of 21.3 ± 0.6°C, humidity of 45.1 ± 8.3%). The progressive increase in altitude to access the Jungfraujoch by train during the HH sessions was simulated during the NH and NN sessions. For 45 min before entering the hypoxic chamber, the subjects breathed either room air (for NN) or hypoxic air (for NH) in a blinded fashion, using a mask connected to a three-way valve to an altitude simulation device (Altitrainer, SMTech, Nyon, Switzerland). The subjects remained in the hypoxic chamber during the whole protocol (including the sleeping period and for meals). Similar standardized meals were provided at the same time under each condition. The same nitrate- and nitrite-free meals and drinks, standardized for nutritional and caloric content, were provided to the subjects at H+0, H+7 and H+19 to control nitrate intake, which can influence oxidative stress levels (see (16) for further details) and performance. The subjects ate in the hypoxic chamber or in the kitchen of the HH laboratory. They were asked to use the restrooms before entering the hypoxic chamber and had a second opportunity to go after the night for a limited time (less than 2 minutes). The schedule and activities were exactly the same for all the conditions. The subjects completed the same diary, which was precisely controlled. They performed preliminary tests of respiration patterns, balance, and baroreflexes. The sleep hours and conditions were also controlled. The bedding was similar among conditions. In addition, the subjects were equipped with earplugs and eye masks during the night. The subjective sleep quality was assessed by a validated questionnaire (Groeningen questionnaire for sleep assessment (22)), and sleep scores for sleep quality and waking state were measured with two visual analogue scales.

Sleep quality was not different between HH and NH (6.6 ± 3.5 vs. 6.5 ± 4.1 for NH vs. HH, respectively, in sleep score; 4.2 ± 1.8 vs. 4.4 ± 2.4 for NH and HH, respectively, in sleep quality; 4.9 ± 2.1 vs. 5.0 ± 2.4 for NH vs. HH, respectively, for waking state).

The sessions consisted of 26 h of exposure to each condition (NN, NH, HH) in a randomized order. Of interest was that a double-blinded protocol was applied between the NN and NH conditions so that neither the subjects nor the experimenters in the hypoxic chamber were informed of the F_iO_2. In each session, the subjects completed the Lake Louise questionnaire (LL) after 10 and 20 h of exposure to assess acute mountain sickness (AMS). The perceived altitude was asked after 20 (H+20) and 26 h (H+26). Blood samples were obtained before and at H+20 during each session. Pulse oxygen saturation (SpO_2) was recorded continuously with a finger pulse oximeter (WristOx2™, Model 3150, Nonin Medical, Inc., Plymouth, MN, USA). A 250-kJ TT was performed after 24 h of exposure using the same cycle ergometer as in the preliminary session. The TT was preceded by a resting period of 5 min, followed by a 3-min warm-up at 70 W. The subjects were then instructed to complete the 250 kJ as rapidly as possible, and they were free to increase/decrease the resistance to adjust the workload as they became familiar with it in the preliminary session. The only visual feedback available for the subjects during the TT was the work performed (from 0 to 250 kJ). The time trial was performed at the same time (*i.e.*, between 1 p.m. and 2 p.m.) of the day in each condition.

Measurements

Gas exchange, heart rate, SpO_2 and perceptual feeling. Ventilation and pulmonary gas exchange were measured breath by breath at rest and during TT using a portable gas analyzer (MetaMax 3B, Cortex, Leipzig, Germany) with an oronasal mask (Vmask™, 7500 series; Hans Rudolph Inc., Shawnee, KS, USA; dead space, 41 mL). This device measures volume using a bidirectional digital turbine. Oxygen uptake and carbon dioxide production are then determined in inspired and expired air successively by an electrochemical cell and an infrared analyzer, respectively, from the air drawn through a Nafion® sampling tube attached to the turbine at the output of the mask. The heart rate and pulse oxygen saturation were continuously recorded during the TT by an additional device, which was the same as that used

for the continuous 26 h recording presented previously (Radical-7®, Masimo Corporation, Irvine, CA, USA) and were stored for offline analyses with commercially available software (Labchart software, AD Instrument, Colorado Springs, CO, USA). At the end of the TT, the subjects were asked to quantify their leg and respiratory discomfort using a visual analogue scale (VAS, 1-10), as well as the rate of perceived exertion (RPE, Borg Scale, 6-20).

Blood lactate. Blood lactate concentration (La) was measured at rest before exercise and immediately after exercise from a capillary finger blood sample (LactatePro, Arkay SAS, Paris, France).

Venous blood sample. Antecubital vein blood samples (4.9 mL EDTA tube, Sarstedt, Nümbrecht, Germany) were obtained before (baseline, *bl)* each session and at H+20. The blood was subsequently analyzed via fluorescent flow cytometry and hydrodynamic focusing (XT-2000i, Sysmex Europe, Norderstedt, Germany), and the following primary hematological parameters were quantified: red blood cells (RBCs), hemoglobin (Hb), hematocrit (Htc), and reticulocyte percentage (RET%). The Sysmex XT-2000i underwent regular internal quality control procedures as required by the standards of laboratory medicine. During the period of our study, the coefficients of variations (CVs), which were determined using internal quality controls, were far less than 1.5% for Hb and 15% for RET% (within CV limits accepted by the manufacturer of the instrument). Changes in the plasma volume (ΔPV in %) between the *bl* and H+20 were calculated using the Dill and Costill equation (14) as follows:

$$\Delta PV(\%) = 100\times\left(\left(\frac{Hb_{bl}}{Hb_{H+20}}\right)\times\left(\frac{100-Htc_{H+20}}{100-Htc_{bl}}\right)-1\right),$$

where Htc is in %, and Hb is in g/dL.

Power, workload and cadence. The power output, total workload and cadence were continuously recorded at 1 Hz during the TT by the cycle ergometer (Cycleops IC 400 Pro, Madison, WI, USA).

Electromyographic recordings. Electromyographic signals (EMG) of the right *vastus lateralis* (*VL*) were recorded using bipolar silver chloride surface electrodes 10 mm in diameter (Kendall Meditrace 100) during the whole exercise. The recording electrodes were taped lengthwise on the skin over the muscle of the belly following SENIAM recommendations, with an inter-electrode distance of 20 mm. The position of the electrodes was marked on the skin so that they could be fixed at the same place for the two other sessions. The reference electrode was attached to the patella. Low impedance (<5 kΩ) at the skin electrode was obtained by shaving and abrading the skin with an abrasive sponge and cleaning it with alcohol. EMG data were recorded with a Biopac system (MP150, Biopac System, Goleta, CA, USA) and were amplified (gain=1000) with a bandwidth frequency ranging from 10 to 500 Hz, digitized at a sampling frequency of 2 kHz, and recorded by the AD conversion system (common mode rejection ratio: 90 dB; input impedance: 100 MΩ; gain: 1000). For data analysis, the integral of the whole EMG activity was calculated using the following formula:

$$iEMG\ (|m(t)|) = \int_0^1 |m(t)|\ dt,$$

where *m* is the raw EMG signal. For each condition and each subject, the iEMG value during TT was normalized to the total duration time of exercise (in s) to obtain an activation ratio per time unit during the time trial.

Data analysis and Statistics. Data are reported as the mean ± standard deviation in the text, tables and figures. The data were tested for homogeneity of variances (Levene's test) and for normality (Shapiro-Wilk test). One-way ANOVA with repeated measures and Tukey's *post-hoc* tests were used to identify differences among conditions (NN, NH, HH) in the mean values for each parameter during the resting period and during the TT. Two-way ANOVA was performed on repeated measures for each condition to determine the time effect for variables measured on several occasions during the sessions with pairwise multiple

comparison procedures (Holm-Sidak method). The correlations between SpO_2 and TT performances were calculated with Pearson's product moment correlation. The null hypothesis was rejected at $P<0.05$. All of the analyses were performed using Sigmaplot software, version 11.0 (Systat Software, San Jose, CA, USA).

Results

Acute mountain sickness and perceived altitudes. No AMS symptoms were observed after 10 h of exposure, and the LL scores were not different between conditions (1.2 ± 0.9, 1.5 ± 2.2 and 2.1 ± 2.1 for NN, NH and HH, respectively; P=0.391, ns). After 20 h of exposure, we reported a positive LL score (*i.e.*, > 3) only for HH but without any significant difference for the other conditions (1.6 ± 1.3, 2.3 ± 2.8 and 3.1 ± 2.6 for NN, NH and HH, respectively; P=0.229, ns). The perceived altitude immediately before the TT (H+20) was not different between NN and NH (1138 ± 922 and 1864 ± 1017 m, for NN and NH, respectively; P=0.09, ns). After the TT (H+26), the perceived altitude was higher for NH and HH than for NN (2752 ± 870 and 3493 ± 27 vs. 1005 ± 702 m, for NH, HH and NN, respectively, $P<0.001$).

Resting values. The SpO_2 at rest was lower in the two hypoxic conditions than in NN ($P<0.001$). In addition, SpO_2 was lower in HH than in NH ($P<0.01$). The heart rate was higher in the two hypoxic conditions ($P<0.001$), as was the $P_{ET}O_2$ ($P<0.001$), with no difference between HH and NH. The $P_{ET}CO_2$ was lower in NH than in the other conditions ($P<0.001$ and $P<0.01$ compared to NN and HH, respectively). All of the resting values are presented in Table 1.

Table 1 Mean cardiorespiratory and metabolic values while subjects were resting on the ergocycle before the time trial, after 24 h in normobaric normoxia (NN), normobaric hypoxia (NH) and hypobaric hypoxia (HH).

	NN	NH	HH
Lactate [mmol. L^{-1}]	2.53 ± 0.65	2.35 ± 0.71	2.7 ± 1.01
SpO_2 [%]	98 ± 1	92 ± 2***	90 ± 2***$^{\#\#}$
HR [bpm]	69 ± 11	77 ± 12***	81 ± 8***
$\dot{V}_E$ [L.min^{-1}]	12.7 ± 4.1	15.7 ± 5.2*	14.1 ± 2.8
V_T [L.min^{-1}]	1.1 ± 0.3	1.2 ± 0.3	1.2 ± 0.3
f [L.min^{-1}]	12.8 ± 3.3	13.6 ± 3.1	13.1 ± 3.3
$P_{ET}O_2$ [mm Hg]	99.9 ± 13.5	70.1 ± 17.8***	58.2 ± 3.2***
$P_{ET}CO_2$ [mm Hg]	30.6 ± 3.3	26.8 ± 2.9***	30.0 ± 2.5$^{\#\#}$
$\dot{V}O_2$ [L.min^{-1}]	0.37 ± 0.09	0.41 ± 0.1	0.39 ± 0.06
$\dot{V}CO_2$ [L.min^{-1}]	0.31 ± 0.12	0.34 ± 0.11	0.35 ± 0.05

SpO_2 pulse oxygen saturation; HR heart rate; $\dot{V}_E$ minute ventilation (BTPS); V_T tidal volume; f breathing frequency; $P_{ET}O_2$ end-tidal O_2 pressure; $P_{ET}CO_2$ end-tidal CO_2 pressure; $\dot{V}O_2$ oxygen uptake; $\dot{V}CO_2$ carbon dioxide produced. Data are mean ± SD; *P<0.05 and ***P<0.001 for differences with NN; $^{\#\#}$P<0.01 for differences between HH and NH.

Blood sample. Differences between the baseline and H+20 were observed in most of the blood parameters for both hypoxic sessions. Plasma volume also decreased to a greater extent for NH and HH than for NN, but this decrease was not different among the hypoxic conditions. All of the values are presented in Table 2.

Time trial performance. Performance time in seconds is presented in Figure 1. Compared to NN (*i.e.*, 1041 ± 151 s), the mean time was 24.1 ± 9.6% and 33.2 ± 12.4% higher for NH and HH, respectively (with both P<0.001). The mean time was 7.5 ± 7.5% higher in HH than in NH (P<0.01).

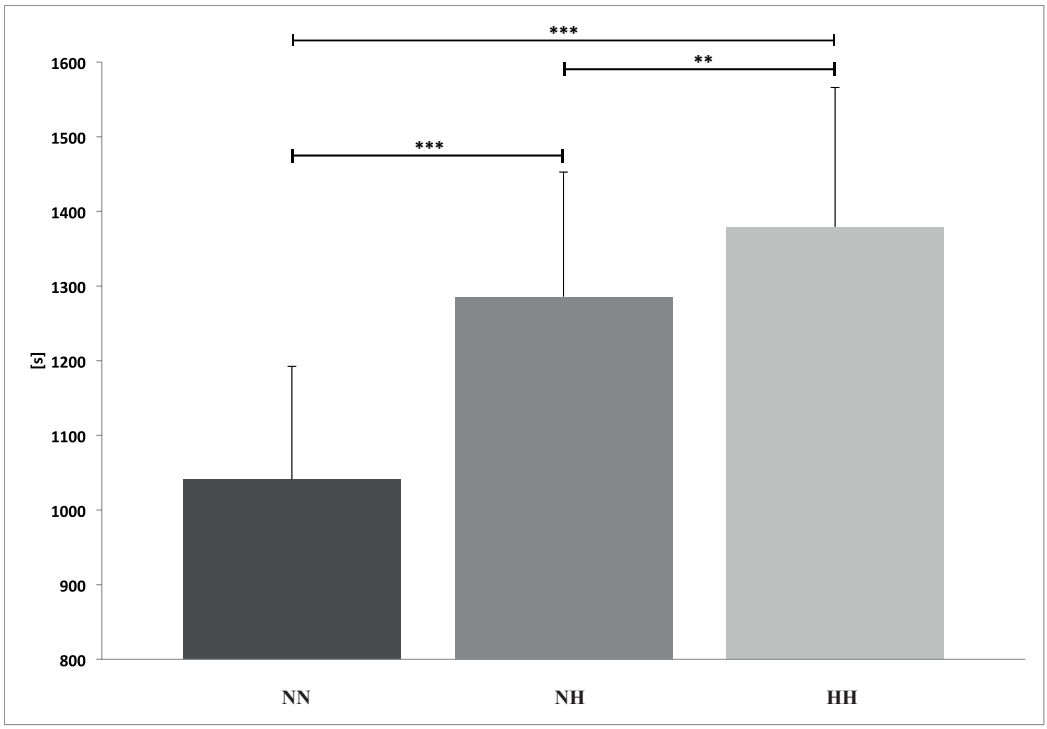

Figure 1. Mean time trial performance in normobaric normoxia (NN), normobaric hypoxia (NH) and hypobaric hypoxia (HH). Data, expressed in seconds (s) are mean ± standard errors. **P<0.01 and ***P<0.001 for differences between conditions.

Pulse oxygen saturation. Mean SpO_2 during the TT was significantly higher in NH than in HH (79.2 ± 3.4 vs. 75.9 ± 5.9%; P<0.05; Figure 2). As expected, SpO_2 was higher in NN (95.3 ± 3.0%) than in the two hypoxic conditions (both P<0.001).

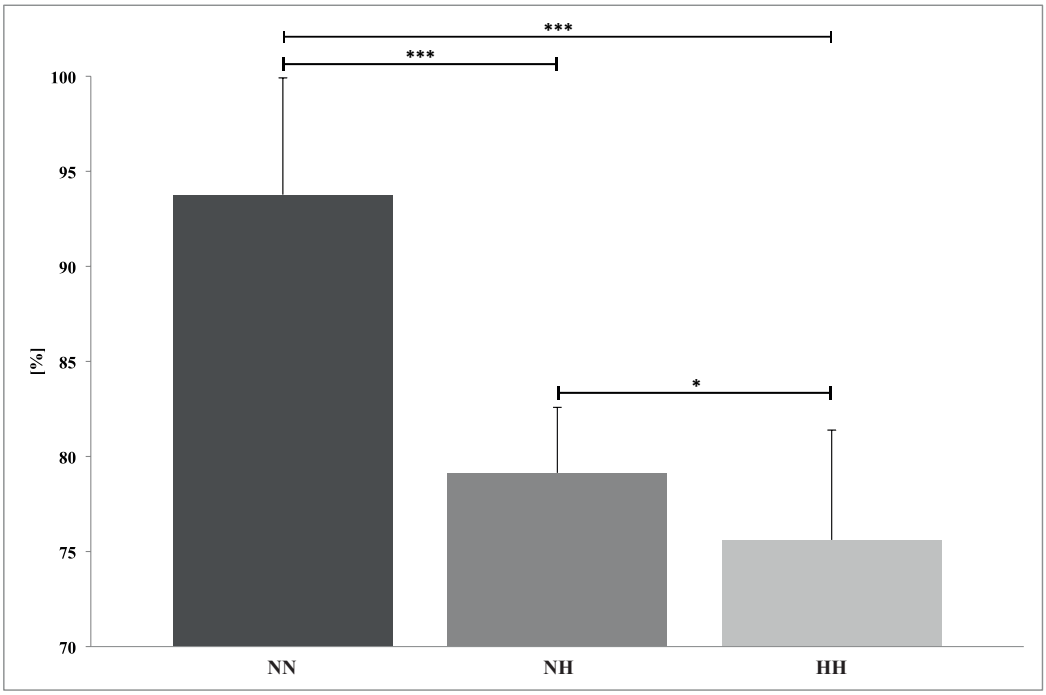

Figure 2. Mean pulse oxygen saturation (SpO_2) during the time trial in normobaric normoxia (NN), normobaric hypoxia (NH) and hypobaric hypoxia (HH). Data are mean ± standard errors. *P<0.05 and ***P<0.001 for differences between conditions.

Cadence. The mean cadence during exercise was lower in HH than in NH (88.4 ± 6.2 vs. 92.5 ± 8.7 rpm; P<0.01) and NN (94.7 ± 6.8 rpm; P<0.001).

Electromyographic activity. The iEMG/time activity was higher in NN than in the two hypoxic conditions (NN>NH, P<0.05 and NN>HH, P<0.001), and it was higher for NH than for HH (P<0.05). The values are presented in Table 3.

Correlations. Negative correlations were observed between VO_{2max} and the performance times in NN (r=-0.56, P=0.06), NH (r=-0.67; P<0.05) and HH (r=-0.58; P<0.05). However, we did not find any correlation between VO_{2max} and the change in performance among conditions. A positive correlation was found between the NN SpO_2 and the TT time (in seconds) in NN (r=0.65; P<0.05), NH (r=0.59; P<0.05) and HH (r=0.56; P<0.05). No correlations were found between the NH or HH SpO_2 and performance or between the changes in SpO_2 (from NN to NH, from NN to HH or from NH to HH) and the change in performance (NN-NH, NN-HH or NH-HH in %).

However, correlations were observed between performance time (in seconds) under all conditions: between NN and NH (r=0.83; P=0.001), between NN and HH (r=0.80; P=0.002) and between NH and HH (r=0.87; P<0.001). In addition, positive correlations were found between the changes in performance time from NN to NH (NN-NH) and from NN to HH (NN-HH) (r=0.64; P=0.03), as well as between the changes from NN to HH (NN-HH) and from NH to HH (NH-HH) (r=0.59; P=0.04) but not between NN-NH and NH-HH (r=-0.24; P=0.46) (Figure 3).

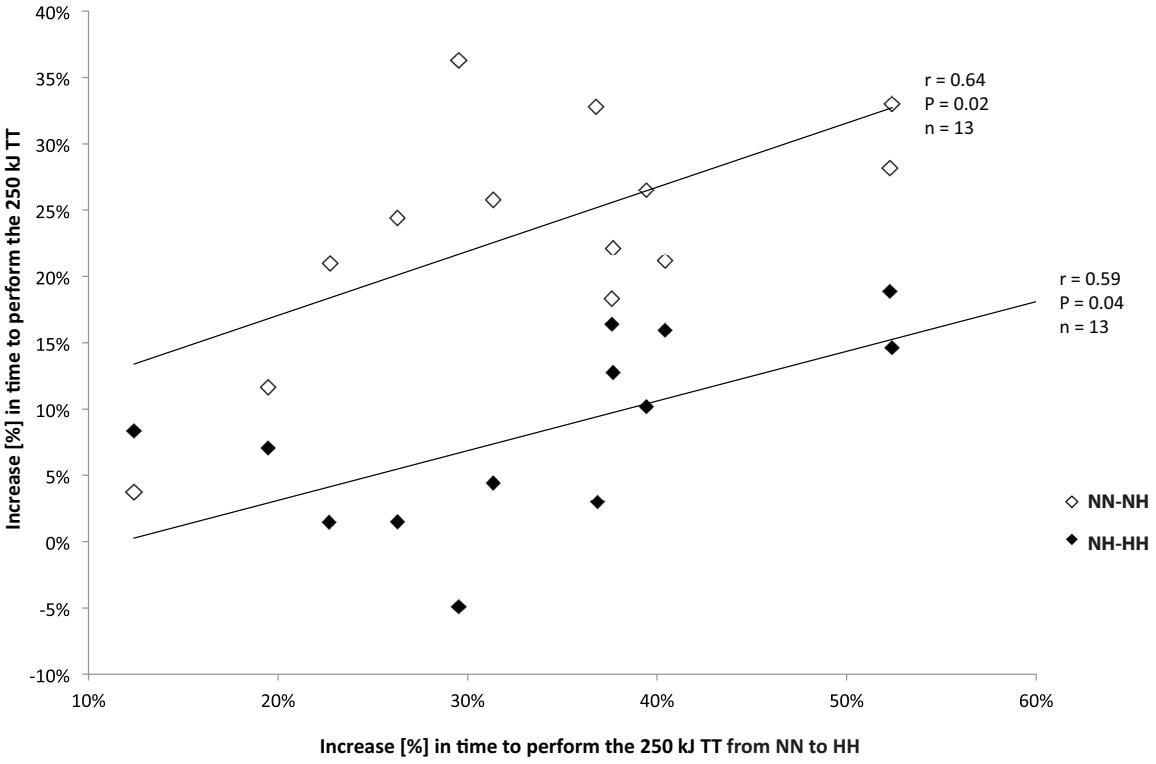

Figure 2. Correlations between the increase (in %) in time to perform the 250 kJ time trial (TT) from normobaric normoxia (NN) to hypobaric hypoxia (HH) with the increases (%) in time to perform the 250 kJ TT either from NN to normobaric hypoxia (NH) or from NH to HH.

Other physiological and subjective feelings parameters. All parameters measured during the time trial are presented in Table 3. The subjective criteria of exhaustion (Borg scale) and perceived exertion were similar among the three conditions. Significant differences in

ventilatory parameters were observed between NN and the two hypoxic conditions ($P<0.05$ to $P<0.001$, see Table 3) but with no difference between NH and HH.

Table 2 Mean hematologic values measured before the sessions (*bl*) and after 20 h (H+20) in normobaric normoxia (NN), normobaric hypoxia (NH) and hypobaric hypoxia (HH).

	NN		NH		HH	
	bl	**H+20**	***bl***	**H+20**	***bl***	**H+20**
RBC [$\times 10^6 \mu l^{-1}$]	4.98 ± 0.35	4.98 ± 0.28	4.88 ± 0.36	5.09 ± 0.39$^{\$}$	5.02 ± 0.36	5.33 ± 0.30$^{*\#\$}$
RET [%]	1.02 ± 0.24	1.05 ± 0.19	1.01 ± 0.39	0.97 ± 0.23	1.07 ± 0.29	1.02 ± 0.30
Htc [%]	43.90 ± 2.54	43.64 ± 1.93	43.28 ± 3.24	44.56 ± 3.27$^{\$}$	44.30 ± 2.76	46.02 ± 2.00$^{*\#\$}$
Hb [$g.dl^{-1}$]	15.16 ± 0.99	15.22 ± 0.88	14.95 ± 1.20	15.57 ± 1.27	15.33 ± 1.05$^{\$}$	16.23 ± 0.67$^{*\#\$}$
EPO [$mU.ml^{-1}$]	3.52 ± 2.25	4.46 ± 1.92	3.74 ± 2.10	9.24 ± 3.69$^{***\$\$\$}$	3.66 ± 2.25	9.44 ± 3.63$^{***\$\$\$}$
Ferritin [$ng.mL^{-1}$]	181.7 ± 111.6	175.9 ± 134.8	251.1 ± 148.4	199.1 ± 136.4$^{\$}$	206.1 ± 154.1	171.5 ± 116.5$^{\$}$
Δ PV [%]	-	-2.9 ± 5.6	-	-8.8 ± 7.5*	-	-8.8 ± 5.3*

RBC red blood cells; RET reticulocytes; Htc haematocrit; Hb haemoglobin; EPO erythropoietin; Δ PV delta plasma volume between H+20 and *bl*; *bl* baseline values; *H+20* values after 20 hours of exposition. Data are mean ± SD; $^{*}P<0.05$ and $^{***}P<0.001$ for differences with NN; $^{\#}P<0.01$ for differences with NH; $^{\$}P<0.05$ and $^{\$\$\$}P<0.001$ for differences with baseline.

Table 3 Mean cardiorespiratory, and electromyographic responses during the time trial and metabolic and perceptual responses at the end of exercise in normobaric normoxia (NN), normobaric hypoxia (NH) and hypobaric hypoxia (HH).

	NN	NH	HH
HR [bpm]	161 ± 8	163 ± 7	163 ± 7
$\dot{V}_E$ [L.min^{-1}]	115.1 ± 30.7	121.3 ± 25.7	120.1 ± 24.1
V_T [L.min^{-1}]	2.87 ± 0.32	2.97 ± 0.35	2.90 ± 0.40
f [1.min^{-1}]	40.0 ± 10.6	41.1 ± 8.8	42.6 ± 11.2
$P_{ET}O_2$ [mmHg]	101.5 ± 12.6	73.6 ± 16.1***	64.7 ± 4.0***
$P_{ET}CO_2$ [mmHg]	31.6 ± 3.7	25.9 ± 4.2***	27.4 ± 4.0**
$\dot{V}O_2$ [L.min^{-1}]	3.5 ± 0.7	3.1 ± 0.4***	2.9 ± 0.2*
$\dot{V}CO_2$ [L.min^{-1}]	3.3 ± 0.6	2.9 ± 0.4**	3.1 ± 0.4
$\dot{V}_E/\dot{V}O_2$	32.1 ± 7.3	37.8 ± 5.6*	40.4 ± 7.0***
$\dot{V}_E/\dot{V}CO_2$	33.1 ± 3.8	41.1 ± 6.4***	37.8 ± 6.0**
iEMG/time *VL*	0.048 ± 0.01	0.040 ± 0.01**	0.032 ± 0.0***$^{\#\#}$
End Lactate [mmol. L^{-1}]	13.3 ± 2.9	10.6 ± 3.0*	10.4 ± 2.3**
Final RPE [6-20]	18 ± 2	19 ± 1	19 ± 1
Final VAS legs [1-10]	8.3 ± 1.6	8.5 ± 2.4	8.2 ± 2.6
Final VAS breath [1-10]	8.7 ± 1.4	9.5 ± 1.2	9.3 ± 1.5

HR heart rate; $\dot{V}_E$ minute ventilation; V_T tidal volume; f breathing frequency; $P_{ET}O_2$ end-tidal O_2 pressure; $P_{ET}CO_2$ end-tidal CO_2 pressure; $V\dot{O}_2$ relative oxygen uptake; $\dot{V}CO_2$ relative carbon dioxide produced; iEMG/time integrated electromyography per total time for the *vastus lateralis (VL)*; RPE rate of perceived exertion; VAS visual analogue scale. Data are mean ± SD; *P<0.05; **P<0.01 and ***P<0.001 for differences with NN. $^{\#}$P<0.05 for differences with NH.

