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Specific effects of hypobaria on physiological responses in pilots exposed to normoxic and hypoxic environments

Aebi Mathias Roland

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Mathias Roland AEBI

Master en Sciences du sport de l'Université de Lausanne

Jury

Prof. Claus Wedekind, Président
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“Mens sana in corpore sano.” – Juvénal

“Persistence can change failure into extraordinary achievement.” – Marv Levy

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List of publications

The publications presented in the thesis are in bold.

1. **Aebi MR, Bourdillon N, Bron D and Millet GP. Minimal influence of hypobaria on heart rate variability in hypoxia and normoxia. *Front. Physiol.* 2020;11:1072.**
2. **Bourdillon N, Aebi MR, Kayser B, Bron D and Millet GP. Both hypoxia and hypobaria impair baroreflex sensitivity but through different mechanisms. (Submitted)**
3. **Aebi MR, Bourdillon N, Kunz A, Bron D, Millet GP. Specific effect of hypobaria on cerebrovascular hypercapnic responses in hypoxia. *Physiol Rep.* 2020;8:e14372.**
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5. **Aebi MR, Bourdillon N, Noser P, Millet GP, Bron D. Cognitive impairment during combined normobaric vs. hypobaric and normoxic vs. hypoxic acute exposure. *Aerosp Med Hum Perform.* 2020 Nov 1;91(11):845-851.**
6. **Aebi MR, Bourdillon N, Bron D and Millet GP. Hypobaric effect in acute hypoxia on physiological responses during exercise. (Submitted)**
7. Aebi MR, Bourdillon N, Meziane HB, Nicol E, Barral J, Millet GP, Bron D. Cardiovascular and cerebral responses during a vasovagal reaction without syncope. *Front Neurosci.* 2019 Dec 10;13:1315.
8. Aebi MR, Willis SJ, Girard O, Borrani F and Millet GP. Active preconditioning with blood flow restriction or/and systemic hypoxic exposure does not improve repeated sprint cycling performance. *Front. Physiol.* 2019; 10:1393.

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Abstract

The main aim of the present thesis was to evaluate the putative influence of hypobaria on physiological responses such as pulse oxygen saturation, heart rate variability, baroreflex sensitivity (BRS), cerebrovascular reactivity to CO_2 (CVR) in hypoxia and in normoxia at rest and during a submaximal exercise. Moreover, cognitive performance was assessed using an arithmetic test. The hypobaric effect was evaluated by comparing two hypoxic (NH: normobaric hypoxia vs. HH: hypobaric hypoxia) and normoxic (NN: normobaric normoxia vs. HN: hypobaric normoxia) conditions with equivalent inspired oxygen pressure.

The results of this thesis suggest an additional influence of hypobaria in hypoxia, which resulted in larger hypoxemia, greater sympathetic activation and increased heart rate at rest. Cognitive performance was altered to the same extent in the two hypoxic conditions, but maintained in normoxia. In both hypobaric conditions, BRS was decreased at rest. Moreover, CVR was significantly modified in hypobaric environments (i.e., HH and HN), which potentially decreased cerebral oxygen delivery. These results imply a specific effect of hypobaria *per se* on BRS (i.e., blood pressure regulation) and cerebrovascular function also in hypoxia, but also in normoxia. At exercise, hypobaria increased the severity of hypoxia, inducing a greater hypoxemia, increases in minute ventilation, heart rate and cerebral / muscular deoxygenation, while it had a negligible influence in normoxia. These changes may be the consequence of a slight change in breathing pattern, mainly due to a larger hypobaria-induced hypocapnia.

The present results are of interest for the Swiss Air Force in their pilots' education, but also of concern for individuals regularly exposed to hypobaric and hypoxic environment, such as astronauts and mountaineers.

Résumé

Cette thèse porte sur les effets de l'hypobarie sur les réponses physiologiques et la performance de concentration de pilotes en hypoxie et en normoxie au repos et durant un exercice sous-maximal. Les effets de l'hypobarie ont été évalués en comparant deux conditions hypoxiques (NH : hypoxie normobare vs. HH : hypoxie hypobare) et normoxiques (NN : normoxie normobare vs. HN : normoxie hypobare) ayant une pression inspirée en oxygène équivalente.

Les résultats de cette thèse suggèrent une influence supplémentaire de l'hypobarie en hypoxie, i.e., une plus grande hypoxémie, une activation du système sympathique augmentée et une plus haute fréquence cardiaque au repos.. La performance de concentration a été diminuée de manière comparable dans les deux conditions hypoxiques mais maintenue en normoxie. Dans les deux conditions HH et HN, la sensibilité du baroréflexe a été diminuée au repos. De plus, la réactivité cérébrovasculaire au CO_2 a été significativement altérée en hypobarie, ce qui a potentiellement impacté la fourniture d'oxygène au niveau cérébral. Ces résultats démontrent un effet spécifique de l'hypobarie sur la régulation de la pression artérielle et la fonction cérébrovasculaire en hypoxie, mais également en normoxie. A l'exercice, l'hypobarie a augmenté la sévérité de l'hypoxie en induisant une plus grande hypoxémie, une augmentation de la réponse ventilatoire, une élévation de la fréquence cardiaque et une plus grande désoxygénation cérébrale/musculaire. Cependant, l'hypobarie en normoxie a eu un effet négligeable sur les réponses physiologiques à l'exercice. Ces changements peuvent être la conséquence d'une légère modification du patron ventilatoire, probablement due à une plus grande hypocapnie induite par l'hypobarie.

Ces résultats sont intéressants pour les forces aériennes suisses dans le cadre de la formation de leurs pilotes, mais également pour les personnes qui sont régulièrement exposées à des environnements hypobariques et hypoxiques comme les astronautes et les alpinistes.

Index of Abbreviations

Bf
Breathing frequency

BP
Blood pressure

CBF
Cerebral blood flow

CDO_2
Cerebral oxygen delivery

CVR
Cerebral vasoreactivity

EEG
Electroencephalography

F_IO_2
Inspired fraction of oxygen

HF
High frequency

HH
Hypobaric hypoxia

[HHb]
Deoxyhemoglobin concentration

HN
Hypobaric normoxia

HR
Heart rate

HRV
Heart rate variability

HVR
Hypoxic ventilatory response

KSS
Karolinska sleepiness scale

LF
Low frequency

MCAv
Middle cerebral artery velocity

MSNA
Muscle sympathetic nerve activity

MVV
Maximal voluntary ventilation

NH
Normobaric hypoxia

NIRS
Near-infrared spectrometry

NN
Normobaric normoxia

$[O_2Hb]$
Oxyhemoglobin concentration

P_AO_2
Alveolar oxygen pressure

P_aO_2
Arterial oxygen pressure

P_B
Barometric pressure

PCO_2
Partial pressure of carbon dioxide

$P_{ET}CO_2$
End tidal partial pressure of carbon dioxide

$P_{ET}O_2$
End tidal partial pressure of oxygen

PO_2
Partial pressure of oxygen

RMSSD
Root mean square of the successive differences

RPE
Rate of perceived exertion

RSA
Respiratory sinus arrhythmia

SpO_2
Pulse oxygen saturation

[tHb]

Total hemoglobin concentration

TOI

Tissue oxygenation index

$\dot{V}E$

Minute ventilation

VT

Tidal volume

Δ

Delta change over time

Chapter 1

Introduction

1 Introduction

1.1 Hypoxic conditions

Hypoxia can be defined as a combination of barometric pressure (P_B) and an inspired fraction of oxygen (F_IO_2) that results in a lower inspired pressure of oxygen (P_IO_2) value (<150 mm Hg) than in normoxia (Conkin & Wessel, 2008a). In fact, P_IO_2 reduction induces a diminution of the alveolar oxygen pressure (P_AO_2) leading to a decrease in arterial oxygen pressure (P_aO_2) and oxygen delivery at the tissue level (West, 1980), which induces physiological responses. The reduction of partial oxygen pressure (PO_2) in hypoxic environment was suggested as the principal physiological stimulus during high-altitude exposure (Self et al., 2011).

The following **Figure 1** illustrates that gas exchange is completed on the flat part of the oxyhemoglobin dissociation curve. However, when exposed to hypoxia, the PO_2 corresponds on the steeper part of the O_2 curve (West et al., 1962, 1983). In consequence, the oxygen dissociation curve is left-shifted through the combined effects of respiratory alkalosis (i.e., hypocapnia and reduced partial pressure of carbon dioxide, PCO_2), and increased production of 2,3 ‑ diphosphoglycerate (2,3-DPG) (Lenfant et al., 1968; Lenfant and Sullivan, 1971). This shift to the left is important as PCO_2 and alkalosis are pronounced at extreme altitude.

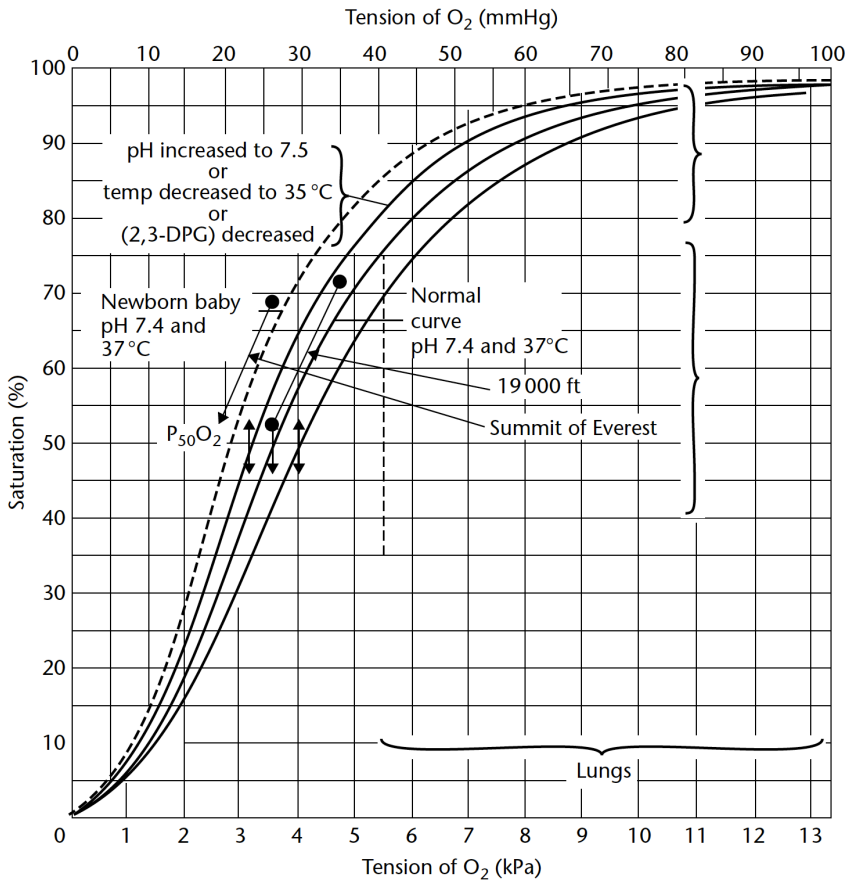

Figure 1: Oxyhemoglobin dissociation curve at sea level and superimposition of data for subjects acclimatised to high altitude superimposed. Left-shift of the curve facilitates gas exchange when exposed to altitude. $P_{50}O_2$ corresponds to the partial pressure of oxygen at which haemoglobin is 50% saturated with oxygen. **Source:** (West et al., 1962, 1983). Recently republished (Milledge, 2020).

Hypoxia can be either simulated in normobaria (normobaric hypoxia, NH) by decreasing the F_IO_2 with a mask or using a hypoxic chamber, or in hypobaria (hypobaric hypoxia, HH), by using a hypobaric chamber and reducing P_B. The hypoxic severity in these two environments, which refers to the inspired oxygen pressure (P_IO_2), is estimated by applying the following equation: $P_IO_2 = (P_B - 47) \times F_IO_2$, where 47 mm Hg is the vapour pressure of water at 37°C. (Conkin and Wessel, 2008). It was first believed that these two hypoxic environments produce the same physiological responses (Bert, 1943). However, a growing body of evidence started to show specific effect of hypobaria in hypoxia, as differences in fluid balance (Loeppky et al., 2005). Conkin highlighted a specific effect of reduced barometric

pressure for equivalent inspired oxygen pressure (P_IO_2), when emphasizing the above-mentioned equation (Conkin, 2016). Nowadays, the scientific community is still debating the putative difference in physiological responses between NH and HH environments. For instance, it was recently proposed that no conclusion can be drawn about the equivalence or not between NH and HH, as substantial errors in the calculation of equivalent P_IO_2 may occur because of great variability of P_B (depending on temperature, season, humidity, etc.) (Richalet, 2020). However, differences in physiological responses when exposed to hypoxia have been recently reported between NH and HH (Millet et al., 2012; Millet and Debevec, 2020).

Investigating the putative differences between NH and HH may have various applications in many fields. For instance, military pilots undertake hypoxic training either in NH (flight simulator) or HH (hypobaric chamber) to learn about their personal symptoms when exposed to acute hypoxic environments. Beside pilots, elite athletes also commonly train in hypoxic environments (NH and HH) in order to stimulate physiological adaptations and enhance their athletic performance after return to sea level using live high-train-low methods (Saugy et al., 2016b; Hauser et al., 2017). Moreover, mountaineers perform altitude pre-acclimatization in NH (i.e. using a hypoxic chamber or sleeping tent with reduced F_IO_2) in preparation for future altitude exposure. Pre-acclimatization in HH environment showed greater efficiency when compared to NH (Fulco et al., 2013). Therefore, putative disparities in physiological responses between NH and HH environments concern various populations, such as military pilots, athletes and mountaineers.

1.2 The effect of hypobaria

Hypobaria is an environmental condition in which barometric pressure is reduced. Early physiologists have been interested in the physiological responses to hypobaria and their implications for life at high altitude and for aviation (Barcroft et al., 1923; Fenn et al., 1946; West, 1981). Hypobaria arises with altitude elevation (**Figure 2**) or can be simulated using a hypobaric chamber. Paul Bert explored and described in "*La pression barometric (1878)*" the relationship between the barometric pressure and the oxygen content in the blood. He mentioned that the pressure was of physiological significance but not the gases concentration in the atmosphere. Then, Bohr considered the exchanges of gas across the capillary membrane (Bohr, 1891).

Physiological responses to lowered barometric pressure without hypoxia have rarely been reported. Isolating the hypobaric effect from the hypoxic one would allow comparing normoxic conditions with equivalent P_IO_2, but different P_B. The effect of hypobaria in normoxia has been explored in early studies (Cerretelli, 1976; Marconi et al., 2004) during chronic exposure at high-altitude while breathing pure enriched O_2 gas mixture. Simulating hypobaric normoxia (HN) (i.e. low P_B and increased F_IO_2 in order to obtain a comparable P_IO_2 than in normobaric normoxia, NN) is therefore of interest to evaluate the putative effect of hypobaria in normoxic condition (Millet and Debevec, 2020).

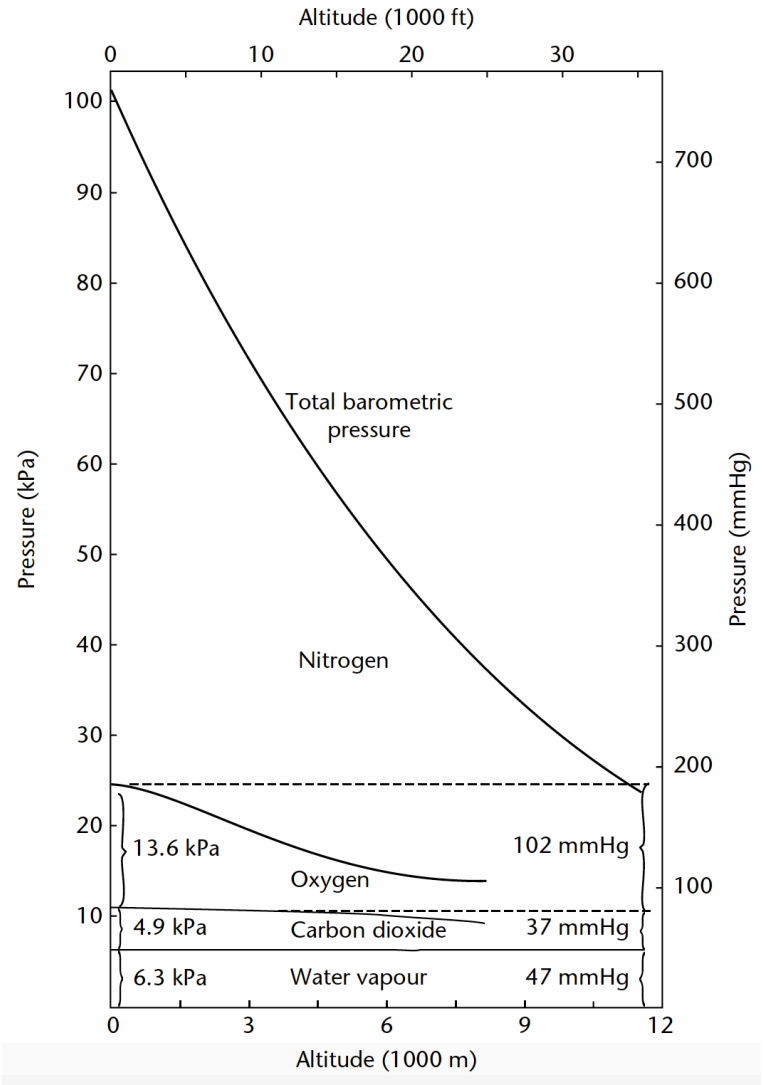

Figure 2: Diagram showing the composition of alveolar gas in non-acclimatized subjects under conditions of reduced barometric pressure. The upper curve shows the barometric pressure, which decreases exponentially with altitude level increase. The distances between the other curves represent estimated partial pressures of the alveolar gases. The horizontal lines represent conditions at sea level; the oxygen curve matches the barometric pressure curve at about 10'300 m (34 000 ft). The alveolar oxygen tension of a subject breathing oxygen at this altitude is equal to the one obtained while breathing air at sea level. **Source:** First published in Committee for Medical Research. Handbook of Respiratory Data in Aviation. Washington DC, 1944. Recently republished (Milledge, 2020).

1.3 Physiological responses to hypoxia

Hypoxia is a strong stimulus, which induces physiological responses in order to adapt to this environmental stress. Physiological changes mostly begin immediately upon hypoxic exposure. Hypobaric hypoxia demonstrated more severe physiological responses when compared to NH during acute exposure (Millet and Debevec, 2020), such as greater hypoxemia, hypocapnia and a lower arterial O_2 saturation (Savourey et al., 2003; Coppel et al., 2015). It was proposed that these different physiological responses may be a consequence of an increased physiological dead space due to the decrease in barometric pressure (Savourey et al., 2003). Moreover, HH induced sleep disturbance (Heinzer et al., 2016), and greater oxidative stress (Faiss et al., 2013b; Ribon et al., 2016) than NH, whilst acute mountain sickness symptoms were more severe (DiPasquale et al., 2016). The following sections mainly focus on physiological responses during acute hypoxic and hypobaric exposures.

1.3.1 Ventilation

Minute ventilation ($\dot{V}E$) elevation is the primary response immediately upon exposure (i.e., 30 minutes), when low oxygen availability in the ambient air is reduced (Fulco et al., 2011; Dempsey and Morgan, 2015). This increased pulmonary ventilation, called the hypoxic ventilatory response (HVR), is necessary to counteract the reduction in oxygen transport induced by hypoxia and maintain O_2 delivery to the tissues (Wagner et al., 1986). Ventilatory response is influenced by the carotid chemoreceptors that react to arterial hypoxemia within a few seconds by causing sympathetic nerve activity and, consequently, hyperventilation. This increase in $\dot{V}E$ largely differs between human individuals (Constantini et al., 2021) and usually occurs when P_IO_2 is decreased below 100 mmHg, which corresponds to an altitude level around 3000 m (Rahn and Otis, 1949). These receptors, that regulate ventilation centrally through the cortical respiratory centres (Teppema and Dahan, 2010), are sensitive to

P_aO_2 decrease but also to an elevation in arterial CO_2 pressure and arterial pH fall. Therefore, there is a potent relation between $\dot{V}E$ and pulse oxygen saturation (SpO_2) when exposed to hypoxia. Hyperventilation in hypoxic environment (*poikilocapnic hypoxia)* is vital to limit the decrease in PaO_2. Ventilatory response is well documented (Ursino et al., 2001) and the **Figure 3** below displays the influence of alveolar PCO_2 at different levels of inspired PO_2 on ventilation (Rebuck & Woodley, 1975). However, hyperventilation induces a reduction in end-tidal carbon dioxide pressure ($P_{ET}CO_2$), called a respiratory alkalosis or hypocapnia, which is due the reduction in CO_2 and H+ concentrations in the plasma (Ursino et al., 2001). Thus, hyperventilation partially counterbalances the P_AO_2 reduction, and therefore P_aO_2 and arterial O_2 saturation (SaO_2). In consequence, the blood pH is increased, which induces a left-shift of the oxyhemoglobin dissociation curve (Calbet et al., 2003). More details about the factors influencing the oxyhemoglobin dissociation curve were previously reported in **section 1.2 "The effect of hypobaria"**. Moreover, hypocapnic state reduces peripheral chemoreceptor sensitivity and lowers central chemoreceptors activity in the medulla (Fitzgerald and Parks, 1971; Easton and Anthonisen, 1988; Cunningham et al., 2011), which lead to a progressive reduction in ventilation in hypoxia.

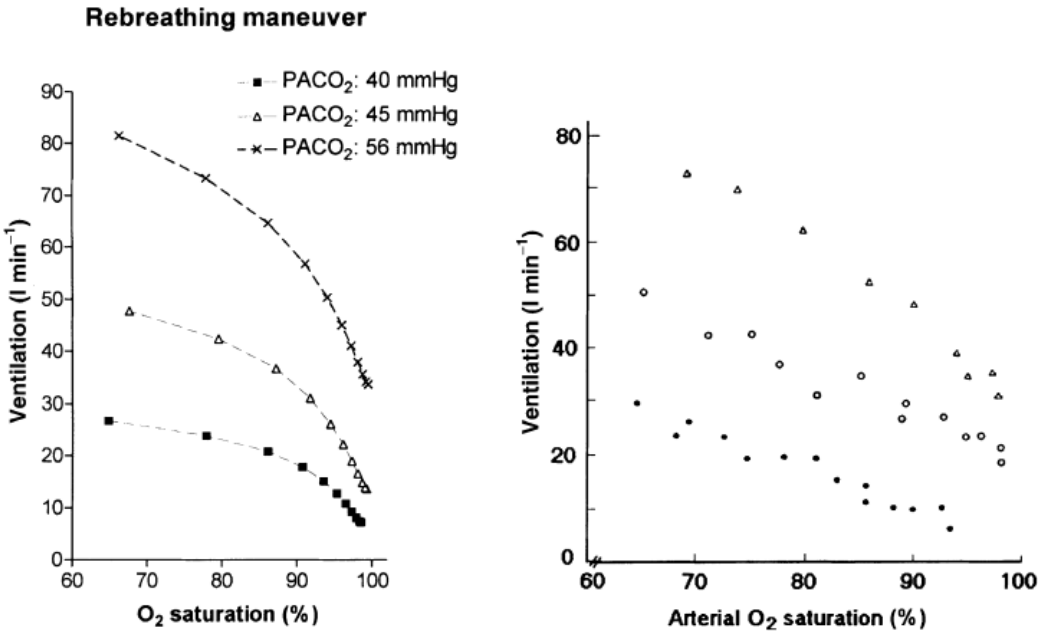

Figure 3: Left panel – Minute ventilation and arterial oxygen saturation obtained during three hypoxic simulations assessed at three constant levels of alveolar CO_2 pressure (P_ACO_2). Each of hypoxic manoeuvre consisted of a decrease in inspired PO_2 from 149 mmHg to 40 mmHg accomplished in 3 minutes. Right panel represents experimental curves measured on a human volunteer at the same levels of P_ACO_2 (Rebuck and Woodley, 1975). **Source:** Clinical Physiology, Volume: 21, Issue: 4, Pages: 465-477, First published: 28 June 2008 (Ursino et al., 2001).

In hypoxia, hyperventilation-induced hypocapnia is enhanced by peripheral respiratory chemoreflex increase (Ogoh, 2019). A recent review, reported ventilatory responses of various studies were reported when exposed to normobaric or hypobaric hypoxia (Coppel et al., 2015). Studies were conducted in long exposure (>1 hour) (Tucker et al., 1983; Roach et al., 1996; Loeppky et al., 1997; Miyagawa et al., 2011; Faiss et al., 2013b) and in acute hypoxia (<1 hour) (Savourey et al., 2003, 2007; Basualto-Alarcón et al., 2012). Minute ventilation in HH was either lower (Tucker et al., 1983; Loeppky et al., 1997; Savourey et al., 2003; Basualto-Alarcón et al., 2012; Faiss et al., 2013b) than in NH or equivalent (Miyagawa et al., 2011; Savourey et al., 2007). More precisely, tidal volume (VT) decrease was reported in many studies (Loeppky et al., 1997; Savourey et al., 2007; Basualto-Alarcón et al., 2012),

up to 0.9 L (Faiss, Pialoux, et al., 2013) in HH. Moreover, breathing frequency (Bf) was higher (Savourey et al., 2003, 2007), lower (Basualto-Alarcón et al., 2012) or unchanged (Loeppky et al., 1997; Miyagawa et al., 2011; Faiss et al., 2013b) in HH than in NH. In addition, larger hypocapnia (decreased $P_{ET}CO_2$) and blood alkalosis were observed when initially exposed to HH in comparison with NH (Savourey et al., 2003), but no difference during prolonged exposure. Overall, disparities regarding ventilatory responses between normobaric and hypobaric hypoxic conditions exist but the putative effect of hypobaria on ventilation remains unclear in hypoxia.

In normoxia, hypobaria may also influence ventilation and induce ventilatory pattern modulations, as inspired gas density is reduced. In fact, the airway resistance is reduced in the lungs and the maximal voluntary ventilation (MVV) is increased. Early studies that have evaluated the effect of hypobaria in normoxia showed that $\dot{V}O_{2max}$ was higher in HN than in NN (Cerretelli, 1976; Marconi et al., 2004). More recently, it was suggested that reduced airway resistance associated with hypobaria would decrease turbulent airflow (Ogawa et al., 2019). More precisely, this study reported changes in ventilatory pattern (i.e. maximal ventilation increase) in HN compared to NN, with lower air density as the putative main factor. The authors demonstrated that $\dot{V}E/\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$ in HN were greater than in NN, which implies that air-flow resistance was modified by hypobaria (Ogawa et al., 2019). Therefore, human individuals have the ability to ventilate greater volume of ambient air at high altitude than at sea level (Milledge, 2020). For instance, increased maximal ventilation in HN was observed when compared to NN (Ogawa et al., 2019). In addition, it was suggested that the ventilatory dead space is increased with hypobaria in normoxia (Ogawa et al., 2019) and hypoxia (Savourey et al., 2003), which may explain the reported differences in the blood gas and the ventilatory parameters. In fact, $P_{ET}CO_2$-P_aCO_2 gradient may be higher when dead

space in increased (Donnellan, 2011). In addition, HN is also used in Chilean miners working at high terrestrial altitude (4200 m) with supplemental oxygen in dormitories for reducing periodic breathing and improving recovery (Moraga et al., 2014).

Reduction in barometric pressure may also increase pulmonary vascular pressure due to the reduced air density in hypobaria (Conkin, 2016). An early study showed blood–brain barrier permeability increase in rabbits exposed to hypobaria without change in PO_2 after 45 minutes of exposure to an equivalent of 30000 ft (around 9140 m) (Chryssanthou et al., 1987). Moreover, lung lymph flow was increased in HN and altitude in sheep, which suggests an increased pulmonary vascular permeability when P_B is reduced maybe due to intravascular microbubbles (Hirai et al., 1988). But contrastingly, it was demonstrated that both hypoxia and hypobaria are needed to induce lymph flow increase (Levine et al., 1988). In addition, pulmonary resistance was increased in HN compared to NN condition, which implies a specific effect of hypobaria possibly affecting pulmonary blood flow (Petrassi et al., 2018a). Moreover, different fluid and acid-base balance responses mediated by the cell-membrane permeability alteration and aldosterone elevation have been suggested as a consequence of hypobaria (Loeppky et al., 2005). Overall, hypobaria may play in important role on ventilation.

1.3.2 Cardiovascular responses

Hypoxic exposure induces ventilatory response (i.e., previous section), but also changes in cardiovascular regulation (Insalaco et al., 1996; Hanada et al., 2003), which is mediated by stimulation of peripheral chemoreceptors sensing a decrease in arterial oxygen partial pressure (Marshall, 1994; Lahiri et al., 2006). It has been previously shown that heart rate was represented by an inverse linear relation to arterial oxygen saturation in isocapnic hypoxia (constant PCO_2), but there was no significant correlation between heart rate and ventilatory

responses to hypoxia (Slutsky and Rebuck, 1978). Moreover, acute systemic hypoxia causes significant increases in heart rate and human skeletal muscle sympathetic nerve activity (MSNA) (Hanada et al., 2003).

Therefore, hypoxia-induced hyperventilation and tachycardia are mediated by the chemoreflex activation in response to P_aO_2 decrease (Marshall, 1994). Acute systemic hypoxia has thus a stimulatory effect on ventilation, heart rate and MSNA (Rowell et al., 1989; Somers et al., 1989; Seals et al., 1991). More recently, it was shown that the MSNA response to hypoxia was not influenced by hypocapnia, which suggests that the interaction occurs only during excitatory chemosensitive stimuli (Jouett et al., 2015). Hypoxic effect on the autonomic cardiac function is detailed in the following sections.

