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Innovations in hypoxic training

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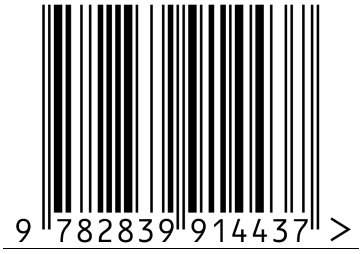

Your attitude, not your aptitude, will determine your altitude.

Zig Ziglar

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A thesis is a unique opportunity to develop your entrepreneurial spirit but also a long process with up and downs. In short you imagine projects, request (and sometimes obtain) funding, realize research protocols, gather experimental data, discuss your results and finally publish your work. All these tasks are supported by a multitude of people giving their time to share their knowledge and provide invaluable help.

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List of publications

1. Ventilation, oxidative stress and nitric oxide in hypobaric vs. normobaric hypoxia. Faiss R., Pialoux V., Sartori C., Faes C., Dériaz O., Millet G.P. Med Sci Sports Exerc. 2013 Feb; 45(2)253-60.

2. Significant molecular and systemic adaptations after repeated sprint training in hypoxia. Faiss R., Léger B., Vesin J.-M., Fournier P.-E., Eggel Y., Dériaz O., Millet G.P., PLoS One. 2013; 8(2):e56522.

3. Advancing hypoxic training in team sports: from intermittent hypoxic training to repeated sprint training in hypoxia. Faiss R., Girard O., Millet G.P. Br J Sports Med 2013 Dec;47 Suppl 1 :i45-i50

4. Responses to exercise in normobaric hypoxia: comparison between elite and recreational ski-mountaineers. Faiss R., von Orelli C., Dériaz O., Millet G.P. Int J Sports Physiol Perf 2014 ; [Epub ahead of print].

5. Repeated double-poling sprint training in hypoxia by competitive cross-country skiers. Faiss R., Willis S., Born D.P., Sperlich B., Vesin J-M., Holmberg H.-C., Millet G.P. Med Sci Sports Exerc 2014 ; in revision.

6. Point: Counterpoint "Hypobaric hypoxia induces / does not induce different responses than normobaric hypoxia" Millet G.P., Faiss R., Pialoux V., Mounier R., Brugniaux J.V., J Appl Physiol. 2012 May; 112(10):1783-4.

7. Last word on Point: Counterpoint: Hypobaric hypoxia induces different responses from normobaric hypoxia. Millet G.P., Faiss R., Pialoux V., J Appl Physiol. 2012 May; 112(10):1795.

8. Hypoxic conditions and exercise:rest ratio are likely paramount. Millet G.P. & Faiss R., Sports Med. 2012 Dec 1; 42(12):1081-3.

9. Evidence for differences between hypobaric and normobaric hypoxia is conclusive. Millet G.P., Faiss R., Pialoux V., Exerc Sci Rev, 2013 Apr; 41(2):133.

10. Hypoxic training and team sports: a challenge to traditional methods? Millet G.P., Faiss R., Brocherie F., Girard O., Br J Sports Med 2013 Dec; 47 Suppl 1 :i6-i7.

11. Hypobaric vs. Normobaric hypoxia: same effects on postural stability? Degache F., Larghi G., Faiss R., Deriaz O., Millet G.P., High Alt Med Biol, 2012 Mar; 13(1):40-5.

12. High-intensity intermittent training in hypoxia: a double-blinded, placebo-controlled field study in youth football players. Brocherie F., Girard O., Faiss R., and Millet G.P., J Strength Cond Res. 2014; submitted.

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Abstract

Athletes seem compelled to include some forms of altitude training in their preparation expecting additional performance gains compared to equivalent training at sea-level. For the general population, altitude training often only consists in spending weeks at altitude to enhance red blood cell production, hemoglobin mass and thus oxygen delivery to the muscles. Over the past two decades, intermittent hypoxic training (IHT), that is, a method where athletes live at or near sea-level but train in hypobaric hypoxia (HH, real altitude) or normobaric hypoxia (NH, simulated altitude) was shown to induce exclusive adaptations directly at the muscular level that may support performance improvements. Our work first demonstrated significant differences between exposure and exercise in HH vs. NH that may help disentangling hypoxia and hypobaria for athletes or mountaineers who use NH to prepare for altitude competitions or expeditions.

Second, we produced a comprehensive review of the strikingly poor and controversial benefits of IHT for performance enhancement in team or racket sports. Using evidence of peripheral muscular adaptations with the recruitment of fast-twitch fibers playing a major role, we then developed and assessed the potential of a new training method in hypoxia based on the repetitions of "all-out" sprints interspersed with incomplete recovery periods, the so called "repeated sprint training in hypoxia" (RSH). We have consequently shown RSH to delay fatigue when sprints with incomplete recoveries are repeated until exhaustion both in cycling and cross-country ski double poling. We definitely outlined RSH as a promising training strategy and proposed new studies to judge the efficacy of RSH in team sports and determine the specific mechanisms that may enhance team game results.

In conclusion, our work allowed updating the panorama over the contemporary hypoxic training possibilities. It provides an overview of the current scientific knowledge about intermittent hypoxic training and repeated sprint training in hypoxia (RSH). This will benefit athletes and teams in intermittent sports looking to include a hypoxic stimulus to their training to gain a specific competitive edge.

Résumé

Athlètes et entraineurs sont généralement tentés d'inclure des formes d'entrainement en altitude à leur préparation, espérant ainsi améliorer leurs performances davantage que par un entrainement en plaine. Pour le grand public, l'entrainement en altitude consiste généralement à passer plusieurs semaines à la montagne pour augmenter le nombre de globules rouges, la masse en hémoglobine et potentiellement améliorer le transport d'oxygène vers les muscles. Toutefois, pendant les deux dernières décennies, des recherches sur diverses méthodes d'entrainement intermittent hypoxique (IHT), où les athlètes vivent en plaine mais s'entrainent en hypoxie hypobarique (HH, altitude réelle) ou en hypoxie normobarique (NH, altitude simulée) ont pu démontrer de réelles adaptations non-hématologiques directement à l'échelle musculaire qui laissent supposer des gains de performance spécifiques.

Notre travail a tout d'abord permis de mettre en évidence des différences significatives lors de l'exposition et l'exercice physique entre HH et NH qui dénouent le lien entre hypoxie et hypobarie avec une importance certaine pour les athlètes ou alpinistes préparant en NH des expéditions ou compétitions sportives. Ensuite, par la publication d'une revue exhaustive nous avons mis en évidence les rares bénéfices controversés de l'IHT pour l'amélioration de la performance en sports d'équipe. Avec l'évidence d'adaptations périphériques musculaires liées au recrutement de fibres rapides, nous avons donc développé une nouvelle méthode d'entrainement en hypoxie reposant sur la répétition de sprints maximaux intercalés de récupérations incomplètes : l'entrainement de sprints répétés en hypoxie (RSH). Nous avons pu démontrer que le RSH permettait de retarder la fatigue tant en cyclisme que lors de la double poussée en ski de fond lors de sprints répétés jusqu'à épuisement. Notre travail a permis de définir le RSH comme méthode prometteuse en proposant de nouvelles études pour juger de son efficacité et des mécanismes sous-jacents dans les sports collectifs

Finalement, notre mise à jour du panorama des méthodes d'entrainement hypoxiques actuelles fournit une vue d'ensemble des outils et méthodes utiles pour les athlètes souhaitant intégrer un stimulus hypoxique à leur entrainement pour gagner un avantage compétitif spécifique.

Index of abbreviations

[HHb]
deoxyhemoglobin

[Mb]
myoglobin concentration

[O_2Hb]
oxyhemoglobin

[tHb]
total hemoglobin

2,3 DPG,
2,3 diphosphoglycerate

AMS,
acute mountain sickness

AOPP
advanced oxidative protein products

CA3,
carbonic anhydrase 3

CHT
continuous hypoxic training

CO_2,
carbon dioxide

CON
control group

CS,
citrate synthase

EPO,
erythropoietin

exNO,
exhaled nitric oxide

F_IO_2,
inspired fraction of oxygen

FT
fast-twitch fibers

GLUT,
glucose transporter

GPX
glutathione peroxidase

H+,
hydrogen ion

Hct,
hematocrit

HH,
hypobaric hypoxia

HIF,
hypoxia inducible factor

HR,
heart rate

HVR,
hypoxic ventilatory response

IHE,
intermittenthypoxic exposure

IHIT,
intermittent hypoxic interval-training

IHT,
Intermittent Hypoxic Training

INT
interval training

kJ,
kilojoule

LH + TH or LHTH
Live High - Train High

LH + TL or LHTL
Live High - Train Low

LHTLH,
Living High - Training Low and High

LL + TH or LLTH
Live Low - Train High

Mb,
myoglobin

MCT
monocarboxylate transporter

MCT-1
monocarboxylate transporter

MCT-4
monocarboxylate transporter

MHC,
myosin heavy-chain

NH,
normobaric hypoxia

NIRS
near-infrared spectroscopy

NO,
nitric oxide

NOS,
nitric oxide synthase

NOx,
nitric oxide blood metabolites

O_2,
oxygen

P_AO_2,
arterial oxygen pressure

PB
barometric pressure

PCO_2
carbon dioxide pressure

PCr
phosphocreatine

$P_{ET}CO_2$,
end-tidal carbon dioxide pressure

PFK,
phosphofructokinase

PGC1α,
peroxisome proliferator-activated receptor gamma coactivator 1-alpha

Pi,
inorganic phosphate

P_IO_2,
inspired pressure of oxygen

Post-
after training

Pre-
before training

$\dot{Q}$
cardiac output

ROS,
reactive oxygen species

RS,
repeated sprints

RSA
repeated sprint ability test

RSH,
repeated sprint training in hypoxia

RSN
repeated sprint training in normoxia

SaO_2,
arterial oxygen saturation

SOD,
superoxide dismutase

ß,
muscle buffer capacity

ST
slow-twitch fibers

TFAM,
mitochondrial transcription factor A

$\dot{V}_E$
minute ventilation

VEGF,
vascular endothelial growth factor

Vt,
tidal volume

Chapter 1

Introduction

1. Introduction

1.1 General introduction

1.1.1 Altitude training

Altitude training is a topic with persistent high interest among athletes, coaches and sport scientists. The effects of critical hypoxic conditions have been previously reported in mountain climbers (Barcroft, 1914; Haldane & Priestley, 1935). The 1968 Mexico Olympics were then a challenge for athletes, trainers, and scientists trying to prepare for competitions at 2300 m. For example, following a symposium on sport at mid altitude in 1965 in Magglingen (Weihe, 1965), a Swiss delegation headed by Dr. Kaspar Wolf sojourned in Mexico in November 1966 during pre-Olympic events where athletes underwent specific performance tests, noticing strong individual differences in the responses to altitude during their prolonged stay at 2300 m (Acquadro *et al.*, 1966). This experience led to recommendations in terms of altitude acclimatization for the Swiss delegation with a 10 days stay in St-Moritz prior to travelling to Mexico, it was also an opportunity to highlight the undeniable lack of scientific knowledge to support an efficient preparation to perform at altitude. All in all, this event acted as a trigger for numerous studies assessing the effect of milder hypoxic conditions and altitude training strategies on physiological responses and athletic performance (Margaria, 1967; Keul & Cerny, 1974; Shephard, 1974).

Nowadays, elite athletes extensively use altitude training in "real" (*i.e.* hypobaric hypoxia, HH) or "simulated" (*i.e.* normobaric hypoxia, NH) conditions in the belief that it will enhance their ability to compete successfully at the international level. Therefore, altitude training has become a widespread concept with numerous training combinations available. Contemporary altitude training possibilities for sea-level performance improvements were first summarized by Wilber (Fig. 1) helping to clarify the actual nomenclature (Wilber, 2007).

In spite of substantial differences between these various forms of hypoxic training and/or exposure, all these methods have the same goal of inducing physiological adaptations and enhancement in athletic performance.

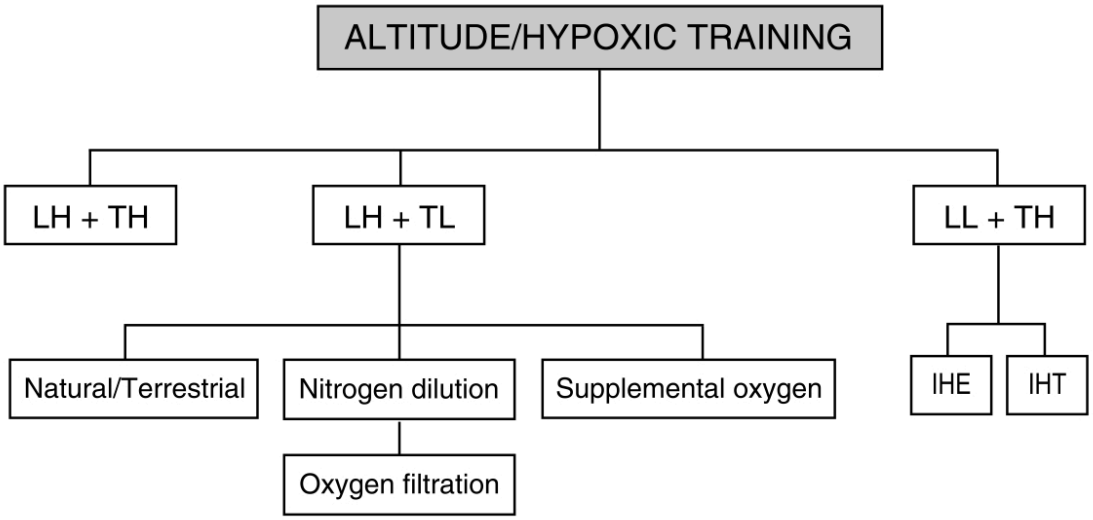

Figure 1 Contemporary altitude training models. IHE, intermittent hypoxic exposure; IHT, intermittent hypoxic training; LH + TH, live high + train high; LH + TL, live high + train low; LL + TH, live low + train high. From Wilber, 2007.

A prolonged stay including training at moderate to high altitude (now known as the "Live High – Train High" LHTH approach) is undoubtedly of interest for subsequent optimal performance at altitude. Further, when considering putative benefits on subsequent sea-level performance the LHTH model has been largely investigated (at least 14 studies to date (Bonetti & Hopkins, 2009)). However, with the decrement of the aerobic capacity at altitude (Wehrlin & Hallen, 2006), maximal training intensity could be limited during a LHTH training camp. The latter led scientists in the 1990s to explore the potential of training models allowing to maintain training power/speed for enhanced sea-level performance such as "live high-train low" (LHTL) (that is when athletes stay for a prolonged sojourn in hypoxic environment but train in normoxia) (Levine & Stray-Gundersen, 1997; Stray-Gundersen *et al.*, 2001; Stray-Gundersen & Levine, 2008). Again, different LHTL strategies have been abundantly analyzed with more than 30 studies reported to date (Bonetti & Hopkins, 2009). In fact, the development of altitude simulation facilities (nitrogen house or hypoxic chamber) or portable devices (hypoxic tents or hypoxicators like the Altitrainer®) allowed a multitude of new combinations to design training

strategies combined with hypoxic exposure. Actually, the LHTL approach could combine night sleep in a normobaric hypoxic tent (or apartment) with training in normoxia (Rusko *et al.*, 2004; Wilber, 2007) or even a prolonged stay at altitude and training with supplemental oxygen (Morris *et al.*, 2000; Wilber *et al.*, 2003). Finally, altitude simulation methods also allowed more recently to explore "Live Low – Train High" (LLTH) modalities. LLTH is characterized by short exposures to a hypoxic environment at rest (that is "intermittent hypoxic exposure" IHE) or during training (that is "intermittent hypoxic training" IHT). Consequently, a recent review (Millet *et al.*, 2010) aggregated current knowledge to update the initial classification of contemporary altitude training models additionally including combinations using advanced technological methods to optimally improve peak performance in elite athletes (Fig. 2).

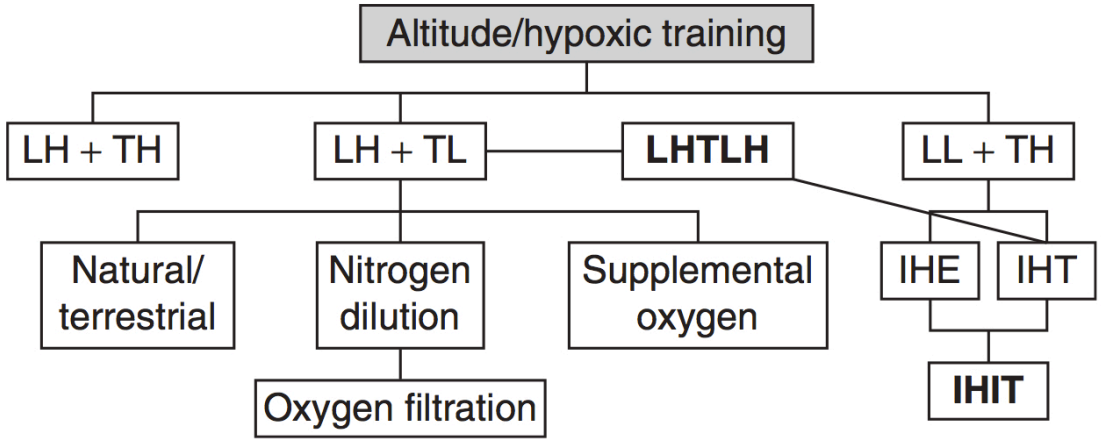

Figure 2 Different hypoxic methods. LH: Living High; TH: Training High; TL: Training Low; LHTLH: Living High, Training Low and High; LL: Living Low; IHE: Intermittent Hypoxic Exposure; IHT: Intermittent Hypoxic Training; IHIT: Intermittent Hypoxic Interval-Training. From Millet *et al.*, 2010.

For instance, athletes in racket or teams sports have shown greater interest lately for IHT since the technological development of simulated altitude facilities allows for good training conditions without the hassle and cost of travelling to altitude sufficiently rapidly not to lose efficient training and/or recovery time. So, IHT in simulated altitude facilities could be of interest in intermittent sports. However, IHE seems probably inefficient and IHT disputable in improving sea-level aerobic performance (Bartsch *et al.*, 2008b; Bergeron *et al.*, 2012). To our knowledge, while IHT is regularly used by professional team sports in football (Liverpool FC, Tottenham Hotspurs), rugby (Wales national team), Australian football (Brisbane Lions, Melbourne FC) or in

combat sports like taekwondo and judo (Japanese and Chinese national teams; Beijing Sport University), it remains unclear whether and how training in hypoxia would enhance athletic performance (Millet *et al.*, 2009; Millet *et al.*, 2010). In addition, the utility of altitude/hypoxic training more specifically in team (or intermittent) sports was discussed very recently by renowned experts and a common statement underlines the lack of current consensus on the optimal strategies to elicit the best results from altitude training in a team-sport population although some approaches could be beneficial (Lundby *et al.*, 2012; Girard *et al.*, 2013a).

Nevertheless, the induced underlying mechanisms for performance enhancement (both in endurance or intermittent sports) are still widely discussed in the scientific community (Gore & Hopkins, 2005; Levine & Stray-Gundersen, 2005; Millet *et al.*, 2010; Billaut *et al.*, 2012; Siebenmann *et al.*, 2012; Jacobs, 2013; Wilber, 2013), and it creates opportunities for well designed specific controlled and blinded studies assessing putative additional effects of IHT (or other methods) compared to the same training in normoxia (Bergeron *et al.*, 2012; Lundby *et al.*, 2012).

1.1.2 Exercise physiology in hypoxia

To interpret potential performance gains obtained by adding some form of hypoxic stress during training, one should understand which mechanisms are involved in the physiological responses to hypoxia. Hypoxic conditions can be defined as a combination of barometric pressure (PB) and an inspired fraction of oxygen (F_IO_2) that results in an inspired pressure of oxygen (P_IO_2) lower than a normoxic value of 150 mm Hg (Conkin & Wessel, 2008). Indeed, hypoxia can be achieved by reducing PB (i.e. hypobaric hypoxia (HH)) or by lowering the F_IO_2 while PB remains stable (i.e. normobaric hypoxia (NH)). For instance, altitude simulation devices developed in the past 20 years are now consistent advanced technological tools reducing P_IO_2 by diluting inspired air with nitrogen, filtering oxygen (O_2), or delivering low oxygen gas mixtures (Wilber, 2007). P_IO_2 is calculated by withdrawing water vapour pressure (47 mmHg) from barometric pressure and multiplying by the inspired oxygen pressure: P_IO_2 (mmHg) = F_IO_2 x (PB – 47).

For example, at an altitude of 3000 m (as in HH), PB approximately equals 525 mmHg and F_IO_2 20.93%. The corresponding P_IO_2 thus equals 0.2093 x (525-47)=100 mmHg. A similar P_IO_2 is achieved in NH at sea level (PB=760 mmHg) by reducing F_IO_2 to 14.1 % (e.g., P_IO_2= 0.141 x (760-45)=100.5 mmHg). Figure 3 illustrates the reduction in P_IO_2 during a 24 h exposure in HH and in NH. The consequence of a reduced P_IO_2 is a diminution of the alveolar oxygen pressure (P_AO_2) and hence of the oxygen distribution to the tissues (Cerretelli, 1980) triggering important and rapid adaptive physiological responses. These concrete responses are the basis of putative additional positive effects expected when adding a hypoxic stress to any training strategy. Any potential benefit can thereafter hypothetically be related to the physiological responses induced by the added hypoxic stress during exercise.

Cardiovascular responses During an acute exposure to a hypoxic environment, the skeletal muscle blood flow is rapidly adjusted to preserve O_2 delivery to respiring tissues during exercise (Gonzalez-Alonso *et al.*, 2002). This mechanism, helped by a nitric oxide (NO) dependent local vasodilation with haemoglobin acting as a hypoxic sensor (Crawford *et al.*, 2006), is easily observable since heart rate (HR) is increased during submaximal exercise (and also at rest) in hypoxia. Actually, the activation of the sympatho-adrenergic axis (with a release of catecholamines) increases the sympathetic tone to raise HR (Koller *et al.*, 1988; Mazzeo *et al.*, 1994). The HR augmentation increases the cardiac output ($\dot{Q}$) and compensates for the decreased stroke volume (mainly due to reduced plasma volume) (Calbet & Lundby, 2009; Fukuda *et al.*, 2010). Then, at maximal exercise intensity, both stroke volume and $\dot{Q}$ are reduced in hypoxia (Wagner, 2000). The latter combined with a reduced P_AO_2 can in turn account for a reduced maximal aerobic performance in hypoxia (Ferretti *et al.*, 1997). An adaptation occurs within days of exposure since $\dot{Q}$ and stroke volume were observed to return to pre-exposure (or even lower) levels, essentially because of a hemoconcentration (Grover *et al.*, 1986). Besides, hypoxic exposure was shown to produce a sustained stimulation of the sympathetic nervous system, which in turn increases resting metabolic rate and stimulates the bone marrow to rise the red blood cell production (Grover *et al.*, 1986).

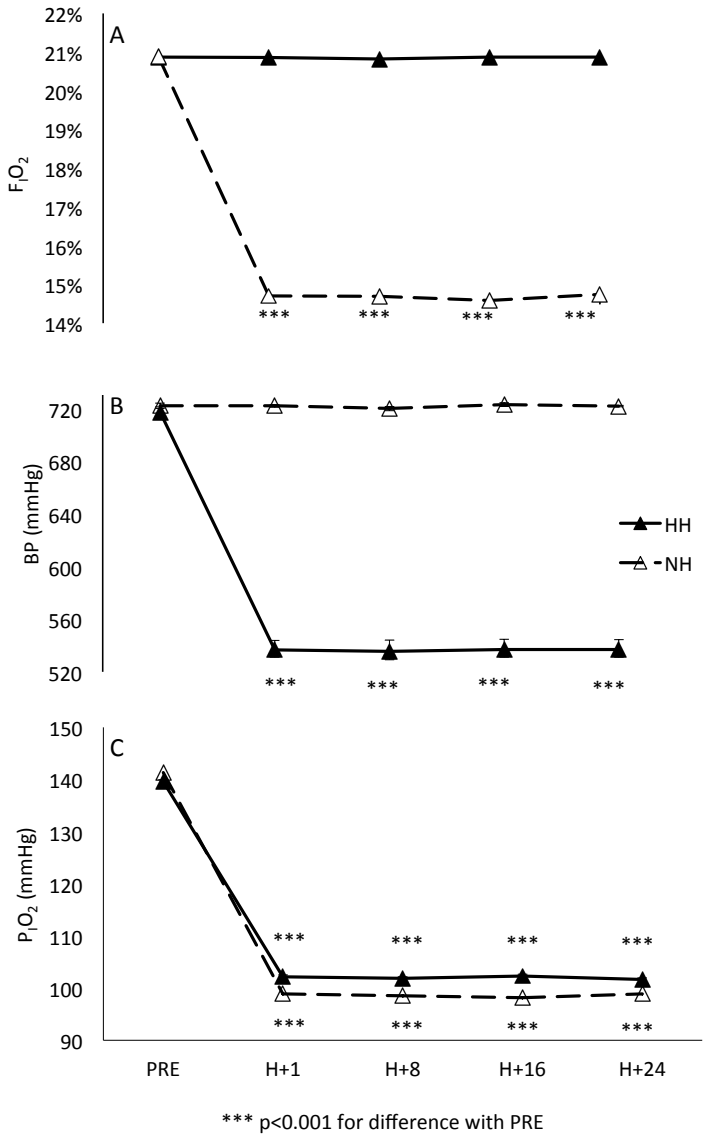

Figure 3 Fraction of inspired oxygen (F_IO_2, %) (A), barometric pressure (BP, mbar) (B) and corresponding pressure of inspired oxygen (P_IO_2, mmHg) (C) before (PRE) and during a 24 h exposure in hypobaric hypoxia (HH) or normobaric hypoxia (NH). From Faiss *et al.*, 2013c.

Hematological responses Indeed, tissue hypoxia is a primary stimuli for the production and release of erythropoietin (EPO), a glycoprotein hormone activating erythropoiesis (Gunga *et al.*, 2007). Thus, a decreased O_2 availability stimulates EPO gene expression and serum EPO levels were shown to i) rise rapidly in acute hypoxia (Eckardt *et al.*, 1989), ii) remain high during a prolonged hypoxic exposure and iii) return to initial values only after 22 days (Milledge & Cotes, 1985). Interestingly, an elevated red blood cell mass was shown to be proportional to oxyhemoglobin saturation (Weil *et al.*, 1968). An increase in red blood cells (and hence in the total

haemoglobin mass) could therefore improve O_2 carrying capacity to the working muscles after sufficient hypoxic exposure (Berglund, 1992). However, one should remember that red blood cells are diluted in plasma and the total erythrocytes volume is expressed as a fraction (in %) of total blood volume by the hematocrit (Hct). Altitude exposure initiates a diminution of plasma volume caused by a fluid transfer outside of the vascular compartment. This leakage is a consequence of increased diuresis, greater respiratory loss of water (due to the dryer air at altitude) and augmented perspiration at altitude (Hogan *et al.*, 1973; Maher *et al.*, 1974; Butterfield *et al.*, 1992). Consequently, [Hb] and Hct are increased, peripheral resistances are augmented due to the higher blood viscosity, and blood circulation is altered (Berglund, 1992). On the other hand, the affinity of oxygen for hemoglobin is reduced at high altitude by the 2,3 diphosphoglycerate (2,3 DPG) to facilitate O_2 delivery to the tissues (Mairbaurl, 1994). The compound 2,3 DPG is produced during a prolonged hypoxic exposure by the red blood cell metabolism (Mines, 1981) to counteract the left-shift of the oxyhemoglobin dissociation curve illustrated hereafter (Fig. 4). For example, an increase of 2,3 DPG was reported in a controlled study in athletes after 13 days of training at 2300 m (Mairbaurl *et al.*, 1986).

Finally, a direct connection between altitude training and any change in hematological parameters is hard to establish. Hence, there is still a vivid debate on the physiological mechanisms mediating performance gains after LHTL: some argue that hematological factors (e.g., augmented red cell mass) are of greatest influence (Levine & Stray-Gundersen, 2005) while others report non-hematological factors (e.g., exercise economy or HIF-mediated changes at the muscular level) to predominate (Gore & Hopkins, 2005). In addition, recent results demonstrated a blunted erythropoietic response following LHTL in athletes with a high initial Hb mass (Siebenmann *et al.*, 2012).

Respiratory responses Another perceived consequence of a diminished P_IO_2 upon hypoxic exposure is an increase in pulmonary ventilation (that is the hypoxic ventilatory response HVR) to maintain O_2 delivery to the tissues (Wagner *et al.*, 1986). The increase in minute ventilation ($\dot{V}_E$) varies greatly individually and usually begins when P_IO_2 is reduced to at least 100 mmHg (approximately 3000 m) (Rahn & Otis, 1949). Actually, exposure to hypoxia stimulates peripheral chemoreceptors in the carotid bodies (sensitive to the drop in arterial O_2 pressure (PaO_2), but also to an increase in arterial CO_2 pressure and decrease in arterial pH) to regulate ventilation centrally through the cortical respiratory centres (Teppema & Dahan, 2010). For instance during an exercise in hypoxia, hyperventilation partially counterbalances the drop of P_AO_2, PaO_2 and arterial O_2 saturation (SaO_2). Concomitantly end-tidal carbon dioxide pressure ($P_{ET}CO_2$) decreases with the consequence of a respiratory alkalosis due to the reduced plasma concentrations of CO_2 and H^+ (Ursino *et al.*, 2001). Blood pH is thus elevated and left shifts the oxyhemoglobin dissociation curve (Fig. 4) (Calbet *et al.*, 2003).

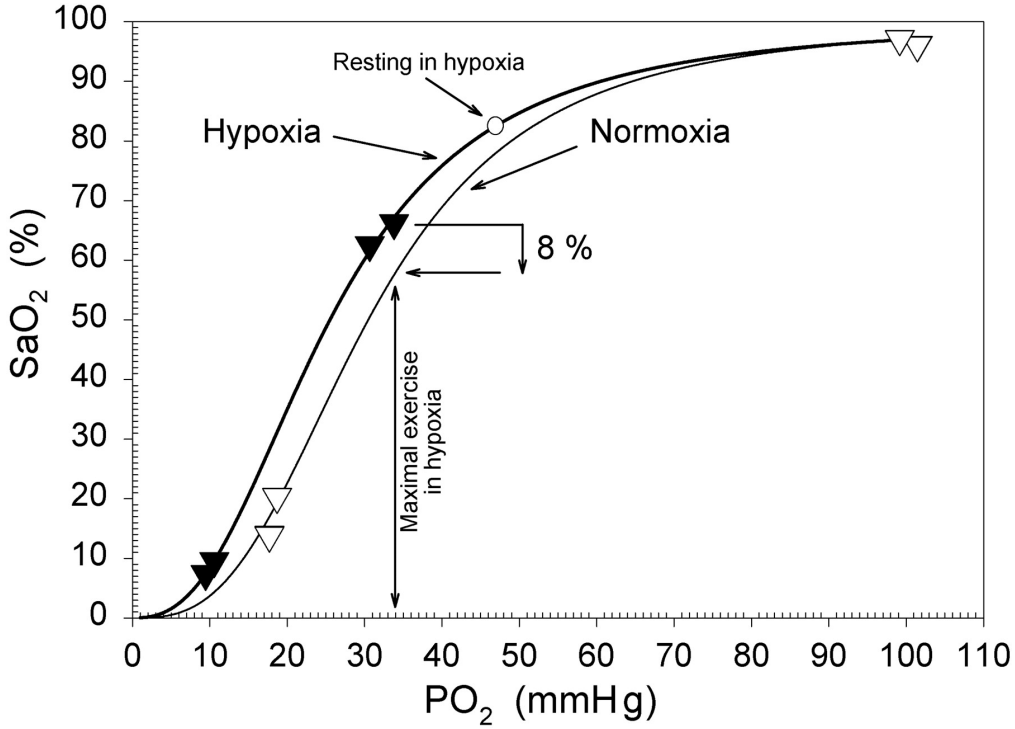

Figure 4 Effect of acute hypoxia on the O_2 dissociation curve of the hemoglobin during exercise in normoxia (white triangles; fine line) and hypoxia (black triangles; thick line). From Calbet *et al.*, 2003.

Interestingly, after training in hypoxia, higher HVR were observed in athletes presenting the highest increase in oxidative stress markers (Pialoux *et al.*, 2009a). The latter corroborates findings in an animal model indicating a reactive-oxygen species (ROS) dependency of an increase of HVR (MacFarlane *et al.*, 2008). Finally, HVR is correlated with $\dot{V}_E$ and SaO_2 during exercise in hypoxia (Benoit *et al.*, 1995), thus, an increase of HVR should be considered as a positive adaptive response with the elevation of $\dot{V}_E$ allowing to finally increase SaO_2 (Huang *et al.*, 1984).

Muscular responses The hypoxic stimulus also induces significant peripheral and muscular adaptations. The aim at the muscular level is essentially to improve O_2 delivery and diffusion to the mitochondria and improve its oxidative capacity to cope with the decreased O_2 availability. A sustained exposure to severe hypoxia may lead to muscle atrophy (Hoppeler & Vogt, 2001). So it was thought that exposing subjects to intermittent hypoxia could potentially dissociate benefits from the hypoxic stimulus (e.g., short exposure only during training in hypoxia) from the negative effects of prolonged exposure to hypoxia. For example, hypoxic training was found to improve muscle capillary density in elite cyclists (Terrados *et al.*, 1988) although such an improvement in capillarization may be due to a diminished muscular volume (Mizuno *et al.*, 1990) thus questioning the presence of an actual angiogenesis after such a hypoxic stimulus. Besides, although it remains unclear whether sustained hypoxia may alter muscle oxidative capacity or improve mitochondrial enzyme activity (Green *et al.*, 1989; Hoppeler *et al.*, 1990; Hoppeler *et al.*, 2003), a combination of hypoxia with specific training modalities may enhance oxidative enzymatic activity more than the same training in normoxia (Terrados *et al.*, 1990). It was also reported that altitude training could lead to an increased concentration in myoglobin, an important component of O_2 transport to the mitochondrial membrane, compared to a similar training at sea level (Reynafarje, 1962; Hoppeler *et al.*, 1990; Terrados *et al.*, 1990).

In addition, skeletal muscles play an important regulatory role in the acid-base balance in the exercising body by buffering the excess H^+ ions. Muscle buffer capacity (ß) (depending on the inorganic phosphate (Pi), HCO_3^- and proteins concentration (Cerretelli & Samaja, 2003)) is affected *inter alia* by exercise type and intensity, subjects' training level, and altitude

acclimatization level. Interestingly, ß was significantly improved in endurance athletes after LHTH (Mizuno *et al.*, 1990) and LHTL (Nummela & Rusko, 2000; Gore *et al.*, 2001) whereas such an adaptation was not observed in other trained cyclists and triathletes after LHTL (Clark *et al.*, 2004). An important issue is however, the individual variation and reproducibility of the responses following altitude training. A large individual variation in physiological and performance changes after normoxic LHTL was thus reported (Robertson *et al.*, 2010). The exact reasons for such individual variability remain to be elucidated but differences in protein synthesis and degradation at the muscular level (Holm *et al.*, 2010) or differences in buffer capacity (Gore *et al.*, 2001; Mizuno *et al.*, 2008) were evoqued. Besides, the current knowledge indicates only minor changes in oxidative enzymes and no skeletal muscle angiogenesis upon prolonged hypoxic exposure (Lundby *et al.*, 2009). Nevertheless, interventional studies in athletes with controlled and blinded designs are still needed to prove that training in hypoxia induces a greater magnitude of the responses at the muscular level and additional effects on athletic performance (Lundby *et al.*, 2012). The latter emphasizes again equivocal benefits of adding hypoxia to a training stimulus in the actual responses at the muscular level.

Molecular responses O_2 sensing has been widely investigated (Taabazuing *et al.*, 2014) and reveals that, in hypoxia, responses occur directly at the gene level. These molecular responses are mediated by O_2-sensitive transcription factors, such as the so-called hypoxia inducible factors (HIF) family. HIF-1α/ß is a master regulator of hypoxia-induced gene expression. While undetectable in normoxic cells because it is degraded in presence of O_2, in hypoxia HIF-1α can enter the cell nucleus, bind to HIF-1ß and constitute the functional transcription factor leading to the regulation of hundreds of genes (Semenza, 2007a). The HIF-1α pathway in normoxia and hypoxia is presented in Figure 5.

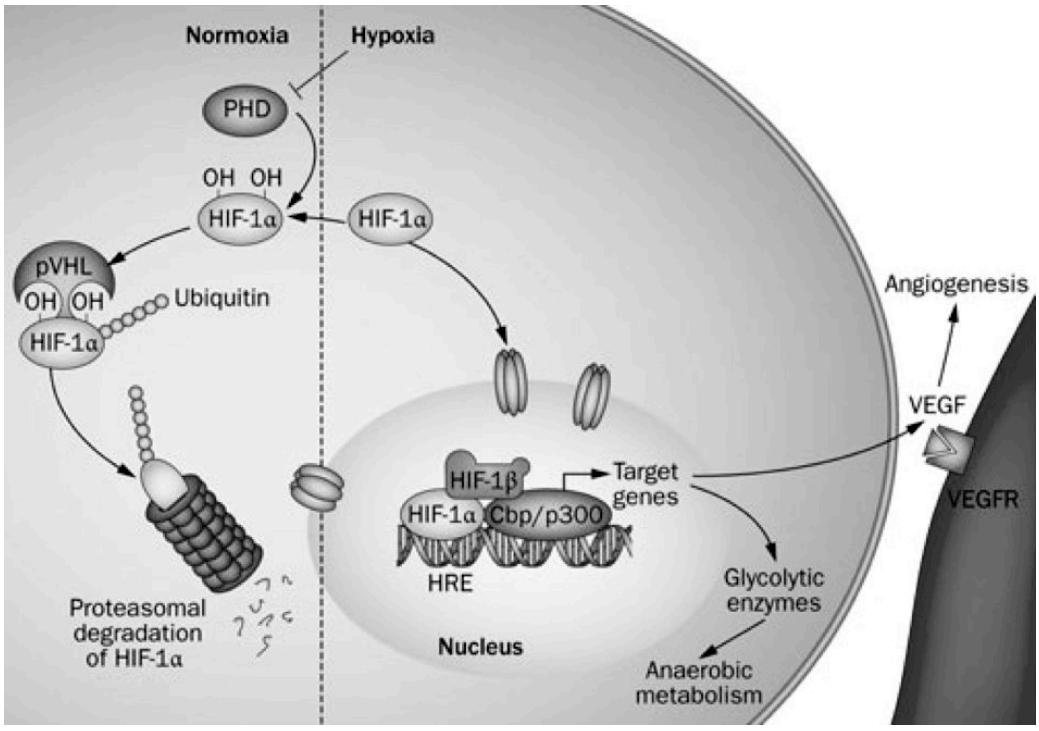

Figure 5 The HIF-1α pathway in normoxia and hypoxia. From Maes *et al.*, 2012.
HIF, hypoxia-inducible transcription factor; HRE, hypoxia response element; PHD, prolyl hydroxylase (Egl nine homologs 1 and 2); pVHL, von Hippel–Lindau protein; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

The hypoxic condition in the muscular tissue accordingly leads to HIF-mediated adaptations at the molecular level (Hoppeler & Vogt, 2001; Zoll *et al.*, 2006) with, for example, the regulation of genes coding for proteins involved in:

-O_2 transport (EPO, myoglobin (Mb) and vascular endothelial growth factor (VEGF))

-Glycolytic metabolism (phosphofructokinase (PFK) and glucose transporters (GLUT)

- pH regulation (carbonic anhydrase 3 (CA3) and monocarboxylate transporters 1 and 4 (MCT1, MCT4))

-Mitochondrial biogenesis/metabolism (peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), mitochondrial transcription factor A (TFAM) and citrate synthase (CS))

-Oxydative stress (superoxide dismutase (SOD))

-Contractile phenotype (myosin heavy chain (MHC).

Although this list is not exhaustive, the latter suggests strong responses to the hypoxic environment at the molecular level (with a key role of HIF) that may significantly influence exercise physiology in hypoxia and account for the tangible responses mentioned above. The means by which HIF initiates a cascade of mechanisms leading to the (up- or down-) regulation of hundreds of genes are well described elsewhere far beyond the scope of this introduction (Semenza *et al.*, 2006; Semenza, 2007a, b). Nevertheless, one should realize that it is now accepted that exercising (and hence training) in hypoxia may lead to substantial responses at the molecular level leading potentially to systemic performance benefits in athletes aiming at a specific competitive edge (Bailey *et al.*, 2000; Bangsbo *et al.*, 2000; Bao *et al.*, 2006; Bailey *et al.*, 2009; Bartsch *et al.*, 2009; He *et al.*, 2012).

1.1.3 Athletic performance capacity in hypoxia

The metabolic dependence on O_2 availability implies that performance capacity may be altered in hypoxia. In HH however reduced aerodynamic resistances might even improve anaerobic performances (Hahn & Gore, 2001). Similarly, performance is certainly maintained in hypoxia for very short efforts relying on the anaerobic glycolysis such as short maximal sprints or repeated sprints (RS) (Bogdanis *et al.*, 1996). RS involve though a progressive rise in the contribution of the aerobic metabolism (Gaitanos *et al.*, 1993) explaining the performance decrement observed in cycling RS even at a moderate altitude (Brosnan *et al.*, 2000). Thus, performance for longer efforts (i.e. aerobic) is clearly degraded at altitude. Numerous studies have reported a decrease in aerobic performance and maximal oxygen uptake ($\dot{V}O_{2max}$) in hypoxia (Squires & Buskirk, 1982; Young *et al.*, 1985; Lawler *et al.*, 1988; Peltonen *et al.*, 1995; Chapman *et al.*, 1999; Peltonen *et al.*, 1999; Friedmann *et al.*, 2005; Mollard *et al.*, 2007; Peltonen *et al.*, 2007; Chapman *et al.*, 2011). In hypoxia, O_2 diffusion is limited in the lung with the consequence of a reduced $\dot{V}O_{2max}$ (Calbet & Lundby, 2009). A linear decrease in $\dot{V}O_{2max}$ was hence shown in endurance trained athletes when exposed to altitudes increasing from 300 to 2800 m (Wehrlin & Hallen, 2006). For instance, in

that study, each 1000 m step was calculated to be associated with about 7.7% decrease in performance (Fig. 6) (Wehrlin & Hallen, 2006). However, such decrease of performance presents a great individual variability, particularly among elite level athletes (Schouweiler & Stray-Gundersen, 2002). Indeed, a more important decrement of absolute $\dot{V}O_{2max}$ was observed in elite *versus* non-elite athletes (Lawler *et al.*, 1988; Koistinen *et al.*, 1995; Mollard *et al.*, 2007) or in endurance trained *versus* sedentary subjects (Gavin *et al.*, 1998; Chapman *et al.*, 1999).