Discussion

The major finding of the present study was that the TT performance decrease from normoxia to hypoxia was 8% greater in HH than in NH for a terrestrial/simulated altitude of 3450 m. A lower SpO_2 value was also observed in HH than in NH. However, there were no relationships between the decrease in saturation and the change in performance from normoxia to either normobaric or hypobaric hypoxia.

Several authors have already compared the performance between NN and either NH or HH. Whereas some have compared them separately (9, 25), only one study (5) has compared performance in normobaric versus hypobaric hypoxic conditions, but the study was neither a direct comparison of the same subjects nor of the same protocol. The large inter-individual differences in hypoxia sensitivity might weaken such protocols when a decrease in performance has been observed even at very low altitudes, such as 580 m above sea level (20). This decrease was reported to be between 10% and 20% at 3000 m (10% (23); 18% (17); 20% (44)) in NH versus 10% at 2100 m in HH (9). At higher altitudes, the performance decrease was approximately 30% (6) in NH versus 40% in HH (41% (19); 44% (37)).

Performance impairment with altitude. Based on several studies conducted at various altitudes, the main mechanism for endurance performance impairment in hypoxia might be the decrease in VO_{2max} with a mean decrease of 7.7% per 1000 m increase in altitude (-4% at 1000 m (8, 31, 46); -7.2% per 1000 m from 200 to 3200 m (11); -10% (31) to -15% (33, 46) at 2500 m; or -30% (31) at 4500 m, all in NH). All of these studies demonstrated that the reduced maximal oxygen uptake was induced by a decrease in arterial oxyhemoglobin saturation, leading to aerobic performance impairment. Obviously, the maximal oxygen uptake was strongly linked to SpO_2 levels also tainted by altitude (7, 29). Indeed, in the present study, SpO_2 was lower in the two hypoxic conditions compared to NN during both the resting period and the TT. The VO_2 was also lower in hypoxia but only during exercise, while

the minute ventilation did not differ between normoxia and hypoxia during exercise. These equivalent ventilation values could be explained by the high intensity of the trial, which was not the case in the study by Faiss *et al.* (16), for instance, in which submaximal exercise was considered so that differences in ventilation between normoxia and hypoxic conditions were found.

Physiological mechanisms. The acute (at H+20) changes in hematologic parameters (*i.e.*, red blood cells, hematocrit and hemoglobin) were greater in HH than in NH. However, the well-known altitude-induced effect of hemoconcentration (21) was similar between the two conditions. Taken together, these latter results were likely of negligible importance to the difference in performance reported between NH and HH. However, the resting $P_{ET}CO_2$ was lower for NH than for HH, as previously described (16, 26, 27), while the SpO_2 was higher in NH than in HH. The $P_{ET}CO_2$ has been described by Reeves *et al.* (36) as 'a good indicator of acclimatization'. The ventilatory acclimatization is a key component of altitude acclimatization and is characterized by an increased hypoxic ventilatory response, resulting in a decreased $P_{ET}CO_2$ associated with an increased SaO_2 (18), in accordance with our results. Altogether, these results suggested that NH is a less demanding condition than HH or that ventilatory acclimatization might occur more rapidly during acute exposure (~24 h) in NH.

However, these slight ventilatory differences observed at rest were no longer observed during the TT. These results were in agreement with those of previous studies that reported similar cardiac responses to both conditions, either at rest or during exercise (5, 39). In contrast, some studies have emphasized an influence of hypoxia type on heart rate (15, 16, 41, 42). These data confirmed that NH and HH cannot be used interchangeably, but their impact on cardiac responses remains up for debate. The cardiac response is influenced by several factors, such as exercise duration, exposure time and SpO_2, and these influences are independent of each factor. In addition, these responses are very individual, and personal monitoring of heart rate

is needed to adjust other training and recovery parameters in hypoxia, whether in NH or HH (13, 15).

Performance in NH versus HH. A greater decrease in endurance performance was noted for the first time in HH compared to NH by direct comparison with the same subjects. Because the mean heart rate and the plasma volume were not different between hypoxic conditions, one might speculate that the performance difference observed in the present study, therefore, should not be attributed to differences in cardiac output. However, a higher SpO_2 was found in NH than in HH, unlike in some reported studies (5, 16, 26) but in agreement with others (40-42). These values have been observed mainly at rest, and only a few recent studies have reported exercise measurements. Thus, the observed higher pulse oxygen saturation in the present study seemed to be one of the main causes of better TT performance in NH than in HH because it is well known that the ability to defend SaO_2 is strongly linked to the ability to maintain performance in aerobic activities at altitudes (7). However, this was not the case for the SpO_2 in NH and HH. In addition, we did not find any relationship between the delta SpO_2 and the delta performance in any of the conditions. Thus, unlike what Chapman *et al.* (7) demonstrated, the two hypoxic conditions did not have a greater impact on the most hypoxemic subjects. Nevertheless, it has been reported that hypocapnea can decrease cerebral blood flow and thus cerebral oxygenation (43) and therefore limit aerobic performance, especially when SaO_2 decreases to less than 82% (1). The fluid circulation and trans-alveolar capillary membrane diffusion differences already cited before (12, 27) could explain the SpO_2 difference between the two hypoxic conditions (24). The barometric pressure might modify the fluid circulation and the trans-alveoli-capillary membrane flux, which might in turn induce greater pulmonary vasoconstriction in HH and then modify the oxygen diffusion by decreasing the pressure gradient between the alveoli and capillaries. In addition, the exaggerated oxidative stress reported in HH might affect NO bioavailability, which could

impair oxygen unloading to tissues (16). Because our SaO_2 values were lower than the aforementioned threshold, one cannot exclude the possibility that a greater degree of hypocapnea was an additional mechanism of the greater performance impairment in HH.

The differences in TT performance between the normoxic and the hypoxic conditions were not correlated with the VO_{2max} measured in NN. Previous studies have reported that elite subjects experienced a greater decrease in VO_{2max} in altitude than their lower level counterparts (23, 32). Despite these findings, we did not measure VO_{2max} in altitude but TT performance, so our results did not support these findings, and none of our subjects was elite. Our results suggested that NH and HH would induce different performance responses across all athletic subjects.

More interesting is that there was a strong correlation between changes in TT performance from NN to NH and from NN to HH, suggesting that the sensitivity to hypoxia determined by chemosensitivity (38), the ventilatory response (34) or cerebral deoxygenation (6) would be a stronger influence than the type of hypoxia per se. In other words, it is likely that being a good or a bad responder to altitude would remain similar independent of exposure to NH or HH. This concept developed by Chapman *et al.* (7, 10) supported that there are good and bad responders to hypoxic stimuli. However, the type of hypoxia did not seem to influence this categorization. This latter point is of great practical importance and indicates that the conclusions of the numerous studies that have examined performance outcomes in hypoxia using NH would likely still have value in HH. However, our results also showed that the severity of the hypoxic stimulus was higher in HH than in NH, and this point must be accounted for when considering the hypoxic dose or when estimating exercise intensities, velocities or power output for HH conditions (*e.g.*, competition in terrestrial altitude) on the basis of NH data.

Muscle activity. Hypoxia per se is known to modify muscle activity during a 5-km cycling TT by decreasing the neural drive, as shown by the greater iEMG activity decrease in hypoxia than in normoxia (2). However, there was a large difference between studies in the modification of iEMG signals in normoxia or hypoxia (3), which might arise from the variability in the hypoxic stimulus, *i.e.*, acute, chronic or severe hypobaric or normobaric hypoxia (45). Thus, both the hypoxic stimulus and the exercise intensity seemed to have impacts on muscle activity. The present study was in agreement with these statements because we found a lower mean iEMG per time in the *vastus lateralis* during the TT in HH compared to both NN and NH. Cerebral perturbations during exercise in hypoxia have been shown to affect motor output and performance deeply (45). Further analyses of cerebral blood flow and cerebral and muscle oxygenation during such the TT are recommended to understand better how much distinct central nervous system adaptations in NH and HH might explain part of the larger performance decrease in the latter.

Subjective feelings. Hypoxia is known to exacerbate the rate of peripheral fatigue development and to increase sensations of difficulty in breathing and limb discomfort during endurance exercise (3). However, in the present study, at the end of the TT, the perceived effort scores, both for the legs and for breathing discomfort, were close to the maximum and were not different among conditions. These results support that the exercise was conducted up to near exhaustion under all of the conditions and that the differences observed were not induced by motivational factors.

This study was conducted with double blinding for the sessions (NN and NH) performed in the hypoxic chamber, and the blinding was effective because we did not find any differences between the perceived altitude of NH and NN immediately before the TT (H+20). However, for obvious practical reasons, blinding was not possible in the HH session. Therefore, one cannot exclude a placebo-nocebo effect on performance. Nevertheless, the 8% difference in

performance between the two hypoxic conditions was greater than the 1 to 5% significant placebo effect attributed to this kind of effort (4).

Perspectives

The present study confirmed that NH and HH did not have equivalent impacts on endurance performance. This result does not question all of the preceding altitude studies conducted only in NH, but it suggests that the results observed would have been more significant had these been conducted in HH. It is true that hypoxic chambers and the use of simulated altitude allow for much greater flexibility and more adjustment possibilities than terrestrial altitude. For athletes and coaches preparing for altitude competitions with NH, it is thus important to benefit from this flexibility and convenience. However, we believe that training intensities or saturation levels should be adjusted when transferred from one type of hypoxia (*e.g.*, testing in NH) to another (*e.g.*, competing in HH). These statements would help coaches and sport physiologists make more accurate recommendations for training intensities or durations at various altitude levels and types.

Limitation

Although our conclusions could, theoretically, be extended to all athletes, the present population remains recreational and does not include elite athletes. Additional studies are needed to confirm how much elite endurance performance may or may not be more affected in HH compared to NH conditions. Moreover, the performance could be influenced by the pacing strategy and, for logistical reasons, it was impossible to blind all the conditions (*i.e.*, subjects and experimenters knew when they were in HH at the top of Jungfraujoch). Therefore, one cannot rule out that slight environmental differences (*e.g.*, during sleep or exercise) might have influenced to a small extent the behavior of the subjects.

Conclusion.

This study showed greater performance impairment during a cycle time trial in hypobaric conditions, compared to normobaric hypoxia. As previously suggested, HH seemed to be a more stressful stimulus, and NH and HH were not interchangeable with endurance performance as the main objective. The main factor of this performance difference between hypoxic conditions seemed to be the lower arterial oxygen saturation in HH at rest, as well as during exercise. Further research is needed to better understand the physiological mechanisms responsible for these differences and for their impacts on muscle and brain function and pacing strategies.

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Chapter Seven

Article 3 – Same performance changes after Live High-Train Low in normobaric versus hypobaric hypoxia.

7. Article 3 - Same performance changes after Live High-Train Low in normobaric versus hypobaric hypoxia.

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Abstract

Purpose: We investigated the changes in physiological and performance parameters after a Live High-Train Low (LHTL) altitude camp in normobaric (NH) or hypobaric hypoxia (HH) to reproduce the actual training practices of endurance athletes using a crossover-designed study. **Methods:** Well-trained triathletes (n=16) were split into two groups and completed two 18-day LTHL camps during which they trained at 1100-1200 m and lived at 2250 m (P_iO_2 = 111.9 ± 0.6 vs. 111.6 ± 0.6 mmHg) under NH (hypoxic chamber; F_iO_2 18.05 ± 0.03%) or HH (real altitude; barometric pressure 580.2 ± 2.9 mmHg) conditions. The subjects completed the NH and HH camps with a one-year washout period. Measurements and protocol were identical for both phases of the crossover study. Oxygen saturation (S_pO_2) was constantly recorded nightly. P_iO_2 and training loads were matched daily. Blood samples and VO_{2max} were measured before (Pre-) and 1 day after (Post-1) LHTL. A 3-km running-test was performed near sea level before and 1, 7, and 21 days after training camps. **Results:** Total hypoxic exposure was lower for NH than for HH during LHTL (230 vs. 310 h; P<0.001). Nocturnal S_pO_2 was higher in NH than in HH (92.4 ± 1.2 vs. 91.3 ± 1.0%, P<0.001). VO_{2max} increased to the same extent for NH and HH (4.9 ± 5.6 vs. 3.2 ± 5.1%). No difference was found in hematological parameters. The 3-km run time was significantly faster in both conditions 21 days after LHTL (4.5 ± 5.0 vs. 6.2 ± 6.4% for NH and HH), and no difference between conditions was found at any time. **Conclusion:** Increases in VO_{2max} and performance enhancement were similar between NH and HH conditions.

Key words: Aerobic exercise, altitude-training camp, crossover study, real altitude, simulated altitude

Introduction

Endurance athletes commonly use altitude-training camps with several hypoxic methods to achieve maximal sea-level performance enhancement (Millet *et al.,* 2010). The "Live High – Train Low" (LHTL) method, where athletes live and sleep at altitudes between 2200 and 2500 m and train under 1200 m (Levine and Stray-Gundersen, 1997;Chapman, 2013), is recognized as an effective method that can improves performance in athletes, despite a large inter-subject variability in response (Lundby *et al.,* 2012). More than 15 years of research have revealed that LHTL is an effective training method to enhance sea-level performance in endurance athletes, and it provided 1-3% additional benefit compared with similar normoxic training, although not confirmed by all studies (Siebenmann *et al.,* 2012). These altitude-training camps are conducted under "real" (*i.e.*, hypobaric hypoxia, HH (Stray-Gundersen and Levine, 2008;Chapman *et al.,* 2014;Saugy *et al.,* 2014)) or simulated altitudes (*i.e.*, normobaric hypoxia, NH (Dehnert *et al.,* 2002;Clark *et al.,* 2009;Garvican *et al.,* 2011;Schmitt and Millet, 2012)). Emerging evidence suggests different physiological responses between these two types of hypoxia (Millet *et al.,* 2012), and it is now admitted that they cannot be used interchangeably (Fulco *et al.,* 2011;Saugy *et al.,* 2014;Coppel *et al.,* 2015;DiPasquale *et al.,* 2015). Short-term exposure in HH seems to induce greater levels of hypoxemia, when compared to NH (Savourey *et al.,* 2003). Likewise, reduced ventilatory responses (Loeppky *et al.,* 1997;Faiss *et al.,* 2013), but higher oxidative stress, combined with impaired nitric oxide bioavailability (Faiss *et al.,* 2013) were reported in HH. Regarding all these differences, pre-acclimatization effectiveness (Fulco *et al.,* 2013) and acute mountain sickness (AMS) scoring (DiPasquale *et al.,* 2015) are logically higher in HH.

Sea-level performance improvement following LHTL may also be different between NH and HH (Bonetti and Hopkins, 2009). Most LHTL studies in HH conditions have reported performance or haematological improvements (Wehrlin *et al.,* 2006;Bonetti and Hopkins,

2009;Chapman *et al.,* 2014;Saugy *et al.,* 2014;Garvican-Lewis *et al.,* 2015), and positive outcomes have been less frequent in NH conditions (Robach *et al.,* 2006b;Bonetti and Hopkins, 2009;Clark *et al.,* 2009;Robertson *et al.,* 2010b), when compared with control (*i.e.* sea-level) group. However, there is not a sufficient body of knowledge to confirm whether NH or HH induces better performance enhancement after LHTL training camps. It is difficult to compare results from studies with different parameters, such as different hypoxic doses, training loads, temperatures and statistical analyses (Millet *et al.,* 2012;Coppel *et al.,* 2015). There are not any crossover experimental designs to reduce the influence of the confounding factors that influence post-altitude responses and directly compare altitude-induced adaptations and performance changes after LHTL in NH and HH conditions in the same subjects. Athletes and coaches generally consider both hypoxic conditions similar, and it is important to clarify within practical and ecological conditions whether these two types of LHTL training camps may be used interchangeably. Consequently, we designed a crossover study to assess physiological and performance responses in trained athletes during and after LHTL camps matched in the inspired pressure of oxygen (P_iO_2) in NH or HH conditions. The first phase of the crossover was published previously (Saugy *et al.,* 2014), and results demonstrated better performance enhancement after the altitude training camp conducted under HH conditions. Groups in the present study were crossed to complete the crossover design and reduce an eventual group effect. We hypothesized that the LHTL intervention conducted under HH conditions would produce better performance improvement and greater physiological adaptations than under NH, which is consistent with the results of the first phase of the crossover.

Methods

Subjects

Twenty-four well-trained male triathletes participated in the first phase of this study, and 21 male triathletes participated in the second phase. We pooled data across phases to obtain a crossover analysis of sixteen subjects who were included in both conditions (n=10 in 2013 and n=6 in 2014 for NH condition, and n=6 in 2013 and n=10 in 2014 for HH condition). The main characteristics of all subjects in the analysis are presented as means ± standard deviation: age 24 ± 4 years, body height 179 ± 5 cm, body weight 70 ± 5 kg, BMI 21.8 ± 1.7 $kg.m^2$, VO_{2max} 66.3 ± 7.5 $mL.kg^{-1}.min^{-1}$, and P_{max} 380 ± 48 w. Subjects were included in the NH and HH group in the first phase and switched to the other hypoxia type for the second phase. The washout period between two phases was one year. The following inclusion criteria for participation and data analysis were used: 1) a minimum of 5 years of endurance training and frequent participation in endurance competitions; 2) initial ferritin levels >30 µg/l; 3) sufficient training loads during the lead-in period; and 4) participation in both parts of the study (*i.e.*, NH and HH altitude camps). All athletes provided written informed consent to participate in the study. The local ethical committees approved the study (Commission Cantonale Valaisanne d'Ethique Médicale, CCVEM; Agreement 051/09 and French National Conference of Research Ethics Committees; N°CPP EST I: 2014/33; Dijon, France), which corresponded to the two training locations. All experimental procedures conformed to the standards set by the Declaration of Helsinki.

Study design

This study includes the first phase of a crossover design published previously (Saugy *et al.,* 2014). Therefore, values presented here are means of the two phases (*i.e.*, 2013 and 2014) of the crossover study unless specified, and more precise details are provided in the statistics

section. Many methodological details are reported elsewhere (Saugy *et al.,* 2014), but these details are also outlined here for the reader's convenience. The experimental design was identical to the first phase of the crossover and consisted of a 33-week period divided into four different phases: 1) 24 weeks of training load quantification at sea level; 2) a 3-week lead-in period also at sea level, where the training loads were quantified, and the training sessions were supervised; 3) an 18-day LHTL training camp under NH or HH conditions; and 4) a 3-week post-altitude period at sea level where the training sessions were also supervised and loads quantified (see Fig. 1). The two phases were performed exactly during the same period of the competitive season (July) for the two consecutive years, with athletes training in the same club under the supervision of the same coaches. Subjects were assigned to the opposite condition (NH or HH) of the condition they underwent during first phase of the study (*i.e.*, in 2013). They were initially matched based on VO_{2max} values that were measured during the Pre-test of the 2013 study. Both groups lived at an altitude of 2250 m under simulated (NH) or real (HH) hypoxic conditions. All subjects trained at an altitude between 1100 and 1200 m. Subjects performed several physiological tests in a well-ventilated laboratory (Prémanon, France, 1150 m) before (Pre-) and immediately after (Post-1) the LHTL camp. Measurements included blood samples, anthropometric measurements, and maximal incremental tests on a cycle ergometer (VO_{2max}). The Pre- and Post-1 tests were performed in the same order at the same time of day with the same materials for both phases of the crossover study. Subjects performed five 3-km running tests at the following times: prior to lead-in, before LHTL (Pre-), one day after LHTL (Post-1), seven days after LHTL (Post-7), and twenty-one days after LHTL (Post-21), in exactly the same manner for 2013 and 2014. All 3-km running tests were performed near sea level (between 100 and 390 m).

Hypoxic exposure

Subjects were exposed to a normobaric hypoxia equivalent to 2250 m during NH conditions, which was obtained by extracting oxygen (*i.e.* oxygen filtration) from ambient air in hypoxic chambers (inspired oxygen pressure (P_iO_2) 111.9 ± 0.6 mmHg; inspired oxygen fraction (F_iO_2) 18.05 ± 0.03%; Barometric pressure (BP) 666.6 ± 3.6 mmHg). The gas composition in each hypoxic chamber was continuously monitored using oxygen and carbon dioxide analysers (FIELDBROOK Ltd., London, UK) connected to a central station under the control of an independent and specialized physiologist. The hypoxic chambers were of medium size (15 ± 1 m^2) and equipped with conventional beds. Two subjects were in each room, and they primarily spent their time sleeping or resting between training sessions. Subjects in NH conditions left chambers 5-6 times daily on average to eat and train. Daily hypoxic dose in NH was 12.7 ± 0.5 h for a total hypoxic exposure of 229.2 ± 5.9 h. The HH group lived in Fiescheralp, Switzerland (2250 m, P_iO_2 111.6 ± 0.6 mmHg; F_iO_2 20.9 ± 0.0%; BP 580.2 ± 2.9 mmHg) and traveled twice daily to the valley (altitude < 1200 m) via cable car for training. The daily hypoxic dose in HH was 17.1 ± 1.7 h for a total hypoxic exposure of 309.9 ± 4.1 h. The hypoxia exposure was monitored daily and recorded manually for both conditions.

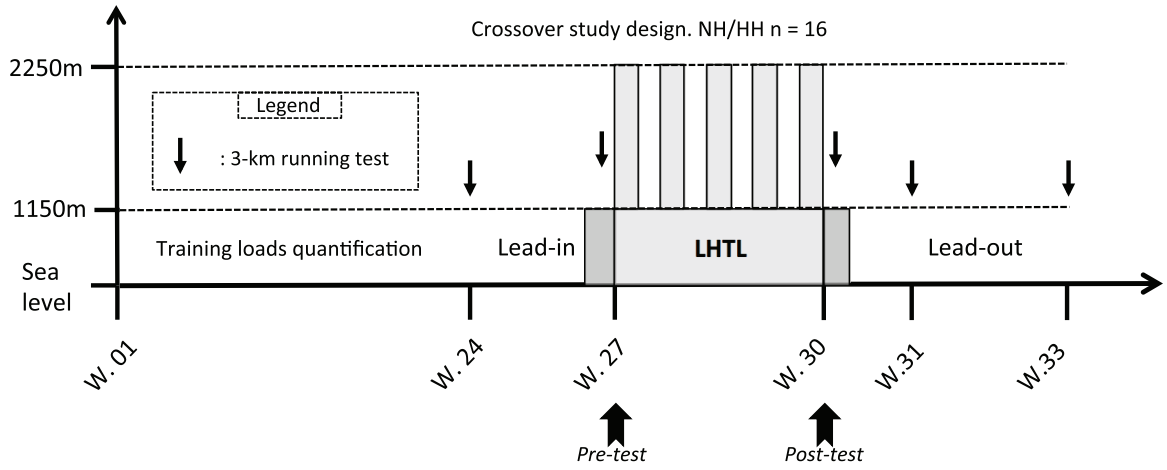

Figure 1. Overview of the whole protocol conducted in a crossover design in two consecutive years. In horizontal axis the protocol duration of each part in weeks (W) and in vertical axis the testing altitude, including: the six months before the lead-in period where the training loads were assessed, the lead-in, the LHTL camp and the lead-out period. With: 3-km test = the 3-km running tests on the track near sea level made on Pre-, Post-1, Post-7 and Post-21; LHTL = Live High Train Low training camp for normobaric hypoxia (NH) and hypobaric hypoxia (HH). The two dark grey slots before and after the LHTL period correspond to the 2 days of Pre- and Post-tests (*i.e.* Pre1, Pre2 and Post1, Post2, respectively) at 1150 m.

Measurements

Training loads. Training consisted of swimming, cycling and running. Two experienced certified coaches supervised and advised athletes during each training session during camps, and intensity and volume were matched for both groups. Training load quantification was performed using "Objective Load Scale" (ECOs) (Cejuela Anta and Esteve-Lanao, 2011), which was specially developed for training quantification in triathlons. Briefly, the ECOs were calculated by multiplying the total duration of a training session (in minutes) with a scoring value between 1 and 50, depending on the heart rate-based training zone (1 to 8) and by a factor of 1.0, 0.75 or 0.5 for running, swimming or biking, respectively. Daily and weekly training loads (ECOs) of each subject were quantified based on each subject's physical characteristics and training program intensity.

Running and maximal oxygen uptake. Running performance was evaluated using 3-km running tests that were completed on a 400-m outdoor synthetic track near sea level. Starts were given individually in a time-trial mode (*i.e.*, 30 s between each subjects) to avoid any group or pacing influences. VO_{2max} was tested before and after LHTL (*i.e.* at Pre-1 or Pre-2 and Post-1, see Fig. 1) using an incremental cycling performance test. Subjects were tested on their own bicycles, which were linked to a computerized ergometer system (Cyclus 2®, RBM elektronik automation GmbH, Leipzig, Germany). Workload was increased by 30 $W.min^{-1}$ after a 5-min warm-up period at a workload of 90 W until voluntary exhaustion was reached. Subjects were strongly encouraged to perform until they reached maximal exhaustion. They wore a nose clip and a mouthpiece for breath collection. Oxygen (O_2) and carbon dioxide (CO_2) levels were continuously measured and monitored as breath-by-breath values in expired gas (Ultima Cardio 2 gas exchange analysis system, MGC Diagnostics with Breezesuite software, Saint Paul, MN, USA). The flow meter and gas analyser were calibrated prior to each test. VO_{2max} was determined as the highest 30 s average value and based on the standard criteria of maximal exhaustion (VO_2 plateau, RER > 1.1 and incapacity to maintain the exercise load). Maximal power output (P_{max}) was considered as the load of the last stage completed.