The human brain is known as a particular vulnerable organ to hypoxia because of its reliance on aerobic metabolism and the absence of oxygen storage (Raichle and Gusnard, 2002). On the other hand, however, skeletal muscles are tolerant to temporary hypoxaemia thanks to their myoglobin content, minor oxygen demand at rest and anaerobic metabolism capacity (Lundby et al., 2009). In order to limit oxygen deprivation, beside tachycardia and hyperventilation, blood flow adjustments (i.e., through vasodilation) occur (Fernandes et al., 2018) in the brain (Kety and Schmidt, 1948) and skeletal muscles (Richards et al., 2017). For instance in the human forearm, it was reported that hypoxia-induced vasodilation was in part mediated by the local action of adenosine released when exposed to hypoxia (Leuenberger et al., 1999). Moreover, hypoxia increased sympathetic drive to the heart and blood vessels (Somers et al., 1989), which result in vasoconstrictive stimulus. However, it has been reported that sympathetic vasoconstriction is superimposed by vasodilatation in hypoxia without interference (Weisbrod et al., 2001; Dinenno et al., 2003). Moreover, vasodilatation and perfusion increases have been reported in cerebral (Willie et al., 2012) and muscular vascular

beds (Richards et al., 2017) when exposed to hypoxia. Regarding blood pressure, a significant relationship between hypoxic ventilatory responses and both systolic and diastolic blood pressure responses to gradual hypoxia has been reported (up to 5050 m) (Insalaco et al., 1996). However, no or minor mean arterial pressure elevation was observed in acute systemic hypoxia with makeable skeletal muscle sympathetic discharge, ventilation and heart rate increases (Saito et al., 1988; Rowell et al., 1989; Seals et al., 1991).

Contrastingly to the hypoxic stimulatory effect, it has been shown that MSNA, heart rate and ventilation were unchanged or reduced with hyperoxia (i.e., when breathing 100 % O_2) (Seals et al., 1991; Hansen and Sander, 2003). Nevertheless, the putative specific effects of hypobaria and hyperoxia on cardiovascular responses remain unclear.

1.3.3 Heart rate variability and baroreflex sensitivity

Heart rate variability is a non-invasive measurement to evaluate the cardiac autonomic control (Buchheit, 2014). It is also commonly used to monitor fatigue and overreaching in athletes (Bourdillon, Schmitt, et al., 2017; Meeusen et al., 2013), despite some debates about the pro and cons of frequency- vs. the time- (Plews et al., 2012) domain heart rate variability (HRV) parameters (Schmitt et al., 2015). The spectral power in low frequency (LF), high frequency (HF) and total power (LF + HF) are the most commonly used parameters of frequency domain, whereas the root mean square of the successive differences (RMSSD) is generally used as the main time domain parameter.

It has been reported that hypoxia has a negative influence on autonomic cardiac responses (Botek et al., 2015) and induces vascular systemic/integrative metabolic and endocrine compensation (Marshall, 1998). More precisely, acute hypoxic exposure induces sympathetic activation (Richalet et al., 1988; Marshall, 1994; Hainsworth et al., 2007) and parasympathetic activity decrease (Wille et al., 2012). Contrastingly, HRV parameters

measured 24h after maximal anaerobic exercise in normobaric hypoxia at 2500 m and 4000 m remained unchanged, when compared to normoxia (Álvarez-Herms et al., 2020). Thus, the timing of the HRV measurement, the duration of hypoxic exposures probably influence HRV modulation, as it seems under the influence of the rate of ascent (Vogel & Harris, 1967).

Respiratory sinus arrhythmia (RSA) is reliable non-invasive quantitative estimates of human vagal cardiac nerve circulation (Katona and Jih, 1975), which corresponds to the normal fluctuation of the heart rate (HR) in relation with the cycle of respiration. It consists of an increase in HR during inspiration and decrease during expiration. RSA magnitude is affected by autonomic balance and its measurement represents the HF parameter (parasympathetic activity) of HRV (Tzeng et al., 2007). At rest, a dominant parasympathetic nervous system activity facilitates RSA, whereas sympathetic nervous system activation decreases its magnitude. Moreover, RSA is the consequence of the modulation of pulmonary ventilation on vagal activity (Eckberg, 1983; Bernardi et al., 1998) and reflects the preferential heart beats distribution during inspiration. Estimation of RSA magnitude may provide an indirect measure of vagal tone and represent the level of mean vagal outflow (Hirsch and Bishop, 1981; Eckberg, 1983; Tzeng et al., 2007).

However, it remains unclear if hypoxia impacts the distribution of the heartbeats throughout the ventilatory cycle in humans. Acute hypoxia may influence RSA, as it induces sympathetic activation and stimulates carotid body, whereas hyperoxia facilitates sympathetic withdrawal (Lazar et al., 2020). Moreover, the increase in sympathetic activity in acute hypoxia may induce both blood pressure and heart rate increases (Hainsworth et al., 2007). In contrast, hypoxia also has an influence on vascular smooth muscle in the systemic circulation (Marshall, 1994), which may cause vasodilation and hypotension. For instance, hypoxia had non-significant effect on RSA, as the HRV frequency parameters remained unchanged in hypoxia when compared normoxia. Moreover, there was no significant change in the

preferential clustering of heartbeats during either the inspiration or expiration phases of ventilation in hypoxia (Brown et al., 2014). It is therefore of importance to quantify changes in HRV when exposed to hypoxia, as its influence on cardiac autonomic control remains unclear.

Hypobaria may play an independent role in hypoxia, as ventilation and cardiac autonomic control differ between NH and HH (Savourey et al., 2003, 2007; Conkin and Wessel, 2008). In addition, the ventilatory response when exposed to acute hypoxia may be reduced by the hyperventilation-induced hypocapnia (Weil et al., 1970). Therefore, hypocapnic state may decrease carotid body stimulation (Lahiri et al., 1978) and have an influence on RSA (Brown et al., 2014).

When exposed to hypobaria, change in ventilatory pattern (Ogawa et al., 2019) and increased intrapulmonary pressure (Conkin, 2016) may influence heart rate variability, as the cardiac autonomic activity is influenced by the respiration through the RSA (Brown et al., 1993) and the pulmonary arterial baroreceptors (Hainsworth et al., 2007). For instance, parasympathetic activity was increased in HN (Prabhakaran & Tripathi, 2011). However, the putative specific influence of hypobaria on HRV, especially in normoxia, has been scarcely explored up to date.

The baroreflex is a strong mechanisms that regulates blood pressure (BP) (Abboud & Thames, 2011; Kirchheim, 1976) and is responsible for adequate blood supply to the brain and all organs. Baroreceptors are located in the carotid sinus and the aorta and sense systemic blood pressure through the stretch of receptors. Baroreceptors detect changes in blood pressure and induce reflex circulatory adjustments that lowers BP variability and its negative consequences, by providing moment-to-moment negative-feedback (Chapleau, 2003). Thus, blood pressure is adjusted by the afferent discharge due to change in arterial baroreceptors. For instance, reduction in arterial pressure decreases baroreceptor afferent discharges, which

lead to a parasympathetic activity reduction and a sympathetic tone increase, triggering an increase in HR, cardiac contractility, and vascular resistance. On the contrary, an elevation in pressure inhibits the sympathetic and stimulates the parasympathetic activity. Various conditions challenge the arterial baroreflex, such as exhaustive endurance exercise (Gratze et al., 2005) or intensive training and altitude exposure (Bourdillon, Saugy, et al., 2017).

Baroreflex sensitivity (BRS) is non-invasive measurement of the arterial baroreflex function. High BRS value underlines a fast change in HR in response to small changes in BP, which suggests a sensitive autonomic control of BP. BRS is directly related to basal parasympathetic activity (Hughson, Maillet, et al., 1994). However, when exposed to severe hypoxia, parasympathetic activity is withdrawn (Hughson, Yamamoto, et al., 1994b; Ponchia et al., 1994). Decreased parasympathetic activity in hypoxia alters the neural control of the heart (Yamamoto et al., 1996) and leads to BRS resetting to higher blood pressures (Raven et al., 2006). Such resetting results in BRS decrease directly upon altitude expose (Roche et al., 2002) in acute and chronic hypoxia (Bourdillon et al., 2018). Resetting of the BRS is clear above 4500 m but remains unclear at lower altitudes (Querido et al., 2011). However, lower BRS values in NH and HH than in normobaric normoxia have been previously reported, but without differences between altitude levels of 2250 m and 3450 m (Bourdillon, Saugy, et al., 2017).

During exercise, the arterial baroreceptors are activated due to changes in cardio-circulatory dynamics caused by the BP elevation (Michelini et al., 2015). However, there is only a moderate increase in mean BP, because of the BRS resetting to higher blood pressures (Bevegård & Shepherd, 1966b; Joyner, 2006; Pawelczyk & Raven, 1989b), which results in BRS reduction during exercise (Vallais et al., 2009).

1.3.4 Cerebrovascular regulation to carbon dioxide

From a clinical point of view, brain vasculature health corresponds to the capacity of the cerebrovascular system to provide sufficient blood flow to match tissue demand (Fisher & Mikulis, 2021). Cerebral blood flow (CBF) is very sensitive to hypoxic stimulus and plays an important role in cerebral oxygen delivery (cDO_2) maintenance. CBF is controlled by complex vasoactive responses of the middle cerebral artery (MCA) (Imray et al., 2014; Willie et al., 2014a), extracranial cerebral vessels (Lewis et al., 2014) and in the pial mater arterioles (Wolff, 1930). PaO_2 and carbon dioxide arterial pressure ($PaCO_2$) play a complex role on CBF. More precisely, each mmHg decrease of $PaCO_2$ induces a decrease in CBF by approximately 3-4% (Brugniaux et al., 2007; Ainslie and Duffin, 2009; Willie et al., 2012). On the opposite, $PaCO_2$ and blood pH elevations are major factors, due to their vasoactive effects, increasing CBF via a common mechanism (Willie et al., 2014b). In fact, resting cerebral blood flow can more than double in case of hypercapnia (Lassen, 1959).

In acute hypoxia (from minutes to hours), cerebral vasodilatation (i.e., increase of MCA diameter) occurs to limit reduction in cDO_2 (Wilson et al., 2011; Imray et al., 2014; Mikhail Kellawan et al., 2017). More precisely, cerebral vasodilation permitted an increase in cDO_2 by 0.5-2.5% of SaO_2 decrease (Cohen et al., 1967; Jensen et al., 1996; Willie et al., 2012). In parallel however, hyperventilation and hypocapnia induced by hypoxia stimulate vasoconstriction, but vasodilation typically dominates as progressive CBF increase was observed at altitude, despite hypocapnia (Willie et al., 2014a). In a recent review (Hoiland et al., 2016), several studies demonstrated the compensatory rise in CBF upon acute exposure to isocapnic hypoxia in order to maintain cDO_2. Cerebral blood flow was increased in hypoxia (Cohen et al., 1967; Mardimae et al., 2012). The figure bellow illustrates the increased CBF according to changes in PO_2 (**Figure 4**), supporting the influence of blood gases as a key

factor in CBF regulation (Duffin et al., 2021; Mardimae et al., 2012). Therefore, cDO_2 in acute hypoxia is associated with the cerebral vasodilation, which compensates the vasoconstriction caused by hypocapnia induced by chemoreflex-driven ventilation (Teppema & Dahan, 2010).

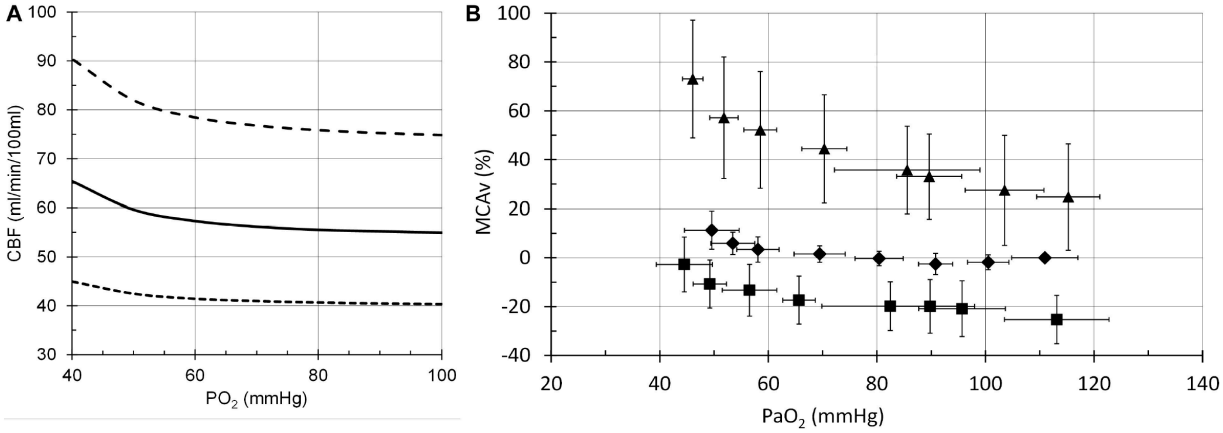

Figure 4: Cerebral blood flow (CBF) responses to PO_2. (A) Model responses at PCO_2 = 35 mmHg (dotted line), PCO_2 = 40 mmHg (solid line) and PCO_2 = 50 mmHg (dashed line). (B) Measurements of middle cerebral artery blood flow velocities using Transcranial Doppler (TCD) at different PCO_2 = 30 mmHg (squares), PCO_2 = 40 mmHg (diamonds) and PCO_2 = 50 mmHg (triangles). **Source:** (Mardimae et al., 2012). Recently republished (Duffin et al., 2021).

One of the methods to evaluate how CBF is regulated by the cerebral vasculature is by measuring cerebrovascular reactivity to CO_2 (CVR, **Figure 5**). CVR is represented with a sigmoidal curve, displayed below. In case of CO_2 increase or O_2 decrease, an elevation in CBF occurs. CVR evaluation is possible by measuring the middle cerebral artery velocity (MCAv) (Ainslie & Ogoh, 2010). CVR is strongly regulated by blood pH (i.e., hydrogen ion concentration). At altitude, the relationship between changes in P_aCO_2 and $[H^+]$ is altered due to buffering capacity alteration (i.e., changes in acid base status), which has consequences on how P_aCO_2 is translated into a vasodilatory stimulus (Hoiland et al., 2019). When CO_2 remains uncontrolled, the magnitude of change in CBF in hypoxia is related to four reflex-

mechanisms factors: (i) hypoxic ventilatory response; (i) hypercapnic ventilatory response at rest; (iii) hypoxic cerebral vasodilation; and (iv) cerebral vasoconstriction due to hypocapnia (Brugniaux et al., 2007). The effect of hypoxia on CVR remains unclear as controversial results were obtained: For instance, CVR in hypoxia was increased during hyperoxic poikilocapnia (Fan et al., 2010) and hyperoxic isocapnia (Subudhi et al., 2010); decreased during hyperoxic poikilocapnia or unchanged during hypoxic poikilocapnia (Ainslie & Burgess, 2008) and uncontrolled hypercapnia (Jansen et al., 1999).

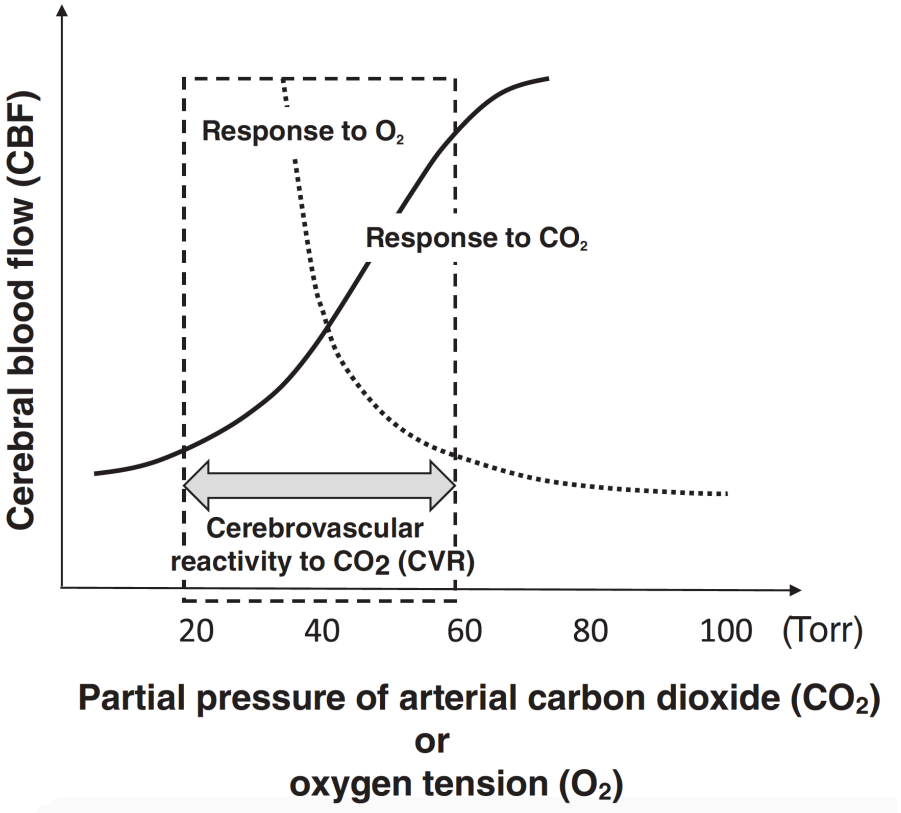

Figure 5: Cerebral vasculature is sensitive to changes in partial pressure of arterial CO_2 (P_aCO_2) and oxygen (P_aO_2). Cerebral blood flow (CBF) increases with decreased PaO_2 or P_aCO_2 elevation. Cerebrovascular reactivity to CO_2 (CVR) corresponds to the response of CBF to changes in $PaCO_2$. **Source:** (Ogoh, 2019).

Although still debated (Millet et al., 2012; Millet and Debevec, 2020), differences in the cerebrovascular regulation may therefore exist between NH and HH. The effects of hypobaria on CVR responses are scarcely explored and to our knowledge, there is no study comparing cerebrovascular reactivity to CO_2 in NN vs. HN and NH vs. HH conditions. Investigating CVR in hypobaric normoxia/hypoxia is therefore of interest in the context of both aviation and high-altitude residents/mountaineers/workers, as these populations may be exposed to hypobaria with or without supplemental oxygen.

1.3.5 Brain activity

Electroencephalography (EEG) is a non-invasive high-resolution measurement that quantifies electrical changes within the brain, which provides temporal resolution in the millisecond range (Gevins et al., 1995) . EEG is appropriated to record rapid changes in regional patterns of neuronal activation using multiple electrodes placed over the scalp (Gevins, 1998). The EEG signal can be divided into "bands" according to the frequency of the brain waves, which corresponds to the rhythmic activity of the brain.

Early studies have been studied EEG in hypoxia since the 1930s (Gibbs et al., 1935; Walter, 1969). In hypoxia, cerebral oxygenation is reduced at rest and neuronal impairment can occur in case of a prolonged misbalance between O_2 supply and demand (Krnjević, 1999). Hypoxia is observable in three different circumstances; when the oxygen supply to the blood is insufficient in case of hypoxic exposure, anaemia or ischemia (Plum & Posner, 1982). When exposed to hypoxia, one adaptive responses is an increased CBF in order to limit oxygen deprivation to the brain (Wilson et al., 2011). Some early studies were interested in EEG during sessions of cerebral hypoxia (Berger, 1931; Gibbs et al., 1935; Walter, 1969) and revealed that the neuronal activity is sensitive to brain's oxygen supply, time exposure and altitude level (Gastaut, 1961; Goodall et al., 2014; Ozaki et al., 1995).

Many studies have investigated the influence of acute hypoxic exposure on brain's electrical activity, when exposed to NH (Burykh, 2005; Rebuck et al., 1976; Rice et al., 2019a, 2019b; Schellart & Reits, 2001) or HH (Kraaier et al., 1988; Ozaki et al., 1995; Papadelis et al., 2007). For instance, frontal and temporal EEGs' phase shift increased in delta- and theta-range, whereas average level of the phase shift decreased in beta-range in NH (i.e., using 8% oxygen content gas mixture) (Burykh, 2005). All frequencies (alpha, beta, gamma, and theta) showed a power decrease for all channels in acute NH (25000 ft, 7620 m) (Rice et al., 2019a). Alpha activity deviated strongly in NH with closed eyes, to greater extent during first 20 minutes of exposure (Schellart & Reits, 2001). Hypobaric hypoxia induced a significant increase in slow activity, with a decrease in alpha activity and a non-significant decrease in beta activity (Kraaier et al., 1988). Alpha power decreased at 6000 m and theta increased at 5000 m and 6000 m in HH (Ozaki et al., 1995). Ozaki and colleagues showed that first stages of HH exposure (from 3000m to 4000m) was not characterised by significant modulations of alpha activity, whereas further elevation above 5000 m led to an increase of alpha power and a significant enhancement in theta activity (Ozaki et al., 1995).

EEG slowing is related to increases in alpha (low frequency) and theta activity (Ozaki et al., 1995). A review showed a slowing of cerebral neuronal activity at rest in acute hypoxia (Goodall et al., 2014). It was also reported in other studies (Ernsting, 1963; Kraaier et al., 1988; Ozaki et al., 1995). Moreover, an increased slowing of the EEG signal in hypobaric hypoxia suggested a reduced neuronal activity (Papadelis et al., 2007). Interestingly, EEG slowing occurred when SpO_2 decreased below 75% (Goodall et al., 2014). On the contrary, no EEG change was observed when SpO_2 remained above 75% (Rebuck et al., 1976). Accordingly, it has been stated that the central nervous system is functionally impaired from an altitude level around 4500 m (Luks et al., 2021).

Microstates, consists of an electrophysiological observation of a given structure of the overall scalp electric field (i.e., topography), also suggested as the “atoms of thought” (Lehmann, 1990). These microstates correspond to synchronized activation of different neuronal configurations (Brunet et al. 2010) and reflect the functional states of neurocognitive networks (Koenig et al 2002). The estimated duration of such conscious brain states is in the range of a few hundred milliseconds, based on EEG measurements (Bressler, 1995). Moreover, microstates measured with multichannel EEG remain stable for periods of approximately 100 milliseconds before switching to a new “cognitive state” (Lehmann et al., 1987). Recently, some studies were interested in EEG resting state topographical analysis (Custo et al., 2014, 2017; Spring et al., 2017, 2018), which are represented by four to seven resting state maps. Each of these maps has been spatially correlated with a specific brain network distribution and a resting state network (Britz et al., 2010). For instance, map A has been associated with the visual, map B with the auditory, map C with the salience, and map D with the attentional resting state networks (Britz et al., 2010). It has been proven that microstates give information about vigilance state and sleep stages (Brodbeck et al., 2012; Bréchet et al., 2020) , cognitive and attentional processes (Milz et al., 2016; Seitzman et al., 2017) and the influence of acute physical exercise (Spring et al. 2017, 2018). It seems that there is no study evaluating microstates in hypoxia. Therefore, the present thesis adds some novelty by evaluating microstates at different altitude levels in acute hypobaric hypoxia at rest.

1.4 Cognitive functions in hypoxic environments

In a review that evaluated cognition in various environmental conditions, cognitive function tended to be altered in acute hypoxia (Taylor et al., 2015). Decreased oxygen availability at moderate and high-altitude (around 1500–7500 m) leads to cognitive function alterations

(Adam et al., 2008; de Aquino Lemos et al., 2012). Moreover, executive function impairment was negatively correlated with arterial oxygen saturation during gradual simulated altitudes in NH (Ochi et al., 2018). In the most recent review evaluating the effect of acute hypoxia on cognition, it was reported that low PaO_2 (35–60 mmHg) was the key predictor of cognitive performance impairment, independently of the type of hypoxia (normobaric or hypobaric) (McMorris et al., 2017). These authors suggested that the CBF increase, when PaO_2 level is low (<60 mmHg), is unable to compensate for the lack of oxygen supply for the maintenance of cognitive performance (McMorris et al., 2017). Many studies have reported cognitive performance impairment in acute HH (Asmaro et al., 2013; Beer et al., 2017; Takács et al., 2017) or NH (de Aquino Lemos et al., 2012; Phillips et al., 2015). For instance, acute hypoxia increased reaction time and short-term memory impairment was noticeable above 6000 m (Virués-Ortega et al., 2004). However, hypobaria may play a negligible role on cognition in hypoxic conditions, as there was no change in cognitive performance between NH and HH (McMorris et al., 2017).

Cognitive tasks include memory (working, spatial, and verbal), attention and executive function (Lezak et al., 2004). When investigating cognitive function, tasks are usually categorized either as “simple” or “complex” (Ramsey & Kwon, 1992), A simplistic task categorization was reported in a recent review on cognition (Taylor et al., 2015). For instance, tasks including short-term memory and simple arithmetic are considered as “simple cognitive tasks”, whereas arithmetic efficiency and working-memory as “complex cognitive tasks” (Taylor et al., 2015). Working-memory, which is the ability to keep and process short-term information long enough to maintain attention when performing a cognitive task (Steiger et al., 2019). Because of inter and intra-individual variations, the independent effect of hypoxia and hypobaria on cognitive tasks remains unclear.

1.5 Implications in pilots

Military personnel and pilots are daily exposed to hypobaric, and in some cases, hypoxic environments when cabin decompression (Auten et al., 2010; Nishi, 2011) or oxygen regulator system failure occur. Beside various stressors during flight, such as noise, vibrations, acceleration, or spatial disorientation, the supposed most hazardous factor when flying at high altitude remains hypoxia (Harding & Mills, 1983). Therefore, it is critical to understand the effect of hypoxia on cognitive responses in pilots. A recent review focused on cognitive performance in HH and reported their implications for training in the field of aviation (Neuhaus & Hinkelbein, 2014). For instance, pilot's performance remained equivalent in simulated hypoxic condition (3810 m) (Peacock et al., 2017). However, working memory was altered in pilots with lower arterial oxygen saturation, when exposed to acute HH at extreme altitude (10'000 m) (Malle et al., 2013). The effects of hypoxia is highly variable between individuals, as it causes mild symptoms and performance alterations at low altitudes (below 10,000 ft, 3048 m) (Cable, 2003; A. Smith, 2005) but may result in rapid loss of consciousness (i.e., hypoxic syncope) at moderate altitudes (e.g. 18,000 ft; PO_2 70 mmHg) (Chiang et al., 2012). Therefore, impaired hypoxia tolerance may cause in-flight incapacitation (Chiang et al., 2012).

Hypoxia remains a flight-safety issue in terms of aviation medicine, as hypoxic episodes are increasingly common in military aviation. Nowadays, hypoxia training is mandatory for military pilots, however evidences regarding the training effects in hypoxia remain low.

Hypoxia awareness training has been used to help aircrew members recognize personal symptoms to hypoxic environments (Chiang et al., 2021). A recent study evaluated the training effect in normobaric hypoxia (Leinonen et al., 2021). These authors showed that hypoxia training improves pilots' ability to recognise their symptoms to hypoxia up to 2.4 years after an initial training session in NH (Leinonen et al., 2021). Traditionally, aircrew

have been trained in hypoxia using hypobaric chamber (i.e., in hypobaric hypoxia) to recognize the symptoms at simulated altitudes of 25,000 ft or more (Cable, 2003). A recent review provided an overview regarding the cognitive responses to hypobaric hypoxia and evaluated relevant implications for aviation training (Neuhaus and Hinkelbein, 2014). The authors also mentioned the major disparities regarding the required quality and quantity of hypoxia training for both military and civilian pilots (Neuhaus and Hinkelbein, 2014). Then, latest review on cognition and application in the military aviation proposed an overview of the basic physiology and implications of hypoxia for military aviation and evaluated the utility of hypoxia recognition training (Shaw et al., 2021). Hypoxia recognition training is paramount, as hypoxia may occur below 10,000 ft (3,048 m) in susceptible individuals in unpressurised aircrafts or at higher altitudes in pressurised cockpits at higher altitudes in case, for instance, of breathing systems failure. Between 10,000 ft (3048 m) and 15,000 ft (4,572 m), hypoxic symptoms are common and cognitive functions are slightly impaired. Both symptoms and cognitive functions are often difficult to be accurately quantified with hypoxia, as they may in part be due to the effects of hyperventilation-induced hypocapnia. With gradual altitude increase above 15,000 ft, brain function exponentially deteriorates until loss of consciousness (Shaw et al., 2021). Overall, hypoxia training is of importance, as hypoxia in flight still remains a serious risk for pilots (i.e., most commonly occurring below 19,000 ft, 5791 m), which can result in fatalities (Cable, 2003).

1.6 Physiological responses at exercise

It is known for long that the aerobic performance is reduced in both acute and chronic exposures to hypoxia (Pugh, 1967). A reduction in P_IO_2 causes a decrease of the P_AO_2 and therefore the oxygen furniture to the tissues (Cerretelli, 1980), which triggers rapid and important adaptive physiological responses. Exercise increases oxygen demands, whereas

hypoxia affects blood oxygenation at the lungs level. During submaximal exercise, some physiological regulations (i.e., increased heart rate and cardiac output; higher skeletal muscle blood flow) occur in order to maintain oxygen delivery by counterbalancing the decreased arterial oxygen concentration (Roach et al., 1999). Hypoxic ventilatory responses is correlated with $\dot{V}E$ and SaO_2 during exercise in hypoxia (Benoit et al., 1995), Increased $\dot{V}E$ allows to increase SaO_2 , hence HVR is suggested as a positive adaptation to hypoxia (Huang et al., 1984). Moreover, cardiac output increase increasing blood volume in circulation (Grover et al., 1986). During exercise in acute hypoxia, the skeletal muscle blood flow is rapidly adjusted to maintain oxygen delivery to the tissues (Gonzalez-Alonso, 2002). In addition, decreased cerebral oxygenation *per se* plays a role on performance alteration and motor drive reduction during moderate-intensity exercise in severe hypoxia (i.e., when arterial O_2 saturation was below 70–75%) (Verges et al., 2012). For instance, decrement in cycling time-trial performance was higher hypobaric than normobaric hypoxia (Saugy et al., 2016a). However, same performance change was observed after hypoxic training methods in normobaria versus hypobaria (Saugy et al., 2016b). Therefore, the putative effect of hypobaria remains unclear on physical performance in hypoxia.

Cerebral and muscular oxygenation can be measured using near-infrared spectroscopy (NIRS). NIRS is a non-invasive technique to measure changes in oxygenation parameters, such as oxy-, deoxy- and total haemoglobin concentration (O_2Hb, HHb and tHb, respectively; changes in $\Delta\mu$mol). At rest, cerebral, tissue showed larger deoxygenation when resting in acute hypoxia, while muscle oxygenation remained unchanged (Ainslie et al., 2007; Peltonen et al., 2007). Moreover, tissue oxygenation index (TOI) in the muscle remained equivalent between normoxia and hypoxia at rest, whereas cerebral TOI decreased in hypoxia (Rupp & Perrey, 2009). NIRS showed great consistency regarding muscle oxygen consumption during

low- to moderate-intensity exercise (Lucero et al., 2018). A recent study showed reduction in prefrontal and motor cortex oxygenation during submaximal exercise upon first day of exposure at 4350 m (Marillier et al., 2021). Moreover, cerebral and muscle oxygenation were both altered (i.e., HHb concentration increased) in NH during a 5-min cycling exercise at 60–70% of normoxic maximal O_2 uptake at an altitude level of 3000 m (Ainslie et al., 2007). Up to date, there is no study that has investigated the effect of hypobaria *per se* on cerebral and muscle oxygenation during exercise. Therefore, it remains unknown if hypobaria has an additional influence on cerebral and muscle deoxygenation at rest and during exercise in hypoxia.