Nevertheless, previous investigations were conducted on lowlanders (i.e. athletes training mostly at low altitude or sea-level) but acclimatization to hypoxia raises performance at altitude (Millet *et al.*, 2010), and especially when it occurs in HH (Beidleman *et al.*, 2004; Fulco *et al.*, 2013)

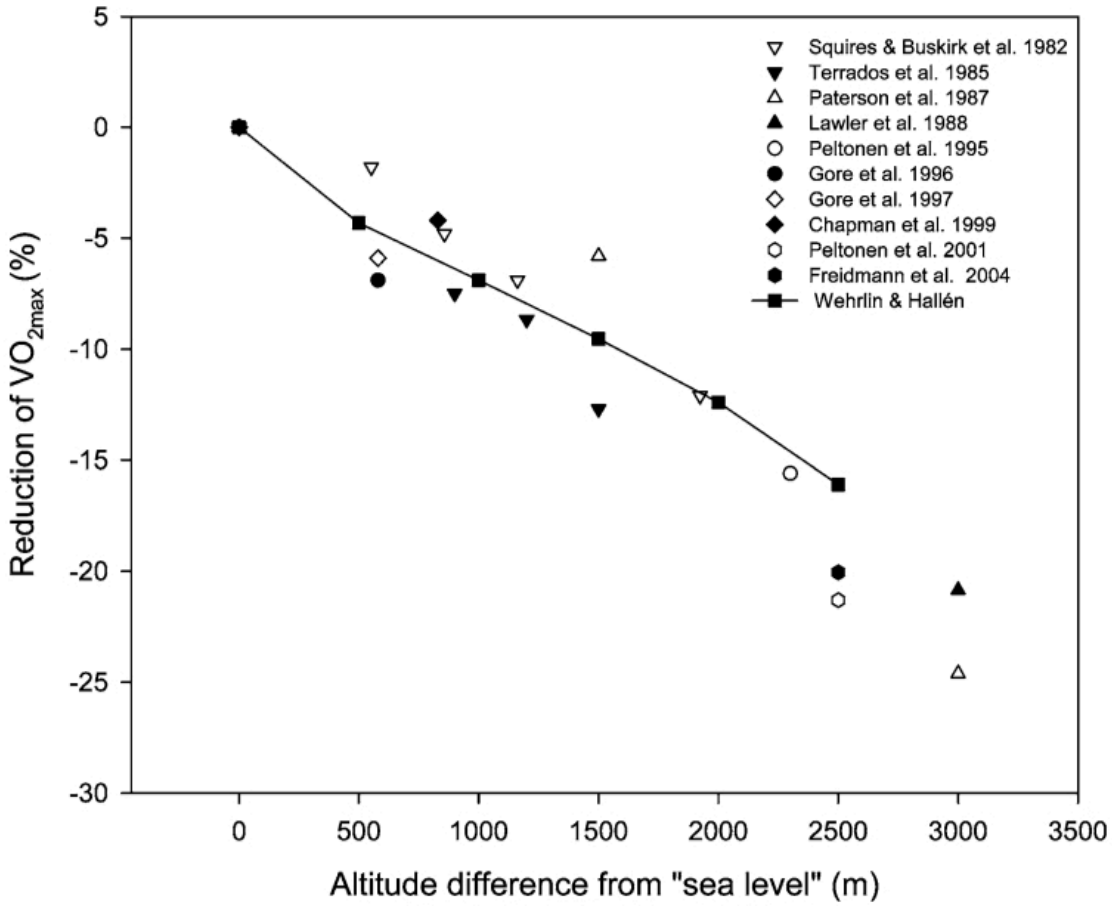

Figure 6 Reduction of maximal oxygen uptake ($\dot{V}O_{2max}$) with altitude increase compared to sea level. From Wehrlin & Hallen, 2006.

Due to the specificity of the discipline, elite ski-mountaineers are thought to be the endurance athletes exposed to the highest "hypoxic dose" (i.e. time spent in hypoxic environment) and

could therefore be better acclimatized to hypoxia because of the repeated altitude exposures (MacNutt *et al.*, 2012). We therefore designed a study (presented in Chapter 5, Article 1) to compare $\dot{V}O_{2max}$ and exercise responses in elite and recreational ski mountaineers in normoxia and hypoxia. We expected that elite and recreational ski mountaineers would have a similar performance decrement at altitude since acclimatization would compensate the larger impairment of aerobic capacity found in elite versus non-elite subjects. This study was particularly useful in better understanding physiological responses to hypoxia in elite athletes with the later aim to develop innovative training strategies for an elite population.

1.2 Responses to hypobaric vs. normobaric hypoxia

As mentioned above, hypoxic conditions are defined by the reduced P_IO_2 obtained either when reducing PB (i.e. hypobaric hypoxia (HH)) or by lowering the F_IO_2 while PB remains stable (i.e. normobaric hypoxia (NH). For long, both HH and NH were thought to produce similar physiological responses accordingly described as an "equivalent air altitude model" (Conkin & Wessel, 2008). However, a growing body of evidence highlights that HH and NH does not seem to induce strictly identical responses. For instance, in HH compared to NH, different ventilatory responses have been observed early with a higher respiratory frequency, lower tidal volume (Vt) and $\dot{V}_E$ during 2 h at 4750 m (Tucker *et al.*, 1983). Similar differences in ventilation between HH and NH were then confirmed during 10 h at 4770 m (Loeppky *et al.*, 1997) and during 40-min exposure at rest at 4500 m (Savourey *et al.*, 2003). A trend for lower $P_{ET}CO_2$ and $P_{ET}O_2$ was also shown. The reduction of PB in HH (and by definition not in NH) could lead to a higher alveolar physiological dead space potentially explaining a specific ventilatory response in HH. PB drop may lead to a larger pulmonary vasoconstriction and thus alter O_2 diffusion because it can modify both fluid circulation (e.g., pulmonary lymph) and the trans-alveoli-capillary membrane flux (Levine *et al.*, 1988). A significant decrease in plasma volume was then observed in NH compared to HH (Loeppky *et al.*, 2005). Again, PB is thought to account partly for this difference with a larger fluid retention (or lower diuresis) in HH. Any modification in plasma volume (e.g.,

hemoconcentration and increased peripheral resistances) could alter O_2 delivery to the muscles, therefore, physiological responses and training adaptations may substantially differ in NH and HH although these have never been investigated to date.

Furthermore, acute mountain sickness (AMS) symptoms were systematically more pronounced in HH compared to NH (Roach *et al.*, 1996; Loeppky *et al.*, 2005; Conkin & Wessel, 2008). It also seems that intermittent HH exposure may be effective in the acclimatization to altitude and reduction of the AMS severity (Beidleman *et al.*, 2004). A recent review reported differences in the relative effectiveness of different pre-acclimatization strategies in HH or NH on physiological responses (e.g., $P_{ET}CO_2$, SaO_2) and coherently concludes that HH and NH cannot be used interchangeably since HH seems far more effective in reducing AMS symptoms or optimizing exercise performance at altitude (Fulco *et al.*, 2013).

Furthermore, slight differences in physiological responses and NO have been reported at rest between HH and NH during short exposures (Hemmingsson & Linnarsson, 2009). NO plays a central role in the physiological responses in hypoxia. As mentioned earlier, when O_2 declines in arterial blood, the carotid chemoreceptors trigger the HVR through the cortical respiratory centers (Dempsey & Forster, 1982). Simultaneously, when O_2 tension falls, a nitrite-nitrate-NO pathway is gradually activated (Lundberg *et al.*, 2008) to support HVR through vasodilatory mechanisms. Interestingly, when nitric oxide synthase (NOS) is inhibited, HVR is reduced (Gozal *et al.*, 1996). Thus, a lower HVR observed in HH could potentially result (at least indirectly) from a lower NO bioavailability. Moreover, although NO in exhaled air (exNO) is known to decrease at altitude (Brown *et al.*, 2006; Donnelly *et al.*, 2011), nitric oxide blood metabolites (NOx) such as nitrate and nitrite had not been assessed in HH vs. NH yet. The exact mechanisms underlying any exNO (and NOx) difference between HH and NH remain unknown.

Oxidative stress is a potential candidate. The oxidative stress appears when ROS production overcomes the cell's capacity to neutralize the free radicals. This in turn defines the antioxidant capacity. The byproducts of the metabolic aerobic respiration are neutralized by active enzymatic antioxdiants (e.g., superoxide dismutase (SOD), catalase, or glutathione peroxidase (GPX) and

passive antioxidants (e.g., vitamin E, vitamin C or glutathione) (Powers & Hamilton, 1999). It is well established that physical exercise activity challenges the antioxidants capacity by overproducing free radicals (Niess & Simon, 2007; Powers *et al.*, 2011). Markers of protein and lipid oxidation (e.g., F2-isoprostanes, carbonyls and advanced oxidative protein products (AOPP) are subsequently elevated (Urso & Clarkson, 2003). Similarly to acute exercise, hypoxic exposure was reported to increase oxidative stress (Pialoux *et al.*, 2009b). In addition, oxidative stress is known i) to decrease the NO bioavailability in the vasculature (Thomas *et al.*, 2008b) and ii) to modulate the acute ventilatory response to hypoxia after chronic hypoxic exposure (Pialoux *et al.*, 2009c). The latter suggests potential links between the oxidative stress, the NO metabolism and the physiological responses during hypoxic exposure. Surprisingly, this was never compared in HH vs. NH although hypothetical differences may help to support the discrepancies reported in both conditions.

Moreover, from a recent meta-analysis (Bonetti & Hopkins, 2009) considering altitude training strategies it is also striking that exposure to HH doesn't seem to produce the same effects than NH since "real" altitude protocols seem to better enhance sea-level performance. Therefore, for the efficiency of hypoxic training methods and especially for intermittent sports, major key-questions related to the necessary hypoxic dose, the most beneficial hypoxic exposure (i.e. NH or HH) and the optimal training protocol for worthwhile adaptations and performance improvement still remain to be answered.

Obviously, disentangling hypoxia and hypobaria is of importance for athletes or mountaineers who use NH to prepare for altitude competitions or expeditions (Kayser, 2009) and further investigations on the differences in mechanisms and the clinical significance of hypobaric vs. normobaric hypoxia are needed.

In that context, we designed a study (presented in Chapter 6, Article 2) in order to determine if previously shown differences in ventilatory and nitrosative responses during short duration exposure in HH vs. NH were maintained over 24 hours at 3000 m in HH vs. NH and if these differences would be associated with different oxidative stress and plasma pH variations. The

experimental design was defined to assess for the first time the cardio-respiratory responses to moderate exercise but also analyze the time course of oxidative stress and NO metabolism at rest to discuss putative mechanisms in different physiological responses between HH and NH.

1.3 Responses to intermittent hypoxic training

The relevance of adding a hypoxic stress to any form of training mainly depends on the expected supplementary benefits in terms of performance. From the existing literature, Millet *et al.* presented the overall efficiency of various hypoxic training methods (Table 1, (Millet *et al.*, 2010). Remarkably, most training strategies allowed an increased performance or at least positive mechanisms while others did not bring additional benefits or even triggered detrimental effects. For instance, negative mechanisms were found in almost one third of the LHTL protocols. Meanwhile, even though IHT often failed to enhance performance, no detrimental effects were reported.

Hypoxic method	Number of studies	Increased performance/positive mechanisms	No additional effect	Negative mechanisms
Nitrogen dilution	19	12	2	5
Oxygen filtration	10	5	2	3
LHTL		**58%**	**14%**	**28%**
IHT	16	8	8	0
		50%	**50%**	**0%**
All	45	56%	18%	18%

Table 1 Overall efficiency of various hypoxic methods. From Millet *et al.*, 2010.
LHTL, live high-train low ; IHT : intermittent hypoxic training.

Interestingly performance improvements after specific altitude training were reported and attributed to non-hematological peripheral factors (e.g., increased muscle buffer capacity (Gore *et al.*, 2007), better locomotion efficiency (Saunders *et al.*, 2009). Then it seems clear that the hypoxic dose during IHT is insufficient to elicit polycythemia (Levine & Stray-Gundersen, 2006). Therefore, any putative additional benefit of adding a hypoxic stress to intermittent training (i.e. IHT) shall be associated with peripheral adaptations at the muscular level rather than improved oxygen transport due to an augmented Hb mass.

Nevertheless, before thinking about further benefits in the subsequent sea-level (or low altitude) performance when adding hypoxia to a training strategy one should find the specific training strategy improving performance in normoxia. Interval training (INT) may be a successful approach. INT methods have been extensively investigated (Billat, 2001a, b) and are widely used by athletes both in endurance and intermittent sports since tangible performance benefits arise after well thought INT. Although it is beyond the scope of this work to detail all mechanisms involved in performance improvement after INT, it challenges by definition the metabolic and neuromuscular systems because INT consists of "repeated short-to-long bouts of rather high-intensity exercise interspersed with recovery periods" (Billat, 2001a). The optimal exercise intensity (Millet *et al.*, 2003b) and work-to-rest ratio (Millet *et al.*, 2003a) need to be determined to bring metabolic, cardiovascular, and other peripheral adaptive mechanisms to their limit.

The rationale of adding a hypoxic stimulus to INT (i.e. IHT) relies on the hypothesis that adaptations at the muscular level surpass those triggered by INT alone, with the evidence that IHT augments the physiological strain (Buchheit *et al.*, 2012) and elicits specific molecular adaptations (Vogt *et al.*, 2001; Kime *et al.*, 2003; Zoll *et al.*, 2006).

Indeed, it has been shown in IHT that training intensity *per se* modulates molecular adaptations at muscular level in hypoxia (Hoppeler & Vogt, 2001) and systemic results seemed to confirm it, because the increase in performance varies extremely depending on the type of IHT used (Dufour *et al.*, 2006; Roels *et al.*, 2007). A significant increase in mRNA expression of genes involved in O_2 transport (e.g., VEGF and Mb) appeared indeed only after high-intensity hypoxic training when compared to the same normoxic training (Vogt *et al.*, 2001). In this way, results assess that IHT induces peripheral adaptations possibly not found with INT alone such as increase in muscle buffer activity or the mentioned specific molecular adaptations at muscular level (Geiser *et al.*, 2001; Hoppeler & Vogt, 2001; Vogt *et al.*, 2001; Hoppeler *et al.*, 2003). In addition, including hypoxia sessions into the usual training of athletes was shown to qualitatively improve mitochondrial function (Ponsot *et al.*, 2006). Hence, different high-intensity training methods in hypoxia could be advantageous for anaerobic glycolysis: significant changes

concerning pH regulation and lactate transportation at the muscular level after a 6-week IHT were found (Zoll *et al.*, 2006). Improved anaerobic performance has previously been described after nine days after IHT with peak and average power output being significantly improved during a specific anaerobic cycling test while a control group performing INT did not improve (Meeuwsen *et al.*, 2001). Thus, we mention here putative benefits of IHT for anaerobic performance. It is striking though that the advantages of IHT for anaerobic performance (in intermittent sports for example) have hardly been studied and that the mechanisms originating performance improvement are obscure. To the same extent, several controversial discussions addressed the usefulness of IHT for improving football performance (Bartsch *et al.*, 2009; Gatterer *et al.*, 2009). Actually, four studies were mentioned showing improvement in anaerobic performance after high altitude training (Bartsch *et al.*, 2008a). However, all of these studies were uncontrolled and thus cannot distinguish between the effects of training and those of hypoxia. To have an insight of the potential of IHT vs. INT, we produced a comprehensive review of the existing IHT studies in the literature presented in Chapter 11, Article 7. The overview of existing LLTH methods was updated to gather data from 21 studies. To consider the recent update of the methods' nomenclature (Millet *et al.*, 2013a), we separated training containing either essentially continuous aerobic training bouts in hypoxia (CHT) (11 studies, Table 2) or intermittent hypoxic training with more intense repeated intervals (i.e., IHT) (10 studies, Table 3). Interestingly, an additional benefit on performance-related variables of LLTH compared to the same training performed in normoxia was present only in one CHT study and three of the IHT studies. It allowed us to highlight the role of training content and intensity in the subsequent performance improvement despite strikingly poor additional benefits of IHT vs. INT in promoting athletic performance (especially in the rare study involving team-sports athletes). It finally evoked the lack of well-designed controlled IHT studies including maximal intensity exercise training and helps to understand the orientation of our research towards a very specific and novel form of intensive hypoxic training, the so called repeated sprint training in hypoxia (RSH).

Author (year)	Subjects	Design (number of training sessions, type, altitude, training content)	Groups	Statistically significant results (P<0.05)
Roskamm *et al.* (1969)	Untrained	24 over 4 wks, cycling, 2250 m (N=6) or 3450 m (N=6) (HH). 30-min aerobic training	CHT, N=12 CNT, N=6	+10-17% VO_{2max} +6% VO_{2max}
Emonson *et al.* (1997)	Untrained	15 over 5 wks, cycling, 2500 m (HH). 45 min at 70% of VO_{2max}	CHT, N=9 CNT, N=9	+12% VO_{2max} +12% VO_{2max}
Katayama *et al.* (1999)	Untrained	10 over 2 wks, cycling, 4500 m (HH). 30 min at 70% of normoxic VO_{2max} level	CHT, N=7 CNT, N=7	+7% VO_{2max} +5% VO_{2max}
Bailey *et al.* (2000)	Runners	4 wks at ~2000 m (NH). Aerobic training, no details	CHT, N=18 CNT, N=14	+15% VO_{2max} +5% VO_{2max}
Geiser *et al.* (2001)	Untrained	30 over 6 wks, cycling, 3850 m (NH). 30 min at 77-85% of max heart rate	CHT, N=18 CNT, H=15	+11% VO_{2max}, +17% 30-min TT mean PO +9% VO_{2max}, +19% 30-min TT mean PO
Karlsen *et al.* (2002)	Cyclists	9 over 3 wks, cycling. 3000 m (NH). 120 min aerobic training	CHT, N=8 CNT, N=8	NS changes in VO_{2max} or 30-min TT NS changes in VO_{2max} or 30-min TT
Hendriksen & Meeuwsen (2003)	Triathletes	10 over 10 days, cycling, 2500 m (HH). 105 min aerobic training	CHT, N=8 CNT, N=8	**+5% PPO cycling Wingate** NS increase
Ventura *et al.* (2003)	Cyclists	18 over 6 wks, cycling, 3200 m (NH). 30 min aerobic training	CHT, N=7 CNT, N=5	NS changes in VO_{2max} or 10-min TT NS changes in VO_{2max} or 10-min TT
Dufour *et al.* (2006)	Runners	12 over 6 wks, running, 3000 m (NH). 24-40 min < VO_{2max}.	CHT, N=9 CNT, N=9	+5% VO_{2max}, +35% T_{lim} at vVO_{2max} NS changes
Hamlin *et al.* (2009)	Cyclists & triathletes	10 over 10 days, cycling, 3200-4400 m (NH). 90 min aerobic training followed by two 30-s Wingate tests	CHT, N=9 CNT, N=7	+3% PO cycling Wingate NS changes
Mao *et al.* (2011)	Active males	25 over 5wks, cycling, 2750 m (NH). 30 min aerobic training	CHT, N=12 CNT, N=12	+16% VO_{2max} +10% VO_{2max}
Holiss *et al.* (2014)	Runners	16 over 8 weeks, running, 2150 m, (NH). 40 min < VO_{2max}	CHT, N=5 CNT, N=7	**-3% submaximal VO_2**, -1% VO_{2max} +2% submaximal VO_2, **+5% VO_{2max}**

Table 2 Summary of current research findings relative only to the use of hypoxic training including continuous aerobic bouts in hypoxia (CHT) updated from (Faiss *et al.*, 2013a). Significant difference between groups is shown in bold (P<0.05). HH, hypobaric hypoxia; NH, normobaric hypoxia; CNT, continuous normoxic training; NS, non significant (P > 0.05); PPO, peak power output; PO, power output; TT, time trial; VO_{2max}, maximal oxygen uptake; T_{lim}, time to exhaustion; vVO_{2max}, velocity associated to VO_{2max} .

Author (year)	Subjects	Design (number of training sessions, type, altitude, training content)	Groups	Statistically significant results (P<0.05)
Terrados *et al.* (1988)	Elite cyclists	12-20 over 3-4 wks, cycling, 2300 m (HH). Aerobic training and some intervals (15 s at 130% of aerobic peak power output)	IHT, N=4 INT, N=4	+33% PPO +22% PPO
Martino *et al.* (1995)	Elite swimmers	Swim sprints at 2800 m (HH) during 21 days at altitude. No details available	IHT, N=20 INT, N=13	**-6% 100 m swim time, +34% PPO arm Wingate** NS changes
Truijens *et al.* (2003)	Swimmers	15 over 5 wks, swimming, 2500 m (NH). 12.5 min > 100% VO_{2max} (30 s or 60 s bouts)	IHT, N=8 INT, N=8	NS changes +6% VO_{2max}
Morton & Cable, (2005)	Team sport players	12 over 4 wks, cycling, 2750 m (NH). 10 x 1-min at 80% of 2-min PPO	IHT, N=8 INT, N=8	+8% cycling Wingate PPO, + 7% VO_{2max} +6.5% cycling Wingate PPO, 8% VO_{2max}
Roels *et al.* (2005)	Cyclists & triathletes	14 over 7 wks, cycling, 3000 m (NH). 6-8 x 2-3 min at 100% of aerobic PPO	IHT, N=11 IHIT, N=11 INT, N=11	+4% 10-min TT mean PO **+9% VO_{2max}**, +5% 10-min TT mean PO +5% 10-min TT mean PO
Dufour *et al.* (2006)	Runners	12 over 6 wks, running, 3000 m (NH). 24-40 min with intervals < VO_{2max}.	IHT, N=9 INT, N=9	**+5% VO_{2max}, +35% T_{lim} at vVO_{2max}** NS changes
Roels *et al.* (2007)	Cyclists & triathletes	15 over 3 wks, cycling, 3000 m (NH). 9 x 60 min at 60% VO_{2max} and 36 min with intervals of 2 min at 100% aerobic PPO (2 min bouts)	IHT, N=10 INT, N=9	+7% aerobic PPO +7% aerobic PPO, +8% 10-min TT mean PO
Lecoultre *et al.* (2010)	Cyclists	12 over 4 wks, cycling, 3000 m (NH). 4 x 12-18 min at 100-120% of aerobic PPO, 4 x 30-48 min < VO_{2max} and 4 x 100-min aerobic training.	IHT, N=7 INT, N=7	+7% 40 km TT mean PO +6% 40 km TT mean PO
Manimmanakorn *et al.* (2012)	Female team-sport players	15 over 5 wks, knee flexion and extension, ~4500 m (NH). 6 sets of low resistance knee extensions and flexions to failure with 30 s between sets	IHT, N=10 INT, N=10	**+15% MVC3, +17% MVC30, +129% REP201RM** + 86% REP201RM
Holliss *et al.* (2013)	Active males	15 over 3 wks, leg-extension, 3000 m (NH). 10 x 60-70 s intense exercise with 20-30 s passive recovery. One leg IHT, the other leg INT.	IHT, N=9 INT, N=9	+25% leg-extension incremental T_{lim} +27% leg-extension incremental T_{lim}

Table 3 Summary of current research findings relative only to the use of intermittent hypoxic training (IHT) updated from (Faiss *et al.*, 2013a).
Significant difference between groups is shown in bold (P<0.05). HH, hypobaric hypoxia; NH, normobaric hypoxia; CNT, continuous normoxic training; IHIT, intermittent hypoxia interval training, CON; control groupNS, non significant (P > 0.05); PPO, peak power output; PO, power output; TT, time trial; VO_{2max}, maximal oxygen uptake; T_{lim}, time to exhaustion; vVO_{2max}, velocity associated to VO_{2max}; MVC3, peak maximum voluntary contraction in 3 s; MVC30, area under the peak 30 s maximal voluntary contraction curve; REPS201RM, repetitions at 20% of 1 repetition maximal load; RSA, repeated sprint ability test to exhaustion; LT4, power output corresponding to 4 mmol blood lactate

1.4 The Repeated Sprint training in Hypoxia (RSH) model

Methodological flaws associated with IHT were recently overcome with studies investigating a new hypoxic training strategy named RSH (Faiss *et al.*, 2013b; Galvin *et al.*, 2013; Puype *et al.*, 2013; Brocherie *et al.*, 2014; Faiss *et al.*, 2014b). Indeed, the major problem related to IHT (compared to similar training in normoxia) is that hypoxia lowers the stimulus for the active musculature because of a lowered oxygen flux and degraded power output (Brosnan *et al.*, 2000; Levine, 2002). By definition, RSH is a training method in hypoxia based on the repetition of short "all-out" efforts (≤30 s) interspersed with short incomplete recoveries. Interestingly, single sprint performance (<10 s) is preserved up to high altitudes (<3800 m) (Bowtell *et al.*, 2013) and fatigue resistance is then decreased when sprints are repeated with large decrement in mechanical work at the maximal effort intensity (Balsom *et al.*, 1994; Smith & Billaut, 2012). RSH therefore substantially differs from IHT with the maximal intensity of the training stimulus forcing the utmost recruitment of fast-twitch fibers (FT) required during all-out efforts. Adding a hypoxic stimulus (i.e. reducing systemic O_2 availability) to this type of training stimulus may not impair the maximal performance being essentially anaerobic but positively stimulate HIF-mediated adaptive mechanisms at the muscular level.

Repeated sprint training in normoxia (RSN) (i.e. traditional repeated sprint training) is very commonly used in team or racket sports. Overall performance during a repeated-sprint ability test (RSA) was shown for example to predict total sprint distance during a professional soccer match (Rampinini *et al.*, 2007). Thus, improving RSA performance may lead to a greater team-sport related global performance (Bishop *et al.*, 2011). Practically, RSA performance will highly depend on i) the ability to maintain energy supply (e.g., phosphocreatine (PCr) resynthesis), ii) metabolite accumulation (e.g., Pi and H^+) and iii) neural factors (e.g., neural drive and muscle activation) (Girard *et al.*, 2011). Among these factors, PCr resynthesis is a key determinant of RSA performance. Thus, the decrease in PCr stimulates the contribution of the anaerobic glycolotic metabolism at the start of a sprint but then the oxidative pathway is determinant in the

PCr resynthesis rate (Haseler *et al.*, 1999). In this context, accumulation of H^+ (and thus the subsequent recovery in muscle pH) may be secondary to the primordial restoration of the energy supply to produce power during RSA (Mendez-Villanueva *et al.*, 2012). Limiting the O_2 supply - with RSH for instance- could thus lead to an adaptation in the mechanisms ensuring energy and O_2 supply in the tissue working in normoxia. In fact, during an effort in hypoxia, a compensatory vasodilation associated with an increase in blood flow ensures an adjusted O_2 delivery to the muscle and this vasodilation is likely maximal during sprinting in hypoxia since exercise intensity modulates its amplitude (Casey & Joyner, 2012). Additionally, FT, preferentially recruited while sprinting (Hautier *et al.*, 1996) may better adjust to a high energetic demand with a greater fractional O_2 extraction than slow twitch fibers (ST) if oxygen tension falls in the muscle (e.g., in hypoxia) (McDonough *et al.*, 2005).

The latter suggests that RSH could be more beneficial than RSN for subsequent performance improvement due to the particular combination of repeated maximal exercise bouts combined with O_2 deprivation. Interestingly, a performance enhancement was confirmed recently by Puype *et al.* showing that power output corresponding to 4 mmol of lactate during an incremental test improved by 7% after RSH but did not change after RSN (Puype *et al.*, 2013). Similarly, an additional benefit of RSH compared to RSN was recently described in rugby players with a 19% gain in high-intensity intermittent running performance (Galvin *et al.*, 2013). This substantially higher performance improvement is particularly interesting since the test used (Yo-Yo IR1 test (Bangsbo *et al.*, 2008)) correlates well with physical performance and amount of high-intensity running in several team sports such as soccer, basketball, rugby and handball (Galvin *et al.*, 2013). Finally, our research group reported that RSH improved sprinting times in young well-trained football players to a greater extent than RSN (Brocherie *et al.*, 2014).

It is important to note that even though these results seem to confirm the potential of RSH vs. RSN, we should mention that our group published the first results assessing the effects of RSH on subsequent normoxic RSA performance improvement and adaptations at the muscular level. This study is presented in Chapter 8, Article 4.

Our blinded design included a large sample size of 20 subjects in a RSH group, 20 subjects in a RSN group and 10 subjects not training (control group (CON)) because we were well aware of potential methodological flaws (e.g., placebo or nocebo effects) affecting hypoxic training studies (Bergeron *et al.*, 2012). We tested RSA performance on a cycle ergometer before (Pre-) and after (Post-) training to determine putative additional benefits of RSH vs. RSN.

To discuss potential mechanisms behind any difference in performance gains, we assessed muscle oxygenation by using a near-infrared spectroscopy (NIRS) technique and molecular responses by analyzing mRNA expression of HIF-mediated genes from muscular biopsies. In brief, NIRS allows having an insight in O_2 utilization in the muscle by measuring concentrations of hemoglobin saturated in O_2 (oxyhemoglobin concentration, [O_2Hb]) or unsaturated in O_2 (deoxyhemoglobin concentration, [HHb]) and total hemoglobin/myoglobin concentration ([tHb]). The up- or down-regulation of the mRNA expression of genes may reflect both the changes in muscular protein content and training based muscle performance improvements (Egan & Zierath, 2013).

We also considered our initial hypothesis of fiber-type selective mechanisms supporting additional benefits of RSH compared to RSN. In other words, if FT and their maximal recruitment play a key role in adaptive mechanism during RSH, activities or exercises involving a higher proportion of FT could potentially benefit more from RSH. Indeed in cross-country double poling, upper body muscles are largely contributing to power production (Holmberg *et al.*, 2005), and upper arms muscle comprise a high proportion of FT (Klein *et al.*, 2003). We consequently tested the effects of RSH vs. RSN on cross-country double poling performance during a RSA test to confirm our first hypothesis and further explore the mechanisms underlying the projected efficiency of RSH. This original research is presented in Chapter 10, Article 6.

Table 4 summarizes the results of all RSH studies to date complementary to traditional IHT studies mentioned before (Tables 2 & 3).

Author (year)	Subjects	Study design	Groups	Satistically significant results (p<0.05)
Puype *et al.* (2013)	Moderately trained cyclists	18 over 6 wks, cycling, 3000 m (NH). 4-9 sprints of 30 s interspersed with 4.5 min recovery	RSH, N=10 RSN, N=10 CON, N=10	+6% sprint PO, +6% VO_{2max}, +6% 10-min PO, **+7% LT4** +5% sprint PO, +6% VO_{2max}, +6% 10-min PO, NS NS changes
Galvin *et al.* (2013)	Rugby players	12 over 4 wks, treadmill running, 3500 m (NH). 10 sprints of 6 s interspersed with 30 s recovery	RSH, N=15 RSN, N=15	**+33% Yo-Yo Intermittent Recovery Test performance** +14% Yo-Yo Intermittent Recovery Test performance
Faiss *et al.* (2013)	Moderately trained cyclists	8 over 4wks, cycling, 3000 m (NH). 3 x 5 all-out 10 s sprints interspersed with 20 s recovery	RSH, N=20 RSN, N=20 CON, N=10	+6% sprint PO, **+38% completed sprints in RSA test** +7% sprint PO, no change in completed sprints NS changes
Brocherie *et al.* (2014)	Football players	10 over 5wks, running, 2900 m (NH). 5 x 4 all-out 5 s sprints interspersed wth 45 s recovery	RSH, N=8 RSN, N=8	**-4% first sprint time, -4% cumulated sprint time** -2% first sprint time, -2% cumulated sprint time

Table 4 Summary of current research findings for repeated sprint training in hypoxia (RSH) adapted from Faiss *et al.*, 2013a.
Altitude described as either hypobaric hypoxia (HH) or normobaric hypoxia (NH). Significant difference between groups is shown in bold ($P<0.05$). PO, power output; VO_{2max}, maximal oxygen uptake, LT4, power output corresponding to 4 mmol blood lactate

A recent review questioned again the relevance of altitude training for performance enhancement in team-sport athletes (Billaut *et al.*, 2012). Although there was a comprehensive review of the scarce literature, our results allowed us to comment it on two points of importance i) the previously mentioned relative differences between HH and NH, ii) the exercise intensity and work-to-rest ratio during IHT (or RSH for instance) since we believe that these two points are paramount for understanding the possible benefits of hypoxic training in team-sport athlete performance (Chapter 9, Article 5; Millet & Faiss, 2012).

Chapter 2

Summary of experimental results

2. Summary of experimental results

2.1 Larger aerobic performance decrement at altitude in elite vs. recreational ski-mountaineers

With a study design comparing physiological responses to normobaric hypoxia at rest and performance decrement during maximal treadmill running, we highlighted a significantly ($p<0.05$) higher HVR in elite (E) vs. recreational (R) ski-mountaineers (1.58 ± 1.9 vs. 0.23 ± 0.49 $l{\cdot}min^{-1}{\cdot}\%^{-1}{\cdot}kg^{-1}$).

At maximal intensity, SpO_2 was significantly lower ($p<0.01$) in E than in R, both in N (91.1 ± 3.3 vs. $94.3 \pm 2.3\%$) and in H (76.4 ± 5.4 vs. $82.3 \pm 3.5\%$). In both groups, SpO_2 was lower ($p<0.01$) in H. Between N and H, $\dot{V}O_{2max}$ decreased to a greater extent ($p<0.05$) in E than in R (-18% and -12%, $p<0.01$). In E only, $\dot{V}O_{2max}$ decrement was significantly correlated with the SpO_2 decrement ($r=0.74$, $p<0.01$) but also with $\dot{V}O_{2max}$ measured in normoxia ($r=0.64$, $p<0.05$). We concluded that despite a probable better acclimatization to altitude, $\dot{V}O_{2max}$ was more reduced in E than in R ski-mountaineers, confirming previous results observed in lowlander E athletes.

2.2 Conclusive differences between hypobaric and normobaric hypoxia

The aim of the study was to investigate the time course in oxidative stress and NO metabolism at rest and cardio-respiratory responses to moderate exercise during 24 hours at 3000 m in HH vs. NH.

When compared to normoxic values before exposure, during exercise, tidal volume (Fig 7A), and minute ventilation (Fig. 7B) were lower ($p<0.05$) while $P_{ET}CO_2$ (Fig. 7C) higher ($p<0.05$) in HH compared to NH. SpO_2, HR, oxygen consumption, breathing frequency, and end-tidal O_2 pressure showed similar adaptations in HH and NH. At rest, exNO and NOx decreased in HH (-21% and -36% after 24 h respectively, $p<0.05$) but remained stable in NH (Fig. 8A and B). By contrast, advanced oxidation protein products and superoxide dismutase were higher in HH than in NH after 24 h (+295%, $p<0.05$ and +37%, $p<0.01$, respectively). Here we demonstrated for the first time that the different adaptations to an equivalent hypoxic stimulus in HH vs. NH were sustained over 24 hours and associated with impaired NO biodisponibility and exaggerated oxidative stress.

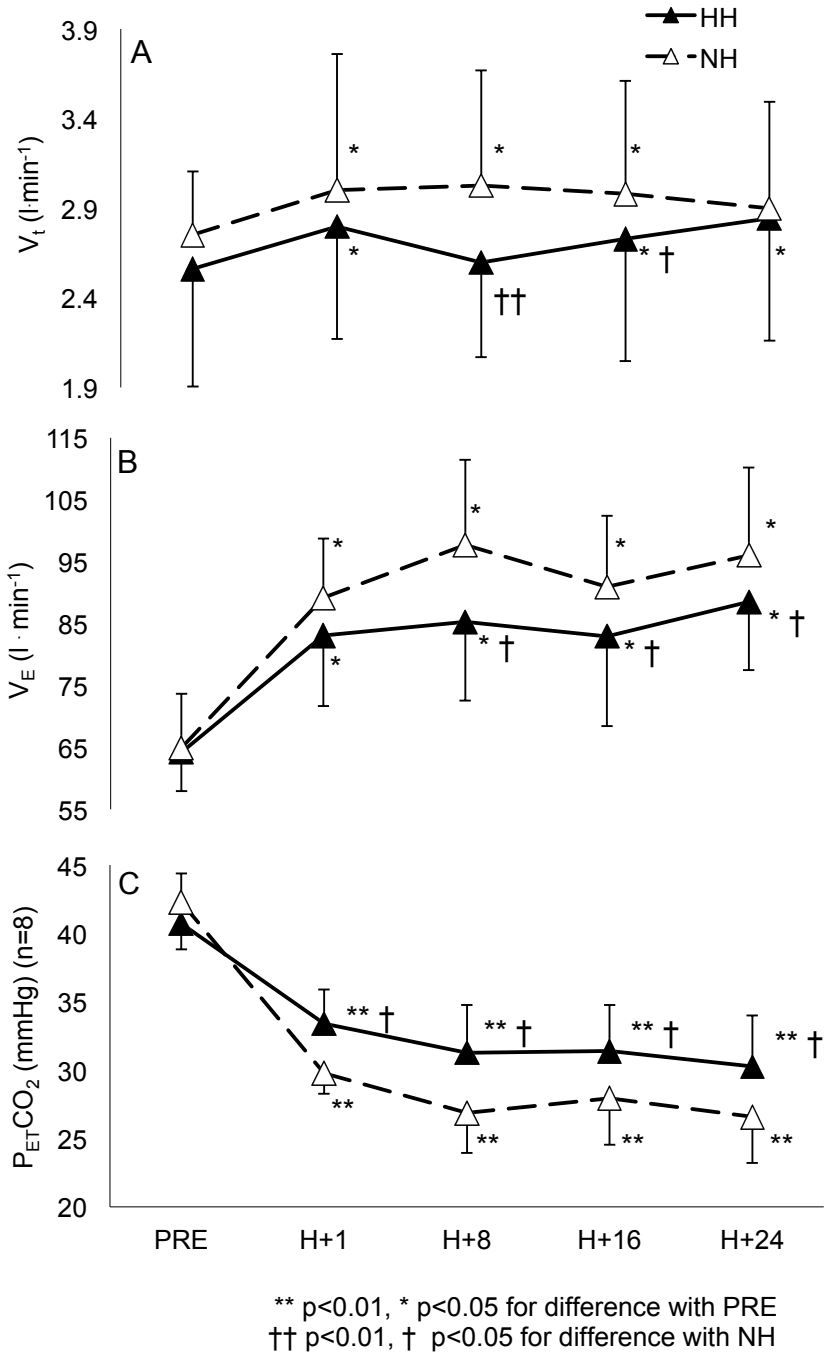

Figure 7 (A) Tidal volume (Vt, l· min^{-1} (BTPS)), (B) minute ventilation (VE, l · min^{-1} (BTPS)) and (C) end-tidal CO_2 pressure ($P_{ET}CO_2$, mmHg) during moderate exercise in hypobaric hypoxia (HH) and normobaric hypoxia (NH).

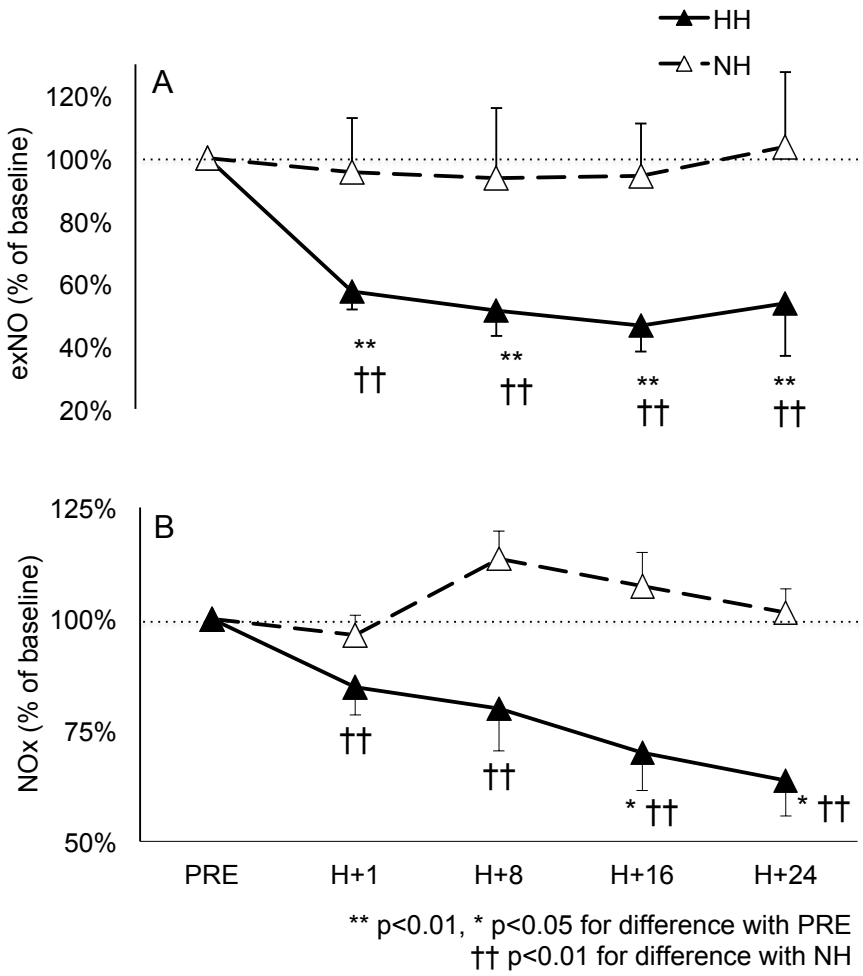

Figure 8 (A) Exhaled nitric oxide (exNO, % of baseline) and (B) Blood nitric oxide metabolites (NOx, % of baseline) at rest during the 24 h in hypobaric hypoxia (HH) and normobaric hypoxia (NH).

2.3 Significant molecular and systemic adaptations after repeated sprint training in hypoxia

We investigated for the first time the additional benefits for repeated sprint performance after repeated sprint training performed in hypoxia (RSH) compared to normoxia. RSH delayed fatigue during a repeated sprint test to exhaustion with larger muscle perfusion variations in active muscles and significant molecular adaptations.

From Pre- to Post-, the average power output of all sprints in RSA was increased ($p<0.01$) to the same extent (+6% vs. +7%, NS) in RSH and in RSN but the number of sprints to exhaustion was increased in RSH (9.4 ± 4.8 vs. 13.0 ± 6.2 sprints, $p<0.01$) but not in RSN (9.3 ± 4.2 vs. 8.9 ± 3.5 sprints, NS) (Fig. 9). mRNA concentrations of HIF-1a (+55%), CA3 (+35%) and monocarboxylate transporter-4 (MCT-4) (+20%) were augmented ($p<0.05$) whereas TFAM (-40%), PGC-1α (-23%) and monocarboxylate transporter-1 (MCT-1) (-36%) were decreased ($p<0.01$) in RSH only (Fig. 10). Further, the successive changes in total hemoglobin variations (Δ[tHb]) during sprints throughout the RSA test increased to a greater extent ($p<0.01$) in RSH (Fig. 11).