Blood samples. Blood samples were taken from the antecubital vein (3 x 4.9 mL EDTA tube®, Sarstedt, Nümbrecht, Germany) either immediately after waking up or before breakfast, twice during each study phase on the first morning during the Pre- and Post-tests (*i.e.* before and after LHTL, see Fig. 1). Blood analyses were conducted using an XT-2000i analyser® (Sysmex Europe, Norderstedt, Germany) in a Lausanne WADA (World Anti-Doping Agency) accredited laboratory (Lamon *et al.,* 2010). All samples were analysed in duplicate, and mean values were used for the study. The following hematological parameters were quantified: red blood cells (RBCs), hemoglobin (Hb), hematocrit (Hct), mean cell

volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), reticulocyte percentage (RET%), and absolute number of reticulocytes (RET#). Regular quality control procedures were applied as required by the standards of WADA-accredited laboratories, and the coefficient of variations (CV) was within the CV limits accepted by the manufacturer for the instrument. Plasma EPO was quantified using an ELISA kit® (Stemcell Technologies, Grenoble, France), and the lower limit of quantification was measured at 1.6 mU/mL. Baseline ferritin was quantified using standard laboratory procedures (Dimension EXL, Siemens Healthcare Diagnostics SA, Zürich, Switzerland) to evaluate subject's iron stores. All athletes were tested for doping by the accredited laboratory according to the biological passport standards to avoid performance enhancement via doping. Determined CVs were always below 15%.

Night assessment. S_pO_2 and HR were recorded nightly from Pre-1 to Post-2 at 0.25 Hz using a wrist oximeter connected to a finger sensor (Wristox 3150® with 8000SM-WO Sensor, Nonin, Plymouth, MN). The oxygen desaturation index (ODI 3%; *i.e.* the number of times per hour of sleep that the blood's oxygen level drops by 3 percent or more) has been calculated throughout the periods.

Data Analysis and Statistics. Subjects' data were pooled for each condition from both phases of the study as follows: the NH condition values considered were the pooled values from the NH subjects in 2013 (n=10) and the NH subjects in 2014 (n=6); the same subjects were considered for the HH condition but reversed (n=6 in 2013 and n=10 in 2014; *i.e.*, n=16 for the whole analysis). Data are reported as means and standard deviations of the sixteen subjects considered for the crossover analysis. Data were tested for equality of variance (Fisher-Snedecor F-test) and normality (Shapiro-Wilk test). When both conditions were met, a two-way ANOVA was performed for repeated measures for each hypoxia condition (NH

and HH). To determine the time effects for variables measured on several occasions during camps, pairwise multiple comparison procedures was used (Holm-Sidak method, applied to the S_pO_2, HR and training loads). NH and HH were subsequently compared across time (Pre-, Post-1, Post-7, and Post-21) using a two-way ANOVA. When equality of variance or normality was not satisfied (differences in blood parameters tests from Pre- to Post-1 conditions), variables were analysed for each condition using a Friedman test for repeated measures. To determine time effects, pairwise multiple comparison procedures was used (Bonferroni test). Differences in percentage changes between conditions were tested using a Wilcoxon signed rank sum test (applied to changes in performance and blood parameters). The statistical power of the performed tests concerning the 3000 m performance with alpha = 0.05 was, for the time effect of 1.000, and for the group effect of 0.469. Differences between NH and HH condition at baseline (Pre-) were tested using a Mann-Whitney rank sum test (applied to incremental cycling test parameters and baseline blood sample parameters). Null hypotheses were rejected at $P<0.05$. All analyses were completed using Sigmaplot 11.0 software (Systat Software, San Jose, CA).

Results

Hypoxic doses, P_iO_2, night peripheral oxygen saturation and heart rate. Daily hypoxic dose (12.7 ± 0.5 vs. 17.1 ± 1.7 h, $P<0.001$) and total hypoxic exposure (229.2 ± 5.9 vs. 309.9 ± 4.1 h, $P<0.001$) were lower in NH than in HH. The average P_iO_2 values were not different between conditions (111.9 ± 0.6 vs. 111.6 ± 0.6 mmHg, for NH and HH). The nightly average of HR was higher for NH than for HH (51 ± 1 vs. 48 ± 2 bpm for NH and HH, $P<0.001$), and these values stayed higher when returning to 1200 m in Prémanon during the two nights of post-test (51 ± 2 vs. 46 ± 2 bpm, for NH and HH, $P<0.001$). Nightly S_pO_2 values were similar between two groups during the control nights (*i.e.*, the two nights at 1150 m before LHTL camps, Pre-1 and Pre-2), but values were higher in NH than in HH during the entire camp

(D1-D18; 92.4 ± 1.2 vs. 91.3 ± 1.0%, for NH and HH, P<0.001). These values remained higher (P<0.05) during the two nights at Post-1 (94.4 ± 0.9 vs. 93.6 ± 0.9%, for NH and HH, P<0.05). All values are presented in Fig. 2. In addition, the ODI 3% was significantly lower for NH than HH throughout the hypoxic nights (9.9 ± 1.6 vs. 15.1 ± 3.5, P<0.001).

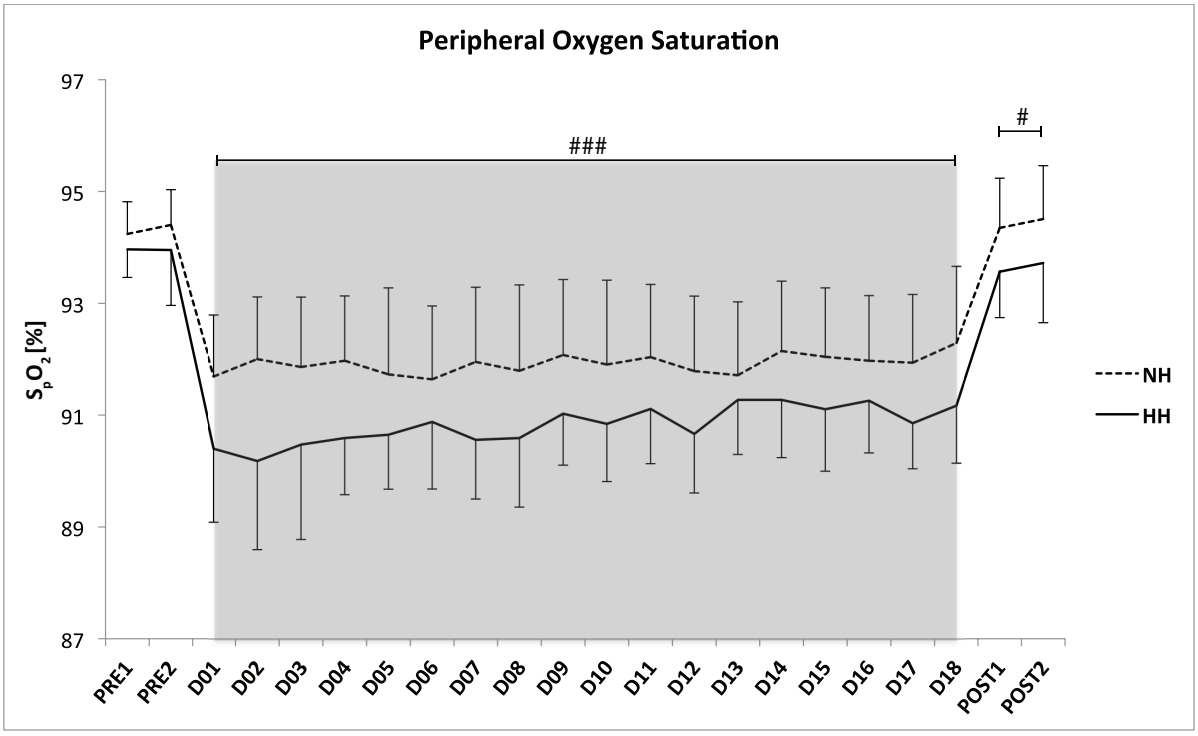

Figure 2. Mean values of night oxygen pulse saturation (S_pO_2) for the crossover data. Data are presented in mean ± standard error. Pre1-Pre2: measurements before the camps (1150 m, Prémanon, France); D01-D18: measurement during the camps (NH: hypoxic room in Prémanon, France; HH: Fiescheralp, Switzerland); Post1-Post2: measurements after the camps (1150 m, Prémanon, France). #P<0.05, ###P<0.001 for differences between conditions.

Training loads. No difference was found in daily training loads during the lead-in (3 weeks prior LHTL camps; 79.8 ± 22.7 vs. 87.4 ± 23.1 ECOs) or the lead-out (3 weeks following the LHTL camps; 164.4 ± 20.2 vs. 173.9 ± 24.1 ECOs; see fig. 1) periods between NH and HH, respectively. No difference was found in daily training loads (226.7 ± 56.5 vs. 214.5 ± 56.4 ECOs for NH and HH groups) between the two groups during the 18-d LHTL camps in both conditions of the crossover. Finally, weekly training loads monitored during the 24 weeks

prior to the study were not different between groups (979 ± 207 vs. 1135 ± 98 ECOs for NH and HH group, respectively). Daily training loads during the LHTL in phase 2 were reduced compared to phase 1 (232.2 ± 27.2 vs. 220.3 ± 31.4 for NH and 217.3 ± 48.1 vs. 211.4 ± 21.0 ECOs for HH, $P<0.05$).

3-km performance test. The 3-km performance was significantly increased to a larger extent in the HH group than in the NH group at Post-21 in the first phase of the study (−1.2 ± 2.9 vs. −3.2 ± 3.8%, for NH and HH, $P<0.05$). Performance in the second phase (*i.e.*, 2014) increased from Pre- to Post-1 (−3.3 ± 2.0 vs. −3.9 ± 2.9%, for NH and HH, $P<0.01$), Post-7 (−2.7 ± 3.1 vs. −2.6 ± 3.6%, for NH and HH, $P<0.05$) and Post-21 (−8.4 ± 4.1 vs. −9.1 ± 6.1%, for NH and HH, $P<0.01$). Performance increased from Post-1 and Post-7 to Post-21 for both conditions. However, no difference was noted between NH and HH groups at any time. The crossover demonstrated that performance increased from Pre- to Post-1 (–1.92%, $P<0.05$) and Post-7 (–2.44%, $P<0.05$) for HH but not in NH (–0.97 and -2.27% from Pre- to Post-1 and Post-7 respectively, ns). And it increased from Pre- to Post-21 ($P<0.001$), Post-1 to Post-21 ($P<0.001$) and Post-7 to Post-21 ($P<0.001$) for both conditions. However, no difference was noted between conditions at any time (Fig. 3). We found important inter-individual differences between both conditions, *i.e.* during two successive years. For example subject n°1 decreased his performance time at Post-21 by −3.6% in NH vs. −7.4% in HH. Subject n°6 increased his performance time at Post-21 by 1.9% in NH and decreased it by −12.1% in HH.

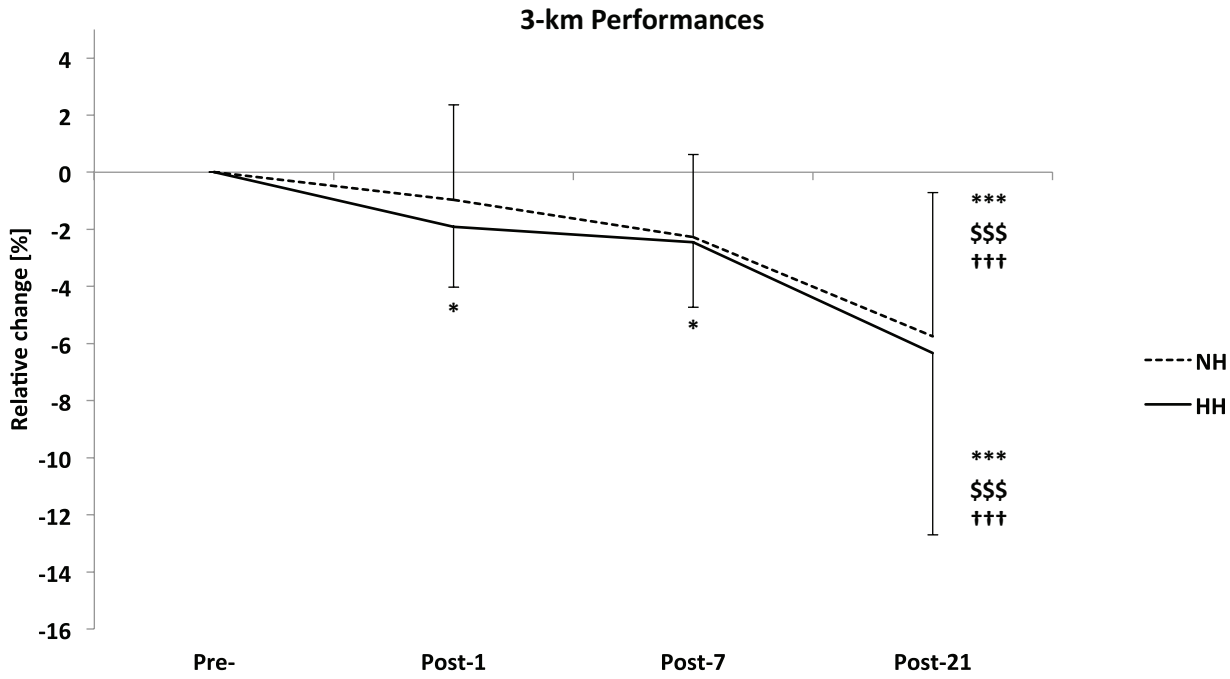

Figure 3. Relative change in 3-km run time from Pre- to Post-1, Post-7, and Post-21 as determined on a running track near sea level for the normobaric hypoxia (NH) and hypobaric hypoxia (HH) conditions (in %) for the crossover (n = 16). Data are mean ± standard error *P<0.05, **P<0.01 and ***P<0.001 for differences with Pre-; $^{\$\$\$}$P<0.001 for differences with Post-1; †††P<0.001 for differences with Post-7.

Maximal test on cycle ergometer. Subjects increased their power output and maximal oxygen uptake values immediately after altitude training camps in both NH and HH, but without any difference between conditions. Table 1 presents all values. Ventilation and heart rate at the submaximal work rate of 190 ± 24 W corresponding to 50% PPO pre- values have been measured. HR decreased to the same extent from Pre- to Post-1 (−6 ± 3 vs. −6 ± 2 bpm) and ventilation did not change significantly (2.5 ± 2.2 vs. −1.7 ± 7.9 L.min^{-1}) in NH vs. HH, respectively.

Table 1 Main parameters measured during the incremental test on ergocycle for normobaric hypoxia (NH) and hypobaric hypoxia (HH) group before (Pre-) and after (Post-) the camps.

		Pre-	Post-	Delta %
VO_{2max} [$ml.O_2.kg.min^{-1}$]	NH	65.2 ± 6.3	68.3 ± 5.0 ***	4.9 ± 5.6
	HH	66.7 ± 8.5	68.8 ± 2.4 **	3.2 ± 5.1
HR_{max} [$bpm.min^{-1}$]	NH	191 ± 8	191 ± 6	0.4 ± 2.2
	HH	190 ± 7	189 ± 6	−0.5 ± 1.7
P_{max} [W]	NH	362 ± 45	385 ± 33 ***	7.1 ± 6.6
	HH	397 ± 46	411 ± 39 ***	4.9 ± 5.1
VE_{max} [$l.min^{-1}$]	NH	180.1 ± 17.4	185.4 ± 15.1	3.6 ± 10.5
	HH	192.3 ± 31.7	195.2 ± 21.7	2.3 ± 7.7

Physiological parameters during the incremental test on ergocycle; VO_{2max} maximal oxygen uptake; HR_{max} maximal heart rate; P_{max} maximal power output; VE_{max} maximal ventilation. Data are mean ± SD; **P<0.01 and ***P<0.001 for differences between Pre- and Post-.

Hematological parameters. EPO values decreased significantly to the same extent from Pre- to Post-1 in both conditions (−30 ± 25 and −36 ± 21% for NH and HH, P<0.001). No significant changes were observed in the other parameters. Table 2 presents all values.

Table 2 Main parameters measured with blood analyses for normobaric hypoxia (NH) and hypobaric hypoxia (HH) group before (Pre-) and after (Post-) the camps.

		Pre-	Post-	Delta %
EPO [mU/mL]	NH	5.12 ± 2.57	3.08 ± 1.55***	−33.6 ± 27.3
	HH	4.48 ± 1.45	3.02 ± 0.71***	−35.7 ± 19.7
RBC [u/µl]	NH	5.21 ± 0.49	5.29 ± 0.46	1.7 ± 7.3
	HH	5.24 ± 0.44	5.29 ± 0.38	1.1 ± 4.6
Hb [g/dl]	NH	15.38 ± 1.23	15.99 ± 1.05	4.3 ± 6.7
	HH	15.44 ± 1.08	15.75 ± 1.01	2.4 ± 5.1
Hct [%]	NH	45.52± 3.31	46.70 ± 3.10	2.9 ± 7.1
	HH	45.51 ± 3.16	46.44 ± 2.59	2.5 ± 3.7
MCV [fl]	NH	87.56 ± 4.10	88.53 ± 3.48	1.15 ± 1.3
	HH	87.19 ± 3.46	87.96 ± 3.30	0.9 ± 1.4
MCH [pg]	NH	29.56 ± 1.02	30.30 ± 1.22	2.5 ± 1.8
	HH	29.50 ± 1.12	29.84 ± 1.26	1.2 ± 1.8
MCHC [g/dl]	NH	33.78 ± 0.81	34.25 ± 0.92	1.4 ± 1.7
	HH	33.83 ± 0.85	33.92 ± 0.91	0.3 ± 2.1
RET [%]	NH	1.14 ± 0.53	1.13 ± 0.27	6.3 ± 31.1
	HH	1.09 ± 0.28	1.09 ± 0.36	3.8 ± 31.8

Blood and hemoglobin parameters; EPO erythropoietin; RBC red blood cells; Hb hemoglobin; Htc hematocrit; MCV mean cell volume; MCH mean cell hemoglobin; MCHC mean cell hemoglobin concentration; RET reticulocytes. Data are mean ± SD; ***P<0.001 for differences between Pre- and Post-.

Discussion

This study is the first crossover study to compare physiological and performance responses during and after an 18-d LHTL altitude camp conducted in NH or HH conditions. The crossover design incontestably increased the statistical power of the present analysis compared with the first phase of this study (Saugy *et al.,* 2014). Lower peripheral oxygen saturation levels during the night and longer hypoxic exposure were noted in HH than in NH. However, no differences in blood parameters, maximal power output or VO_2max were observed between hypoxic conditions. Both conditions directly induced performance enhancement seven or twenty-one days after the camps. However, no differences were observed between NH and HH in sea level performances.

The second phase (*i.e.*, 2014) and crossover 3-km running performances were not different between conditions at any measurement point (Fig. 3), which is unlike the first phase of the study. Both conditions induced performance enhancements Post-1, Post-7 and Post-21 in 2014 and at Post-21 when considering the crossover. VO_{2max} and performance increased to the same extent for NH and HH conditions. So the VO_{2max} increase itself could explain at least partly the enhanced performance. Moreover, there is currently a large debate about running economy and efficiency when returning from altitude camps (Schmitt *et al.,* 2006;Lundby *et al.,* 2007;Chapman, 2013). In the present study VO_2 and ventilation were unchanged while HR decreased to the same extent during a submaximal cycling test in both groups. These later results show that cycling efficiency was not modified. It is therefore likely that running economy did not change significantly and did not influence the running performance enhancement. This evolution is consistent with the established insight for the development of performance after altitude training camps described by Sinex et al. (Sinex and Chapman, 2015) and drawn from Millet et al. (Millet *et al.,* 2010). These studies have demonstrated initial improvements in performance (days 1-7) and a higher plateau in performance (days 18-20 or more). The result that improvements were significant for only HH at Post-1 and Post-7

in the crossover was clearly influenced by NH data of 2013. A group effect was observed in the first phase, and NH subjects in 2013 did not assimilate the combination of training load and hypoxic dose as well as the other group. The training content and load were strictly similar between the two groups during each phase but were adjusted from phase 1 to phase 2 by coaches (probably from their experience of phase 1). The main change consisted in a reduced training load in phase 2 (232.2 ± 27.2 vs. 220.3 ± 31.4 for NH and 217.3 ± 48.1 vs. 211.4 ± 21.0 ECOs for HH, in phase 1 vs. phase 2) and a different periodization. Of interest is that a similar training adjustment has been performed between successive studies conducted in a chronological order at the same location; *i.e.* with elite Nordic skiers (Robach *et al.,* 2006a), swimmers (Robach *et al.,* 2006b) and distance runners (Brugniaux *et al.,* 2006). The crossover design tone down this tendency, but it highlights the considerable importance of inter-individual variations in responses to altitude training (Friedmann *et al.,* 2005;Garvican *et al.,* 2010;Chapman, 2013;Sinex and Chapman, 2015). Of interest is the observed intra-individual variability between successive years, in line with a previous case study (Garvican *et al.,* 2007). This inter- or intra-subjects variability between the two phases raises questions about the physiological basis of highly variables findings from previous published LHTL studies (Pialoux *et al.,* 2009;Robertson *et al.,* 2010a;Nordsborg *et al.,* 2012;Robach *et al.,* 2012;Siebenmann *et al.,* 2012;Garvican-Lewis *et al.,* 2013). With small effects and sample sizes, added to the large among of confounding factors (*i.e.* training loads, subjects training level, food supplies, sleep…), the probability of type 2 errors has been often under-considered.

The daily exposures are consistent with previous studies in normobaric or hypobaric hypoxia with 8-12 $h.d^{-1}$ (Roberts *et al.,* 2003;Saunders *et al.,* 2004) and 18 $h.d^{-1}$ (Levine and Stray-Gundersen, 1997;Wehrlin and Marti, 2006) in NH and HH, respectively. Night peripheral oxygen saturation was lower for HH than for NH from the beginning to the end of the camps,

and it remained lower after returning to Prémanon (1150 m) for the Post-tests (Fig. 2). This result confirmed the results of the first phase of the crossover, and it is consistent with previous studies (Savourey *et al.,* 2003;Savourey *et al.,* 2007;Self *et al.,* 2011) using short exposures and higher altitudes (less than 1 h, 4500 to 7620 m). No difference was reported in longer exposure (up to 24 h, from 3000 m to 4564 m) studies (Roach *et al.,* 1996;Loeppky *et al.,* 2005;Miyagawa *et al.,* 2011;Faiss *et al.,* 2013). However, the hypoxic exposure was always shorter than in the present study. To our knowledge, no study has directly compared NH and HH conditions in LHTL camps using a crossover design. Potential mechanisms underlying this difference in the nocturnal S_pO_2 found in the present study were reported previously (Saugy *et al.,* 2014). Briefly, a stronger pulmonary vasoconstriction in HH, which was induced by the modified fluid circulation and trans-alveoli-capillary membrane flux under the influence of barometric pressure, may lead to decreased pressure gradient and oxygen diffusion (Levine *et al.,* 1988;Loeppky *et al.,* 2005;Millet *et al.,* 2012). In addition, the ODI 3%, a reliable indicator of apnea/hypopneas index was calculated throughout all nights and indicated larger sleep disordered breathing in HH than NH. This is in line with previous results from Heinzer et al. (Heinzer *et al.,* 2013) who has reported more hypopneas with polysomnography analyses in HH compared to NH. However, the present difference in ODI 3% seems not clinically relevant (much lower than reported values in clinical groups) for inducing difference in performance enhancement between conditions. Moreover, Goodall et al. (Goodall *et al.,* 2014) recently found that the integrity of the corticospinal system is modified after 2 weeks at 5260 m, which potentially reduces fatigue level observed in acute hypoxia and might be a contributor to increased performance following acclimatization. Moreover, adaptive changes have been observed after 3 h of NH exposure (Rupp *et al.,* 2012). Thus, it seems that a time-dependent effect on the central nervous system exists. These central nervous system adaptations are likely influencing the observed changes in performance.

However, these mechanisms have not been investigated in the present study conducted under lower altitudes.

Differences in daily and total hypoxic exposures were found between conditions (13 vs. 17 $h.d^{-1}$ and 230 vs. 310 h for NH and HH), but our aim was to compare the two LHTL camps in "real conditions" in ecological ways. Moreover, this hypoxic doses difference is not the main factor for the S_pO_2 difference between NH and HH since S_pO_2 was lower for HH from the first night of exposure and remained stable throughout the camps (see Fig. 2).

Hematological parameters evolved to the same extent in both conditions, which is consistent with recent studies using natural (Garvican *et al.,* 2012;Garvican-Lewis *et al.,* 2013;Saugy *et al.,* 2014;Garvican-Lewis *et al.,* 2015) or simulated altitudes (Wehrlin *et al.,* 2006;Gore *et al.,* 2013;Saugy *et al.,* 2014) in LHTL protocols, but none of these studies have directly compared NH and HH. Interestingly, most of the studies on "altitude acclimatization" (*e.g.* conducted with untrained lowlanders) failed to demonstrate altered erythrocyte volume for up to 3 weeks below 4000 m (Sawka *et al.,* 2000). These authors reported that "physical activity modulates the erythrocyte volume expansion during altitude acclimatization" and that elite athletes engaged in aerobic training, despite a large inter-individual variability, might have larger benefits from the same hypoxic dose, due to genetically inherited factors that may modulate the hypoxic ventilatory drive, Hb P50, or erythropoietin responsiveness to hypoxia. Most of these points remain unresolved and were beyond the scope of the present study. Nevertheless, the LHTL camps in NH or HH did not affect hematological parameters, except serum EPO concentrations (table 2). However, the higher the Hbmass value at the start of the hypoxic exposure, the lower the Hbmass increase (Robach and Lundby, 2012). This result is consistent with previous studies from Dehnert et al. (Dehnert *et al.,* 2002) and Robach et al. (Robach *et*

al., 2006b), who have observed no changes in primary hematological parameters. EPO concentrations significantly fell when the subjects returned to 1150 m for the Post-tests, but this drop was not different between conditions in the crossover, unlike the first phase of the study. A drop in EPO after return to normoxia following continuous hypoxic exposure was reported previously (Milledge and Cotes, 1985;Savourey *et al.,* 1996;Savourey *et al.,* 2004;Risso *et al.,* 2007;MacNutt *et al.,* 2013). The lower serum EPO concentration found after the camp in the present study may be explained by the oscillating nature of LHTL, which was suggested by Garvican et al. (Garvican *et al.,* 2012), but the underlying mechanisms are not clear. However, MacNutt et al. (MacNutt *et al.,* 2013) has provided indirect evidence of neocytoloysis and an assumption of the mechanisms in a study with mice: a decrease in EPO mRNA within 1 h of hypoxia cessation combined with neocytolysis, whereby the most recently formed erythrocytes are targeted for destruction and phagocytized by macrophages in the spleen.