1.7 Aims and hypotheses

The main aim of this thesis was to evaluate the putative specific effect of hypobaria in hypoxia and normoxia on physiological responses and cerebral regulation at rest and during submaximal cycling exercise in young healthy pilot trainees. A minor aim consisted of evaluating the altitude severity at altitude levels of 3000 and 5500 m in hypobaric hypoxia.

To investigate the putative effect of hypobaria in hypoxia, comparison was made between acute exposure in NH and HH with equivalent P_IO_2. Then, in order to isolate the putative influence of hypobaria, comparison between NN and a hypobaric normoxic condition (HN) with comparable P_IO_2 was assessed. Detailed methods regarding protocol and measurements can be found in the following section entitled “Summary of experimental results” and in articles at the end of the present thesis.

The hypotheses are described and summarized below:

1) HH would induce greater ventilatory stimulus and physiological responses (i.e., increased heart rate and hypoxemia) than NH.
2) Hypoxia would induce sympathetic activity to greater extent with hypobaria.
3) Hypoxia and hypobaria would decrease baroreflex sensitivity. Moreover, BRS would also decrease during exercise.
4) Cerebrovascular reactivity to carbon dioxide would be increased in acute hypoxia with a putative specific effect of hypobaria-induced hypocapnia.
5) Slowing of the EEG signal would occur in hypobaric hypoxia when hypoxemia is observed. Then, the increase of altitude level would affect EEG signal to a greater extent and potentially alter microstates in acute hypobaric hypoxia.
6) Cognitive performance would be affected in hypoxic conditions, potentially to larger extent with hypobaria. However, cognitive performance would be maintained in hypobaric normoxia when compared to normobaric normoxia.
7) Hypobaria would increase symptoms severity in hypoxia, whereas it would play a negligible role in normoxia.
8) Exercising in hypobaric hypoxia would lead to greater physiological compensatory cardiorespiratory mechanisms (i.e., increased heart rate, minute ventilation and middle cerebral artery velocity) in order to limit the oxygen deprivation in the cerebral and muscular compartments.
9) At exercise, cerebral and muscular oxygenation would decrease to greater extent during a submaximal cycling exercise with hypobaria in hypoxia.
10) Hypobaria would have a negligible influence in normoxia. However, changes in breathing pattern may be observed in HN due to the reduced barometric pressure.

The present thesis adds novelty by evaluating the effect of hypobaria on heart rate variability, baroreflex sensitivity, cerebrovascular responses and cognitive performance in hypoxia and normoxia. Moreover, it evaluated electroencephalography at different altitude levels in hypobaric hypoxia. Finally, it investigated the putative additional influence of hypobaria on physiological and cardiorespiratory responses, baroreflex sensitivity, cerebral regulation and oxygenation parameters during submaximal cycling exercise. Finally, the present thesis has potential applications for pilots, aircrews, mountaineers and athletes, as they are regularly exposed to hypobaria, but also hypoxia when performing hypoxic training.

Chapter 2

Summary of experimental results

2 Summary of experimental results

The present section summarizes the main findings from the experimental studies that were conducted at the Swiss Aeromedical Center in Dübendorf (Zürich). Experiments were conducted in the hypobaric chamber of the Swiss Air Force (**Figure 6**).

Figure 6: Hypobaric chamber of the Swiss Air Force at the Aeromedical Center in Dübendorf (Zürich). **Copyright:** Fabrice Ducrest, University of Lausanne.

The first study was performed according to the Declaration of Helsinki and was approved by the Swiss Ethic Committee of Zürich (Swissethics, BASEC ID: 2017-00752). This clinical trial can be found on ClinicalTrials.gov (ID: NCT03303118). This study assessed physiological responses in fifteen healthy pilot trainees (26±4 years, 177±7 cm, 71±9 kg) at rest only in various hypoxic and hypobaric conditions. It first evaluated the altitude severity on physiological responses in hypobaric hypoxia at two different altitude levels (P_B:

523.8±6.9 and 381.7±9.2 mmHg for 3000 m and 5500 m, respectively). P_IO_2 between NN vs. HN (141±1 vs. 133±3 mmHg) and NH vs. HH (74±1 vs. 70±2 mmHg) were matched by adjusting the barometric pressure in the hypobaric chamber or F_IO_2 using the known equation ($P_IO_2=(P_B-47)\times F_IO_2$), when 47 mmHg corresponds to water vapour pressure at 37°c (Conkin, 2016). Participants inhaled ≈11% and ≈40% O_2 gas mixture (0.03% CO_2) concentration for NH and HN, respectively while P_B remained similar between NH and NN, but was decreased to similar extent in HN and HH. In each condition, participants performed: (1) Five min of acclimatization; (2) 7-min rest period seated with eyes closed for electroencephalography and heart rate variability recordings; (3) Cognitive test (duration: 4 minutes); and (4) Cerebrovascular reactivity to CO_2 assessment.

The second study assessed physiological responses at rest, but also during a submaximal cycling exercise (6 minutes, 1W/kg). It was performed according to the Declaration of Helsinki and was approved by the Swiss Ethic Committee of Zürich (Swissethics, BASEC ID: 2018–00006). This clinical trial can be found on ClinicalTrials.gov (ID: NCT03439202). The primary focus was to evaluate the putative hypobaric effect in hypoxia and normoxia. Therefore, condition in HH at 3000 m was removed from the protocol. In addition, altitude level (i.e., barometric pressure) in hypobaric conditions (HH and HN) was adjusted to 5000 m in order to perfectly match P_IO_2 (**Table 1**) between NN vs. HN (141.2±0.8 vs. 141.5±1.5 mm Hg) and NH vs. HH (75.7±0.4 vs. 74.3±1.0 mm Hg). Each condition consisted of: (1) Five minutes of condition acclimatization; (2) Cognitive test assessment; (3) 7-min rest period seated with eyes closed for electroencephalography and heart rate variability recordings; (4) A 6-min submaximal cycling exercise (1W/kg); (5) 7-min rest period post-exercise for EEG and HRV recordings; and (6) Cognitive test post-exercise. In this second study, eighteen healthy pilot trainees (14 men and 4 women, age 26±3 years; height 177±9 cm; weight 70±11 kg)

participated voluntarily. The main novelty of this second study is that blood pressure was measured in each condition, in order to evaluate the putative separate effect of hypoxia and hypobaria on blood pressure regulation (i.e., BRS). Moreover, cerebral and muscular oxygenation was monitored during the entire protocol.

Table 1: Barometric pressure and inspired pressure in oxygen (P_IO_2)

	NN	**HN**	**NH**	**HH**
Barometric pressure (mmHg)	723 ± 4	406 ± 4 *	723 ± 4	403 ± 5 *
P_IO_2 (mmHg)	141 ± 1	142 ± 2	76 ± 1 *#	74 ± 1 *#

Normobaric normoxia (NN); hypobaric normoxia (HN); normobaric hypoxia (NH); hypobaric hypoxia (HH). * $p<0.05$ for difference with NN; # $p<0.05$ for different with HN. **Source: Article 2**, submitted.

2.1 Ventilatory and cardiovascular responses at rest

During the resting period, $\dot{V}E$, HR, mean systolic and diastolic blood pressures were equivalent between all conditions. Data regarding HR are described in the following section. Blood pressure data are detailed in the section "baroreflex sensitivity". Resting $P_{ET}CO_2$ was lower in HN and HH compared to NN and NH ($p<0.001$ for all). As expected, end tidal partial pressures of oxygen ($P_{ET}O_2$) were lower in hypoxic conditions (NH and HH) than in the two-normoxic conditions (NN and HN). In addition, $P_{ET}O_2$ was lower in HH than in NH and in HN than in NN (both $p<0.05$). In the present project, no vascular data were recorded (i.e., artery diameter and blood flow). Ventilatory and cardiovascular data at rest and during exercise are detailed in **Articles 2, 3 and 6.**

2.2 Minimal influence of hypobaria on heart rate variability

Heart rate variability was recorded using a heart rate monitor (Polar RS800CX, FI-90440 Kempele, Finland). HRV measurement was assessed according to previous findings (Bourdillon et al., 2017), (i.e., around 300 beats were analysed during the last four minutes of a 6-min rest period seated). HRV data analysis was performed using specific software (Kubios HRV Standard, V 3.0). Analyses of the time domain HRV index (RMSSD) and spectral power values for frequency bands for: HF (0.15 to 0.50 Hz), LF (0.04–0.15 Hz) and total power (LF+HF) were assessed. Finally, the LF/HF ratio was calculated to evaluate the sympathovagal balance.

HR was higher in both NH and HH when compared to NN and HN ($p<0.001$), but to larger extent in HH than in NH. RMSSD was reduced in NH and HH likewise in comparison to NN and HN ($p<0.001$, **Figure 7**). LF (ms^2) was lower in NH and HH ($p<0.01$ and $p<0.001$, respectively) than NN. Moreover, HF (ms^2) was decreased in HH when compared to HN ($p=0.025$). More precisely, HF decreases were greater in NH (-35 %, $p=0.048$) and HH (-60 %, $p<0.001$) than in HN (+8 %), in comparison with NN. Moreover, HF reduction also was larger in HH than in NH ($p=0.048$). Overall, total power decreased in NH ($p=0.035$) and HH ($p=0.004$) in comparison with HN and NN ($p<0.001$). Regarding LF/HF ratio, it was greater in HH than NH. It was also higher in HH than HN ($p<0.001$). Finally, LF/HF ratio was lower in HN than NN ($p=0.041$). Detailed HRV results are reported in **Article 1** (Aebi et al., 2020a).

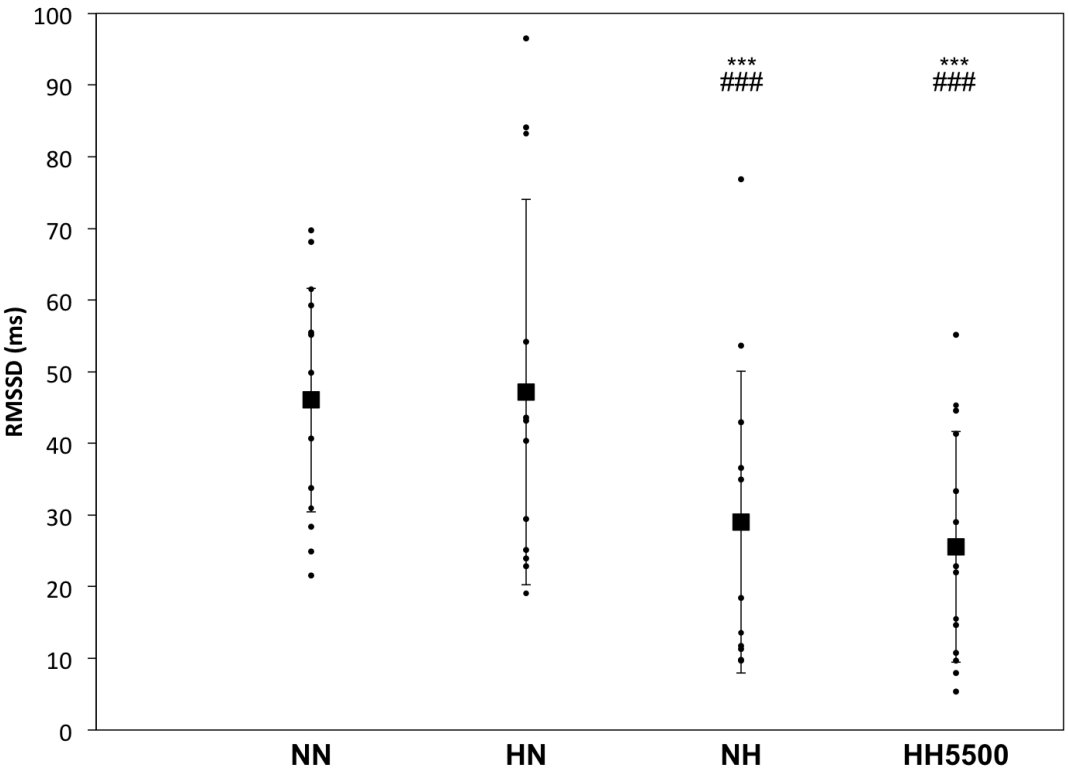

Figure 7: The root mean square of the successive differences (RMSSD) for each subject. Bold squares represent absolute means ± SD for conditions: NN, Normobaric normoxia; HN, hypobaric normoxia; NH, normobaric hypoxia; HH5500, hypobaric hypoxia at 5500 m. ***p<0.001 for difference with NN. ###p<0.001 for difference with HN. **Source:** (Aebi et al., 2020a)

2.3 Baroreflex sensitivity impairment in hypoxia and hypobaria

In this study, we also recorded continuous blood pressure and calculated baroreflex sensitivity (BRS) at rest, during submaximal cycling exercise and its recovery period. BP was recorded continuously using a double cuff installed on the index and the middle fingers and at a sampling frequency of 1,000 Hz using a photoplethysmography device combined to a double cuff (NIBP100D, Biopac Systems, Inc. Goleta, CA, USA). Detailed explanations of assessments and data analysis can be found in **Article 2**.

At rest, there was an equivalent decrease in BRS in HN, NH and HH compared to NN ($p<0.01$, $p<0.05$ and $p<0.001$, respectively) whereas no differences in HR, mean, systolic and diastolic blood pressures between these conditions were observed. At the end of exercise, BRS was reduced in NN, HN and NH ($p<0.001$, $p<0.01$ and $p<0.05$, respectively) but not in HH, in comparison with resting period. During recovery, BRS returned to basal values, showing equivalent values between conditions. Detailed results are displayed in the **Figure 8**. Additional figures and detailed data are represented in **Article 2**.

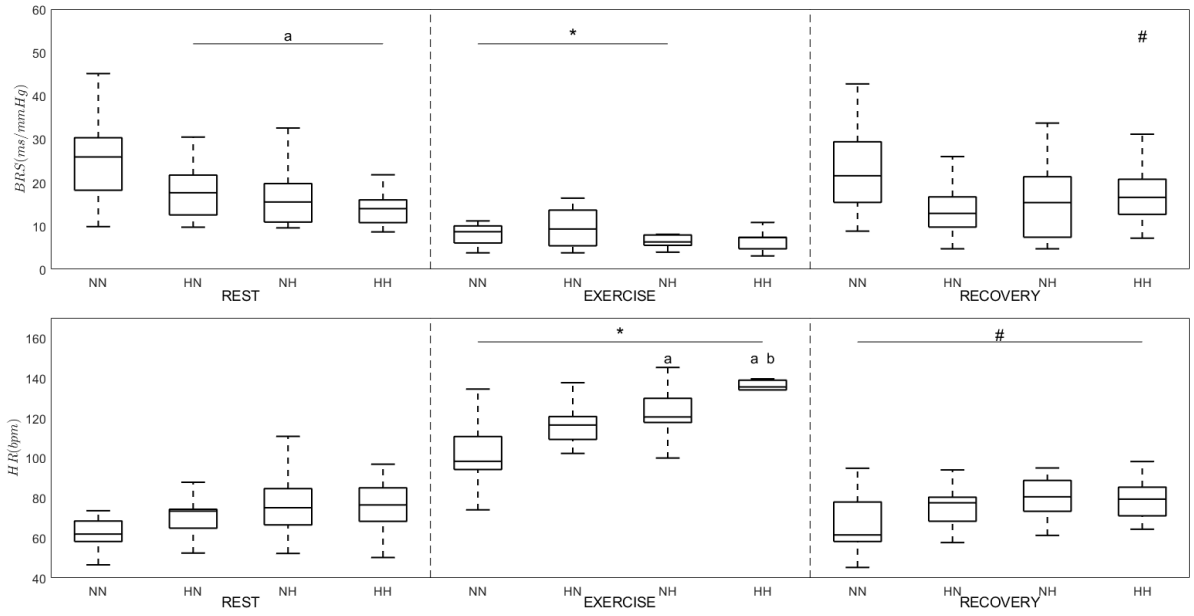

Figure 8: Baroreflex sensitivity (BRS) and heart rate (HR) in normobaric normoxia (NN), hypobaric normoxia (HN), normobaric hypoxia (NH) and hypobaric hypoxia (HH), during rest, exercise and recovery. **Source: Article 2**, submitted.

a $p<0.05$ for difference with NN
b $p<0.05$ for difference with HN
c $p<0.05$ for difference with NH
***** $p<0.05$ for difference with rest
# $p<0.05$ for difference with exercise

2.4 Specific effect of hypobaria on cerebrovascular reactivity to CO_2

The first study assessed the effect of hypobaria in hypoxia on CVR. It added novelty by also evaluating CVR in a hypobaric normoxic condition. Isolating the hypobaric effect from the hypoxic one would allow comparing comparable normoxic conditions, but with different barometric pressures. During CVR assessment, participants wore a mask and breathed through a two-way Y-valve, which permitted switching from ambient air (i.e., in the hypobaric chamber) to a separate hermetic bag that was filled with a hypercapnic gas mixture (20.9% O_2, 5% CO_2). For NH and HN conditions, participants were switched from a first gas mixture (≈11% O_2, 0.03% CO_2 or ≈40% O_2, 0.03% CO_2 respectively). MCAv was monitored during the entire protocol in the left middle cerebral artery using a pulsed Doppler ultrasound system (ST3, Spencer technology, Seattle, WA, USA). Gas exchanges data were recorded using a portable gas analyser (K5, Cosmed, Roma, Italy). Detailed methods are explained in **Article 3**.

There was a significant increase in CVR with increased altitude levels (**Figure 9**) in HH conditions. Midpoint was significantly lowered (i.e., left-shifted) at 3000 m (27.3 ± 2.0 mmHg) and 5500 m (19.6 ± 2.0 mmHg), compared to NN (35.7 ± 3.3 mmHg, $p<0.001$). Midpoint was decreased to greater extent at 5500 m than at 3000 m ($p<0.001$). Compared to NN (0.23 ± 0.12), the slope of sigmoid curve was significantly increased at 3000 m (0.52 ± 0.27, $p=0.007$) and 5500 m (0.66 ± 0.33, $p<0.001$) in HH. However, there was no significant change in slope between 3000 m and 5500 m HH.

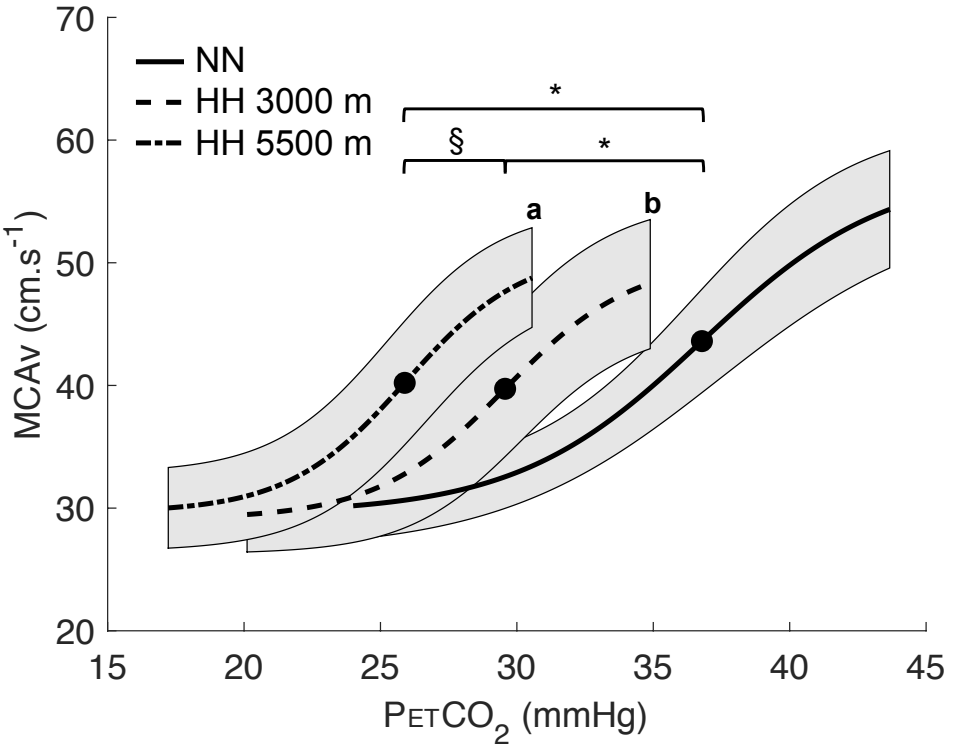

Figure 9: Mean sigmoidal curves of all subjects (n=9): In Normobaric Normoxia (NN, Dübendorf 440m); 3000 m and 5500 m in hypobaric hypoxia (HH) conditions. Bold point represents midpoint. * $p<0.05$ midpoint different than NN; § $p<0.05$ midpoint different than 3000 m; a $p<0.05$ slope different between 5500 m and NN; b $p<0.05$ slope different between 3000 m and NN. Shaded areas surrounding the sigmoid curves represent the 95% confidence interval. **Source**: (Aebi et al., 2020b).

There was a decrease in midpoint (left shift) with decreased barometric pressure (**Figure 10**). Midpoint was significantly lower in 5500 m HH and HN (21.6 ± 1.9 mmHg), when compared to NN ($p<0.001$). Slope was increased in HH in comparison with normobaric conditions; NH (0.35 ± 0.19, $p=0.003$) and NN ($p<0.001$). Slope was unchanged with hypoxia for the equivalent barometric pressure values, when comparing NN vs. NH and HH vs. HN, respectively. In normoxia, slope in HN tend to be increased when compared to NN ($p=0.069$).

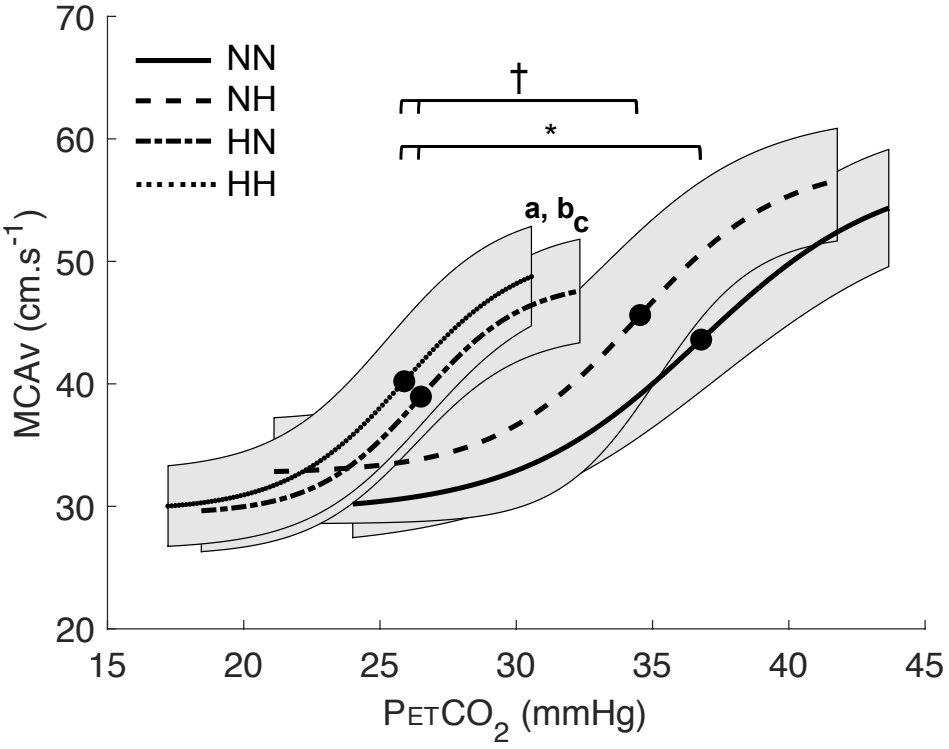

Figure 10: Mean sigmoidal curves of all subjects (n=9) in: normobaric normoxia (NN); normobaric hypoxia (NH); hypobaric hypoxia (HH) and hypobaric normoxia (HN) conditions. Bold point represents midpoint. † $p<0.05$ midpoint different between HH/HN and NH; * $p<0.05$ midpoint different between HH/HN and NN; a $p<0.05$ slope different between 5500 m HH and NN; b $p<0.05$ slope different between 5500 m HH and NH; c $p=0.069$ slope tend to be different between HN and NN. Shaded areas surrounding the sigmoid curves represent the 95% confidence interval. **Source**: (Aebi et al., 2020b).

2.5 Brain activity and microstates in hypobaric hypoxia

In the first study, electroencephalography was recorded in twelve healthy pilot trainees (10 men and 2 women, age 26±4 years; height 175±8 cm; weight 68±8 kg) in HH at two different altitude levels, 3000m and 5500 m. Continuous recording of the EEG was assessed at a sampling rate of 200 Hz with a 21-channels EEG cap (Waveguard connect, eemagine, Germany) mounted according to the International 10-20 recommendations. Electrodes were connected to a portable EEG system (Trackit, Lifelines, USA). The impedance (<8 kΩ) was checked before each EEG data collections. Detailed data analysis is explained in **Article 4**.

Detailed changes in power values are displayed in **Figure 11.** After the Bonferroni corrections, there was no significant change in power values regarding delta, theta, alpha and beta values. However, beta power increased significant only at 5500 m in HH (31% of the electrodes) after Bonferroni corrections. Overall, the present data indicate only a significant power values in the beta band increase at 5500 m.

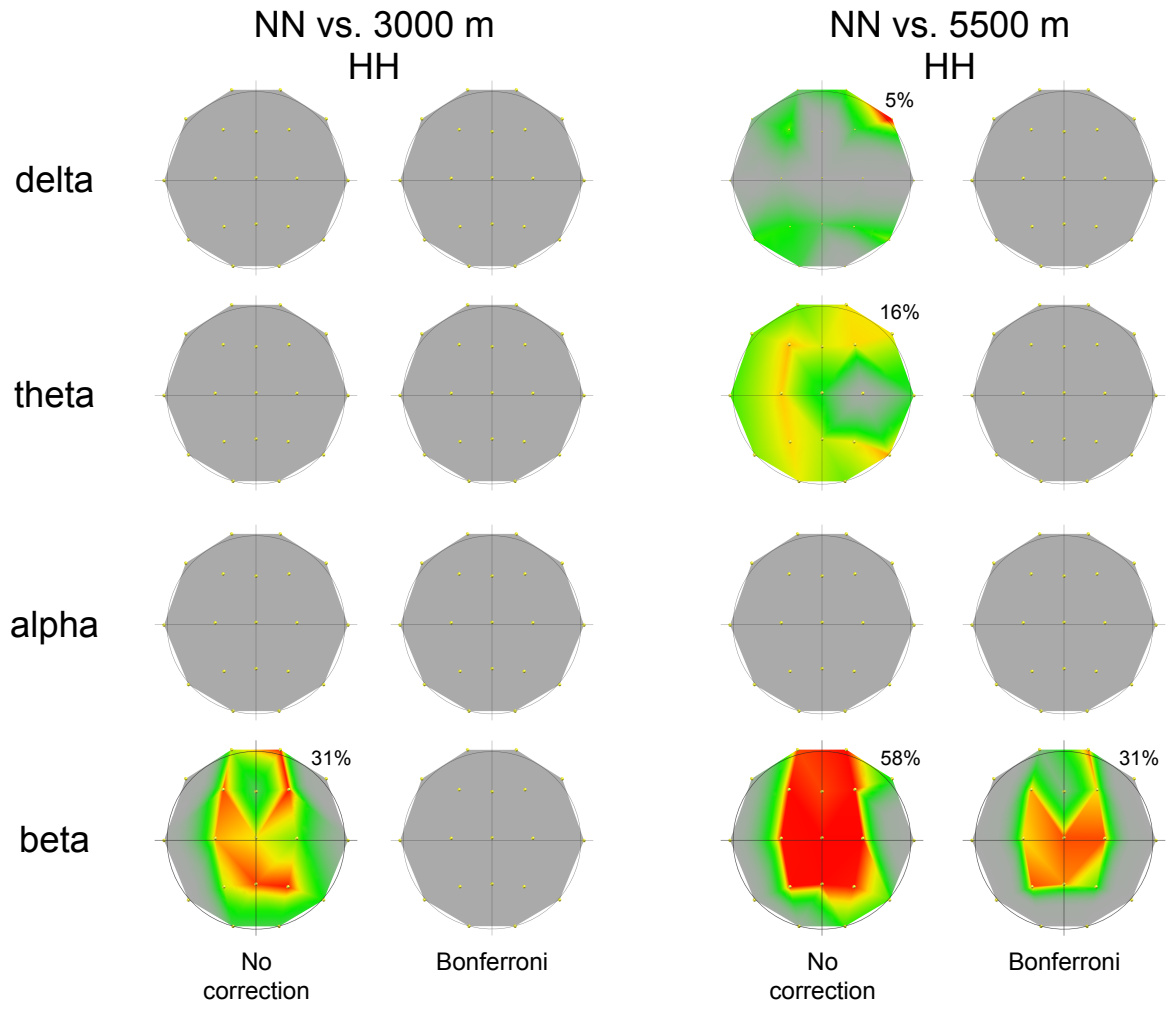

Figure 11: Change in Power values in beta (13.5-30 Hz), alpha (8-13 Hz), theta (4-7 Hz) and delta (0.5-4 Hz) frequency bands between control condition in normobaric normoxia (NN) and hypobaric hypoxic (HH) conditions (for altitude levels of 3000 m and 5500 m). **Source: Article 4** in preparation.

Regarding microstates analyses, the ANOVA repeated-measures did not show any main effect nor interaction between the two conditions in HH and the six maps for the delta global explained variance (GEV, which reflects the relative time coverage) and delta time coverage. Tukey post-hoc test revealed that the significant but uncorrected interaction is explained by a decrease of mean duration at 5500 m HH as compared to baseline for the map C only. Moreover, the data reveal that the frequency of occurrence slightly increased at 5500 mm HH as compared to the baseline for the map C. Detailed results are reported in **Article 4**.