Our findings resulted in larger improvement in cycling repeated sprint performance in RSH than in RSN with significant molecular adaptations and larger blood perfusion variations in active muscles.

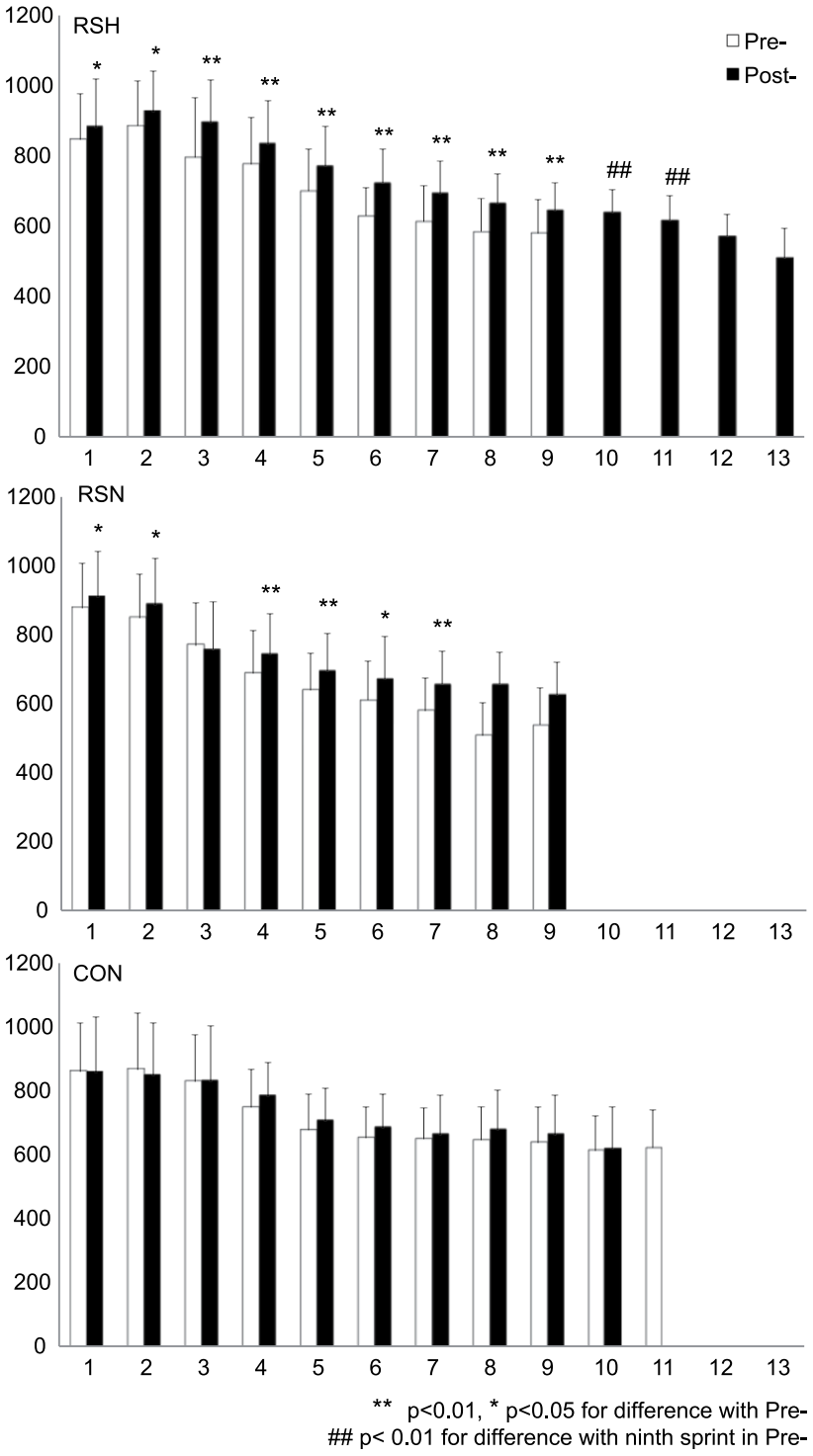

Figure 9 Average power output (W) in successive sprints during the repeated sprint test before (Pre-) and after (Post-) the specific repeated sprint training in hypoxia (RSH), in normoxia (RSN) or in control group (CON).

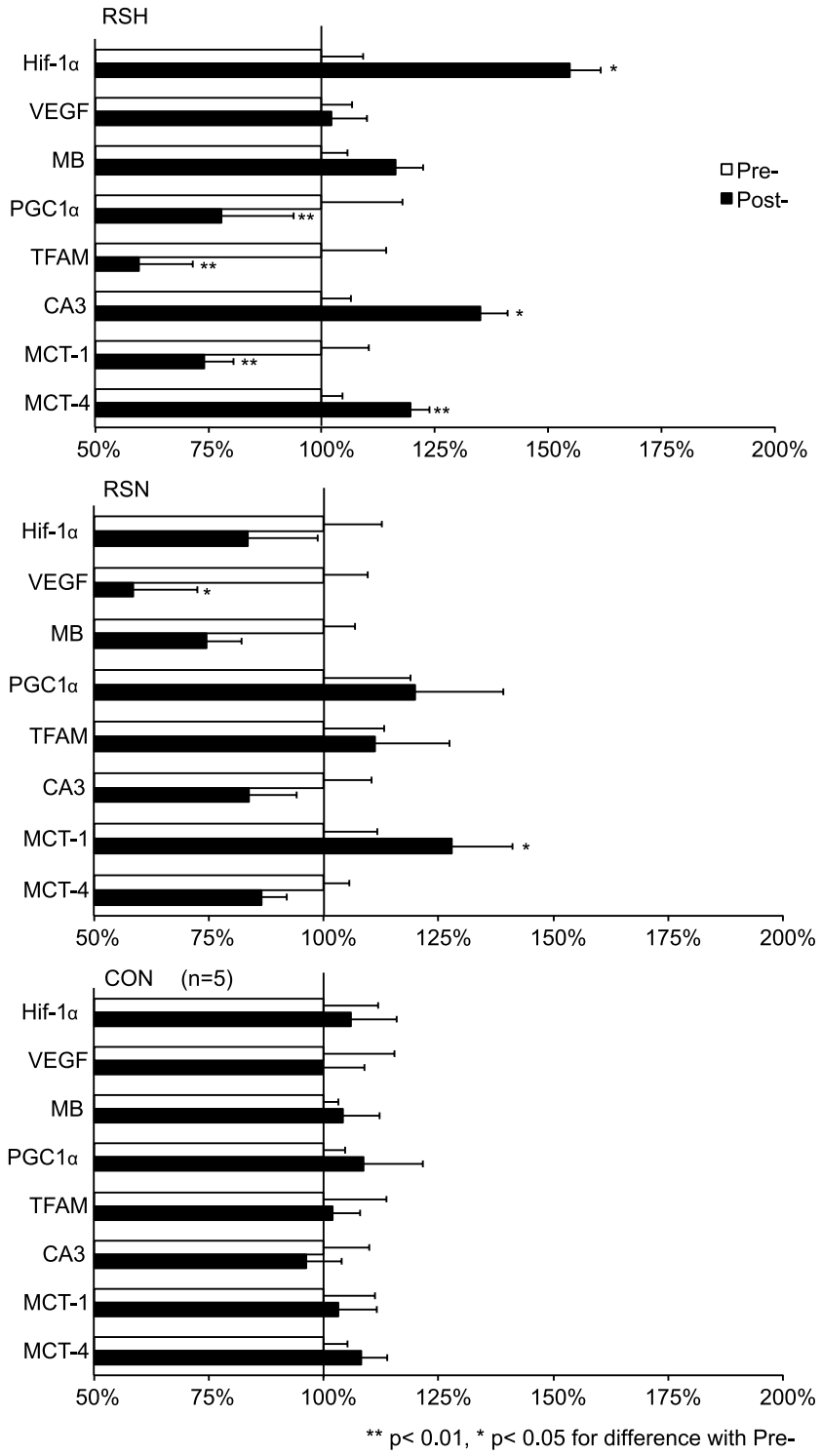

Figure 10 Relative mRNA expression of selected gene transcripts after 4 weeks of specific repeated sprint training before (Pre-) and after (Post-) the specific repeated sprint training in hypoxia (RSH), in normoxia (RSN) or in control group (CON). Open bars represent Pre- values and solid bars Post- values of mRNA concentrations in *vastus lateralis* muscle. Post- values were expressed in % compared to Pre- values (set to 100%). HIF-1a, hypoxia inducible factor-1a; VEGF, vascular endothelial growth factor; MB, myoglobin; PGC1-a, proliferator-activated receptor gamma coactivator-1a; TFAM, mitochondrial transcription factor A; CA3, carbonic anhydrase III; MCT-1, monocarboxylate transporter-1; MCT-4, monocarboxylate transporter-4.

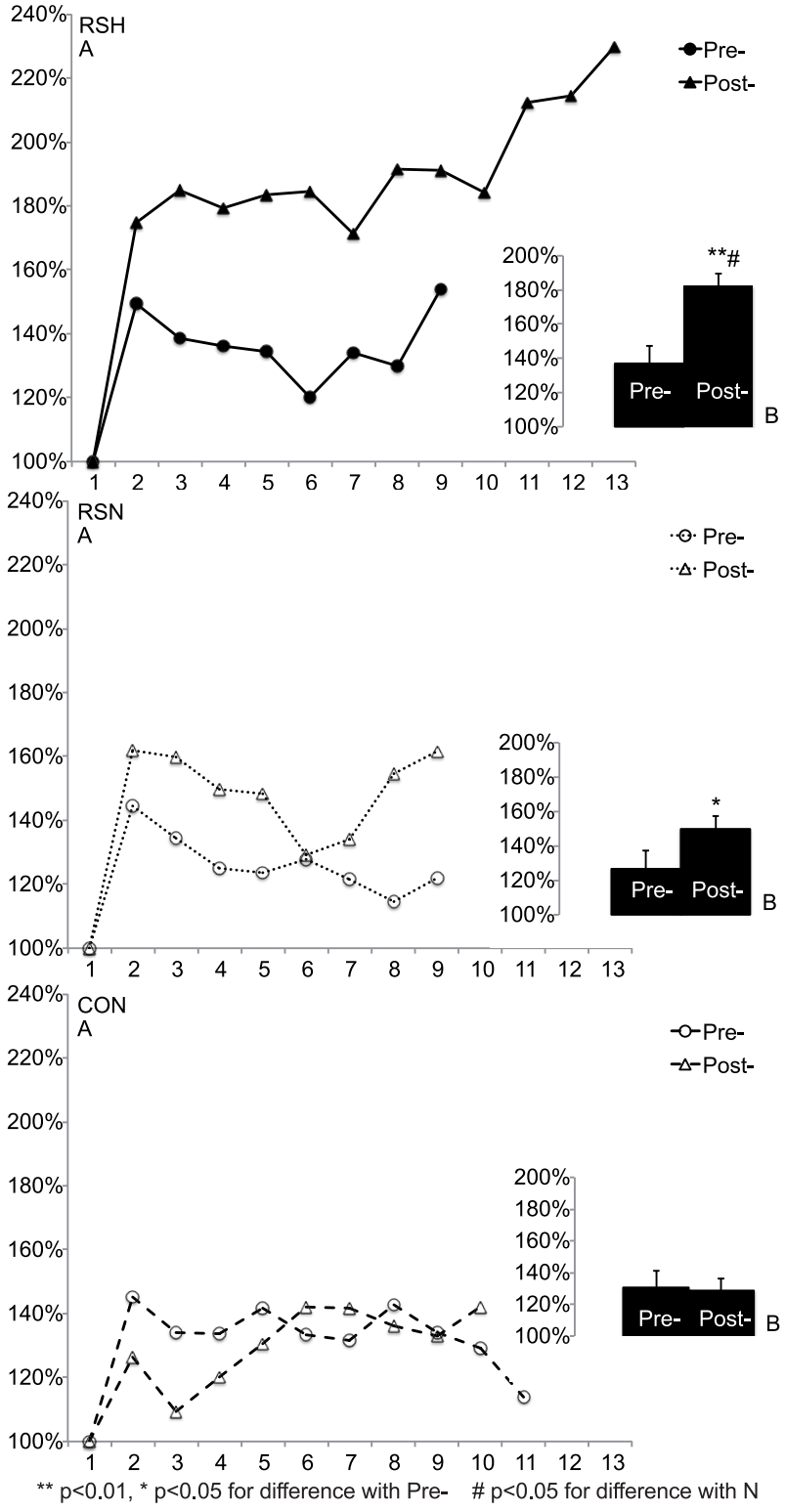

Figure 11 (A) Δ[tHb]: successive changes in total hemoglobin concentrations' amplitude during sprints (expressed in percent compared to the first sprint) measured by near infrared-spectroscopy and (B) $\Delta[tHb]_{av}$: average of all changes during the repeated sprint test to exhaustion before (Pre-) and after (Post-) the specific repeated sprint training in hypoxia (RSH), in normoxia (RSN) or in control group (CON).

2.4 Repeated sprint training in hypoxia in upper-body muscles

Successively, we designed a second research protocol to evaluate the efficiency of RSH in cross-country skiing double poling performance. We demonstrated that RSH was a potent training stimulus to improve sprinting and aerobic performance in cross-country ski double poling: fatigue was delayed during repeated sprints in normoxia after RSH but not after RSN.

From Pre- to Post-, power output in the RSA was increased ($p<0.01$) to the same extent (25% vs. 21%, NS) in RSH and in RSN while the number of sprints performed was enhanced in RSH (10.9 ± 5.2 vs. 17.1 ± 6.8, $p<0.01$) but not in RSN (11.6 ± 5.3 vs. 11.7 ± 4.3) (Fig. 12). After both types of training, average power output during a 3 times 3 min "all-out" team sprint was improved by 10%.

Successive changes in total hemoglobin concentrations' amplitude during sprints (Δ[tHb]) are then displayed in Figure 13. After training, $\Delta[tHb]_{av}$ increased to a greater extent ($F=35.9$, $p<0.001$) in RSH than in RSN (Figure 5B). From Pre- to Post-, $\Delta[HHb]_{av}$ increased significantly in RSH (+225%, $p<0.01$) but decreased in RSN (-27 %, $p<0.01$). There was a significant training group x time interaction ($F=551.8$, $p<0.001$).

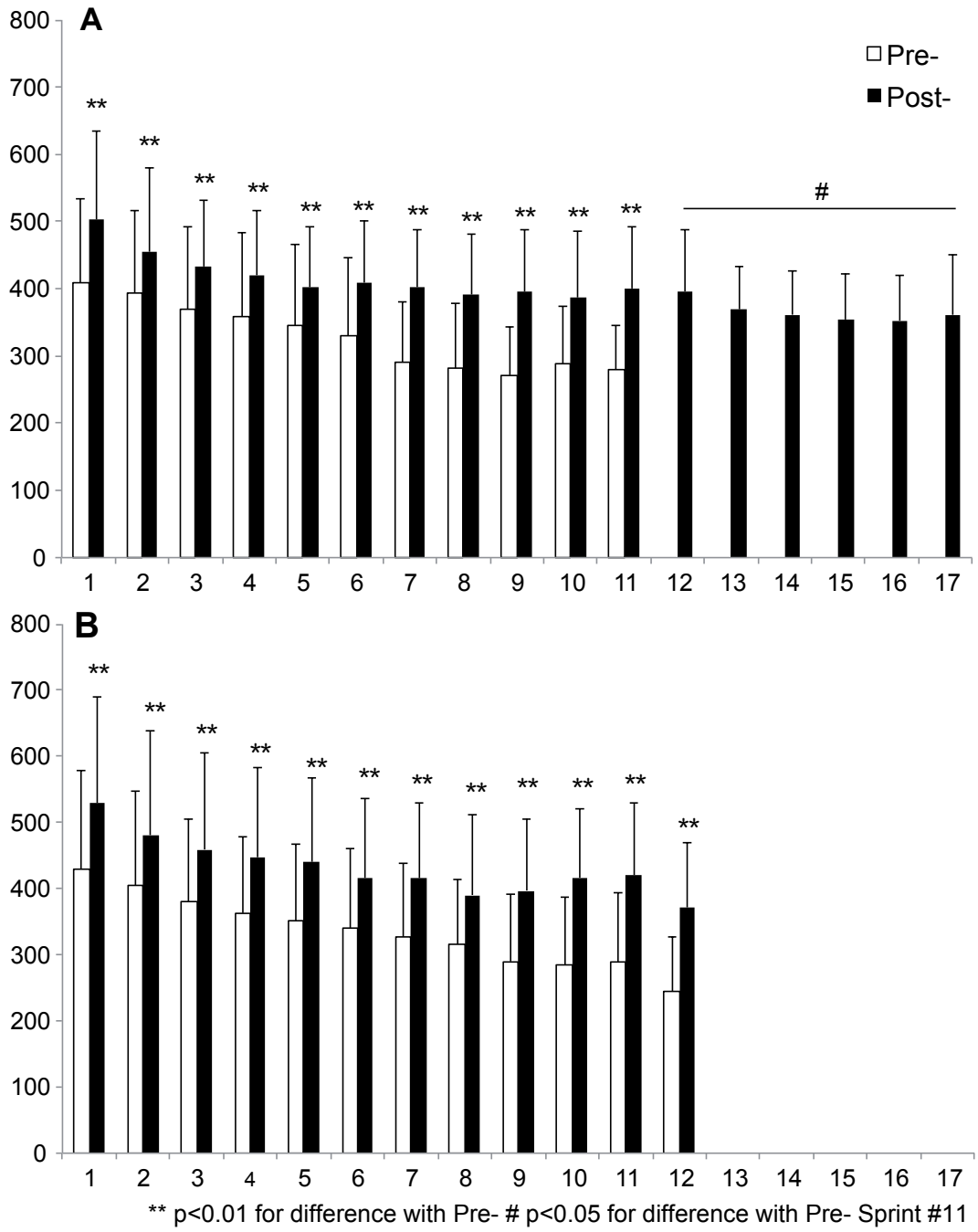

Figure 12 Peak power output (W) in successive sprints during the repeated sprint test before (Pre-) and after (Post-) specific repeated sprint training in hypoxia (RSH, A) or in normoxia (RSN, B).

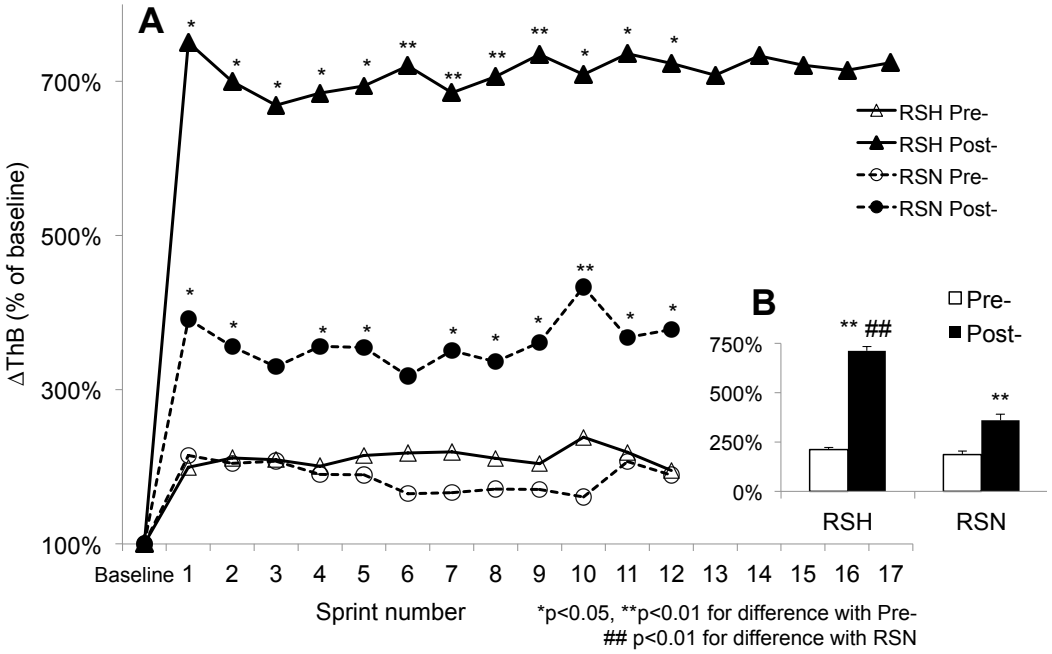

Figure 13 Δ[tHb]: successive changes in total hemoglobin concentrations' amplitude during sprints (expressed in percent compared to the resting baseline set to 100%) measured by near-infrared spectroscopy (A) and $\Delta[tHb]_{av}$: average of all changes during the repeated sprint test to exhaustion (B) before (Pre-) and after (Post-) the specific repeated sprint training in hypoxia (RSH), in normoxia (RSN)

Chapter 3

Discussion and perspectives

3. Discussion and perspectives

The difference between winning and losing in competitive sports is often minuscule. Athletes engage in assiduous training and sacrifices to gain the worthwhile performance improvement that will bring them from the shade to the light. Adding a hypoxic stress to regular training programs is accordingly often thought to make the significant positive difference.

Scientific evidence however tends to support only minor benefits of altitude training strategies unless the employed training paradigms specifically aim at particular adaptive mechanisms supported by correct fundamental theoretical concepts and an appropriate methodology (Lundby *et al.*, 2012).

Our work therefore first focused on physiological responses to hypoxia that may support the rationale for altitude training strategies. With a particular interest for putative different individual responses in physiological adaptations between an elite and recreational population, we highlighted that elite ski-mountaineers had a larger impairment in maximal aerobic performance than their lower level counterparts despite markers of a better acclimatization status (e.g., higher HVR at rest) (Faiss *et al.*, 2014a). We had hypothesized that elite and recreational ski mountaineers would exhibit similar performance decrement at altitude since the effects of acclimatization would compensate the diffusion impairment of aerobic capacity. Our results confirmed that the desaturation level observed both in normoxia (e.g., exercise-induced hypoxemia) and hypoxia is likely the main trigger of aerobic performance decrement at altitude. Although we may only speculate on the underlying mechanisms, our results for such a specific elite population support the relationship between a drop in SpO_2 and a proportional decrement in aerobic performance in hypoxia in accordance with Chapman *et al.* (Chapman *et al.*, 1999). Indeed, diffusion limitations in the lungs impairs pulmonary gas exchanges (Torre-Bueno *et al.*, 1985; Wagner, 1996) and account for the lower SpO_2 observed because of interstitial edemas or too short transit times of the red blood cells through the pulmonary capillaries (due to an

elevated cardiac output) (Dempsey *et al.*, 1982; Wagner *et al.*, 1986; Schaffartzik *et al.*, 1992; Hopkins *et al.*, 1996). Nevertheless, other factors should be mentioned that may explain the lower SpO_2 observed like intra- and extra-pulmonary shunts (Dempsey *et al.*, 1984; Powers *et al.*, 1992) or ventilation-perfusion inequalities (Torre-Bueno *et al.*, 1985; Hammond *et al.*, 1986; Wagner *et al.*, 1986; Schaffartzik *et al.*, 1992; Hopkins *et al.*, 1994). The reported decrease in $\dot{V}O_{2max}$ was negatively correlated with the decrement of $\dot{V}_{Emax}$. In other words, athletes with lower maximal minute ventilation increase in hypoxia compared to normoxia presented lower $\dot{V}O_{2max}$ decrements in hypoxia. This latter result suggests that too large of an increase in minute ventilation in hypoxia may be inefficient for inducing the classicaly associated increases in P_AO_2 and alveolar–arterial pressure gradient and consequently for limiting the drop of SpO_2 and $\dot{V}O_{2max}$. Eventually, besides the remarkable variability in terms of HVR observed among subjects, our study confirmed the rather unpredictable individual effect of altitude in terms of performance decrement during hypoxic exposures (Bartsch & Swenson, 2013).

Second, we questioned the equivalent altitude air model (Conkin & Wessel, 2008) with the evidence for differences between HH and NH in terms of ventilatory responses, fluid balance, AMS symptoms and NO metabolism (Millet *et al.*, 2013b). We concluded that the exercise-induced increase in ventilation throughout 24h of hypoxic exposure was less in HH than NH and associated with a decrease in NO bioavailability in HH (Faiss *et al.*, 2013c).

Indeed, the important new point is that plasma nitric oxide (i.e. nitrate and nitrite) concentration was lower in HH than in NH, suggesting the altered systemic NO bioavailability by HH.

Nitrate and nitrite are physiologically converted back in blood and tissues to form NO and, remarkably, the nitrate–nitrite–NO pathway is gradually activated as oxygen tensions falls (Lundberg *et al.*, 2008). Simultaneously, a decline of O_2 in arterial blood and respiratory cortical centers stimulates the carotid chemoreceptors and triggers HVR (Dempsey & Forster, 1982). Interestingly, lower HVR was observed in rats when NO production was inhibited (Gozal *et al.*, 1996). Similarly, Haxhiu et *al.* found that NO synthase (NOS) blockade attenuated the hypoxia-induced increase in respiratory activity in rats and that the increased respiratory output observed

in HVR resulted from the oxygen deprivation leading to the activation of NO-cGMP dependent pathway in the central nervous system (Haxhiu *et al.*, 1995).

The results of these studies suggest that the lower minute ventilation in HH in our subjects could be due to a decrease in NO bioavailability. NOx was lowered in HH and it could be explained by the increase in oxidative stress in HH. Indeed, the larger oxidative stress revealed by higher plasma concentration of AOPP and SOD in HH might inhibit NO formation and reduce its bioavailability. This phenomenon of NO metabolism down-regulation associated with oxidative stress increase was already known (Pialoux *et al.*, 2011). Mechanistically, reactive oxygen species (ROS) inducing oxidative stress impair the bioavailability of NO (Thomas *et al.*, 2008a). Then, plasma pH was higher in NH compared to HH. Free radicals are likely overproduced in case of a lower pH (Bernheim, 1963; Siesjo, 1985). The increased ventilatory drive in NH (as revealed by the lower $P_{ET}CO_2$ during exercise) may decrease the blood H^+ concentration. The proton being known as a pro-oxidant particle, that respiratory alkalosis in NH may accordingly reduce the ROS production and explain the higher plasma oxidative stress measured in HH.

Undeniably, the new findings we reported may have important potential consequences for adaptations and physical performance at altitude, since different responses in ventilation and oxidative stress may influence benefits to athletes training or living at altitude for performance improvement (Millet *et al.*, 2012b). However, it is still unclear whether the differences we reported in pH, ventilatory responses as well as nitrosative and oxidative stress between HH and NH have a clinical relevance (Millet *et al.*, 2012b). With the recent report showing HH to be more effective than NH as pre-acclimatization treatment in order to minimize AMS symptoms (Fulco *et al.*, 2013), we believe that further clinical investigations are worth of interest in the differences between HH and NH.

Our findings also led us to highlight differences in postural control between HH and NH with a degraded postural stability only in HH that was likely attributed to hypobaria (Degache *et al.*, 2012). In addition, our group currently explores further mechanisms at the origin of differences during a prolonged exposure to either HH (Jungfraujoch, 3470 m) or NH (hypoxic chamber)

with additional measurements (e.g., cerebral activation and oxygenation, muscular oxygenation, fatigue and performance) to have a better insight of putative mechanisms involved in the differences we observed.

In addition, the efficiency of the LHTL paradigm in HH vs. NH is presently being analyzed. Preliminary results underline higher performance gains 21 days after LHTL in HH compared to NH (Saugy *et al.*, 2014).

Athletes and mountaineers should definitely consider the individual adaptive responses to the different HH and NH environments when including altitude training to their preparation.

The second major goal of our work was aimed at innovation in altitude training and development of original training strategies oriented towards intermittent sports performance. With increasing media coverage presenting high profile clubs or national squads in team-sport disciplines including altitude training in their preparation, it was striking that only 3 studies assessed the effects of hypoxic exposure or training in a team-sport population (Morton & Cable, 2005; Hamlin *et al.*, 2008; Manimmanakorn *et al.*, 2013). Although we did not analyze a team sport-specific population, our work contributed to advance hypoxic training methods from more traditional IHT to the innovative RSH method (Faiss *et al.*, 2013a). Only few studies assessed the potential and mechanisms related to RSH (Faiss *et al.*, 2013b; Galvin *et al.*, 2013; Puype *et al.*, 2013; Faiss *et al.*, 2014b; Goods *et al.*, 2014), therefore, there is an urgent need for mechanistic and applied studies to investigate performance changes following RSH.

Nevertheless, the latter studies may provide some clues on the mechanisms underpinning superior performance improvement after RSH rather than RSN. Our results, for instance, suggested an improved vascular conductance in repeated sprints to exhaustion where fast-twitch fibers are likely better utilized (Faiss *et al.*, 2013b). RSH may therefore be potent because it recruits by definition FT fiber maximally. In fact, an increased sympathetic vasoconstrictor activity directed towards skeletal muscle (Hanada *et al.*, 2003) occurs in hypoxia but to a greater extent within FT (glycolytic type II fibers in rat) (Ferguson *et al.*, 2012). But a NO-mediated

compensatory vasodilation prevails over this vasoconstriction to increase blood flow and match O_2 delivery to the working muscle (Casey & Joyner, 2012). Though muscle fatigue attenuates the vasodilatory responsiveness (Jacobs & Segal, 2000), a delayed fatigue during RSA test after RSH could then partly be related with an improved responsiveness of the vascular bed. Interestingly, blood flow and vascular conductance were shown to be augmented mostly in FT after dietary nitrate supplementation (Ferguson *et al.*, 2012).

To further, the proposed mechanism based on the preferential recruitment and modified behavior of FT with an enhanced vasodilatory compensation induced during RSH is appealing as it could also explain why previous IHT studies (Truijens *et al.*, 2003; Roels *et al.*, 2007; Lundby *et al.*, 2012) with lower exercise intensities failed to demonstrate additional benefits to endurance performance when compared to similar normoxic training. If FT fibers recruitment is not sufficient (like in the latter studies), hyoxic training may not trigger (or only partially) the improvement in muscle blood perfusion observed both in upper- and lower-body muscles after RSH (Faiss *et al.*, 2013b; Faiss *et al.*, 2014b). Conclusively, fatigue could potentially be delayed after RSH through FT working with less anaerobic energy dependence.

Furthermore, modifications at the molecular level support a shift towards an improved anaerobic glycolytic activity following RSH only, in parallel to the increased blood perfusion and potentially better waste metabolites removal. Besides the expected upregulation of HIF-1α, we observed a decrease in mRNA expression of genes implicated in mitochondrial biogenesis such TFAM and PGC-1a while LDH concentration was increased. Moreover, citrate synthase activity was not different between RSH and RSN suggesting that oxidative capacity was not different. Our results therefore indicated a shift from aerobic to anaerobic glycolytic activity in the muscle not in line with the previously suggested enhanced oxidative capacity after IHT (Vogt *et al.*, 2001; Zoll *et al.*, 2006; He *et al.*, 2012). In addition, we observed an upregulation of MCT-4 and a downregulation of MCT-1 in RSH. MCT-4 is predominantly expressed in FT where it mediates lactic acid efflux (Ullah *et al.*, 2006) from the cells whereas MCT-1 rather supplies lactate to the cells for aerobic energy production (Dimmer *et al.*, 2000). Again, as MCT-4 (but not MCT-1) is upregulated by

hypoxia (Ullah *et al.*, 2006), our results attributed the delayed fatigue after RSH mainly to modified FT behavior and improved anaerobic glycolysis.

Interestingly, Puype *et al.* showed that the phosphofructokinase activity was markedly increased (+59%), quite likely reflecting an upregulation of muscle glycolytic capacity only after RSH (Puype *et al.*, 2013).

Then, maintenance of power production during sustained RSA performance may be associated with the rate of PCr resynthesis. If RSH allows increasing muscle blood perfusion and vascular conductance as mentioned above, it may in turn also elevate locally microvascular PO_2 that is known to reduce PCr breakdown and speed up PCr resynthesis (Haseler *et al.*, 1999). Since hypoxia *per se* was shown to modulate PCr recovery (Holliss *et al.*, 2013), RSH may accordingly optimally delay fatigue during RSA being by definition the combination of repeated sprint efforts and hypoxia. In addition, the exercise-to-rest ratio during high-intensity or maximal exercise likely modifies the energetic contribution of glycolysis (Balsom *et al.*, 1992; Tabata *et al.*, 1997). Since it substantially varies between activities (e.g., in team or racket sports), exercise-to-rest is thought to be a key component associated to the relative putative effect of RSH in sports and should be further investigated. Measuring PCr recovery kinetics by ^{31}P magnetic resonance spectroscopy on an isolated muscle during repeated bouts of maximal efforts with incomplete recoveries would thus allow a direct insight to the mechanisms potentially influenced by RSH *in vivo*. Although this remains to be investigated, it could strengthen the RSH as a promising training strategy influencing directly team-sport performance and supporting indirectly performance optimization in other sports. RSH efficiency is likely to induce fiber-type selective and intensity dependent adaptations at the muscular level and therefore triggers different mechanisms than those usually expected with traditional IHT (e.g., modification of the oxidative capacity or buffer capacity).

Our review of the IHT studies to date indicated that the "IHT" acronym refers to numerous training methods in hypoxia from continuous low-intensity to very long exercise *versus* interval training in hypoxia (Faiss *et al.*, 2013a; Millet *et al.*, 2013a).

Hence, in the literature of altitude training strategies, more clarity may be needed to meet the athletes' and trainers' expectations that may substantially differ depending on their performance goals. Our work therefore finally allowed to propose an updated classification of the training methods currently available (Fig. 14) (Millet *et al.*, 2013a).

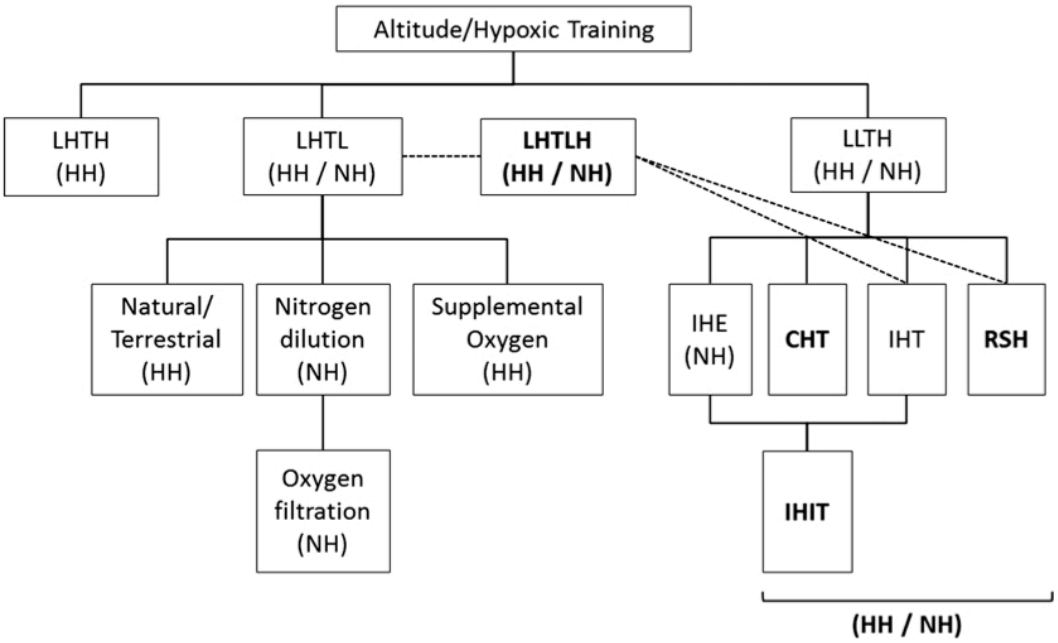

Figure 14 Updated panorama of the contemporary hypoxic methods currently available. From Millet *et al.*, 2013a. LHTH, live high–train high; LHTL, live high–train low; LHTLH, live high–train low and high; LLTH, live low–train high; IHE, intermittent hypoxic exposure; CHT, continuous hypoxic training; IHT, interval hypoxic training; RSH, repeated sprint training in hypoxia; IHIT, IHE during interval-training; NH, normobaric hypoxia; HH, hypobaric hypoxia.

The new proposed classification clearly distinguishes LLTH methods depending on the mechanisms thought to alter performance gains: increased oxidative capacity for continuous hypoxic training (CHT), buffering capacity for IHT or modified behavior of FT for enhanced O_2 utilization (RSH).

Moreover LHTL and LHTH may enhance performance in some (but not all) endurance athletes (Lundby *et al.*, 2012). Resulting sea-level performance improvements may differ when done in HH or NH (Saugy *et al.*, 2014) and this consequently remains to be further investigated in that context. If the difference between "natural/terrestrial" and simulated altitude was mentioned for the LHTL/LHTH strategies in the initial classification of altitude training methods (Fig. 1)

(Wilber, 2007) this distinction was not made for the LLTH methods. With the increasing evidence for different physiological responses to HH and NH our work contributed to support (Millet *et al.*, 2012b, a; Faiss *et al.*, 2013c; Millet *et al.*, 2013b), we believe its paramount to consider these differences in designing or analyzing hypoxic training related data.

It is still safe to conclude that IHT does not enhance sea-level performance in endurance athletes further than the same training in normoxia (Lundby *et al.*, 2012). The role of exercise intensity *per se* in the training induced responses in hypoxia should now be recognized and RSH may indeed potentiate the effects of adding a hypoxic stress to a training stimulus by challenging at most the adaptive mechanisms of the tissue working in hypoxia to promote subsequent performance gains in normoxia. RSH may irrefutably be considered as a promising hypoxic training strategy to enhance anaerobic as well as team sport performance.

In conclusion, our work intended to bring innovations in contemporary altitude training. We first highlighted conclusive differences between HH and NH, so that the type of hypoxia (hypobaric or normobaric) may now systematically be reported and taken into consideration in the analysis and preparation of altitude training or prolonged hypoxic exposure. Secondly, we developed and investigated a new and very innovative form of training with the promising "repeated sprint training in hypoxia" method. This model defied traditional altitude training strategies by hypothesizing that the benefits of exercise training and those of adding a hypoxic environment may not cumulate unless exercise intensity during training is maximal in order to challenge muscular adaptive mechanisms. Overall, our experimental results supported this hypothesis and enabled us to suggest causal mechanisms with fiber-type selective optimization of O_2 delivery to the working muscles. Nonetheless there is only limited evidence for the benefits of RSH in team or racket sports. Our findings however legitimize further well-designed controlled and blinded studies. We are for instance using recent technological advances with mobile inflatable hypoxic marquees to investigate RSH directly in team sport activities (Girard *et al.*, 2013b). Our investigations are now also exploring the potentially paramount role of the exercise-to-rest ratio during RSH. The implementation of RSH in professional athletes (e.g., cyclists of the IAM

professional cycling team; Wales's rugby team, Orlando Magic NBA Basketball team) with positive feedbacks from trainers and athletes embodies the potential of this original and innovative method. Then, with regards to the strong metabolic adaptive responses at the muscular level, our work undeniably leads to clinical implications and therapeutical opportunities with RSH if the underlying mechanisms can be better comprehended.

In future perspectives, adding RSH to traditional LHTL models or performing RSH in HH may later on enable us to further materialize the benefits of that method.

Finally, we hope to substantiate and validate our innovations in altitude training with our continued exploration and description of the putative mechanisms behind additional performance benefits of RSH.

Chapter 4

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4. References

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Chapter 5

Article 1 - Responses to exercise in normobaric hypoxia: comparison between elite and recreational ski-mountaineers.

5. Article 1 - Responses to exercise in normobaric hypoxia: comparison between elite and recreational ski-mountaineers.

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Abstract

Purpose Hypoxia is known to reduce maximal oxygen uptake ($\dot{V}O_{2max}$) more in trained than in untrained subjects in several lowland sports. Ski mountaineering is practiced mainly at altitude. So elite ski-mountaineers spend significantly longer training duration at altitude than their lower level counterparts. Since acclimatization in hypobaric hypoxia is effective, we hypothesized that elite would exhibit a similar $\dot{V}O_{2max}$ decrement in hypoxia than recreational ski-mountaineers.

Methods Eleven elite (E, Swiss national team) and twelve recreational (R) ski-mountaineers completed an incremental treadmill test to exhaustion in normobaric hypoxia (H, 3000 m, F_IO_2 14.6 ± 0.1%) and in normoxia (N, 485m, F_IO_2 20.9 ± 0.0%). Pulse oxygen saturation in blood (SpO_2), $\dot{V}O_{2max}$, minute ventilation ($\dot{V}_E$) and heart rate (HR) were recorded.

Results At rest, hypoxic ventilatory response was higher ($p<0.05$) in E than in R (1.58 ± 1.9 vs. 0.23 ± 0.49 $l \cdot min^{-1} \cdot \%^{-1} \cdot kg^{-1}$).

At maximal intensity, SpO_2 was significantly lower ($p<0.01$) in E than in R, both in N (91.1 ± 3.3 vs. 94.3 ± 2.3%) and in H (76.4 ± 5.4 vs. 82.3 ± 3.5%). In both groups, SpO_2 was lower ($p<0.01$) in H. Between N and H, $\dot{V}O_{2max}$ decreased to a greater extent ($p<0.05$) in E than in R (-18% and -12%, $p<0.01$). In E only, $\dot{V}O_{2max}$ decrement was significantly correlated with the SpO_2 decrement ($r=0.74$, $p<0.01$) but also with $\dot{V}O_{2max}$ measured in normoxia ($r=0.64$, $p<0.05$).

Conclusion Despite a probable better acclimatization to altitude, $\dot{V}O_{2max}$ was more reduced in E than in R ski-mountaineers, confirming previous results observed in lowlander E athletes.