Strength and limitations

The use of a crossover design increases statistical power, and it is of great importance because of the large inter-subject variations due to hypoxia (Coppel *et al.,* 2015). It is even more important when comparing normobaric and hypobaric hypoxia because of the slight nature of their physiological differences. However, considering the statistical power (*i.e.* 0.469) for the group effect on the 3000 m performances, we cannot exclude the presence of type-2 error. Thus, there is still a possibility of performance difference between NH and HH, despite the crossover-designed protocol. This study is the first crossover study to compare prolonged altitude training in NH and HH. The present study compared the physiological and performance differences between NH and HH during and after a three-week LHTL conducted under “real” conditions (*i.e.*, daily exposures based on the literature and real training sessions supervised by coaches). The aim of this study was not to test the effectiveness of LHTL

training alternatives. It is likely that the large performance enhancement following the LHTL period was due to the intensified training during both LHTL and lead-out periods. The high training loads during the LHTL camp could be the main stimulus leading to performance gain during the lead-out period. Given the increase in training loads and the lack of a control group, we cannot evaluate whether hypoxic exposure/acclimatization actually contributed to the performance enhancement. Moreover, considering the fact that there was no control group, we cannot rule out that there is a strong placebo effect that would influence partly the performance enhancement in the athletes. It is a serious limitation in the present study since we cannot appreciate the magnitude of this placebo effect and if it was different between NH and HH conditions. In addition, considering the statistical power above-mentioned, further studies are needed with larger sample sizes to completely answer the research question.

Athletes were well trained, and training loads were not different between conditions. Training loads were quantified six months prior the study and supervised during the whole protocols for both phases of the crossover. The “real life” parameters of this study induced significant hypoxic dose differences between conditions. One cannot exclude that the slight physiological differences found between NH and HH would also appear with same hypoxic doses. On the other hand, we have to consider that simulated vs. real altitude might have produced different results if we had compared them at equal hypoxemia doses. Since the condition x time interaction was not significant, one cannot report from a statistical point of view that a condition was more efficient than the other one. However, from a practical and coaching point of view, the 0.95, 0.17 and 0.58% larger performance improvement in HH compared to NH (from Pre- to Post-1, Post-7 and Post-21, respectively) are not negligible. Nevertheless the aim of the present study was not a true comparison between these two hypoxic conditions, which would have requires equal stimulus levels. This crossover study confirmed that NH and HH involve different physiological adaptations but elicit similar performance improvements

when using LHTLs of the same duration. The hypoxic dose and/or the altitude level should be adjusted to individual athlete responses, *e.g.*, the night S_pO_2, to achieve the highest performance improvements. Obviously, the NH condition is more convenient for this purpose. Further investigations should focus on the individualization of the training and hypoxic exposure. Nevertheless, it is important to take into consideration that the group effect from the larger NH cohort in 2013 could be driving the results. Since the crossover is not perfectly balanced (with 10 and 6 instead of 8 and 8) the crossover design could not "cancel" this tendency.

Conclusion

The present crossover study provided a further step to compare normobaric and hypobaric hypoxia. The results confirmed that NH and HH are definitely not interchangeable when several physiological responses are considered, as suggested previously (Fulco *et al.,* 2011;Millet *et al.,* 2012;Saugy *et al.,* 2014;Coppel *et al.,* 2015). Despite differences in the stimulus that occur when using these two different methods in real life, we report no observable differences in responses to NH vs. HH.

However, the hematological responses and performance improvements post-LHTL were similar. Each hypoxic condition has advantages and drawbacks from a practical point of view. The HH condition leads to longer hypoxic doses for a given training period (*i.e.*, 18 d in this study), which may fit easier into a complex training plan with elite athletes, but the logistical constraints, and the cost may be detrimental. In contrast, NH condition allows athletes and trainers to individualize the hypoxic stimulus. It may be interesting to adjust the hypoxic dose by modifying the time spent in the room or the altitude setting to athletes' physiological responses and training level. However, training camps conducted under normobaric hypoxia require longer periods of time to achieve sufficient hypoxic doses because of the lower amount of time spent in hypoxia than under HH.

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Author contributions

JS, LS and GM made substantial contributions to the conception and design of the work, the acquisition, analysis, and interpretation of data for the work; they drafted the work and revising it critically for important intellectual content. AH, GC, RC, RF, JW, JR, NR made the acquisition, analysis, or interpretation of data for the work and revising it critically for important intellectual content. JS, LS, AH, GC, RC, RF, JW, JR, NR and GM gave their final approval of the version to be published and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Chapter Eight

Article 4 – Comparison of sleep disorders between real and simulated 3450-m altitude.

8. Article 4 - Comparison of sleep disorders between real and simulated 3450-m altitude.

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Abstract

Study objectives: Hypoxia is known to generate sleep disordered-breathing but there is a debate about the pathophysiological responses to two different types of hypoxic exposure: normobaric hypoxia (NH) and hypobaric hypoxia (HH), which have never been directly compared. Our aim was to compare sleep disorders induced by these two types of altitude.

Design/Setting: Subjects were exposed to 26h of simulated (NH) or real altitude (HH) corresponding to 3450m and a control condition (NN) in a randomized order. The sleep assessments were performed with nocturnal polysomnography (PSG) and questionnaires.

Subjects: Thirteen healthy trained males subjects volunteered for this study (mean±SD; age 34±9 yrs, body weight 76.2±6.8 kg, height 179.7±4.2 cm).

Results: Mean nocturnal oxygen saturation was further decreased during HH than in NH (81.2±3.1 vs. 83.6±1.9%; $P<0.01$) when compared to NN (95.5±0.9%; $P<0.001$). Heart rate was higher in HH than in NH (61±10 vs. 55±6 bpm; $P<0.05$) and NN (48±5 bpm; $P<0.001$). Total sleep time was longer in HH than in NH (351±63 vs. 317±65 min, $P<0.05$), and both were shorter compared to NN (388±50 min, $P<0.05$). Breathing frequency did not differ between conditions. Apnea-hypopnea index was higher in HH than in NH (20.5[15.8-57.4] vs. 11.4[5.0-65.4]; $P<0.01$) and NN (8.2[3.9-8.8]; $P<0.001$). Subjective sleep-quality was similar between hypoxic conditions but lower than in NN.

Conclusions: Our results suggest that HH has a greater impact on nocturnal breathing and sleep structure than NH. In HH, we observed more periodic breathing, which might arise from the lower saturation due to hypobaria, which needs to be confirmed.

Introduction

Sleep has been recognized as an essential component for athlete preparation and has been suggested to be the single best recovery strategy available to an athlete [1, 2]. On the opposite, sleep impairment has been shown to negatively influence the athletes' performance [3], mainly through an impairment of their aerobic performance, which was shown to be directly related to sleep quantity and quality [4, 5]. A drawback of altitude training might be a lower sleep quality since high-altitude exposure is known for altering the sleep quality: West *et al.* [6] reported significant sleep impairment at high altitude based on climbers subjective account and objectively through polysomnography (PSG) recordings. Szymczak *et al.* used two standardized scales (the Pittsburgh Sleep Quality Index and the Athens Insomnia Scale) to assess the subjective sleep quality alterations at high altitude and found significant impairments in general sleep quality and sleep induction [7]. However, the precise mechanisms causing sleep disturbances at high altitude remain unclear.

Recreational mountain sports in altitude such as trekking, ski touring or alpinism are becoming increasingly popular. Altitude/Hypoxic training is also commonly used by athletes for enhancing their sea-level performance through an improved oxygen transport capacity as a result of a hypoxia-induced erythropoietic response [8]. Hypoxia can be produced by a combination of reduced barometric pressure (PB) and/or a reduced inspired fraction of oxygen (F_IO_2) resulting in an inspired partial pressure of oxygen (P_IO_2) less than 150 mmHg [9]. There is currently a large debate on the patho-physiological responses to hypoxia in two different types of exposure: hypobaric hypoxia (HH; F_IO_2 = 20.9%; PB < 760 mmHg) or normobaric hypoxia (NH; F_IO_2 < 20.9%; PB = 760 mmHg) [10]. These two types of hypoxia seem to trigger different physiological responses with, for instance a greater increase in oxidative stress markers [11] and a decrease in the nitric oxide (NO) bioavailability in HH, compared to NH condition [12, 13]. In addition, breathing pattern has been shown to be affected

in a different way between HH and NH with a lower tidal volume, lower minute ventilation, a higher physiological dead space and a higher respiratory frequency during HH exposure [9, 14, 15]. Several previous studies have also demonstrated that the severity of acute mountain sickness (AMS) is higher in HH compared to NH [16-18].

During sleep, altitude also seems to have an impact on respiratory physiology in both HH and NH: Oxygen transport has been shown reduced in continuous [19] and intermittent NH [20] as well as in HH [21]. Moreover, Nespoulet *et al.* showed that O_2 and CO_2 chemosensitivity are closely related to ventilation during NH[22]. Kinsman *et al.* largely studied the respiratory and sleep disturbances during sleep in NH and observed periodic breathing (irregular respiratory pattern marked by alternating periods of rapid and slow respirations and by apneic periods lasting 15 sec or less) at a simulated altitude of 2650 m[23-25]. In addition, they also determined that the rate of rapid eye-movement sleep (%REM) increased with simulated hypoxic exposure compared to normoxia and that longer exposure time was associated with worse sleep quality. In HH conditions, several authors observed the development of periodic nocturnal breathing with increasing altitude. Moreover, Sargent *et al.* and Roach *et al.* [26, 27] reported a reduction of REM sleep and the presence of periodic breathing during sleep in young athletes after rapid ascent to 3600 m. This occurrence of nocturnal periodic breathing with accompanying cyclical intermittent hypoxia (IH [19, 28]) means that the sleeping athlete would experience varying severities of cyclical, intermittent arterial hypoxemia, that contain many of the features of maladaptive IH associated in sleep apneas (*e.g.*, reactive oxygen species producing cyclical HbO_2 desaturation/resaturation, transient arousals, repeated swings in cerebral vascular resistance and blood flow and intra-thoracic pressures coinciding with apneic/hyperpneic phases of the periodic cycles) [29]. Furthermore, it was speculated [30] that these maladaptive effects of the altitude-induced nocturnal IH would persist during the

athlete's daily training sessions and negatively impact exercise performance even in normoxia through elevated sympathetic vasoconstrictor outflow and pulmonary vascular remodeling reduced plasma volume or high ventilatory drive. Thus, sleeping in altitude would lead to alterations in breathing physiology during sleep and/or subsequently to maladaptive effects altering directly exercise performance even at sea level. On the other hand, recent studies also suggest that periodic breathing at altitude may be a protective/adaptive mechanism: It has been shown that for a given stimulus (either normobaric or hypobaric) mean nocturnal S_pO_2 mean can be higher in subjects exhibiting a large amount of apneas compared to those who don't [22]. In addition, periodic breathing seems not to play a predominant role in the pathogenesis of acute mountain sickness [31] and may even have a protective role [22]. This supposes a possible disconnection between sleep quality (high number of apneas would be deleterious) and adaptive efficiency (high number of apneas could be positive despite a poor sleep quality). This was recently shown in heart failure patients in whom treatment of Cheyne stokes breathing with adaptive servoventilation increased significantly cardiovascular mortality [32]. To date, sleep pattern has been observed in either NH or HH, but never directly compared between one another. The purpose of the present study was therefore to compare the magnitude of sleep and breathing disturbances between these two hypoxic conditions in order to better understand the underlying pathophysiological mechanisms.

Methods

Participants

Thirteen healthy trained males subjects volunteered for this study (mean ± SD; age 34 ± 9 yrs, body weight 76.2 ± 6.8 kg, height 179.7 ± 4.2 cm). All subjects provided a written informed consent before participation. The experiment was approved by a Medical Ethics Committee (Commission Cantonale Valaisanne d'Ethique Médicale, CCVEM; Agreement 051/09) and performed in accordance with the Declaration of Helsinki.

Protocol

After a first visit to the sleep laboratory, the participants were asked to sleep under three different conditions in random order, 12 to 20 days apart. Two nights were spent in a hypoxic chamber (ATS Altitude, Sydney, Australia) at an altitude of 485 m (Sion, Switzerland). One night recording was performed in the chamber under normobaric normoxia (NN) conditions with a F_IO_2 of 20.9% (control night, PB of 718.1 ± 3.8, mmHg P_IO_2 140.5 ± 0.6 mmHg, a temperature of 23 ± 1°C and a humidity of 42.8 ± 4.4%), and a second night in the chamber under normobaric hypoxia (NH) with a F_IO_2 of 13.6% to simulate an altitude of 3450 m (PB of 715.8 ± 3.8 mmHg, P_IO_2 of 91.0 ± 0.6 mmHg, a temperature of 22.7 ± 0.8°C and a humidity of 41.0 ± 4.8%). Oxygen level was controlled with an electronic oximeter (GOX 100 oximeter, Greisinger, Regenstauf, Germany). In order to blind the subjects to normobaric hypoxic or normoxic condition, the hypoxic system was also running during the NN night but normoxic airflow was spread into the chamber. The third nocturnal recording was performed in hypobaric hypoxia (HH) at the Jungfraujoch High Altitude Research Station (3450 m, F_IO_2 of 20.9%, PB of 481.8 ± 4.7 mm Hg, P_IO_2 of 90.9 ± 1.0 mmHg, temperature of 21.3 ± 0.6°C, humidity of 45.1 ± 8.3%). Each session consisted in a 26-h exposure to each condition (NN, NH, HH) in a randomized order. The schedule and activities during the whole sessions were exactly the same for each condition. The bedding conditions were similar among conditions. Sleep hours and conditions were very well controlled. Subjects were equipped with the PSG and then directly went to bed at 10 pm. The light were turned off at this moment for the whole night and turned on at 6 am. Thus the time spent in bed and the night duration were exactly the same among conditions. Moreover, subjects were wearing earplugs and eye masks during the nights, to avoid any external (*i.e.* noise and/or light) perturbations. The travel duration to access the Jungfraujoch by train during the HH sessions was approximately 50 minutes. This gradual gain in altitude was simulated during the NH and NN sessions. For 45 min before

entering the chamber, subjects breathed either hypoxic air (for NH) or room air (for NN) in a blinded fashion, using a mask connected to a three-way valve to an altitude simulation device (Altitrainer, SMTech, Nyon, Switzerland).

The hypoxic chamber is a well-ventilated 30 m^3 room (2.4 m×5.0 m×2.5 m) with transparent glass panels. The system consists in a compressor storing air in pressurized tanks with serial connection to air filters allowing oxygen reduction (altitude simulation) in the chamber. Temperature inside the chamber was maintained at 23° (23 and 22.7° for NN and NH respectively) in average by an internal air conditioning system.

During each session, participants were asked to fill three questionnaires upon waking-up in the morning including 1) The ESQ (Environmental Symptoms Questionnaire, divided in two parts: ESQ C for cerebral symptoms and ESQ R for respiratory symptoms out of 67 questions[33]) developed to help researchers quantifying symptoms experienced by individuals exposed to extreme environmental conditions, 2) the Lake Louise score questionnaire (LLS), a scoring system developed by the 1991 International Hypoxia Symposium consensus committee, which is widely used today to assess the severity of AMS [34] and 3) the Groningen Sleep Quality Scale (GSQS)[35], which was used to evaluate high altitude sleep (HAS) disturbances.

Twice per session, 4 hours before sleep and 4 hours after waking up, the Eupneic end-tidal CO_2 pressure ($P_{ET}CO_2$) was recorded on a breath-by-breath basis at the mouth with a Pitot tube (Medgraphics CPX, Loma Linda, USA). This parameter is a good indicator of arterial pressure in CO_2 ($PaCO_2$).

Sleep was recorded using polysomnography (PSG). A trained sleep technician equipped the subjects with the PSG recorder (Titanium, Embla® Flaga, Reykjavik, Iceland) between 7 and 9 PM. All sleep recordings included six electroencephalography, two electrooculography, three surface electromyography (one submental, two for right and left anterior tibialis

muscles) channels, electrocardiogram (composed of two electrodes), nasal pressure, thoracic and abdominal belts, body position, oxygen saturation and pulse rate.

All PSG recordings were scored by a trained sleep technicians (NT) using Somnologica software (Version 5.1.1, by Embla® Flaga, Reykjavik, Iceland) and reviewed by certified sleep physicians. Sleep stages, leg movements and arousals were scored according to the 2007 AASM criteria [36]. Apneas/hypopneas were scored according to the AASM 2012 rules [37]. The average number of apneas/hypopneas per hour of sleep (apnea-hypopnea index [AHI]) was calculated. The oxygen desaturation indexes represent the number of oxygen saturation drops ($\geq$ 3% and $\geq$ 4% for ODI 3% and ODI 4%, respectively) per hour of sleep.

Statistic

Data are reported as means and standard deviation for all parameters except for AHI and ODI where the medians and the 1st and 3rd quartile are reported. Data were tested for equality of variance (Levene's Test for Equality of Variances) and for normality (Shapiro-Wilk test). One-way ANOVAs with Student-Newman-Keuls post-hoc tests for all pairwise comparisons were used to identify differences between conditions (NN, NH, HH) in all respiratory, sleep and questionnaires data. AHI and ODI were not normally distributed, so ANOVAs were done on log-transformed data and values are presented with medians and confidence intervals. Null hypothesis was rejected at $P<0.05$. A two-ways ANOVA with repeated measures (condition x time) was used to identify differences in $P_{ET}CO_2$ between before and after the night as well as between conditions; Holm-Sidak post-hoc test was used for all pairwise comparisons to identify differences between conditions (NN, NH, HH). Pearson correlation was used to determine correlations between S_pO_2, hypopneas and LLS. All analyses were made using Sigmaplot 11.0 software (Systat Software, San Jose, CA). We estimated that 13 subjects were needed to have a 90% power with and alpha of 0.05 to detect 10±10 respiratory events per hour of sleep between the different conditions.

Results

Sleep variables in the three different conditions are presented in table 1. Total sleep duration was decreased under both hypoxic conditions compared with the normoxic condition. The time spent in deep sleep (N3) and in REM sleep was also reduced in both hypoxic conditions (ANOVA $P<0.05$ and $P<0.001$ respectively), with an additional reduction in HH compared to NH ($P<0.05$ for both).

Table 1: sleep indexes and indicators measured with night polysomnography					
Indices	**Unity**	**NN**	**NH**	**HH**	**ANOVA P**
TST	**[min]**	388 ± 50	317 ± 65**	351 ± 63*$^{\#}$	0.031
Sleep efficiency	**[%]**	92.8 ± 4.8	85.3 ± 12.5	84.4 ± 12.2	0.094
WASO	**[min]**	29 ± 19	55 ± 45	63 ± 48	0.083
AI	**[1.min^{-1}]**	17.3 ± 6.8	26.2 ± 14.3	24.6 ± 13.9	0.150
SOL	**[min]**	7.4 ± 7.1	9.4 ± 9.7	5.6 ± 3.6	0.407
NREM 1	**[min]**	28.5 ± 11.3	26.9 ± 16.0	32.01 ± 12.8	0.620
NREM 1	**[%]**	7.4 ± 2.8	8.6 ± 4.9	9.2 ± 4.1	0.502
NREM 2	**[min]**	217.9 ± 38.0	192.2 ± 40.8	207.7 ± 41.7	0.271
NREM 2	**[%]**	56.3 ± 7.5	61.1 ± 7.1	59.1 ± 6.5	0.235
NREM 3	**[min]**	66.7 ± 17.5	55.8 ± 18.5*	49.2 ± 15.1*$^{\#}$	0.043
NREM 3	**[%]**	17.1 ± 3.4	17.6 ± 4.6	14.3 ± 4.2	0.098
REM	**[min]**	75.0 ± 19.9	42.2 ± 20.9**	62.4 ± 27.1*$^{\#}$	0.003
REM	**[%]**	19.2 ± 4.0	12.7 ± 4.9***	17.4 ± 5.8*$^{\#}$	0.006
Position transition	**[n]**	26.0 ± 26.6	26.7 ± 15.3	34.9 ± 22.6	0.522

With NN: Normobaric normoxia, NH: normobaric hypoxia and HH: hypobaric hypoxia. TST total sleep time; Sleep efficiency is the ration of time spent asleep (*i.e.* TST) to the amount of time spent in bed; WASO wakefulness after sleep onset; AI total arousal index; SOL sleep onset latency; NREM non rapid eyes movements during sleep in different stages of sleep (1, 2, 3); REM rapid eyes movements. Data are mean ± SD. *$P<0.05$, **$P<0.01$, ***$P<0.001$ for differences with NN; $^{\#}P<0.05$ for differences with NH.

Respiratory parameters during sleep are displayed in table 2.

Table 2: respiration indexes and indicators measured with the night polysomnography.					
Indices	**Unity**	**NN**	**NH**	**HH**	**ANOVA P**
AHI	**[n]**	8.2 [3.9-8.8]	11.4 [5.0-65.4]*	20.5 [15.8-57.4]*$^{\#}$	0.006
AHI in REM	**[n]**	0 [0-6.8]	1.2 [0-17.0]	6.5 [0.6-11.5]	0.291
AHI NREM	**[n]**	6.9 [3.2-8.2]	10.2 [5.4-69.1]*	20.9 [12.2-58.9]*$^{\#}$	0.010
AHI Supine	**[n]**	9.4 [2.4-18.1]	17.9 [7.9-61-7]*	40.7 [17.5-76.2]*$^{\#}$	0.003
AHI Non-supine	**[n]**	5.2 [3.1-7.0]	11.9 [2.8-51.0]	23.5 [7.3-40.4]	0.116
ODI 3%	**[n]**	4.4 [2.2-4.8]	22.7 [13.1-73.8]*	47.6 [22.1-82.2]*$^{\#}$	<0.001
ODI 4%	**[n]**	0.9 [0.5-1.2]	9.1 [5.7-59.2]*	29.2 [8.8-57.1]*$^{\#}$	<0.001
RAI	**[n]**	5.3 ± 3.9	14.2 ± 17.8	13.1 ± 13.4	0.185
Hypopnea	**[n]**	7.2 ± 2.4	18.3 ± 15.9*	25.3 ± 18.8**$^{\#}$	0.005
Mean S_pO_2	**[%]**	95.5 ± 0.9	83.6 ± 1.9***	81.2 ± 3.1***$^{\#\#}$	<0.001
Min S_pO_2	**[%]**	92.0 ± 1.5	74.7 ± 7.0***	72.6 ± 4.2***$^{\#}$	<0.001
Heart rate	**[beat.min^{-1}]**	48 ± 5	55 ± 6*	61 ± 10***$^{\#}$	<0.001
%TST S_pO_2 < 90%	**[%]**	0.2 ± 0.7	99.79 ± 0.3***	99.84 ± 0.6***$^{\#}$	<0.001
Breathing freq	**[breath.min^{-1}]**	9.7 ± 3.2	10.5 ± 3.4	10.9 ± 3.7	0.748

With NN: Normobaric normoxia, NH: normobaric hypoxia and HH: hypobaric hypoxia. AHI apnea/hypopnea index; AHI in REM apnea/hypopnea index in rapid eyes movements; AHI NREM apnea/hypopnea index in non-rapid eyes movements; AHI Supine apnea/hypopnea index in supine position; AHI Non-Supine apnea/hypopnea index in Non-Supine position; ODI 3% oxygen desaturation index of 3%; ODI 4% oxygen desaturation index of 4%; RAI respiratory arousal index; Mean S_pO_2 mean of oxygen saturation level; Min S_pO_2 minimal oxygen saturation level; %S_pO_2<90% % of oxygen saturation under 90%; *f* breathing frequency. Data are median ± IC for all AHI and ODI and mean ± SD for RAI, HI, Mean S_pO_2, Min S_pO_2, HR and %S_pO_2 < 90%. *P<0.05, **P<0.01 and ***P<0.001 for differences with NN; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 for differences with NH.

In HH and NH, there was a decrease in mean nocturnal S_pO_2 (P<0.001) and an increase in ODI 3% or ODI 4% compared to NN condition (P<0.001). We also observed a further significant alteration in these parameters in HH compared to NH (P<0.05 for all). In the same line, the frequency of respiratory events (AHI), increased in NH compared to NN and was even significantly higher in HH compared to NH (P<0.05). These differences in AHI were however absent in REM sleep. The type of respiratory events observed included a great

majority of hypopneas, which were almost exclusively of central type. There was no significant difference in the types of apnea (central, obstructive or mixed) events between the three conditions, which were almost exclusively of central type. Breathing frequency did not differ between the 3 conditions.

The LLS were not statistically different between conditions (1.6 ± 1.3, 2.3 ± 2.8 and 3.1 ± 2.6 for NN, NH and HH, respectively), but showed mild AMS (score > 3) only in HH.

The results of the questionnaires assessing subjective sleep quality and sleep related complaints are displayed on table 3. Only the Goeningen questionnaire showed a significant decrease in sleep quality in NH and HH compared to NN, without difference between the two hypoxic conditions.

Table 3: questionnaires scores

Questionnaire	Score	NN	NH	HH	ANOVA P
LLS	**[0-15]**	1.6 ± 1.3	2.3 ± 2.8	3.1 ± 2.6	0.229
ESQ C	**[0-5]**	0.1 ± 0.1	0.3 ± 0.5	0.5 ± 0.6	0.096
ESQ R	**[0-5]**	0.2 ± 0.2	0.2 ± 0.3	0.3 ± 0.4	0.268
SLS	**[0-14]**	3.8 ± 3.4	6.6 ± 3.5*	6.5 ± 4.1*	0.016
VAS SQ	**[0-10]**	5.5 ± 2.1	4.0 ± 2.1	4.7 ± 2.2	0.194
VAS WS	**[0-10]**	5.9 ± 1.8	4.9 ± 2.1	5.0 ± 2.4	0.329

Questionnaires filled in the morning after each night in normobaric normoxia (NN), normobaric hypoxia (NH) or hypobaric hypoxia (HH). LLS Lake Louise score; ESQ C environmental syndrome questionnaire for cerebral symptoms of acute mountain sickness; ESQ R environmental syndrome questionnaire for respiratory symptoms of acute mountain sickness; SLS sleep score (Groeningen); VAS SQ visual analogic scale for sleep quality; VAS WS visual analogic scale for waking state. Data are mean ± standard deviations. *P<0.05 for differences with NN.

The $P_{ET}CO_2$ before sleep was not different between HH and NH (34.9 ± 5.5 vs. 35.1 ± 2.7 mmHg for HH and NH respectively) but they were both significantly lower compared to NN (38.7 ± 3.2 mmHg, P<0.05). After the sleep the $P_{ET}CO_2$ remained higher for NN (39.3 ± 3.1 mmHg, P<0.001) compared to the hypoxic conditions (33.2 ± 2.2 and 33.5 ± 4.8 mmHg for

NH and HH, respectively) with both hypoxic conditions being similar (condition by time interaction F = 2.003, P = 0.167).