2.6 Hypoxic effect on cognitive performance

The ‘‘Konzentrations-Leistungs-Test - Revidierte Fassung’’ (KLT-R) is a concentration-performance-test, which evaluates both quantity and quality of the capacity of concentration (Düker & Lienert, 2001b). KLT-R test consists of blocks each including 20 separate arithmetic tasks. In the first study, subjects performed two blocks in each condition. More detailed regarding cognitive assessment can be found in **Article 5** (Aebi et al., 2020c).

Number of calculations assessed was lower only in HH when compared to NN ($p=0.018$) and HN ($p=0.011$). Number of correct answer decreased to the same extent in NH and HH when compared to normoxic conditions (NN and HN, $p<0.001$). Percentage of error increased in the two hypoxic conditions when compared to normoxic conditions. There was no significant difference between NN vs. HN and NH vs. HH regarding cognitive performance.

SpO_2 decreased in NH and HH in comparison with normoxic conditions, with significant lower value in HH than NH ($p=0.008$). HH induced higher HR value than NH ($p=0.026$). MCAv was greater only in HH than in all other conditions. Interestingly, there was a significant correlation between ΔMCAv and ΔSpO_2 in HH ($r=-0.741$, $p=0.008$). Estimated cDO_2 was significantly decreased in NH ($p=0.033$) and HH ($p=0.016$) in comparison with NN. Complete results regarding cognitive performance are reported in **Article 5**.

2.7 Symptoms in hypoxia

Interestingly, some symptoms were more represented in hypoxic conditions (NH and HH), such as: dizziness, tiredness and calculation difficulties. Subjects reported being dizzy, having postural alterations, cold hands and being nauseous in NH and HH only. Globally, subjects reported more symptoms in HH than NH (symptoms are displayed in **Figure 12**). Detailed results regarding the symptoms are reported in **Article 5**.

Regarding subjective sleepiness state of the subjects, karolinska sleepiness scale (KSS) score was higher in NH and HH when compared to NN ($p<0.001$) and HN ($p=0.022$ and $p=0.006$ for NH and HH, respectively). However, KSS score remained equivalent between NN and HN ($p=0.664$).

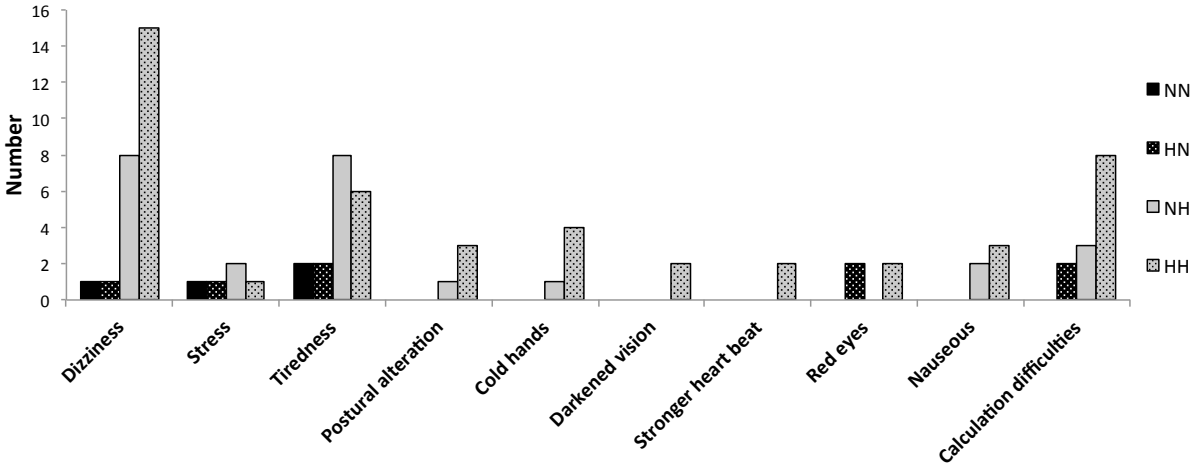

Figure 12: Representation of the types of symptoms (x-axis) and number of symptoms reported by the subjects (Y-axis) for each condition: Normobaric normoxia (NN), hypobaric normoxia (HN), normobaric hypoxia (NH) and hypobaric hypoxia (HH). **Source:** (Aebi et al., 2020c).

2.8 Influence of hypobaria in hypoxia on physiological responses during submaximal exercise

The second experimental investigation assessed physiological responses at rest and during a 6-min submaximal cycling exercise (1W/kg). Physiological parameters such as SpO_2, HR, MCAv and gas exchanges were measured at rest seated on the bike (baseline), during exercise and recovery. Moreover, cerebral and muscular oxygenation parameters were recorded using a NIRO-200NX (Hamamatsu Photonics, Hamamatsu City, Japan). One probe was positioned on the participants' forehead horizontally on the left side. Another probe was placed on the left *vastus lateralis* muscle. For more details, see **Article 6**.

Resting HR was higher in HH than in NN ($p=0.024$). At exercise, HR values were higher in hypoxic conditions than in normoxic ones, with higher value in HH than in NH ($p=0.002$). SpO_2 was similar between NN and HN at rest and during cycling exercise but was higher than in the two hypoxic conditions ($p<0.001$). Moreover, HH showed lower SpO_2 values than in NH at rest ($p=0.027$) and during exercise (-10%, $p<0.001$).

MCAv was higher in hypoxia and increased during exercise (+8 cm/s) in HH only when compared to normoxic conditions (NN and HN, $p=0.01$) at rest.

During the resting period, $\dot{V}E$ was similar between the four conditions. However, during exercise, $\dot{V}E$ was greater in HH than in NN and HN ($p<0.001$, **Figure 13**), as well as in NH ($p<0.024$). VT and Rf significantly increased during exercise compared to rest in all conditions, and did not differ between conditions during exercise. $P_{ET}CO_2$ was lower ($p<0.001$) in HH (23±1 mmHg) and HN (23±3 mmHg) and when compared to NN and NH (36±4 and 33±4 mmHg, respectively). As expected, $P_{ET}O_2$ was lower in hypoxia (NH and HH) when compared to in NN and HN, but with an additive effect of hypobaria (HN<NN; 92±13 vs. 101±4 mmHg; $p<0.001$ and HH<NH; 41±2 vs. 50±7 mmHg; $p<0.001$).

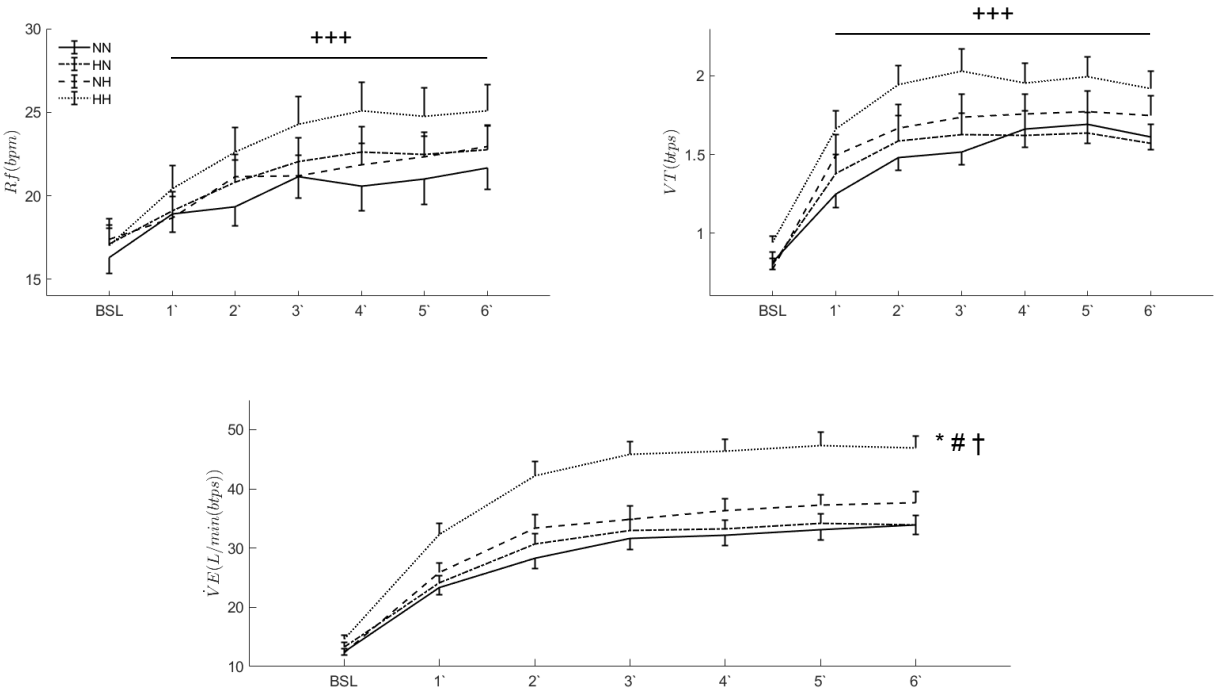

Figure 13: Respiratory frequency (Rf), tidal volume (VT) and minute ventilation (V̇E) in normobaric normoxia (NN), hypobaric normoxia (HN), normobaric hypoxia (NH) and hypobaric hypoxia (HH), during rest (baseline, BSL) and exercise. * $p<0.05$ for difference with NN, # $p<0.05$ for difference with HN, † $p<0.05$ for difference with NH, +++ $p<0.001$ for difference with BSL values in all conditions. **Source: Article 6**, submitted.

Cerebral and muscular tissue oxygenation index were similar between normoxic conditions (NN and HN) at rest and exercise. At rest, TOI in the VL was similar between the two hypoxic conditions and NN but was lower than in HN. During exercise, TOI in VL was lower in hypoxia when compared to normoxic conditions as well as in HH vs. NH ($p<0.001$). In addition, change in [O_2Hb] was lower ($p=0.002$) and [HHb] greater ($p<0.001$) in HH compared to all other conditions in the VL. There was no significant difference in [tHb] between conditions. Detailed data are showed in **Article 6.**

Chapter 3

Discussion

3 Discussion

The main findings of the present PhD thesis are listed below:

- Minute ventilation increased in HH, inducing larger hypocapnia than in normobaric hypoxia at rest.
- Significant increase in sympathetic activity in hypoxic conditions only (NH and HH).
- Large and specific effect of hypobaria *per se* on baroreflex sensitivity, at rest in normoxia despite no specific effects on HR or blood pressure. This influence of hypobaria on baroreflex sensitivity was demonstrated at rest but not during exercise and recovery and was less evident in hypoxia.
- Cerebrovascular reactivity CO_2 was increased and reset to a lower $P_{ET}CO_2$ pressure in hypobaric conditions (HH and HN), with a potential negative impact on cerebral oxygen delivery.
- The Beta power increased in acute HH. No change in microstates was found in HH.
- Reduction in cognitive performance was similar between NH and HH.
- Larger symptoms diversity in HH, which implies a greater severity in hypoxia with hypobaria.
- Larger physiological (heart rate increase and pulse oxygen saturation decrease) and ventilatory (increased minute ventilation) responses at exercise in HH than in NH. Moreover, hypobaria induces larger cerebral and muscular deoxygenation at exercise in hypoxia, whereas it played a negligible role in normoxia.

These findings are displayed in the schematic **Figure 14**. Overall, when compared to control condition (NN), some physiological responses were more exaggerated in hypoxia with hypobaria (HH), such as pulse oxygen saturation decrease, heart rate increase, middle cerebral artery velocity increase, cerebrovascular reactivity to CO_2 and lower cerebral oxygen

delivery. For instance, the arrow between NH and HH indicates larger responses observed in HH when compared to NH. However, some responses were equivalent between NH and HH, as cognitive performance, baroreflex sensitivity and rated perceived exertion during exercise, as described in the space between these two conditions.

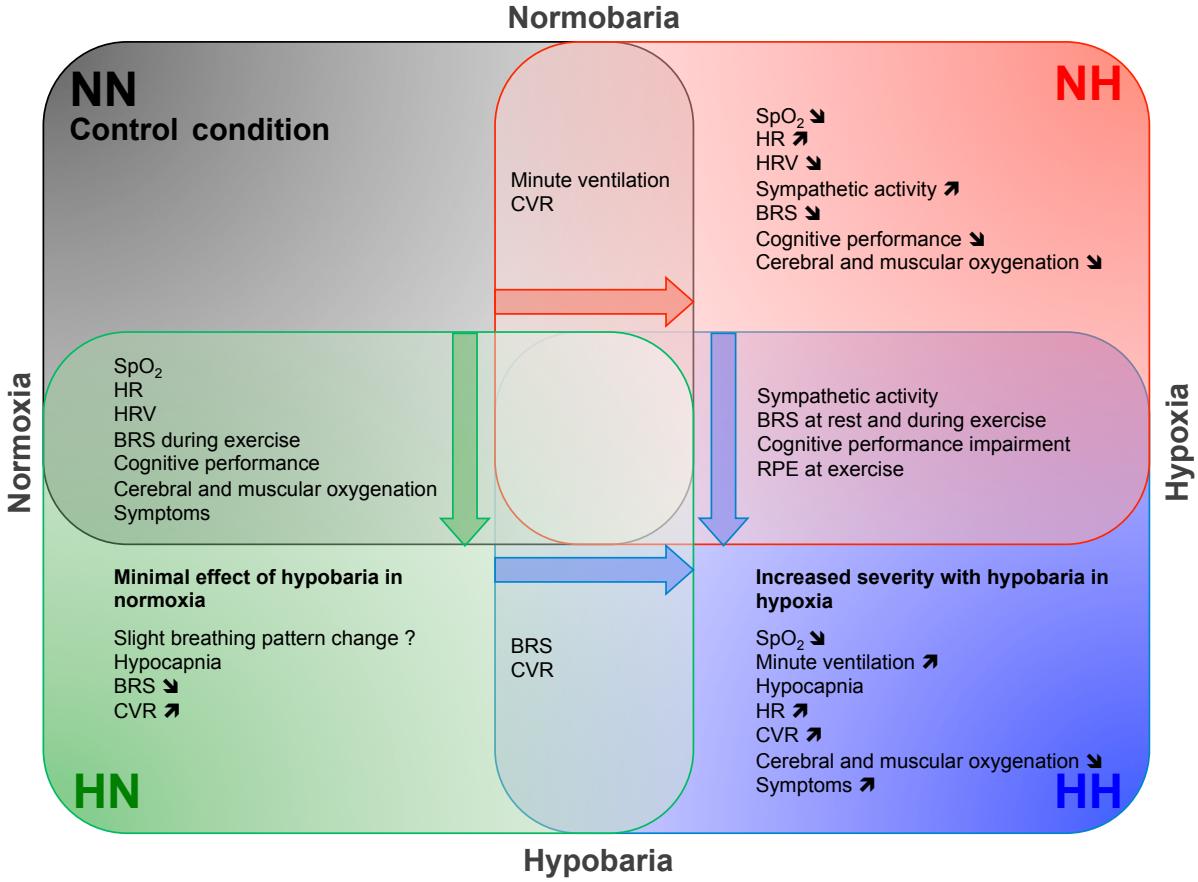

Figure 14: Summary of the findings of the present PhD thesis. Pulse oxygen saturation (SpO_2), heart rate (HR), heart rate variability (HRV), baroreflex sensitivity (BRS), cerebrovascular reactivity to CO_2 (CVR) and rated perceived exertion (RPE).

On the left side of the diagram, hypobaria played a minimal role in normoxia (HN). In HN, hypocapnia was observed. Baroreflex sensitivity decreased in HN when compared to NN at rest. Cerebrovascular reactivity to CO_2 was slightly increased. Nevertheless, there was no difference regarding pulse oxygen saturation, heart rate, symptoms, and cerebral or muscular oxygenation at exercise.

3.1 Specific effect of hypobaria in hypoxia and normoxia

3.1.1 Ventilatory and cardiovascular responses

Resting ventilation did not change between conditions; despite large decreases in $P_{ET}CO_2$ in hypobaric conditions (HN and HH) or large decreases in $P_{ET}O_2$ in hypoxic conditions (NH and HH). $P_{ET}O_2$ decrease was probably not sufficient to trigger a hyperventilation at rest that usually occurs when SpO_2 drops below 60% (74% in the present HH condition). In line, ventilation showed similar values between NH and HH (Savourey et al., 2007; Miyagawa et al., 2011). Contrastingly, effect of hypobaria on ventilation has previously been shown (Loeppky et al., 1997). As indirectly shown by the lower S_pO_2 in hypobaric hypoxia, these differences may be related to a greater physiological dead-space with hypobaria (Savourey et al., 2003) or a greater hypoxic pulmonary vasoconstriction that could result alveolar dead space changes and ventilation–perfusion ratio alteration (i.e., O_2 gradient reduction from the alveoli to the pulmonary circulation) (Loeppky et al., 1997).

In hypobaric normoxia, the gradient pressure in O_2 between the gas and the pulmonary alveolus is similar to sea level, whereas the CO_2 gradient pressure is greater than in normobaria (i.e., NN and NH), which may have caused the $P_{ET}CO_2$ decrease in the present thesis. $P_{ET}CO_2$ values were comparable between hypobaric conditions (HN and HH), but were lower than in normobaric conditions (NN and NH). Hypocapnia likely induced pulmonary vasoconstriction, potentially affecting the O_2 diffusion capacity from the alveolar compartment to the blood in hypobaric normoxia. Thus, hypobaria did not have any additional influence on S_pO_2 in normoxia, which could explain equivalent cardiorespiratory responses between both normoxic conditions (NN and HN).

At exercise, the hypocapnia-induced cerebral vasoconstriction may in part be compensated by the hypoxemia- and exercise-induced vasodilation. In the present thesis, minute ventilation increased at exercise in HH only when compared to all other conditions. Moreover, HR was around 10 bpm higher in HH than in NH, which implies a greater cardiorespiratory response at exercise in acute hypoxia with hypobaria. The results of this thesis are in line with previous studies (Savourey et al., 2003; Boos et al., 2016; Rupp et al., 2019) or a review (Coppel et al., 2015). Potential mechanisms has been reported that may be affected by hypobaria, as greater ventilation/perfusion disparity and intravascular bubble formation, greater alveolar dead space as well as changes in alveolar fluid permeability and chemosensitivity in HH when compared to NH (Faiss et al., 2013a; Coppel et al., 2015). However, further studies are needed for investigating the putative effect of hypobaria on respiratory responses at exercise in these two hypoxic conditions.

Overall, minute ventilation was equivalent between conditions at rest but was higher in HH when compared to NH during exercise, which implies a slight additive influence of hypobaria on ventilation when exposed to acute severe hypoxia at 5000 m.

3.1.2 Heart rate variability

Regarding HRV, RMSSD was similarly decreased in hypoxic conditions (NH and HH) compared to NN. The present HRV results suggest a hypoxic effect and no hypobaric effect on RMSSD for comparable P_IO_2 conditions (NN vs. HN and NH vs. HH). LF/HF ratio was increased in HH compared to HN, suggesting a hypoxic effect on sympathetic activity with equivalent barometric pressure. Our results are in line with a decrease of overall variability and a predominance of sympathetic activity in hypoxia (Chen et al., 2008; Perini et al., 1996).

Acute hypoxia is considered as an important sympathetic activity activator (Richalet et al., 1988; Marshall, 1994; Hainsworth et al., 2007). Moreover, it has been previously shown that the cardiac autonomic nerve activity is influenced by ventilation (Brown et al., 1993). Minute ventilation and breathing frequency significantly increased in HH, but not NH, when compared to NN at rest (Aebi et al., 2020b). Moreover, tidal volume tended to be higher in HH than NN, while it remained unchanged in NH (Aebi et al., 2020b). In addition, MSNA increased when exposed to acute hypoxia (Duplain et al., 1999; Hansen and Sander, 2003), due to the hypoxia-induced sympathetic activation (Marshall, 1994). The present results confirmed the hypoxia-induced sympathetic activity elevation, as LF/HF ratio was higher in HH than HN ($p<0.001$) (i.e. with similar barometric pressure between HN and HH). Of interest, LF/HF was higher in HH than in NH, implying a slight additional influence of hypobaria on sympathetic activation in hypoxia. Nevertheless, LF/HF change is not strong enough for such statement regarding the influence of hypobaria on HRV in hypoxia since there was no other significant difference in HRV parameters between HH and NH.

In normoxia, HR was similar between NN and HN. However, some differences were found between NN and HN regarding some HRV components, which suggests a slight influence of hypobaria on HRV in normoxia at rest: LF/HF ratio was lower in HN than NN ($p=0.041$), which suggests predominance of parasympathetic activity in HN. In line with this observation, parasympathetic increase was observed in subjects breathing hyperoxic gas mixture when exposed at 4574 m (Prabhakaran and Tripathi, 2011). Breathing a hyperoxic gas mixture may be related to a MSNA reduction (Querido et al., 2010). More precisely, peripheral chemoreceptors seem inhibited with hyperoxic stimulus leading to decrease in MSNA (Querido et al., 2010). In addition, breathing pattern changes due to lower air density in hypobaria is an additional factor to take into account (Ogawa et al., 2019). One limitation

of the present thesis is that respiratory parameters were not recorded during HRV measurement. However, ventilatory data non-significant, but lower ventilation value in HN than NN, which may suggest a slight change in breathing pattern. Decrease of LF/HF ratio in HN compared to NN may imply a slight hypobaric influence normoxia (NN vs. HN).

Overall, the present thesis confirmed the decrease in HRV when exposed to acute hypoxia and adds novelty by suggesting a slight influence of hypobaria in both hypoxia and normoxia on HRV modulations through ventilation pattern differences.

3.1.3 Baroreflex sensitivity

As discussed in the above-section, direct effect of hypobaria *per se* on ventilation has previously been shown (Loeppky et al., 1997; Savourey et al., 2003). In the present thesis, BRS was likely affected by hypocapnia in the hypobaric conditions (HN and HH), which may be related to vascular tone modifications through sympathovagal balance changes (Aebi et al., 2020a). However, there was no significant difference in HRV between NN and HN, which suggest that hypobaria does not seem to be the main trigger of the BRS alteration (Aebi et al., 2020a). In hypobaric normoxia, the pressure gradient in O_2 between the lung alveoli and the gas is comparable to sea level, but the pressure gradient in CO_2 is greater in HN than in NN or NH, which induced a $P_{ET}CO_2$ reduction. $P_{ET}CO_2$ values were comparable between hypobaric conditions (HN and HH) but were lower than in normobaric ones (NN and NH). This hypocapnic state in HN probably reduced afferent traffic from the chemoreceptors, which induced decrease in BRS. This latter observation emphasizes the essential role of the central chemoreceptors in the BRS decrease (Dempsey et al., 2014; Smith et al., 2015). Moreover, it has been shown that central chemoreceptors may also be affected by blood pH increase observed in HN when compared to NN and NH, which is highly dependent on blood PCO_2 (Aebi et al., 2020b).

It was previously suggested that pulmonary blood flow was decreased by hypobaria, independent of the hypoxia severity (Petrassi et al., 2018b). Interestingly, supplemental inspired CO_2 in hypoxic conditions or in case of hypocapnia is a therapeutic methods used to diminish the hypoxic pulmonary vasoconstriction (Chuang et al., 2010). A previous study has reported that hypobaria increases total lung capacity, functional residual capacity, closing capacity, and residual volume (Coates et al., 1979), which could be related to a larger volume of air trapped in the alveoli when atmospheric pressure is reduced. This results in increased CO_2 diffusion form the blood capillaries to the alveoli. Moreover, lung volume elevation increases alveolar capillaries compression (Simmons et al., 1961; Hakim et al., 1982), which could lead to the decreased $P_{ET}CO_2$ in the HN. However, $P_{ET}CO_2$ decrease in hypobaric normoxia is debated. For instance, there was no differences in P_aCO_2 between rest and high intensity exercise at 5260 m, whereas hyperventilation was observed (Petrassi et al., 2018b). Therefore, further studies are needed to better understand the putative effect of hypobaria on carbon dioxide diffusion.

3.1.4 Cerebrovascular reactivity

In hypoxia, hyperventilation-induced hypocapnia is enhanced by an increase in peripheral respiratory chemoreflex (Ogoh, 2019). In addition, it has been previously reported that HH induced greater hypocapnia and blood alkalosis when compared to NH (Savourey et al., 2003). In the present thesis, midpoint of sigmoidal curve was reset to a lower $P_{ET}CO_2$ value suggesting a hypocapnia-induced blood alkalosis and vasoconstriction in hypoxic conditions (Willie et al., 2015), thus less efficiency of cerebral blood vessels to regulate CBF in acute hypoxia. Many studies have evaluated the CVR in humans exposed to high altitude (Jensen et al., 1996; Jansen et al., 1999; Ainslie and Burgess, 2008; Fan et al., 2010, 2015; Lucas et al., 2011; Flück et al., 2015; Willie et al., 2015). Nevertheless, the effect of hypoxia on CVR

remains unclear, as some results are controversial. For instance, CVR increased in hypoxia during hyperoxic poikilocapnia (Fan et al., 2010) and hyperoxic isocapnia (Subudhi et al., 2010); decreased during hyperoxic poikilocapnia (Ainslie and Burgess, 2008) or unchanged during hypoxic poikilocapnia (Ainslie and Burgess, 2008) and uncontrolled hypercapnia (Jansen et al., 1999). However, it has been shown that response of CBF to CO_2 is blunted in hypoxia, which potentially limits dilatory responses (Leffler et al., 1986; McPherson et al., 1987; Fan et al., 2013). The present thesis showed an increase of the sigmoid curve slope and midpoint left-shift in HH compared to NH, which imply a specific effect of hypobaria *per se* on CVR when exposed to hypoxia. However, NH induced smaller left shift, higher partial CO_2 pressure and lower pH values compared to HH, probably due to minor hyperventilation. As midpoint was left-shifted to a lower $P_{ET}CO_2$ value (hypocapnia) in HH and HN, it suggests that vascular reserve to dilate may be blunted in hypobaria (HH vs. NH and HN vs. NN), in both, hypoxia or normoxia. If this vascular dilation reserve is decreased, it might negatively affect cerebral oxygen delivery. Detailed discussed results regarding cerebral oxygen delivery are reported in **Article 3**.

CO_2 sensitivity analysis is based on the subjects' exposure to a range of arterial CO_2 going from hypocapnia to hypercapnia. In the results, hypocapnia was induced by voluntary hyperventilation of the participants as described in a previous study (Fan et al., 2015). During hyperventilation, MCAv was logically decreased due to the hypocapnia-induced vasoconstriction (Kaur et al., 2018). Then, hypercapnia (i.e., induced when subjects breathing was switched to the enriched carbon dioxide gas mixture) triggered cerebral vasodilation, which result in MCAv elevation. During hypercapnia, MCAv increases in order to wash out CO_2 from the brain tissue to regulate and maintain cerebrospinal fluid pH (Xie et al., 2006). In fact, it seems that hypocapnia plays an important role on CVR. A recent review that has

been focused on CVR reported the importance of $PaCO_2$ change as a mediator of cerebral microvascular hemodynamic function (Ogoh, 2019). Moreover, it has been reported that decrease or increase of MCAv(Caldwell et al., 2021) induced by cerebral vaso-constriction or -dilation, when $PaCO_2$ was low or high (i.e., during hypo- or hypercapnia, respectively) (Markwalder et al., 1984). Cerebral autoregulation was enhanced (faster) or diminished (slower) by hypocapnia or hypercapnia, respectively (Aaslid et al., 1989). Therefore, it is likely that myogenic tone of cerebral vasculature is influenced by $PaCO_2$ changes, which may affect cerebral autoregulation dynamic (Ogoh, 2019). Recently, it has been concluded that CBF is regulated by $PaCO_2$ rather than arterial pH (Caldwell et al., 2021). Moreover, it has been reported that ventilation *per se* does not influence CVR independent of $PaCO_2$ changes (Carr et al., 2021). In the present thesis, blood gas measurements showed $PaCO_2$ decrease in hypobaric conditions (see data in **Article 3**), when CVR was increased. However, because of temporal dissociation between blood gas measurement and CVR assessment (around 15 minutes), these data were not used to discuss CVR differences. However, CVR showed a left-shift of the midpoint in hypobaric conditions (i.e., HH and HN), which indicate a resetting to a lower $P_{ET}CO_2$ values. Moreover, CVR was increased in HH (i.e., steeper sigmoid slope). One may speculate that CVR increase in acute hypobaric conditions (i.e., HH and HN) may be mediated by the respiratory alkalosis-induced hypocapnia. Of interest, a recent study developed a model, which assumes that smooth muscle vasoconstriction and vasodilation and hence cerebral blood flow, are proportional to the intracellular hydrogen ion concentration (Duffin et al., 2021). Therefore, this model can be used to predict CBF regulation based on the independent or combined effects of hypoxia, hypercapnia and anemia (Duffin et al., 2020, 2021).

Overall, the present thesis showed i) A left-shift in $P_{ET}CO_2$-MCAv sigmoid curve with an increase in CVR with altitude level in HH. This latter observation was also observed under

hypobaric conditions for a similar P_IO_2 in hypoxia. In addition, an influence of hypobaria *per se* on CVR, mediated by hypocapnia was observed (i.e., sigmoid midpoint left-shift); ii) No hypoxic effect on CVR for equivalent barometric pressure (NN vs. NH) and (HN vs. HH).

3.1.5 Symptoms

Participants reported more symptoms in hypoxic conditions (NH and HH). Based on their occurrence, HH probably was more stressful as more symptoms were reported in HH when compared to NH. Regarding subjective sleepiness state, KSS score was higher in NH and HH when compared to NN and HN. KSS score remained similar between NN and HN, which implies no additive effect of hypobaria on subjective fatigue in normoxia.

One aim of the present thesis was to collect qualitative data in order to evaluate the individual sensitivity and subjects' feeling, when exposed to various acute hypoxic and hypobaric conditions. The subjects reported the symptoms they have experienced at the end of each condition. Some symptoms were mainly represented in hypoxic conditions (NH and HH), such as: Dizziness, tiredness and calculation difficulties. Moreover, subjects reported being dizzy, having postural alterations, cold hands and being nauseous in NH and HH only. . Interestingly, subjects reported more symptoms in HH than NH. Moreover, a few symptoms were reported in HH only (i.e., darkened vision, feeling of a stronger heart beat), which suggest a putative additive effect of hypobaria on symptoms in hypoxia. These observations are in line with previous studies, where symptoms seemed qualitatively different in HH (DiPasquale et al., 2015) and acute mountain sickness was increased in HH when compared to NH (Roach et al., 1996). Therefore, symptoms may qualitatively differ between NH and HH during acute exposures.