Introduction

Since 2002 and the first World championships of ski mountaineering, the number of competitions and the level of the elite (E) athletes have constantly increased. At a competitive level, it requires a high degree of physical fitness due to the important cardiopulmonary strain of the effort with more than 20% of the race time spent near maximal intensity[1]. Due to the necessary equipment, energy cost of ski-mountaineering was shown to be higher compared to walking or snowshoeing[2] and, more interestingly, racing performance was shown to be correlated with maximal oxygen uptake ($\dot{V}O_{2max}$)[3]. Besides, ski mountaineering requires a mountainous and snowy environment; it is therefore usually practiced at an altitude over 2000 m. Numerous studies have reported a decrease in performance and $\dot{V}O_{2max}$ with altitude[4-13]. In hypoxia, O_2 diffusion is limited in the lung with the consequence of a reduced $\dot{V}O_{2max}$[14]. In addition, a strong relationship between $\dot{V}O_{2max}$ in normoxic conditions and its decline in hypoxic conditions has been described[15,16]. More recently, a linear decrease in $\dot{V}O_{2max}$ was shown in endurance-trained athletes when exposed to altitudes increasing from 300 to 2800 m[16]. For instance, in that study, each 1000 m step was associated with about 6.3% decrease in performance (ranging from 4.6% to 7.5%), close to the 7.7% decrease per 1000 m the same authors calculated from other existing studies[16]. Accordingly ski mountaineers competing at 3000 m could then expect a decrement of their aerobic performance or $\dot{V}O_{2max}$ of approximately 22-25%. However, this latter variable presents a great individual variability, particularly among elite level athletes[17]. Interestingly, a more important decrement of absolute $\dot{V}O_{2max}$ was observed in elite versus non-elite athletes[7,8,18] or in endurance trained versus sedentary subjects[4,19]. For instance, Koistinen et al observed the largest reduction of $\dot{V}O_{2max}$ in the fittest ice-hockey players and cross-country skiers[18]. Lawler et al. underlined likewise a more severe impairment of $\dot{V}O_{2max}$ in endurance-trained cyclists, triathletes and distance runners compared to untrained subjects[7]. Such difference was also reported in male[20] and female triathletes[21] exercising at moderate to high altitude.

Mollard et al. reported, for example, a larger decrement of $\dot{V}O_{2max}$ in trained triathletes vs. untrained subjects (-20% vs. -15%, respectively, $p<0.05$) at 3500 m[20]. Interestingly, in that study, the drop of arterial oxygen saturation in (SaO_2) was a strong predictor of the $\dot{V}O_{2max}$ decrement in hypoxia. A relative hypoventilation (i.e. lower increase of ventilation) in response to hypoxia supports the higher sensitivity observed in trained athletes[8,22]. Actually, this putative hypoventilation during exercise[23] combined with a higher limitation of O_2 diffusion in pulmonary capillaries when cardiac output is near maximal[24,25] could explain the higher decrease in SaO_2 during maximal exercise at altitude in trained vs.

untrained athletes but also the individual variability in $\dot{V}O_{2max}$ decrement at altitude[26]. Despite these observations, all previous investigations were conducted on lowlanders (i.e. athletes training mostly at low altitude or sea-level), which suggests that highly trained ski mountaineers would exhibit more severe impairment of their aerobic capacity compared to their recreational counterparts.

However, it is also known that acclimatization to hypoxia optimizes the performance at altitude[27]. Due to the specificity of their discipline, elite ski-mountaineers are thought to be the endurance athletes exposed to the highest "hypoxic dose" (i.e. time spent in hypoxic environment) amongst all sports. For example, an elite athlete can be expected to train for up to 1000 h yearly and for more than 50% of the time above 1500 m (trail running and ski mountaineering). Elite ski-mountaineers could therefore be better acclimatized to hypoxia because of the repeated altitude exposures[28] and finally an important total hypoxic dose[29] that may induce a lower decrease in $\dot{V}O_{2max}$ than in lowland elite athletes. Of interest is that the larger "hypoxic dose" in E is due to training in hypobaric hypoxia and acclimatization to hypobaric hypoxia has been shown recently to be more effective than in normobaric hypoxia[30,31]. Taken together all these results, the $\dot{V}O_{2max}$ of elite endurance ski-mountaineers was postulated to decrease less in acute hypoxia than their recreational counterparts.

The aim of the present study was to compare $\dot{V}O_{2max}$and exercise responses in elite and recreational ski mountaineers in normoxia and in hypoxia. We expected that elite and recreational ski mountaineers would have a similar performance decrement at altitude since better acclimatization would compensate the larger impairment of aerobic capacity found in elite versus non-elite subjects.

Methods

Subjects Eleven elite ski mountaineers (E, 5 males, 6 females) and twelve healthy lowland recreational ski mountaineers (R, 6 males, 6 females) volunteered in the study. Participants in the E group were all member of the Swiss national ski mountaineering team and competing at an international level (7 of them could boast of at least one world cup or world championship victory). Subjects in the R group were recruited among participants of the World most prestigious ski-mountaineering competition, Patrouille des Glaciers (www.pdg.ch) in the Swiss province of Valais. Inclusion criteria were: 1. to be finisher but in the second half of the ranking; 2. to have a $\dot{V}O_{2max}$ not exceeding 50 or 55 $ml \cdot kg^{-1} \cdot min^{-1}$ for females and males, respectively. Subject characteristics are shown in Table 1. Since the aim of our study was to compare E and R athletes, the training and anthropometrical characteristics are representative of each group. Subjects were all non-smokers and not

recently exposed to an altitude above 1600 m for more than 6 hours per day (i.e. no overnight sleep at altitude) during the three weeks preceding the testing procedure. Volunteers signed an informed written consent. The experiment was approved by the local Medical Ethics Committee (CVEM, agreement 007/10, Sion, Switzerland) and performed in accordance with the Declaration of Helsinki.

	Elite		Recreational	
	Males (n=5)	Females (n=6)	Males (n=6)	Females (n=6)
Age (yrs)	28 ± 6	28 ± 10	27 ± 5	27 ± 3
Height (cm)	177 ± 3	169 ± 3	183 ± 4*	167 ± 6
Body mass (kg)	67.6 ± 5.1	58.6 ± 5.5	77.7 ± 8.6*	61.0 ± 5.3
$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	69.3 ± 4.0	57.8 ± 8.8	48.6 ± 4.5**	46.0 ± 3.9**
Body fat (%)	10.1 ± 3.2	20.1 ± 8.1	14.9 ± 7.1	24.7 ± 6.1
Altitude of residence (m)	818 ± 367	837 ± 321	597 ± 154	742 ± 304

Table 1 Subjects' characteristics

$\dot{V}O_{2max}$, maximal oxygen uptake. * $p < 0.05$, ** $p < 0.01$ for difference with E. Means ± SD

Study design Experimental design consisted of two incremental running test to exhaustion on a motorized treadmill (HP Cosmos Mercury, Nussdorf-Traunstein, Germany) performed in a random order either in normoxia (N, 485 m, F_IO_2 20.9 ± 0.0%, T 25 ± 1 °C) or in normobaric hypoxia (H, 3000 m, F_IO_2 14.6 ± 0.1%, T 27 ± 2 °C) in a hypoxic chamber (ATS Altitude, Sydney, Australia) built in our laboratory (Sion, Switzerland). For each subject, tests were performed at the same time of the day and separated by 14 days in order to avoid any acclimatization effect. Recruited subjects were familiar with treadmill running and physiology tests with gas exchange measurements to prevent any learning effect during the protocol. Twenty-four hours prior to each trial, subjects were asked to refrain from any physical training and caffeine intake. A self-reported training diary and a questionnaire were used to estimate training volume and training time spent above 1500 m for eight weeks outside of the competitive season in autumn (October-December). Body fat percentage was determined during the first visit to the laboratory by air-displacement plethysmography (Bod Pod, Concord, USA).

Experimental procedure After 5 min measurement at rest seated, the incremental running test started at a speed of 1.5 $m \cdot s^{-1}$ with further increments of 0.5 $m \cdot s^{-1}$ every 3 min until 4 $m \cdot s^{-1}$

with the slope of the treadmill set at 2%. When 4 $m \cdot s^{-1}$ was reached, only the slope was increased by 2% for every additional step until exhaustion.

Measurements The following measurements were performed at rest and maximal intensities in normoxic and hypoxic conditions:

Gas exchanges Subjects were equipped with an oro-nasal mask (VmaskTM, 7500 series, Hans Rudolph Inc., Shawnee, KS; deadspace 41 ml) to measure gas exchange with an indirect calorimeter (Metamax 3B, Cortex, Leipzig, Germany)[32] calibrated according to manufacturers' recommendations before each measurement. This device measured volume using a bidirectional digital turbine. Oxygen uptake and carbon dioxide production were determined in inspired and expired air successively with an electrochemical cell and an infrared analyser, respectively, from the air drawn through a Nafion® sampling tube attached to the turbine at the output of the mask. Minute ventilation ($\dot{V}_E$) and end-tidal carbon dioxide pressure ($P_{ET}CO_2$) at rest were calculated the average values of the three last minutes of the resting period. Maximal minute ventilation ($\dot{V}_{Emax}$) and $\dot{V}O_{2max}$ were recorded as the highest 30 s averages measured.

Hypoxic ventilatory response (HVR) was calculated at rest according to the following formula as defined in Richalet et al.[33]: Hypoxic ventilatory response (HVR)= (($\dot{V}_{EH}$ - $\dot{V}_{EN}$)/(SpO_{2N} – SpO_{2H})) / BM) x 100 ($l \cdot min^{-1} \cdot \%^{-1} \cdot kg^{-1}$). Where BM stands for body mass (kg) and the H and N indices indicate hypoxic or normoxic condition.

Heart rate was recorded continuously at 1 Hz with a heart rate belt and receiver (Suunto T6D, Vantaa, Finland). Pulse oxygen saturation (SpO_2) was recorded continuously at 1 Hz during the whole test; subjects were equipped before the test with a wrist worn fingertip pulse oximeter (Nonin Wristox 3100 with 8000SM-WO Sensor, Plymouth, USA). The recorded SpO_2 signal was visually checked to exclude putative artefacts and HR measured with the pulse oximeter was controlled and compared to HR measured by the HR monitor.

Data analysis and statistics Data are presented as means (standard deviations). After data were tested for normality (Shapiro-Wilk test) and equality of variance (Fisher-Snedecor F-test), independent two-sample t-tests and paired t-tests were used to compare variables of each group in both conditions. To investigate the group x condition interaction two-way analyses of variance for repeated measures were then performed with all pairwise multiple comparison procedures (Holm-Sidak method). Pearson's product-moment correlation coefficient was used to evaluate the associations between variables. Null hypothesis was rejected at $p<0.05$. All analyses were made using Sigmaplot 11.0 software (Systat Software, CA, USA).

Conflicts of interest We have no conflict of interest or any financial tie to disclose.

Results

Table 1 displays subject's characteristics. Altitude of residence was not different between groups. Weekly training volume was significantly higher in E compared to R (12.2 ± 2.4 h and 4.6 ± 1.6 h, respectively, $p<0.01$). Training was mostly performed at moderate to high altitude in E but not in R (74 ± 19% and 27 ± 23% above 1500 m, respectively, $p<0.01$). The yearly exposure to altitude above 1500 m (including training camps and overnights) was estimated to be of 2285 ± 446 h vs. 589 ± 306 h in E and R, respectively ($p<0.01$).

Table 2 summarizes all variables measured at rest and during incremental tests at maximal intensity.

At rest

In H compared to N, only SpO_2 varied significantly at rest where it was lowered similarly in E and R (-5% and -6%, respectively, $p<0.01$). HVR was significantly higher in E compared to R (1.4 ± 1.9 vs. 0.3 ± 0.6 $l \cdot min^{-1} \cdot \%^{-1} \cdot kg^{-1}$, $p<0.05$)

At maximal intensity

SpO_2 was significantly lower in E compared to R both in N (3.5% lower, $p<0.01$) and in H (7.2% lower, $p<0.01$) where it was significantly decreased (17% and 13% lower for E and R respectively, $p<0.01$). In H compared to N, $\dot{V}O_{2max}$ was significantly lowered in E and R (-18% and -12%, respectively, $p<0.01$) but to a greater extent in E ($F=5.6$, $p=0.02$). In E, the decrement of $\dot{V}O_{2max}$ in H was significantly correlated with the SpO_2 decrement in H ($r=0.74$, $p<0.01$) (Figure 1A) but also with $\dot{V}O_{2max}$ measured in normoxia ($r=0.64$, $p=0.03$) (Figure 1B). In addition, a significant negative correlation was found between the relative decrement of $\dot{V}O_{2max}$ in H and the decrement of maximal minute ventilation ($\dot{V}_{Emax}$) in H ($r=-0.60$, $p=0.049$) (Figure 1C).

		Elite (n=11)		Recreational (n=12)	
		N	H	N	H
Rest	$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	4.8 ± 0.8	4.7 ± 4.7	4.4 ± 0.8	4.6 ± 0.6
	$\dot{V}_E$ ($l \cdot min^{-1}$)	10.4 ± 2.75	10.9 ± 3.4	9.8 ± 2.6	10.7 ± 1.9
	$P_{ET}CO_2$ (mmHg)	34.8 ± 2.6	30.8 ± 3.6##	33.5 ± 2.6	30.9 ± 2.2##
	SpO_2 (%)	99.2 ± 0.9	94.2 ± 3.2##	98.8 ± 1.2	93.1 ± 3.4##
	HR ($b \cdot min^{-1}$)	66 ± 14	66 ± 10	71 ± 12	75 ± 15
Maximal effort	$\dot{V}O_2$max ($ml \cdot kg^{-1} \cdot min^{-1}$)	63.0 ± 9.0	51.8 ± 7.0##	47.3 ± 4.3**	41.8 ± 6.6##**
	$\dot{V}_E$max ($l \cdot min^{-1}$)	141.2 ± 31.7	143.7 ± 27.4	122.7 ± 28.1	128.3 ± 30.5
	SpO_2 (%)	91.1 ± 3.3	76.4 ± 5.3##	94.3 ± 2.3*	82.3 ± 3.6##**
	HRmax ($b \cdot min^{-1}$)	185 ± 13	180 ± 12	193 ± 11	187 ± 11

Table 2 Variables measured in normoxia (N) and hypoxia (H) at rest and during submaximal and maximal efforts in Elite (E) and Recreational (R) ski mountaineers

$\dot{V}O_2$, oxygen uptake ; V_E, minute ventilation ; $P_{ET}CO_2$, end-tidal carbon dioxide pressure; SpO_2, pulse oxygen saturation ; HR, heart rate. Means ± SD

# p<0.05, ## p<0.01 for difference with N * p<0.05, ** p<0.01 for difference with E

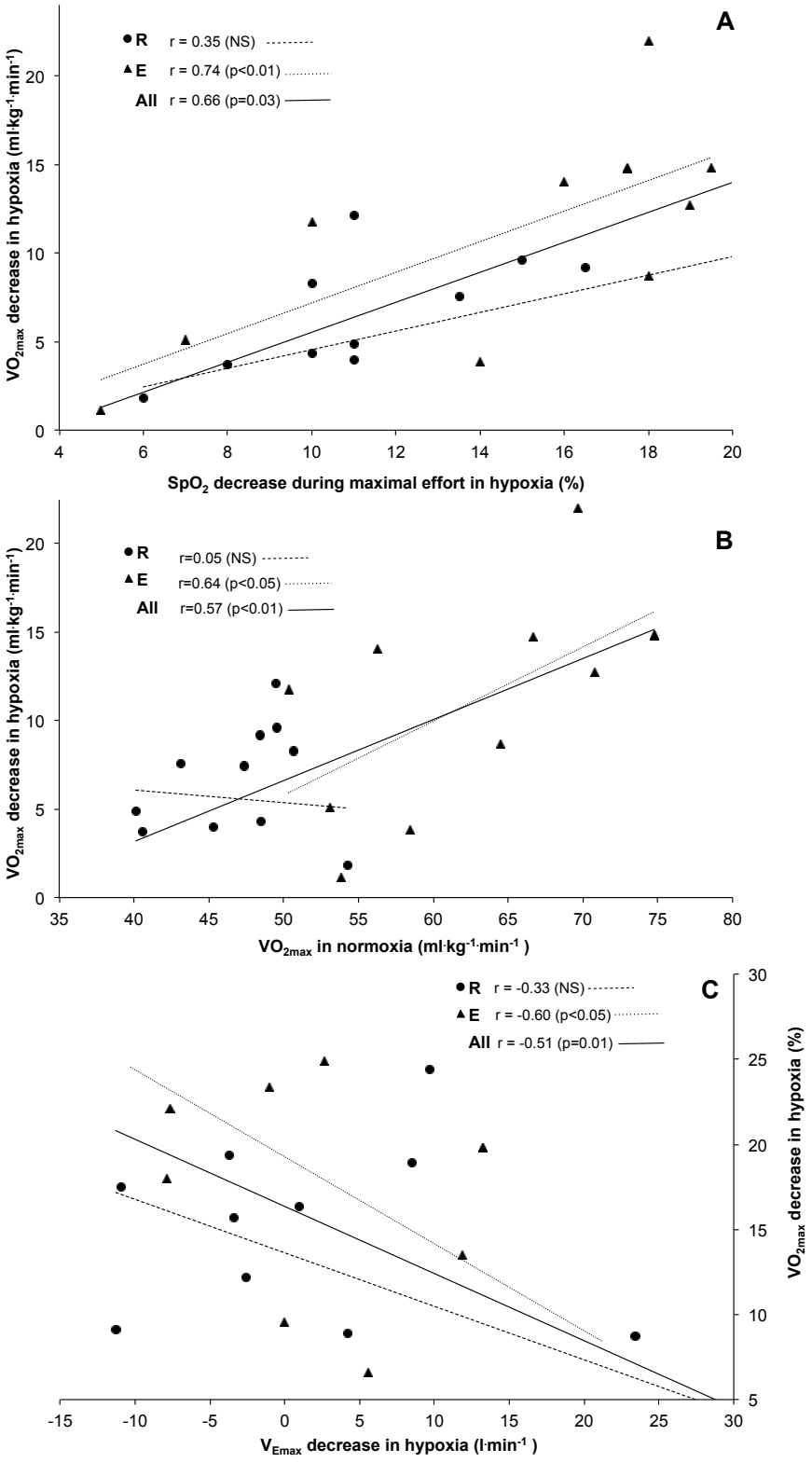

Figure 1 Correlations in elite (E) and recreational (R) ski mountaineers between $\dot{V}O_{2max}$ decrease in hypoxia and (A) SpO2 decrease (B) $\dot{V}O_{2max}$ in normoxia and (C) V_{Emax} decrease in hypoxia

Discussion

The main finding of this study is that, in contrast to our hypothesis, despite a better acclimatization status at rest, elite ski-mountaineers had a larger impairment in maximal aerobic performance than their lower level counterparts; that is $\dot{V}O_{2max}$ decreased both in E and R, but to a greater extent in E compared to R when performing a maximal intensity effort in normobaric hypoxia. In accordance with existing results[7,8,20,34], this decrement was correlated with the level of desaturation (i.e. drop of SpO_2 in hypoxia) but only in E (fig. 1A). Overall, our results confirm that the extent of desaturation may trigger of aerobic performance decrement in altitude. Moreover, it appears that altitude acclimatization may not compensate for such desaturation, strongly influenced by alveolar diffusion limitations, already observed in normoxia in elite endurance athletes. In accordance with earlier results[7,18], we observed that elite athletes developed a larger arterial hypoxemia at maximal intensity than recreational athletes. Interestingly, Chapman *et al.* underlines that the decrease in $\dot{V}O_{2max}$ in well-trained athletes during an effort in hypoxia may also be related to the degree of exercise-induced arterial hypoxemia in normoxia[4]. Actually 7 athletes out of 11 in our E group presented a $SpO_2 \leq 92\%$ (defined as exercise-induced hypoxemia[35]) during the maximal effort in normoxia and interestingly these subjects presented also the highest decrease in $\dot{V}O_{2max}$ (up to -32%) and the lowest SpO_2 during hypoxic exercise. It is well known that in hypoxemic athletes (with SpO_2 on the steeper part of the HbS_pO_2 curve) a drop in PO_2 has a larger influence on the decrease in SpO_2. Although we may only speculate on the underlying mechanisms, our results for this specific elite population (Fig. 1A) support the relationship between a drop in SpO_2 and a proportional decrement in aerobic performance in hypoxia in accordance with Chapman *et al.*[4]. Mechanistically, when exercising in hypoxia (or when arterial oxygen pressure drops below 60 mmHg[24]), oxygen sensing by the peripheral chemo-receptors in the carotid body[36] triggers a pulmonary hyperventilation that improves blood oxygenation[14]. This hyperventilation shifts to the left the O_2 dissociation curve and with a resulting increase in SpO_2 (reflecting SaO_2)[37]. However, during exercise at high altitude, respiratory factors may limit exercise performance capacity[38]. Diffusion limitation in the lungs impairs pulmonary gas exchanges[39,40] because of interstitial edema or too short transit times of the red blood cells through the pulmonary capillaries (due to an elevated cardiac output)[25,41-43]. Since maximal cardiac output sets a limit to $\dot{V}O_{2max}$ [44], such a diffusion limitation may account for the lower SpO_2 values observed in the elite ski mountaineers in our study performing by definition at a higher absolute intensity compared to the recreational ones. Still, the lower SpO_2 in E could also depend on other mechanisms, such as intra- and extra-pulmonary

shunts[45,46] or ventilation-perfusion inequalities[25,40,42,47,48]. Besides, it is striking to note that the relative decrease in $\dot{V}O_{2max}$ was negatively correlated with the decrement of $\dot{V}_{Emax}$ (Fig. 1C). In other words, athletes with lower maximal minute ventilation increase in hypoxia compared to normoxia presented lower $\dot{V}O_{2max}$ decrements in hypoxia. This latter result suggests that a too large increase in minute ventilation in hypoxia might be inefficient for inducing the classicaly associated increases in P_AO_2 and alveolar–arterial pressure gradient *and consequently for limiting the drop of* SpO_2 *and* $\dot{V}O_{2max}$. This is supported by the negative correlation between SpO_2 and $\dot{V}O_{2max}$ decrements (Fig. 1A).

A similar mechanism was however not observed in recreational subjects presenting lesser hypoxemia during maximal efforts and may consequently partly explain the difference between E and R.

We hypothesized that elite and recreational ski mountaineers would exhibit similar performance decrement at altitude since the effects of acclimatization would compensate the diffusion impairment of aerobic capacity. If ventilation increases within seconds upon exposure to hypoxia (acute ventilatory response to hypoxia) the altitude acclimatization process takes several days but then persists for several years when residing in hypoxic environment[49]. This process results in work performance enhancement at altitude[29] due to physiological adaptive mechanisms such as increased ventilation[50]. In this context, HVR measured as a ratio between the ventilation increase and the SpO_2 decrease in hypoxia[51], can be considered as a good index of peripheral chemosensitivity to hypoxia[49], despite the fact that ventilatory responses to normobaric hypoxia is likely different than to hypobaric hypoxia[30,52]. Interestingly, Katayama et al. underlined that HVR is increased after high altitude sojourns or intermittent hypoxic exposures[53] although endurance training during intermittent hypoxia may blunt this increase[54]. For instance, the elite ski mountaineers presented notably higher training volume mostly performed at moderate to high altitudes thus indicating repeated hypoxic exposures. This suggest a long lasting enhanced activation of the chemosensitive carotid bodies[55] and that such athletes could retain some acclimatization benefits upon altitude re-exposure[56] by maintaining a high level of aerobic performance concomitantly to their improved HVR[57]. Consequently, our belief is that elite ski mountaineers would benefit from prior recurrent hypoxic exposures. Our results confirmed that HVR at rest was significantly higher in E compared to R. However, in contrast to our hypothesis, we observed a larger decrease in maximal aerobic performance in E compared to R associated with larger desaturation levels. The latter suggest that a high resting HVR as a characteristic of acclimatization has little effect on the decrement of maximal aerobic performance in hypoxia. The increased cardiac output during exercise in

hypoxia when SpO_2 is lowered[58] may account for shortened alveolar transit time, limitation of oxygen diffusion and hence large decrease in SpO_2. The higher $\dot{V}O_{2max}$ decrease observed in E compared to R may thus essentially depend on this limitation of alveolar O_2 diffusion in hypoxia that directly influences the level of hypoxemia. As a consequence, in elite mountaineers, prior recurring exposures to hypobaric hypoxia (during training for instance) do not seem to overcome the pulmonary limitations. The fact the acclimatization was due to larger exposure to hypobaric hypoxia reinforces this latter point. Acclimatization was shown to be more effective in hypobaric than normobaric hypoxia and the ventilatory adaptations are specific to the hypoxic conditions[31,59]. Elite mountaineers are a unique elite athletes group exposed to extremely large training in hypobaric hypoxia. However, this seems to have rather limited influence to the differences with sub-elite athletes in responses to hypoxia already reported in lowland athletes.

Practical applications

Besides the remarkable variability in terms of hypoxic ventilatory response observed among subjects, our study confirms the rather unpredictable individual effect of altitude in terms of performance decrement during hypoxic exposures[60]. This also precludes the translation of our results in other subjects since a "selection" bias may exist in the participants' panel with ski mountaineers maybe engaging with pleasure in such an activity because they know by own experiences that they tolerate altitude exposures well. In fact, it may also explain the relatively low $\dot{V}O_{2max}$ decrement observed in our study (that is -18% and -12% in E and R, respectively) while a higher drop had been observed in lowland athletes (up to -23%[16]). Nevertheless our study supports a screening of athletes' SpO_2 (or SaO_2) and ventilatory responses during efforts at altitude that may help athletes and coaches preparing competitions at high or rather moderate altitudes depending on their individual sensibility[61].

Conclusions

In conclusion, $\dot{V}O_{2max}$ decrement at altitude and acute responses to hypoxia in elite vs. recreational ski-mountaineers are described for the first time in this study. Our results confirm that the desaturation level observed both in normoxia (e.g., exercise-induced hypoxemia) and in hypoxia is likely the main trigger of aerobic performance decrement at altitude. Despite potential acclimatization due to large dose of training in hypoxia, elite ski mountaineers presented a larger impairment in aerobic performance than their recreational level counterparts; e.g., $\dot{V}O_{2max}$ decreased to a greater extent in E compared to R when performing a maximal intensity effort in normobaric hypoxia.

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Author Contributions

Study design: Faiss, von Orelli and Millet.

Acquisition of data: Faiss and von Orelli.

Analysis and interpretation of data: Faiss, von Orelli and Millet.

Drafting of the manuscript: Faiss and Millet.

Critical revision of the manuscript for important intellectual content: Faiss, von Orelli, Deriaz and Millet.

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Chapter 6

Article 2 - Ventilation, oxidative stress and nitric oxide in hypobaric vs. normobaric hypoxia.

6. Article 2 - Ventilation, oxidative stress and nitric oxide in hypobaric vs. normobaric hypoxia.

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Abstract

Purpose Slight differences in physiological responses and nitric oxide (NO) have been reported at rest between hypobaric hypoxia (HH) and normobaric hypoxia (NH) during short exposure Our study reports nitric oxide and oxidative stress at rest and physiological responses during moderate exercise in hypobaric hypoxia (HH) vs. normobaric hypoxia (NH).

Methods Ten subjects were randomly exposed for 24 h to HH (3000 m, F_IO_2 20.9 %, BP 530 ± 6 mmHg) or to NH (F_IO_2 14.7 %, BP 720 ± 1 mmHg). PRE- and every 8 h during the hypoxic exposures, pulse oxygen saturation (SpO_2), heart rate (HR) and gas exchanges were measured during a 6-min submaximal cycling exercise. At rest, partial pressure of exhaled NO [exNO], blood nitrate and nitrite [NOx], plasma levels of oxidative stress and pH were additionally measured.

Results During exercise, minute ventilation was lower in HH compared to NH (-13% after 8 h, $p<0.05$). End-tidal CO_2 pressure was lower ($p<0.01$) than PRE both in HH and NH but decreased less in HH than in NH (-25% vs. -37%, $p<0.05$).

At rest, exNO and NOx decreased in HH (-46% and -36% after 24 h respectively, $p<0.05$) whilst stable in NH. By contrast, oxidative stress was higher in HH than in NH after 24 h ($p<0.05$). Plasma pH was stable in HH but increased in NH ($p<0.01$). When compared to PRE-normoxic values, SpO_2, HR, oxygen consumption, breathing frequency and end-tidal O_2 pressure showed similar changes in HH and NH.

Conclusion Lower ventilatory responses to a similar hypoxic stimulus during rest and exercise in HH vs. NH were sustained over 24 hours and associated with lower plasma pH, exaggerated oxidative stress and impaired NO bioavailability.

Introduction

Exposure to hypoxia triggers rapid and important adaptive physiological responses. Hypoxic conditions can be defined as a combination of barometric pressure (BP) and an inspired fraction of oxygen (F_IO_2) that results in an inspired pressure of oxygen (P_IO_2) lower than a normoxic value of 150 mmHg (5). The subsequent diminished arterial oxygen pressure (P_aO_2) induces an increased pulmonary ventilation ($\dot{V}_e$) in order to maintain O_2 delivery to the tissues (42). It has been suggested that exposure to hypobaric hypoxia (HH) or normobaric hypoxia (NH) inducing the same P_IO_2 may elicit different physiological responses (5, 18, 25, 28, 36).

Savourey et al. reported greater breathing frequency (f), lower tidal volume (V_t) and $\dot{V}_e$ in HH compared to NH during a 40-min acute hypoxic exposure at rest (36). Similar differences in $\dot{V}_E$ and V_t between HH and NH have been shown during 2 hours at 4750 m (40) or during 10 hours at 4770 m (25). However, the differences in cardio-ventilatory responses to exercise between HH and NH during longer exposure (i.e. more than a few hours) are not known.

Nitric oxide (NO) plays a significant role in the physiological responses to hypoxia. Partial pressure of exhaled NO (exNO) was shown to decrease at high altitude (4, 8) although the putative effect of this decrease on pulmonary vasoconstriction remains debated (8, 9). Accordingly, recent work reported lower levels of NO in exhaled air when measured at high-altitude (HH) compared to an equivalent simulated altitude (NH) (18). The mechanisms underlying the observed differences are not known. Oxidative stress is a potential candidate.

Hypoxic exposure increased oxidative stress (33). Oxidative stress is known i) to decrease the NO bioavailability in the vasculature (39) and ii) to increase the acute ventilatory response to hypoxia (31) after chronic hypoxic exposure. However, to date, differences in oxidative stress between HH and NH have never been studied.

The aim of the present study was therefore to extend prior work demonstrating different ventilatory and nitrosative responses during short duration exposure in HH vs. NH by determining if putative changes in ventilation and NO metabolism over 24 hours at 3000 m in HH vs. NH could be associated with different oxidative stress and pH variations.

Methods

Subjects Ten healthy lowland well-trained men (35 ± 8 years, 179 ± 7 cm, 74 ± 8 kg and $\dot{V}O_2max$ 60.9 ± 6.6 $ml \cdot kg^{-1} \cdot min^{-1}$ range [48-70 $ml \cdot kg^{-1} \cdot min^{-1}$]) active in recreational and endurance sports (2-5 training sessions per week) participated in the study.

Volunteers signed an informed written consent. Subjects were all non-smokers, and neither acclimatized nor recently exposed to altitude. The experiment was approved by the local Medical Ethics Committee (CVEM, agreement 051/09, Sion, Switzerland) and performed in accordance with the Declaration of Helsinki.

Study design Experimental design consisted of two different 24-hour periods of exposure to hypobaric hypoxia (HH), normobaric hypoxia (NH), in a random order with two groups of 3 subjects and one group of 4 subjects. For each subject, experiments were separated by 23 days in average (range 15-32) allowing sufficient elimination of any acclimatization effect.

Measurements at altitude were taken either at 3000 m in a quiet and warmed room in the mountains (HH, F_IO_2 20.9 ± 0.04%, BP 530 ± 6 mmHg, P_IO_2 102 ± 0.3 mmHg, temperature (T) 25 ± 2 °C, Col des Gentianes, Nendaz, Switzerland) or in a hypoxic chamber (ATS Altitude, Sydney, Australia) built in our laboratory (NH, F_IO_2 14.7 ± 0.13%, BP 720 ± 1 mmHg, P_IO_2 99 ± 0.4 mmHg, T 27 ± 2 °C, Sion, Switzerland). Normoxic measurements were completed outside the hypoxic chamber at an altitude of 485 m (F_IO_2 20.9%, BP 720 ± 1 mmHg, P_IO_2 140 ± 1.2 mmHg, T 24 ± 1 °C, Sion, Switzerland). BP and F_IO_2 were controlled every hour using precise electronic oximeter (GOX 100, Greisinger, Regenstauf, Germany) and barometer (GPB 2300, Greisinger, Regenstauf, Germany), respectively.

Prior to any hypoxic trial, participants performed an incremental test with 3 min exercise steps on a cycle ergometer (Ergoline 900, Sensormedics, USA) to determine maximal aerobic power (Pmax) and maximal oxygen consumption ($\dot{V}O_2max$) using a portable indirect calorimeter (Metamax 3B, Cortex, Leipzig, Germany) (41).

Pulse oxygen saturation (SpO2) was recorded continuously at 0.25 Hz at the finger (Wristox 3100^{TM} with 8000SM-WO Sensor, Nonin, Plymouth, MN) and averaged for the last 3 minutes preceding each moderate exercise (in seated position, at rest) and the last 3 minutes of each 6-min cycling bout (at exercise).

Experimental procedure 5 testing sessions were performed in HH and NH:

- PRE: baseline testing in normobaric normoxic condition in the morning before exposure to altitude.
- H+1, H+8, H+16 and H+24: measurement after respectively 1, 8, 16 and 24 h of exposure at altitude.

In HH condition, H+1 measures were performed upon arrival after 1 h of travel by car and cable car between the laboratory (485m) and the building at altitude (3000 m) approximately 2 hours after PRE measurement, corresponding to an exposure of about 45 minutes above 2000 m and 30 minutes at 3000 m. In NH condition, H+1 measures were performed after 60 min in the hypoxic chamber where the corresponding altitude was 2000 m on entering and 3000 m (F_IO_2 14.7%) after 15 min.

The same nitrate- and nitrite-free meals and drinks, standardized for nutritional and caloric content were provided to the subjects and ingested after H+1, 90 min before H+8 and 180 min before H+24. Twenty-four hours prior to each trial, subjects were asked to refrain from any physical training and caffeine intake and reported their food intake (being replicated before the

second trial). They were instructed to follow a low nitrate/nitrite diet for 4 days, avoiding fruits, salads and cured meats as recommended by Wang et al. (43). Subjects started each 24 hours hypoxic exposure exactly at the same time at approximately 1 pm. They were allowed to talk, read, watch television and shortly walk inside the room but did not have any other physical activity than during the tests. Sleep time was asked to be of 8 hours but only within the 10 pm – 9 am timeframe.

Based on previous research (27) we estimated that the prevalence of acute mountain sickness (AMS) would be relatively low (less than 13%), at the altitude of the experiment (3000 m) with our subjects exposed effortless to each hypoxic condition and exercising moderately only during 4 x 6 min over 24 h. Consequently, we did not include an evaluation of AMS symptoms. However, few subjects (2 out of 10) reported some symptoms of light discomfort (e.g., headache and dizziness) in HH whereas none in NH. Since this difference might be of clinical relevance, future research at this altitude or higher should definitely assess the AMS severity.

Measurements at rest and during a 6 min submaximal cycling exercise in HH or NH condition at PRE, H+1, H+8, H+16 and H+24 were as follows:

***Heart rate and gas* exchanges** Subjects were equipped with an oro-nasal mask (Vmask^{TM}, 7500 series, Hans Rudolph Inc., Shawnee, KS; deadspace 41 ml) to measure gas exchange with the same analyser than during preliminary testing. This device measures volume using a bidirectional digital turbine. Oxygen uptake and carbon dioxide production are then determined in inspired and expired air successively with an electrochemical cell and an infrared analyser, respectively, from the air drawn through a Nafion® sampling tube attached to the turbine at the output of the mask. F_iO_2 and F_iCO_2 are continuously measured, in order to account for putative deviation in ambient conditions. Distorted O_2 and CO_2 measurement for 2 subjects were excluded due to defective calibration of the gas sensor prior to measurement. Heart rate (HR) was recorded (RS800, Polar Electro, Finland). After a 6 min seated measurement period in a quiet environment, subjects started pedaling for 6 min on the subject-adjusted bicycle ergometer at a workload equal to 50% of their previously determined Pmax. Parameters at exercise were calculated as the average of the last 3 min of the 6 min cycling bout. At the 5th minute of exercise, the rate of perceived exertion (RPE) was evaluated using a 6-20 Borg scale.

Blood pressure Blood pressure was measured at rest seated using an automatic device (BP A100, Microlife AG, Wildnau, Switzerland). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were manually reported.

Biochemical analyses

Exhaled Nitric Oxide (exNO) Fraction of NO in the exhaled air was measured in standing position with a handheld electrochemical analyzer (NIOX MINO®, Aerocrine, Solna, Sweden) (19) following standard quality criteria (1) and a previously published procedure (8). For

comparison across conditions, fraction of exhaled NO displayed by the device (in ppb) was expressed as partial pressure of exhaled NO (in nmHg) (8). According to recent recommendations for measurements at altitude (17, 20), data obtained in HH at 3000 m were additionally corrected by applying a correction factor to adjust for the mass flow deviation and the higher sensitivity of the sensor at high altitude (see table 2 in Donnelly et al. (8) and table 1 in Hemmingsson et al. (17)).

Plasma nitric oxide, pH, oxidative stress and enzymatic antioxidants

A 5 ml blood sample was taken at rest from the antecubital vein at PRE, H+1, H+8, H+16 and H+24. After centrifugation, 400 uL aliquots of plasma were immediately frozen and stored at -80 °C until blinded analysis less than 6 month after the experiment in the same laboratory. This warrants the perfect reproducibility of the analytic methods. Plasma total nitric oxide end-products (NOx) (nitrate + nitrite + nitrosothiols) were measured with a chemiluminescence NO analyzer (Sievers 280 NOA, General Electric, Boulder, USA) after reduction of NOx to NO using VCl_3 in hydrochloric acid at 90 °C (3) as previously performed in our laboratory (6).

Plasma pH was measured at 37° C using the laboratory pH-meter inoLab 720 (WTW, Weilheim, Germany). The accuracy of this pH meter is ± 0.004 (according to the manufacturer).

Plasma advanced oxidation protein products (AOPP) were measured according to the semi-automated methods developed by Witko-Sarsat et al (44). The plasma concentrations were determined by spectrophotometry and were calibrated with a chloramine-T solution that absorbs at 340 nm in the presence of potassium iodide. The absorbance of the reaction was read at 340 nm. AOPP concentrations were expressed as μmol L^{-1} of chloramine-T equivalents. The intra-assay coefficient of variation is 5.4%. The quantitative determination of the Superoxide dismutase (SOD) activity was performed using the method described by Oberley and Spitz (29). SOD activity was determined by the degree of inhibition of the reaction between superoxide radicals, produced by a hypoxanthine - xanthine oxydase system, and nitroblue tetrazolium. The intra-assay coefficient of variation is 5.6%.

Glutathione Peroxidase (GPX) activity was determined by the modified method of Paglia and Valentine (30) as the rate of oxidation of NADPH to $NADP^+$ after addition of glutathione reductase (GR), reduced glutathione (GSH) and NADPH, using H_2O_2 as a substrate. The intra-assay coefficient of variation is 4.6%. Concentrations of plasma malondialdehyde (MDA), as thiobarbituric reactive substances, were determined as previously described (34). The pink chromogen was extracted with *n*-butanol and its absorbance was measured at 532 nm by spectrophotometry using 1,1,3,3-tetraethoxypropan as standard. The intra-assay coefficient of variation is 2.2%.

Concentrations of plasma nitrotyrosine, as end product of protein nitration by $ONOO^-$, were measured by ELISA as previously described (12). The intra-assay coefficient of variation is 6.8%

Our research team routinely performs oxidative stress and antioxidants measurements as previously published (32).

Data analysis and statistics

Data are reported as means and standard deviations. Data were tested for normality (Shapiro-Wilk test) and equality of variance (Fisher-Snedecor F-test). When both conditions were met, a one-way analysis of variance for repeated measures was performed for each condition (HH and NH) for the time effect with all pairwise multiple comparison procedures (Holm-Sidak method). Differences to baseline between HH and NH at the same time were then compared using a paired t-test. When normality or equality of variance were not met, variables were analyzed in each condition using a Friedman test for repeated measures on ranks for the time effect with all pairwise multiple comparison procedures (Bonferroni test). In this case, differences to baseline between NH and HH at identical times were then compared using a Mann-Whitney rank sum test. Null hypothesis was rejected at $p<0.05$. All analyses were made using Sigmaplot 11.0 software (Systat Software, CA, USA).

Results

Maximal aerobic power (Pmax) determined during pre experimental tests was Pmax 354 ± 50 W, ranging from 280 to 420 W.

Ventilatory and cardiovascular parameters At rest, minute ventilation ($\dot{V}_e$) increased significantly in HH only after 1h (+24%, $p<0.05$) and in NH after 1h, 8h and 24h (+29% +45% and +38%, respectively, $p<0.05$). Tidal volume (V_t) increased significantly only in NH after 8h and 24h (+24% and +25%, respectively, $p<0.05$). Eupneic end tidal CO_2 pressure ($P_{ET}CO_2$) decreased immediately after 1h in HH and NH (-13 % and -20%, $p<0.05$, respectively) and remained lower than baseline until 24 h in both conditions. Parameters at the different times at rest are summarized in Table 1.

During moderate exercise, $\dot{V}_e$ and V_t were lower in HH compared to NH after 8h and 16h ($p<0.05$) (Fig. 1 A & B). $P_{ET}CO_2$ was lower ($p<0.01$) than PRE both in HH and NH after 1h until 24h (Fig. 1 C). In addition, $P_{ET}CO_2$ decrease was lower in HH than in NH (-25% vs. -37%, $p<0.05$).

HR during exercise was not significantly different between HH and NH and higher than PRE from H+1 to H+24 ($p<0.05$). Breathing frequency, end tidal O_2 pressure and SpO2 were not significantly different in HH and NH, both at rest and during exercise. Parameters at the different times during moderate exercise are summarized in Table 2.

Nitric oxide During the 24-hour exposure exNO decreased ($p<0.01$) in HH (-43%, -49%, 53% and -46% after 1 h, 8 h, 16 h and 24 h) but remained stable in NH (Fig. 2 A). Compared to NH, exNO was lower in HH after 1 h (-36%, $p<0.01$), 8h (-38%, $p<0.01$), 16h (-46%, $p<0.01$) and 24h (-43%, $p<0.05$).

Similarly, NO_x decreased over 24 h in HH (-37% at 24h, $p<0.01$) whilst stable in NH (+1%, NS). (Fig. 2 B).

Oxidative stress, enzymatic antioxidants and pH During the 24-hour exposure, advanced oxidation protein products (AOPP) were higher ($p<0.05$) in HH (+120% and +260% after 1 h and 24 h) than in NH (+13% and +88% after 1 h and 24 h) (Fig. 3 A). SOD was significantly higher in HH than in NH after 24 h (37%, $p<0.01$) (Fig. 3 B).

Plasma pH was stable in HH but increased in NH ($p<0.01$) where it remained higher than HH ($p<0.01$) until 24 h (Fig. 3 C).