A negative correlation was noted between S_pO_2 and the hypopneas (r = -0.442, P = 0.024; NH and HH data pooled). When considering the conditions separately, the relationship was significant only in NH (r = -0.555, P = 0.049 vs. R = -0.326, P = 0.277, for NH and HH respectively). Another correlation was observed between S_pO_2 and the LLS (r = -0.416, P = 0.035; NH and HH data pooled) but was significant only in HH (r = 0.006, P = 0.984 vs. r = -0.659, P = 0.014; for NH and HH respectively).

Discussion

To our knowledge, this is the first study comparing directly the effects of normobaric vs. hypobaric hypoxia with matched inspired pressure of oxygen on sleep structure and sleep disordered breathing in the same group of subjects. Our results show that both hypoxic conditions are associated with decreased sleep quality and increased nocturnal breathing disturbances. The major finding is that the addition of hypobaria to hypoxia (*i.e.* HH) further alters nocturnal breathing and sleep quality compared to hypoxia alone (*i.e.* NH).

The decrease in mean S_pO_2 found in both hypoxic conditions were expected but we observed a further decrease in HH compared to NH despite a similar oxygen pressure level (P_IO_2 91.0 ± 0.6 mmHg vs. 90.9 ± 1.0 for NH and HH). These data are in line with previous ones [15, 18, 38]. Although differences in physiological dead space between NH and HH have been previously suggested[14], this mechanism remains highly speculative. In the present study, breathing frequency was not significantly higher in HH than in NH, but differences in breathing pattern between the two hypoxic conditions have been already reported[15, 39] with a higher breathing frequency in HH, even if this point is still debated [39].

The lower S_pO_2 in HH compare to NH could also be due to a decrease in NO bioavailability, yielding a pulmonary capillary vasoconstriction and impaired alveolar/capillary gas exchange and modifying O_2 diffusion by decreasing the pressure gradient [9, 40, 41]. Barometric pressure per se can modify the fluid circulation (*e.g.* pulmonary lymph) and the trans- alveoli-capillary membrane flux [40]. It may also influence the N_2 and O_2 concentration in the cerebrospinal fluid and therefore partly change the central regulation of ventilation [9]. Moreover, it has been speculated that apnea/hypopneas have a protective effect during sleep at altitude by preserving a better oxygenation, probably due to a more pronounced hyperventilation following apnoeic events [22]. It was not the case in the present study: the lower the S_pO_2, the higher the number of hypopneas. In addition, the relationship between S_pO_2 and hypopneas was significant only in NH, suggesting that the difference in mean nocturnal S_pO_2 between NH and HH was not only due to the difference in the ventilatory events.

The increase in respiratory events (central apneas and hypopneas) found in both hypoxic conditions are believed to be related to a hypoxia-induced increase in chemoreceptor sensitivity (higher loop gain)[42]. When loop gain increases, the ventilatory response to mild increases in arterial CO_2 level generated by hypopneas or apneas tends to be excessive during sleep. This ventilatory "overshoot" at the end of the respiratory events will in turn generate a drop in $PaCO_2$ level (and re-increase S_pO_2) in the following seconds. During sleep, ventilation drive is highly dependent on the blood CO_2 level: when P_aCO_2 drops below a certain level called the "apnea threshold", breathing slows down or stops generating a hypopnea or an apnea until P_aCO_2 builds up and stimulates breathing again, and so on.

Another factor influencing the increase in respiratory events could be the decreased mean S_pO_2 which increases the amplitude of the oxygen desaturations (ODI 3% and ODI 4%) for a given decrease in P_aO_2, since the hemoglobin dissociation curve is much steeper for the S_aO_2

values of 81%-84% that we observed in hypoxic conditions. A decrease in arousal threshold (increased arousability) associated with hypoxia could also induce respiratory instability and increase the frequency of nocturnal respiratory events. However, we do not believe that this was a major factor since the arousal index was only mildly increased in hypoxic conditions compared with normoxia.

The decrease in respiratory events found during REM sleep is a well-known phenomenon occurring at low and high altitude due to a lesser CO_2 sensitivity in this sleep stage. The increase in sleep disordered breathing observed in HH compared to NH is however more difficult to explain. The possible increase in physiological dead space in HH is probably not the only cause since previous studies by our group and others showed that an increase in dead space (using a face mask) could significantly decrease altitude-induced central respiratory events [43, 44] (even though this experimental increase in dead space was probably much larger). Altered environmental conditions (comfort, temperature…) at high altitude compared with simulated altitude could play a role but the arousal index was not different between both conditions. Of importance was that the environmental factors were strictly controlled, with the particular attention to humidity, temperature and sleep conditions (bedtime and rise, noise and light conditions…) and, obviously, inspired oxygen fractions. A measurement of end-tidal CO_2 during sleep in both conditions could help to better understand these differences.

We also found decreased sleep duration and a lower proportion of deep sleep (N3) and REM sleep in both hypoxic conditions compared with normoxic condition. These sleep structure alterations have been reported before in young athletes at high altitude (3600 m) with a resumption of after two weeks except for lower deep sleep [26]. The same pattern was also reported in mountaineers at higher altitude (4559 m) with an early improvement after 3 nights

already[31]. The most likely explanation for these sleep structure alterations is the increase in sleep-disordered breathing, which increases sympathetic nervous system activity. Our results do however not support direct impact of environmental conditions such as temperature or discomfort of the bed since we saw the same decrease in REM sleep, SWS and TST in both hypoxic conditions whereas NN recordings took place in the exact same chamber as NH; with F_IO_2 being the only difference. Surprisingly, the sleep duration was more impaired in NH condition than in HH condition despite a lower AHI in NH. We could speculate that hypobaric conditions (HH) may be more exhausting for the brain and could thus generate a greater sleep need than in NH but this hypothesis would need to be confirmed in a specific study.

Despite the fact that no statistical difference in LLS was reported between conditions, a score > 3 was only present in HH, showing a mild AMS in this condition only. Hypobaric hypoxia seems to induce more severe acute mountain sickness than normobaric hypoxia, which is in line with a recent study from DiPasquale *et al.* [18]. Moreover the relationship between S_pO_2 and LLS was significant in the pooled data of NH and HH, but only in HH and not in NH. The lower the S_pO_2, the higher the AMS, in line with previous studies.

The larger "maladaptive response" [30] of sleeping in HH (*i.e.* induced by lower S_pO_2 and positive LLS), when compared to NH, is of practical importance for altitude training in athletes. First, it confirms that the severity of hypoxia is higher in HH. Secondly it might explain that these detrimental effects may counteract partly the expected positive ones: recently, we reported that the increase in hemoglobin mass [45] and the performance enhancement [46] were similar following 18 days of "Live High Train Low" in NH or HH, despite a more severe hypoxic stimulus (larger sleeping desaturation) and longer hypoxic dose (300 vs. 220 h) in HH. However, the present study was performed at real and simulated altitude of 3450 m and it is likely that the physiological consequences of various degrees of

hypoxia might differ. Therefore, the conclusions from the current study might not apply to studies performed at lower or higher altitudes. Further studies at different altitudes are needed to assess the potential sleep differences between NH and HH. Many fields are concerned by the differences between normobaric and hypobaric hypoxia: national and international teams and athletes are now using altitude or hypoxic training to improve their preparation[47]; several military forces are also using pre-acclimatization strategies to prepare for high altitude missions or assess the impact of hypobaric vs. normobaric hypoxia for space and aviation applications[48, 49].

Conclusion

Our results demonstrate for the first time that hypobaric hypoxia (*e.g.* "real altitude") has a greater impact on nocturnal breathing and sleep structure than normobaric hypoxia (*e.g.* "simulated altitude") conditions. Primarily, hypobaric hypoxia induces lower nocturnal oxygen saturation and more AHI compared to normobaric hypoxia. The main differences between these conditions could be NO metabolism altering pulmonary capillaries vasodilation or an increased physiological dead space due to hypobaria, but these hypotheses will need to be confirmed in further studies. Further researches are required to determine individually the duration and severity of inspired PO_2 (*i.e.* the degree of hypoxia) for achieving an optimal combination of positive (erythropoietic and peripheral) effects without significantly inducing maladaptive consequences for recovery and performance. However, the present study explores only one (the first) night. A valuable perspective would be to extend the comparison between conditions over longer duration, in order to assess the different adaptations during and after the acclimatization period. Another perspective would be to extend the comparison at lower or higher altitudes, as the present results are only applicable at the tested altitude of 3450 m.

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Chapter Nine

Article 5 – Sleep disordered breathing during Live High-Train Low in Normobaric versus Hypobaric Hypoxia.

9. Article 5 - Sleep disordered breathing during Live High-Train Low in Normobaric versus Hypobaric Hypoxia.

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Abstract

The present study aimed to compare sleep disordered breathing during Live High-Train Low (LHTL) altitude camp using normobaric (NH) and hypobaric (HH) hypoxia. Sixteen highly trained triathletes completed two 18-d LHTL camps in a crossover-designed study. They trained at 1100-1200 m while they slept either in NH at a simulated altitude of 2250 m or in HH. Breathing frequency and oxygen saturation (SpO_2) were recorded continuously during all nights and oxygen desaturation index (ODI 3%) calculated. Breathing frequency was lower for NH than HH during the camps (14.6 ± 3.1 vs. 17.2 ± 3.4 breath.min^{-1}, P<0.001). SpO_2 was lower for HH than NH (90.8 ± 0.3 vs. 91.9 ± 0.2, P<0.001) and the number of ODI 3% was higher for HH than NH (15.1 ± 3.5 vs. 9.9 ± 1.6, P<0.001). Sleep in moderate hypobaric hypoxia is more altered than in normobaric hypoxia during a LHTL camp.

Keywords: Exercise, hypobaric hypoxia, normobaric hypoxia, sleep quality.

Introduction

The "Live High-Train Low" (LHTL) training method, introduced in the 1990s (Levine and Stray-Gundersen 1997), where athletes sleep at moderate altitude while training at lower altitudes or near sea-level, is now used successfully by many endurance athletes. Nevertheless, it involves sleeping at altitude, which has been shown to impair sleep quality both at high (Sargent *et al.*, 2013) and moderate altitude (Latshang *et al.*, 2013). Furthermore, sleep was recognized as an essential component for athletes' preparation and is suggested to be the best recovery strategy available (Halson 2008). In addition, lower sleep quantity and quality impairment were shown to negatively influence athletes' performance (Cook *et al.*, 2011), mainly through a decrease in their aerobic performance (Davenne 2008; Oliver *et al.*, 2009).

Several possibilities to induce hypoxia could currently be used for LHTL. Simulated altitude, called normobaric hypoxia (NH), with reduction of the inspired fraction of oxygen (F_iO_2) can be used instead of the natural and terrestrial altitude, so called hypobaric hypoxia (*e.g.*, a lower barometric pressure inducing a lower oxygen partial pressure, HH). Several slight physiological differences between NH and HH exposure have been reported (Millet *et al.*, 2012; Richard and Koehle 2012). Indeed, the minute ventilation (V_E) is higher in NH than in HH with a combination of a higher tidal volume and lower respiratory frequency (Conkin and Wessel 2008; Savourey *et al.*, 2003). Acute mountain sickness (AMS) symptoms have been shown to be less severe in NH than in HH (Loeppky *et al.*, 2005) and the pre-acclimatization in 'real' altitude (HH) resulted in significant decrease in severity of AMS when traveling to HH conditions, while it was not the case with NH pre-acclimatization (Fulco *et al.*, 2013). Differences in fluids balance have also been observed with a higher diuresis and larger decrease in plasma volume for NH, while HH induced larger fluid retention (Conkin and Wessel 2008; Loeppky *et al.*, 2005). The nitric oxide (NO) in exhaled air or in plasma and the

oxidative stress markers were also shown to be lower in NH than in HH after 10h of exposure (Ribon *et al.*, 2016), 24h of exposure (Faiss *et al.*, 2013) or 18-d of LHTL training (Debevec *et al.*, 2015). Taken together, these differences suggest different physiological responses to hypoxic exposure in 'simulated' (NH) versus 'real' altitude (HH) given similar inspired oxygen partial pressure (P_IO_2). Therefore, we can then speculate that the type of hypoxia (*e.g.* normobaric or hypobaric hypoxia) could influence the sleep in altitude in a different way between conditions, even with P_IO_2 strictly matched. Which could be the potential mechanisms underlying differences between sleep in normobaric and hypobaric hypoxia and their influence on the athletes' recovery? Several protocols have already studied the sleep disturbances either with NH (Fulco *et al.*, 2011; Hoshikawa *et al.*, 2013; Nespoulet *et al.*, 2012) or HH (Latshang *et al.*, 2013; Roach *et al.*, 2013; Sargent *et al.*, 2013) but never with a direct comparison on the same subjects. The purpose of the present study was then to compare the sleep disordered breathing during a LHTL altitude training camp of 18 days either in NH or HH, with a crossover design including a one-year washout period between the two campaigns. Regarding all precedents studies on the differences between the two types of hypoxia, we hypothesized that HH would impair sleep breathing to a greater extent than NH.

Methods

Ethics statement. The study was approved by the regional medical ethics committee (Commission Cantonale Valaisanne d'Ethique Médicale, CCVEM; Agreement 051/09 and French National Conference of Research Ethics Committees; N°CPP EST I: 2014/33; Dijon, France). All experimental procedures conformed to the standards set by the declaration of Helsinki. This study was part of a comprehensive research program investigating physiological, psychological and performance adaptions of endurance athletes to normobaric

and hypobaric LHTL protocol. Many methodological details are reported elsewhere (Saugy *et al.,* 2014), but are also outlined here for the convenience of the reader.

Participants. Sixteen subjects participated to the study (data are mean ± SD: age 24 ± 4 years, body height 179 ± 5 cm, body weight 70 ± 5 kg, BMI 21.8 ± 1.7 $kg.m^2$, VO_{2max} 66.3 ± 7.5 $mL.kg^{-1}.min^{-1}$, and P_{max} 380 ± 48 w). All participants were informed about the experimental procedures and gave a written informed consent prior start of the study. The participants were non-smokers, sea near sea-level residents (< 600 m) and were not exposed to altitudes above 2000 m for at last one month prior to the study.

Study design. This crossover designed study comprised of two main experimental campaigns. Before the first campaign the participants were equally assigned to either the NH or the HH LHTL protocol. A one-year washout period was implemented between the campaigns to avoid any carry-over effect from the first intervention. During the second campaign the participants' conditions were crossed in order to complete the study crossover design. The intervention consisted of an 18-day LHTL phase performed in either NH or HH preceded by Pre-test and ending with Post-test. During the LHTL phase all participants performed supervised training sessions at altitudes between 1100 – 1200 m and resided at natural or simulated altitude of ~ 2250 m. Peripheral oxygen saturation (SpO_2) was measured during each night throughout the LHTL phase using a fingertip portable oximeter. During the LHTL phase, the participants were asked to spend a minimum of 12 $hrs \cdot day^{-1}$ in 1-3 person hypoxic rooms with conventional beds. The reduction of the O_2 fraction (F_IO_2) was achieved and maintained using an oxygen extraction system (OBS, Husøysund, Norway) that delivered the oxygen-depleted air to the designated rooms. The system was calibrated before the start of the study using precision calibration gases. The levels of O_2 and carbon dioxide were

continuously monitored using designated probes in each room (OBS, Husøysund, Norway). The simulated altitude in the rooms was kept constant throughout the protocol in order to maintain a simulated altitude of 2250 m (F_IO_2 = 18.05 ± 0.03%; Barometric pressure (PB) = 666.6 ± 3.6 mmHg; P_IO_2 = 111.9 ± 0.6 mmHg; simulated altitude ~ 2250 m). The HH participants resided in a ski resort in the Swiss Alps (F_IO_2 = 20.9 ± 0.0%; PB = 580.2 ± 2.9 mmHg; P_IO_2 = 111.6 ± 0.6 mmHg; altitude = 2250 m) throughout the terrestrial LHTL phase in 1-5 person rooms equipped with conventional beds (similar as in the NH rooms).

Measurements

Anthropometrics values. Athletes' body weight and height were measured in the morning before the breakfast and main body composition parameters were measured during Pre- and Post-tests and twice per week during the LHTL camp.

Questionnaire. Daily Lake Louise questionnaires were filled directly after waking up in rooms (hypoxic rooms for NH and normal room in HH, but all in hypoxia) during the whole protocol.

Sleep breathing assessment. SpO_2 and HR were recorded during all nights at 0.25 Hz with a wrist oximeter connected to a finger sensor (Wristox 3150 with 8000SM-WO Sensor, Nonin, Plymouth, MN). In addition, the number of times per hour of sleep that SpO_2 level drops by 3 percent or more has been calculated throughout these nights recordings (*i.e.* oxygen desaturation index = ODI 3%). Subjects were wearing an instrumented t-shirt (model SEW, CSEM, Neuchâtel, Switzerland) during all nights (including the 2 Pre- and Post- nights). This device made of comfortable fabric measured the breathing frequency with an elastic sensor included in the textile and the sleeping position with accelerometers.

Data Analysis and Statistics. Data are reported as means and standard deviations. Data were tested for equality of variance (Fisher-Snedecor *F-test*) and for normality (Shapiro-Wilk test). When both conditions were met, a two-way ANOVA for repeated measures was performed with the two conditions (NH and HH). To determine the time effect for variables measured several times during the camps, all pairwise multiple comparison procedures was used (Holm-Sidak method). Differences between means in NH and HH were then compared using a paired t-test. Null hypothesis was rejected at $P<0.05$. All analyses were made using Sigmaplot 11.0 software (Systat Software, San Jose, CA).

Results

Peripheral oxygen saturation. The peripheral oxygen saturation was higher for NH than HH during the whole camps (91.9 ± 0.2 vs. 90.8 ± 0.3, P<0.001) and remained higher at Post (94.4 ± 0.1 vs. 93.6 ± 0.2, P<0.05). ODI 3% mean was significantly higher for HH than NH throughout the hypoxic nights (15.1 ± 3.5 vs. 9.9 ± 1.6, P<0.001). The nightly ODI 3% was significantly higher in HH than NH from D2 to D14. No difference was found before and after the camp. The evolution of the ODI 3% during the camps is presented in Figure 1.

Heart rate. The mean nocturnal heart rate during the whole camp was higher for NH than HH (51 ± 1 vs. 48 ± 2 bpm, P<0.001).

Night breathing frequency. Although the night breathing frequencies were similar between the conditions during the two nights prior the camps (14.4 ± 2.4 vs. 15.1 ± 2.9 breath.min^{-1} for NH and HH, respectively), they were lower for NH than HH (14.6 ± 3.1 vs. 17.2 ± 3.4 breath.min^{-1}, P<0.001) during the camps (D1-D18), and stayed lower the two nights following the camps (14.2 ± 3.4 vs. 15.6 ± 3.1 breath.min^{-1}, P<0.01). These data are presented in Figure 2.

Lake Louise Questionnaire

No positive Lake Louise score and no difference between conditions were found (1.2 ± 1.4 vs. 1.2 ± 1.6 for NH and HH, respectively).

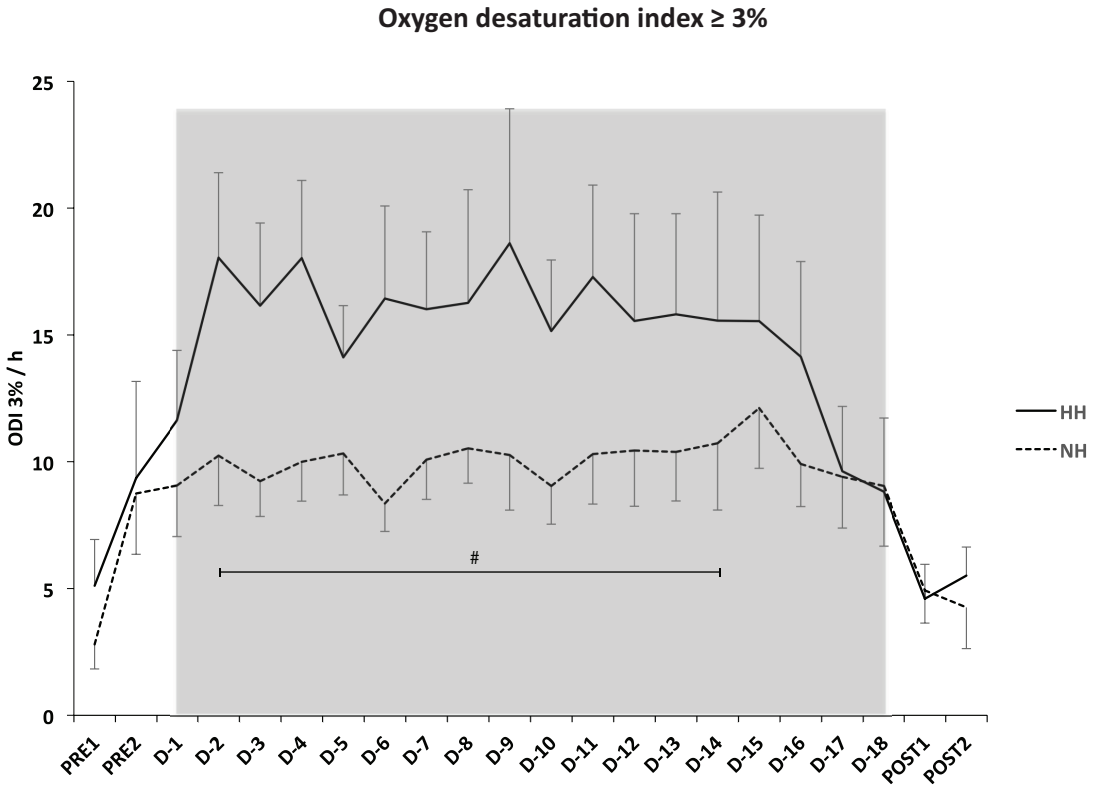

Figure 1. Values (mean ± standard error) of night oxygen desaturation index larger than 3% ≥per hour (ODI > 3%). Pre1-Pre2: measurements before the camps (1150 m); D01-D18: measurement during the camps (NH: hypoxic room; HH: ski resort). $^{\#}P<0.05$ for differences between conditions.

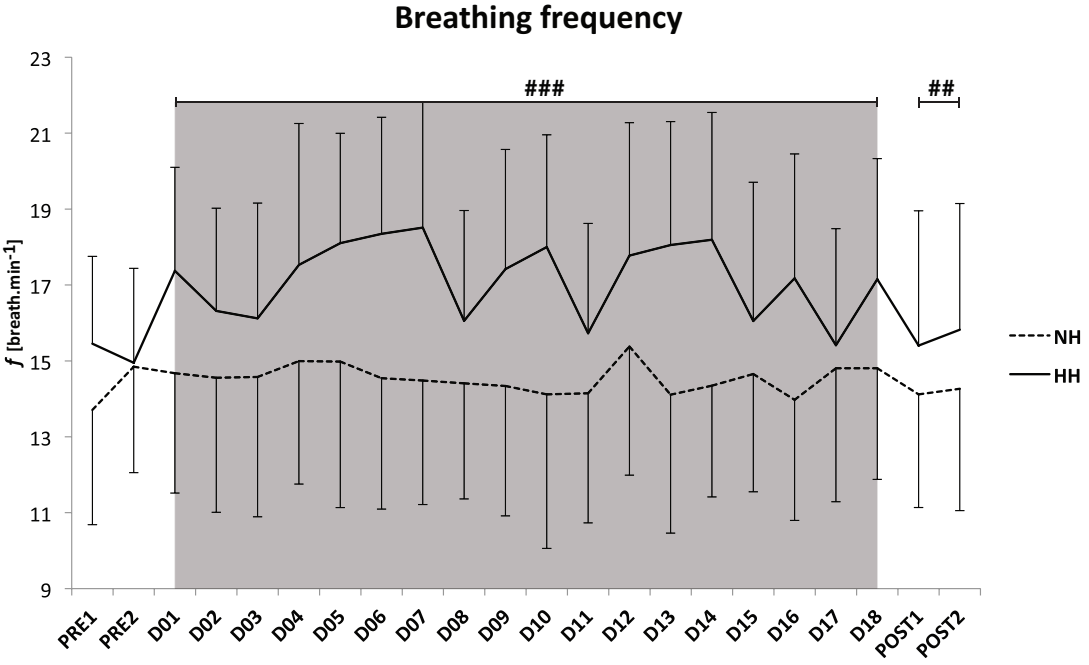

Figure 2. Values (mean ± standard error) of breathing frequency (*f*) measured during the night with instrumented t-shirts for NH and HH groups. Pre1-Pre2: measurements before the camps (1150 m); D01-D18: measurement during the camps (NH: hypoxic room; HH: ski resort). $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ for differences between conditions.

Discussion

To our knowledge, this is the first crossover study comparing the sleep breathing during an 18-d LHTL altitude camp conducted either in NH or HH conditions. Lower night peripheral oxygen saturation levels and HR were noted in HH compared to NH. Moreover, a higher ODI 3% has been observed during the nights in HH. In addition, a lower nocturnal breathing frequency has been shown in NH compared to HH.

As stated before, sleep quality is known to be disturbed in hypoxia either with NH (Fulco *et al.,* 2011; Hoshikawa *et al.,* 2013; Kinsman *et al.,* 2003; Nespoulet *et al.,* 2012) or HH (Kinsman *et al.,* 2003; Latshang *et al.,* 2013; Roach *et al.,* 2013; Sargent *et al.,* 2013). The decreased oxygen fraction leads to hypoxemia, which is known to induce sleep perturbations (Fulco *et al.,* 2011). In addition, Fulco et al. (Fulco *et al.,* 2011) stated that sleep either in NH or HH characterized the altitude acclimatization as a result from numerous inter-related

physiological adjustments that compensate hypoxemia. The progressive decrease in the end-tidal PCO_2 ($P_{ET}CO_2$) leads to an increase in peripheral oxygen level (SpO_2) during the first days of moderate altitude residence in HH (Young and Reeves 2002). A comparable degree of acclimatization was induced in NH with daily exposure to hypoxia (Muza 2007).

Logically, SpO_2 was lower in hypoxia than in the two control nights. Of interest is that SpO_2 remained lower in HH compared to NH during and after the camps. These differences were also found at higher altitude with shorter expositions (Saugy *et al.,* 2015). The reasons of this lower peripheral oxygen saturation in HH are still unclear. However, one may speculate that the barometric pressure modifies the fluid circulation and the transalveoli–capillary membrane flux, which might in turn induce greater pulmonary vasoconstriction in HH and then modify the oxygen diffusion by decreasing the pressure gradient between the alveoli and capillaries. In addition, the exaggerated oxidative stress generally reported in HH might affect nitric oxide bioavailability, which could impair oxygen unloading to tissues (Faiss *et al.,* 2013; Ribon *et al.,* 2016).