Military pilots often train in a flight simulator in NH and perform hypoxia awareness training in HH. For instance, a study showed cognitive and flight performance impairment during

training in NH (Varis et al., 2019). Therefore, as previously recommended (Smith, 2008; Singh et al., 2010; Johnston et al., 2012), it remains of importance and necessary to regularly assess hypoxia awareness training, in order to train military and civilian pilots to recognize their personal symptoms, especially in hypobaric hypoxia.

3.1.6 Cognitive functions

Concentration performance was only altered in hypoxic conditions (NH and HH: i.e. greater percentage of error). Therefore, both hypoxic conditions comparably affected cognitive performance in the present thesis. Nowadays, there is little amount of studies that have investigated cognitive performance comparison in NH versus HH. Long ago, a study showed similar decrease in visual attention in NH and HH at simulated altitude level of 3450 m, when compared to sea level (Schlaepfer et al., 1992). More recently, it was suggested, that NH may be associated with greater cognitive function reduction than HH (McMorris et al., 2017). However, NH induced comparable percentage of errors than HH in the present thesis. In addition, calculations number decreased in HH only, when compared to NN, which suggests speed reduction in HH to perform the arithmetic task. For instance, completion time was longer at 5334 m and more than doubled at 7620 m in HH when compared to sea level (Asmaro et al., 2013), which is in line with our results (i.e., decreased calculation number in HH at 5500 m). How acute hypoxia negatively alters cognitive function is not completely understood, although it is likely a combination of mechanisms, which may include fatigue (Virués-Ortega et al., 2004) and neuronal damage (Bjursten et al., 2010). Moreover, some physiological changes occur in the brain in HH, which can alter tasks including working-memory (Ma et al., 2019). Interestingly, a recent study reported beneficial effects of acute moderate exercise in hypoxia on cognitive performance (Ando et al., 2020). Therefore, the effect of hypoxia on cognitive functions remains complex and further studies are warranted to

evaluate the physiological factors that modulate cognitive performance at rest and during exercise when exposed to hypoxia.

The present thesis showed an equivalent deleterious effect of hypoxia on cognitive performance in NH and HH. Moreover, cognitive performance was maintained in HN when compared to NN. These results thus confirm the detrimental effect of hypoxia on cognitive performance and add new insights regarding the negligible influence of hypobaria.

3.1.7 Physiological responses at exercise

During exercise, it remains unclear how hypobaria influences ventilation and CBF regulation in acute hypoxia. It has been reported that cerebral oxygenation decreases while MCAV increases during submaximal exercise after prolonged exposure to high altitude (Imray et al., 2005). In the present thesis, estimated cDO_2 remained equivalent between conditions during submaximal exercise, but MCAv significantly increased in HH only. NIRS measurements showed a significant cerebral oxygenation reduction in hypoxic conditions during submaximal exercise. Moreover, a larger [O_2Hb] decrease and [HHb] increase was observed in HH in comparison with NH, which suggests a greater cerebral deoxygenation in HH. Moreover, cerebral TOI significantly decrease in hypoxic conditions, to greater extent in HH versus NH, which confirms the proposed larger cerebral deoxygenation in HH when compared to NH at exercise.

At the muscular level, TOI was equivalent between control (NN) and hypoxic conditions at rest, but then significantly decreased in NH and HH during cycling exercise. Of interest, muscle TOI decreased to larger extent in HH when compared to NH, which implies – once again - an additional effect of hypobaria on muscle deoxygenation in hypoxia. It has been previously shown that cerebral, but not muscle, tissue oxygenation decreased when exposed to acute hypoxia at rest (Ainslie et al., 2007; Peltonen et al., 2007). Moreover, muscle TOI

remained equivalent between normoxia and hypoxia, whereas cerebral TOI and SpO_2 were reduced in hypoxia after a 15-min rest period (Rupp and Perrey, 2009). The results of the present thesis are therefore in line with previous findings (Ainslie et al., 2007; Peltonen et al., 2007; Rupp and Perrey, 2009). However, the present thesis adds more insights by showing an additional hypobaric effect on muscle deoxygenation (i.e., muscle TOI and [O_2Hb] decreases, with [HHb] increase) during submaximal cycling exercise in acute hypoxia. This latter difference may be due to the larger hypoxemia (i.e., lower SpO_2) and greater ventilatory response, which induced a more prominent hypocapnia in HH, potentially affecting oxygen diffusion. Contrastingly, the influence of hypobaria on muscle oxygenation seems negligible in normoxia, as no difference in muscle oxygenation was observed between NN and HN.

Overall, hypobaria induced larger decreases in cerebral and muscular oxygenation during submaximal cycling exercise in hypoxia, whereas it had negligible influence in normoxia.

Chapter 4

Conclusion and perspectives

4 Conclusion and perspectives

The present PhD thesis evaluated the putative specific influence of hypobaria on physiological responses in normoxia (i.e., between NN and HN) and in hypoxic (i.e., between NH and HH) at rest and during submaximal cycling exercise. Hypobaria increased severity regarding physiological responses in hypoxia (such as greater hypoxemia, hypocapnia, cardiorespiratory responses, etc.), whereas it showed minimal influence in normoxia.

4.1 Application in aviation

Swiss military pilots regularly perform both flight trainings with hypoxia in normobaria (i.e., NH in flight simulator with decreased inspired oxygen fraction) and hypoxia awareness training in hypobaria (HH, using the hypobaric chamber of the Swiss Air Force). The present results are thus of interest for the Swiss Air Force to highlight specific differences in physiological responses in young healthy pilot trainees during acute exposure to "simulated" (NH) and "real" (HH) altitude. Therefore, the findings of the present thesis could be helpful in the education of future Swiss military pilots. Moreover, there is a practical interest since hypobaric normoxia occurs in the aviation field; i.e., for pilots exposed to hypobaria in cockpit using supplemental oxygen. In fact, pilots may be exposed to hypobaria during flights at high-altitude in unpressurized cabin aircraft or in case of sudden cabin depressurization. The present results showed minimal effect of hypobaria in normoxia, but some slight changes were observed regarding the breathing pattern and cerebrovascular responses. Additional studies are needed to evaluate the potential hypobaric effect in normoxic condition (i.e., in HN), as it is barely explored up today.

The present thesis aimed to be as specific as possible to flight and training conditions for pilots. For instance, pilots are daily exposed to hypobaric environment in-flights either in normoxia (HN) or hypoxia (HH), in case of cabin decompression (Muehlemann et al., 2013) or unpressurized cabins (Nishi, 2011). Moreover, military pilots perform training in flight simulator (i.e., in NH condition) and assess hypoxia-awareness training in HH. It is therefore paramount to evaluate how cerebral functions may be affected in various environments, such as NH, HH and HN conditions.

4.2 Relation with space physiology

Recently, a review focused on the nominal and expected challenges of space flight and provide medical and environmental challenges of the expected future increase in civilian space flight (Stepanek et al., 2019a). In response, the thematic of hypoxia was introduced as an important factor for astronauts (Millet, 2019). For instance, the hypoxic stimulus in future habitats to the moon or Mars would corresponds to altitude levels of 3000 to 4000 m (Bodkin et al., 2006). In fact, severe altitude exposure in hypobaric hypoxia may induce high-altitude illnesses such as high-altitude pulmonary or cerebral oedema and acute mountain sickness (Bärtsch et al., 2005; Bärtsch and Swenson, 2013). For astronauts, severe hypoxia or decompression illnesses could occur in case of loss of integrity of a pressurization system (vehicle or space suits) (Stepanek et al., 2019a). However, hypoxia seems to play a marginal role on skeletal-muscle function alteration, which is mainly due to microgravity and immobility (Salvadego et al., 2018). Moreover, future commercial suborbital flights will be pressurized at a cabin altitude of 8000 ft (2438 m) or less (Stepanek et al., 2019b). Therefore, hypobaric hypoxia is not expected as clinically significant in the case of future civilian space flights (Stepanek et al., 2019b).

This thesis demonstrated a specific effect of hypobaria *per se* on baroreflex sensitivity and cerebrovascular reactivity to carbon dioxide, which were attributed primarily to hypocapnia in hypobaric environment (in normoxia and hypoxia). This finding is of interest in space physiology since it has direct implications for astronauts with large clinically significant physiological alterations when exposed to microgravity.

4.3 Perspectives

Overall, the present thesis adds more insights regarding the effects of hypobaria either in hypoxic and normoxic conditions. Based on the present results, individuals exposed to hypobaric normoxia, such as military aircraft pilots, could be supplemented in CO_2 (in addition to O_2 of course) in order to avoid hypocapnia and subsequent baroreflex sensitivity alteration, impaired cerebrovascular reactivity and vasoconstriction, which may affect cerebral perfusion. However, future studies are needed to determine the adequate amount of inspired CO_2 to avoid these adverse consequences. Moreover, the relationship between pulmonary O_2 and CO_2 diffusion, blood content (blood arterial gases) and baroreflex function should be investigated in normoxia and hypoxia with or without hypobaria involved. This would help to further dissociate the baro- and chemoreflex arcs to better understand the mechanisms of blood pressure regulation in a large variety of environmental conditions.

Chapter 5

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5 References

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Article 1 – Minimal influence of hypobaria on heart rate variability in hypoxia and normoxia

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Minimal Influence of Hypobaria on Heart Rate Variability in Hypoxia and Normoxia

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Introduction: The present study evaluated the putative effect of hypobaria on resting HRV in normoxia and hypoxia.

Methods: Fifteen young pilot trainees were exposed to five different conditions in a randomized order: normobaric normoxia (NN, P_B = 726 ± 5 mmHg, F_IO_2 = 20.9%), hypobaric normoxia (HN, P_B = 380 ± 6 mmHg, F_IO_2≅40%), normobaric hypoxia (NH, P_B = 725 ± 4 mmHg, F_IO_2≅11%); and hypobaric hypoxia (HH at 3000 and 5500 m, HH3000 and HH5500, P_B = 525 ± 6 and 380 ± 8 mmHg, respectively, F_IO_2 = 20.9%). HRV and pulse arterial oxygen saturation (SpO_2) were measured at rest seated during a 6 min period in each condition. HRV parameters were analyzed (Kubios HVR Standard, V 3.0) for time (RMSSD) and frequency (LF, HF, LF/HF ratio, and total power). Gas exchanges were collected at rest for 10 min following HRV recording.

Results: SpO_2 decreased in HH3000 (95 ± 3) and HH5500 (81 ± 5), when compared to NN (99 ± 0). SpO_2 was higher in NH (86 ± 4) than HH5500 but similar between HN (98 ± 2) and NN. Participants showed lower RMSSD and total power values in NH and HH5500 when compared to NN. In hypoxia, LF/HF ratio was greater in HH5500 than NH, whereas in normoxia, LF/HF ratio was lower in HN than NN. Minute ventilation was higher in HH5500 than in all other conditions.

Discussion: The present study reports a slight hypobaric effect either in normoxia or in hypoxia on some HRV parameters. In hypoxia, with a more prominent sympathetic activation, the hypobaric effect is likely due to the greater ventilation stimulus and larger desaturation. In normoxia, the HRV differences may come from the hyperoxic breathing and slight breathing pattern change due to hypobaria in HN.

Keywords: normobaric normoxia, normobaric hypoxia, hypobaric normoxia, hypobaric hypoxia, heart rate variability

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INTRODUCTION

Environmental hypoxia is a condition characterized by a decrease in the inspired oxygen pressure (P_IO_2) (Millet et al., 2012), which *per se* has a negative influence on autonomic cardiac response (Botek et al., 2015) and induces systemic/integrative metabolic, endocrine and vascular compensation (Marshall, 1998). More precisely, acute hypoxic exposure induces decreases in heart

rate variability (HRV) and parasympathetic activity (Wille et al., 2012), whereas sympathetic activity increases (Richalet et al., 1988; Marshall, 1994). Contrastingly, HRV parameters 24 h after maximal anaerobic exercise comparing normoxic with two normobaric hypoxic conditions (equivalent to 2500 and 4000 m) remained unchanged (Álvarez-Herms et al., 2020). Therefore, the duration of hypoxic exposures as well as the timing of the HRV measurement probably influence HRV modulation. However, it is well established that high-altitude leads to sympathetic activation (Hainsworth et al., 2007), also under the influence of the rate of ascent (Vogel and Harris, 1967).

Heart rate variability is a non-invasive method to assess the cardiac autonomic control (Buchheit, 2014) and is commonly used to monitor fatigue and overreaching in athletes (Meeusen et al., 2013; Bourdillon et al., 2017), despite some debates about the pro and cons of the time (i.e., root mean square of the successive differences, RMSSD) (Plews et al., 2012) vs. frequency [i.e., spectral power in low frequency (LF), high frequency (HF) and total power (LF + HF)] domain HRV parameters (Schmitt et al., 2015).

Environmental hypoxia can be provoked either by lowering inspired oxygen fraction (F_IO_2; normobaric hypoxia, NH) or using a hypobaric chamber by reducing the barometric pressure (P_B; hypobaric hypoxia, HH). For long, it was believed that all responses to hypoxia were only caused by the alveolar oxygen pressure (P_AO_2) decrease (Conkin, 2016). Contradictory, HH is suggested as a more severe environmental condition than NH (Millet et al., 2012). Several differences between NH and HH were reported, such as minute ventilation (Savourey et al., 2003), oxydative stress (Faiss et al., 2013), sleep disturbance (Saugy et al., 2016), and cerebrovascular function (Aebi et al., 2020). Therefore, NH and HH are not interchangeable (Conkin, 2016) but the clinical significance of the difference remains highly debated (Millet and Debevec, 2020; Richalet, 2020).

Isolating the hypobaric effect from the hypoxic one would allow comparing similar normoxic conditions with different P_B. A hypobaric normoxic (HN) condition (i.e., low P_B and hyperoxic breathing in order to obtain a comparable P_IO_2 than in normobaric normoxia, NN) is therefore of interest for evaluating the putative hypobaric effect in normoxia (Millet and Debevec, 2020). Moreover, there is a practical interest of the present study since hypobaric normoxia occurs in the context of aviation; i.e., for pilots exposed to hypobaria in cockpit using supplemental oxygen. More precisely, pilots during flights at high-altitude may be exposed to hypobaria in unpressurized cabin aircraft, in case of sudden cabin depressurization during commercial flights or in military aircraft while breathing hyperoxic gas mixture. Hypobaric normoxia is also used for workers (i.e., miners in Chile) exposed to high terrestrial altitude with supplemental oxygen for example in dormitories for reducing periodic breathing and improving recovery (Moraga et al., 2014). Due to lower air density, a recent study showed ventilatory pattern change (i.e., increased maximal ventilation) in such environment (Ogawa et al., 2019). Moreover, increase in intrapulmonary pressure has been reported (Conkin, 2016). These physiological changes may impact HRV parameters, as the cardiac autonomic activity is influenced by the respiration (Brown et al., 1993) and the pulmonary arterial baroreceptors (Hainsworth et al., 2007). It was also suggested that parasympathetic influence increases in HN (Prabhakaran and Tripathi, 2011).

The present study evaluated first the altitude level influence on HRV during acute HH exposure at 3000 and 5500 m when compared to NN. More importantly, we investigated the putative effect of hypobaria on HRV during acute exposure in hypoxia (NH vs. HH) and in normoxia (NN vs. HN).

MATERIALS AND METHODS

Participants and Protocol Design

Fifteen healthy pilot trainees (26 ± 4 years, 177 ± 7 cm, 71 ± 9 kg) were exposed to five different conditions in a randomized order: NN (440 m, P_B = 726 ± 5 mmHg, F_IO_2 = 20.9%); NH (simulated altitude of 5500 m, $P_B = 725 \pm 4$ mmHg, $F_IO_2 \cong 11\%$); HN (depressurization at 5500 m with hyperoxic breathing to avoid hypoxia in hypobaria, $P_B = 380 \pm 6$ mmHg, $F_IO_2 \cong 40\%$) and HH ($P_B = 525 \pm 6$ and 380 ± 8 mmHg, for 3000 m (HH3000) and 5500 m (HH5500) respectively, F_IO_2 = 20.9%). Gas mixtures employed for NH and HN conditions were prefilled in cylinders. Participants breathed 100% of oxygen during altitude elevation (i.e., during atmospheric pressure reduction in the hypobaric chamber). Decompression lasts for around 2 min in the hypobaric conditions (HH and HN). A physician screened the participants during a familiarization visit to ensure they were healthy and did not report any medical or altitude related issues.

Twenty-four hours before test visit, participants were asked to avoid physical exercise and consuming a heavy meal, alcohol and caffeine. Participants remained at rest, seated, during the entire experimental procedures. Each tested condition consisted 5 min of condition acclimatization followed by 6 min seated at rest. Then, participants also performed a concentration test [arithmetic tasks including working memory, KLT-R test (Düker and Lienert, 2001)] and hypercapnic breathing protocol to assess cerebrovascular reactivity to CO_2 (Aebi et al., 2020). Each period lasted for 30 min, interspaced by a 30 min rest period in NN, for total session duration of 5 h.

In order to evaluate putative hypobaric effect between normoxic and hypoxic conditions with comparable P_IO_2: NN vs. HN (141 ± 1 vs. 133 ± 3 mmHg) and NH vs. HH5500 (74 ± 1 vs. 70 ± 2 mmHg) were compared by adjusting P_B in the hypobaric chamber or F_IO_2 (i.e., ≈11% and ≈40% O_2 gas mixture for NH and HN, respectively) based on known equation ($P_IO_2 = (P_B - 47) \times F_IO_2$), when the water vapor pressure at 37°C is 47 mmHg (Conkin, 2016).

Measurements

Heart rate variability was recorded with heart rate monitor (Polar RS800CX, FI-90440 Kempele, Finland). HRV measurement was performed according to previous findings of our research group (Bourdillon et al., 2017), during the last 4 min of a 6 min rest period seated (i.e., around 300 beats were analyzed). HRV data were analyzed using specific software (Kubios HVR Standard, V 3.0). Time domain HRV index (RMSSD) and spectral power for frequency bands for: HF (0.15–0.50 Hz), LF (0.04–0.15 Hz) and

total power (LF + HF) were analyzed. LF/HF ratio was calculated to evaluate the sympathovagal balance.

Pulse oxygen saturation (SpO_2,%) was monitored at the left earlobe using an oximeter (3100 pulse oximeter, Nonin, Plymouth, MN) and acquired at 0.5 Hz. Mean SpO_2 was calculated during the last minute of rest period in each condition.

Gas exchanges data were recorded using a gas analyzer (K5, Cosmed, Roma, Italy) that was calibrated outside of the hypobaric chamber before each session. Flow volume was calibrated with a 3L syringe. After calibrating zero CO_2 with scrubber, reference gas was assessed using a certified Cosmed gas concentration (16% O_2 and 5% CO_2). Ventilatory data were recorded by the analyzer and exported in Cosmed software for later analysis (OMNIA, Cosmed, Roma, Italy).

Statistical Analysis

Repeated measures ANOVA were assessed for condition comparison for absolute values. Greenhouse-Geisser sphericity correction was applied when Mauchly's test statistic was significant ($p < 0.05$). Then, Tukey *Post hoc* test was performed for condition comparison. Statistical analysis was performed separately for altitude comparison (NN, HH3000 and HH5500) and for conditions comparison (NN, HN, NH, and HH5500). Repeated measures ANOVA (non-parametric, Friedman) were performed for relative (%Δ) changes from NN values. Statistical analysis was assessed using Jamovi software (Jamovi project 2018, version 0.9). Significant difference was set for $p < 0.05$.

Ethical Approval

This study was performed according to the Declaration of Helsinki and was approved by the Swiss Ethic Committee of Zürich (Swissethics, BASEC ID: 2017-00752). This clinical trial can be found on ClinicalTrials.gov (ID: NCT03303118). All participants were informed about all procedures of this study and gave their written informed consent before participating to this study.

RESULTS AND DISCUSSION

Altitude Level Influence in Hypobaric Hypoxia

All absolute physiological data for HH conditions are displayed in **Table 1**. As expected, HR gradually increased with altitude level in HH3000 ($p = 0.014$) and HH5500 ($p < 0.001$) when compared to NN. RMSSD decreased in HH3000 ($p = 0.013$) and HH5500 ($p < 0.001$) when compared to NN. LF and total power absolute values were lower in HH5500 and HH3000 than in NN. Moreover, relative changes in LF and total power were greater in HH5500 vs. HH3000 (-59 vs. -41%, $p = 0.047$ and -61 vs. -44%, $p = 0.047$, for LF and total power, respectively). Previously, decrease in total power was also observed at high altitude (Hughson et al., 1994; Sevre et al., 2001), in line with the present results. Moreover, total power reduction indicates a reduced autonomic heart rate control (Kautzner and John Camm, 1997). Despite a significant HR increase, HF (ms^2) did not significantly decrease in HH3000 ($p = 0.17$). However, HF (ms^2) significantly decreased in HH5500 ($p = 0.004$), when compared to NN. Several studies have suggested a shift in the balance of the autonomic nervous system toward relatively less parasympathetic and more sympathetic activity at high altitude (Hughson et al., 1994; Perini et al., 1996; Sevre et al., 2001). Overall, HRV and parasympathetic activity decreased (i.e., RMSSD, HF, LF and total power reduction) with altitude elevation in acute HH, therefore to greater extent at 5500 m. The present results suggest a larger predominance of the sympathetic activity in hypobaric hypoxia.

TABLE 1 | Absolute values are means ± *SD*.

	NN	HH3000	HH5500
HR (bpm)	73.4 ± 7.0	81.4 ± 10.2*	93.0 ± 14.2***§§§
RMSSD (ms)	46.1 ± 15.6	37.5 ± 20.1*	25.5 ± 16.1***
LF (ms^2)	2381 ± 1311	1405 ± 1162**	783 ± 536***
HF (ms^2)	816 ± 442	606 ± 531	311 ± 314**
LF (n.u.)	73.9 ± 9.4	72.3 ± 13.5	75.1 ± 14.1
HF (n.u.)	25.8 ± 9.5	25.5 ± 10.7	24.9 ± 14.0
LF/HF ratio	3.3 ± 1.2	3.4 ± 1.8	4.4 ± 2.9
Total Power (LF + HF)	3197 ± 1629	1703 ± 112**	999 ± 602***
SpO_2 (%)	99.5 ± 0.4	95.4 ± 2.7**	81.3 ± 5.5***§§§

*Physiological variables (n = 15): HR, Heart rate; RMSSD, root mean square of the successive differences; LF, low frequency; HF, high frequency; SpO_2, pulse oxygen saturation in NN, normobaric normoxia in hypobaric hypoxia at 3000 m and 5500 m (HH3000 and HH5500). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for difference with NN. §$p < 0.05$, §§§$p < 0.001$ for difference with HH3000.*

Slight Additional Effect of Hypobaria in Both Hypoxia and Normoxia

HR increased in both NH and HH5500 when compared to NN and HN ($p < 0.001$), but to larger extent in HH5500 than NH (**Table 2**), which confirms previous findings (Savourey et al., 2003; Self et al., 2011). However, RMSSD decreased in NH and HH5500 likewise in comparison to NN and HN ($p < 0.001$, **Figure 1**). Moreover, LF (ms^2) was lower in NH and HH5500 ($p < 0.01$ and $p < 0.001$, respectively) than NN. HF (ms^2) was lower in HH5500 than in HN ($p = 0.025$). More precisely, decreases in HF were greater in NH (-35%, $p = 0.048$) and HH5500 (-60%, $p < 0.001$) than in HN (+ 8%), when compared to NN. Moreover, reduction in HF was also larger in HH5500 than in NH ($p = 0.048$), which implies a greater parasympathetic activity reduction. Total power decreased in NH ($p = 0.035$) and HH5500 ($p = 0.004$) when compared to HN and NN ($p < 0.001$). Sevre et al. (2001) demonstrated a transient reduction in parasympathetic and sympathetic activity (i.e., decreased total power, LF and HF power) during stepwise exposure to high altitude. The present results confirm previous findings suggesting HRV reduction (Wille et al., 2012), sympathetic activity elevation (Richalet et al., 1989; Marshall, 1994), and sympathetic predominance during acute exposure to hypoxia (Chen et al., 2008; Wille et al., 2012).

Acute hypoxia is considered as a potent activator of sympathetic activity (Richalet et al., 1988; Marshall, 1994; Hainsworth et al., 2007). When exposed to acute hypoxia, the muscle sympathetic nerve activity (MSNA) increases (Duplain et al., 1999; Hansen and Sander, 2003), due to the hypoxia-induced sympathetic activation (Marshall, 1994). LF/HF ratio was

TABLE 2 | Absolute values are means ± *SD.*

	Normoxia		Hypoxia	
	NN	**HN**	**NH**	**HH5500**
HR (bpm)	73.4 ± 7.0	77.4 ± 10.7	86.9 ± 13.2*** ###	93.0 ± 14.2*** ###††
RMSSD (ms)	46.1 ± 15.6	47.1 ± 26.9	29.0 ± 21.1*** ###	25.5 ± 16.1*** ###
LF (ms^2)	2381 ± 1311	1908 ± 1833	1176 ± 1178***	783 ± 536*** ##
HF (ms^2)	816 ± 442	987 ± 1058	661 ± 885	311 ± 314#
LF (n.u.)	73.9 ± 9.4	61.0 ± 12.7	68.8 ± 10.4	75.1 ± 14.1
HF (n.u.)	25.8 ± 9.5	39.0 ± 12.7	31.2 ± 10.4	24.9 ± 14.0
LF/HF ratio	3.3 ± 1.2	1.6 ± 0.9*	2.6 ± 1.3	4.4 ± 2.9###†
Total Power (LF + HF)	3197 ± 1627	2895 ± 2719	1433 ± 1466** #	999 ± 602*** ##
SpO_2(%)	99.5 ± 0.4	98.4 ± 1.8	86.0 ± 4.5*** ###	81.3 ± 5.5*** ###††

*Physiological variables (n = 15). HR, Heart rate; RMSSD, root mean square of the successive differences; LF, low frequency; HF, high frequency and SpO_2, pulse oxygen saturation in each condition: NN, Normobaric normoxia; HN, hypobaric normoxia; NH, normobaric hypoxia; HH5500, hypobaric hypoxia at 5500 m. *$p < 0.05$, **$p = 0.002$, ***$p < 0.001$ for difference with NN. #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ for difference with HN. †$p < 0.05$, ††$p < 0.01$ for difference with NH.*

higher in HH5500 than HN ($p < 0.001$), which confirms the hypoxia-induced sympathetic activity elevation (i.e., with similar barometric pressure between HN and HH5500). Interestingly, LF/HF was greater in HH5500 than NH, which may imply a slight hypobaric additional influence on sympathetic activation commonly reported in hypoxia. However, since there was no other significant difference in HRV parameters between HH5500 and NH, we assume that the present experimental evidences are not strong enough for such statement about the influence of hypobaria on HRV in hypoxia.

In normoxic conditions, HR was similar between NN and HN. Nevertheless, some differences were found between NN and HN for some HRV indices, suggesting a slight hypobaric influence on HRV in normoxia at rest: LF/HF ratio was lower in HN than NN ($p = 0.041$), suggesting parasympathetic activity predominance in HN. However, HF was similar and LF did not significantly decrease in HN ($p = 0.105$) when compared to NN. Parasympathetic increase was observed in subjects exposed to 4574 m breathing enriched O_2 gas mixture (Prabhakaran and Tripathi, 2011). This may be related with a decreased MSNA when breathing a hyperoxic gas mixture (Querido et al., 2010). In fact, peripheral chemoreceptors seem inhibited with hyperoxic stimulus leading to MSNA reduction (Querido et al., 2010). Moreover, change in breathing pattern due to lower air density in hypobaria, may be an additional factor to take into account (Ogawa et al., 2019). Despite non-significant difference, our data pointed this breathing pattern change in hypobaria, with lower ventilation value in HN than NN.

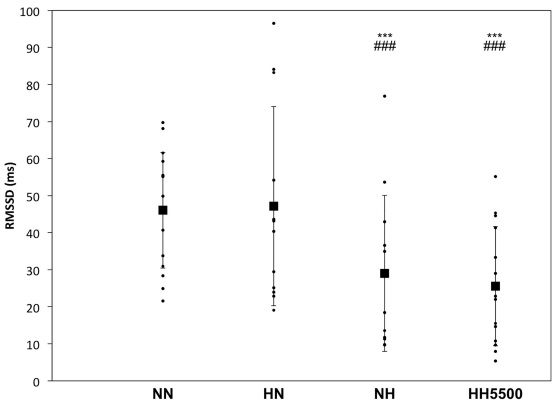

FIGURE 1 | The root mean square of the successive differences (RMSSD) for each subject. Bold squares represent absolute means ± *SD* for conditions: NN, Normobaric normoxia; HN, hypobaric normoxia; NH, normobaric hypoxia; HH5500, hypobaric hypoxia at 5500 m. ***$p < 0.001$ for difference with NN. ###$p < 0.001$ for difference with HN.

Influence of Ventilation on Heart Rate Variability

It is known that the cardiac autonomic nerve activity is influenced by ventilation (Brown et al., 1993). In a parallel article from our laboratory, minute ventilation and breathing frequency significantly increased in HH5500, but not NH, when compared to NN at rest (Aebi et al., 2020). Moreover, tidal volume tended to be higher in HH5500 than NN, while it remained unchanged in NH (Aebi et al., 2020). Gas exchanges data were collected on nine of the fifteen participants for measuring the hypercapnic response to CO_2. These data were collected 10 min following HRV measurement (**Table 3**). In fact, they should be interpreted with cautious, as it may not reflect accurately the gas exchanges during HRV recording, but it gives us insights of the ventilatory responses in each condition. We did not record ventilation and HRV during the same time period on purpose since wearing the mask may be a cofounding factor for resting HRV. The severity of the respiratory hypoxic response probably influenced HRV modulations. Therefore, HH5500 may induce slight greater cardiovascular stress than NH, possibly due to higher respiratory stimulus in addition to the hypoxic stimulus (i.e., greater minute ventilation). In the context of hypoxia, respiratory "stress" (i.e., hyperventilation in HH in the present study) may activate directly sensors and regulators of other integrative systems as endocrine-metabolic and cardiovascular. More precisely, gene expression and their immediate and long-term expression may also individually influence HRV. Hundreds

TABLE 3 | Absolute values are means ± *SD* (*n* = 9).