Variable	Condition	PRE	H+1	H+8	H+16	H+24
SpO_2	HH	98 ± 1	93 ± 1**	91 ± 3**	92 ± 2**	93 ± 2**
(%)	NH	97 ± 1	90 ± 3**	91 ± 2**	91 ± 2**	92 ± 1**
SBP	HH	126 ± 8	124 ± 9	124 ± 9	121 ± 9	131 ± 10
(mmHg)	NH	126 ± 10	129 ± 13	123 ± 7	118 ± 9	129 ± 9
DBP	HH	75 ± 9	75 ± 5	78 ± 9	77 ± 7	76 ± 6
(mmHg)	NH	81 ± 8	74 ± 5	77 ± 6	77 ± 7	77 ± 7
HR	HH	57 ± 7	62 ± 8	68 ± 13*	61 ± 10	65 ± 9
(bpm)	NH	57 ± 9	63 ± 10	69 ± 13*	66 ± 7	71 ± 10*
$\dot{V}_e$	HH	11.0 ± 1.8	13.6 ± 1.8*	11.8 ± 1.9#	10.7 ± 1.8†	12.7 ± 2.3#
($l.min^{-1}$)	NH	10.3 ± 1.6	13.3 ± 3.3*	14.9 ± 3.5*	12.2 ± 1.6*	14.2 ± 1.5*
V_t	HH	0.75 ± 0.15	0.88 ± 0.21	0.75 ± 0.21†	0.75 ± 0.23#	0.86 ± 0.25†
($l.min^{-1}$)	NH	0.76 ± 0.18	0.89 ± 0.26	0.94 ± 0.3*	0.84 ± 0.24	0.95 ± 0.23*
f	HH	15.5 ± 3.1	16.8 ± 3.4	16.8 ± 2.7	16.1 ± 3	16.8 ± 3.8
(1/min)	NH	14.3 ± 2.7	15.9 ± 4.2	17.1 ± 4.4	15.8 ± 3.7	16.2 ± 3.8
$P_{ET}O_2$	HH	97.6 ± 4.0	66.4 ±4.1**	61.9 ±6.0**	65.0 ±5.4**	65.6 ±5.5**
(mmHg) (n=8)	NH	98.6 ± 2.8	62.3 ±2.8**	61.6 ±2.2**	62.7 ±2.6**	65.6 ±2.8**
$P_{ET}CO_2$	HH	38.5 ± 3.5	33.4 ± 2.5*#	33.8 ± 2.1*††	33.1 ± 1.3*††	30.8 ± 1.4*††
(mmHg) (n=8)	NH	36.8 ± 1.4	29.4 ± 2.4*	27.5 ± 1.3*	27.9 ± 0.9*	26.5 ± 1.5*
$\dot{V}O_2$	HH	4.6 ± 0.6	6.7 ± 1.2	7.0 ± 1.2*	5.9 ± 0.4*	5.9 ± 1.2
($mlO_2.min^{-1}.kg^{-1}$)	NH	4.5± 0.6	5.4 ± 0.9	6.1 ± 1.2*	5.1+ 0.4	5.4±0.6
(n=8)						
$\dot{V}CO_2$	HH	3.5 ± 0.7	5.2 ± 0.9	4.9 ± 1.0	4.6 ± 0.4	4.4 ± 1.2
($mlO_2.min^{-1}.kg^{-1}$)	NH	3.7± 0.7	4.1 ± 0.8	4.2 ± 0.8	3.9 ± 0.2	4.1 ± 0.6
(n=8)						
RER	HH	0.76 ± 0.14	0.78 ± 0.06	0.71 ± 0.03	0.77 ± 0.02	0.74 ± 0.07
(n=8)	NH	0.82 ± 0.05	0.76 ± 0.06	0.70 ± 0.05*	0.76 ± 0.06	0.76 ± 0.04*
Displayed exNO	HH	25 ± 14	27 ± 14	25 ± 15	22 ± 13	25 ± 15
(ppb)	NH	23 ± 13	22 ± 14	21 ± 11	22 ± 13	23 ± 9
Corrected exNO	HH	16.9 ± 9.8	9.5 ±5.0**††	8.8 ±5.3**††	7.9 ±4.5**††	8.9 ±5.4**††
(nmHg)	NH	15.2 ± 8.5	14.9 ± 9.2	14.1 ± 7.4	14.7 ± 8.6	15.7 ± 8.7
NOx	HH	40.6 ± 25.1	31.6 ±19.6††	28.1 ±18.9††	24.2 ±16.3*††	22.85 ±16.2*††
(uM)	NH	29.6 ± 9.7	27.7 ± 7.3	32.7 ± 9.7	30.2 ± 7.1	28.9 ± 6.9
GPX	HH	100	114 ± 26	85 ± 27	105 ± 43	103 ± 41
(% of baseline)	NH	100	111 ± 30	123 ± 23	107 ± 21	121 ± 33
MDA	HH	100	117 ± 40	103 ± 62	111 ± 56	108 ± 52
(% of baseline)	NH	100	92 ± 36	111 ± 35	116 ± 55	97 ± 51
Nitrotyrosine	HH	100	86 ± 16	77 ± 35	91 ± 20	75 ± 40
(% of baseline)	NH	100	105 ± 26	75 ± 37	98 ± 16	87 ± 25

Table 1 Time course of measured parameters at rest over 24 h in hypobaric hypoxia (HH) and normobaric hypoxia (NH)

SpO_2, pulse oxygen saturation; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; $\dot{V}_e$, minute ventilation (BTPS); V_t, tidal volume; *f*, breathing frequency; $P_{ET}O_2$, end tidal O2 pressure; $P_{ET}CO_2$, end tidal CO2 pressure; $\dot{V}O_2$, relative oxygen uptake; $\dot{V}CO_2$, relative carbon dioxide produced; RER, respiratory exchange ratio; exNO, exhaled nitric oxide; NOx, blood nitric oxide metabolites, GPX, glutathione peroxidase; MDA, malondialdehyde.
n=10 unless otherwise stated
**p<0.01, * p<0.05 for difference with PRE
†† p<0.01 , † p<0.05, # p<0.1 for difference with NH

Variable	Condition	PRE	H+1	H+8	H+16	H+24
HR	HH	116 ± 6	127 ± 7**	134 ± 11**	129 ± 9**	133 ± 8**
(bpm)	NH	117 ± 9	130 ± 6**	138 ± 9**	133 ± 7**	136 ± 6**
f	HH	26.8 ± 7.9	31.0 ± 6.0	34.0 ± 6.9*	32.0 ± 7.7*	32.8 ± 8.4*
(1/min)	NH	24.1 ± 2.9	31.2 ± 6.6*	33.4 ± 6.3*	31.6 ± 6.9*	34.2 ± 6.5*
$P_{ET}O_2$	HH	97.0 ± 3.4	64.9 ± 2.5 **	66.1 ± 4.1**	66.2 ± 2.4**	66.1 ± 4.4**
(mmHg) (n=8)	NH	95.1 ± 3.5	65.0 ± 1.5**	67.0 ± 2.9**	65.9 ± 3.4**	67.8 ± 3.4**
$\dot{V}O_2$	HH	34.0 ± 7.7	37.0 ± 6.3	41.4 ± 5.0§	40.5 ± 5.2*	40.9 ± 5.3§
($mlO_2.min^{-1}.kg^{-1}$)	NH	36.2 ± 5.7	37.4 ± 5.0	39.2 ± 5.6*	38.0 ± 4.4*	39.3 ± 4.3*
(n=8)						
$\dot{V}CO_2$	HH	32.2 ± 7.0	31.1 ± 8.2	35.8 ± 6.2§	35.5 ± 8.0§	35.0 ± 4.9
($mlCO_2.min^{-1}.kg^{-1}$)	NH	33.2 ± 5.8	31.9 ± 5.3	32.3 ± 5.2	32.3 ± 4.2	32.5 ± 3.5
(n=8)						
RER	HH	0.95 ± 0.03	0.83 ± 0.09*	0.86 ± 0.06#	0.87 ± 0.11	0.85 ± 0.03*
(n=8)	NH	0.92 ± 0.04	0.82 ± 0.06*	0.82 ± 0.05*	0.85 ± 0.06*	0.83 ± 0.03*
SpO_2	HH	97.7 ± 0.7	82.6 ± 4.2**	78.6 ± 5.5**	82.1 ± 4.6**	82.4 ± 4.1**
(%)	NH	97.8 ± 0.8	83.1 ± 3.4**	78.1 ± 3.3**	81.5 ± 2.0**	82.6 ± 3.2**
RPE	HH	11 ± 1	13 ± 1*	14 ± 1 *†	13 ± 1*	13 ± 1*
	NH	11 ± 1	13 ± 1*	15 ± 1*	13 ± 1*	13 ± 1*

Table 2 Time course of measured parameters during moderate exercise in hypobaric hypoxia (HH) and normobaric hypoxia (NH)

HR, heart rate; *f*, breathing frequency; $P_{ET}O_2$, end tidal O_2 pressure; $\dot{V}O_2$, relative oxygen uptake; $\dot{V}CO_2$, relative carbon dioxide produced; RER, respiratory exchange ratio; SpO_2, pulse oxygen saturation; RPE, rate of perceived exertion (Borg scale 6-20)
n=10 unless otherwise stated
**p<0.01 , * p<0.05, § p<0.1 for difference with PRE
† p<0.05, # p<0.1 for difference with NH

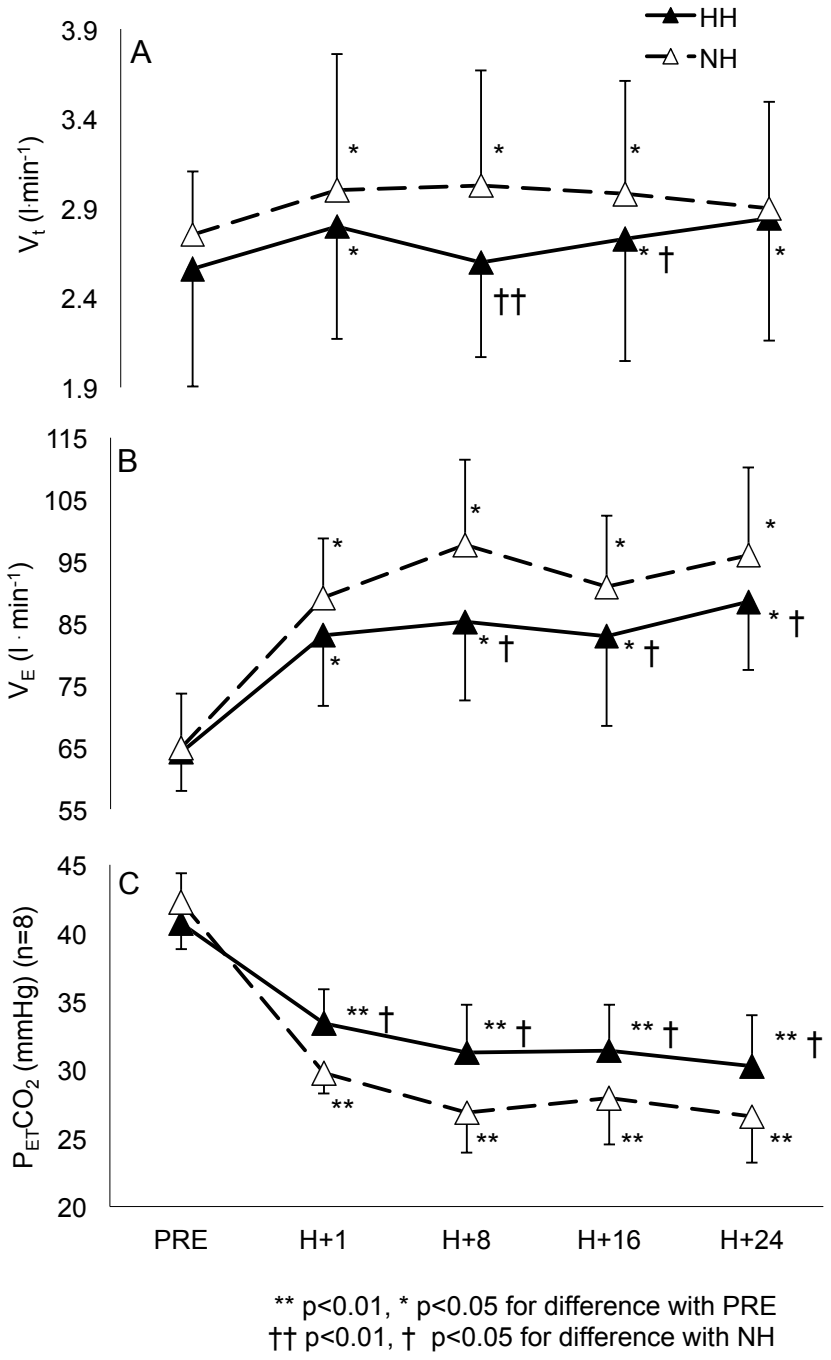

Figure 1 (A) Tidal volume (Vt, l · min^{-1} (BTPS)), (B) minute ventilation (VE, l · min^{-1} (BTPS)) and (C) end-tidal CO_2 pressure ($P_{ET}CO_2$, mmHg) during moderate exercise in hypobaric hypoxia (HH) and normobaric hypoxia (NH). n=10 unless otherwise stated

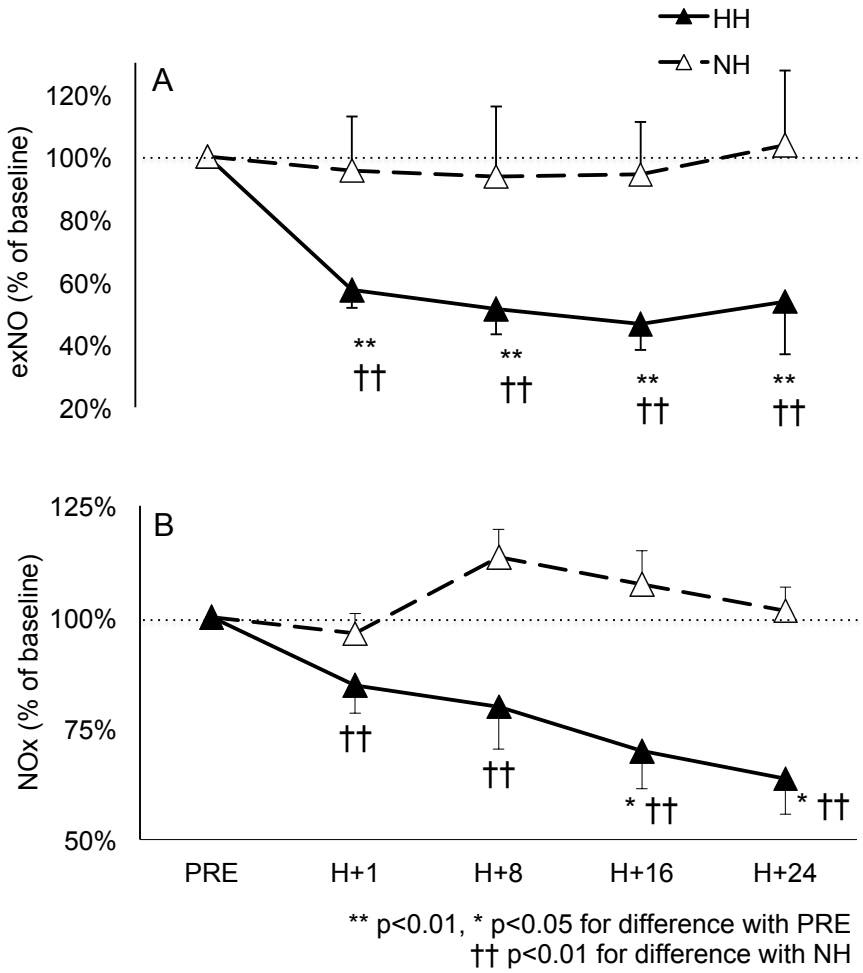

Figure 2 (A) Exhaled nitric oxide (exNO, % of baseline) and (B) Blood nitric oxide metabolites (NOx, % of baseline) at rest during the 24 h in hypobaric hypoxia (HH) and normobaric hypoxia (NH)

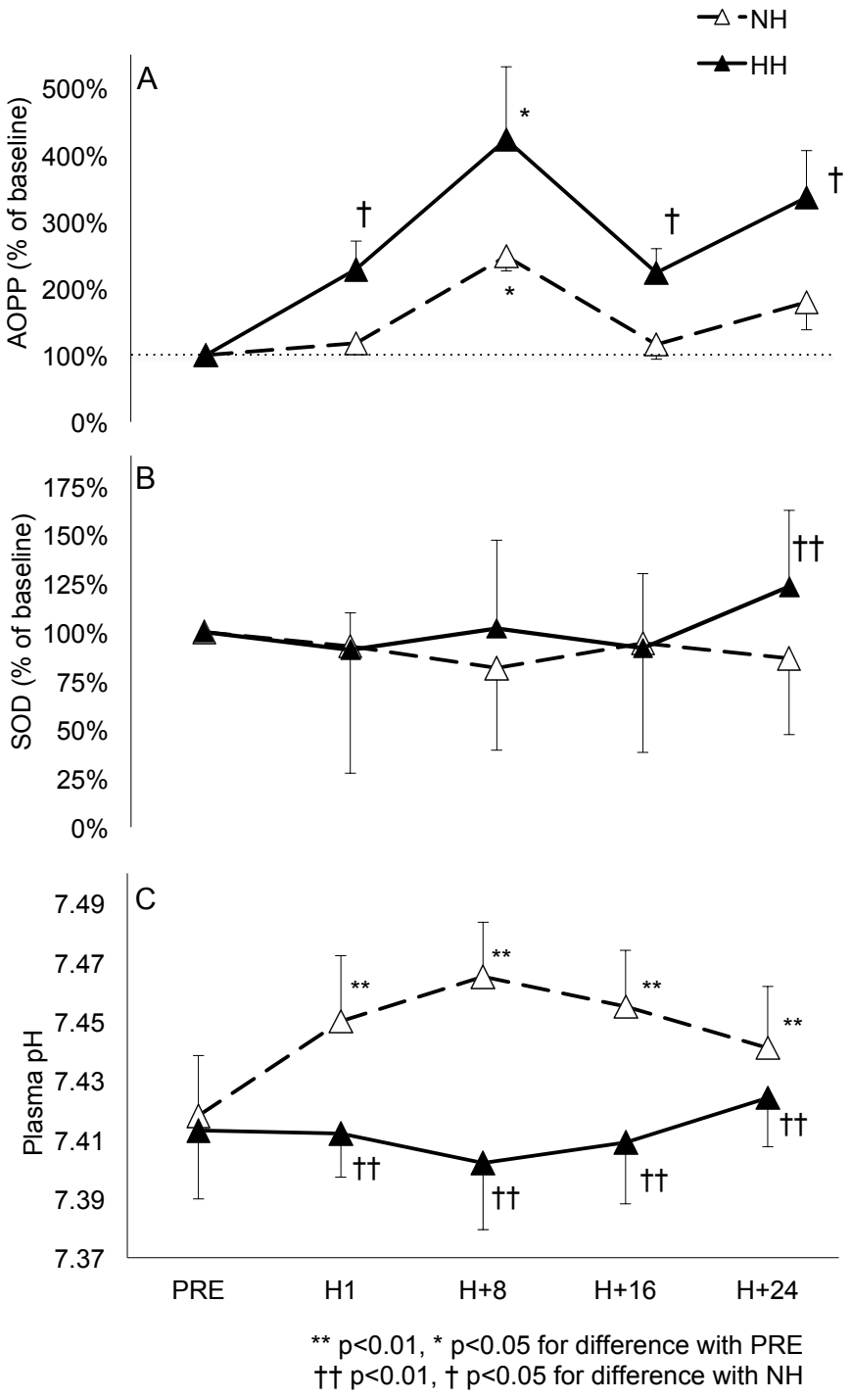

Figure 3 (A) Blood advanced oxidation protein products (AOPP, % of baseline), (B) Superoxide dismutase (SOD, % of baseline) at rest during the 24 h in hypobaric hypoxia (HH) and normobaric hypoxia (NH) and (C) Plasma pH at rest during the 24 h in hypobaric hypoxia (HH) and normobaric hypoxia (NH)

Discussion

Here we show for the first time different responses to an equivalent hypoxic stimulus in HH vs. NH sustained over 24 hours and associated with impaired NO bioavailability and exaggerated oxidative stress.

Primarily, the acute ventilatory response to hypoxia (AHVR), usually associated with rapid ascents to high altitude (21), was lower in HH compared to NH in our study, in agreement with previous observations done during shorter hypoxic exposures (36).

Secondly, lower amounts of NO in the exhaled air (exNO) have also been reported in HH compared to NH and ascribed to differences in barometric pressure (10, 18, 20). Accordingly, our data show significantly less exNO in HH compared to NH. By comparison with the results of Hemmingson et al. (18) who reported lower exNO in HH than in NH during an acute exposure to very high altitude (10 min at 5000 m), the present data additionally show that the exNO can decrease in HH up to 24 hours of exposure at an altitude of 3000 m. As proposed, the increased axial back diffusion of NO in the alveolar compartment when BP is decreased (37) coupled with the strong affinity of NO to hemoglobin (23) or pressure-induced suppression of the NO formation in the airways (20) may explain the lower exNO observed in HH. However, exNO should be interpreted with care because values were corrected for flow deviation and sensor sensitivity with correction factors of Hemmingsson et al. (17) that may vary due to different subject and/or sensor. Nevertheless, our results are in accordance with previously published results (18) but suggest other mechanisms for differences in ventilatory responses between HH and NH based on the interplay between exaggerated oxidative stress and impaired NO bioavailability in blood and tissue.

Indeed, the important new point is that plasma nitric oxide (i.e. nitrate and nitrite) concentration was lower in HH than in NH, suggesting altered systemic NO bioavailability by HH.

Nitrate and nitrite are physiologically converted back in blood and tissues to form NO and, remarkably, the nitrate–nitrite–NO pathway is gradually activated as oxygen tensions falls (26). Simultaneously, a decline of O_2 in arterial blood and respiratory cortical centers stimulates the carotid chemoreceptors and triggers AHVR (7). Interestingly, lower AHVR was observed in rats when NO production was inhibited (13). Similarly, Haxhiu et *al.* found that NO synthase (NOS) blockade attenuated the hypoxia-induced increase in respiratory activity in rats and that the increased respiratory output observed in AHVR resulted from the oxygen deprivation leading to the activation of NO-cGMP dependent pathway in the central nervous system (16).

Taken together, the results of these studies suggest that the lower minute ventilation in HH in our subjects could be due to a decrease in NO bioavailability. Yet, our results show an increased oxidative stress in HH compared to NH supporting the speculation that exaggerated oxidative stress may both affect ventilation and NO bioavailability.

In the present study, NOx decreased in HH and remained stable in NH. This lowered NOx observed in HH could be explained by the increase in oxidative stress. Indeed, the increased oxidative stress revealed by higher plasma concentration of AOPP and SOD in HH might inhibit NO formation and reduce its bioavailability. This phenomenon of NO metabolism down-regulation associated with oxidative stress increase was observed recently (32). Mechanistically, reactive oxygen species (ROS) inducing oxidative stress impair the bioavailability of NO (39). More specifically, the superoxide anion ($O_2^{\circ -}$) likely overproduced during hypoxia (14) can react with NO to form peroxynitrite ($ONOO^-$) thereby inducing NO inactivation and eNOS uncoupling. In this context, it was clearly shown that oxidative stress was increased during a similar normobaric hypoxic stimulus (12 h at a $P_{ET}O_2$ of 60 mmHg) (31) strongly suggesting an overgeneration of ROS.

Interestingly, plasma pH was higher in NH compared to HH and the higher SOD activity and levels of AOPP during HH suggests that ROS is likely more generated during HH by comparison with NH. This is in accordance with the enhanced free radical formation in case of a lower pH (2, 38). A relative acidosis can induce oxidative stress by causing protonation of the peroxynitrite anion (ONOO-) leading to radical hydroxyl (OH•) generation and by promoting delocalization of protein-bound iron stores thereby accentuating redox stress induced by Fenton reaction (15, 22). Actually, the increased ventilatory drive (as shown by the lower $P_{ET}CO_2$ during exercise) observed in NH likely decreases the blood H^+ concentration in line with our reported pH values. The proton being known as a pro-oxidant particle, this respiratory alkalosis in NH may thus reduce the ROS production and explain the higher plasma oxidative stress measured in HH.

We also acknowledge that only half of the oxidative stress markers suggest higher ROS production. However, different oxidative pathways specific for each marker with respect to hypoxia exposure could explain these discrepancies. MDA was shown to be much less sensitive than other oxidative stress markers (24) since it represents the end product of the polyunsaturated fatty acid (PUFA) oxidation pathway. In the context of our slight physiological difference between HH and NH, it would be unlikely that MDA was able to detect such difference. Similarly, we already reported such observation (AOPP increase without MDA change) in athletes submitted to hypoxic training (vs. identical normoxic training) (35). In addition AOPP and MDA do not reflect the same oxidative stress pathways, AOPP resulting from the myeloperoxidase pathway (44) via monocytes activation while MDA from PUFA oxidation. These results may suggest that hypoxia-induced oxidative stress could be more related to superoxide generation from NADPH oxidase activation than to an extracellular ROS production. In this present study SOD was significantly higher at H+24 but GPX was not affected during the 24h of hypoxic exposure. SOD represents the first line of antioxidant

enzyme in reducing the superoxides produced in the cell whereas GPX share with catalase the reduction of H_2O_2. In addition, H_2O_2 is less reactive than superoxide. These antioxidant enzyme characteristics may suggest that SOD could be less stimulated than GPX for a similar superoxide production.

Finally, the lack of nitrotyrosine change despite higher oxidative stress in HH could result of the observed decreased NO bioavailability (demonstrated by lower NOx and exNO).

In conclusion, we strongly believe that increase of AOPP and SOD may reflect a higher ROS overgeneration in HH compared to NH.

In conclusion, the exercise-induced increase in ventilation throughout 24h of hypoxic exposure was less in HH than NH and was associated with a decrease in NO bioavailability in HH. Furthermore, we observed increased oxidative stress and enzymatic antioxidants in HH. It can be hypothesized that the difference in oxidative stress and NO observed between HH and NH could explain the higher normobaric ventilatory responses to hypoxia. Although further investigation is needed to clarify how decreased NO bioavailability and increased oxidative stress could impact the lower ventilatory responses to hypoxia observed in HH, These new findings may have important potential consequences for adaptation and physical performance at altitude, since different responses in ventilation and oxidative stress may influence benefits to athletes training or living at altitude for performance improvement (28)

It is still unclear if the reported differences in pH, ventilatory responses as well as nitrosative and oxidative stress between HH and NH have a clinical relevance (28). However, since it was recently shown that HH was more effective than NH as pre-acclimatization treatment in order to minimize AMS symptoms (11), we believe that further clinical investigation are worth of interest.

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Chapter 7

Article 3 - Evidence for differences between hypobaric and normobaric hypoxia is conclusive.

7. Article 3 - Evidence for differences between hypobaric and normobaric hypoxia is conclusive.

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Letter to the editor

In a recent review (3) on the relative effectiveness of different pre-acclimatization strategies on physiological responses (e.g., $P_{ET}CO2$, SaO_2) and endurance performance of previously non-acclimatized subjects, Fulco et al. provided sets of data highlighting the potential differences in physiological mechanisms of acclimatization and exercise between hypobaric (HH) and normobaric (NH) hypoxia.

The authors are to be commended for the consistency (e.g., investigators' team, subject characteristics, assessment protocols, target altitude) among dozens of studies (most of them conducted at Pikes Peaks Laboratory, CO, 4300 m) over two decades, which enabled a robust comparison of benefits and effectiveness of different HH vs. NH pre-acclimatization strategies. Their conclusions are convincing enough to recommend HH instead of NH for pre-acclimatization due to the more developed ventilatory acclimatization and lesser decrease in performance when assessed at 4300 m.

However, stricto sensus, the review by Fulco et al. did not clarify if the differences observed between HH and NH (4, 6) are clinically relevant and lead to a higher severity of acute mountain sickness (AMS) in HH than in NH, a topic highly debated [(4) vs. (7)] where, to date, there is not well-controlled comparative cross-over study.

To our view, the most striking and novel finding arising from Fulco et al. (3) is the very low (or lack of) transfer of the benefits induced by the NH-acclimatization to the HH condition: When transported to 4300 m (HH), subjects who were pre-acclimatized in NH had a minimal benefit (e.g., no or <1 mmHg decrease in $P_{ET}CO2$ or AMS prevalence of 50%-64% instead of 80- 100% in non-acclimatized subjects). In addition, the NH pre-acclimatization did not induce any acclimatization can be effective in NH, but not in HH, and that the light ventilatory and AMS benefits retained in HH for the NH pre-acclimatized groups did not translate to performance benefits.

These data raise many questions about the mechanisms because they "remain elusive" (3) and cannot be explained by the slight differences in ventilation, fluid balance and nitric oxide metabolism already described between NH and HH (4, 6). In a recent study (2), we confirmed the differences in ventilation (e.g., higher $P_{ET}CO2$) and the impaired NO bioavailability in HH vs. NH, but reported also a higher oxidative stress that may be explained by a lower plasma pH.

Obviously, disentangling hypoxia and hypobaria is of importance for athletes or mountaineers who use NH to prepare for altitude competitions or expeditions. Moreover, the role of barometric pressure on oxidative and nitrosative stress during exposure to hypoxia might have interesting medical implications, as suggested by recent developments of therapeutic intermittent hypoxic methods (1, 8) in several pathologies (e.g., ischemic heart disease, stroke, cancer, chronic

lung disease, hypertension). Indeed, oxidative stress pathway has been recognized to regulate the Hypoxia Inducible Factor 1 (5), known to have a pivotal role in such disease progression/treatment. In conclusion, the review by Fulco et al. suggests further investigations on the differences in mechanisms and therapeutic use of hypobaric vs. normobaric hypoxia are needed.

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Chapter 8

Article 4 - Significant molecular and systemic adaptations after repeated sprint training in hypoxia.

8. Article 4 - Significant molecular and systemic adaptations after repeated sprint training in hypoxia.

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Abstract

While intermittent hypoxic training (IHT) has been reported to evoke cellular responses via hypoxia inducible factors (HIFs) but without substantial performance benefits in endurance athletes, we hypothesized that repeated sprint training in hypoxia could enhance repeated sprint ability (RSA) performed in normoxia via improved glycolysis and O_2 utilization. 40 trained subjects completed 8 cycling repeated sprint sessions in hypoxia (RSH, 3000 m) or normoxia (RSN, 485 m). Before (Pre-) and after (Post-) training, muscular levels of selected mRNAs were analyzed from resting muscle biopsies and RSA tested until exhaustion (10-s sprint, work-to-rest ratio 1:2) with muscle perfusion assessed by near-infrared spectroscopy. From Pre- to Post-, the average power output of all sprints in RSA was increased ($p<0.01$) to the same extent (6% vs 7%, NS) in RSH and in RSN but the number of sprints to exhaustion was increased in RSH (9.4 ± 4.8 vs. 13.0 ± 6.2 sprints, $p<0.01$) but not in RSN (9.3 ± 4.2 vs. 8.9 ± 3.5). mRNA concentrations of HIF-1α (+55%), carbonic anhydrase III (+35%) and monocarboxylate transporter-4 (+20%) were augmented ($p<0.05$) whereas mitochondrial transcription factor A (-40%), peroxisome proliferator-activated receptor gamma coactivator 1α (-23%) and monocarboxylate transporter-1 (-36%) were decreased ($p<0.01$) in RSH only. Besides, the changes in total hemoglobin variations (Δ[tHb]) during sprints throughout RSA test increased to a greater extent ($p<0.01$) in RSH.

Our findings show larger improvement in repeated sprint performance in RSH than in RSN with significant molecular adaptations and larger blood perfusion variations in active muscles.

Introduction

Hypoxic conditions can be characterized in working muscle by a decreased oxygen tension (e.g., lower myoglobin oxygen saturation and intramyocellular oxygen partial pressure) [1]. There are some evidences that exercising in hypoxia affects muscular functions [2] and a large number of genes mediated by hypoxia-inducible factors (HIFs) [3]. These transcription factors have been demonstrated to control the expression of over 70 targets in response to a reduction in oxygen concentration. Intermittent hypoxic training (IHT) has been reported to evoke cellular responses via HIF1-α [4,5] but without demonstrating substantial performance benefits in endurance athletes [6-8]. Actually, training intensity in hypoxia *per se* modulates molecular adaptations at muscular level with "adaptations that compensate for the reduced availability of oxygen during exercise" [9]. Then, any limitation in O_2 availability (e.g., exercising in hypoxia) induces a compensatory vasodilation shifting blood flow upward to keep constant O_2 delivery to the muscle. This latter mechanism is also influenced by the exercise intensity [10]. For example, during supramaximal efforts, an increase in skeletal muscle blood flow allows matching the augmented O_2 demand tightly [11].

Indeed, repeated sprints (RS) consist in maximal intensity exercise bouts with incomplete recoveries and could potentially challenge these adaptive mechanisms if performed in hypoxia. RS require huge energy amounts over short periods of time provided primarily by anaerobic glycolysis [12] with a progressive rise in the energy contribution of the aerobic metabolism [13]. One may therefore speculate that HIF-mediated molecular adaptations induced by repeated maximal efforts in hypoxia may challenge the functional reserve in muscle oxygen diffusing capacity likely utilized in hypoxia [14].

Besides, fast-twitch fibers (FT), preferentially recruited while sprinting [15], may better adjust to a high energetic demand with a greater fractional O_2 extraction than their slow twitch counterparts [16] if oxygen tension falls in the muscle (e.g., in hypoxia). Consequently it can be hypothesized that RS training in hypoxia (RSH) could induce beneficial adaptations at the muscular level with an improved blood perfusion level inducing an enhanced O_2 utilization by FT.

Nonetheless, to our knowledge, these mechanisms and the potential associated benefits for the ability to repeat sprints in normoxia have never been investigated in a randomized blind controlled study. The goal of our study was therefore to test putative additional benefit on systemic repeated sprint ability performance of RS training in hypoxia vs. normoxia and to assess blood perfusion and molecular responses at the muscular level.

Methods

Subjects. 50 moderately trained male cyclists (age 35 ± 7 years, height 179 ± 5 cm, mass 75 ± 9 kg) volunteered in the study and provided their written informed consent after the state Medical Ethics Committee approved the protocol (Commission Cantonale Valaisanne d'Ethique

Médicale, CCVEM, Agreement 07/10, Sion, Switzerland). Subjects were all non-smokers lowlanders. None of the subjects were acclimatized or recently exposed to altitude and were asked to avoid any exposure to an altitude of more than 1500 m during the protocol.

Study design. Experimental protocol consisted in two testing sessions before (Pre-) and after (Post-) a four-week supervised specific training period (8 training sessions). Subjects were randomly assigned to one of the two treatment groups with repeated sprint training either in normoxia (RSN, n=20) or hypoxia (RSH, n=20). A control group (CON, n=10) completed only Pre- and, after four weeks without specific training, Post-. Subjects were not specifically accustomed with 10 s cycling sprints but they were familiar with laboratory testing and longer high intensity cycling intervals. Protocol was run in single-blind fashion, as subjects both in RSH and RSN were told that all training sessions were held at altitude (without any accurate information on the altitude level). When asked, 95% of the subjects in RSH and 85% in RSN declared that their training was performed in hypoxia. Analyzes of blood samples and muscle biopsies were performed double-blinded.

Supervised training protocol. Volunteers completed eight specific cycling training sessions with repeated sprints during four weeks starting the week after Pre- and finishing the week before Post-. Two sessions per week were completed in a hypoxic chamber (ATS Altitude, Sydney, Australia) built in our laboratory at an altitude of 485 m (Sion, Switzerland). The hypoxic chamber is a well-ventilated 30 cu. m room (2.4 m x 5.0 m x 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. For RSH, inspired oxygen fraction (F_iO_2) was set to 14.6 % to simulate an altitude of 3000 m and controlled regularly with an electronic device (GOX 100 oximeter, Greisinger, Regenstauf, Germany). In order to blind subjects to altitude, the system was also run for RSN with a normoxic airflow into the chamber. Air input flow (up to 1000 l/min) was sufficient for safe, comfortable and stable training conditions. Temperature inside the chamber was maintained at 25° in average and three fans and crushed ice buckets were used to cool subjects.

Training sessions. Following 10 min warm-up at 120 W, all training sessions consisted in 3 sets of 5 x 10 s all-out repeated sprints with a 5 min recovery period at 120 W between sprints and sets and ending with 10 min recovery at 120 W (Fig. 1). Subjects were instructed to perform all-out sprints trying to reach and maintain the highest power output for every sprint. Each session lasted 36 min 30 s and subjects spent ~40 min in the chamber for every training session (320 min during the 4-weeks training period). Up to four subjects trained simultaneously in the chamber using their own road bicycle equipped with a power meter (Powertap Pro+ Wheel, Cycleops, Madison, USA) mounted on a stationary trainer with magnetic resistance (Powertap Supermagneto Pro, Cycleops, Madison, USA). Power output and heart rate were recorded for

each training session. Electrolytes-carbohydrates sports drink (Isotonic, Sponser, Wollerau, Switzerland) and water were provided to ensure appropriate hydration and carbohydrate intake during training. After each session, subjects reported their rate of perceived exertion (RPE, Borg Scale, 6-20) and ticked the level of pain in the legs on a continuous visual analogic scale (VAS). Training sessions were scheduled with at least one day of rest in between for optimal recovery. Outside of the laboratory, volunteers were asked to maintain their usual endurance training avoiding any intense training and to fill a training diary.

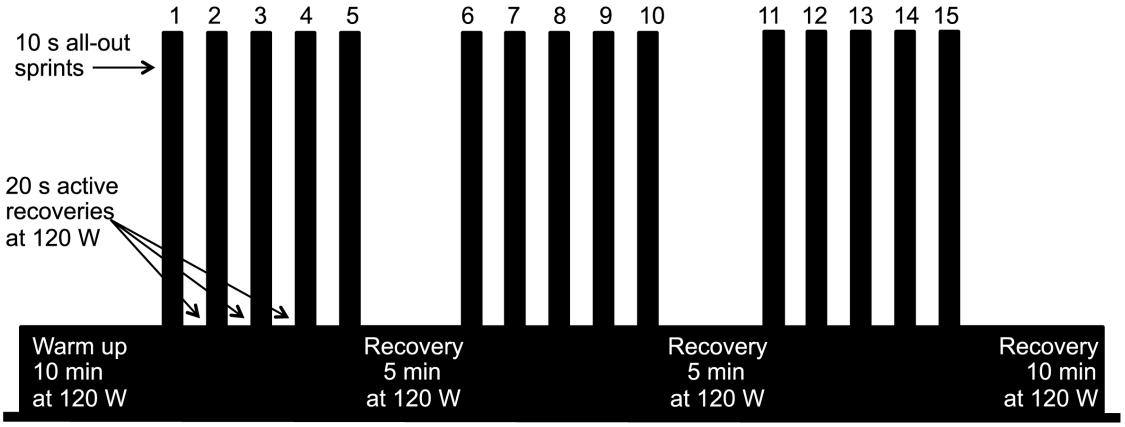

Figure 1 Description of a typical training session.

Baseline (Pre-) and final (Post-) tests. During the 48 h prior to each testing session, subjects were asked to refrain from any training or exhaustive activity. Due to the extreme intensity of the tests, subjects were asked not to report to the laboratory on an empty stomach. A standard breakfast (cornflakes or muesli with milk, bread with jam and water) was therefore advised. In all cases, subjects were asked to fill a nutritional diary for three days before each test and reproduce the last meals avoiding alcohol and caffeine intakes during the 24 h before each test. Tap water was provided *ad libitum* during the tests. Pre- and Post- trials were performed in the exact same sequence in normoxia in the well-ventilated laboratory at a constant temperature of 24 °C.

Blood sampling and muscle biopsies. Upon arrival in the morning, subjects were weighed and a 2,5 ml blood sample was then taken from the antecubital vein for immediate hematocrit and hemoglobin concentration ([Hb]) evaluation. Gold standard hematocrit measurement was done by centrifugation of heparinized capillaries during 5 min at 13000 rpm (Haematokrit 210 Centrifuge, Hettich, Germany). [Hb] was assessed with a photometry device (Hemocue 201, Ängeholm, Sweden). Blood lactate concentration ([La]) was measured from a capillary fingertip sample (Lactate Pro, Arkray, Japan) at rest and immediately after each test.

Resting skeletal muscle microbiopsy samples were taken under local anaesthesia (Rapidocaine®, 1% plain) of the left leg from the belly of the *vastus lateralis* muscle using percutaneous needle biopsy technique (Pro-Mag, Medical Device Technologies Inc., Gainsville, USA) [17]. Three individual muscle samples (between 60 and 90 mg of muscle) were taken within less than 1 min from one single incision. The muscle samples were washed in saline solution then immediately frozen in liquid nitrogen for RNA and protein extraction. For practical reasons muscle biopsies were taken in 5 subjects only in CON.

RNA extraction and real time quantitative PCR. RNA from skeletal muscle (approximately 25 mg of muscle) was extracted using a commercially available preparation, peqGOLD Tri-Fast (Peqlab, Germany). Five micrograms of RNA were reverse transcribed to cDNA using Random Hexamer primers (Promega AG, Switzerland) and AffinityScript Multiple Temperature Reverse Transcriptase (Agilent Technologies Inc, Santa Clara, CA, USA), while quantitative PCR was performed using an MX3000p thermal cycler system and Brilliant SYBER Green QPCR Master Mix (Agilent Technologies). PCR primers sequences are listed in Table 1 and PCR conditions were used as published previously by our research group [17,18]. To control for any variations due to efficiencies of the reverse transcription and PCR, acidic ribosomal phosphoprotein P0 (RPLP0 or 36B4) was used as an internal control. All PCR runs were performed in triplicate.

Enzyme activities. 20 mg of tissue were used to measure enzyme activities of interest in RSH and RSN but not in CON. Lactate dehydrogenase (LDH) activity was determined using a colorimetric method developed by BioAssay system (QuantiChrom) according to manufacturer's instructions. The non-radioactive colorimetric LDH assay is based on the reduction of the tetrazolium salt MTT in a NADH-coupled enzymatic reaction to a reduced form of MTT which exhibits an absorption maximum at 565 nm [19]. Measurement of citrate synthase (CS) activity was performed by linking the release of Coenzyme-A to the colometric agent DTNB 5,5-dithiobis-2-nitrobenzoate. Changes in absorbance were followed at 412 nm [20].