The oxygen desaturation index (ODI 3%) was significantly higher for HH compared to NH (Figure 1) denoting a more disturbed sleep breathing than in NH. ODI 3% is considered as a reliable indicator of sleep apnea (Hang *et al.,* 2015). This episodic breathing observed in HH might lead to intermittent hypoxia and consequently have clinical consequences: the desaturation-reoxygenation sequence is a typical pattern coupled with the majority of respiratory events defining intermittent hypoxia leading to oxidative stress with production of reactive oxygen species (ROS) (Lavie 2003). This increase in ROS could generate vascular endothelial damage and dysfunction (Lavie 2008). Altered baroreflex activity, increased pulmonary arterial pressure, changes in heart structure are also potential deleterious causes of sleep disordered breathing induced by episodic breathing in intermittent hypoxia (Lavie 2008).

The increase in ventilation is one of the first physiological adaptations when exposed to either normobaric or hypobaric hypoxia (Dempsey and Morgan 2015; Fulco *et al.,* 2011). This rise counteracts partly the reduction in oxygen transport induced by hypoxic environments. Ventilation and its associated parameters (*i.e.* $P_{ET}O_2$, $P_{ET}CO_2$, breathing frequency and physiological alveolar dead space) have already been shown different between NH and HH (Conkin and Wessel 2008; Loeppky *et al.,* 2005; Savourey *et al.,* 2003). In the present study, the breathing frequency was higher in NH compared to HH from the first to the last night in hypoxia and remained higher during the two following nights (14.6 ± 3.1 vs. 17.2 ± 3.4 breath.min^{-1} for NH and HH, respectively, $P<0.001$). These later findings confirmed that "*NH is not a surrogate to HH"* (Conkin 2016) and that NH and HH cannot be used interchangeably (Coppel *et al.,* 2015; DiPasquale *et al.,* 2015; Fulco *et al.,* 2011; Saugy *et al.,* 2015; Saugy *et al.,* 2014).

It can be assumed that these differences in nocturnal SpO_2 and ventilation should have an influence on acute mountain sickness, but no AMS has been reported. It is well known that AMS is not a problem for the majority of athletes between 2000 and 2500 m (Schommer *et al.,* 2012) and that under 3000 m the risk of developing acute altitude illness is very low (Luks *et al.,* 2010).

The present study is unique: In a "real-word" setting, well-trained endurance athletes slept for 2 phases of 18-days in different hypoxia conditions, with a finger oximeter and an instrumented t-shirt in order to measure saturation and breathing frequency without disturbing the sleep quality and the training program. By signal analysis, ODI 3% were extracted. The use of polysomnography is not possible in these conditions because it would disturb the recovery of the athletes. Of interest is that the present results at a moderate altitude and with prolonged exposure confirm our results measured with polysomnography at higher altitude (3450 m) during one night where lower SpO_2 and higher amount of ODI 3% were observed

for HH compared to NH (Heinzer *et al.,* 2016)

Conclusion

Our results demonstrate for the first time with a crossover study that sleeping in moderate altitude during LHTL altitude camp involved different physiological adaptations between NH and HH. First, HH induces lower nocturnal oxygen saturation compared to NH, and a larger amount of ODI 3%. The present study adds elements to the growing body of evidences stating that NH and HH cannot be used interchangeably (Coppel *et al.,* 2015; Fulco *et al.,* 2011; Millet *et al.,* 2012; Saugy *et al.,* 2015; Saugy *et al.,* 2014). However, results should be handle with care considering the very individual side of altitude exposures responses (Chapman 2013). Moreover, the oxygen desaturation index used is calculated from the raw SpO_2 measurements and is an indicator of sleep apneas, but not as relevant as the parameters measured by polysomnography analysis.

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Chapter Ten

Article 6 – Pacing strategies during cycle time trial in normobaric versus hypobaric hypoxia.

10. Article 6 – Pacing strategies during cycle time trial in normobaric versus hypobaric hypoxia.

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Abstract

Hypoxia is one major environmental factor, which is supposed to mediate central motor command as well as afferent feedbacks at rest and during exercise. We examined the potential differences between normobaric and hypobaric hypoxia on the pacing strategies during a self-paced aerobic exercise. Sixteen healthy subjects performed three cycling time trials (250 kilojoules) in three different conditions: hypobaric hypoxia (HH), normobaric hypoxia (NH) and normobaric normoxia (NN) after 24 h of exposure. Cerebral and muscular oxygenation was assessed by near infrared spectroscopy. Power output and peripheral oxygen saturation were also measured during the time trial. The tests were divided in 25 slices of 10 kilojoules to assess the pacing strategies.

Running title: Self-paced exercise at simulated and terrestrial altitude.

Introduction

Prolonged self-paced exercise, *i.e.* time trial, is more and more used in sport sciences for its relevance to real competitions (Periard & Racinais, 2016). With growing interest in the last decades, different models have been proposed to explain the complex regulation of such performances implying pacing – distribution of work or energy expenditure throughout a time- or distance-fixed exercise – based on several integrative and/or independent factors. Most of them refer to central and peripheral regulations through different sensitive feedbacks that could be selected by the central nervous system (St Clair Gibson *et al.*, 2006; Edwards & Polman, 2013; Roelands *et al.*, 2013; Renfree *et al.*, 2014). One of the most known pacing theory has been established by Tucker (Tucker, 2009), called *the anticipatory feedback rate of perceived exertion (RPE) model,* in which the aim is to maintain the homeostasis of the athlete in order to finish exercise using the brain as a central governor. Even though discrepancies exist between authors, pacing is commonly accepted as a conscious process depending on anticipatory/feedforward components and informed by the balance between the central motor command (*i.e.*, effort) and afferent feedbacks from multiple physiological systems (*i.e.*, exertion). Hence, the RPE would drive behavioral adjustments in power output (Abbiss *et al.*, 2015) to both promote optimal performance intensity and prevent catastrophic changes in the monitored physiological variables (bodily harm), what is particularly critical when external/environmental conditions are challenging.

One major environmental factor, which is supposed to mediate central motor command as well as afferent feedbacks at rest and during exercise, is hypoxia. Cerebral perturbations when exercise is conducted under hypoxic conditions compared to normoxia have been reviewed recently (Verges *et al.*, 2012; Goodall *et al.*, 2014) and authors concluded that several aspects of the supraspinal function such as cerebral blood flow or central motor command play a significant role in the hypoxia-induced impaired performance. Particularly, a major role of the

prefrontal and/or motor cortex oxygenation trends has been underlined with near-infrared spectroscopy measurements during constant (Amann *et al.*, 2007) or incremental (Subudhi *et al.*, 2009) workload exercise to exhaustion in hypoxia.

Besides, decreased arterial oxygenation has been supposed to influence motor output via an effect on peripheral locomotors muscles (Amann *et al.*, 2006b; Amann *et al.*, 2007; Amann & Calbet, 2008). Enhanced afferent feedbacks (*i.e.* peripheral fatigue) from respiratory muscles exercising in hypoxia are also likely to impact performance through brain integration (Verges *et al.*, 2010; Walker *et al.*, 2016).

Overall, it is known that hypoxia acutely decreases peak oxygen consumption (VO_2max) and aerobic performance, in particular with large muscular groups involved like in cycling activities (Verges *et al.*, 2012), but how pacing may be affected remains uncertain. Only a few studies investigated self-paced exercise when arterial oxygenation is manipulated, suggesting marginal differences (Clark *et al.*, 2007; Beidleman *et al.*, 2014; Bourdillon *et al.*, 2014) or similar trends (Amann *et al.*, 2006b; Periard & Racinais, 2016) in pacing between normoxia and hypoxia, despite reduced average power output in the later. A major concern regarding these studies is that subjects where exposed to simulated altitude for only 5 to 40 minutes before time trials. As oxygen delivery to the tissues (*e.g.* muscle, brain) progressively evolves in the first hours of exposition to hypoxia (*e.g.* time delay in changes between arterial and cerebral oxygenation, (Rupp *et al.*, 2013c), pacing strategies adopted after a 24-h period may strongly differ from what has been observed in artificially acute models of exposition.

Discrepancies in the literature regarding altered pacing in hypoxia may result from differences in time trial duration targets (*e.g.*, 5 min for (Clark *et al.*, 2007) *versus* 50-60 min for (Periard & Racinais, 2016)) but also from the type of hypoxia used in these models (hypobaric *versus* normobaric, respectively). Indeed, there is now a large debate on the use of diverse methods to induce hypoxia (Millet *et al.*, 2012) as numerous differences have been recently found in

subjects resting and exercising at a given pressure of inspired oxygen (P_iO_2), but among normobaric (NH) or hypobaric (HH) hypoxic conditions (Coppel *et al.*, 2015). For instance, a combination of a higher tidal volume and lower respiratory frequency leading to higher minute ventilation in NH compared to HH (Conkin & Wessel, 2008) has been reported and markers of oxidative stress have recently been shown lower in NH compared to HH (Faiss *et al.*, 2013; Ribon *et al.*, 2016). The mechanisms underlying these slight physiological differences are not so clear yet, but we have just shown that they are great enough to significantly emphasize performance impairment in HH compared to NH on a 250-kJ cycling time trial at 3450 m (Saugy *et al.*, 2016b). A potential explanation of this greater decrement could be the lower arterial saturation (SpO_2) found at rest and during exercise in HH, despite an iso-P_iO_2. However, the extent to which underlying physiological responses driving pacing strategies would be differentially affected in HH versus NH remains unknown.

The influencing factors of pacing may provide insight into how or why performance becomes impaired. In this study, we therefore examined pacing strategies in relation to arterial saturation, muscle activity, cerebral hemodynamics and rate of perceived exertion (*i.e.* subjective feelings) during a 250-kJ cycling time trial conducted after 24 h in 1) normobaric hypoxia (NH) at a simulated altitude of 3450 m, 2) in hypobaric hypoxia (HH) at a terrestrial altitude of 3450 m and, 3) in normobaric normoxia as a control condition. It was hypothesized that a 24-h exposure to either normobaric or hypobaric hypoxia would affect pacing strategy as a way to avoid critical exercise-induced brain harm. It was also anticipated that SpO_2 and cerebral hemodynamics would decline to a greater extent during time trial in HH, leading to suboptimal pace management and a greater performance loss compared to NH.

Methods

Experimental design and part of the methods have already been presented in a previous paper (Saugy *et al.*, 2016b) focusing on global exercise performance in HH *versus* NH but with no mention to the present interests (*i.e.* exercise-induced physiological adaptations and related pacing strategies). For the convenience of the reader, key methodological informations are redefined in the present paper.

Subjects

Sixteen healthy, trained males subjects volunteered to participate to this study (mean ± SD; age 34.7 ± 9.5 years, body weight 75.2 ± 7.2 kg, height 180 ± 6 cm, VO_{2max} 60.2 ± 9.9 $ml.kg^{-1}.min^{-1}$). Written informed consent was obtained from each participant before participation. Subjects were all non-smokers, and neither acclimatized nor recently exposed to altitude. All procedures conformed to the standards set by the *Declaration of Helsinki* and the study was approved by a Medical Ethics Committee (Commission Cantonale Valaisanne d'Ethique Médicale, CCVEM; Agreement 051/09).

Experimental Design

The experimental design consisted in a preliminary visit and three testing sessions. During the first meeting to the laboratory, subjects completed the baseline anthropological measurements, filled the consent form and the preliminary questionnaires. Participants then performed (*i*) a maximal incremental exercise test (F_iO_2: 0.21; 60 W + 30 $W.min^{-1}$) to determine VO_{2max} and the peak workload on a braked cycle ergometer including a Powertap sensor (Cycleops IC 400 Pro, Madison, Wisconsin, USA); and (*ii*) a familiarization test with the 250-kilojoules time trial on the same ergocycle.

The experimental design was then composed of three different sessions in a randomized order separated by at least 12 days. One session was performed in a hypobaric

hypoxia (HH) environment at the Altitude Research Station in Jungfraujoch (3450 m, F_iO_2: 20.9%, BP: 478.5 ± 3.2 mmHg, P_iO_2: 100.2 ± 1.4 mmHg). Two sessions were conducted in a hypoxic chamber (ATS Altitude, Sydney, Australia) built in our laboratory (Sion, 485 m, Switzerland). The hypoxic chamber is a well-ventilated 30-m^3 room (2.4 m × 5.0 m × 2.5 m) with transparent glass panels. The system consists in a compressor storing air in pressurized tanks with serial connection to air filters allowing oxygen reduction (altitude simulation) in the air input flow to the chamber. Temperature inside the chamber was maintained at 22°C in average maintained by an internal air conditioning system. One of the two sessions completed in the chamber was performed in normobaric hypoxia (NH) with a F_iO_2 of 13.8% (BP: 716.5 ± 4.4 mmHg, P_iO_2: 100.3 ± 1.8 mmHg) corresponding to a simulated altitude of 3450 m. The other session was performed in normobaric normoxia (NN) with a F_iO_2 of 20.9% (BP: 719.1 ± 4.0 mmHg, P_iO_2: 150.5 ± 1.6 mmHg). These parameters were controlled regularly with an electronic device (GOX 100 oximeter, Greisinger, Regenstauf, Germany). In order to blind subjects to altitude, the system was also running normoxic airflow into the chamber during the NN sessions. To simulate the progressive rise in altitude to reach Jungfraujoch by train in HH sessions, subjects breathed either hypoxic air (for NH) or room air (for NN) provided via a mask connected with a three-way valve to an altitude simulation device (Altitrainer, SMTech, Nyon, Switzerland) before each session in the hypoxic chamber. It was done in a total blinded way.

Each session consisted of 26 hours of exposure in each condition (NN, NH, HH) in a randomized blinded order. After 24 h of exposure, subjects completed a self-paced 250 kJ time trial, being free to set the resistance as they did it in the familiarization session. All trials were preceded by a rest period of 5 min, followed by 3 min warm-up period at 70W. Work completed from 0 to 250 kJ was the only information (visually) provided to subjects during the time trial. In order to mimic real world cycling environmental conditions and to prevent

excessive thermal stress, a fan providing a high wind speed was placed directly in front (~80 cm) of the subjects during the trials.

Measurements

Gas exchanges, heart rate and SpO_2. Breath-by-breath pulmonary gas exchange, minute ventilation (VE) and respiratory frequency (R*f*) were measured at rest and throughout time trial using a portable gas analyzer (MetaMax 3B, Cortex, Leipzig, Germany) with an oronasal mask (Vmask™, 7500 seires; Hans Rudolph Inc., Shawnee, KS; dead space, 41 mL). The volumes were measured using a bidirectional digital turbine. Oxygen uptake (VO_2), carbon dioxide production and end-tidal carbon dioxide partial pressure ($P_{ET}CO_2$) were determined with an electrochemical cell and an infrared analyzer, respectively, from the air drawn through a Nafion® sampling tube attached to the turbine at the output of the mask. Heart rate (HR) and peripheral oxygen saturation (SpO_2) were continuously recorded (Radical-7®, Masimo Corporation, Irvin, CA, USA) and stored for offline analyzes with commercially available software (Labchart software, AD Instrument, Colorado Springs, CO, USA).

Power, workload and cadence. Power output, speed, cadence and total workload were continuously recorded during the time trial by the cycle ergometer (Cycleops IC 400 Pro, Madison, Wisconsin, USA).

Electromyographic recordings. Quadriceps electromyography (EMG) was continuously recorded from the right *vastus lateralis* (VL) using bipolar silver chloride surface electrodes of 10-mm diameter (Kendall Meditrace 100). Recording electrodes were taped lengthwise on the skin over the muscle belly following SENIAM recommendations, with an interelectrode distance of 20 mm. Positions of the electrodes were marked on the skin so ensure precise replacement in other sessions. Reference electrode was attached on the patella. Low impedance (<10 kΩ) at the skin-electrode was obtained by shaving and abrading the skin with an abrasive sponge and cleaning with alcohol. EMG data were recorded with Biopac system

(MP150, Biopac System, Goleta, CA) and amplified (gain = 1000) with a bandwidth frequency ranging from 10 to 500 Hz, digitized at a sampling frequency of 2 kHz, and recorded by the AD conversion system (common mode rejection ratio: 90 dB, input impedance: 100 MΩ; gain: 1000). For the data analysis, the integral of the EMG activity was calculated over 10-kJ time-periods throughout time trial using the formula:

$$iEMG\ (|m(t)|) = \int_0^1 |m(t)|\ dt,$$

where *m* is the raw EMG signal.

Cerebrovascular variables. Mean middle cerebral artery blood flow velocities (MCAv) were measured bilaterally using a 2-MHz pulsed Doppler ultrasound system (ST3, Spencer technology, Seattle, USA). The Doppler ultrasound probes were positioned over right and left temporal windows and held firmly in place with an adjustable headband (Marc 600 Headframe, Spencer technology, Seattle, USA). The signals were at depths ranging from 44 to 58 mm. Signal quality was optimized using an M-mode screen shot and insonation depth, probes and headband locations were marked to ensure within-subject repeatability. Bilateral MCAv were averaged to represent an index of global cerebral blood flow at rest and during exercise. Cerebral O_2 delivery (cDO_2) before and during exercise was calculated using the equation: cDO_2 = mean MCAv x CaO_2, where CaO_2 refers to the oxygen content of the arterial blood estimated as follows: CaO_2 = [hemoglobin concentration assessed in each condition after 20 h of exposure x 1.36 x SpO_2/100], oxygen dissolved in plasma being neglected. cDO_2 was then expressed as a percentage of the resting normoxic (NN) pre-exercise value.

Near-infrared Spectroscopy measurements. Cerebral oxygenation in the left prefrontal (PFC) and motor (MC) cortex was assessed by monitoring changes in oxy- and deoxy- hemoglobin (O_2Hb and HHb, respectively) obtained with spatially resolved, continuous wave near-

infrared spectroscopy (NIRS) (Artinis, Oxymon MkIII, Zetten, The Netherlands). Theoretical and performance details of NIRS have been previously described (Perrey, 2008). PFC NIRS probes were centered between Fp1 and F3 locations according to the international 10-20 EEG system, with 3.5-cm interoptode distance. MC NIRS data were expressed as the average of a 4-channel square setting (3-cm interoptode distance) fixed with headbands between Cz and C3 locations. Muscle oxygenation was assessed from the right *vastus lateralis (at mid thigh)* using a 4-cm interoptode distance. For PFC and muscle, probe holders were secured to the skin using double-sided adhesive tape to minimize any change in its relative position and all optodes were covered with black sweatbands for them to be shield from ambient light. Total hemoglobin changes (THb = O_2Hb + HHb) were calculated to reflect the changes in tissue blood volume within the illuminated areas and difference in hemoglobin (HbDiff = HbO_2 - HHb) was calculated as a reliable estimator of change in tissue (de-) oxygenation status (Hoshi *et al.*, 2001; Rooks *et al.*, 2010). NIRS data were recorded at 10 Hz, filtered with a 2-s moving Gaussian window smoothing algorithm and expressed as relative changes (Δμmol) from the stable baseline preceding each time trial.

Diet and perceptual variables

Diet was standardized for nutritional and caloric content. Same nitrate- and nitrite-free meals and drinks were provided to the subjects (at the same times of day in the 24 h preceding the tests for the three conditions) to control nitrate intake, which can influence performance and oxidative stress levels (see (Faiss *et al.*, 2013; Ribon *et al.*, 2016) for further details). Subjects ate in the hypoxic chamber or in the kitchen of the HH laboratory and were allowed to drink water ad libitum during the whole protocol.

To evaluate acute mountain sickness (AMS) symptoms, subjects completed self-reported questionnaires in the morning before each time trial (after 20 h of exposure). These

evaluations comprised 4 sections of the Lake Louise AMS questionnaire (LLS, *i.e.* "headache", "gastrointestinal distress", "fatigue/weakness", "dizzy/light-headedness") (Roach *et al.*, 1993) and the neurologic part of the Environmental Symptom Questionnaire (ESQ-III ESQc, 11 items graded from 0 to 5 covering "lightheadedness" "faintness" "weakness" "nausea" "I feel hung-over" and so on) (Sampson *et al.*, 1983). AMS is defined as a LLS score of more than 3 or an ESQc score ≥ 0.7.

Finally, subjects were regularly asked to qualify their rate of perceived exertion (RPE, Borg Scale, 6-20) during the time trial. Legs and breathing feelings were also assessed with visual analog scales (VAS, 0-10), ranging from "no difficulty" to "extremely difficult".

Statistics. Data are reported as means and standard deviations with 95% confidence intervals. Data were tested for equality of variance (Fisher-Snedecor *F-test*) and for normality (Shapiro-Wilk test). To investigate the pacing strategies we divided the time trial in 25 slices of 10 kJ (increments of 4% of the total work completed). One-way ANOVA were used to determine if systemic (SpO_2, VO_2), cerebrovascular (MCAv, cDO_2) and AMS (LLS, ESQc) variables were different between conditions before the time trial (*i.e.*, at baseline, BL). When significant interaction effects were found, Bonferroni *post-hoc* tests were used to localize differences between conditions (NN, NH, HH). Two-way ANOVA (time x condition) with repeated measures were used for each parameter during the time trial. When significant main or interaction effects were found, Bonferroni *post-hoc* tests were used to localize differences between conditions (NN, NH, HH) and/or time (each 10 kJ slice from 0 to 250 kJ). Null hypothesis was rejected at $P<0.05$. All analyses were made using Sigmaplot 11.0 software (Systat Software, San Jose, CA).

Results

Time trial performance and pacing. Compared to NN (*i.e.*, 1041 ± 151 [955.9-1126.9] s), the mean time required to complete 250 kJ was 24.1 ± 9.6 [18.7-29.5] % and 33.2 ± 12.4 [26.2-40.2] % higher for NH and HH, respectively (with both $P < 0.001$). The mean time was 7.5 ± 7.5 [3.2-11.7] % higher in HH than in NH ($P < 0.01$) (Saugy *et al.*, 2016b). Compared to NN the whole time trial pace was reduced for both hypoxic conditions (Fig. 1A, $P<0.001$). The HH power output was significantly reduced compared to NH from 140 to 220 kJ ($P<0.05$). When expressed as a function of the average power sustained in each respective condition (Fig. 1B), an interaction effect was observed ($P<0.05$) showing inverse trends between NN and both NH and HH, despite a comparable range of variation (~30%, ~21% and ~23%, for NN, NH and HH, respectively). In HH, normalized power output followed a similar pattern to NH.

Pulse oxygen saturation. As presented in Fig. 2A, SpO_2 was significantly higher at baseline (97.7 ± 1.2 [97.1-98.4] %; $P<0.001$) and during cycling in NN compared to both hypoxic conditions (0-250 kJ; $P<0.001$) and SpO_2 was significantly lower in HH compared to NH at rest (92.2 ± 2.1 [91.1-93.3] vs. 89.9 ± 1.9 [88.9-91.1] %; $P<0.01$) and during the first half of the time trial (0-140 kJ; $P<0.05$).

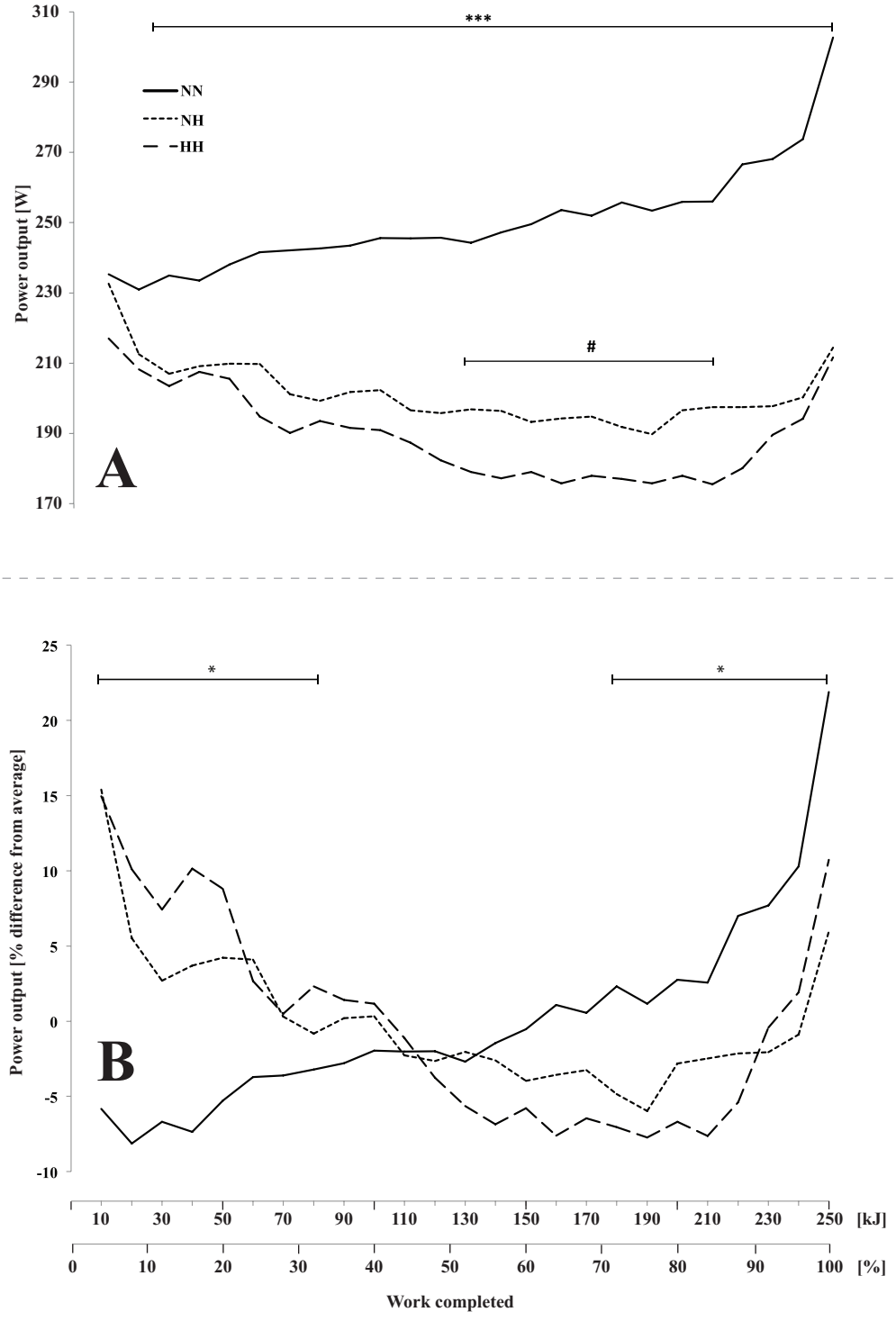

Figure 1. Power output (A) and normalized power output (B) during the self-paced 250-kJ time trial in normobaric normoxia (NN), normobaric hypoxia (NH) and hypobaric hypoxia (HH). *P<0.05, ***P<0.001 for difference between NN and both hypoxic conditions; #P<0.05 for difference between NH and HH.