	NN	HH3000	HN	NH	HH5500
V_E (L/min)	12.1 ± 1.4	12.5 ± 1.4	10.3 ± 1.4	12.1 ± 2.7	16.0 ± 2.7*** $ ### ††
BF (cycle/min)	15.9 ± 2.6	16.7 ± 2.8**	17.9 ± 3.0	17.0 ± 3.6	17.9 ± 2.7***
VT (L)	0.82 ± 0.21	0.79 ± 0.20	0.62 ± 0.21	0.79 ± 0.27	0.98 ± 0.31* $ ##

*Ventilatory parameters: Minute ventilation (VE), breathing frequency (BF) and tidal volume (VT) at rest. In normobaric normoxia (NN, Dübendorf altitude level of 440 m), hypobaric hypoxia (HH, at altitude level of 3000 m and 5500 m, HH3000 and HH5500), hypobaric normoxia (HN, altitude level of 5500 m in normoxia), and normobaric hypoxia (NH, altitude simulation of 5500 m in normobaria). Statistical analysis was performed separately for altitude comparison in HH (NN, HH3000 and HH5500) and for conditions comparison (NN, HN, NH, and HH5500). *p = 0.061, **p < 0.01, and ***p < 0.001 different from NN. $ p < 0.05 different from HH3000. # p < 0.05, ## p < 0.01, and ### p < 0.001 different from HN. † p < 0.05 different from NH.*

of genes targeted in the HIF1-α pathway are potential candidates but it is beyond the scope and the results of the present study to further speculate on it.

HRV analysis requires caution, as it may not reflect pure autonomic tone and is influenced by several regulation loops as baroreflex or respiratory sinus arrhythmia. In addition, interpretation in LF/HF is popular but controversial (Williams et al., unpublished). HF component of the power spectral analysis of HRV is affected by the respiratory modulation of the vagal nerve activity. LF component and how it relates to the sympathetic tone and the baroreflex remains controversial (Williams et al., unpublished). HRV spectral analysis appears more sensitive and helpful than time-domain HRV indices (Schmitt et al., 2015). As frequency spectral power parameters did not show significant changes, we believe that supplemental time-domain parameters (i.e., pNN50 and SDNN) would not add value to the present manuscript.

Overall, HRV decreased in hypoxic conditions, which is in line with a previous elegant study that showed a decrease in spectral components of heart rate variability (i.e., total power, LF and HF) when exercising in acute hypoxia (F_IO_2 = 11.5%) in comparison with exercise in normoxia (Povea et al., 2005). The present study adds novelty by suggesting a slight influence of hypobaria in both hypoxia and normoxia on HRV modulations through ventilation pattern differences. The alveolar air equation shows that the coupled alveolar O_2 (P_AO_2) and carbon dioxide partial pressures (P_ACO_2) for NH and HH are not identical when P_IO_2 is equivalent (Fenn et al., 1946; Rahn and Otis, 1949; Rahn and Fenn, 1962). Therefore, physiological responses to NH cannot be identical to the responses to HH given only equivalent hypoxic P_IO_2. An integrated mechanism should start with the alveolar air equation, especially the contribution of N_2 in setting the coupled P_AO_2 and P_ACO_2 partial pressures (Conkin, 2016).

Heart Rate Variability and Its Potential Relation With Hypoxemia

As expected, SpO_2 decreased in HH3000 (p = 0.003) and HH5500 ($p < 0.001$) when compared to NN, but to a greater extent in HH5500 (**Table 1**.). SpO_2 was higher in normoxic conditions (NN and HN, $p < 0.001$) than in NH and HH5500 (**Table 2**). Moreover, SpO_2 was lower in HH5500 than in NH (p = 0.002), which confirmed the greater hypoxemia induced by HH, when compared to NH, in line with several previous studies (Savourey et al., 2003; Saugy et al., 2016). It was previously shown that ΔSpO_2 interacts with ΔLF/HF ratio (Botek et al., 2015). Moreover, ΔSpO_2 was correlated with delta RMSSD using natural logarithm transformation (ΔLn RMSSD) during first 5 min of NH exposure (Krejèí et al., 2018). In the present study, %Δ HR was negatively correlated with%Δ SpO_2 (r = -0.594, p = 0.046) in HH5500. Last but not least, %Δ SpO_2 was positively correlated (r = 0.629, p = 0.032) with total power in HH5500 only. Therefore, the present results confirm potential relation between HRV modulations and SpO_2 in acute hypoxia (i.e., during the first 10 min of exposure). In addition, time-dose may also play an important role in individual hypoxemic state and HRV modulation. Duration of flights often differ depending on the mission, which may influence stress perceived and tolerated by the pilot. A recent study demonstrated that hypoxic stimulus may improve the tolerance to discomfort in athletes during high – intensity exercise (Álvarez-Herms et al., 2016). It would thus be of interest to investigate how hypoxia and hypobaria would modulate HRV during different exposure durations.

In conclusion, the present study reports a slight hypobaric effect either in normoxia or in hypoxia. In normoxia, this effect is related to an increase of parasympathetic activation, likely due to the hyperoxic breathing in HN. In hypoxia, where hypobaria induced a more prominent sympathetic activation, the hypobaric effect is likely due to the greater ventilation stimulus and larger desaturation in HH5500 than in NH.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Swiss Ethic Committee of Zürich, BASEC ID: 2017-00752. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MA, NB, DB, and GM were part of the conception of the protocol. MA conducted the experiments, was responsible for

data acquisition, and wrote the manuscript. MA conducted the analysis and interpreted the results with NB and GM. GM revised the manuscript critically and gave advices to MA for corrections. All authors reviewed and approved the manuscript prior to submission.

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Conflict of Interest: MA and NB were employed by the companies Armasuisse and Be.care, respectively.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Article 2 – Both hypoxia and hypobaria impair baroreflex sensitivity but through different mechanisms

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Both hypoxia and hypobaria impair baroreflex sensitivity but through different mechanisms

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Abstract

Introduction

Baroreflex is a vital mechanism for the regulation of adequate blood supply to all organs, especially the brain. Baroreflex sensitivity (BRS) is a measure of baroreflex function and is lower in normobaric and hypobaric hypoxia compared to normobaric normoxia. The aim of this study was to assess the effects of hypobaria on BRS in both normoxic and hypoxic conditions.

Methods

Continuous blood pressure and ventilation were recorded in eighteen participants at rest, during moderate intensity cycling exercise and during post-exercise recovery in four conditions: normobaric normoxia (NN), hypobaric normoxia (HN), normobaric hypoxia (NH) and hypobaric hypoxia (HH). Barometric pressure was matched between normobaric (NN vs. NH, 723±4 mmHg) and hypobaric (HN vs. HH, 406±4 vs. 403±5 mmHg) conditions while inspired oxygen pressure (P_iO_2) was matched between normoxic (NN vs. HN, 141.2±0.8 vs. 141.5±1.5 mmHg) and hypoxic (NH vs. HH, 75.7±0.4 vs. 74.3±1.0 mmHg) conditions. BRS was assessed using the sequence method.

Results

At rest, BRS decreased similarly in HN, NH and HH compared to NN ($p < 0.01$, $p < 0.05$ and $p < 0.001$, respectively). Heart rate (HR), mean, systolic and diastolic blood pressures did not differ between the four conditions. During exercise, HR was higher ($p < 0.001$) in NH and HH than in NN whilst ventilation (VE) was higher in HH than in NN ($p < 0.001$). During recovery HR and VE evolved similarly between all conditions. BRS decreased during exercise and returned toward basal values during recovery similarly in all conditions.

At rest, the end-tidal pressure in O_2 ($PetO_2$) was lower in hypoxia than in normoxia with a cumulative effect of hypobaria (HN < NN; 92±13 vs. 101±4 mmHg; $p < 0.001$ and HH < NH; 41±2 vs. 50±7 mmHg; $p < 0.001$). $PetCO_2$ was impacted by hypobaria with lower values ($p < 0.001$) in HN and HH (23±3 and 23±1 mmHg) than in NN and NH (36±4 and 33±4 mmHg).

Conclusion

The novel finding of this study is the specific effect of hypobaria on BRS at rest in normoxia, i.e., BRS was lower in HN than in NN. The hypoxic and hypobaric effects do not add to each other resulting in comparable BRS decreases in HN, NH and HH. The decrease in BRS in HN may be attributed to hypocapnia, via stimulation of the central chemoreceptors, whilst the decrease in hypoxia likely originates from both carotid body (hypoxia) and central (hypocapnia) chemoreceptors. During exercise, the increases in HR and VE seem to blunt the effects of hypobaria and hypoxia on BRS that were observed at rest.

INTRODUCTION

The physiological effects of altitude in humans are often studied in normobaric hypoxia (NH) according to the air equivalent model. This model posits that the inspired oxygen pressure (PiO_2) matters without any influence of the barometric pressure *per se* (Conkin and Wessel, 2008). However, in recent years, differences between NH and "real altitude" (hypobaric hypoxia, HH) have been reported (Millet et al., 2012). In HH compared to NH arterial oxygen saturation was lower (Saugy et al., 2014; Coppel et al., 2015), sleep more disturbed (Heinzer et al., 2016), and oxidative stress more pronounced (Ribon et al., 2016), whilst acute mountain sickness symptoms were more severe (DiPasquale et al., 2016). Subtle effects on heart rate variability were also reported (Aebi et al., 2020a). Although still subject of debate (Millet and Debevec, 2020; Richalet, 2020), at equivalent PiO_2 HH appears as a stronger stimulus than NH, which suggests an influence of the decreased barometric pressure *per se*, at least in hypoxia.

But it takes an additional hypobaric normoxic condition (HN) to isolate the effect of hypobaria in both normoxic (NN vs. HN) and hypoxic (NH vs. HH) environments. Direct comparison of "simulated" (in a normobaric hypoxic chamber, NH) and "real" altitude (in a mountainous environment, HH) is limited by the difficulty to match environmental factors (e.g., radiation, temperature, humidity) and the impossibility to "blind" the experiments (i.e., in HH, the participants know that they are at altitude). These limitations can be overcome with a pressure regulated chamber, by varying both pressure and inspired oxygen fraction. The HN condition requires lowering barometric pressure combined with increasing inspired oxygen so that the PiO_2 remains similar to NN values (akin to what may occur in aviation when breathing 100% oxygen in a depressurized cabin). By comparing NN, NH, HN and HH it becomes possible to further disentangle the effects of hypoxia and hypobaria.

The arterial baroreflex is involved in the regulation of blood pressure (BP) and ultimately defends the adequate blood supply to all organs, especially the brain. Baroreceptors sense systemic blood pressure through the stretch of receptors in the carotid sinus and the aorta. Changes in arterial baroreceptor afferent discharge lead to adjustments in blood pressure. A decrease in arterial pressure reduces baroreceptor afferent discharge leading to a decrease in parasympathetic activity and an increase sympathetic tone, triggering an increase in HR, cardiac contractility, and vascular resistance. A rise in pressure does the contrary, inhibiting the sympathetic and activating the parasympathetic activity. The arterial baroreflex is challenged in numerous conditions such as after exhaustive endurance exercise (Gratze et al., 2005) or during exercise and at altitude (Bourdillon et al., 2017).

Baroreflex sensitivity (BRS) is a measure of arterial baroreflex function. The faster the changes in heart rate (HR) in response to small changes in BP, the more sensitive the autonomic control of BP and the higher the BRS. BRS is directly linked to basal parasympathetic activity (Hughson et al., 1994a). In hypoxic conditions, there is a parasympathetic withdrawal (Hughson et al., 1994b; Ponchia et al., 1994) that alters the neural control of the heart (Yamamoto et al., 1996), leading to the resetting of BRS to higher blood pressures (Raven et al., 2006). This resetting results in decreased BRS (Roche et al., 2002) in acute and chronic hypoxia (Bourdillon et al., 2018). This resetting of BRS is clear above 4,500 m but is less evident for lower altitudes (Querido et al., 2011). However, we previously reported lower BRS values in NH and HH than in normobaric normoxia (NN) but without differences between the two hypoxic conditions at 2,250 and 3,450 m (Bourdillon et al., 2017).

During exercise, changes in cardio-circulatory dynamics cause an increase in BP that activates the arterial baroreceptors (Michelini et al., 2015). Yet, mean blood pressure only increases moderately, because there is a resetting of BRS to higher blood pressures (Bevegård and Shepherd, 1966; Eckberg et al., 1975; Pawelczyk and Raven, 1989; Joyner, 2006), which logically results in decreased BRS during exercise (Vallais et al., 2009).

In order to better assess the respective influence of hypoxia and hypobaria on BRS, the aim of the present study was to investigate the potential effects of decreased barometric pressure *per se* on the cardiovagal

baroreflex sensitivity at rest and during moderate-intensity exercise in normoxia (NN vs HN) and severe hypoxia corresponding to an altitude of 5,000 m (NH vs HH).

METHODS

Ethics

This study was performed according to the Declaration of Helsinki and was approved by the Swiss Research Ethics Committee of Zürich (Swissethics, BASEC ID: 2018–00006). The trial was registered on ClinicalTrials.gov (ID: NCT03439202). The participants were informed about all procedures of this study and gave their written informed consent before participation.

Participant recruitment and screening

Eighteen healthy pilot trainees (14 men and 4 women, age 26 ± 3 years; height 177 ± 9 cm; weight 70 ± 11 kg) participated voluntarily in this study. None of the participants were exposed to hypoxia before enrolment in the present study and/or no relevant altitude exposure was reported in the preceding four weeks preceding the trials. A physician screened the participants during a familiarization visit to ensure they were healthy and did not report any medical or altitude-related issues. None of the participants were on medication during this study.

Study design

This study was conducted at the Aeromedical Center (AeMC) of the Swiss Air Force, in Dübendorf, Switzerland. During a single visit the participants were exposed to four conditions: normobaric normoxia (NN, Dübendorf, 440 m, barometric pressures in Table 1), hypobaric normoxia (HN), normobaric hypoxia (NH) and hypobaric hypoxia (HH), in a randomized order and single-blind. Each condition lasted 30 min and was carried out at local barometric pressure or at a simulated altitude of 5,000 m in the Swiss army hypobaric chamber. Each condition was preceded by 30 min of rest in NN. Decompression to 5,000m took about 2 min in the two hypobaric conditions (HN and HH).

During the twenty-four hours before the visit, the participants were asked to avoid physical exercise or heavy meals, and to refrain from alcohol and caffeine consumption. Each condition started with a 5-min adaptation period followed by a concentration test (KLT-R test (Düker and Lienert, 2001) including arithmetic and working memory tasks) and 6 min seated at rest. The participants then sat on a cycle ergometer (eBike II basic, GE medical systems, Germany) and remained at rest during 3 min (data analyzed for the resting period further in this article). The participants then exercised at 1 Watt per kilogram of bodyweight (73 ± 15 Watts at 80 rpm) for 6 min and recovered seated on the bike for 3 min. The participants gave their rating of perceived exertion (RPE, using the 6-20 Borg scale) at the end of exercise.

Conditions

Barometric pressure was matched between the two normobaric (NN vs. NH) and between the two hypobaric (HN vs. HH) conditions, whilst the inspired oxygen pressure (PiO_2) was matched between the normoxic (NN and HN) and between the hypoxic (NH and HH) conditions (cf. Table 1). Matching was achieved by adjusting the barometric pressure in the hypobaric chamber or the inspired oxygen fraction (FiO_2) using tanks of gas mixtures of known concentrations (Conkin, 2016). Participants breathed 11.2% or 39.4% O_2 (0.03% CO_2, balance N_2) during NH and HN, respectively, whilst the barometric pressure was decreased comparably in HN and HH (cf. Table 1). For blinding, the altimeter in the hypobaric chamber was hidden and changes in pressure and gas concentrations administered through the mask were not communicated to the participants.

Blood pressure recording

Blood pressure was recorded at a sampling frequency of 1,000 Hz using a photoplethysmography device combined to a double cuff (NIBP100D, Biopac Systems, Inc. Goleta, CA, USA). Blood pressure was recorded continuously from the double cuff installed on the index and the middle fingers, throughout rest, exercise, and recovery. The device was connected to a computer for data storage using dedicated software (Acqknowledge, Biopac Systems, Inc. CA, USA). Signal processing was performed offline using custom Matlab routines (MATLAB, R2019b, MathWorks, Natick, MA, USA).

Ventilatory data

The gas analyzer (K5, Cosmed, Rome, Italy) was calibrated outside of the hypobaric chamber before each session. This procedure was recommended by the manufacturer and gives reliable results for ventilation ($\dot{V}E$), tidal volume (VT), respiratory frequency (Rf), end-tidal O_2 and CO_2 pressures $PetO_2$ and $PetCO_2$, but not for oxygen consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$). Flow was calibrated with a 3L syringe. Zero CO_2 calibration was performed using a scrubber. A second point calibration was performed using a certified gas mixture (16% O_2 and 5% CO_2). Ventilatory data were recorded breath-by-breath and exported with proprietary software for later analysis (OMNIA, Cosmed, Roma, Italy) as instructed by the manufacturer (Perez-Suarez et al., 2018; Crouter et al., 2019). The PiO_2 measured with the gas analyzer were as expected given the known concentrations of the gas tanks which increases confidence in the measures realized in the challenging HN condition.

Blood pressure and baroreflex analyses

Systolic blood pressure (SBP) peaks, and heart beat-to-beat time intervals, defined as the time intervals between successive systolic peaks (inter-beat intervals, IBI), were extracted directly from the BP recordings. the sequence method was used to compute BRS. It is based on the identification of at least three consecutive beats in which an increase (or decrease) in SBP is followed by an increase (or decrease) in IBI. Three conditions were necessary for a SBP-IBI sequence to be qualified for BRS computations: 1) a minimum change of 1 mmHg between two consecutive SBP values and a minimum change of 5 ms for IBI (Bernardi et al., 2010) ; 2) the minimum correlation coefficient between changes in SBP and changes in IBI was 0.85 and 3) at least five sequences were necessary to validate a BRS estimate. The slope of the regression line between changes in SBP and IBI was used as BRS estimates and all the computed slopes were averaged to obtain the BRS.

This method allows a direct interpretation of the causal link between blood pressure and heart rate changes (Parati et al., 1988). It is one of the most used. The computations are automatic and standardized, which virtually eliminates intra- and inter-participant measurement variability (La Rovere et al., 2008). The baroreflex nature of these spontaneous beat-to-beat interval systolic pressure sequences was demonstrated by showing in cats that the number of sequences markedly dropped (−89%) after the surgical opening of the baroreflex loop by sinoaortic denervation (Di Rienzo et al., 2001). Consistency of the various methods for BRS computation in hypoxia has been shown recently (Bourdillon et al., 2019).

Beat-to-beat heart rate (HR) was directly computed from the IBI intervals. Mean, systolic, and diastolic blood pressure were extracted from the continuous blood pressure recordings.

Heart rate variability analysis

RR intervals were recorded in parallel with the continuous blood pressure trace using a chest strap (watch RS800CX + sensor H7 + chest belt, Polar, Kempele, Finland). The RR intervals from the resting period were first inspected to remove ectopic beats from the recordings. Ectopic beats were then compensated by means of interpolation to calculate normal-to-normal (NN) intervals. From the NN intervals, the following heart rate variability (HRV) parameters were extracted: the root mean square of

the successive differences (RMSSD); the spectral power in the low-frequency (pLF, 0.04 – 0.15 Hz) and high-frequency bands (pHF, 0.15 – 0.40 Hz) in ms^2; and the values (expressed in normalized units) for LF and HF, respectively. However, since these two indexes are perfectly correlated, only normalized HF (nHF) are presented and discussed. The spectral power was estimated using a fast Fourier transform on the resampled NN intervals (4 Hz) using a window length of 250 data points and an overlap of 50%. All computations were performed using custom MATLAB routines.

Statistical analysis

Data are presented as mean +/- SD except in the figures for clarity purposes SEM is plotted. Three-way repeated measures ANOVAs were performed to assess the effects of hypobaria, hypoxia and exercise on BRS, HR, mean, systolic and diastolic blood pressure. This resulted in NN, HN, NH and HH conditions during rest, exercise, and recovery. The p level for significance was set at 0.05. Values for p are presented < 0.05, or < 0.01 or < 0.001. The Tukey-Kramer *post hoc* test was performed when appropriate. All analyses were completed using custom MATLAB routines.

RESULTS

Barometric pressure was well matched between the two pairs of conditions, NN vs. NH and HN vs. HH. Also, PiO_2 was well matched between NN vs. HN and NH vs. HH (Table 1).

At rest, BRS decreased comparably in HN, NH and HH compared to NN ($p < 0.01$, $p < 0.05$ and $p < 0.001$, respectively, Figure 1) whilst there were no differences in heart rate (HR), mean, systolic and diastolic blood pressures between the four conditions. During exercise, BRS decreased in NN, HN and NH ($p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively) but not in HH, when compared to rest. During recovery, BRS returned toward basal values, without differences between conditions.

During the resting period, HR and $\dot{V}E$ were not different between the four conditions. HR increased during exercise in all conditions ($p < 0.001$ for all) and more so in NH and HH than in NN ($p < 0.001$ for both). There was a tendency for a difference in HR between NN and HN ($p = 0.066$, Figure 1). Exercise $\dot{V}E$ was higher in HH than in NN ($p < 0.001$). There was no difference in HR and $\dot{V}E$ during the recovery period between the four conditions. VT and RF significantly increased during exercise compared to rest in all conditions.

Results for $\dot{V}E$, VT, Rf, $PetO_2$ and $PetCO_2$ are detailed in Figure 2. Resting $PetCO_2$ was lower in HN and HH compared to NN and NH ($p < 0.001$ for all). During exercise, $PetCO_2$ increased in NN, NH and HN ($p < 0.001$ for all) but not in HH, when compared to rest. In addition, $PetCO_2$ was lower in HN, NH and HH ($p < 0.001$ for all) than in NN as well as in NH and HH ($p < 0.001$ for all) compared to HN and in HH compared to NH ($p < 0.001$). During recovery, $PetCO_2$ returned toward resting values in all conditions and was significantly lower in HN and HH compared to NN and NH ($p < 0.001$ for both).

At rest, as expected, $PetO_2$ was lower in the two hypoxic conditions (NH and HH) than in the two normoxic conditions (NN and HN). Moreover, $PetO_2$ was lower in HN than in NN and in HH than in NH (both $p < 0.05$). During exercise, $PetO_2$ remained lower in HN, NH and HH than in NN ($p < 0.001$) and was still lower in HN than in NN as well in HH than in NH (both $p < 0.05$). Comparable effects were observed between the four conditions during recovery.

There was a positive correlation between $PetCO_2$ and BRS at rest ($p < 0.001$ and $R = 0.43$) as illustrated in Figure 3.

Blood pressure parameters are detailed in Table 2. Mean, systolic and diastolic blood pressures were comparable between conditions and did not significantly change during the moderate-intensity exercise.

Table 3 summarizes the HRV results. RMSSD significantly decreased in HH compared to NN and HN ($p < 0.01$ for both) and there was a tendency for a decrease in NH compared to NN ($p < 0.10$). There was a tendency for decreases in HF and nHF in HH compared to NN ($p < 0.10$ for all).

DISCUSSION

This study investigated the effect of hypobaria on baroreflex sensitivity in both normoxic and hypoxic conditions. The main result is a large and specific effect of hypobaria *per se*, at rest in normoxia despite that no specific effects were found on HR or blood pressure. This influence of hypobaria on BRS was demonstrated at rest but not during exercise and recovery and was less evident in hypoxia.

Decreased BRS in hypoxia

The comparable decrease in BRS at rest in the two hypoxic conditions (NH and HH, when compared to NN) confirms previous findings in the literature (Roche et al., 2002; Bourdillon et al., 2018). The known hypoxic effect did not add to the hypobaric effect observed in HN resulting in values similar between HH, NH and HN values.

The reduction in BRS in acute hypobaric hypoxia is probably mediated by the carotid body chemoreceptors (Mozer et al., 2016). Previous studies suggested that acute hypobaric hypoxia initiates a persistent increase in chemo-afferent activity to the rostro-ventrolateral medulla via the nucleus tractus solitarius, which results in long-lasting sympathoexcitation, likely accompanied by a parasympathetic withdrawal (Guyenet, 2000; Prabhakar and Kumar, 2010). These modifications of the autonomic balance are probably one of the triggers that lead to an altered BRS (Raven et al., 2006). Accordingly, there was a decrease in RMSSD, a tendency for a decrease in HF (both markers of parasympathetic activity) and a tendency for a shift in the autonomic balance toward sympathetic dominance (decreased nHF) in the HH conditions (Table 3). Another important trigger may be the central chemoreceptors, which are known to be more responsive to CO_2 than the peripheral ones (Dempsey et al., 2014; Smith et al., 2015) and therefore may also play a pivotal role in BRS decrease.

These classical explanations are directly linked to the changes in blood gases (and potentially in the cerebrospinal fluid), affecting the chemoreceptors. In humans, the baro- and chemo-reflex arcs coincide, so that sensory information regarding BP and arterial blood gas homeostasis converge in an integrative fashion (Somers et al., 1991). There is a negative relationship between the baro- and chemo-reflexes; i.e., the baroreflex activation inhibits the chemoreflex and vice versa (Cooper et al., 2005). Therefore, heightened activation of the chemoreceptors in hypoxic conditions likely decreased the baroreflex function, which presently resulted in the decreased BRS (Mozer et al., 2016; Bourdillon et al., 2018). There was a positive correlation between $PetCO_2$ and BRS (Figure 3), indicating that reduced blood CO_2 in HN and HH conditions likely contributed significantly to the decreased BRS. Hypocapnia in the HH condition was likely due to hyperventilation and is well described in the literature. The mechanisms leading to hypocapnia the HN condition remain to be elucidated since there was no changes in ventilation, VT, Rf, inspired and expired times, indicating that the breathing pattern did not change.

The present work used a spontaneous cardiovagal BRS, which only estimates sensitivity or gain around the operating point of the baroreflex stimulus-response curve. A potential resetting of the arterial baroreflex generally occurs with exercise, the carotid baroreflex control of HR reduced progressively as exercise workload increased (Raven et al., 2006). In HH conditions, a downward resetting of cardiovagal baroreflex, coupled with an upward resetting of sympathetic vascular baroreflex, without any alterations in BRS, was observed (Simpson et al., 2019). The main variable of interest of the present study was the cardiovagal baroreflex and there was no assessment of the sympathetic vascular baroreflex component.

Effect of hypobaria on BRS: large in normoxia and minimal in hypoxia

A direct effect of hypobaria *per se* on ventilation has previously been shown (Loeppky et al., 1997; Savourey et al., 2003). In the HN condition, the O_2 pressure gradient between the lung alveoli and the gas is maintained around sea level values, but the CO_2 pressure gradient is larger than in NN or NH, which caused a decrease in $PetCO_2$. The latter was comparable between HN and HH but was lower than in NN and NH (Figure 2). Therefore, in HN, hypocapnia presumably decreased afferent traffic from the chemoreceptors, which resulted in a decreased BRS. This finding emphasizes the pivotal role of the central chemoreceptors in the BRS decrease (Dempsey et al., 2014; Smith et al., 2015). Also, blood pH is highly dependent on blood CO_2 partial pressure and was reported to increase in HN compared to NN and NH, which again emphasizes the role of central chemoreceptors by modifying the pH of the cerebrospinal fluid (Aebi et al., 2020b).

PiO_2 were well matched between NN and HN conditions (Table 1). As it has been demonstrated in the present article, BRS was likely affected by hypocapnia in the HN and HH conditions, which probably occurred via modifications in the sympathovagal balance and therefore modifications in the vascular tone (Aebi et al., 2020a). However, as previously reported (Aebi et al., 2020a), hypobaria *per se* does not seem to be the main trigger in the observed BRS alteration since there was no significant difference in HRV between NN and HN. Hypocapnia likely triggered vasoconstriction in the pulmonary circulation, which may have affected the O_2 diffusion capacity from the alveoli to the blood in the HN condition. In the HH condition, both decreased O_2 gradient from the alveoli to the pulmonary circulation, and the pulmonary hypoxic vasoconstriction decreased arterial O_2 content. Additional inspired CO_2 in hypocapnic or hypoxic conditions is a therapeutic means used to attenuate the hypoxic pulmonary vasoconstriction (Chuang et al., 2010).

Previous study suggested that pulmonary blood flow through intrapulmonary arteriovenous anastomoses, was decreased by hypobaria, independent of the hypoxia severity (Petrassi et al., 2018). Previous work has shown that hypobaric decompression increased total lung capacity, functional residual capacity, closing capacity, and residual volume (Coates et al., 1979) which may be attributed to a greater volume of air trapped in the alveoli at lower atmospheric pressure therefore increasing CO_2 diffusion form the blood capillaries to the alveoli. An increase in lung volume increases compression of alveolar capillaries (Simmons et al., 1961; Hakim et al., 1982) and may contribute to decrease $PetCO_2$ in the HN condition. Decreased $PetCO_2$ in the HN condition is a subject of debate. At 5,260 m, no differences in P_aCO_2 were reported between rest and high intensity exercise (as shown by RER ~.99) despite induced hyperventilation (Petrassi et al., 2018).

Ventilation and heart rate

Resting ventilation and heart rate did not change throughout the conditions, even in HH, which was expected, despite large decreases in $PetCO_2$ in HN and HH conditions or large decreases in $PetO_2$ in NH and HH conditions. The decrease in PetO2 was not sufficient to trigger an increase in ventilation, that usually happens when SpO_2 drops below 60% (74% in the present HH condition). The exercise intensity was very light (1 W/kg) and likely not sufficient to elicit the differences in ventilation and heart rate usually seen at higher intensities in hypoxic conditions. In addition, although PiO_2 was matched among the conditions, the differences in SpO_2 observed between NH and HH but not between NN and HN is likely to differentially affect PaO_2 and peripheral chemoreflex, blood flow /O_2 delivery, as well as group III/IV skeletal muscle afferents (Wan et al., 2020), thereby influencing the resetting of cardiovagal baroreflex (Hureau et al., 2018). This mechanism would be clearer at higher intensities requiring higher muscle activation, but the present work was expected to be of practical significance for aircraft pilots who are not performing exercises at high intensities. Moreover, the feasibility of using high intensity exercise is questionable due to the narrow range of tolerable intensities at high altitude.

Limitations

Respiration is a confounding factor for the characterization of the baroreflex control from spontaneous fluctuations (Porta et al., 2012). The impact is particularly deleterious at rest and during experimental situations imposing a strong influence of respiration such as during exercise. In the present study no change in ventilation or breathing pattern was observed, therefore limiting the effects of respiration as a confounding factor.