Gene	Sequence 5–3	Temperature
MCT-1	Sense CCA AGG CAG GGA AAG ATA AGT CT	60
	Anti ATC TTT TTT CAC ACC AGA TTT TCC A	
MCT-4	Sense GCA CCC ACA AGT TCT CCA GT	60
	Anti CAA AAT CAG GGA GGA GGT GA	
CA3	Sense GTC CTC TCC CTG GAC CCT AC	60
	Anti TTG TCC AAT GCA TCA AGG AA	
Tfam	Sense CCA AAA AGA CCT CGT TCA GCT TA	60
	Anti TGC GGT GAA TCA CCC TTA GC	
Mb	Sense GCA TGC CAC CAA GCA CAA G	60
	Anti TGA TGC ATT CCG AGA TGA ACT C	
PGC-1a	Sense GGT CTC TCC TTG CAG CAC AAG	60
	Anti CTG GGA TGA CCG AAG TGC TT	
VEGF	Sense CCT TGC TGC TCT ACC TCC AC	60
	Anti ATC TGC ATG GTG ATG TTG GA	
HIF-1a	Sense TCC ATG TGA CCA TGA GGA AA	60
	Anti CCA AGC AGG TCA TAG GTG GT	
RPLP0	Sense GTG ATG TGC AGC TGA TCA AGA CT	60
	Anti GAT GAC CAG CCC AAA GGA GA	

Table 1 Quantitative PCR primer sequences.
MCT-1, monocarboxylate transporter 1; MCT-4, monocarboxylate transporter 4; CA3, carbonic anhydrase III ; Tfam, transcription factor A mitochondrial ; Mb, myoglobin ; PGC-1a, peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; VEGF, vascular endothelial growth factor A; HIF-1, hypoxia inducible factor 1, alpha subunit; RPLP0, ribosomal protein large P0.

Exercise performance tests and measurements. An electronically braked cycling ergometer (Lode Excalibur Sport, Groninge, The Netherlands) equipped with clip-on pedals was used for all performance tests. Seating and handlebar positions were adjusted individually for all subjects and reproduced for all tests. For each performance test, maximal instantaneous power output and pedaling frequency (PF) were recorded and average power output calculated. All tests were performed against an individual fixed torque of 0.8 $Nm \cdot kg^{-1}$ with the ergometer in manufacturer's "Wingate mode" unless otherwise specified. RPE and leg pain were assessed as described above.

Near-infrared spectroscopy measurements. Muscle oxygenation was assessed using a near-infrared spectroscopy (NIRS) technique well described elsewhere [21]. The NIRS device (Oxymon Mk III, Artinis, Zetten, The Netherlands intramuscular oxygenation) was used to measure changes in muscle oxygenation by placing a double optode sensor on *m. vastus lateralis* on

the right leg, at mid thigh with an interoptode spacing of 40mm. The probe was attached to the skin with double-sided tape and firmly fastened with an opaque cotton elastic band wrapped around subjects' thigh. Position of the probe was marked during Pre- with a permanent pen for accurate repositioning in the Post-trial. A standard differential pathlength factor (DPF) of 4.0 was used in lack of any clear standard value for human quadriceps muscle during cycling sprints [22,23]. All signals were recorded with a sampling frequency of 50 Hz. They were down sampled at 10 Hz using Matlab (Matlab Software, Nattick (MA) USA) routine resample. Then a 10th-order low-pass zero-phase Butterworth filter (cutoff frequency 0.1 Hz) was applied to the resampled signals in order to remove possible artifacts and smooth the pedaling-induced perturbations. Automatic detection of the starting and end times of the successive sprints was obtained by estimating the filtered desoxyhemoglobin signal upper and lower envelopes using local minima and maxima in a sliding window of length 400 samples (4 s). The starting and end times were obtained as the times of contact between the envelopes and the signal. This allowed the determination of maximum and minimum for each signal during the successive sprint and recovery phases. Concentrations for oxyhemoglobin ([O_2Hb]), desoxyhemoglobin ([HHb]) and total hemoglobin/myoglobin ([tHb]) were determined. Figure 2 illustrates the typical signals recorded by NIRS. Since [HHb] values were proposed to be less sensitive than [O_2Hb] to blood flow variations [24] and changes in O_2Hb signals might be confounded by rapid blood volume changes during sprints [25] only [HHb] and [tHb] were analyzed for relevant interpretations. Differences between maximum and minimum concentrations were defined as the amplitude of the variation for each sprint (Δ[tHb] and Δ[HHb]). Thus, for example, at the beginning of each sprint a minimum value for [HHb] is measured whereas [HHb] reaches a maximum at the end of each sprint. Conversely, a maximum in [tHb] is observed at the beginning of each sprint (i.e. end of each recovery period) and [tHb] decreases to reach a minimum value at the end of each sprint. The amplitudes of the first sprint were standardized as 100%. Amplitudes during the following sprints and the average value for all sprints throughout the RSA test (i.e. $\Delta[HHb]_{av}$ and $\Delta[tHb]_{av}$) were calculated. The same analysis was performed during the successive recovery phases. In addition, the Δ[tHb]/Power was calculated as the ratio between Δ[tHb] and mean power for each sprint as an index of blood perfusion relative to the intensity of each sprint.

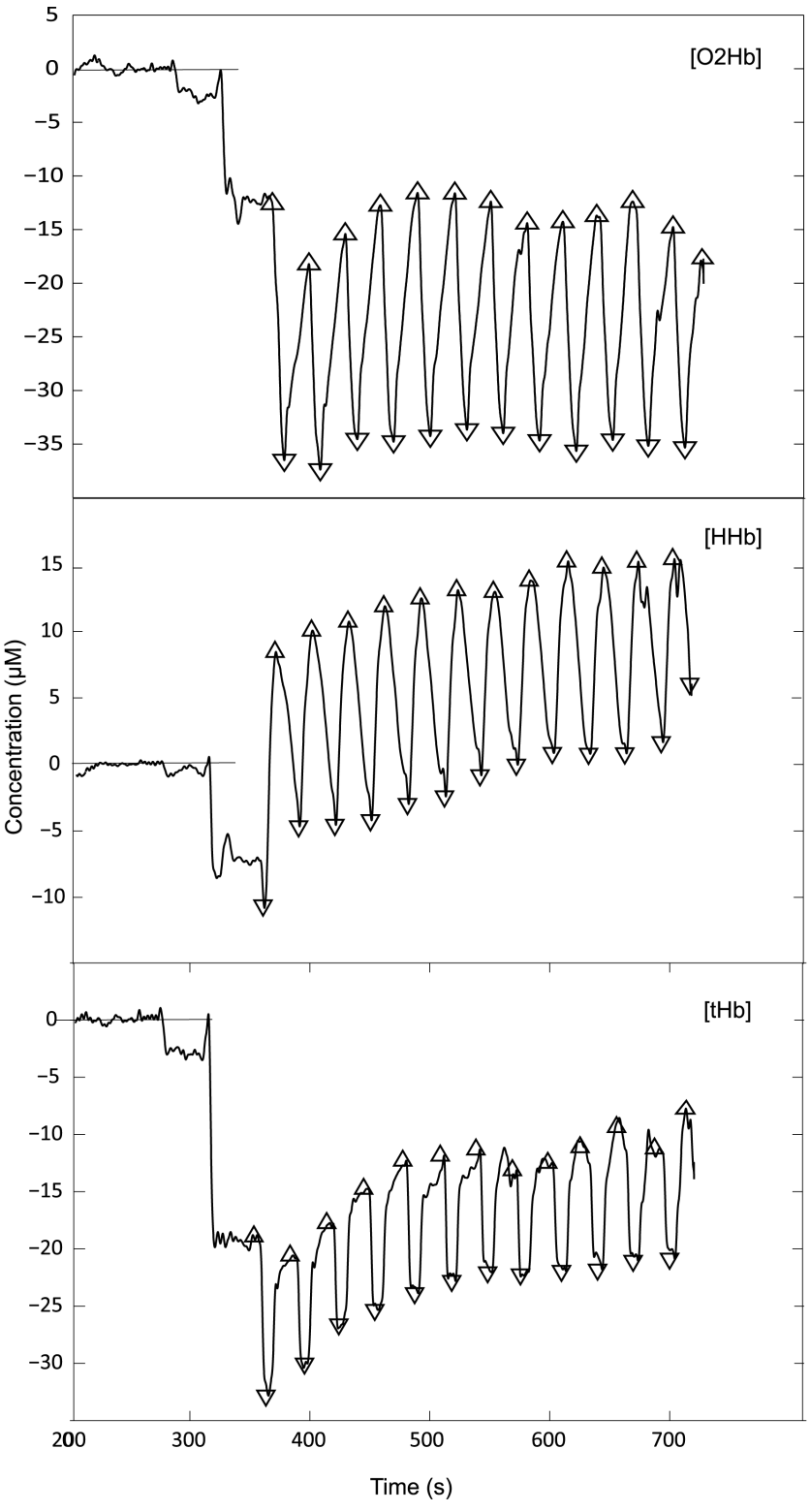

Figure 2 Typical signals recorded by near-infrared spectroscopy during repeated sprint ability test after signal treatment, maximum and minimum determination and smoothing. Concentrations of O_2Hb, HHb and tHb are expressed as changes in µM from resting baseline set to 0 µM.

Electromyography acquisition and analysis. Surface EMG of the vastus lateralis (VL) and biceps femoris (BF) muscles on the right leg were recorded during the tests using MP36 hardware (Biopac Systems Inc., Santa Barbara, CA) with specific electrodes (EL-503, Biopac) with a diameter of 35 mm pasted longitudinally on the distal part of the muscle belly with an center-to-center distance of 25 mm. A ground electrode was located on the patella. Prior to electrode placement, skin on the surface of these muscles was carefully shaved, abraded and cleaned (impedance < 5 kΩ). EMG cables running inside subjects' cycling tights prevented cables movement. The myoelectric signal was collected at 2000 Hz, amplified and filtered (pass band 5–500 Hz, gain = 1000). EMG parameters were then computed in a dedicated software (AcqKnowledge 4.1.0, Biopac Systems Inc., Santa Barbara, CA). During RS, each muscle activity was determined by calculating the mean value of the root mean square (RMS) of the signal for each sprint. Average values were then calculated for the entire duration (10 s) of each sprint.

Repeated sprint ability test. Approximately 20 min after the resting biopsy, subjects performed a 5 min warm-up at 120 W at their preferred PF followed by an isolated 10 s sprint, 4 min 50 s recovery at 120 W and a second isolated 10 s sprint. 15 s after this second sprint, subjects were asked to stop pedaling and rest keeping the right leg motionless and straight during 5 min for NIRS baseline measurement. Subjects then started pedaling for 45 s against a 20 W resistance at a PF of approximately 85 rpm followed immediately by the repeated sprint ability (RSA) test with a 1:2 work-to-rest ratio (10 s all-out sprint and 20 s rest). Immediately after each sprint, the ergometer switched automatically to a resistance of 20 W during the 20 s active recovery phase. Subjects were given very strong verbal encouragement and performed as many sprints as possible until exhaustion (i.e. task failure). To avoid any protective pacing strategy, average power during the first two sprints was controlled to reach at least 95% of the best average of the two isolated sprints, which was the case in all subjects. Subjects were never given any indication on the number of sprints performed. A minimal PF of 70 rpm after 5 s or less of sprinting was set as a criterion to stop subjects' test. However, volitional exhaustion was observed in all subjects before this criterion was reached. Unfinished sprints (subjects not being able to turn pedals anymore) where not taken into consideration.

3 min All-Out test. 45 min after the RSA test, subjects performed a 3-min all-out test following a protocol well described elsewhere [26] in order to determine 3-min average power output. Strong verbal encouragement was given throughout the test and PF was instructed to be as high as possible all time to elicit maximal all-out performance. Information about elapsed time was only given during the final minute every 15 seconds in order to prevent pacing and further motivate subjects. A peak in oxygen consumption (VO_{2peak}) was calculated as the highest 30 s average value during the test with a gas exchange analyzer (Metalyzer, Cortex Medical, Leipzig, Germany). Although residual fatigue from the RSA test was probable, this test was performed for

all subjects at the same moment of the testing sequence allowing for a good comparison of subjects' aerobic capacity (average power during 3 min). For this test, the ergometer was set in linear mode (power output increases as PF increases).

30 s Wingate test. Prior to the first and last training sessions, subjects performed a standard 30 s Wingate test [27] after a 6 min warm-up at 120 W and the average power during the 30 s was recorded.

Statistical analyses. Data are presented as mean (SD). Performance and blood perfusion changes during RSA test were first evaluated with a 2-way (training group x sprint number) general linear model repeated-measures ANOVA with all pairwise multiple comparison procedures (Holm-Sidak method). Performance improvement, muscle oxygenation during RSA, mRNA concentrations, blood lactate and other single variables test were then evaluated with 2-way (training group x time (Pre- vs. Post-)) general linear model repeated-measures ANOVAs with all pairwise multiple comparison procedures (Holm-Sidak method). All analyses were made using Sigmaplot 11.0 software (Systat Software, CA, USA). Null hypothesis was rejected at $p<0.05$.

Results

Total work and training intensity during supervised RS training were similar during RSN and RSH, with only the mean heart rate being higher in RSH (Table 3). In addition to specific training in the laboratory, subjects trained similarly for 5.0 ± 2.5 h and 5.1 ± 2.2 h for the RSH and RSN groups, respectively.

	RSN		RSH		CON	
	Pre-	Post-	Pre-	Post-	Pre-	Post-
Body mass (kg)	73.7 ± 7.7	73.7 ± 8.1	76.8 ± 10.4	76.5 ± 10.1	76.4 ± 11.0	75.8 ± 11.3
Hematocrit (%)	45.6 ± 2.7	45.4 ± 2.2	44.2 ± 2.7	44.8 ± 2.4	44.9 ± 2.6	45.7 ± 2.7
Hemoglobin ($g \cdot l^{-1}$)	152 ± 12	154 ± 12	153 ± 12	154 ± 16	154 ± 8	157 ± 9
VO_{2peak} ($l \cdot min^{-1}$)	3.8 ± 0.5	4.0 ± 0.4	3.8 ± 0.4	3.8 ± 0.4	3.9 ± 0.5	3.9 ± 0.5

Table 2 Subjects' characteristics before (Pre-) and after (Post-) repeated sprint training in hypoxia (RSH), in normoxia (RSN) or in the control group (CON).

VO_{2peak}, peak in oxygen consumption measured during 3-min all-out test.

	RSH	RSN
Total work (kJ)	2728 ± 242	2738 ± 335
Mean peak power output (W)	960 ± 117	1003 ± 146
Mean heart rate (bpm)	151 ± 12	141 ± 12#
Mean RPE	16.2 ± 1.4	16.7 ± 1.5
« Pain in the legs » VAS	7.0 ± 1.9	7.6 ± 0.9

Table 3 Total work and measured parameters during all training sessions in the hypoxic (RSH) and normoxic (RSN) training groups

RPE, Rate of perceived exertion (Borg 6-20 scale); VAS, Visual analogic 1-10 scale

# $p < 0.05$ for difference with RSH

Performance. From Pre- to Post-, the average power of all sprints during the RSA test increased ($p<0.01$) to the same extent (6 ± 7% vs. 7 ± 8%, NS) in RSH and in RSN, respectively, but not in CON (2 ± 5%, NS). The number of sprints prior to exhaustion was increased in RSH (9.4 ± 4.8 vs. 13.0 ± 6.2, $p<0.01$) but not in RSN (9.3 ± 4.2 vs. 8.9 ± 3.5, NS) nor in CON (11.0 ± 7.1 vs. 10.3 ± 6.2, NS). In Post- compared to Pre-, 10-s average power in the successive sprints was significantly improved till the ninth sprint in RSH and till the seventh in RSN. In RSH, this 10-s power output was significantly better ($p<0.01$) in the 10th and 11th sprints in Post- than the ninth sprint in Pre- (Fig. 3). Significant group (RSH vs. RSN) by time (Pre- vs. Post-) interactions were found in the number of sprints ($F=24.22$; $p<0.001$) and total work ($F=27.07$; $p<0.001$) performed during the RSA test. Subjects reached volitional exhaustion at the same relative power output (e.g., % of the best sprint) in RSH (67 ± 11% vs. 69 ± 8% for

Pre- vs. Post-, respectively, NS) and in RSN (65 ± 7% vs. 68 ± 9%, NS). From Pre- to Post-, average PF during the last 5 s of all successive sprints was improved to the same extent ($p<0.01$) in RSH (96 ± 14 vs. 104 ± 11) and in RSN (100 ± 11 vs. 110 ± 10) but not in CON (103 ± 2 vs. 103 ± 3, NS). The maximal PF of each sprint was higher ($p<0.01$) in Post- both in RSH (124 ± 11 vs. 130 ± 8) and in RSN (125 ± 8 vs. 131 ± 8) but not in CON (129 ± 3 vs. 130 ± 4, NS).

The average power during the 3-min all-out test as well as hematocrit and hemoglobin concentration at rest were unchanged between Pre- and Post- in all groups (Tables 3 and 4). RSH and RSN improved single 10-s sprint and Wingate performances ($p<0.01$) similarly whereas it did not change in CON (Table 4).

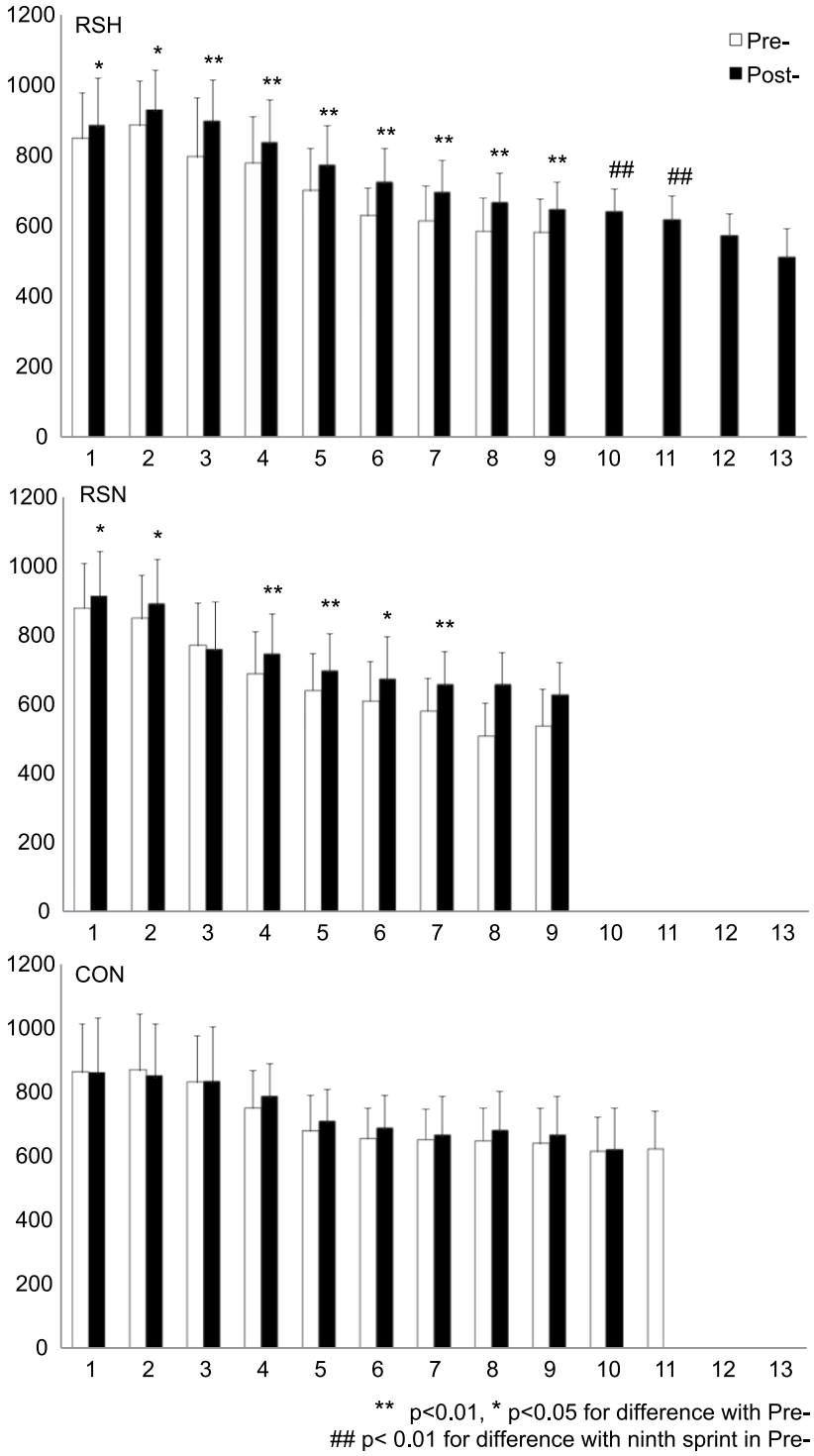

Figure 3 Average power output (W) in successive sprints during the repeated sprint test before (Pre-) and after (Post-) the specific repeated sprint training in hypoxia (RSH), in normoxia (RSN) or in control group (CON).

	RSH		RSN		CON	
	Pre-	Post-	Pre-	Post-	Pre-	Post-
Single 10-s sprint average power (W)	870 ± 132	925 ± 120**	879 ± 131	940 ± 131**	890 ± 151	877 ± 163
RSA Peak Power (W)	1365 ± 311	1500 ± 217**	1351 ± 226	1427 ± 275*	1357 ± 275	1377 ± 322
RSA Peak heart rate (bpm)	173 ± 14	175 ± 11	172 ± 8	172 ± 8	171 ± 10	171 ± 11
RSA Best average power (W)	870 ± 133	925 ± 120**	879 ± 131	940 ± 131**	870 ± 151	877 ± 163
RSA Mean power of all sprints (W)	699 ± 97	737 ± 84**	693 ± 120	734 ± 104**	726 ± 115	747 ± 125
RSA [La] ($mmol \cdot l^{-1}$)	15.0 ± 2.3	15.4 ± 2.1	14.2 ± 1.7	14.8 ± 1.6	14.8 ± 2.0	13.8 ± 1.5
RSA Average RPE	17.3 ± 1.5	17.4 ± 1.7	17.8 ± 1.4	17.8 ± 1.5	17.8 ± 1.0	17.6 ± 0.8
RSA Average « Pain in the legs » VAS	5.2 ± 2	6.7 ± 1.9**	5.8 ± 1.9	7.7 ± 1.7**	6.4 ± 2.3	6.1 ± 1.8
30 s Wingate average power (W)	699 ± 102	718 ± 94**	688 ± 75	723 ± 86**	670 ± 86	689 ± 105
30 s Wingate [La] ($mmol \cdot l^{-1}$)	11.0 ± 2.2	12.0 ± 2.0	11.3 ± 2.3	11.0 ± 2.2	10.8 ± 3.0	11.4 ± 2.5
30 s Wingate Average RPE	15.3 ± 2.0	16.1 ± 1.9	16.3 ± 1.6	17.0 ± 1.6	15.8 ± 1.7	16.0 ± 0.9
3-min all-out average power (W)	368 ± 45	383 ± 39	371 ± 49	382 ± 47	385 ± 48	378 ± 48
3-min all-out [La] ($mmol \cdot l^{-1}$)	14.7 ± 2.1	15.0 ± 1.9	14.5 ± 2.0	15.1 ± 2.0	14.2 ± 2.6	13.8 ± 2.4
3-min all-out RPE	17.5 ± 1.7	18.0 ± 1.7	18.1 ± 1.8	18.7 ± 1.1	17.8 ± 1.0	17.9 ± 1.0

Table 4 Performance results before (Pre-) and after (Post-) repeated sprint training in hypoxia (RSH), in normoxia (RSN) or in the control group (CON).
RSA, repeated sprint ability test; [La] Blood lactate concentration after repeated sprint test; RPE, rate of perceived exertion (Borg 6-20 scale); VAS, visual analogic scale (1-10) score.
** $p < 0.01$, * $p < 0.05$ for difference with Pre-

Muscle oxygenation. **Repeated sprints.** After training, $\Delta[tHb]_{av}$ increased to a greater extent (F= 15.8, p<0.01) in RSH than in RSN and was not different in Pre- and in Post- in CON (Fig. 4B).

Meanwhile, $\Delta[HHb]_{av}$ increased similarly in RSH (from 79 ± 6% to 98 ± 5%, p<0.01) and in RSN (from 106 ± 3% to 120 ± 10% , p<0.01) but was not different in Pre- and in Post- in CON (118 ± 9% vs. 121 ± 13%, NS).

The average Δ[tHb]/Power ratio increased to a greater extent (F=9.1, p<0.05) in RSH (from 1.64 ± 0.25 to 2.23 ± 0.35, p<0.01) than in RSN (from 1.68 ± 0.15 to 1.98 ± 0.30, p<0.05) and was not different Pre- compared to Post- in CON (1.61 ± 0.26 vs. 1.56 ± 0.33, NS).

Recovery phases. After training, $\Delta[tHb]_{av}$ increased to a similar extent in RSH and in RSN but was not different Pre- compared to Post- in CON (Fig. 5B).

Meanwhile, $\Delta[HHb]_{av}$ increased significantly only in RSH (from 98 ± 7 to 115 ± 12%, p<0.05) but not in RSN (105 ± 12% vs. 116 ± 10%, NS) nor in CON (109 ± 8% vs. 113 ± 13%, NS).

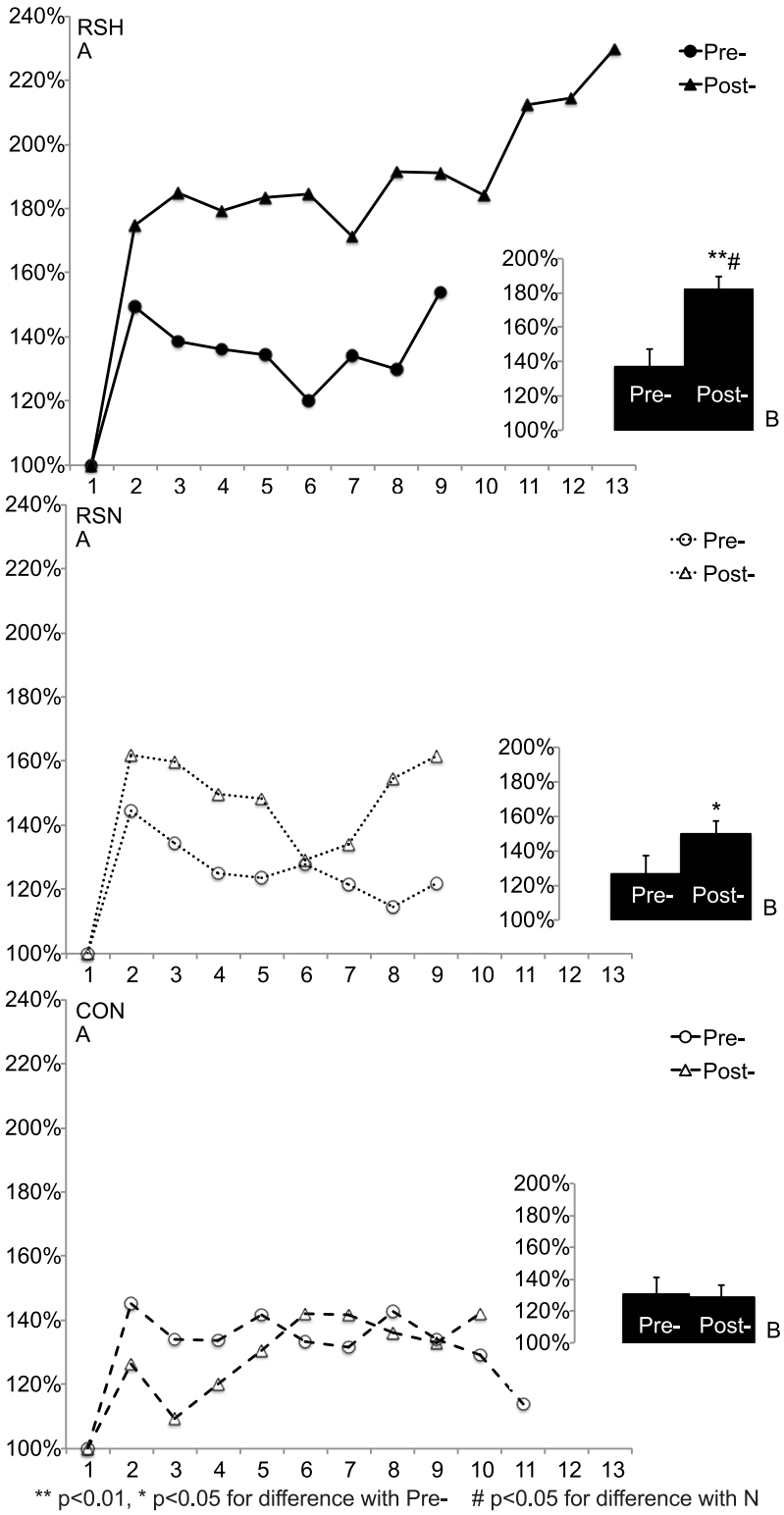

Figure 4 Δ[tHb]: successive changes in total hemoglobin concentrations' amplitude during sprints (expressed in percent compared to the first sprint) measured by near infrared-spectroscopy (A) and $\Delta[tHb]_{av}$: average of all changes during the repeated sprint test to exhaustion (B) before (Pre-) and after (Post-) the specific repeated sprint training in hypoxia (RSH), in normoxia (RSN) or in control group (CON).

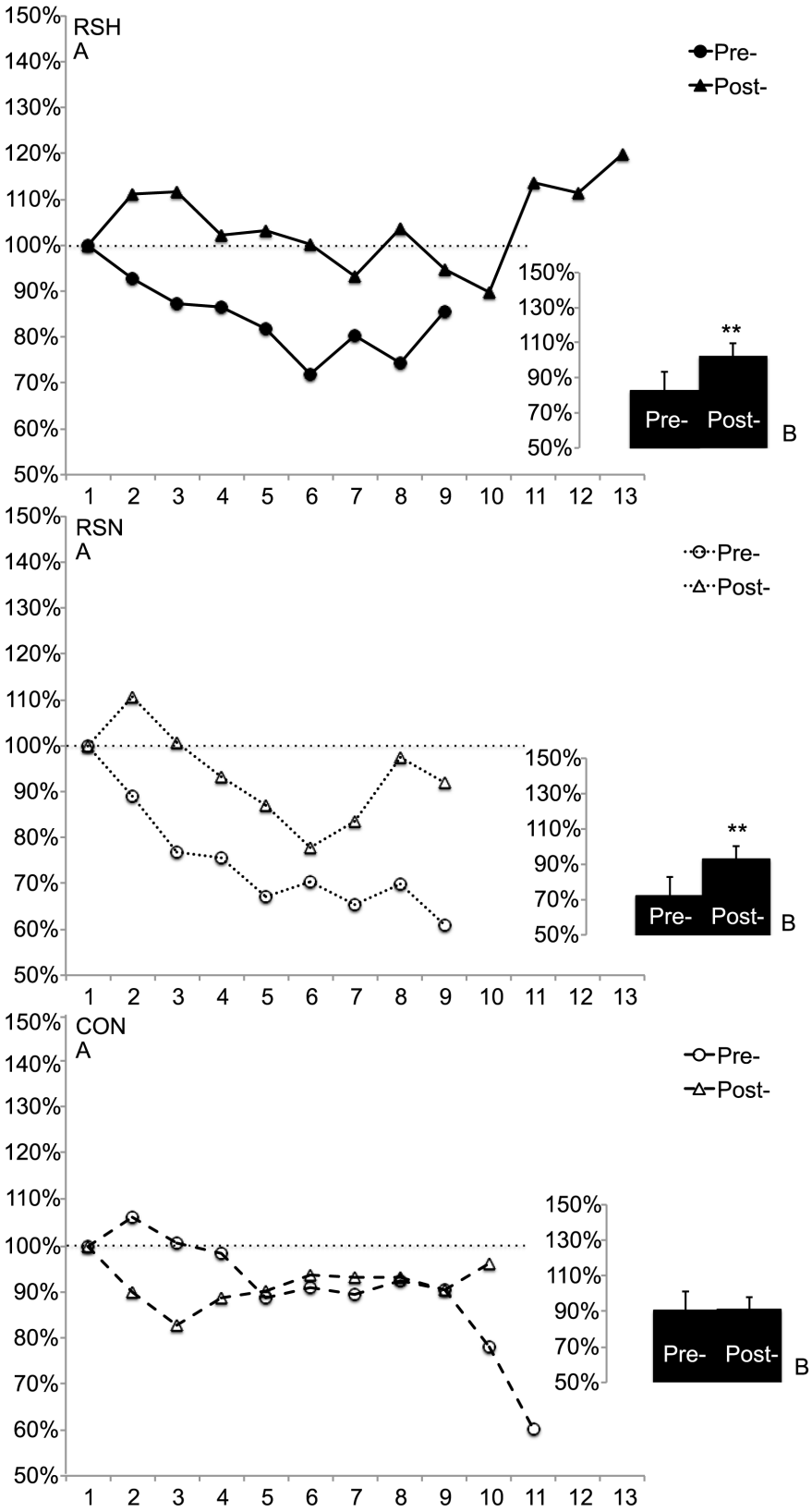

Figure 5 Δ[tHb]: successive changes in total hemoglobin concentrations' amplitude during recovery phases between sprints (expressed in percent compared to the first recovery phase) measured by near infrared-spectroscopy (A) and $\Delta[tHb]_{av}$: average of all changes during the repeated sprint test to exhaustion (B) before (Pre-) and after (Post-) the specific repeated sprint training in hypoxia (RSH), in normoxia (RSN) or in control group (CON).

Elextromyographic activity. From Pre- to Post-, average RMS activity of the VL muscle was not different in RSH (0.53 ± 0.23 vs. 0.50 ± 0.16 mV, NS), in RSN (0.49 ± 0.15 vs. 0.51 ± 0.12 mV, NS) or in CON (0.53 ± 0.21 vs. 0.51 ± 0.19 mV). Similarly, from Pre- to Post-, average RMS activity of the BF muscle was not different in RSH (0.22 ± 0.09 vs. 0.24 ± 0.10 mV, NS), in RSN (0.20 ± 0.08 vs. 0.22 ± 0.09 mV, NS) or in CON (0.22 ± 0.05 vs. 0.24 ± 0.18 mV, NS). RMS activity of the VL across the RSA test decreased relatively to the first sprint (set to 100%) but to a similar extent in RSH (91 ± 8% vs. 89 ± 9%, NS), RSN (86 ± 11% vs. 87 ± 12%, NS) or in CON (89 ± 10% vs. 88 ± 6%, NS) in Pre- vs. Post-, respectively

mRNA selected gene transcripts and enzyme activity. Figure 6 displays Pre- and Post- mRNA expression levels in RSH, RSN and CON groups. In Post- compared to Pre-, mRNA gene concentrations of hypoxia inducible factor (HIF-1α, +55%, $p<0.05$), carbonic anhydrase III (CA3, +35%, $p<0.05$), monocarboxylate transporter-4 (MCT-4, +20%, $p<0.05$) and lactate dehydrogenase (LDH, +12%, $p<0.05$) were augmented in RSH only. Conversely, mitochondrial transcription factor A (TFAM), peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α) and monocarboxylate transporter-1 (-36%, $p<0.01$) concentrations were decreased in RSH only (-40% and -23% respectively, $p<0.01$) (Fig. 6). From Pre- to Post-, LDH activity was increased in RSH (63.6 ± 13.8 vs. 71.3 ± 14.2, $p<0.05$) but not in RSN (56.3 ± 14.8 vs. 61.7 ± 16.2, NS). Citrate synthase activity was not different and did not vary significantly in RSH (8.6 ± 2.5 vs. 10.6 ± 2.7, NS) and in RSN (8.5 ± 3.1 vs. 8.5 ± 3.8).

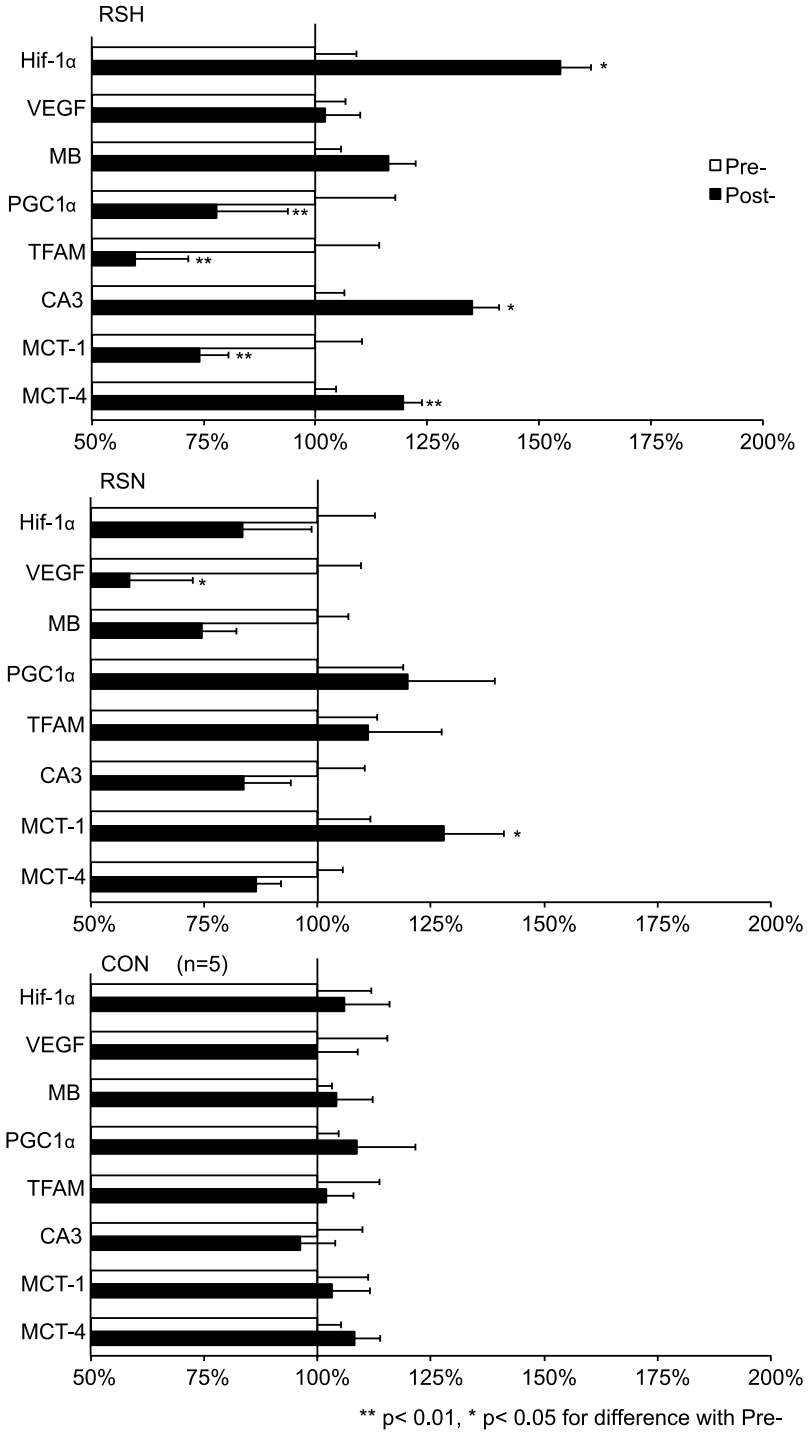

Figure 6 Relative mRNA expression of selected gene transcripts after 4 weeks of specific repeated sprint training before (Pre-) and after (Post-) the specific repeated sprint training in hypoxia (RSH), in normoxia (RSN) or in control group (CON). Open bars represent Pre- values and solid bars Post- values of mRNA concentrations in *vastus lateralis* muscle. Post- values were expressed in % compared to Pre- values (set to 100%). HIF-1α, hypoxia inducible factor-1α; VEGF, vascular endothelial growth factor; MB, myoglobin; PGC1-α, proliferator-activated receptor gamma coactivator-1α; TFAM, mitochondrial transcription factor A; CA3, carbonic anhydrase III; MCT-1, monocarboxylate transporter-1; MCT-4, monocarboxylate transporter-4; LDH, lactate dehydrogenase.

Discussion

The principal novel finding of this investigation is that repeated sprint training in hypoxia allows further enhancement of repeated-sprint performance to exhaustion than the same training in normoxia. Second, in RSH, the amplitude of blood flow variations during sprint phases was increased. Third, the increased mRNA expression of factors involved in pH regulation and glycolysis as well as the decrease in factors involved in mitochondrial biogenesis after RSH suggest a potential enhancement of the glycolytic (but not oxidative) activity in muscle.

Repeated sprint ability and cycling performance improvement. RSH and RSN elicited similar significant power improvements (~6-7%) but hypoxic training only additionally delayed the decrease of power output and therefore extended the number of sprints performed. The ability to reach a higher PF and maintain it high during the last 5 s of the successive sprints explains the power improvements both in RSH and RSN. Indeed, maximal power production (e.g., during repeated sprints) is related to the ability to recruit more FT at very high PF [28] and FT are known to be essential in the power production when intensity increases [29]. Consequently, the observed increase in power can be attributed to an improved muscle recruitment strategy (e.g., additional FT motor units [30]) and inter-muscle coordination [22]. Noteworthy, like RSH, RSN improved average and maximal power output of the sprints; so the specific training performed is useful compared to CON in improving power during repeated sprints.

In addition, the aerobic performance (3-min all-out) was not modified after training (Table 4). Similarly, improvement of single 10-s sprint and Wingate performance did not differ between RSH and RSN. This was expected since intermittent training in hypoxia is known to be rather inefficient for additional performance improvements in endurance athletes, when compared to similar training performed in normoxia [6].

Blood flow variations and delayed exhaustion during RSA test.

Δ[tHb] represents changes in blood volume in the muscle (i.e. blood perfusion) [31] so it is expected to vary widely during the RSA test. Since PF was higher in Post- and Δ[tHb] is influenced by cycle frequency [32], the increase in $\Delta[tHb]_{av}$ was expected and could contribute to higher power production both in RSH and RSN. Then, during the repeated sprint test, fatigue appeared rapidly, highlighted by the power decrement (~33-35%, Fig. 3) throughout the set. At the muscular level, waste metabolites accumulation and energy supply are certainly essential limiting factors in RSA performance [33]. During RS phosphocreatine breakdown is very high [12] and inorganic phosphate (P_i) accumulates in muscle. Since increased P_i levels may decline force, especially in FT recruited during such fatiguing exercise [34], an improved waste metabolites removal when blood flow is raised [35] might delay fatigue during a RSA test. This is in accordance with the greater increase in $\Delta[tHb]_{av}$ during sprints in RSH compared to RSN and

the increase of the Δ[tHb] to power ratio, so it can be argued that blood perfusion was improved in RSH and could explain additional sprints performed in RSH.