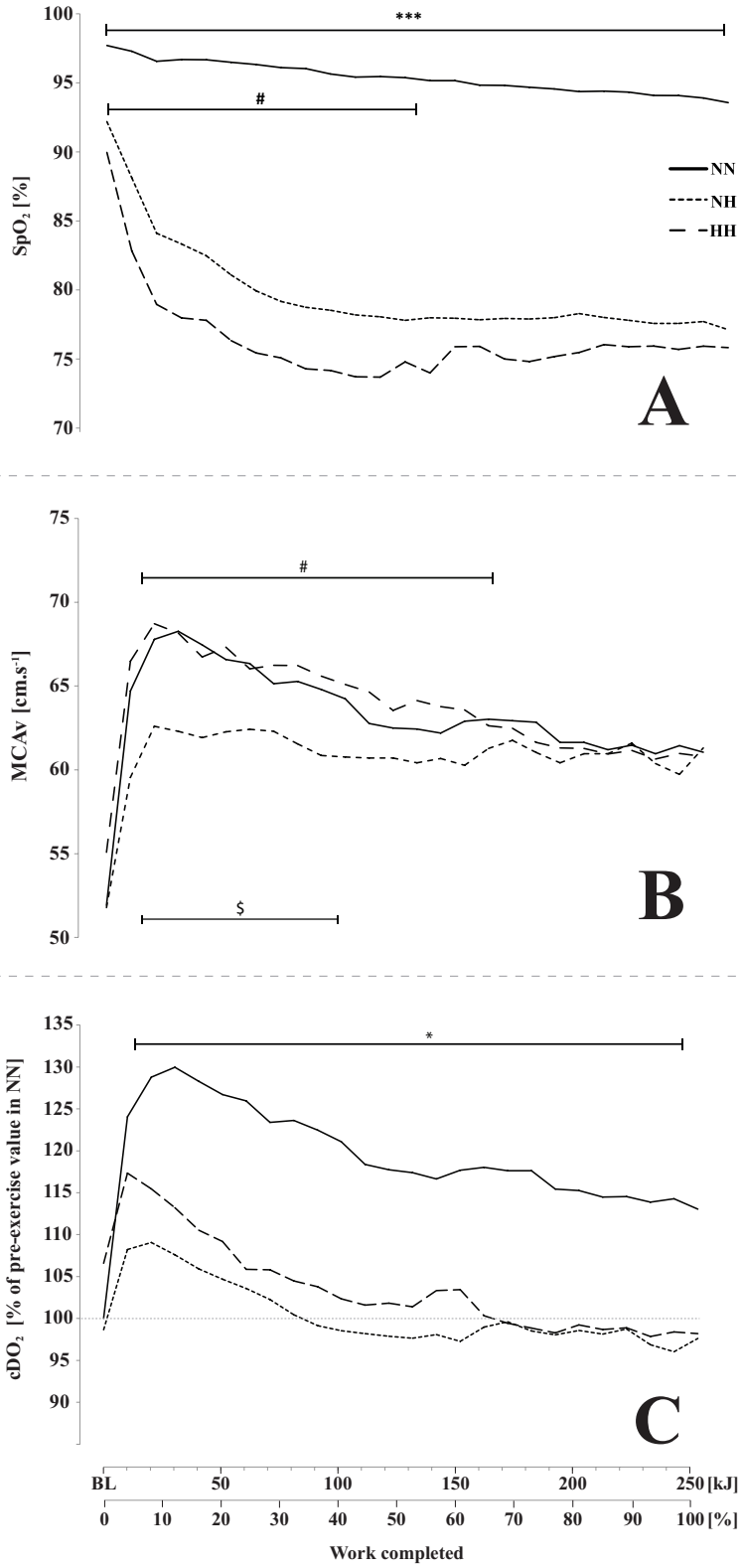

Figure 2. Peripheral oxygen saturation (SpO_2, panel A), middle cerebral artery blood flow mean velocity (MCAv, panel B) and cerebral oxygen delivery (cDO_2, panel C) during the self-paced 250-kJ time trial in normobaric normoxia (NN), normobaric hypoxia (NH) and hypobaric hypoxia (HH). *P<0.05, ***P<0.001 for difference between NN and both hypoxic conditions; $^{\$}$P<0.05 for difference between NN and NH; $^{\#}$P<0.05 for difference between NH and HH.

Cerebrovascular variables. MCAv at rest was not different between conditions. MCAv was increased in the first quarter of the time trial in both NN, NH and HH (+ 31%, + 15% and +25%, respectively), the increase being lower in NH compared to HH (from 10 to 150 kJ, $P<0.05$) and NN (from 10 to 100 kJ, $P<0.05$) while no difference was found along time trial between HH and NN (Fig 2B), in which conditions MCAv decreased from 40 kJ onward. Rest cDO_2 values were not different between conditions (Fig 2C). In contrast, during time trial cDO_2 was lower in both NH and HH compared to NN ($P<0.05$), with no difference between NH and HH, in which conditions cDO_2 decreased to near baseline values at ~100 kJ while it remained elevated in NN ($P<0.05$).

Cardio-respiratory parameters. VO_2 was higher for NN compared to both hypoxic conditions (from 70 to 250 kJ; $P<0.001$) and trend to be lower for HH compared to NH before the end-spurt (from 70 to 210 kJ; $P=0.08$)(Fig. 3A). No difference was observed between conditions in VO_2 relative to VO_{2max} (Fig. 3B). Heart rate was higher at rest for both hypoxic conditions (78 ± 12 [71-84] and 81 ± 8 [76-85] bpm for NH and HH, respectively) compared to NN (69 ± 11 [62-75] bpm) but no difference was observed during the time trial (Fig. 3C). $P_{ET}CO_2$ was lower for NH (26.8 ± 2.9 [25.1-28.4] mmHg) than NN (30.6 ± 3.3 [28.7-32.5] mmHg; $P<0.05$) and HH (29.9 ± 2.5 [28.5-31.4] mmHg; $P<0.05$) at rest but no statistical difference was reached during the time trial (Fig. 3D). VE was higher in NH than NN at rest (15.8 ± 5.2 [12.8-18.8] and 12.8 ± 4.2 [10.4-15.1] $L.min^{-1}$, respectively, $P<0.05$) but no difference was found between NH and HH at rest or between conditions during exercise (Fig. 3E). No difference was found neither at rest nor during the exercise for R*f* (Fig. 3F).

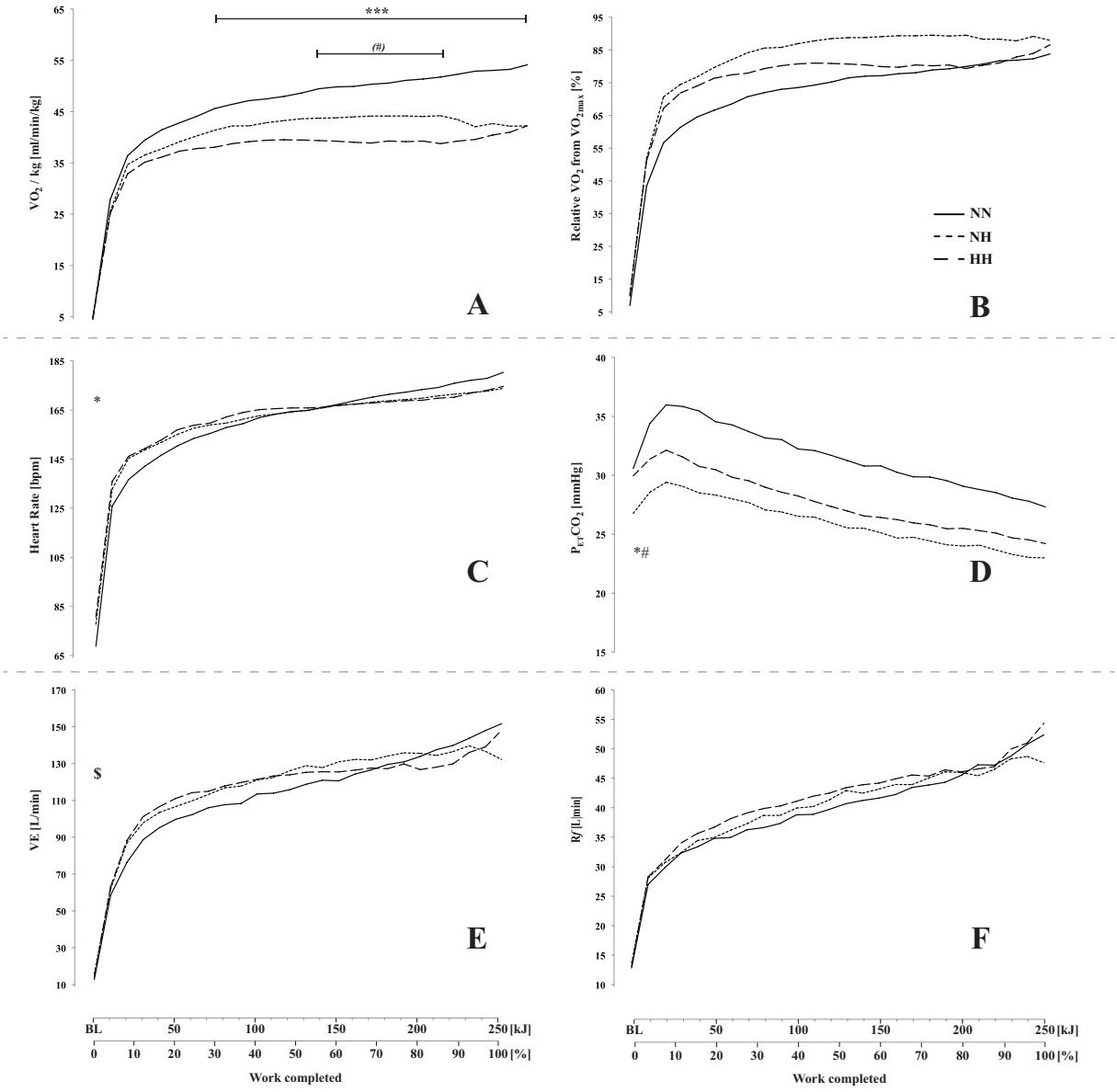

Figure 3. Absolute oxygen uptake (VO_2, panel A), oxygen uptake relative to VO_{2max} (panel B), heart rate (panel C), end-tidal CO_2 pressure ($P_{ET}CO_2$, panel D), minute ventilation (VE, panel E) and respiratory frequency (R*f*, panel F) during the self-paced 250-kj time trial in normobaric normoxia (NN), normobaric hypoxia (NH) and hypobaric hypoxia (HH). *P<0.05; ***P<0.001 for difference between NN and both hypoxic conditions; $^{\$}$P<0.05 for difference between NN and NH; $^{(\#)}$P=0.08; $^{\#}$P<0.05 for difference between NH and HH.

Cerebral oxygenation. These results are presented in Figure 4A-F. PFC and MC HbDiff were higher for NN than for the two hypoxic sessions throughout the time trial (P<0.001, fig. 4A and 4B). Lower values were found in HH compared to NH in PFC HbDiff at the beginning of the time trial (20-120 kJ, P<0.05, fig. 4A), while higher values were observed in HH compared to NH in MC HbDiff in the second part of the time trial (160-240 kJ, P<0.05, fig.

4B). Cerebral HHb exercise-induced increase was lower for NN than the two hypoxic conditions in both PFC and MC (P<0.001, fig. 4C and 4D), this increase being particularly limited during the first half of the time trial. MC HHb was also slightly lower for HH compared to NH in the last part of exercise (P<0.05). Increase in cerebral THb along exercise was similar in the three conditions in PFC (Fig. 4E) but was higher for NN in MC during the second half of the time trial (from 100 kJ onward, P<0.001, fig. 4F).

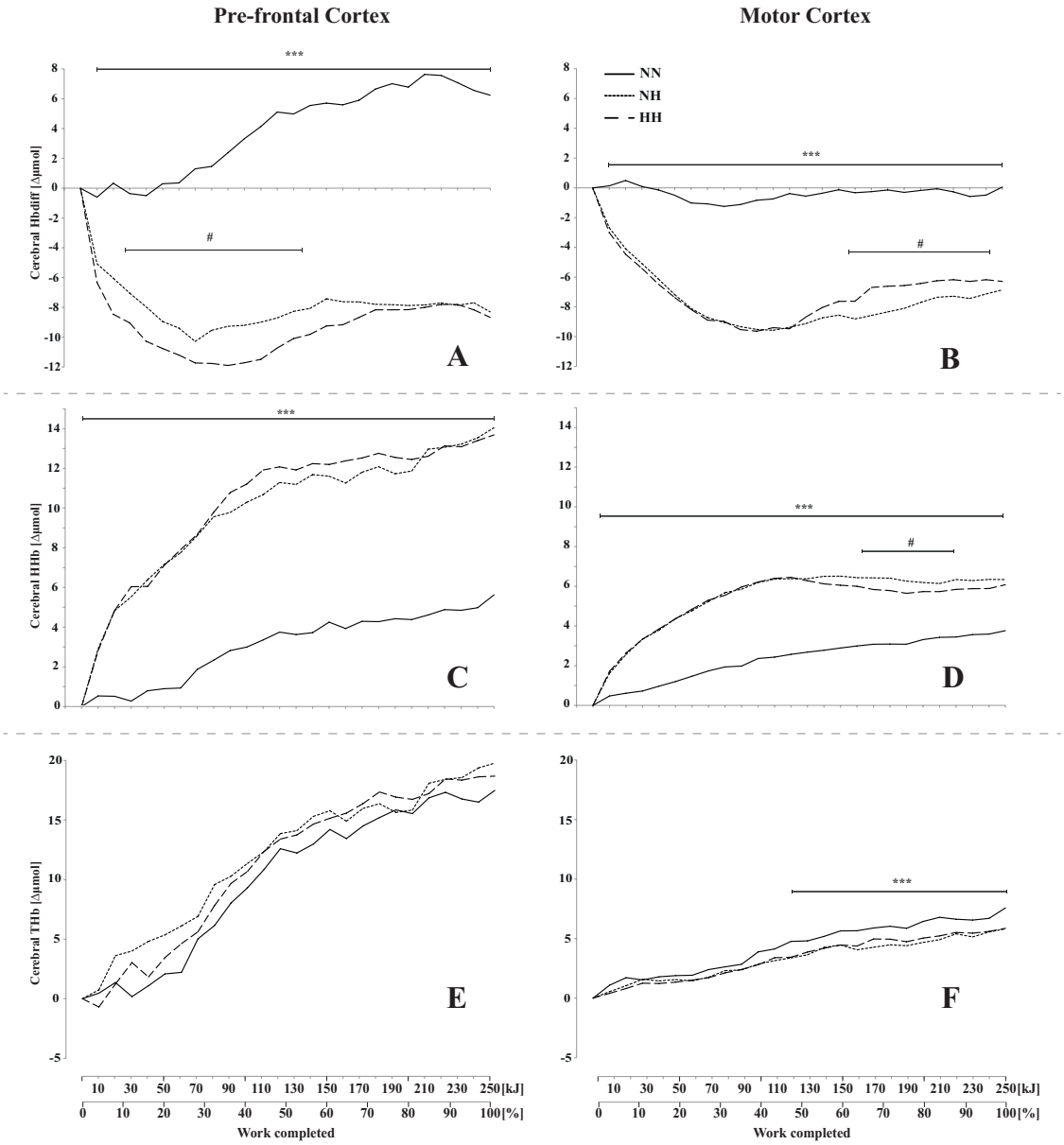

Figure 3. Mean changes in cerebral hemoglobin difference (HbDiff = O_2Hb-HHb), deoxy-(HHb), and total-hemoglobin (THb), during the self-paced 250-kJ time trial in normobaric normoxia (NN), normobaric hypoxia (NH) and hypobaric hypoxia (HH). Data are shown for the prefrontal cortex (PFC, panels A to C) and for the

motor cortex (MC, panels D to F). ***P<0.001 for difference between NN and both hypoxic conditions; #P<0.05 for difference between NH and HH.

Muscle oxygenation. Muscle HbDiff was lower for NN compared to NH from 20 to 210 kJ (P<0.05) and lower for HH compared to NN from 190 to 240 kJ (P<0.05)(Figure 5A). HbDiff was also lower in NH compared to HH from 50 kJ onward (P<0.05). No difference has been found in muscle HHb increase during time trials between conditions (Figure 5B). Muscle THb were higher in HH compared to both NN and NH conditions from 90 kJ onward (P<0.05) (Figure 5C) with no difference between NN and NH.

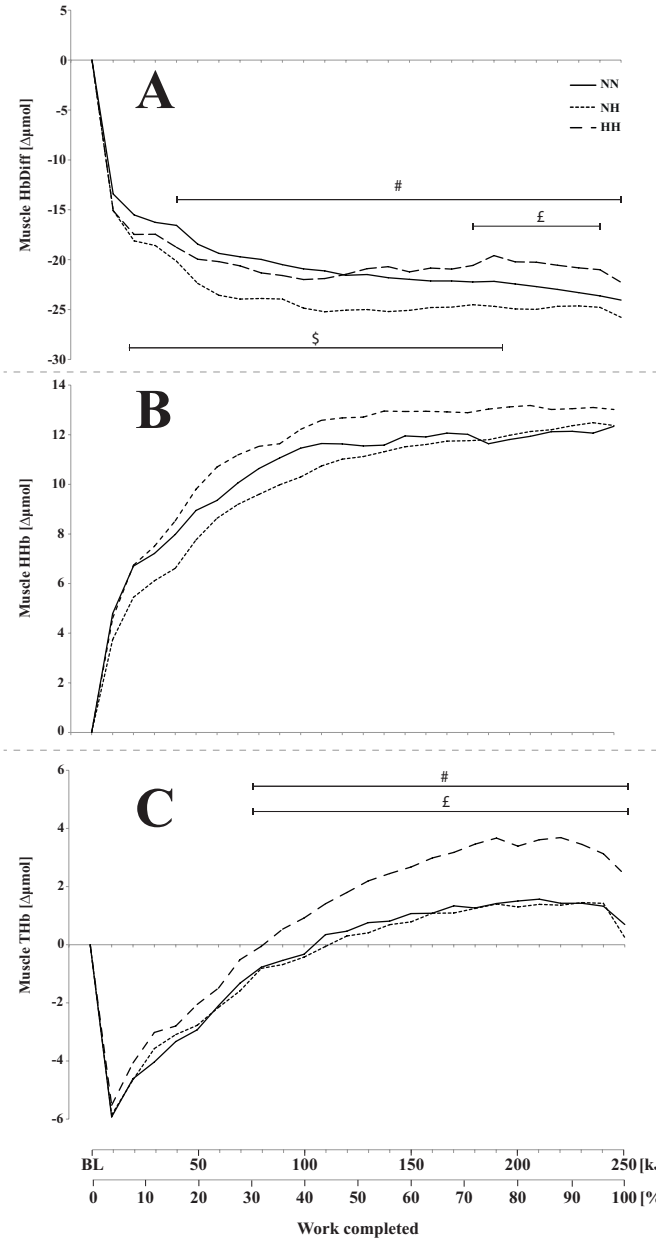

Figure 5. Mean changes in muscle hemoglobin difference (HbDiff = O_2Hb-HHb, panel A), deoxy-(HHb, panel B), and total-hemoglobin (THb, panel C), during the self-paced 250-kJ time trial in normobaric normoxia (NN), normobaric hypoxia (NH) and hypobaric hypoxia (HH) for the *vastus lateralis*. $P<0.05 for difference between NN and NH; £P<0.05 for difference between NN and HH; #P<0.05 for difference between NH and HH.

Electromyographic activity. EMG activity was higher for NN compared to both hypoxic conditions from 60 kJ onward (Fig 6, P<0.001) and it was lower for HH compared to NH from 130 to 210 kJ (P<0.05).

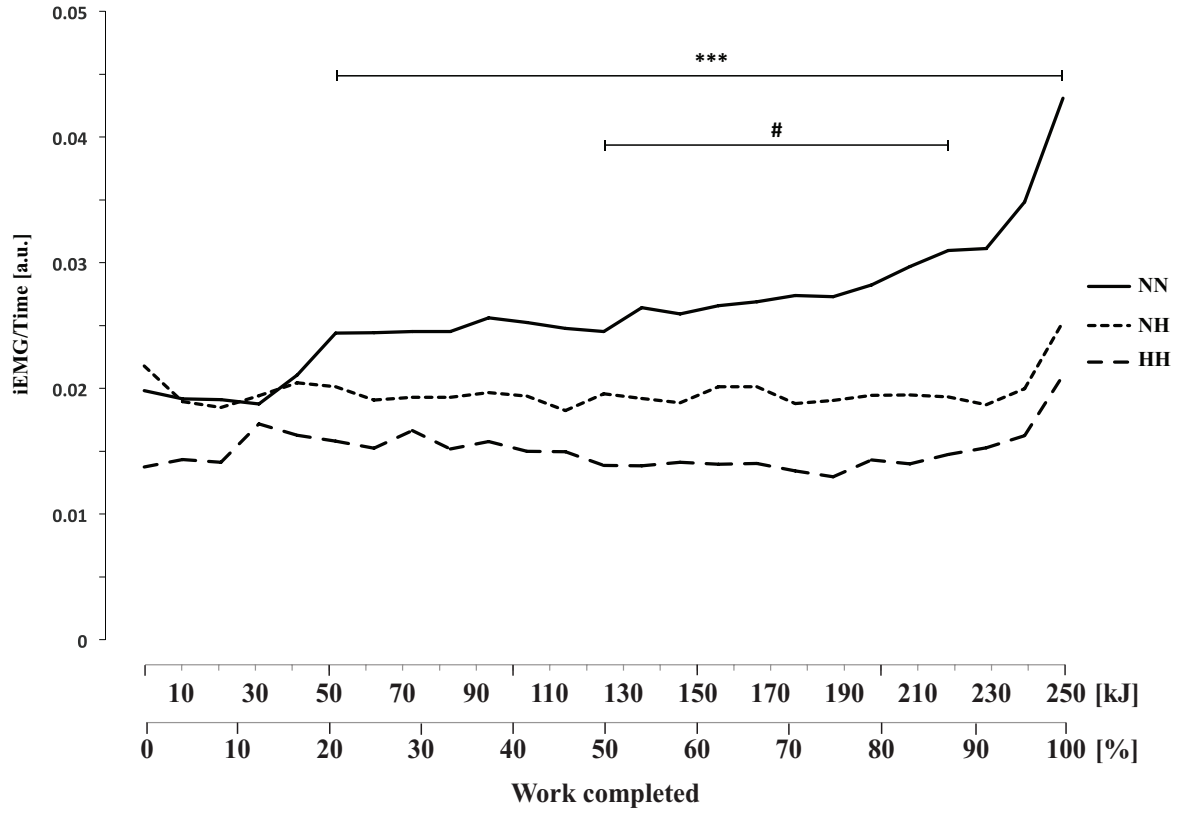

Figure 6. Electromyographic activity (iEMG) measured for the *vastus lateralis* during the self-paced 250-kJ time trial in normobaric normoxia (NN), normobaric hypoxia (NH) and hypobaric hypoxia (HH). ***P<0.001 for difference between NN and both hypoxic conditions; #P<0.05 for difference between NH and HH.

Subjective feelings. The LLS was not statistically different between conditions (1.6 ± 1.4 [0.9-2.4], 2.3 ± 2.8 [0.8-3.8] and 3.2 ± 2.7 [1.8-4.7] for NN, NH and HH, respectively), but showed mild AMS (score > 3) only in HH. ESQc was also higher in HH only (0.48 ± 0.65 [0.13-0.84]) compared NN (0.06 ± 0.07 [0.02-0.01], P<0.05), with no difference versus NH (0.27 ± 0.53 [0-0.56], P>0.05).

Perceived exertion (Fig. 7A) and legs discomfort (Fig 7B) were not different between conditions along cycling. However, breathing discomfort (Fig 7C) was higher for both

hypoxic conditions from the beginning to the end of the time trial (P<0.001), with no difference between NH and HH. In addition, a biphasic evolution from 0 to 70 kJ and from 70 to 250 kJ was observed with a time effect on both legs and breathing discomfort (both P<0.001, fig. 7B-C).

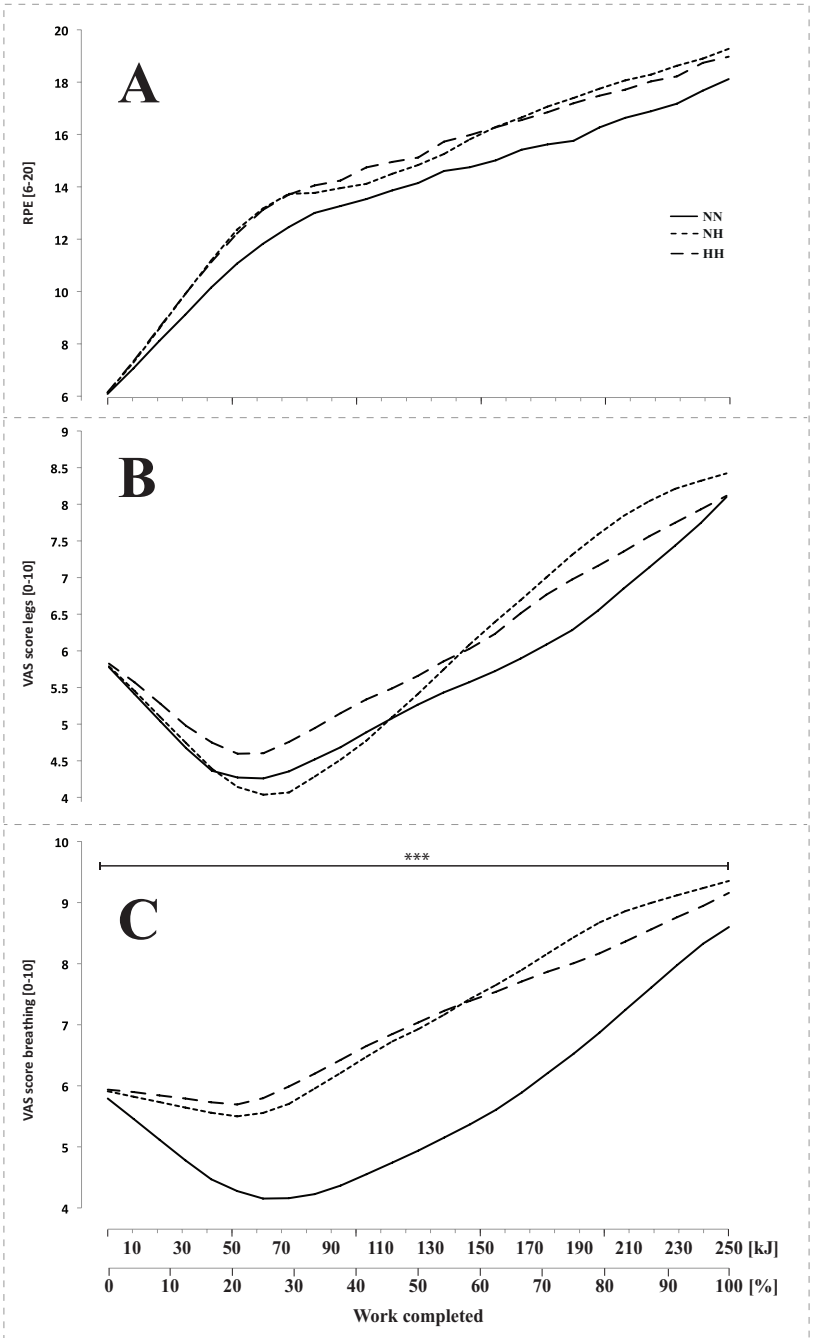

Figure 7. Rate of perceived exertion (RPE, panel A) and visual analog scale score (VAS) for legs feelings (panel B) and breathing difficulty (panel C) during the self-paced 250-kJ time trial in normobaric normoxia (NN), normobaric hypoxia (NH) and hypobaric hypoxia (HH). VAS, visual analog scale. ***P<0.001 for difference between NN and both hypoxic conditions.