In this paper, BRS is reported according to the sequence method, which is the most commonly used and which allows a direct interpretation of the causal link between blood pressure and heart rate changes. However, Bernardi's ratio of the standard differences, the frequency and the transfer function methods were also used (Bourdillon et al., 2019). The conclusion of this work would not have been different with the other methods.

Eighteen participants may be seen as a rather small sample size in regards of the number of factors of the analysis (effect of hypoxia, hypobaria and exercise). However, each participant underwent all the conditions in a randomized order thereby minimizing the inter-individual variability. In addition, our group of participants was rather homogeneous (all military aircraft pilot trainees), therefore despite a small sample size the statistical results remain interesting and contains original data.

Perspectives

Overall, our results indicate that humans exposed to HN conditions, such as military aircraft pilots, should be supplemented in CO_2 (in addition to O_2 of course) to avoid hypocapnic conditions and subsequent decreased BRS and vasoconstriction, that may impact the cerebral perfusion. Future studies need to determine the adequate amount of inspired CO_2 to avoid impaired physical and cognitive performances (Aebi et al., 2020c). Future studies should focus on the relationship between pulmonary O_2 and CO_2 diffusion, blood content and baroreflex function in the four conditions, attempting to further disentangle the chemo- and baro-reflex arcs to better understand the mechanisms of blood pressure regulation in conditions of hypobaria and/or hypoxia.

CONCLUSION

This study was the first to demonstrate a specific effect of hypobaria *per se* on BRS, which we attribute primarily to hypocapnia in normoxic environment. This finding is of interest in space physiology since it has direct consequences for astronauts exposed to microgravity with large clinically significant physiological alterations. The latter, by stimulating the CO_2 chemoreceptors triggered the decrease in BRS in the HN condition. The effects of hypocapnia and hypoxia do not add to each other so that the decrease in BRS is comparable between HN, NH and HH conditions. The hypothesis that adequate additional inspired CO_2 in hypobaria-induced hypocapnic conditions would prevent impaired BRS requires further investigation.

FIGURE LEGEND

Figure 1

Baroreflex sensitivity (BRS) and heart rate (HR) in normobaric normoxia (NN), hypobaric normoxia (HN), normobaric hypoxia (NH) and hypobaric hypoxia (HH), during rest, exercise and recovery.

a $p < 0.05$ for difference with NN, b $p < 0.05$ for difference with HN, c $p < 0.05$ for difference with NH, * $p < 0.05$ for difference with rest, # $p < 0.05$ for difference with exercise.

Figure 2

Ventilation ($\dot{V}E$), end-tidal oxygen pressure ($PetO_2$) and end-tidal carbon dioxide pressure ($PetCO_2$) in normobaric normoxia (NN), hypobaric normoxia (HN), normobaric hypoxia (NH) and hypobaric hypoxia (HH), during rest, exercise and recovery

a $p<0.05$ for difference with NN, b $p<0.05$ for difference with HN, c $p<0.05$ for difference with NH, * $p<0.05$ for difference with rest, # $p<0.05$ for difference with exercise.

Figure 3

Linear correlation between end-tidal carbon dioxide pressure ($PetCO_2$) and baroreflex sensitivity (BRS) in normobaric normoxia (NN), hypobaric normoxia (HN), normobaric hypoxia (NH) and hypobaric hypoxia (HH), at rest.

DISCLOSURE

NB, MRA, BK, DB and GPM have no conflicts of interest, sources of funding, or financial ties to disclose and no current or past relationship with companies or manufacturers who could benefit from the results of the present study. NB is an employee of be.care SA. MRA is an employee of armasuisse. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABLILTY

The data that support the findings of this study are openly available in Zenodo at http://doi.org/10.5281/zenodo.4297460, reference number https://zenodo.org/record/4297460#.X8TAb7fjKUk.

AUTHOR CONTRIBUTION

GPM designed the study. MRA collected the data. NB and MRA analyzed the data. NB did the signal processing. NB wrote the article and prepared the figures. GPM and BK reviewed the article. All the authors approved the final version of the manuscript and declare no conflict of interest.

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Table 1. Barometric pressure, inspired pressure in oxygen (PiO_2) and pulse saturation (SpO_2) at rest before exercise.

	NN	HN	NH	HH
Barometric pressure (mmHg)	723 ± 4	406 ± 4 a	723 ± 4	403 ± 5 a
PiO_2 (mmHg)	141.2 ± 0.8	141.5 ± 1.5	75.7 ± 0.4 ab	74.3 ± 1.0 ab
SpO_2	99.4 ± .5	98.3 ± 2.1	83.5 ± 6.0 ab	74.7 ± 5.1 abc

Normobaric normoxia (NN); hypobaric normoxia (HN); normobaric hypoxia (NH); hypobaric hypoxia (HH).

a $p < 0.05$ for difference with NN; b $p < 0.05$ for different with HN; c $p < 0.05$ different from NH.

Table 2. Blood pressure data.

Parameter	Time	NN	HN	NH	HH
BPmean (mmHg)	Rest	93 ± 14	98 ± 14	91 ± 13	89 ± 11
	Exercise	97 ± 12	97 ± 16	98 ± 13	89 ± 11
	Recovery	98 ± 11	101 ± 15	96 ± 14	93 ± 10
BPsys (mmHg)	Rest	126 ± 23	130 ± 22	125 ± 18	125 ± 15
	Exercise	135 ± 20	136 ± 30	141 ± 22	132 ± 27
	Recovery	134 ± 16	135 ± 21	130 ± 24	123 ± 15
BPdia (mmHg)	Rest	77 ± 11	85 ± 14	79 ± 13	75 ± 12
	Exercise	78 ± 11	77 ± 16	80 ± 12	67 ± 11
	Recovery	81 ± 10	84 ± 15	85 ± 11	79 ± 10

Normobaric normoxia (NN); hypobaric normoxia (HN); normobaric hypoxia (NH); hypobaric hypoxia (HH).

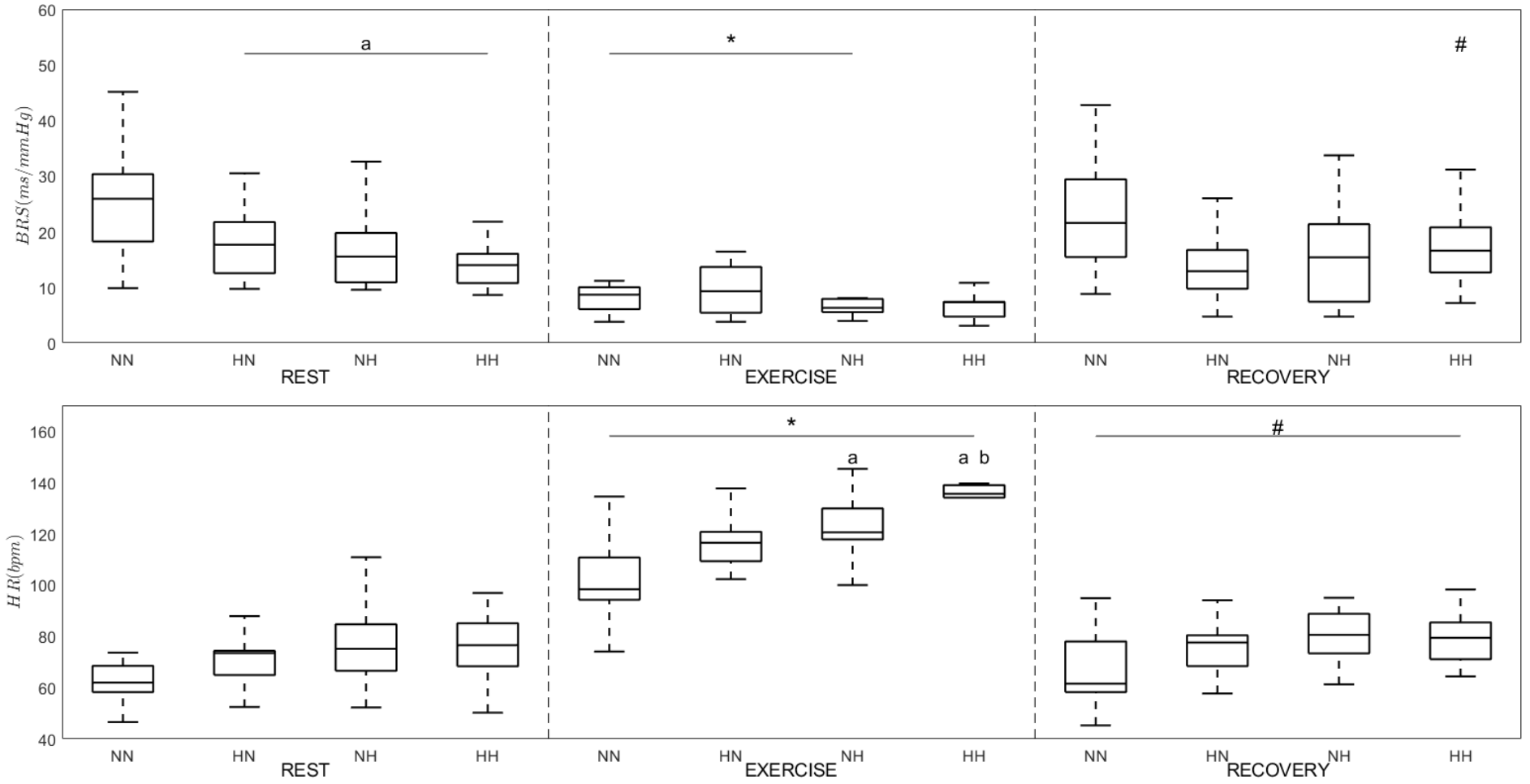
Table 3 - HRV data at rest before exercise.

	NN	HN	NH	HH
RMSSD (ms)	49 ± 20	37 ± 17	30 ± 20 (a)	18 ± 15 ab
LF (ms^2)	1317 ± 732	1374 ± 1263	1184 ± 1665	559 ± 637
HF (ms^2)	1068 ± 771	911 ± 775	752 ± 979	329 ± 530 (a)
nHF (%)	42 ± 14	41 ± 11	38 ± 8	30 ± 13 (a)

Normobaric normoxia (NN); hypobaric normoxia (HN); normobaric hypoxia (NH); hypobaric hypoxia (HH).

(a) $p < 0.10$; a $p < 0.05$ for difference with NN; b $p < 0.05$ for different with HN.

Figure 1


BRS(ms/mmHg)
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50
40
30
20
10
0
a
*
#
NN
HN
NH
HH
REST
EXERCISE
RECOVERY
HR(bpm)
160
140
120
100
80
60
40
a
a b

Figure 2

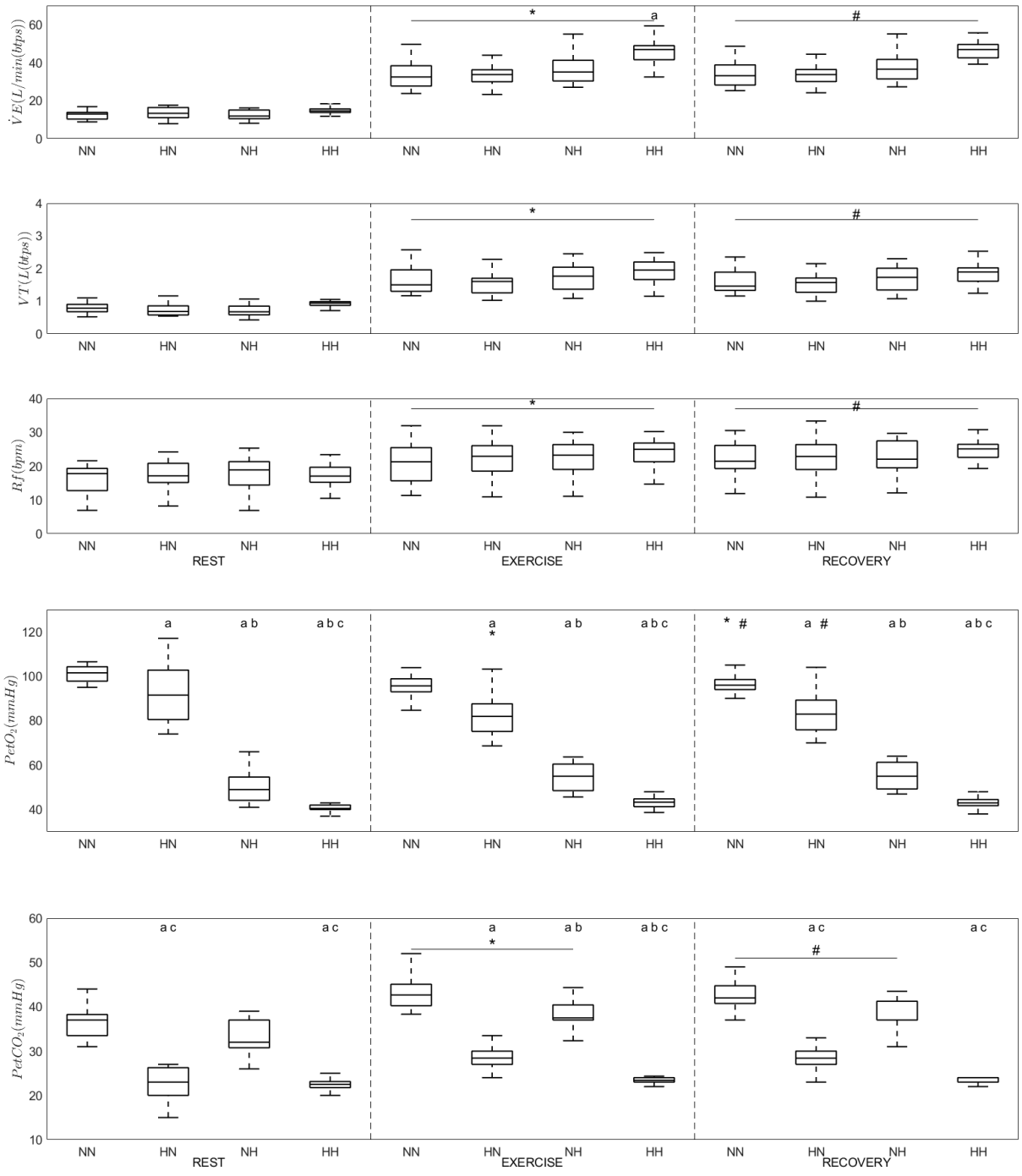
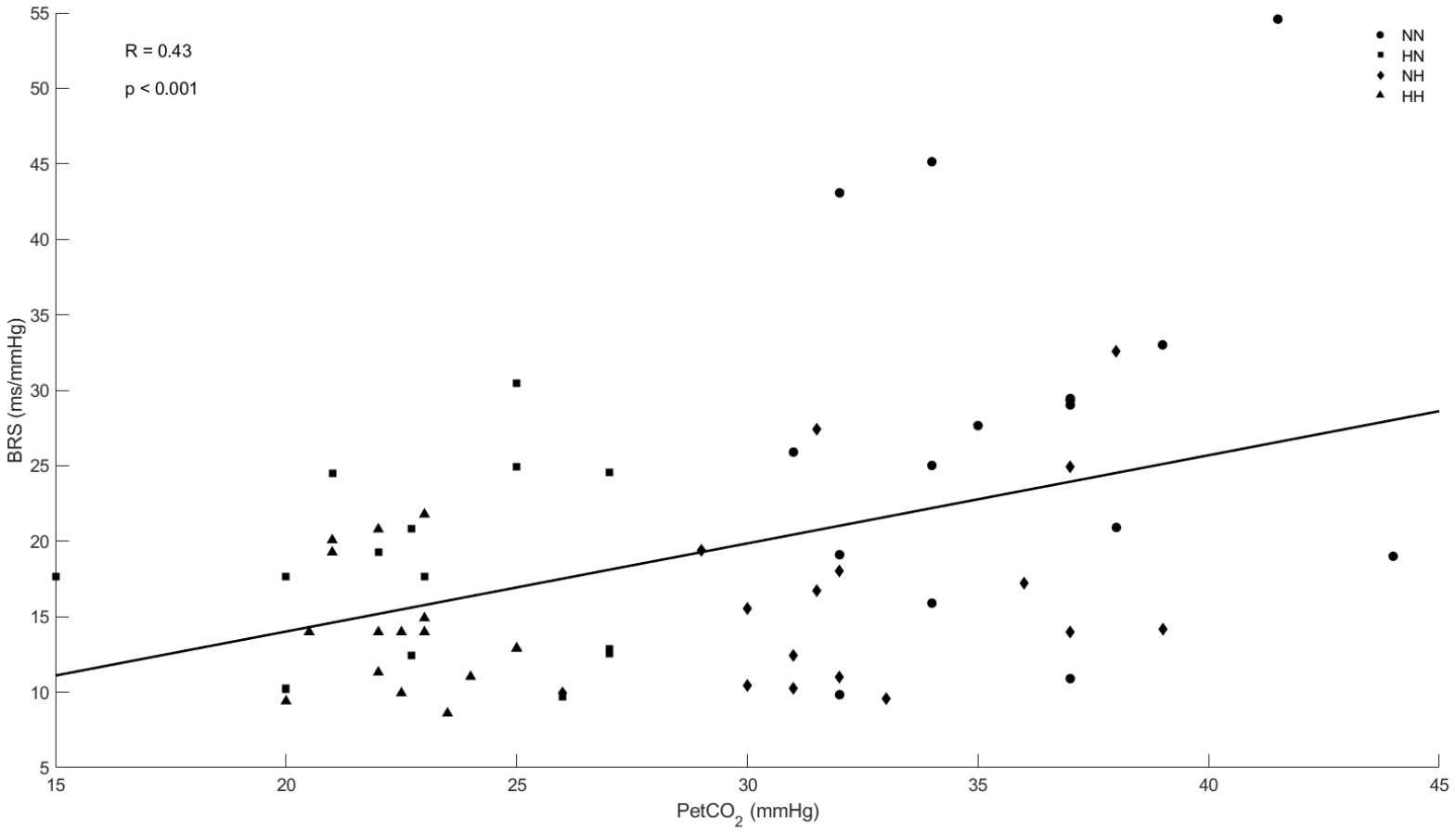

$\dot{V}E(L/min(btps))$
$VT(L(btps))$
$Rf(bpm)$
$PetO_2(mmHg)$
$PetCO_2(mmHg)$
NN
HN
NH
HH
REST
EXERCISE
RECOVERY

Figure 3


R = 0.43
p < 0.001
NN
HN
NH
HH
BRS (ms/mmHg)
PetCO2 (mmHg)
55
50
45
40
35
30
25
20
15
10
5
15
20
25
30
35
40
45

DISCLOSURE

NB, MRA, BK, DB and GPM have no conflicts of interest, sources of funding, or financial ties to disclose and no current or past relationship with companies or manufacturers who could benefit from the results of the present study. NB is an employee of be.care SA. MRA is an employee of armasuisse. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Article 3 - Specific effect of hypobaria on cerebrovascular hypercapnic responses in hypoxia

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ORIGINAL RESEARCH

Physiological Reports

Specific effect of hypobaria on cerebrovascular hypercapnic responses in hypoxia

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Abstract

It remains unknown whether hypobaria plays a role on cerebrovascular reactivity to CO_2 (CVR). The present study evaluated the putative effect of hypobaria on CVR and its influence on cerebral oxygen delivery (cDO_2) in five randomized conditions (i.e., normobaric normoxia, NN, altitude level of 440 m; hypobaric hypoxia, HH at altitude levels of 3,000 m and 5,500 m; normobaric hypoxia, NH, altitude simulation of 5,500 m; and hypobaric normoxia, HN). CVR was assessed in nine healthy participants (either students in aviation or pilots) during a hypercapnic test (i.e., 5% CO_2). We obtained CVR by plotting middle cerebral artery velocity versus end-tidal CO_2 pressure ($P_{ET}CO_2$) using a sigmoid model. Hypobaria induced an increased slope in HH (0.66 ± 0.33) compared to NH (0.35 ± 0.19) with a trend in HN (0.46 ± 0.12) compared to NN (0.23 ± 0.12, $p = .069$). $P_{ET}CO_2$ was decreased (22.3 ± 2.4 vs. 34.5 ± 2.8 mmHg and 19.9 ± 1.3 vs. 30.8 ± 2.2 mmHg, for HN vs. NN and HH vs. NH, respectively, $p < .05$) in hypobaric conditions when compared to normobaric conditions with comparable inspired oxygen pressure (141 ± 1 vs. 133 ± 3 mmHg and 74 ± 1 vs. 70 ± 2 mmHg, for NN vs. HN and NH vs. HH, respectively) During hypercapnia, cDO_2 was decreased in 5,500 m HH ($p = .046$), but maintained in NH when compared to NN. To conclude, CVR seems more sensitive (i.e., slope increase) in hypobaric than in normobaric conditions. Moreover, hypobaria potentially affected vasodilation reserve (i.e., MCAv autoregulation) and brain oxygen delivery during hypercapnia. These results are relevant for populations (i.e., aviation pilots; high-altitude residents as miners; mountaineers) occasionally exposed to hypobaric normoxia.

KEYWORDS

cerebral blood flow autoregulation, cerebral oxygen delivery, hypobaria, hypoxia

Denis Bron and Grégoire P. Millet have contributed equally to this work.

Clinical Trial Registrations: This clinical trial can be found on ClinicalTrials.gov (ID: NCT03303118).

1 | INTRODUCTION

Cerebral blood flow (CBF) regulation is very sensitive to hypoxia and regulates the cerebral oxygen delivery (cDO_2) maintenance. CBF is regulated by complex vasoactive responses of the middle cerebral artery (MCA) (Imray et al., 2014; Willie, Smith, et al., 2014; Willie, Smith, Tzeng, Fisher, & Ainslie, 2014), extracranial cerebral vessels (Lewis, Messinger, Monteleone, & Ainslie, 2014) and in the pial mater arterioles (Wolff, 1930). There is a complex effect of oxygen arterial pressure (PaO_2) and carbon dioxide arterial pressure ($PaCO_2$) on CBF. More precisely, CBF is lowered by around 3%–4% for each mmHg of $PaCO_2$ decrease (Ainslie & Duffin, 2009; Brugniaux, Hodges, Hanly, & Poulin, 2007; Willie et al., 2012). On the contrary, increases in $PaCO_2$ and in blood pH are major factors increasing CBF via a common pathway, due to their vasoactive effects (Willie, Smith, et al., 2014; Willie, Tzeng, et al., 2014). When exposed to acute hypoxia (from minutes to hours), cerebral vasodilatation (i.e., increase in MCA diameter) occurs to limit the cDO_2 decrease (Imray et al., 2014; Mikhail Kellawan, Harrell, Roldan-Alzate, Wieben, & Schrage, 2017; Wilson et al., 2011). This regulation leads to an increase in cerebral oxygen delivery by 0.5%–2.5% of SaO_2 decrease (Cohen, Alexander, Smith, Reivich, & Wollman, 1967; Jensen, Sperling, Severinghaus, & Lassen, 1996; Willie et al., 2012). On the other hand, hypoxia-induced hyperventilation and hypocapnia result in a vasoconstrictor stimulus, but vasodilation typically prevails as consistent increase in CBF were observed at altitude, despite hypocapnia (Willie, Smith, et al., 2014; Willie, Tzeng, et al., 2014). There are several studies demonstrating the compensatory rise in CBF upon acute exposure to isocapnic hypoxia to maintain cDO_2 (for review see, Hoiland, Bain, Rieger, Bailey, and Ainslie (2016). cDO_2 in acute hypoxia is thus related to cerebral vasodilation, which compensates the hypocapnic vasoconstriction induced by chemoreflex-driven ventilation (Teppema & Dahan, 2010).

Although still debated, hypobaric hypoxia (HH) may be more severe than normobaric hypoxia (NH) at a given inspired oxygen pressure (Millet, Faiss, & Pialoux, 2012). As an example, HH induces greater hypocapnia and blood alkalosis when compared to NH during acute exposure, which may be the consequence of an increase in ventilatory dead space (Savourey, Launay, Besnard, Guinet, & Travers, 2003). These differences between NH and HH may therefore induce changes in the cerebrovascular regulation.

One of the ways to assess how the cerebral vasculature regulates CBF is through measuring reactivity to CO_2 (CVR) and can be measured by the blood velocity in the middle cerebral artery (Ainslie & Ogoh, 2010). CVR is regulated by hydrogen ion concentration (i.e., pH). At altitude, with changes in acid–base status, the relationship between changes in P_aCO_2 and $[H^+]$ is altered due to altered buffering capacity, which has implications for how P_aCO_2 is transduced into a vasodilatory stimulus (Hoiland, Fisher, & Ainslie, 2019). The magnitude of change in CBF in hypoxia is related to four reflex mechanisms factors when CO_2 is uncontrolled: (I) hypoxic ventilatory response; (II) hypercapnic ventilatory response at rest; (III) hypoxic cerebral vasodilation; and (IV) hypocapnic cerebral vasoconstriction (Brugniaux et al., 2007). CVR in hypoxia is still unclear as controversial results were obtained: CVR in hypoxia was increased during hyperoxic poikilocapnia (Fan et al., 2010) and hyperoxic isocapnia (Subudhi, Panerai, & Roach, 2010); decreased during hyperoxic poikilocapnia or unchanged during hypoxic poikilocapnia (Ainslie & Burgess, 2008) and uncontrolled hypercapnia (Jansen, Krins, & Basnyat, 1999). To our knowledge, no study has investigated the putative effect of hypobaria on CVR when exposed to acute hypoxia (i.e., NH vs. HH).

The present study adds novelty by also evaluating CVR in a hypobaric normoxic (HN) condition. Isolating the hypobaric effect from the hypoxic one would allow comparing similar normoxic conditions with different barometric pressures (P_B). The HN condition is when low P_B is combined with hyperoxic breathing to obtain an inspired pressure of oxygen (P_IO_2) similar to normobaric normoxia (NN). When exposed to hypobaria, the air density is reduced (Conkin, 2016), which may reduce air flow resistance and work of breathing (Loeppky et al., 1997; Ogawa, Fujii, Kurimoto, & Nishiyasu, 2019).This may lead to change in ventilatory pattern (i.e., increased maximal ventilation in HN compared to NN) (Ogawa et al., 2019). Moreover, it was suggested that the ventilatory dead space is increased by hypobaria in hypoxia (Savourey et al., 2003) and normoxia (Ogawa et al., 2019), which could underlie the reported differences in the ventilatory and blood gas parameters. When dead space is greater, $P_{ET}CO_2$-P_aCO_2 gradient may be increased (Donnellan, 2011). Decrease in barometric pressure has been reported to also increase pulmonary vascular vasoconstriction pressure due to the lower air density in hypobaria (Conkin, 2016). More precisely, pulmonary resistance was increased in hypobaria (HN and HH), independent of oxygen tension, suggesting that pulmonary blood flow may be changed in hypobaria (Petrassi et al., 2018). Moreover, different fluid and acid–base balance responses mediated by augmentation of aldosterone and altered cell-membrane permeability have been suggested as a consequence of hypobaria (Loeppky et al., 2005). Nevertheless, the effects of hypobaria on the ventilatory responses and CVR responses using HN conditions are scarcely explored and to our knowledge, there is no study comparing CVR in NN versus HN and NH versus HH conditions.

The implications of CVR in hypobaric normoxia/hypoxia are therefore of interest in the context of both aviation (pilots

and passengers) and high-altitude residents/mountaineers/workers, as these populations may be exposed to hypobaric environment with supplemental oxygen. In the present study, we aimed to evaluate the putative effect of hypobaria during acute exposure between conditions with comparable P_IO_2 (NH vs. HH and NN vs. HN) on CVR. We also aimed to investigate the hypoxic effect on CVR for conditions with same P_B (NN vs. NH and HN vs. HH). We hypothesized that acute hypoxic exposure would induce a left shift and increase in CVR, which would be more exaggerated in hypobaria. This CVR regulation would be effective for maintaining cDO_2 in all conditions.

2 | MATERIALS AND METHODS

2.1 | Ethical approval

This study was performed according to the Declaration of Helsinki and was approved by the Swiss Ethic Committee of Zürich (Swissethics, BASEC ID: 2017–00752). This clinical trial can be found on ClinicalTrials.gov (ID: NCT03303118). All participants were informed about all procedures of this study and gave their written informed consent before participating to this study.

2.2 | Subject recruitment and screening

Nine healthy pilot trainees (seven men and two women, age 28 ± 4 years; height 176 ± 5 cm; weight 70 ± 10 kg) participated voluntarily in this study. None of the participant was exposed to hypoxia before enrolment in the present study and/or to altitude in the days preceding the trials. A physician screened the participants during a familiarization visit to ensure they were healthy and did not report any medical- or altitude-related issues. Moreover, none of the participants was on medication during the present study. After obtaining written informed consent, participants were enrolled and took part to the test visit.

2.3 | Study design

This study was conducted at the Aeromedical Center (AeMC), medical center of the Swiss Air Force, in Dübendorf in Switzerland. Participants came for a test visit and underwent experimental trials at sea level (Dübendorf, 440 m, P_B: 726 ± 5 mmHg) and hypobaric and/or hypoxic conditions. Material was first installed on the subjects, and then participants underwent a pre-test in normobaric normoxia (Pre-). In a randomized order, participants undertook four experimental conditions of 30 min (3,000 m HH; 5,500 m HH; NH to simulate 5,500 m of altitude and 5,500 m HN) in the Swiss army hypobaric chamber interspersed with three periods of 30 min in normoxia for total session duration of 5 hr. Twenty-four hours before all visits, participants were asked to avoid physical exercise, heavy meal, and alcohol or caffeine consumption. Participants remained at rest, seated, during the entire experimental procedures. Each period consisted of (a) 5 min of acclimatization; (b) capillary blood gas sample; (c) 7 min seated at rest with eyes closed for electroencephalography and heart rate variability measurement; (d) 4 min to assess a cognitive test; and (e) hypercapnic modified breathing test. The hypercapnic modified breathing test was performed after 20 min of condition exposure.

2.4 | Conditions comparison

To evaluate putative hypobaric effect between normoxic and hypoxic conditions, P_IO_2 between NN versus HN (141 ± 1 vs. 133 ± 3 mmHg) and NH versus HH (74 ± 1 vs. 70 ± 2 mmHg) were compared by adjusting P_B in the hypobaric chamber or F_IO_2 based on known equation ($P_IO_2 = (P_B\text{-}47) \times F_IO_2$), when 47 mmHg corresponds to water vapor pressure at 37°c (Conkin, 2016). Participants breathed ≈11% and ≈40% O_2 gas mixture (0.03% CO_2) concentration for NH and HN, respectively, while P_B remained similar between NH and NN, but was decreased similarly in HN and HH.