Moreover, during sprinting, due to the high PF, FT are predominantly recruited [28,36]. FT are mainly glycolytic [37] and contribute highly to the energy supply during RS [33]. More sprints in RSH could then be explained by an improved behavior of FT although it requires to optimize the contribution of anaerobic glycolysis known to be impaired as sprints are repeated [33]. Indeed, due to the poorer O_2 delivery to working FT (compared to slow twitch (ST)) and their greater fractional O_2 extraction if highly perfused [16], these fibers are likely to benefit more from the higher blood perfusion during sprints in RSH. This could help enhancing microvascular O_2 delivery to FT, minimizing substrate level phosphorylation and intracellular perturbation (e.g., P_i accumulation and decreased pH), thereby "making FT to behave more like their oxidatively efficient ST counterparts" [38]. Then, with more FT recruited and working with less anaerobic energy dependence, fiber fatigue could potentially be diminished [39].

Interestingly, a recent study underlined a higher decrease in power output but with similar [tHb] kinetics during a RSA test performed in hypoxia compared to normoxia [40]. However, during our supervised training, RS power output did not decrease more in RSH than in RSN (since total work was similar (Table 3)). This could be explained by the training including only sets of 5 repeated sprints and therefore limiting power decrements. Then the improved [tHb] kinetics (i.e. Δ[tHb]) we observed might have been elicited by the greater exercise:rest ratio used (1:2) inducing shortened recoveries.

When exercising, the O_2 availability to demand ratio is lowered and muscle tissue oxygen delivery is maintained by an increased extraction of O_2 in blood [41]. A more severe reduction of the ratio (e.g., during repeated sprints) requires an adapted arterial flow control (e.g., vasodilatation) to ensure adequate tissue perfusion [10]. In addition, during exercise in hypoxia the compensatory vasodilatation (with an increase in blood perfusion) aims at maintaining constant the total O_2 delivery to the muscle [10]. Alongside, in hypoxia there is an increased sympathetic vasoconstrictor activity directed towards skeletal muscle [42] that occurs to a greater extent within FT (glycolytic type II fibers in rat) [43]. However, "the compensatory vasodilatation prevails over the vasoconstrictor response" and exercise intensity is essential in the amplitude of this compensatory vasodilatation [10]. So, maximal exercise intensities (e.g., in sprints) may maximize this amplitude. Though muscle fatigue attenuates the vasodilatory responsiveness [44] and the delayed fatigue during RSA test in RSH could then partly be due to an improved responsiveness of the vascular bed. Interestingly, blood flow and vascular conductance were shown to be augmented mostly in FT after dietary nitrate supplementation [43]. In accordance with the increase in $\Delta[tHb]_{av}$ in RSH (Fig. 4B), one may finally hypothesize that RSH enhancing predominantly FT behavior could participate to the improved blood perfusion through nitric

oxide mediated vasodilatation mechanisms [10]. However, it must be admitted that data collected by NIRS might only represent muscle perfusion indirectly and should be interpreted with care. In addition, we did not observe significant differences in thigh muscle electromyography between groups. Nevertheless voluntary activation was reported not to differ during short-term hypoxia even at a higher altitude [45].

Still, the proposed mechanism based on the preferential recruitment and modified behavior of FT with an enhanced vasodilatory compensation induced in RSH is appealing as it could also explain why previous IHT studies [6-8] with lower exercise intensities failed to demonstrate additional benefits to endurance performance when compared to similar normoxic training. In these latter studies, FT fibers recruitment might not have been high enough since intensity was 2-4 fold lower than with the present RS training.

Molecular adaptations after specific training. Besides, performance and blood perfusion results are in accordance with the expression levels of some mRNAs. Our protocol including only brief periods of hypoxic training was sufficient for inducing a significant upregulation of HIF-1α. Such elevation in HIF-1α mRNA leads to a downstream activation of HIF-1 dependent pathway [46]. The subsequent increase in mRNA expression of CA3 and improved activity of LDH additionally suggests an increased capacity for pH regulation [47], although no significant difference in blood lactate was observed between Pre- and Post-. Indeed we also observed a decrease in mRNA expression of genes implicated in mitochondrial biogenesis such TFAM and PGC-1α while LDH concentration was increased. Moreover, citrate synthase activity was not different between RSH and RSN suggesting that oxidative capacity was not different. Our study therefore indicates a shift from aerobic to anaerobic glycolytic activity in the muscle not in line with the previously suggested enhanced oxidative capacity after IHT [4,5,48]. In addition, we observed an upregulation of MCT-4 and a downregulation of MCT-1 in RSH. MCT-4 is mostly expressed in glycolytic tissues (i.e. FT) where it mediates lactic acid efflux [49] from the cells whereas MCT-1 is adapted to supply lactate to the cells for energy production [50]. Again, as MCT-4 (but not MCT-1) is upregulated by hypoxia [49], our results are in line with the hypothesis that more sprints performed during RSA were due mainly to modified FT fibers behavior. Although this study confirms an elevated HIF1-α mRNA expression after exercising at high-intensity during short exposures in hypoxia (and not in normoxia), the observed changes are largely different than the previously reported HIF-1 mediated muscle adaptations after IHT [5,48] but underlines specific adaptations in RSH. Yet we also confirm the key role of exercise intensity *per se* during hypoxic training [9]. Overall, the upregulation of genes involved in oxygen signaling (HIF-1a), oxygen carrying (Mb) and pH regulation (CA3) and the concomitant downregulation of genes implicated in mitochondrial biogenesis (TFAM and PGC-1α) suggest a shift from aerobic to anaerobic glycolytic activity in the muscle.

Conclusions

This study is the first to observe larger performance improvement after repeated sprint training in hypoxia than for to the same training in normoxia. Our main novel findings were that repeated sprint training in hypoxia leads to i) increased variations of blood perfusion possibly delaying fatigue during a RSA test and ii) specific molecular adaptations large enough for inducing further improvement in systemic RSA performance. Our results suggest an improved vascular conductance in repeated sprints to exhaustion where fast-twitch fibers are likely better utilized. Fatigue could potentially be delayed through FT working with less anaerobic energy dependence. In parallel to the increased blood perfusion and potentially better waste metabolites removal, modifications at the molecular level support a shift towards improved anaerobic glycolytic activity following RS training in hypoxia only.

In conclusion, this study reports the effectiveness of RSH for improving intermittent exercises performed in normoxia. The mechanisms are likely largely different than those previously associated to the inefficient IHT.

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Chapter 9

Article 5 - Hypoxic conditions and exercise:rest ratio are likely paramount.

9. Article 5 - Hypoxic conditions and exercise:rest ratio are likely paramount.

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We read with great interest the recent review of our colleagues, Francois Billaut et al.,[1] on the highly debated topic of 'altitude training in team sports'. While we acknowledge the authors for their excellent comprehensive review of the scarce literature that confirms, to a large extent, some of our previous views,[2] we wish to comment on two points of importance (i) the relative differences between hypobaric (HH) and normobaric (NH) hypoxia; and (ii) the difference between intermittent hypoxic training (IHT) and repeated sprints (RS).

We believe that these two points are para- mount for understanding the potential benefits of hypoxic training on RS and, more generally, on team-sport athlete performance and, therefore, that further investigation of these points should also be recommended prior to any evidence-based recommendations being made.

1. Physiological Differences between Hypobaric and Normobaric Hypoxia?

Although an increasing body of literature[3-10] suggests that physiological responses both to exercise and at rest are slightly different between HH and NH, and despite the obvious consequences in terms of periodization and practical use of the various types of the presented hypoxic methods, this point has been completely ignored in the review,[1] and therefore assumes that HH and NH can be used interchangeably, since the same inspired oxygen pressure (P_IO_2) would potentially produce the same physiological responses and benefits. However, differences in cardio-respiratory responses, fluid balance and nitric oxide metabolism have been described between NH and HH.[3-10] Importantly, for an equivalent partial pressure in O_2, the reduction in O_2 arterial content (C_aO_2) seems to be more marked in HH than in NH.[11] Regarding 'living high-training low' (LHTL), one may observe that the recommended minimal 'altitude' is generally higher for NH than for HH[2,12,13] and that some adjustments are sometimes integrated in experimental design.[14] We did report[5] that most of the LHTL studies conducted in NH did not induce any additional performance benefits (i.e. significant difference between the experimental hypoxic and the control normoxic groups),[14-20] in opposition to the LHTL studies conducted in HH[21-24] where, generally, benefits in both erythropoietic responses and/or performance were noted. The results of a meta-analysis[25] support the significantly larger additional benefits in performance (estimated by change in power output) in elite athletes for LHTL in 'terrestrial' HH than that in 'artificial' NH (4.0% vs 0.6%). However, since there is no data supporting any clinical difference regarding the efficiency of either 'living low-training

high' (LLTH) or LHTL in HH when compared with NH, any evidence-based recommendation would not be relevant at this stage.

Based on the recent literature,[3,26] another point to be questioned is the recommendation that IHT might "enhance endurance performance when subsequent exercise is conducted in hypoxia"[1] and the efficiency of pre-acclimatization strategies using LLTH or LHTL in NH for preparing matchplay at altitude. Fulco et al.[3] reported that pre-acclimatization in NH (assumed to be effective as shown by large increase in arterial saturation in O_2 [S_aO_2] or decrease in end-tidal pressure in O_2 [$P_{ET}CO_2$] between the first and last pre-acclimatization exposure in NH) induced only minimal benefits (e.g., negligible decrease in $P_{ET}CO_2$ or reduction in acute mountain sickness prevalence) in HH (Pikes Peak, 4300 m). More importantly, NH pre-acclimatization did not induce any reduction in the acute performance decrement (cycling or treadmill time trial) observed during the first 24 hours after arrival at 4300 m, whereas the improvement is ~15–20% in HH pre-acclimatized athletes (when compared with non-pre-acclimatized or sham groups, for whom impairment in endurance performance can be 60% or greater, when compared with sea level). To our knowledge, there are no well-controlled crossover studies comparing the efficiency of any HH versus NH pre-acclimatization on the change in performance during acute or longer exposure to real altitude in elite athletes. However, the influence of the barometric pressure is highlighted by both the study of Fulco et al.[3] showing a very low transfer of the benefits induced by an NH-acclimatization to HH responses and our recent study[10] where nitric oxide bioavailability was impaired while oxidative stress was increased in HH versus NH.

2. Intermittent Hypoxic Training or Repeated Sprints?

Our group recently showed that RS training in hypoxia allowed further enhancement of RS performance than the same training in normoxia.[27] By using a sprint to rest ratio of 1 : 2, we confirmed some molecular adaptations at the skeletal muscle level after hypoxic training cited by Billaut et al.[1] but showed for the first time that these adaptations are large enough for inducing further improvement in systemic RS performance. Our data highlight an upregulation of genes involved in oxygen signalling (HIF-1a), oxygen carrying (Mb) and pH regulation (CA3) and the concomitant downregulation of genes implicated in mitochondrial biogenesis (TFAM and PGC-1a) suggesting a shift from aerobic to anaerobic glycolytic activity in the muscle and a more efficient use of fast twitch (FT) muscle fibres after hypoxic RS training. This type of maximal-intensity intermittent training (e.g., RS) induces a large recruitment of the FT fibres. It is known that, relative to slow twitch (ST) muscle, FT muscle fibres experienced a greater

fractional O_2 extraction because of a poorer O_2 delivery to working FT fibres.[28] We believe that RS in hypoxia would lead to an enhanced microvascular O_2 delivery to FT, minimizing substrate level phosphorylation and intracellular perturbation (e.g., inorganic phosphate accumulation and pH), thereby making FT fibres behave more like their oxidatively efficient ST counterparts.[29] Then, with FT work- ing with less anaerobic energy dependence, fibre fatigue could potentially be diminished[30] and RS performance augmented. This hypothesis is appealing, as it could also explain why most of the previous IHT studies[31-34] conducted at non-maximal intensity (and therefore with a lower FT recruitment) failed to demonstrate any additional positive effects when compared with similar normoxic training. Despite the prudence of the authors "there is no clear trend about the effect of IHT on endurance performance,"[1] we question the fact that team-sport performance could be enhanced with IHT by improving endurance. So far, "it is safe to conclude that IHT does not increase exercise performance at sea level in endurance athletes any more than simply training at sea level."[34]

Since the exercise-to-rest ratio influencing, to a large extent, the oxidative versus glycolytic component[35,36] during RS is different between team sports (e.g., football vs ice hockey), one may hypothesize that the effectiveness of RS training in hypoxia will differ between these sports, but this remains to be investigated!

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Chapter 10

Article 6 - Repeated double-poling sprint training in hypoxia by competitive cross-country skiers.

10. Article 6 - Repeated double-poling sprint training in hypoxia by competitive cross-country skiers.

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Abstract

Purpose Repeated sprint training in hypoxia (RSH) was recently shown to improve repeated sprint ability (RSA) in cycling. This phenomenon is likely to reflect fiber-type dependent, compensatory vasodilation and, therefore, our hypothesis was that RSH is even more beneficial for activities involving upper-body muscles, such as double-poling during cross-country skiing.

Methods In double-blinded fashion, 17 competitive cross-country skiers performed 6 sessions of repeated sprints (each consisting of 4 sets of 5 10-s sprints, with 20-s intervals of recovery) either in normoxia (RSN, 300 m, F_iO_2 = 20.9%, n=8) or normobaric hypoxia (RSH, 3000 m, F_iO_2 = 13.8 %, n=9). Before (Pre-) and after (Post-) training, performance was evaluated with a RSA test (10 s all-out sprints - 20 s recovery, until peak power output declined by 30%) and a simulated team sprint (TS, 3 x 3 min all-out with 3 min rest) on a double-poling ergometer. *Triceps brachii* oxygenation was measured by near-infrared spectroscopy.

Results From Pre- to Post-, peak power output in the RSA was increased ($p<0.01$) to the same extent (29 ± 13% vs. 26 ± 18%, NS) in RSH and in RSN while the number of sprints performed was enhanced in RSH (10.9 ± 5.2 vs. 17.1 ± 6.8, $p<0.01$) but not in RSN (11.6 ± 5.3 vs. 11.7 ± 4.3). Additionally, the amplitude in total hemoglobin variations during sprints throughout RSA rose more in RSH ($p<0.01$). During TS, power output improved by 11 ± 9% in RSH and 15 ± 7% in RSN

Conclusion Our findings reveal greater improvement in the performance of repeated double-poling sprints, together with larger variations in the perfusion of upper-body muscles in RSH compared to RSN.

Keywords: altitude training, repeated sprints, cross-country ski, performance

Introduction

For many years now, coaches and athletes have combined the stress of training with hypoxia to elicit even greater physiological adaptations (11, 14, 26, 38, 40). First, using low-intensity aerobic exercise designed to enhance oxygen transport; and, more recently, with high-intensity interval-training or repeated sprints.

However, a recent review concludes that the additional benefits for sea-level performance of intermittent hypoxic training (IHT) compared to similar training under normoxic conditions are strikingly small (13). At the same time, three interesting investigations reveal that a novel approach involving repeated sprint training in hypoxia (RSH) does provide additional systematic benefits in comparison to the same training under normoxic conditions (14, 16, 32).

Since RSH is based on repetition of "all-out" efforts of short (≤30 s) duration separated by short periods of incomplete recovery, the efficiency of this strategy probably depends on the maximal intensity of the successive bouts of sprinting (separated by very short periods of recovery) to evoke potent adaptations at the molecular level and, possibly, in the delivery of O_2 to the mitochondria (14). Indeed, the intensity of hypoxic training *per se* appears to modulate muscle performance at the molecular level with "adaptations that compensate for the reduced availability of oxygen during exercise"(24). Moreover, the limitation of O_2 delivery during exercise triggers compensatory vasodilation, shifting blood flow upwards in attempt to maintain sufficient delivery of O_2 to muscles to an extent dependent on the intensity of the exercise (9).

Repeated sprints under hypoxic conditions may benefit from such adaptive mechanisms, perhaps stimulating the diffusion of O_2 into working muscles (9, 14). We have reported that the repeated sprint ability (RSA) of trained cyclists improved significantly after as few as 8 sessions of RSH (14). In that study, the additional benefit of RSH was thought to involve improved O_2 extraction by the fast-twitch fibres (FT) (which are those predominantly recruited during sprints (21)) as a result of the high-intensity repeated sprinting (27). Greater amplitudes of muscle blood perfusion variations suggesting enhanced muscle blood flow supported this hypothesis of a greater O_2 utilization by FT after RSH.

Accordingly, physical activity involving extensive recruitment of FT may benefit most from RSH. Of interest in this context is the fact that upper-arm muscles contain a high proportion of FT (25). For example, in professional tennis players, 2/3 of all the fibers in the *m. triceps brachii* are fast-twitch, while the corresponding value for the *m. vastus lateralis* is only 1/3 (33). Moreover, in elite cross-country (XC) skiers the proportions of FT in the *triceps brachii* and *vastus lateralis* are 48% and 32%, respectively (31).

Consequently, if RSH improves the extraction of O_2 by FT, the benefits seen in trained cyclists should certainly occur in XC skiers as well.. During double-poling XC skiing, upper-body muscles contribute substantially to power production (23) and even if this sport is thought to be primarily aerobic, recent developments include the introduction of shorter racing formats, such

as individual and team-sprints. Such events require greater anaerobic capacity and upper-body power (34) and, together with the repeated changes in velocity and intermittent sprints that occur during mass-start races, underline the decisive role of sprinting ability for the outcome of a competition. Clearly, improving RSA may give XC skiers (including top athletes) a competitive edge.

Nonetheless, the potential associated benefits for the ability to repeat sprints in normoxia after specific RSH vs. RSN have never been investigated in a randomized, double-blind controlled study in XC skiers. We hypothesized that repeated sprint training in hypoxia improves the performance of competitive XC skiers more than the same training under normoxic conditions.

Methods

Subjects. 17 highly trained XC skiers (11 males and 6 females – Table 1) were recruited within Swedish national and regional cross-country and biathlon teams to volunteer in this study. Subjects were familiar to the double-poling ergometer and to high intensity exercise bouts from their usual training practice and competitive background. Subjects were all non-smokers lowlanders. None of the subjects were acclimatized or recently exposed to altitude and were not exposed to an altitude of more than 500 m during the protocol. Subjects provided their written informed consent after the state medical ethics committee approved the experiment (Regionala etiksprövningsnämden, Agreement 2013-72-31M, Umeå, Sweden) performed according to the Declaration of Helsinki.

Study design. Experimental protocol consisted in two testing sessions before (Pre-) and after (Post-) a specific repeated-sprints training period of two weeks (3 sessions per week). Subjects were assigned into a specific training group: repeated sprint training in hypoxia (RSH, n=9, 6 males, 3 females) or repeated sprint training in normoxia (RSN, n=8, 5 males, 3 females).

Training protocol Training consisted in six sessions of repeated double-poling sprint over 2 weeks on a XC ski ergometer including each time 4 sets of 5 sprints of 10 s interspersed with 20 s of recovery (Figure 1). Warm up and recovery periods between sets were performed with an individualized resistance of 1 $W \cdot kg^{-1}$.

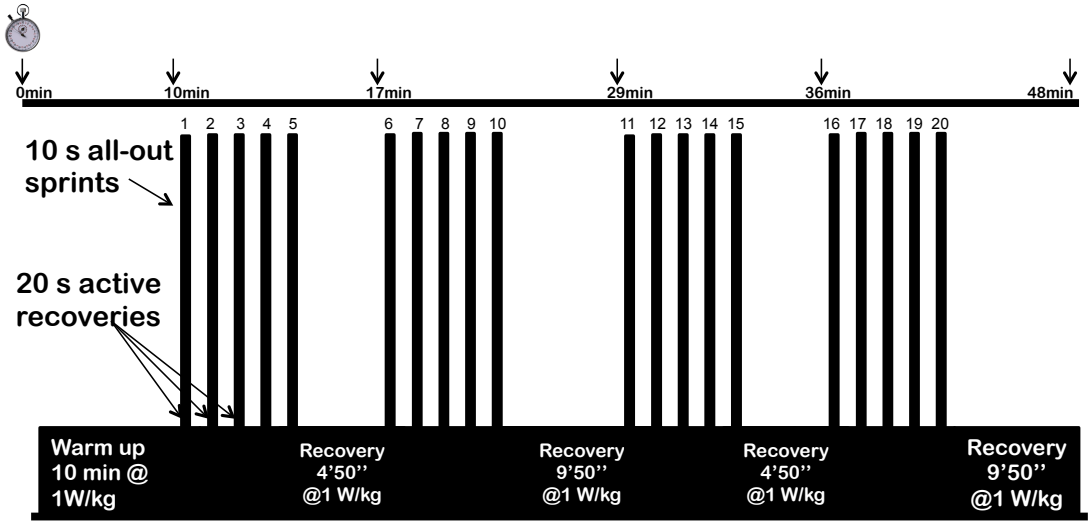

Figure 1 Description of one training session

For all training sessions, subjects wore a comfortable oro-nasal mask allowing normal breathing. Subjects breathed either room air (for RSN) or hypoxic air (for RSH) provided via the mask through a hose (suspended to the ceiling by a supportive elastic band) connected with a three-way valve to an altitude simulation device (Altitrainer, SMTech, Nyon, Switzerland). For RSH, ambient air was mixed with nitrogen (from pressurized tanks) and inspired oxygen fraction was reduced to 13.8 ± 0.1% in order to simulate an altitude of 3000 m. Two independent researchers assigned subjects randomly to RSN or RSH with groups matched upon subjects' peak oxygen uptake measured during the team sprint simulation aerobic test in Pre-. Helpers then handled the altitude simulation device to provide either normal air or hypoxic air during training by turning the three-way valve so that two subjects training simultaneously inspired either air from the altitude simulator or from the room in the well-ventilated laboratory at a constant temperature of ~24 °C. This way, the altitude simulator (with the nitrogen outlet sounding) was running during all training sessions. Subjects and investigators were subsequently unable to know if training was performed in hypoxia or normoxia and the protocol was run double-blinded. Subjects were asked to avoid any strenuous training outside of the protocol but to keep their usual aerobic training sessions that were reported in a training diary.

Subjects filled a questionnaire reporting their physical activity and food and drinks intake during the 48 h prior each testing session. During these 48 h, subjects were asked to refrain from any training or exhaustive activity. Due to the extreme intensity of the tests, subjects were asked not to report to the laboratory on an empty stomach. A standard breakfast (bread with jam and water) was therefore advised. Subjects were asked to reproduce the last meals avoiding alcohol and caffeine intakes during the 24 h before each test. A preliminary visit allowed subjects to familiarize with the ergometer by completing a 15 min trial including two times 3 repeated sprints of 10 s.

Performance tests

Repeated sprint ability test RSA was tested with 10 s all-out double-poling sprints repeated with incomplete recoveries of 20 s until task failure.

Subjects first performed a 4 min 50 s aerobic warm-up at 1 W·kg^{-1}followed by an isolated 10 s all-out sprint, 4 min 50 s active recovery at 1 W·kg^{-1}and a second isolated 10 s all-out sprint. After 10 min passive recovery seated (including 5 min with the right arm kept still to calibrate the NIRS device) the RSA test started with 1 min at 1 W·kg^{-1}before the first sprint.

Subjects were given very strong verbal encouragement to perform as many sprints as possible until task failure. Task failure was set at 70% of the highest peak power reached. Subjects were given a warning when they did not reach that value the first time and the test was ended the second time. Subjects were not told the criterion for task failure or any indication on the number of sprints performed. In addition, to avoid any protective pacing strategy, peak power during the first two sprints was requested to reach at least 95% of the best peak power of the two isolated sprints, which was the case in all subjects. Unfinished sprints (subjects not being able to pull anymore) were not taken into consideration.

Team sprint simulation aerobic test Exactly 45 minutes after the end of the RSA test, subjects were asked to warm-up again by completing three incremental steps of 4 min at 1, 1.5 and 2 W·kg^{-1}. Then, after additional 5 min passive rest, subjects were asked to perform a team sprint simulation (TS) consisting of 3 bouts of 3 min all-out interspersed with 3 min active recovery at 0.5 W·kg^{-1}. Strong verbal encouragement was given throughout the test and poling frequency was instructed to be as high as possible all time to elicit maximal all-out performance. Information about elapsed time was only given during the final minute every 15 seconds in order to prevent pacing and further motivate subjects. A peak in oxygen consumption ($\dot{V}O_{2peak}$) was calculated as the highest 30 s average value during the test. Although residual fatigue from the RSA test was probable, TS was performed for all subjects at the same moment of the testing sequence allowing for a good comparison of subjects' aerobic capacity (average power of all 3 min bouts).

Equipment and measured variables A specially designed double-poling ergometer (SkiErg, Concept2, Morrisville, USA) was used for all training sessions and performance tests. The ergometer is equipped with cross-country ski handles and straps (Leki, Kirchheim, Germany). Power is produced by pulling cords spinning a wind resistance flywheel. A damper located on the flywheel housing controls the airflow and thus the necessary work to accelerate the flywheel during each stroke. The flywheel deceleration rate (called drag factor by the manufacturer) displayed digitally on the ergometers interface was used to set and reproduce the resistance individually for each test and training session. Drag factor was set at 130% and 110% of body weight in male and female subjects, respectively. To simulate natural XC skiing double-poling movement subjects stood with their own cross-country ski boots in bindings screwed in a

wooden plate. The distance between the bindings and the ergometer was freely chosen by the subjects to mimic their habitual position and reproduced for each test and training session. A wide belt around subjects' hips was additionally attached to the wall behind them with an elastic band in order to induce some resistance at the hips to better simulate the double-poling sprinting movement (Figure 2).

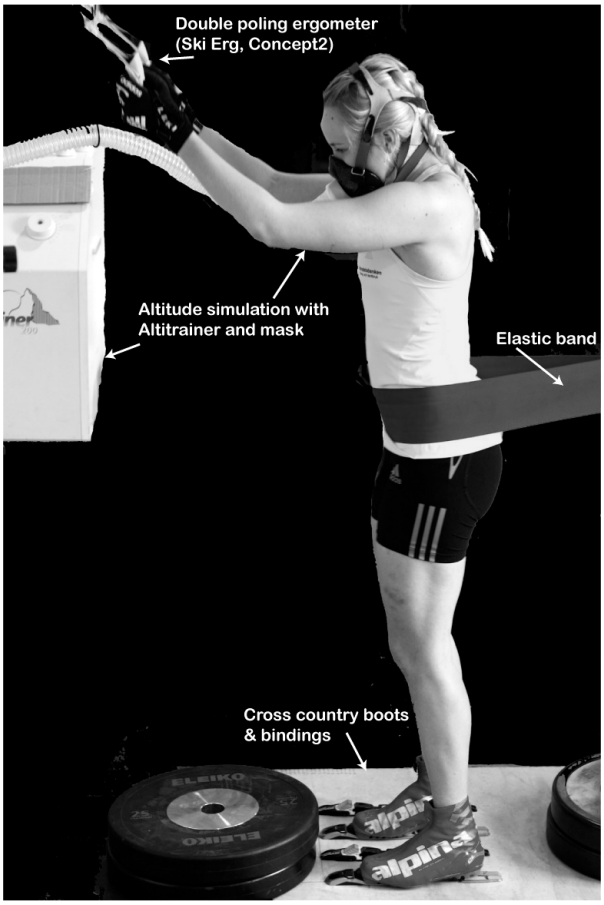

Figure 2 Ergometer setup for cross-country ski double-poling

The ergometer displays instantaneous power output and cycle frequency for each stroke that were recorded externally in a spreadsheet (Excel, Microsoft, Redmond, USA) using a Microsoft ActiveX® software component to extract data live.

A peak in oxygen consumption ($\dot{V}O_{2peak}$) was determined during TS in Pre- and Post- with an ergospirometry device (AMIS 2001, Innovision A/S, Odense, Denmark) as the highest 30 s average measured.

Muscle oxygenation, blood lactate, blood pH, hematocrit, blood hemoglobin concentration, arterial oxygen saturation, heart rate and rate of perceived exertion were measured at rest and at the end of each performance test as well as during the first and sixth training session (at rest and after the final sprint).

Muscle oxygenation was measured using a near-infrared spectroscopy (NIRS) technique as described elsewhere (5). The NIRS device (Portamon, Artinis, Zetten, The Netherlands) was used to measure changes in muscle oxygenation by placing a triple optode sensor on the muscle belly of the right arms *m. triceps brachii* with an interoptode spacing of 40 mm. The probe was protected with a thin transparent plastic sheet and then attached to the skin with double-sided tape and firmly fastened with an opaque cotton elastic band wrapped around subjects' arm. Position of the probe was marked during Pre- with a permanent pen for accurate repositioning. Differential pathlength factor (DPF) was set to 3.79 and 4.67 in male and females respectively (12). All signals were recorded with a sampling frequency of 20 Hz. They were down sampled at 10 Hz using Matlab (Matlab Software, Nattick (MA) USA) routine resample. Then a 10th-order low-pass zero-phase Butterworth filter (cutoff frequency 0.1 Hz) was applied to the resampled signals in order to remove possible artifacts and smooth the movement-induced perturbations. Automatic detection of the start and end times of the successive sprints was obtained by estimating the filtered deoxyhemoglobin signal upper and lower envelopes using local minima and maxima in a sliding window of length 400 samples (4 s). The starting and end times were obtained as the times of contact between the envelopes and the signal. This allowed the determination of maximum and minimum for each signal during the successive sprint and recovery phases. Concentrations for oxyhemoglobin ($[O_2Hb]$), deoxyhemoglobin ([HHb]) and total hemoglobin/myoglobin ([tHb]) were recorded. Since [HHb] values were proposed to be less sensitive than $[O_2Hb]$ to blood flow variations (10) and changes in O_2Hb signals might be confounded by rapid blood volume changes during sprints (6) only [HHb] and [tHb] were analyzed for relevant interpretations. Differences between maximum and minimum concentrations were defined as the amplitude of the variation for each sprint (Δ[tHb] and Δ[HHb]) and Δ[tHb] was used as an index of blood perfusion (14). Thus, for example, at the beginning of each sprint a maximum in [tHb] is observed at the beginning of each sprint (i.e. end of each recovery period) and [tHb] decreases to reach a minimum value at the end of each sprint. The concentration recorded during a 3-min seated rest with no arm movement was set as 100% for standardization (Fig. 4). So amplitudes for each sprint (i.e. Δ[tHb] and Δ[HHb]) and the average value for all sprints throughout the RSA test (i.e. $\Delta[tHb]_{av}$ and $\Delta[HHb]_{av}$) were calculated.

Surface electromyography (EMG) signals were recorded from the *triceps brachii*, *latissimus dorsi*, *rectus abdominis*, and *soleus* muscles from one side of the body (randomized, right or left) using surface electrodes (Ambu A/S, Ballerup, Denmark), TeleMyo 2400 T G2™ data logger (Noraxon, Cologne, Germany) and MyoResearch XP Master Edition® Software V.1.08.27 (Noraxon, Scottsdale, USA). Surface electrodes (Ambu® Blue Sensor N, Ambu A/S, DK) were positioned with 20 mm of inter-electrode distance on the prepared skin (impedance<5kΩ) after

muscle belly identification by palpation of the contracted muscle and marked for proper repositioning during Post- testing. Athletes performed a series of previously practiced 5 s maximal isometric voluntary contractions (MVC) to normalize the data to each individual. Prior to calculation of EMG variables, all raw signals were processed first to determine the MVC using the IIR filter with a bandpass 20 – 400 Hz Butterworth approximation, then treated with a EMG reduction in the *latissimus dorsi* channel, followed by RMS smoothing with a window of 100ms, and amplitude normalization to the peak value with a window of 250ms. Calculations were performed to assess the mean amplitude expressed in % of MVC during both performance tests. Analyses of the RSA included calculation of the average of all cycles performed during each 10 s sprint period. 30 s averages were used for the analysis of the TS data.

Blood lactate concentration was measured using a Biosen lactate measurement device (C-Line Sport, EKF Industrial Electronics, Magdeburg, Germany) from 20 µL fingertip capillary blood. Blood pH, haematocrit, haemoglobin concentration and arterial oxygen saturation (SO_2) were assessed by collecting 20 µL earlobe capillary blood analysed immediately in a blood analyser (ABL800 CO-OX Flex, Radiometer, Copenhagen, Denmark). Heart rate was recorded at 1 Hz by telemetry (RS800, Polar OY, Kempele Finland). Rate of perceived exertion was assessed for the legs, the arms and breathing using a 6-20 Borg scale.

Statistical analyses Data are presented as mean (SD). Performance and blood perfusion changes during RSA test were first evaluated with a 2-way (training group x sprint number) general linear model repeated-measures ANOVA with all pairwise multiple comparison procedures (Holm-Sidak method). Performance improvement, muscle oxygenation during RSA, blood lactate and other single variables test were then evaluated with 2-way (training group x time (Pre- vs. Post-)) general linear model repeated-measures ANOVAs with all pairwise multiple comparison procedures (Holm-Sidak method). All analyses were made using Sigmaplot 11.0 software (Systat Software, CA, USA). Null hypothesis was rejected at $p<0.05$.

Results

Total work and training intensity during supervised training were similar during RSN and RSH (Table 2). In addition to specific training in the laboratory, subjects completed a similar amount of aerobic training between Pre- and Post- of 16.6 ± 6.6 h and 16.3 ± 6.5 h in RSH and RSN, respectively. Total hypoxic exposure was 4.8 ± 0.2 h in RSH.

RSA Performance. Performance results at Pre- and Post- are summarized in Table 3. No significant differences between groups were observed in any variable in Pre-. During the RSA test, peak power output (29 ± 13% vs. 26 ± 18%, NS) and likewise the average power of all sprints (18 ± 16% vs. 22 ± 14%, NS) increased ($p<0.01$) to the same extent from Pre- to Post- in RSH and in RSN. The number of sprints prior to exhaustion was increased in RSH (from 10.9 ± 5.2 to 17.1 ± 6.8, $p<0.01$) but not in RSN (11.6 ± 5.3 and 11.7 ± 4.3, NS) (Figure 3). In Post-, compared to Pre-, 10-s peak power in the successive sprints was significantly improved till the fifteenth sprint in RSH and till the eleventh in RSN. Significant group (RSH vs. RSN) by time (Pre- vs. Post-) interactions were found in the number of sprints ($F=11.22$; $p=0.004$) and total work ($F=8.11$; $p=0.01$) performed during the RSA test.

Isolated 10-s sprint peak power output and TS performance increased ($p<0.01$) similarly in RSH and RSN (Table 3).

Muscle oxygenation. Successive values in Δ[tHb] during the RSA test are displayed in Figure 4. After training, $\Delta[tHb]_{av}$ increased to a greater extent ($F=35.9$, $p<0.001$) in RSH (from 212 ± 12% to 713 ± 21%, Pre- to Post-) than in RSN (from 186 ± 19% to 361 ± 30%, Pre- to Post-) (Figure 4B). From Pre- to Post-, $\Delta[HHb]_{av}$ increased significantly in RSH (+225%, $p<0.01$) but decreased in RSN (-27 %, $p<0.01$). There was a significant training group x time interaction for $\Delta[HHb]_{av}$ ($F=551.8$, $p<0.001$) .

Surface Electromyography. From Pre- to Post-, the mean amplitude of the activity of all muscles taken together (expressed as the sum of the % of MVC of all muscles) did not change in RSA test neither in RSH (119.1±22.2 vs. 121.6±17.7%, NS) nor in RSN (118.2±27.7 vs. 121.3±76.2%, NS). Similarly, the mean amplitude (all 3-min bouts taken together) did not change during TS neither in RSH (205.8 ± 76.0 vs. 216.9 ± 69.6%, NS) nor in RSN (204.0 ± 49.8 vs. 176.0 ± 34.0%, NS).

	RSN (n=8)		RSH (n=9)	
	Pre-	Post-	Pre-	Post-
Body mass (kg)	74.7 ± 7.8	74.7 ± 7.6	72.2 ± 7.0	72.3 ± 7.1
Hematocrit (%)	46.0 ± 3.2	45.7 ± 2.3	46.5 ± 2.0	46.1 ± 1.8
Hemoglobin ($g \cdot l^{-1}$)	154 ± 13	152 ± 15	156 ± 7	152 ± 12
VO_{2peak} ($l \cdot min^{-1}$)	4.2 ± 0.8	4.0 ± 0.8	4.1 ± 0.6	4.2 ± 0.7

Table 1 Subjects' characteristics before (Pre-) and after (Post-) repeated sprint training in normoxia (RSN) or in hypoxia (RSH).

VO_{2peak}, peak oxygen consumption

	RSN	RSH
Total work of all training sessions (kJ)	1290 ± 197	1335 ± 137
Mean peak power output (W)	482 ± 149	481 ± 131
Mean heart rate (bpm)	149 ± 11	144 ± 13
Mean SO_2 after last sprint of each session (%)	94.3 ± 1.3	86.0 ± 4.2#
Mean RPE « Arms »	17.6 ± 0.7	16.9 ± 1.1
Mean RPE « Legs »	16.7 ± 1.4	17.0 ± 1.8
Mean RPE « Breathing»	18.3 ± 0.4	18.0 ± 1.0

Table 2 Parameters measured during supervised training in the normoxic (RSN) and hypoxic (RSH) training groups

SO_2, Arterial oxygen saturation ; RPE, Rate of perceived exertion (Borg 6-20 scale). $p<0.01$ for difference with RSN

	RSN		RSH	
	Pre-	Post-	Pre-	Post-
Single 10-s sprint average power (W)	441 ± 153	539 ± 158**	421 ± 122	534 ± 123**
RSA Peak Power (W)	437 ± 156	532 ± 158**	415 ±117	528 ± 121**
RSA Best average power (W)	365 ± 124	440 ± 128**	350 ± 91	437 ± 98**
RSA Mean power of all sprints (W)	315 ± 107	376 ± 111**	308 ± 94	352 ±78**
RSA Total work of all sprints (kJ)	33.1 ± 11.9	42.0 ± 16.6	30.6 ± 12.8	58.8 ± 24.4**#
RSA [La] ($mmol \cdot l^{-1}$)	11.0 ± 2.5	12.6 ± 2.1	11.1 ± 2.0	12.3 ± 2.9
RSA SO_2 (%)	93.2 ± 0.8	93.6 ± 1.4	94.2 ± 1.2	93.5 ± 2.2
RSA Blood pH	7.23 ± 0.04	7.22 ± 0.06	7.21 ± 0.05	7.22 ± 0.04
3x3- average power (W)	218 ± 62	239 ± 64**	219 ± 49	240 ± 46**
3x3- average power during first minute (W)	239 ± 64	264 ± 69**	243 ± 49	272 ± 50**
3x3- stroke rate (‘min^{-1})	60 ± 4	57 ± 4	64 ± 8	62 ± 9
3x3- [La] ($mmol \cdot l^{-1}$)	11.7 ± 3.0	11.9 ± 2.8	13.1 ± 2.2	14.1 ± 2.9

Table 3 Performance results before (Pre-) and after (Post-) repeated sprint training in normoxia (RSN) or in hypoxia (RSH)

RSA, repeated sprint ability test; [La] Blood lactate concentration; SO_2 Oxygen saturation. RSA parameters measured immediately after repeated sprint test.

3x3, 3 times 3-min all-out team sprint simulation

** $p < 0.01$ for difference with Pre- # $p<0.05$ for difference with RSN

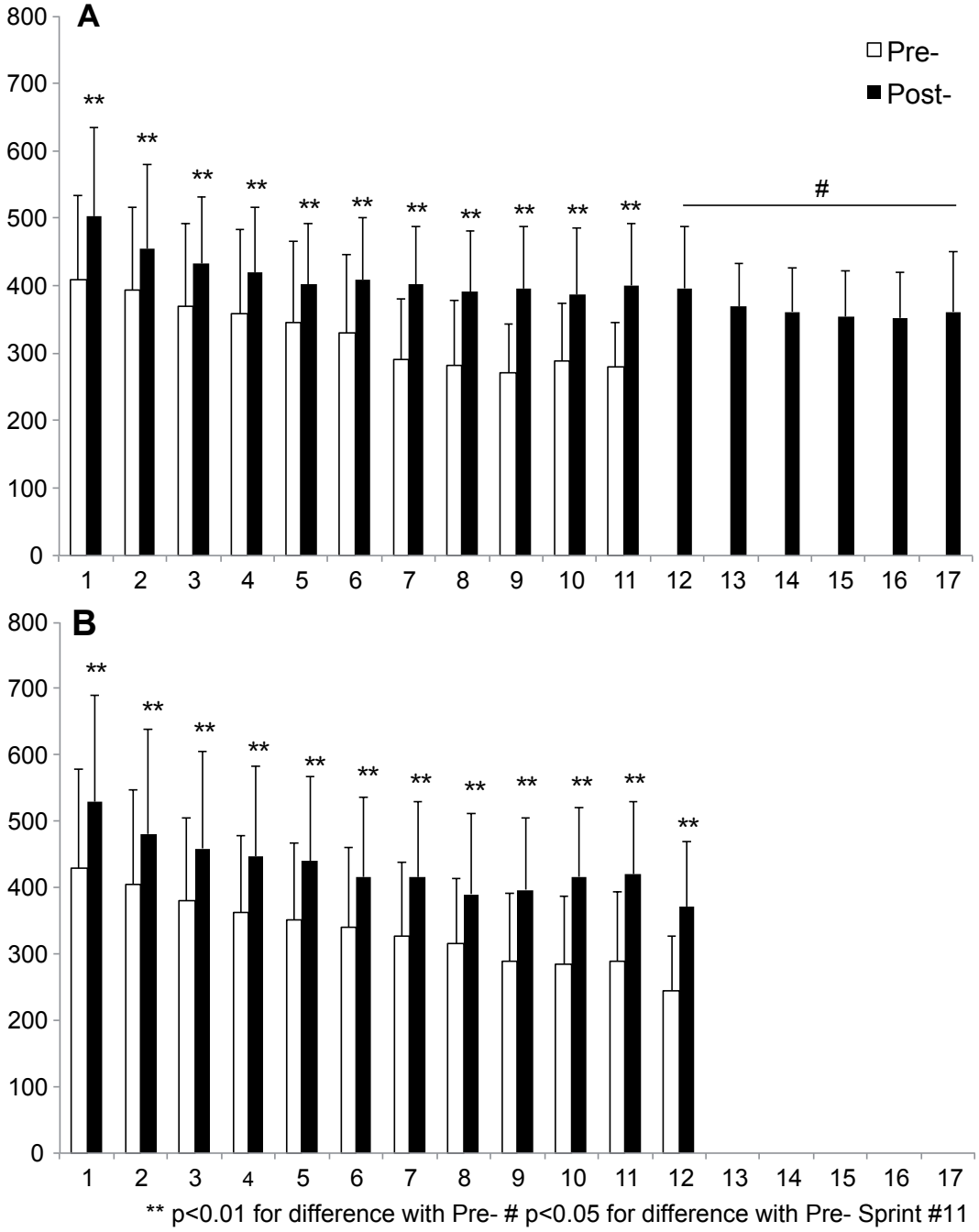

Figure 4 Peak power output (W) in successive sprints during the repeated sprint test before (Pre-) and after (Post-) specific repeated sprint training in hypoxia (RSH, A) or in normoxia (RSN, B)

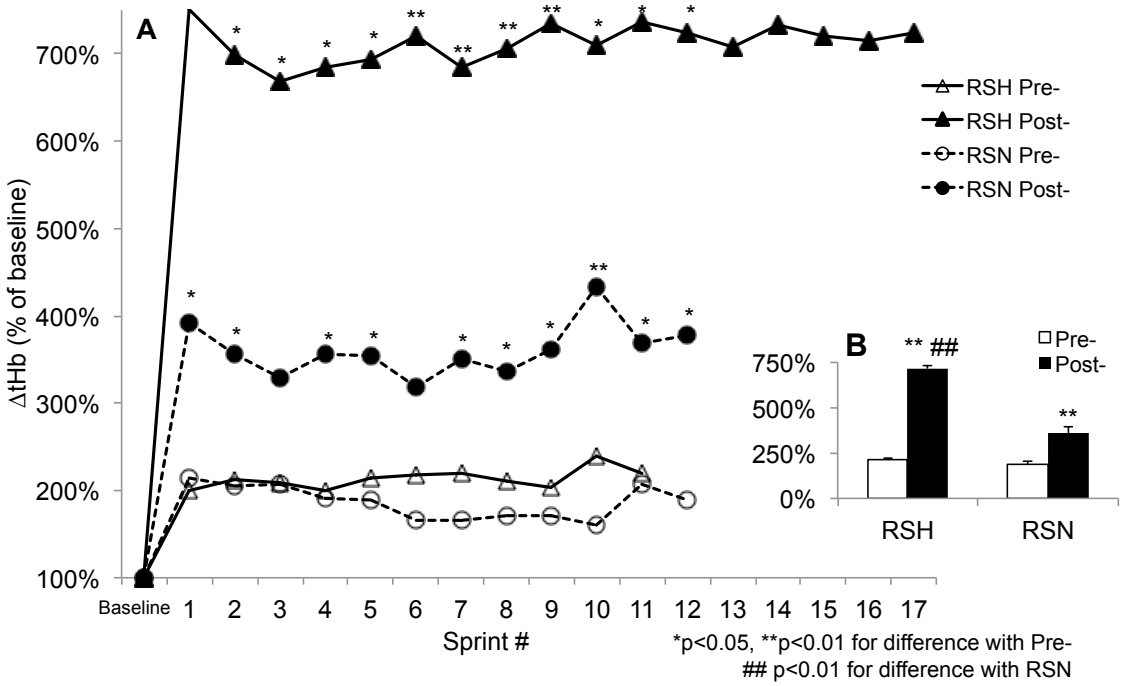

Figure 5 Δ[tHb]: successive changes in total hemoglobin concentrations' amplitude during sprints (expressed in percent compared to the resting baseline set to 100%) measured by near infrared-spectroscopy (A) and $\Delta[tHb]_{av}$: average of all changes during the repeated sprint test to exhaustion (B) before (Pre-) and after (Post-) the specific repeated sprint training in hypoxia (RSH), in normoxia (RSN)

Discussion

The main finding of the present investigation was that with competitive XC skiers repeated double-poling sprint training in hypoxia (involving large upper body muscle groups) resulted in greater improvement in repeated sprint performance than analogous normoxic training (RSN). Secondly, the amplitude of variations in blood perfusion during sprints increased to a greater extent in RSH than in RSN. And third, these differences were specific to repeated sprinting, i.e., performance in connection with an aerobic simulated team sprint is enhanced to a similar degree by RSH and RSN.