Discussion

The major findings of the present study was that, after a 24-h exposure, decreased time trial performance was observed in both normobaric or hypobaric hypoxia with an inverse and

more variable pacing pattern compared to normoxia, in conjunction with depressed cerebrovascular function for a same relative intensity of exercise (*i.e.*, $\%VO_{2max}$, RPE). The present study also demonstrates for the first time that, despite lower power output during time trial in HH versus NH, similar pacing pattern and relative exercise intensity were observed for very distinctive physiological adaptations, emphasizing, and explaining at least in part, subtle differences between HH and NH at exercise, which have been suggested in the past.

Pacing in hypoxia. Hypoxia-induced impairments of aerobic performance have been extensively studied by the past but much fewer studies have described how pacing (*i.e.*, distribution of power output or energetic reserves) or pacing strategy (*i.e.*, self-selected tactic adopted by the athlete) may help to understand why we fatigue when oxygen availability becomes challenging.

Previous reports examined exclusively time trials performed after very short exposure to hypoxia, ranging from only 3-40 min (Amann *et al.*, 2006a; Clark *et al.*, 2007; Fan & Kayser, 2013; Van Cutsem *et al.*, 2015; Periard & Racinais, 2016) up to 2 h (Beidleman *et al.*, 2014). Sudden and acute hypoxic exposure provides an interesting model that stresses oxygen transport systems but in real-world training/competing (particularly at terrestrial altitude), exposure time preceding exercise is usually longer and is prone to deeply influence how pace would be regulated (Tucker, 2009). Indeed we previously showed that the first hours at altitude progressively affect motor cortical excitability (Rupp *et al.*, 2013a) or muscle and cerebral oxygenation (Rupp *et al.*, 2013c) for instance. In the present experiment, the prooxidant/antioxidant balance became impaired from 10 h in hypoxia (Ribon *et al.*, 2016), hematologic parameters were affected after 20 h in hypoxia (Saugy *et al.*, 2016b) and sleep quality was significantly disturbed in hypoxia the night before time trial (Heinzer & Saugy, 2016). This might, at least partly explain why available evidence suggests that the acute

reduction in both maximal aerobic power and endurance performance is maximal within 16–24 h of exposure (2300 m, (Schuler *et al.*, 2007)), and resorbs progressively thereafter.

To our knowledge this is the first study to investigate time trial performance in conjunction with systemic, muscle and cerebrovascular variables after a 24-h exposure to hypoxia. However, for duration longer than ~5 min, the strategy most commonly displayed in literature consist in an initial fast start over the first 10–15 % of the trial, followed by a slight reduction in power output with an even pace until the final 10-15 % of the time trial, where there is an end-spurt in which the power output returns to the initial values (Roelands *et al.*, 2013). Our results show that subjects started exercise at the same workload (~226 W) in normoxia and hypoxia but acutely reduced power output until end-spurt in hypoxia whereas a slightly positive trend was seen in normoxia over the same period. Variation in power outputs (as a function of mean power during the trial) from the start to 85% of time trial was twice higher in hypoxia compared to normoxia (~22% and ~11%, respectively). Hence, the pacing pattern adopted during ~17-23 min time trials was significantly different in NN *versus* after 24 h in either NH or HH. This observation is supported by a previous experiment examining comparable time trial duration (15-km cycling, 25-35 min) but at higher acute altitude (~5000m, NH; (Fan & Kayser, 2013; Bourdillon *et al.*, 2014)). However most of the studies reporting self-paced exercise performance at lower but acute altitude for shorter (5-min or 5-km cycling, ~3200 m in HH or ~2700 m in NH; (Clark *et al.*, 2007) and (Amann *et al.*, 2006a), respectively), similar (30-min cycling, 3800 m, HH; (Van Cutsem *et al.*, 2015)) or longer (750-kJ cycling, ~3000 m, NH; (Periard & Racinais, 2016)) time trial durations failed to demonstrate any difference between normoxic and hypoxic pacing profiles. These discrepancies may suggest an effect of hypoxic dose (higher altitude or longer pre-exposure duration) on the ability to regulate efficiently power output along exercise. Yet, it should be stressed that comparisons with the cited studies are questionable because i) time trials <7-8

min may follow a particular trend compared to longer exercises (Roelands *et al.*, 2013), ii) some of the studies were time-delimited rather than total work-delimited and compel to a predefined initial power output with the risk to affect subsequent pacing (Van Cutsem *et al.*, 2015), and iii) some results relate to remarkably well-trained subjects (*e.g.*, VO_{2max} >4.5 L/min in NN; (Periard & Racinais, 2016)) what is not the case in our study (VO_{2max} = 4.2 L/min in NN).

Moreover, the distinct regulation of the pace in normoxia and hypoxia was associated with distinct systemic, cerebrovascular and muscle adaptations but for a similar RPE and similar normalized (relative) VO_2 throughout time trials. It has been proposed that at the onset of time trial exercise, afferent information from various physiological systems and external/environmental cues is used by the brain to generate a conscious RPE and then to regulate work rate to ensure that this conscious RPE does not increase excessively at any stage during exercise, reaching maximal tolerable RPE at the moment exercise is completed (mandatory for optimal performance), but not before. The concept of "teleoanticipation" refers to both a conscious and unconscious prediction of a pacing pattern, whereby the rate of energy expenditure is regulated in a way that allows the performer to complete a task as fast as possible while controlling the magnitude of homeostatic disturbance (Tucker *et al.*, 2004; Tucker, 2009; Tucker & Noakes, 2009). This concept also relies on the existence of an anticipatory component (*i.e.*, "exercise template" dependent from the previous experience and expected endpoint) and the constant interplay between afferent feedbacks from the periphery and a thin regulation/modulation of the central drive (*i.e.*, active neural calculations in the brain) (St Clair Gibson & Noakes, 2004).

Initial phase. Athletes are supposed to self-select an initial exercise intensity, which is anticipated to be optimal for the expected exercise format, but this was not the case in

hypoxia as expressed from the rapid decrease in power output over that period, with a -15% variation in normalized power output compared to a +3% variation only in normoxia (Fig. 1A). Interestingly, similar low levels and high rate of increase in RPE were observed in all conditions in the present study in the first 30% of completed work (0-70 kJ). These RPE were however generated from a very different balance between central drive and afferent feedbacks (physiological adaptations) in normoxia and hypoxia. A wide range of variables can influence pacing but recently, tactical adaptations in the chosen pacing strategies have been incredibly manipulated with the use of central nervous system drugs and selective block of the central projection of ascending sensory pathways (*e.g.*, fentanyl, opioid analgesic) (Amann *et al.*, 2009; Roelands & Meeusen, 2010), so that the role of neurophysiological processes has grown.

Cortical representation of muscle involved in cycling is served dominantly by MCA (Jorgensen *et al.*, 1992) and transcranial Doppler as we used can provide quantitative information on cerebral hemodynamic changes at the macrovascular level (*i.e.*, cerebral arteries) but is unable to assess directly the qualitative repercussions of such changes for the tissue at the microvascular level. How much reductions in SpO_2 and cDO_2 translate into changes in cerebral tissue oxygenation when self-paced exercise is performed in hypoxia remains largely unknown. In this way, NIRS is increasingly used to measure the (mis)balance between oxygen supply and utilization directly in tissue microvessels (venules, arterioles and capillaries), with a predominant venous contribution (70-80%) (Hamaoka *et al.*, 2007; Wolf *et al.*, 2007).

Interestingly, we found that the cerebrovascular responses in NN over the first part of exercise appeared appropriate (*e.g.*, cDO_2 maintained at ~125% from baseline value, preserved brain oxygenation), while it is likely that the brain function was threatened in

hypoxia over that period from a rapid decrease in SpO_2, cDO_2 (Fig. 2) and cerebral oxygenation towards low levels (Fig. 3).

Main phase. From 30 to 85% of the time trial mean power output difference between NN and both hypoxic conditions continued to grow (Fig. 1A), still without any difference in RPE and relative exercise intensity as expressed from $\%VO_{2max}$, HR or ventilation parameters (Fig. 7).

RPE as an expression of the somatosensory experience (Hampson *et al.*, 2001), is supposed to be a key component of a regulatory system that protects against bodily harm (Tucker, 2009) and it achieves the regulation of the physiological perturbations occurring during exercise. The lower work-rate in hypoxia was achieved by the alteration in the degree of skeletal muscle recruitment (*i.e.*, lower iEMG) in accordance with Peltonen *et al.* (Peltonen *et al.*, 1997). This is corroborated for the first time by a significant cerebral hypoperfusion at the micro-vascular level (from THb with NIRS, Fig. 3F) over the motor cortex, which directly drives the muscles.

With growing interest in the last decade, NIRS is now widely used as a suitable tool for studying muscle and cerebral hemodynamics and oxygenation responses to exercise with challenging environmental conditions as altitude (Verges *et al.*, 2012), but previous research almost exclusively considered the prefrontal cortex (PFC). PFC synthesizes information from a wide range of brain systems and exerts control over cognitive and executive behavior (*e.g.* task-related memory, sensory information integration, decision-making, movement planning, pacing strategies and motivation), so that these associative areas play a central role in the orchestration of thoughts and actions in accordance with internal goals (Miller & Cohen, 2001; Ramnani & Owen, 2004). PFC is of particular interest in our study but the central motor drive is ultimately conducted from the premotor and primary motor areas, which have never been investigated during self-paced exercise before. From the study of Subudhi *et al.*

(Subudhi *et al.*, 2009) it has been often argued that there is a good correlation between prefrontal, premotor and motor cortices oxygenation measurements during exercise. However, this has been shown during a short, maximal incremental exercise, where pacing strategy was minimal as power output was compelled throughout the test. We recently demonstrated that PFC and MC oxygenation profiles can differ during submaximal fatiguing exercise (Rupp *et al.*, 2013b) and to our knowledge the present study is the first to present simultaneous macro-circulation in MCA and both PFC and MC micro-hemodynamics and oxygenation during a self-paced exercise.

In the present study, PFC and MC oxygenation were both markedly depressed in hypoxia in the first part of the time trial (Fig. 4), confirming what has been reported before almost exclusively during progressive maximal exercises in hypoxia and in the PFC (Subudhi *et al.*, 2009; Vogiatzis *et al.*, 2011). Presumably, exercise-induced increased in cerebral cortex oxygenation reflects progressive increase in oxygen metabolic demand (Secher *et al.*, 2008) with increased neuronal networks activation. Despite lower central drive and muscle activity during time trial in hypoxia, it is likely that the challenging environmental conditions (cf., low F_iO_2) blunted the ability of the neurovascular coupling to increase or even maintain cerebral oxygenation to habitual levels aiming at preserving a positive balance between oxygen supply and consumption. An hypothesis might be that power output is steadily decreased in hypoxia to prevent the body to be exposed to unacceptable levels of SpO_2, cDO_2 and cerebral oxygenation or, at least, to values which would be considered as incompatible with the remaining expected time before exercise completion. The diminished power output certainly allowed cDO_2 and cerebral hemodynamics not to decrease further (cf. baseline values for cDO_2). On the other hand it is important to stress that PFC and MC oxygenation would be only one of many afferent signals influencing complex regulation of motor drive during self-paced exercise (St Clair Gibson & Noakes, 2004).

In the present study, CBF declined during the second part of time trial in both NN and HH despite increased versus decreased power output, respectively. This adaptation mirror the $P_{ET}CO_2$ decrease over the same period and might thus be triggered by hyperventilation-induced hypocapnia (breathing fatigue increase). This assumption is corroborated by the results from Periard & Racinais (Periard & Racinais, 2016) who observed a similar decrease in MCAv during a 750-kJ time trial in normoxia. Conversely, other results demonstrated a maintain MCAv (in normoxia) or an increased MCAv (and almost maintained cDO_2 in hypoxia) during a 15-km time trial (Fan & Kayser, 2013). To explain these results, authors underlined a higher RPE during the time trial in hypoxia (5000 m) and suggested a greater sensori-motor activation compared to normoxia.

End-spurt. In accordance with well-known field observations and as previously described in the literature (Albertus *et al.*, 2005; Roelands *et al.*, 2013) our results indicated a characteristic end-spurt phenomenon in the last 10-15% of the time trial. Indeed, it is assumed that energetic resources would be efficiently used in the case all energy stores are used before finishing race, but not so far from the end to avoid any meaningful slowdown to occur (Foster *et al.*, 1993; Roelands *et al.*, 2013). The level of uncertainty becoming smaller when endpoint approaches, protective motor unit and metabolic reserve throughout exercise can be delivered. The distance (work load) that remains is a crucial "anchor" against which the RPE is interpreted. This might explain why linear increase in RPE was not affected by the end-spurt phenomenon (*i.e.*, steep increase in power output and muscle activity but no steeper increase in RPE). This end-spurt was seen whatever the condition, even if it was delayed and reduced in NH. Our results further support the concept of a central governor, the subjects having the drive and/or motivation in normoxia but also in hypoxia to augment power output when

approaching the end-point (what is not the case anymore after administration of a serotonin reuptake inhibitor in normoxia, (Roelands *et al.*, 2009)).

Normobaric versus hypobaric hypoxia. As previously mentioned, several studies assessed performance and pacing in either normobaric (Bourdillon *et al.*, 2014) or hypobaric (Richalet, 2010; Fulco *et al.*, 2011) hypoxia in comparison with normoxia, resulting in indirect comparisons between these conditions. However, to date only one study was conducted aiming to compare these two hypoxic conditions (Beidleman *et al.*, 2014), but it has been done with independent groups and subjects with relatively low endurance ability during the baseline tests (even after a familiarization session, *i.e.* between 47.5 and 49.5 ml/min/kg). Moreover, due to the small sample size in each groups (*i.e.* n = 6) statistical power was not sufficient to reach significant difference in power output but only a trend (*i.e.* lower power output in HH, P=0.08). The short pre-exposure duration (*i.e.* 2h) might also be a potential reason. In another side, our study is the first to directly compare both hypoxic conditions in a controlled crossover design with the same subjects. Thus, similarly with Beidleman *et al.* (Beidleman *et al.*, 2014) the trends were comparable between NH and HH, but with a significant lower power output at the end of the second part for hypobaric hypoxia, emphasizing different physiological adaptations.

When comparing NH and HH, it should be noted that power output at the immediate onset of the time trial (0-20 kJ, 8%) was similar and also similar to NN, suggesting that the initial selection of work rate was more likely based on previous experience and expectations of exercise duration, rather than on an instantaneous (baseline) afferent input from hypoxemic or disturbed organ/tissue homeostasis (*e.g.*, modified baseline HR, SpO_2, $P_{ET}CO_2$). Indeed, even higher AMS and the knowledge of the condition (no possibility to blind HH) had no influence on the initial power output. However, the tendency towards lower power output in

HH in the first part of the time trial appeared significant in the second part (from ~30% of total work onward). Trends and normalized variation in power output (Fig. 2A) were similar in NH and HH throughout the time trial, but for the first time we were able to identify subtle differences in physiological adaptations with a potential explanation to explain the greater mis-performance in HH.

From an initial "exercise template" similar power outputs were produced in the initial part of exercise but subsequent physiological adaptations progressively differed in NH and HH. Part of the explanation may arise from distinct baseline status in NH and HH after the 24-h exposure.

The 24-h exposure time-window adopted in the present study coincides for instance with the earliest symptoms of AMS encountered after several hours (*i.e.*, 4-8h) of hypoxic exposure (Wilson *et al.*, 2009; Subudhi *et al.*, 2010) and which incidence is >40% above 3000 m depending on the rate of ascent, the altitude reached and individual physiology (Hackett & Roach, 2001; Richalet & Herry, 2006). In our study, AMS was observed to be slightly higher in HH compared to NH (LLS > 3, and ESQc higher in HH only compared to NN). AMS degree has been correlated to degree of hypoxemia in the first hours of exposition (Fulco *et al.*, 2011). Accordingly baseline SpO_2 before the time trial was significantly lower in HH despite similar PiO_2 (Saugy *et al.*, 2016b).

This might impact subjective feelings during exercise so that power output had to be reduced in HH compared to NH to match equivalent relative exercise intensity (%VO_2max, HR, RPE, leg feelings…). Besides, underlying mechanisms associated with the same relative exercise intensity were different likely explaining lower power output in HH along time trial.

Mass oxygen delivery to the brain is dependent on cerebral blood flow (cDO_2 assessed from MCA in the present study) and on arterial content in oxygen, which is itself a function of

hemoglobin concentration and arterial saturation in oxygen. It seems consistent to think that the rate of cDO_2 is important information integrated by the central nervous system to regulate central drive. We demonstrated that cDO_2 was extremely reduced in both NH and HH compared to NN, already in the first part of the time trial. It is particularly interesting to see how the progressive decrease in power output mirrored the plateau in the decrease in cDO_2, apparently preventing any further decrease under dramatically low (baseline) values in hypoxia.

This was observed in a very similar extent in both NH and HH but we show here for the first time that the similar cDO_2 levels achieved, resulted from distinct mechanisms in NH and HH and were seen in conjunction with significantly different cerebral oxygenation in both conditions.

First SpO_2 was significantly lower at rest and during the first half of the time trial in HH compared to NH, what may explain *i*) the higher hemoconcentration measured before exercise in HH (+6%, (Saugy *et al.*, 2016b)) as a compensatory mechanism and, *ii*) the higher MCAv values in HH (+ 7.5% over 10-150 kJ) throughout exercise due to a higher hypoxic-stimulus-induced cerebral vasodilation (Casey & Joyner, 2012).

Diuresis is common during the first few days of exposure to even moderate hypoxia. Hence rapid increase in hemoglobin concentration has been reported (including after a single night, (Ashenden *et al.*, 2000)) with the magnitude differing markedly between individuals. Hemoconcentration being triggered by hypoxemia, it serves an adaptive purpose by elevating arterial oxygen content, so that more O_2 can be delivered to the tissues per liter of cardiac output.

At the same time, exercise-induced decrease in $P_{ET}CO_2$ was approximately the same in NN, NH and HH (-8 mmHg on average over 20-250 kJ with similar VE and R*f* kinetics, Fig. 3), but the latter started from a significant hypocapnic state only in HH. Accordingly, MCAv

appeared to be much more affected by hypocapnia-induced vasoconstriction in HH compared to NH (Fig. 2B). All together, cDO_2 was maintained at comparable levels in NH and HH, but from significantly lower values of CaO_2 (despite a slight hemoconcentration) and significantly higher values of MCAv in HH.

However, impaired cDO_2 is only part of the equation and explains (*i.e.* reduction in the ability of CNS to voluntarily activate skeletal muscle, (Goodall *et al.*, 2014)) mainly the decreased performance in both NH and HH compared to NN. Cerebral oxygenation profiles described in the present study also help to understand how and why pacing might be down-regulated in HH compared to NH. From our data, a comparable central drive was produced in HH and NH in the initial part of the time trial (*e.g.*, same MC deoxygenation, iEMG, power output, muscle deoxygenation, absolute VO_2). However PFC deoxygenation was significantly higher in HH (Fig. 4A) during that period. As cDO_2 was the same in NH and HH along the time trial, the lower PFC oxygenation may result from higher extraction rate of oxygen (*i.e.*, higher neuronal activity) in this part of the brain. Whatever the explanation (*e.g.*, greater integrative process, higher planning activity) the fact is that lowering power output in HH allowed PFC HbDiff to progressively "restore" to NH levels. Finally these observations suggest similar PFC activity in the second part of the time trial for lower power output in HH than in NH. Consistently, the lower power output in HH was seen in conjunction with a lower degree of muscle recruitment (*i.e.*, lower MC and muscle deoxygenation, lower iEMG).

With simultaneous measurements of systemic parameters, cerebrovascular function, muscle activity and subjective feelings during time trial, this study provides new insight into the mechanisms underlying the distinctive magnitude of pacing down-regulation in HH versus NH. Hence, HH appears to be a more stressful stimulus (*e.g.*, lower SpO_2 and higher PFC deoxygenation for a given power output, higher "adaptive cost") than NH and induces a subjective feeling that exercise is more difficult (same RPE, leg feelings and $\%VO_{2max}$ for a

lower power output). As a consequence, the system was more "protective" in HH and the end-spurt was greater (starting earlier and reaching same absolute power output than in NH).

Methodological considerations.

Although the results of the present investigation could, theoretically, be extended to all athletes, the present recreational population does not include individuals with elite physiology. It remains to be confirmed how much high-level endurance performance may exhibit similar specific pacing adaptations in HH compared with NH.

To express VO_2 as relative values, a similar 22.7% decrement in VO_{2max} was assumed at 3450 m in both HH and NH (based on the 7.7% decreased per 1000m accordingly with (Wehrlin & Hallen, 2006)). This decrement might however be hypoxemia-dependent and subject to inter-individual variability (Young *et al.*, 1985) and if VO_{2max} has been decremented to a larger extent in HH than NH, the volunteers may have finally performed the time trial at slightly higher relative VO_2 in NH and HH than what has been presented here.

As terrestrial rather than simulated altitude was chosen for the HH condition, it was logistically impossible to blind it to the subjects. However, they started the time trial with similar subjective feelings and power output in NH and HH, so that even if it cannot be totally ruled out throughout exercise, it is likely that the knowledge of the condition didn't drastically impact the behavior of the subjects.

The cycle ergometer we used in our experiment was stationary, whereas reduced air density in HH reduces the drag forces opposing the forward motion of the practitioner outside of the laboratory setting. It should be recognized that although time trial performance was decreased at moderate altitude, cycling (up to 40-km time trial at 3400 m) (Olds, 2001) in a real open-field competitive format would probably be improved in HH for high-class athletes despite a decrease in absolute VO_{2max} and marked cerebrovascular perturbations as were

reported here. Also, such "ecological" situations might allow the subject to cope with additional important afferent information, the relative wind speed exerted on the face for instance. The high wind speeds used in the present study likely prevented the development of hyperthermia but the exact role this parameter on the rate of perceived exertion and/or pacing processes (especially when it is cycling-speed-dependent) has probably been underestimated in models aiming to better understand exercise tolerance and its regulation in hypoxia.

Some limitations inherent to NIRS measurement should be noted. Part of the detected NIR light may be affected by the changes in optical properties of superficial tissue layers between the optode and the investigated tissue (*e.g.* scalp and skull for the brain; skin and fat for the muscle)(Cooper *et al.*, 2010; Takahashi *et al.*, 2011). Thus, although we sought to minimize the effects of near-surface blood flow by controlling room air temperature, by giving attention to ensure NIRS setup to be non-compressive and by using enlarged inter-optode distances (3 cm for the brain and 4 cm for the muscle) to reach the maximal light path providing a sufficient signal-to-noise ratio of the optical density measurements (Rolfe, 2000), we cannot rule out the possibility that superficial layers blood flow slightly weighted in the observed chromophore concentration changes. Whether changes in blood gases affect skin blood flow similarly at sea level and after several hours in NH *versus* HH remain unknown, but taken together we are confident that the behaviour of the present oxygenation changes most probably reflected the cortical and muscle tissue oxygenation changes. NIRS penetration depth (*i.e.* ≈ half the inter-optode distance) limits its sensitivity to the upper 1 cm of the cerebral cortex (Boas *et al.*, 2004), so that it is important to consider that the measurements obtained are regional and strictly confined to the zone beneath the sensors. Moreover, CBF may be heterogeneously distributed at exercise and under hypoxia (Pagani *et al.*, 2011), we investigated MCA macrocirculation plus multiple sites of interest by NIRS (prefrontal and

motor cortices) and we acknowledge that observed tissue oxygenation cannot be generalized to whole brain (or to other muscles from NIRS on vastus lateralis).

Finally, the validity of transcranial Doppler to measure variation in CBF (and subsequent estimation of cDO_2) depends critically on the assumption of a constant diameter of the investigated artery. We contend that MCAv is a reliable index of changes in global cerebral blood flow during exercise in normoxia and hypoxia, as the cross-sectional area of the MCA has been shown unchanged within a wide range of changes in $P_{ET}CO_2$ (Valdueza *et al.*, 1997) and thanks to previous reports suggesting that under the conditions of the present study (<5000 m), MCA diameter remains constant (Poulin & Robbins, 1996).

Conclusion

This study showed that pacing strategy during a 250-kJ cycling time trial is impaired after 24 h in hypoxia (different trend and higher variability) compared to normoxia and this is likely the result from a compromised ability of the central nervous system to voluntarily activate skeletal muscles, owing to inadequate oxygen delivery to the brain. Data from the present study also strengthen the fact that performance and pacing is significantly down-regulated in hypobaric versus normobaric hypoxic condition, in conjunction with altered systemic (*e.g.* SpO_2) and prefrontaland motor cortex oxygenation during exercise. Same relative exercise intensity and physiological disturbances are achieved in hypobaric condition from the production of lower absolute power when compared to normobaria, emphasizing definitively that exercise at terrestrial and simulated altitude cannot be carelessly interchangeable.

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