The present study confirms the potential of RSH as an innovative hypoxic training in line with similar benefits and underpinning mechanisms recently reported in cyclists (14). Interestingly, RSH in XC skiers appeared to delay fatigue during a RSA test with 58% more sprints performed after training. This represents a 20% greater benefit in sprint repetition compared to the results reported in cycling in a strictly similar RSA test (10s:20s) (14). While the cyclists in the latter performed sprints to exhaustion, XC skiers in this study were stopped during RSA when maximal power output during sprinting did not reach 70% of the best sprint anymore. In fact, this threshold was similar in the cycling study so it can be argued that RSH in XC skiers was more efficient than in cyclists. This was expected since we hypothesized that the maximal solicitation of FT during sprints in RSH would challenge adaptive mechanisms at the muscular level for sufficient O_2 extraction/delivery. With a higher proportion of FT in upper arms muscles (31, 33) contributing greatly to power production during double-poling (23), the mechanism of compensatory vasodilation particularly important in FT when O_2 tension falls (9) is enhanced after RSH and may explain why fatigue was more delayed during RSA in XC skiers than in cyclists.

Interestingly, a recent update of the current panorama of the hypoxic training methods now distinguishes RSH from traditional IHT methods, predominantly based on other expected adaptive mechanisms (e.g., improvement of the muscle oxidative or buffering capacities) (29). Indeed, a current review questioned the efficiency of IHT and highlighted that out of 20 studies involving IHT, only 4 bring additional benefits in performance-related variables compared to similar training in normoxia (13).

We hypothesized that cross-country double-poling would substantially benefit from fiber-type selective mechanisms allowing a better O_2 extraction by FT after RSH since upper body muscles are largely contributing to power production (23) and especially upper arms muscle comprising a high proportion of FT (25, 31, 33).

Mechanistically, the increase in cardiac output ($\dot{Q}$) tightly matches the O_2 delivery by elevating blood flow and interestingly the rise in $\dot{Q}$ is mainly regulated by peripheral vasodilation changes (with less importance of HR increase) (2). Then, high-intensity intermittent training (e.g., knee-

extension) was reported to increase the capacity for maximal exercise vasodilation by 20-30% (8). The latter may in turn explain partly the two-fold higher amplitude of blood perfusion variations observed after RSN (Fig. 4B). While vascular conductance may not be a limitation to O_2 transport to the thigh muscle (1), arm muscles may incur a blood flow limitation when other muscles are involved during the exercise (39) like during intense double-poling exercise known to recruit greatly upper body muscles but also trunk and leg muscles (23). This hypothesis is in accordance with the vasoconstrictor signals opposing the vasodilatory metabolites observed during upright whole body exercise (7).

Furthermore, hypoxia *per se* was shown to increase blood flow and vascular conductance during exercise with a regulation by the carotid chemoreceptors (35). Consequently, the three-fold increase in arm muscle (*triceps brachii*) perfusion variations during repeated sprinting observed after RSH (Fig. 4B) indicates the cumulative role of hypoxia possibly challenging to a further extent the adaptations observed in RSN. Accordingly, a recent study reported a 16% increase in arm blood flow and 6% in arm O_2 extraction after prolonged low-intensity (60% of max HR) training (4). Since FT have been shown to optimally increase their fraction O_2 extraction during intensive exercise (27), RSH may thus recruit FT maximally and maximize vascular conductance and O_2 extraction to delay fatigue during subsequent RSA in normoxia.

However, the NIRS measurement methods allowed to record local variations in total hemoglobin/myoglobin content (Δ[tHb[) in the muscle, thus reflecting blood perfusion and possibly blood flow variations (19, 37). So this method provided only an indirect estimation of muscle perfusion variations (and thus blood flow) and our interpretations should be considered with care. Nevertheless, the gains in the amplitude of the variations after RSH were far higher when measured here in *m. triceps brachii* compared to *m. vastus lateralis* we reported recently (14). Eventually, the latter is in accordance with our hypothesis that performing RSH in activities soliciting more FT fibers (e.g., cross country double-poling with the upper body comprising more FT (21)) would potentiate larger benefits in RSA performance gains.

In addition, we stated that the exercise-to-rest ratio play an important role in the putative benefits following RSH (28) because it modifies the energetic contribution of glycolysis and the FT recruitment/activation during high intensity exercise (3, 36). Then the rate of post-sprint phosphocreatine (PCr) resynthesis is paramount for maintaining power production during sustained RSA (17). If RSH allows increasing muscle blood perfusion and vascular conductance as mentioned above, it may in turn also elevate locally microvascular PO_2 that is known to reduce PCr breakdown and speed up PCr resynthesis (20). Since hypoxia *per se* was shown to modulate PCr recovery (22), RSH may accordingly optimally delay fatigue during RSA if an optimal exercise-to rest ratio is found. In addition, the lack of differences in both groups from Pre- to Post- in the surface electromyography measurements supports the hypothesis of an improved metabolic glycolytic activity rather than higher power outputs due to greater recruitment of

motor units. Besides, the lack of difference in the aerobic team sprint test between RSH and RSN illustrated that a prolonged intense effort (that is more aerobic by definition) does not benefit to the same extent from RSH with the lesser activation of FT. However, we point out that both isolated sprint and team sprint were improved similarly after RSH and RSN and that there is no down-regulation in maximal anaerobic alactic or aerobic exercises after RSH.

Finally, a recent study investigated different severity of the hypoxic stress (e.g., at simulated altitude of 2000, 3000 and 4000 m) highlighting that "the higher may not be the better" in the training quality for RSH to improve of repeated sprint performance (18). Further studies could consequently focus on the exercise-to-rest ratio to optimize RSH benefits and find the optimal balance between maximal exercise intensity and the added hypoxic stress. So far, we can only observe that the chosen simulated altitude (e.g., normobaric hypoxia) of 3000 m appears providing a stimulus large enough for inducing additional benefits in RSH. One cannot rule out that in hypobaric hypoxia the adaptations would be even higher (30) since we reported different responses in nitrosative and oxidative stress between normobaric and hypobaric hypoxia at 3000 m (15).

In conclusion, our present findings demonstrate for the first time that greater improvement in the performance of repeated double-poling sprints can be attained with RSH compared with RSN with larger variations in the perfusion of active muscles in the former. This adds to the accumulating evidence that RSH represents a promising and potent training strategy promoting repeated-sprint performance enhancement by adding a hypoxic stress to repeated-sprint training. However, further examination is required to confirm that the underlying mechanisms are fiber-specific as well as to firmly establish the potential of such training to improve the performance of team sports. We propose that by including RSH sessions in their training regimen, XC skiers can improve their work capacity during sessions of repeated sprints.

Acknowledgments

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Chapter 11

Article 7 - Advancing hypoxic training in team sports: from intermittent hypoxic training to repeated sprint training in hypoxia.

11. Article 7 - Advancing hypoxic training in team sports: from intermittent hypoxic training to repeated sprint training in hypoxia.

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Abstract

Over the past two decades, intermittent hypoxic training (IHT), that is, a method where athletes live at or near sea level but train under hypoxic conditions, has gained unprecedented popularity. By adding the stress of hypoxia during 'aerobic' or 'anaerobic' interval training, it is believed that IHT would potentiate greater performance improvements compared to similar training at sea level. A thorough analysis of studies including IHT, however, leads to strikingly poor benefits for sea-level performance improvement, compared to the same training method performed in normoxia. Despite the positive molecular adaptations observed after various IHT modalities, the characteristics of optimal training stimulus in hypoxia are still unclear and their functional translation in terms of whole-body performance enhancement is minimal. To overcome some of the inherent limitations of IHT (lower training stimulus due to hypoxia), recent studies have successfully investigated a new training method based on the repetition of short (<30 s) 'all-out' sprints with incomplete recoveries in hypoxia, the so-called repeated sprint training in hypoxia (RSH). The aims of the present review are therefore threefold: first, to summarise the main mechanisms for interval training and repeated sprint training in normoxia. Second, to critically analyse the results of the studies involving high-intensity exercises performed in hypoxia for sea-level performance enhancement by differentiating IHT and RSH. Third, to discuss the potential mechanisms underpinning the effectiveness of those methods, and their inherent limitations, along with the new research avenues surrounding this topic.

Introduction

Prolonged altitude sojourns using the 'live high-train high' or the 'live high-train low' models[1,2] have been increasingly used in athletes involved in endurance and, more recently, in intermittent (eg, team and racket sports) disciplines in an attempt to gain a competitive edge.[2,3] However, the question as to how effectively prolonged altitude exposure can improve athletic performance and its underpinning physiological mechanisms and signalling pathways remains contentious.[4,5]

Over the past two decades, intermittent hypoxic training (IHT), that is, a method where athletes live at or near sea level but train under hypoxic conditions, has gained large popularity. Hence, IHT presents the advantages of minimal travel and relatively low expense and causes limited disruption to the athletes' normal training environment and lifestyle. Another advantage is that it also avoids the deleterious effect (decreased muscle excitability) of an extended stay in altitude.[6] By adding the stress of hypoxia during 'aerobic' or 'anaerobic' interval training (INT), it is believed that IHT would potentiate greater performance improvements compared to similar training at sea level. For long, erythrocytosis was believed to be the primary factor benefiting putative sea-level performance improvement after a sufficient (several weeks) hypoxic stimulus.[4,5,7] However, IHT viewed this from a new perspective with evidence that exercising even for a short period in hypoxia affects a large number of genes mediated by hypoxia-inducible factors (HIFs)[8] and the exercise performance with muscular adaptations arising (and not necessarily an improved oxygen carrying capacity).[9–12] Nevertheless, in other IHT studies, any potentiating effect of hypoxia in addition to training was ambiguous.[3,13–16] Although an improvement in anaerobic performance after IHT has been mentioned in four studies,[17–20] it is noteworthy that these studies were 'uncontrolled', and therefore the effects of training cannot be distinguished from those of hypoxia.13 As such, it seems that after decades of research, "IHT does not increase exercise performance at sea level in endurance athletes any more than simply training at sea level."[21]

Until now, only scarce literature has assessed the potential benefits of altitude training in intermittent sports.[16,22,23] Therefore, the relevance of altitude training in team-sport athletes for improving players' specific fitness (repeated sprint ability (RSA)) has not been scientifically sounded as yet. Team-sport players (eg, football) perform a large number of high-intensity actions, including numerous sprints, often with incomplete recoveries, during the course of a game. As a consequence, developing their ability to repeatedly perform intense exercise bouts for sustained periods is important for crucial match actions.[24] For example, failure to recover after a sequence of intense actions may leave the team more vulnerable defensively by decreasing the chances to reach passes or increasing the time to take up a defensive position (tackles). Sport-specific training methods for team sports using the stress of hypoxia as a strong additional stimulus with specifically designed training models are arguably promising methods. For instance, repeated sprint training in hypoxia (RSH), defined as the repetition of several short (≤30 s) 'all-

out' exercise bouts in hypoxia interspersed with incomplete recoveries (exercise-to-rest ratio <1:4), could be considered as such a sport-specific training strategy. Although RSH could be considered as a form of IHT, its efficacy is presumably based on different mechanisms than on the existing IHT methods (discussed below), therefore justifying the addition of the RSH modality in the altitude training nomenclature.[25]

The aims of the present review are threefold: first, to summarise the main mechanisms for INT and repeated sprint training in normoxia (RSN). Second, to critically analyse the results of the studies involving high-intensity exercises performed in hypoxia for sea-level performance enhancement by differentiating IHT and RSH. Third, to discuss the potential mechanisms underpinning the effectiveness of those methods, and their inherent limitations, along with new research avenues surrounding this topic.

A computer-based literature search was conducted in April 2013 using the PubMed electronic database using combinations of specific keywords: 'altitude', 'hypoxic', 'training', 'intermittent hypoxia', 'repeated sprints', 'interval training', 'exercise' and 'performance'. Recently, an international consensus group of the IOC[26] underlined the further need "to study the effects of training in hypoxia and live high-train low modalities on performance at sea level, low and moderate altitude using a placebo-controlled double-blind design." Well aware of the methodological issues (ie, importance of ruling out placebo effects[21,27]) pertaining to the conclusion of some altitude training studies, the present review is limited to studies including a control (CON) group in their experimental design, allowing the effects of training and hypoxic stimulus to be clearly differentiated.

INT versus RSN

The efficiency of INT[28,29] has been investigated extensively. It can broadly be subdivided into (1) short or long aerobic[28] versus anaerobic[29] INT and (2) short or long intervals versus sprint intervals.[30] INT consists of 'repeated short-to-long bouts of rather high-intensity exercise interspersed with recovery periods'.[28] While any INT session will naturally challenge the metabolic and neuromuscular systems, it is beyond the scope of this review to detail all the stressed factors.[24,30–32] However, we support the recent statement that "the cardiorespiratory (ie, VO_2) data, but also cardiovascular work, stored energy and cardiac autonomic stress responses are the primary variables of INT", whereas "anaerobic glycolytic energy contribution and neuromuscular load/musculoskeletal strain are secondary."[30] Indeed, the expected benefits of INT are primarily to maximise VO_{2max} and therefore cardiac output and the arterial-mixed venous oxygen difference[32] as well as the VO_2 kinetics,[31] which are important determinants of endurance performance. Overall, INT performed at intensities[33] and exercise-to-rest ratios[34] that elicit maximal volume and pressure overloads on the myocardium and VO_2 responses near maximal oxygen uptake (VO_{2max}) are quite likely optimal in terms of cardiac output, blood flow, shear

stress, recruitment and increased oxidative capacity of fast twitch (FT) fibres. This imposes the need to maintain the longest time >90% $VO_{2max.}$[35]

During repeated sprints in normoxia, the factors responsible for the performance decrements (eg, decline in sprint speed/power across repetitions) include limitations to energy supply (eg, phosphocreatine (PCr) resynthesis and aerobic and anaerobic glycolysis), metabolite accumulation (eg, inorganic phosphate, Pi; hydrogen ion, H^+) and neural factors (eg, neural drive and muscle activation).[36,37] Among these factors, the ability to resynthesise PCr is probably the central determinant of RSA. Hence, the oxidative pathway is essential for the PCr resynthesis rate,[38] and the decrease in PCr concomitant to the rise in Pi and AMP stimulates the anaerobic glycolytic contribution at the start of a sprint. If the increase in H^+ accumulation is also known to impair RSA, recent findings[39] suggest that this fitness component is determined to a larger extent by the muscle energy supply (eg, short-term (<1 min) PCr resynthesis rate) than by the H^+ removal.

In team sports, the clinical relevance of improving RSA is debated,[40] but it is a common belief that such adaptations would be beneficial for improved match-related physical performance. For instance, the mean time recorded during an RSA test predicts the distance of high-intensity running and the total sprint distance during a professional football match.[41] Furthermore, football players experience temporary fatigue during a game (eg, lower amount of sprinting, high-intensity running and distance covered after a sequence of repeated and intense actions), which may determine the outcome of crucial situations (eg, decreased technical and tactical behaviour and wrong cognitive choices).[42,43] This suggests that improving RSA would maximise team-sport physical performance and that it is important to better understand training strategies that can enhance this fitness component.

Although the brief description above of the main determinants of INT versus RSN highlights that those training methods aim at developing predominantly the aerobic pathway and RSA, respectively, the practical question of their optimal combination in team sports is widely debated[37,44] with two diverging approaches[45]; that is, an integrative 'mixed' method mainly based on IHT/RSN[37] contrasting with an 'isolated' method based on the parallel development of maximal aerobic speed and maximal sprinting speed.[44] The same debate was translated within the area of the optimal use of hypoxic training in team sports[3,15] and needs to better describe the main adaptive mechanisms of IHT and RSH. This is the objective of the next sections.

Current trends: Is it time to move beyond IHT?

Performance improvement with IHT

In table 1, we report 23 controlled studies (ie, 20 IHT and 3 RSH) including training protocols performed in hypoxia versus normoxia. Interestingly, an additional benefit on performance-related variables of IHT compared with the same training performed in normoxia is present in

only four of those studies. First, Martino *et al*[48] reported a faster 100 m swim time and larger improvement in peak power output during an arm Wingate test after 21 days of training including swim sprints at an altitude of 2800 m, compared to sea level. Since a detailed description of the training sessions is not available, the mechanisms inducing additional hypoxia-related benefits cannot be ascertained. Second, Hendriksen and Meeuwsen[54] highlighted a 5% increase in peak power output during a Wingate cycling test after 10 days of aerobic training in hypobaric hypoxia, while performance in the normoxic training group did not change. Third, Dufour *et al*[59] reported an improved endurance performance capacity in competitive distance runners after 6 weeks of high-intensity aerobic training at 3000 m (ie, 5% increase in their VO_{2max} and 35% longer time to exhaustion running at a speed associated with VO_{2max}), but not performance change in the group training in normoxia. Finally, Manimmanakorn *et al*[23] reported in one of the few studies conducted with team-sport athletes that a knee extension/flexion IHT performed over a 5-week period provided an additional benefit for improving maximum voluntary contraction torque during prolonged leg extensions. A remarkable observation across the above-listed studies is that the additional benefits of IHT seem to be partly related to an upregulation of the glycolytic potential and to an increased anaerobic capacity (eg, larger increase in Wingate performance). These adaptations might help athletes engaged in intermittent sports to improve their match-related performance.

Author (year)	Subjects	Design (number of training sessions, type, altitude, training content)	Groups	Statistically significant results (P<0.05)
Roskamm *et al* (1969)[46]	Untrained	24 over 4 wks, cycling, 2250 m (N=6) or 3450 m (N=6) (HH). 30-min aerobic training	IHT, N=12 INT, N=6	+10-17% VO_{2max} +6% VO_{2max}
Terrados *et al* (1988)[47]	Elite cyclists	12-20 over 3-4 wks, cycling, 2300 m (HH). Aerobic training and some intervals (15 s at 130% of aerobic peak power output)	IHT, N=4 INT, N=4	+33% PPO +22% PPO
Martino *et al* (1995)[48]	Elite swimmers	Swim sprints at 2800 m (HH) during 21 days at altitude. No details available	IHT, N=20 INT, N=13	**-6% 100 m swim time, +34% PPO arm Wingate** NS changes
Emonson *et al* (1997)[49]	Untrained	15 over 5 wks, cycling, 2500 m (HH). 45 min at 70% of VO_{2max}	IHT, N=9 INT, N=9	+12% VO_{2max} +12% VO_{2max}
Katayama *et al* (1998)[50]	Untrained	10 over 2 wks, cycling, 4500 m (HH). 30 min at 70% of normoxic VO_{2max} level	IHT, N=7 INT, N=7	+7% VO_{2max} +5% VO_{2max}
Bailey *et al* (2000)[51]	Runners	4 wks at ~2000 m (NH). Aerobic training, no details	IHT, N=18 INT, N=14	+15% VO_{2max} +5% VO_{2max}
Geiser *et al* (2001)[52]	Untrained	30 over 6 wks, cycling, 3850 m (NH). 30 min at 77-85% of max heart rate	IHT, N=18 INT, H=15	+11% VO_{2max}, +17% 30-min TT mean PO +9% VO_{2max}, +19% 30-min TT mean PO
Karlsen *et al* (2002)[53]	Cyclists	9 over 3 wks, cycling. 3000 m (NH). 120 min aerobic training	IHT, N=8 INT, N=8	NS changes in VO_{2max} or 30-min TT NS changes in VO_{2max} or 30-min TT
Hendriksen and Meeuwsen (2003)[54]	Triathletes	10 over 10 days, cycling, 2500 m (HH). 105 min aerobic training	IHT, N=8 INT, N=8	**+5% PPO cycling Wingate** NS increase
Truijens *et al* (2003)[55]	Swimmers	15 over 5 wks, swimming, 2500 m (NH). 12.5 min > 100% VO_{2max} (30 s or 60 s bouts)	IHT, N=8 INT, N=8	NS changes **+6% VO_{2max}**
Ventura *et al* (2003)[56]	Cyclists	18 over 6 wks, cycling, 3200 m (NH). 30 min aerobic training	IHT, N=7 INT, N=5	NS changes in VO_{2max} or 10-min TT NS changes in VO_{2max} or 10-min TT
Morton and Cable (2005)[16]	Team sport players	12 over 4 wks, cycling, 2750 m (NH). 10 x 1-min at 80% of 2-min PPO	IHT, N=8 INT, N=8	+8% cycling Wingate PPO, + 7% VO_{2max} +6.5% cycling Wingate PPO, 8% VO_{2max}
Roels *et al* (2005)[57]	Cyclists & triathletes	14 over 7 wks, cycling, 3000 m (NH). 6-8 x 2-3 min at 100% of aerobic PPO	IHT, N=11 IHIT, N=11 INT, N=11	+4% 10-min TT mean PO **+9% VO_{2max}**, +5% 10-min TT mean PO +5% 10-min TT mean PO
Roels *et al* (2007)[58]	Cyclists & triathletes	15 over 3 wks, cycling, 3000 m (NH). 9 x 60 min at 60% VO_{2max} and 36 min with intervals of 2 min at 100% aerobic PPO (2 min bouts)	IHT, N=10 INT, N=9	+7% aerobic PPO +7% aerobic PPO, +8% 10-min TT mean PO
Dufour *et al* (2006)[59]	Runners	12 over 6 wks, running, 3000 m (NH). 24-40 min < VO_{2max}.	IHT, N=9 INT, N=9	**+5% VO_{2max}, +35% T_{lim} at vVO_{2max}** NS changes
Hamlin *et al* (2010)[22]	Cyclists & triathletes	10 over 10 days, cycling, 3200-4400 m (NH). 90 min aerobic training followed by two 30-s Wingate tests	IHT, N=9 INT, N=7	+3% PO cycling Wingate NS changes
Lecoultre *et al* (2010)[60]	Cyclists	12 over 4 wks, cycling, 3000 m (NH). 4 x 12-18 min at 100-120% of aerobic PPO, 4 x 30-48 min < VO_{2max} and 4 x 100-min aerobic training.	IHT, N=7 INT, N=7	+7% 40 km TT mean PO +6% 40 km TT mean PO
Mao *et al* (2011)[61]	Active males	25 over 5wks, cycling, 2750 m (NH). 30 min aerobic training	IHT, N=12 INT, N=12	+16% VO_{2max} +10% VO_{2max}
Manimmanakorn *et al* (2013)[23]	Female team-sport players	15 over 5 wks, knee flexion and extension, ~4500 m (NH). 6 sets of low resistance knee extensions and flexions to failure with 30 s between sets	IHT, N=10 INT, N=10	**+15% MVC3, +17% MVC30, +129% REP201RM** + 86% REP201RM
Holliss *et al* (2013)[62]	Active males	15 over 3 wks, leg-extension, 3000 m (NH). 10 x 60-70 s intense exercise with 20-30 s passive recovery. One leg IHT, the other leg INT.	IHT, N=9 INT, N=9	+25% leg-extension incremental T_{lim} +27% leg-extension incremental T_{lim}
Puype *et al* (2013)[63]	Moderately trained cyclists	18 over 6 wks, cycling, 3000 m (NH). 4-9 sprints of 30 s interspersed with 4.5 min recovery at 50 W	RSH, N=10 RSN, N=10 CON, N=10	+6% sprint PO, +6% VO_{2max}, +6% 10-min PO, **+7% LT4** +5% sprint PO, +6% VO_{2max}, +6% 10-min PO, NS NS changes
Galvin *et al* (2013)[64]	Rugby players	12 over 4 wks, treadmill running, 3500 m (NH). 10 sprints of 6 s interspersed with 30 s recovery	RSH, N=15 RSN, N=15	**+33% Yo-Yo Intermittent Recovery 1 Test performance** +14% Yo-Yo Intermittent Recovery 1 Test performance
Faiss *et al* (2013)[65]	Moderately trained cyclists	8 over 4wks, cycling, 3000 m (NH). 3 x 5 all-out 10 s sprints interspersed with 20 s recovery at 120 W	RSH, N=20 RSH, N=20 CON, N=10	+6% sprint PO, **+38% completed sprints in RSA test** +7% sprint PO, no change in completed sprints NS changes

Table 1 Summary of current research findings relative to the use of intermittent hypoxic training (IHT) or repeated sprint training in hypoxia (RSH)

This table is limited to investigations with a group training in hypoxia (intermittent hypoxic training (IHT), intermittent hypoxia interval training (IHIT) or repeated sprint training in hypoxia (RSH)) and a group training in normoxia (intermittent training in normoxia (INT) or repeated sprint training in normoxia (RSN)). Control group without training (CON) present in two studies. Altitude described as either hypobaric hypoxia (HH) or normobaric hypoxia (NH). Significant difference between groups is shown in bold (P<0.05). NS, non significant (P > 0.05); PPO, peak power output; PO, power output; TT, time trial; VO_{2max}, maximal oxygen uptake; T_{lim}, time to exhaustion ; vVO_{2max}, velocity associated to VO_{2max} ; MVC3, peak maximum voluntary contraction in 3 s; MVC30, area under the peak 30 s maximal voluntary contraction curve; REPS201RM, repetitions at 20% of 1 repetition maximal load; RSA, repeated sprint ability test to exhaustion; LT4, power output corresponding to 4 mmol blood lactate.

Besides, in another study conducted with team-sport athletes, similar improvements in aerobic and anaerobic power outputs were observed when training was performed in hypoxia and normoxia.[16] Other well-designed controlled studies highlighted the benefits of IHT on aerobic performance but failed to demonstrate an additional benefit of conducting the training in a hypoxic environment.[46 ,55 ,57 ,58 ,66] With the many different training strategies and methods available, the possibility that IHT might "enhance endurance performance when subsequent exercise is conducted in hypoxia" in football players as stated in a recent comprehensive review3 was therefore questioned by our team.[15]

Physiological mechanisms and limitations of IHT

IHT is quite likely to have a minimal effect on erythropoiesis since a large 'hypoxic dose' is required for significantly "stimulating the erythropoietic pathway to the point that it enhances post-altitude sea-level endurance performance."[4 ,5] In support of this assumption, previous IHT studies failed to observe any significant change in the total haemoglobin mass, red cell volume or any other red cell indices compared with a CON group[62 ,67] (see ref. 2 for further discussions).

Compared with sea-level training, IHT has the potential to induce a further physiological strain68 and specific molecular adaptations,[11 ,12 ,69] though not necessarily associated with improved exercise capacity. The rationale of using IHT relies on the hypothesis that these muscle adaptations surpass those triggered by normoxic exercise. In particular, the lower partial pressure of oxygen (PO_2) in muscle tissue during IHT when compared with INT would lead to a larger upregulation of HIF-1α.[11 ,12 ,62] In untrained or moderately-trained participants, muscular adaptations occurring in response to IHT include—but may not be limited to—an increased citrate synthase activity, mitochondrial density, capillary-to-fibre ratio and fibre cross-sectional area as well as upregulation of factors of mitochondrial biogenesis or enzymes implicated in carbohydrate and mitochondrial metabolism, oxidative stress defence and pH regulation.[10 ,11 ,47 ,52 ,59 ,70 ,71] However, as stated recently,[9] one may question the functional significance of these physiological adaptations (eg, larger increase in citrate synthase activity in IHT than in INT) since the effects of IHT on endurance performance measured in normoxia are 'minimal and inconclusive in trained athletes'.[21]

Several authors have reported additional adaptations potentially favourable to high-intensity exercises. These include improvements in muscle O_2 homeostasis and tissue perfusion induced by improved mitochondrial efficiency, control of mitochondrial respiration,[71 ,72] angiogenesis[73] and muscle buffering capacity.[74] However, the translation into enhanced performance is not always observed and when it does occur, it may be irrelevant for team sports. Hence, non-specific IHT protocols or inappropriate performance tests—that is, evaluating endurance

capacity (with VO_{2max} tests or time trials) but neglecting indices of match-related performance such as RSA—have been mainly conducted so far.

With the exception of studies performed at an intensity corresponding to the second ventilatory threshold,[12,59,72] where the increased expression of factors involved in glucose uptake, oxidative stress defence and pH regulation was associated with an increased endurance performance capacity, most of the IHT studies (including those with some muscle adaptations) did not report any additional performance benefit of IHT over INT. In untrained participants, the effect of training seems to predominate, overwhelming any additional effect of hypoxia.[75] Furthermore, Levine[75] convincingly argued that, compared to similar training in normoxia, IHT quite likely induces a lower stimulus for the active musculature since the lowered power output[76] and the reduced oxygen flux resulting from hypoxia would be associated with a downregulation of muscle structure and function.

Performance improvement with RSH

Some of the methodological limitations related to IHT have been overcome in recent studies investigating a new hypoxic training strategy named RSH.[63–65] RSH is based on the repetition of 'all-out' efforts of short (≤30 s) duration interspersed with short incomplete recoveries. This model differs from IHT since the intensity of the training stimulus is maximal and therefore allows one to maintain high FT recruitment so that positive results can be expected when adding hypoxia to training. RSH is particularly interesting since, under hypoxic conditions (<3800 m), a single sprint performance of short duration (<10 s) is generally preserved, whereas fatigue resistance during RSA tests is reduced with earlier and larger decrements in mechanical work.[77–79]

Recently, we[65] showed that RSH delays fatigue during a repeated sprint test to exhaustion. In that study, 50 trained athletes were randomly dispatched in three different intervention groups (RSH: 3000 m, FiO_2 14.5%; RSN: 485 m, FiO_2 20.9% and CON: no specific sprint training) and tested twice (before and after a 4-week training protocol including two repeated sprint training sessions per week) for the determination of endurance performance, anaerobic capacity and RSA. If endurance performance (during a 3 min 'all-out' time trial) was not increased, RSN and RSH improved the average power output during 10 s sprints (by 6–7%) and a 30 s Wingate test (by 3–5%), although a major additional benefit of RSH compared with RSN was found. The number of sprints completed during an RSA test to exhaustion was improved by 40% only after RSH: an average of 9 sprints was performed before training in both groups but 13 after RSH and still 9 after RSN. The relevance of the observed improvement in RSA in team-sport athletes is unanswered yet since the direct translation of RSA to the team game result is questionable.[40]

Puype *et al*[63] then showed that RSH improved by 7% the power output, corresponding to 4 mmol blood lactate during a maximal incremental test, while it did not change after RSN. However, in that study, the gains in power output during a 10 min time trial (6–7%) or VO_{2max} during an

incremental test (6%) were similar after RSH and RSN. Interestingly, the phosphofructokinase activity was markedly increased (59%) only after RSH, quite likely reflecting an upregulation of muscle glycolytic capacity. Since the performance tests were limited to longer aerobic efforts, they cannot be linked directly to physical performance improvement.

Furthermore, Galvin *et al*[64] recently showed in rugby players a 19% additional benefit of RSH compared with RSN in high-intensity intermittent running performance (Yo-Yo IR1 test[80]). This substantially higher performance improvement has important practical implications since the Yo-Yo test correlates very well with physical performance and the amount of high-intensity running in several team sports such as soccer, basketball, rugby and handball.[64]

Thus, RSH was shown to be as efficient as RSN in improving power output on a single sprint (5–7%) when including 10 s sprints interspersed with 20 s recoveries[65] or 30 s sprints with 270 s recoveries[63] (table 1). Additionally, but only after RSH, cycling power output corresponding to 4 mmol of lactate during an incremental test[63] and high-intensity intermittent running performance were significantly improved[64] only after RSH while fatigue development was delayed during a repeated cycling sprint test performed until exhaustion.[65]

Physiological mechanisms and promises of RSH

We hypothesised that RSH would induce beneficial adaptations mainly due to the improved blood perfusion level inducing an enhanced O_2 utilisation and an improved behaviour of FT fibres. With maximal effort intensities, specific skeletal muscle tissue adaptations (molecular level) may arise through the oxygen-sensing pathway (ie, capillary-to-fibre ratio, fibre cross-sectional area, myoglobin content and oxidative enzyme activity such as citrate synthase) that either do not occur in normoxic conditions or, if they do, they do so to a lesser degree.[10–12] Additionally, exercising in hypoxia is known to trigger a compensatory vasodilation to match an increased oxygen demand at the muscular level.[81]

Increasing evidence indicates that neuromuscular (muscle contractility and/or activation), biomechanical (running economy) and metabolic (muscle and/or cerebral deoxygenation/reoxygenation kinetics) factors may also play key roles in the hypoxia-induced mechanisms in response to maximal-intensity intermittent exercises. For instance, it is generally accepted that neuromuscular transmission and action potential propagation along with muscle fibres (sarcolemma excitability) remain unchanged with acute hypoxia in relaxed muscles or during brief contractions.[82] Indirect evidence rather suggests that the increased rate of fatigue seen at altitude may be the result of a more rapid accumulation of Pi during each sprint and a reduced rate of its removal during recovery.[38,83] Repeated sprints result in large changes in PCr and H^+ concentrations. However, the restoration of power output during repeated sprints seems to be influenced more by the muscle energy supply (eg, PCr resynthesis) than by the recovery of

muscle pH.[39] However, enhanced buffer capacity or upregulation of genes involved in pH control has also been reported after RSH[63,65]

Moreover, performance decrements are also likely to be explained by a reduced neural drive to the active musculature, (estimated by surface electromyography) arising secondary to a stronger reflex inhibition due to brain hypoxia[84] or a hypoxia-induced increased level of intramuscular metabolites known to stimulate group III–IV muscle afferents.[83] Furthermore, larger cerebral deoxygenation levels[77] and slower reoxygenation rates during recoveries[85]—which strongly correlate with the exacerbated reduction in mechanical work in hypoxia during an RSA test—have also been observed with acute altitude exposure. As exercise intensity increases, glycolytic FT muscle fibres are preferentially recruited,[86] while at lower intensity (eg, <VT2) oxidative slow twitch (ST) and FT muscle fibres are solicited.

During sprints in hypoxia, the compensatory vasodilation (with an increase in blood flow) that aims at maintaining constantly the total O_2 delivery to the muscle is quite likely maximal since exercise intensity is essential in the amplitude of this compensatory mechanism.[81] FT fibres are quite likely to benefit more than ST fibres from the higher blood perfusion. Hence, owing to their greater fractional O_2 extraction if highly perfused,[87] the enhanced microvascular O_2 delivery to FT would 'make FT to behave more like their oxidatively efficient ST counterparts'.88 So, RSH efficiency is likely to be fibre-type selective and intensity dependent and therefore based on mechanisms presumably different from those associated with IHT. We speculate here that the improved responsiveness of the vascular bed and the improved blood perfusion through nitric oxide (NO)-mediated vasodilation mechanisms81 could be paramount in RSH. Further investigations into the NO pathway (neuronal NO synthase (nNOS) and endothelial NO synthase (eNOS)) are required in RSH to determine whether mechanisms other than NO-mediated vasodilation are also involved. Moreover, fibre-type selective peripheral vascular effects of nNOS-derived NO have been reported during high-speed treadmill running, whereas these effects were not seen at slower speeds.[89] It is, however, striking to note in two recent studies[89,90a] similar fibre-type mechanism on dietary nitrate (NO_3^-) supplementation that enhances blood flow. With NO_3^- supplementation, blood flow and vascular control were indeed augmented mostly in FT,[90] partly due to the lower microvascular PO_2 in contracting FT.[87] Interestingly, an elevated microvascular PO_2 is known to reduce PCr breakdown[38] and speed PCr recovery kinetics.

Adding a hypoxic stimulus to training can modulate the PCr resynthesis during exercise. In support of this suggestion, Holliss *et al*[62] reported that single leg-extension IHT results in a faster PCr recovery from high-intensity exercise in hypoxia (with only a tendency observed in normoxia). However, exercise tolerance during an incremental test to the limit of exhaustion either in normoxia or hypoxia was not different between IHT and INT. The authors speculated

that the faster PCr resynthesis observed after IHT was probably not due to an enhanced mitochondrial biogenesis but most likely due to a greater enhancement of muscle O_2 delivery. Overall, a faster PCr resynthesis resulting from RSH would manifest because of better maintenance of power production (better recovery between efforts) during intermittent, high-intensity exercises.

The latter could arguably contribute to the increased RSA performance observed in normoxia after RSH.65 By challenging the functional reserve in the muscle oxygen diffusing capacity most likely utilised in hypoxia,[91] repeated maximal efforts in hypoxia have the potential to stimulate beneficial adaptations in terms of PCr resynthesis and oxygen utilisation mediated by HIFs at the muscular level. By extension, the positive impact of RSH on glycolytic performance and skeletal muscle adaptations may lead to putative strong benefits for team sports like football, rugby union or Australian football, where the ability to repeat high-speed runs during an entire game is essential for overall performance.[92] At this stage, however, specific mechanisms that may enhance performance with RSH are still to be determined with further studies.

Conclusion

A thorough analysis of studies that have used IHT leads to strikingly poor benefits for sea-level performance improvement, compared to the same training protocol performed in normoxia.

Despite the positive molecular adaptations observed after various IHT modalities, the characteristics of optimal training stimulus in hypoxia are still unclear and their functional translation in terms of whole-body performance enhancement is minimal.

To overcome some of the inherent limitations of IHT (lower training stimulus due to hypoxia), recent studies have investigated a new training method based on the repetition of 'all-out' sprints in hypoxia, the so-called RSH. The succession of maximal efforts under hypoxic conditions was shown to be beneficial for maximal performance improvement and especially to delay fatigue when sprints with incomplete recoveries were repeated until exhaustion.

RSH is therefore proposed as a promising training strategy in intermittent sports to eventually improve match-related performance. Since team sports are characterised by intense exercise bouts repeated throughout a game, delaying fatigue and improving the ability to repeat sprints are crucial for the improved physical involvement of players.

Until now, there is scant evidence of the additional benefits of high-intensity training performed in hypoxia compared to the same training in normoxia on RSA. Until new evidence is provided, it is felt that compared to IHT, RSH is based on different fundamental mechanisms that are likely to be fibre-type selective, while the positive adaptations are probably dependent on the compensatory vasodilatory effects on the behaviour of FT fibres.

Yet, further studies with large sample sizes and double-blinded designed protocols are needed to endorse the efficacy of RSH. Then, in order to robustly assess the true benefits of RSH versus traditional IHT, both training strategies must be directly compared in the same experimental test setting. Judging the impact of RSH on athletic performance in various team sports could be best improved by testing, for example, specific work-to-rest ratios or the efficacy of different 'hypoxic doses'. Finally, if the efficacy of RSH is confirmed in more ecological situations (including overground sprints in hypoxic marquees rather than cycling an ergometer), it could then be readily implemented in the yearly periodisation of intermittent disciplines.

What are the new findings

- This review critically analyses the results of the studies involving high-intensity exercises performed in hypoxia for sea-level performance enhancements by differentiating intermittent hypoxic training (IHT) and repeated sprint training in hypoxia (RSH).
- IHT leads to strikingly poor benefits for sea-level performance improvement, compared to the same training protocol performed in normoxia.
- RSH is a promising training strategy that has been shown to delay fatigue when sprints with incomplete recoveries are repeated until exhaustion.

How might it impact on clinical practice in the near future

- This review will help athletes and teams in intermittent sports by providing an overview of the current scientific knowledge about intermittent hypoxic training and repeated sprint training in hypoxia (RSH).
- New studies are proposed to judge the efficacy of RSH in team sports and to determine the specific mechanisms that may enhance the team game results with RSH.

Footnotes

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