2.5 | Experimental procedure

2.5.1 | Modified hypercapnic breathing

Participants wore a mask and breathed through a two-way Y-valve, which allowed switching from ambient air in the hypobaric chamber to a hermetic bag filled with a hypercapnic gas mixture (20.9% O_2, 5% CO_2). For NH and HN conditions, participants were switched from a first gas mixture (≈11% O_2, 0.03% CO_2 or ≈40% O_2, 0.03% CO_2 respectively) to the hypercapnic gas mixture (respectively ≈11% O_2, 5% CO_2 or ≈40% O_2, 5% CO_2). As a baseline before hypercapnia, participants were asked to hyperventilate for 1 min to lower their end-tidal partial pressure of CO_2 ($P_{ET}CO_2$). This over-breathing period was sufficient to induce the same level of $P_{ET}CO_2$ than with 3 min in a previous study (~18 mmHg at 5,260 m; Fan et al. 2016). Then, subjects breathed normally for 30 s and were switched to the hypercapnic mixed gas for 3 min. Participants were instructed to breathe ad libitum. After completing the hypercapnic breathing test, subjects were finally switched back to the initial gas mixture.

2.5.2 | Pulse oxygen saturation

Earlobe pulse oxygen saturation was monitored using an oximeter (3100 pulse oximeter, Nonin) and acquired at 0.5 Hz.

2.5.3 | Cerebral blood flow velocity

Middle cerebral artery velocity (MCAv, an index of cerebral blood flow) was measured in the left middle cerebral artery using a 2-MHz pulsed Doppler ultrasound system (ST3, Spencer technology). The Doppler ultrasound probe was positioned over the left temporal window and held in place with an adjustable plastic headband (Marc 600 Headframe, Spencer technology). The signal was acquired at depths ranging from 43 to 54 mm. Signal quality was optimized and basal MCAv characteristics were recorded to facilitate subsequent probe placements.

2.5.4 | Respiratory variables

Gas exchanges data were recorded using a gas analyzer (K5, Cosmed) that was calibrated outside of the hypobaric chamber before each session. Flow volume was calibrated with a 3L syringe. After calibrating zero CO_2 with scrubber, reference gas was assessed using a certified Cosmed gas concentration (16% O_2 and 5% CO_2). Ventilatory data were recorded by the analyzer and exported in Cosmed software for later analysis (OMNIA, Cosmed, Roma, Italy).

2.5.5 | Cerebral oxygen delivery

Cerebral oxygen delivery (cDO_2) was calculated based on MCAv and estimated arterial oxygen content (CaO_2) with known equation ($cDO_2 = MCAv_{mean} \times CaO_2$). CaO_2 can be estimated with hemoglobin concentration ([Hb]) and pulse oxygen saturation (SpO_2) values with following equation ($CaO_2 = [Hb] \times 1.36 \times SpO_2/100$). [Hb] was measured with same device as blood gases described above. cDO_2 was estimated in each conditions for three periods: (a) last 30-s baseline, (b) last 30-s hyperventilation, and (c) last 30-s hypercapnic gas breathing.

2.5.6 | Capillary blood gases

Capillary blood samples were taken at rest on distal part of a finger at the end of the acclimatization phase (i.e., 5 min after exposure). After cleaning up with alcohol, finger extremity was pitched using a lancet and blood sample was acquired in a capillary tube. Following standardized calibration, all blood samples were directly analyzed with a capillary blood-gas analyzing system (OPTI CCA-TS, OPTI Medical Systems, Roswell, GA, USA) for capillary blood parameters: Hemoglobin concentration ([Hb]); capillary O_2 saturation (SO_2, %); pH; partial pressure of capillary O_2 (PO_2); and CO_2 (PCO_2).

2.6 | Data analysis

2.6.1 | Cerebrovascular CO_2 reactivity analysis

Individual fit of each sigmoid curve and the associate parameters (i.e., midpoint and slope) were calculated (Figure 1). Representing CVR using a sigmoid model allows the determination of a midpoint, which corresponds to the optimal operating point of vessels capacity to dilate and constrict (i.e., reserve of cerebral vessels) (Fan et al., 2016). Previous studies have fitted CVR using a sigmoid model (Ainslie & Duffin, 2009; Fan et al., 2016). Some physiological parameters may be missed using a linear model: optimal operating point and physical constraints of the cerebral vasculature (i.e., vascular reserve) (Battisti-Charbonney, Fisher, & Duffin, 2011). Moreover, CVR is sigmoidal with a linear portion between $PaCO_2$ of 25–65 mmHg under constant arterial blood pressure (Madden, 1993). For these reasons, a sigmoidal model was used for CVR analysis in the present study. Midpoint is the middle between minimal and maximal values when the range of $P_{ET}CO_2$ is large enough to elicit maximal vasodilatory response. However, the midpoint is also the inflexion point (i.e., where the slope is maximal). It is found where the first derivative is maximal. In this study, the max slope in all cases was detected using the first derivative (independently of the min and max values). If $P_{ET}CO_2$ elicited

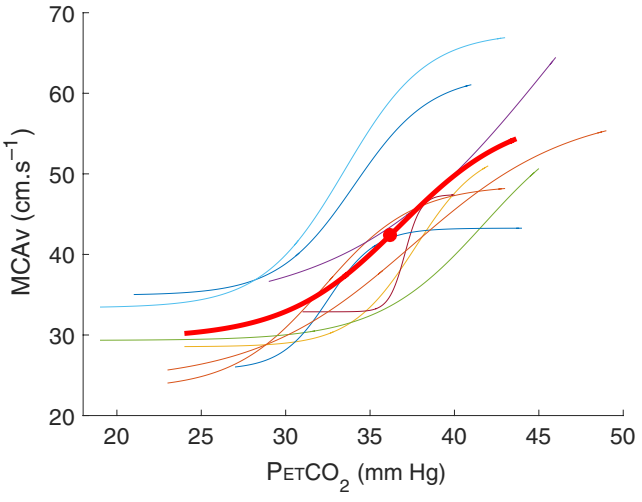

FIGURE 1 A representative example of sigmoidal curves of all subjects ($n = 9$, i.e., in colors) with mean value (bold curve) during hypercapnic test in normobaric normoxia (NN, Dübendorf 440 m). Bold point represents midpoint

the minimal and maximal values of the sigmoid shape, the midpoint would not have changed. The maximum slope of the sigmoid curve is a reasonable assumption for CO_2 sensitivity (Ainslie & Duffin, 2009; Fan et al., 2016). In a sigmoidal curve, the maximum slope is found at the inflexion point, which is also the midpoint. It is found at the maximum of the derivative. As the slope increases, CVR is more sensitive (i.e., greater capacity to constrict and dilate), but in a smaller range of $P_{ET}CO_2$.

2.7 | Statistical analysis

One-way repeated measures ANOVA was assessed for all parameters (SpO_2, MCAv, $P_{ET}O_2$, $P_{ET}CO_2$, and cDO_2 absolute values) to test significance between altitude level (NN, 3,000 m and 5,500 m in HH) and each conditions (NN, 5,500 m HH, NH, and HN) using Jamovi software (Jamovi project (2018, version 0.9). Statistical analysis for sigmoid parameters (midpoint and slope) using mixed model (R, R Foundation for Statistical Computing). Significant difference was set for $p < .05$.

3 | RESULTS

3.1 | Hypoxic effect in hypobaric hypoxia

There was a significant increase in CVR with increased altitude levels (Figure 2) in HH conditions. Data of the sigmoid curves for each condition are represented in Table 1. Midpoint was significantly lowered at 3,000 m (27.3 ± 2.0 mmHg) and 5,500 m (19.6 ± 2.0 mmHg), compared to NN (35.7 ± 3.3 mmHg, $p < .001$). Midpoint was decreased at 5,500 m compared to 3,000 m ($p < .001$). Compared to NN (0.23 ± 0.12), the slope of sigmoid curve was significantly increased at 3,000 m (0.52 ± 0.27, $p = .007$) and 5,500 m (0.66 ± 0.33, $p < .001$) in HH. However, there was no significant change in slope between 3,000 m and 5,500 m HH.

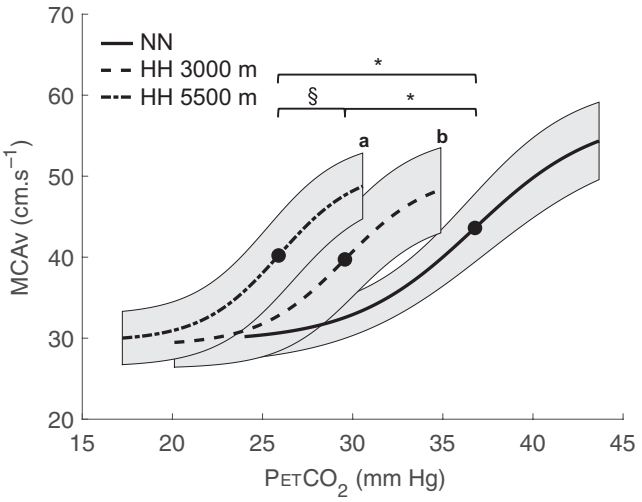

FIGURE 2 Mean sigmoidal curves of all subjects ($n = 9$): In normobaric normoxia (NN, Dübendorf 440 m); 3,000 m and 5,500 m in hypobaric hypoxia (HH) conditions. Bold point represents midpoint. *$p < .05$ midpoint different than NN; §$p < .05$ midpoint different than 3,000 m; (a) $p < .05$ slope different between 5,500 m and NN; (b) $p < .05$ slope different between 3,000 m and NN. Shaded areas surrounding the sigmoid curves represent the 95% confidence interval

SpO_2 and MCAv are represented in Table 2. SpO_2 during baseline was lower in 5,500 m HH than 3,000 m HH and NN ($p < .001$). MCAv during baseline was increased in 5,500 m HH when compared to NN and 3,000 m HH ($p < .001$).

Ventilatory data are presented in Table 3. Minute ventilation resting values were increased in 5,500 m HH (16.0 ± 2.7 L/min) compared to all other conditions. $P_{ET}O_2$ and $P_{ET}CO_2$ were decreased in HH conditions compared to NN, with lower values at 5,500 m when compared to 3,000 m during baseline.

cDO_2 values during CVR assessment (for baseline, hyperventilation, and hypercapnia periods) are displayed in Figure 4. cDO_2 absolute value was similar during baseline period in NN with HH conditions (3,000 m and 5,500 m).

Capillary blood samples data are shown in Table 4. SO_2 gradually decreased at 3,000 m (87.9 ± 1.6%) and 5,500 m (75.0 ± 4.0%) in HH when compared to NN (95.3 ± 1.1%, $p < .001$) after 5 min of condition exposure.

3.2 | Hypobaric effect

There was a decrease in midpoint (left shift) with decreased barometric pressure (Figure 3). Midpoint was significantly lower in 5,500 m HH and HN (21.6 ± 1.9 mmHg), when compared to NN ($p < .001$). Slope was increased in HH compared to normobaric conditions in NH (0.35 ± 0.19, $p = .003$) and NN ($p < .001$). Slope did not change with hypoxia for the same barometric pressure values, when comparing NN versus NH and HH versus HN, respectively. In normoxia, slope in HN tends to be increased when compared to NN ($p = .069$).

During baseline and hypercapnia, SpO_2 was decreased in hypoxic conditions (NH and 5,500 m HH) when compared to normoxic conditions (NN and HN). MCAv elevation between hyperventilation and the end of hypercapnia (i.e., relative delta, %Δ) tended to be lower in 5,500 m HH (+50.9 ± 18.5%) and HN (+58.6 ± 20.6%) than NN (+77.5 ± 9.5%, $p = .065$).

cDO_2 was similar during baseline and decreased to the same extent ($p < .001$) during hyperventilation in all conditions (Figure 4a). Interestingly, cDO_2 during hypercapnia was higher than baseline values only in the normobaric conditions (NN and NH), but not in the hypobaric conditions (HN and HH, Figure 4b). When compared to NN, cDO_2 during

	NN	HH 3000 m	HN	NH	HH 5500 m
Midpoint	35.7 ± 3.3	27.3 ± 2.0*	21.6 ± 1.9*	33.7 ± 1.7#	19.6 ± 2.0*§†
Slope	0.23 ± 0.12	0.52 ± 0.27*	0.46 ± 0.12(*)	0.35 ± 0.19	0.66 ± 0.33*†

TABLE 1 Absolute values are means ± *SD* ($n = 9$). Mean sigmoidal curve data: Midpoint (mmHg) and inclination (slope) of the sigmoid curve

Note: In normobaric normoxia (NN, Dübendorf altitude level of 440 m), hypobaric hypoxia (HH, at altitude level of 3,000 m and 5,500 m), hypobaric normoxia (HN, altitude level of 5,500 m in normoxia), and normobaric hypoxia (NH, altitude simulation of 5,500 m in normobaria). Statistical analysis was performed separately for altitude comparison in HH (NN, 3,000 m and 5,500 m HH) and for conditions comparison (NN, HN, NH, and 5,500 m HH). (*) $p = .069$, *$p < .05$ different from NN conditions; §$p < .05$ different from 3,000 m HH; #$p < .05$ different from HN; and †$p < .05$ different from NH.

TABLE 2 Absolute values are means ± *SD* ($n = 9$)

	Period	NN	HH 3000 m	HN	NH	HH 5500 m
SpO_2 (%)	Baseline	99.3 ± 1.0	93.5 ± 3.7 (*)	98.2 ± 2.0	80.9 ± 5.2*#	78.1 ± 8.7*§#
	Hyperventilation	99.7 ± 0.6	98.6 ± 1.3	99.1 ± 1.5	94.0 ± 4.4*(#)	92.6 ± 5.5*§#
	Hypercapnia	99.6 ± 0.7	96.4 ± 3.4	98.6 ± 2.1	90.3 ± 5.2*#	85.5 ± 5.5*§#
MCAv (cm/s)	Baseline	45.7 ± 7.9	43.8 ± 9.9	47.0 ± 9.2	50.0 ± 8.2	51.6 ± 11.8*§
	Hyperventilation	29.7 ± 4.5	29.9 ± 5.6	31.5 ± 5.5	34.4 ± 7.2	33.1 ± 6.1*(§)
	Hypercapnia	52.5 ± 8.0	47.1 ± 9.1	50.0 ± 11.5	55.4 ± 7.3	49.4 ± 7.7

Note: Pulse oxygen saturation (SpO_2), middle cerebral artery velocity (MCAv), minute ventilation (VE), breathing frequency (BF), tidal volume (VT), end-tidal pressure in carbon dioxide ($P_{ET}CO_2$) and oxygen ($P_{ET}O_2$). For time period: baseline, hyperventilation, and hypercapnia (5% CO_2). In normobaric normoxia (NN, Dübendorf altitude level of 440 m), hypobaric hypoxia (HH, at altitude level of 3,000 m and 5,500 m), hypobaric normoxia (HN, altitude level of 5,500 m in normoxia), and normobaric hypoxia (NH, altitude simulation of 5,500 m in normobaria). Statistical analysis was performed separately for altitude comparison in HH (NN, 3,000 m and 5,500 m HH) and for conditions comparison (NN, HN, NH, and 5,500 m HH). (*) $p = .081$, *$p < .05$ different from NN conditions; (§) $p = .053$, §$p < .05$ different from 3,000 m HH; (#) $p = .069$, #$p < .05$ different from HN. No significant difference between conditions with comparable P_IO_2: NH versus HH and NN versus HN.

TABLE 3 Absolute values are means ± *SD* ($n = 9$)

	Period	NN	HH 3000 m	HN	NH	HH 5500 m
V_E (L/min)	Baseline	12.1 ± 1.4	12.5 ± 1.4	10.3 ± 1.4	12.1 ± 2.7	16.0 ± 2.7*§#†
	Hyperventilation	39.5 ± 7.7	35.0 ± 8.0	35.6 ± 9.2	35.4 ± 6.9	40.4 ± 10.5†
	Hypercapnia	15.2 ± 4.4	13.6 ± 3.0	11.4 ± 2.0	17.8 ± 4.0#	14.1 ± 2.9†
BF (cycle/min)	Baseline	15.9 ± 2.6	16.7 ± 2.8*	17.9 ± 3.0	17.0 ± 3.6	17.9 ± 2.7*
	Hyperventilation	12.7 ± 3.9	12.0 ± 2.9	12.0 ± 2.5	15.0 ± 4.8	11.5 ± 1.5
	Hypercapnia	16.1 ± 2.4	16.4 ± 2.1	17.2 ± 2.8	17.3 ± 2.8	16.0 ± 3.4
VT (L)	Baseline	0.82 ± 0.21	0.79 ± 0.20	0.62 ± 0.21	0.79 ± 0.27	0.98 ± 0.31 (*)§
	Hyperventilation	3.35 ± 0.99	3.04 ± 0.90	3.12 ± 1.02	2.52 ± 1.00*	3.62 ± 0.80§†
	Hypercapnia	1.04 ± 0.23	0.84 ± 0.17*	0.68 ± 0.16*	1.06 ± 0.24#	0.93 ± 0.26 (†)
$P_{ET}O_2$ (mmHg)	Baseline	99.4 ± 8.0	59.7 ± 6.7*	85.3 ± 9.4*	45.0 ± 3.3*#	36.1 ± 4.7*§#
	Hyperventilation	125.8 ± 4.7	81.4 ± 5.4*	102.6 ± 12.7*	58.9 ± 9.1	50.0 ± 7.1*§#
	Hypercapnia	117.2 ± 7.1	70.0 ± 5.8*	86.8 ± 12.9*	58.6 ± 6.2*#	41.4 ± 4.5*§#†
$P_{ET}CO_2$ (mmHg)	Baseline	34.5 ± 2.8	28.5 ± 2.5*	22.3 ± 2.4*	30.8 ± 2.2*#	19.9 ± 1.3*§†
	Hyperventilation	24.0 ± 3.9	20.8 ± 3.0	17.0 ± 3.2*	24.2 ± 4.3#	15.5 ± 2.6*§†
	Hypercapnia	42.0 ± 2.8	31.4 ± 3.3*	25.1 ± 1.7*	40.5 ± 2.1#	22.1 ± 1.7*§#†

Note: Ventilatory parameters: Minute ventilation (VE), breathing frequency (BF), tidal volume (VT), end-tidal pressure in oxygen ($P_{ET}O_2$) and carbon dioxide ($P_{ET}CO_2$). For time period: baseline, hyperventilation, and hypercapnia (5% CO_2). In normobaric normoxia (NN, Dübendorf altitude level of 440 m), hypobaric hypoxia (HH, at altitude level of 3,000 m and 5,500 m), hypobaric normoxia (HN, altitude level of 5,500 m in normoxia), and normobaric hypoxia (NH, altitude simulation of 5,500 m in normobaria). Statistical analysis was performed separately for altitude comparison in HH (NN, 3,000 m and 5,500 m HH) and for conditions comparison (NN, HN, NH, and 5,500 m HH). (*) $p = .061$, *$p < .05$ different from NN conditions; §$p < .05$ different from 3,000 m HH; #$p < .05$ different from HN; and (†) $p = .058$, †$p < .05$ different from NH.

hypercapnia was decreased in 5,500 m HH ($p = .046$) but not in NH. As participants were in normoxia (i.e., breathing hyperoxic gas mixture) in HN condition, cDO_2 was similar during hypercapnia between NN and HN. Our data suggest no significant difference in cDO_2 during hypercapnia between conditions with similar P_IO_2 (i.e., NH vs. HH and NN vs. HN).

TABLE 4 Absolute values are means ± *SD* ($n = 9$). Capillary blood data for hemoglobin concentration ([Hb], g/dl); capillary oxygen saturation (SO_2, %); capillary blood pH; partial pressure of capillary O_2 (PO_2) and CO_2 (PCO_2). In normobaric normoxia (NN, Dübendorf altitude level of 440 m), hypobaric hypoxia (HH, at altitude level of 3,000 m and 5,500 m), hypobaric normoxia (HN, altitude level of 5,500 m in normoxia), and normobaric hypoxia (NH, altitude simulation of 5,500 m in normobaria). Statistical analysis was performed separately for altitude comparison in HH (NN, 3,000 m and 5,500 m HH) and for conditions comparison (NN, HN, NH and 5,500 m HH)

	NN	HH 3000 m	HN	NH	HH 5500 m
[Hb] (g/dl)	16.2 ± 1.9	16.9 ± 2.0	16.4 ± 1.4	16.5 ± 1.9	17.1 ± 1.6
SO_2 (%)	95.3 ± 1.1	87.9 ± 1.6*	92.1 ± 2.4	81.1 ± 4.0*#	75.0 ± 4.0*§#†
PO_2 (mmHg)	77.0 ± 3.9	50.9 ± 2.2*	57.0 ± 4.5*	45.0 ± 4.7*#	34.1 ± 2.5*§#†
PCO_2 (mmHg)	36.2 ± 2.0	29.4 ± 2.8*	30.3 ± 4.2*	35.0 ± 2.7#	24.4 ± 2.2*§#†
pH	7.460 ± 0.015	7.513 ± 0.037*	7.515 ± 0.037*	7.475 ± 0.013#	7.580 ± 0.023*§#†
Hct (%)	48.7 ± 5.8	50.7 ± 6.1	49.1 ± 4.4	49.5 ± 5.8	51.6 ± 5.0

Note: *$p < .05$ different from NN conditions; §$p < .05$ different from 3,000 m HH; #$p < .05$ different from HN; and †$p < .05$ different from NH.

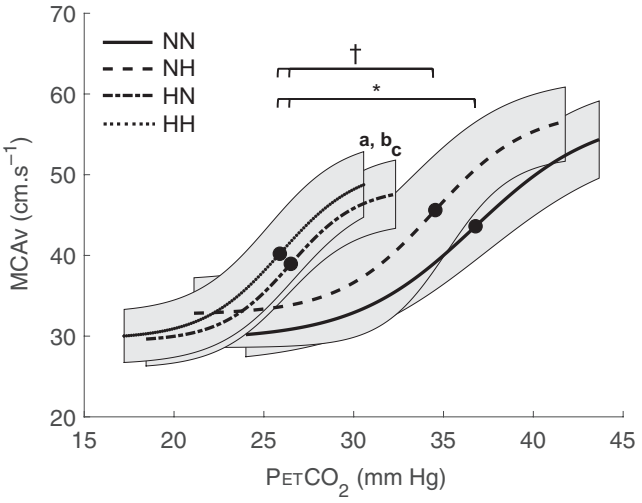

FIGURE 3 Mean sigmoidal curves of all subjects ($n = 9$) in: normobaric normoxia (NN); normobaric hypoxia (NH); hypobaric hypoxia (HH); and hypobaric normoxia (HN) conditions. Bold point represents midpoint. †$p < .05$ midpoint different between HH/HN and NH; *$p < .05$ midpoint different between HH/HN and NN; (a) $p < .05$ slope different between 5,500 m HH and NN; (b) $p < .05$ slope different between 5,500 m HH and NH; (c) $p = .069$ slope tend to be different between HN and NN. Shaded areas surrounding the sigmoid curves represent the 95% confidence interval

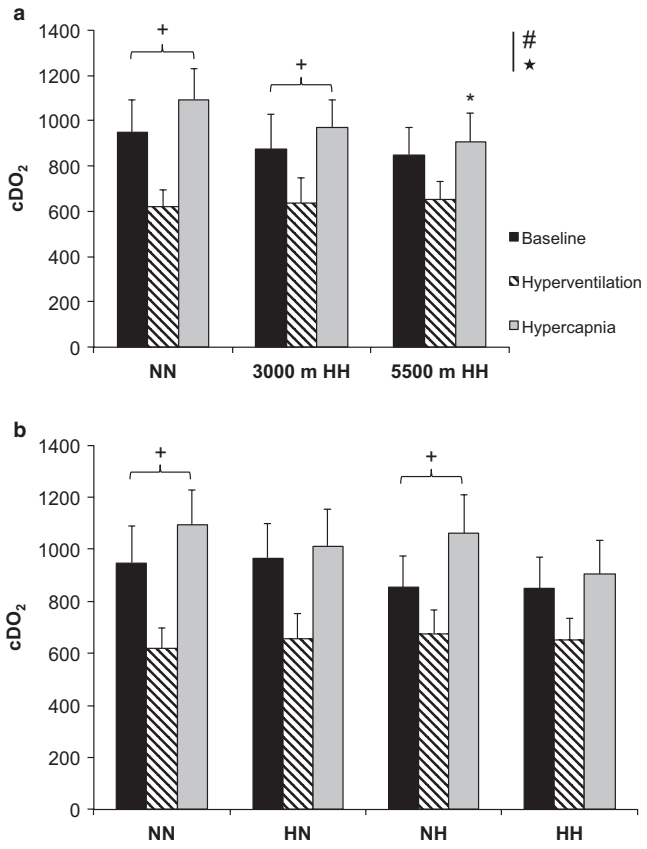

FIGURE 4 Cerebral oxygen delivery (cDO_2, absolute values) of all subjects ($n = 9$), Mean ± *SD*. (a) Normobaric normoxia (NN) and hypobaric hypoxia (HH) conditions at 3,000 m and 5,500 m. (b) NN; normobaric hypoxia (NH); hypobaric hypoxia (HH), and hypobaric normoxia (HN) conditions. Left histograms represent cDO_2 baseline values, middle cDO_2 during hyperventilation, and right cDO_2 at the end of hypercapnia. #$p < .05$ for difference between baseline and hyperventilation values in all conditions; ★$p < .05$ for difference between hyperventilation and hypercapnia values in all conditions; +$p = .014$, +$p < .05$ for difference with baseline values; and *$p = .046$ for difference with NN during hypercapnia

Capillary blood sample showed a lower SO_2 ($p < .001$) in NH (81.1 ± 4.0%) and 5,500 m HH (74.0 ± 4.0%) compared to normoxic conditions (NN and HN: 92.1 ± 2.4%). Moreover, SO_2 was lower in NH than HH ($p = .013$).

4 | DISCUSSION

In the present study, we investigated cerebrovascular changes during CO_2 breathing comparing parameters of sigmoid curve in various normobaric versus hypobaric and normoxic versus hypoxic conditions. We also calculated cDO_2 in all

conditions for three successive periods (baseline, hyperventilation, and hypercapnia) during CVR assessment. The main results are as follows: (a) A left shift in $P_{ET}CO_2$-MCAv sigmoid curve with an increase in CVR with altitude level in HH. The same observation was observed under the influence of hypobaria for a similar P_IO_2 (i.e., significant for HH vs. NH and a trend for HN vs. NN). We observed also an influence of hypobaria per se on CVR, mediated by hypocapnia (i.e., sigmoid midpoint left-shift); (b) No hypoxic effect on CVR for equivalent barometric pressure (NN vs. NH) and (HN vs. HH); and (c) cDO_2 was maintained during baseline in all conditions, but the cerebrovascular reserve was reduced in the hypobaric conditions (HH and HN) compared to the normobaric ones (NN and NH). This resulted in decreased cDO_2 in 5,500 m HH condition during hypercapnia.

4.1 | Increased cerebrovascular reactivity to CO_2 in hypobaria

Under hypoxia, hyperventilation-induced hypocapnia is accelerated by an increase in peripheral respiratory chemoreflex (Ogoh, 2019). Moreover, it has been previously shown that there were greater hypocapnia and blood alkalosis when exposed to HH than NH (Savourey et al., 2003). To our knowledge, there is no study comparing the CVR during acute exposure in HH versus NH. The present results showed a left shift in CVR sigmoid curve in HH, in line with a previous study at high altitude while breathing hyperoxic mixed gas (Fan et al., 2010). Many studies have evaluated the cerebrovascular reactivity to CO_2 in humans exposed to high altitude (Ainslie & Burgess, 2008; Fan et al., 2016, 2010; Flück, Siebenmann, Keiser, Cathomen, & Lundby, 2015; Jansen et al., 1999; Jensen et al., 1996; Lucas et al., 2011; Willie et al., 2015). However, CVR in hypoxia remains unclear with controversial results. For instance, CVR in hypoxia was during hyperoxic poikilocapnia (Fan et al., 2010) and hyperoxic isocapnia (Subudhi et al., 2010); decreased during hyperoxic poikilocapnia (Ainslie & Burgess, 2008) or unchanged during hypoxic poikilocapnia (Ainslie & Burgess, 2008) and uncontrolled hypercapnia (Jansen et al., 1999). Nevertheless, it is known that CBF response to CO_2 is blunted in hypoxia by potentially limiting dilatory responses (Fan, Bourdillon, & Kayser, 2013; Leffler, Busija, Beasley, Fletcher, & Green, 1986; McPherson, Eimerl, & Traystman, 1987). In the present study, the slope of the sigmoid curve was significantly increased in 5,500 m HH compared to NH, suggesting a specific effect of hypobaria on CBF response to CO_2 when exposed to hypoxia. Reduced reactivity results in less central CO_2 washout and greater ventilatory stimulus (Peebles et al., 2007). In fact, minute ventilation was greater in NH than HH during hypercapnia, whereas reactivity was increased in HH in the present study. However, due to its interaction with hypoxia, cerebrovascular responses to CO_2 in such environment should be interpreted with caution. In hypoxia, CVR may not reflect true vasoreactivity (Fan et al., 2016), as CVR may be affected by the hypoxia-induced vasodilation (Gupta, Menon, Czosnyka, Smielewski, & Jones, 1997).

In the present study, capillary blood samples showed a lower SO_2 in 5,500 m HH than NH after 5 min of condition exposure, which is in line with the larger hypoxemia observed in HH than NH (Savourey et al., 2003). However, these values should be evaluated with cautious, as capillary blood sample was not measured during the peak ventilatory response that occurs during the first 2 min of poikilocapnic hypoxia exposure (Steinback & Poulin, 2007). Interestingly, SO_2 (at 5 min) showed a difference between NH and HH of ~ 6% while SpO_2 difference (measured at 20 min during CVR) was 2%–3% during baseline of the hypercapnic test (Table 2). Collectively, these results support that hypoxemia may influence CVR. However, because of temporal dissociation between measurements, blood gas values (shown in Table 4) were not use to discuss CVR differences. MCAv resting values during baseline were only significantly increased in 5,500 m HH when compared to NN and 3,000 m HH (Table 2). MCAv was logically decreased during hyperventilation due to the hypocapnia-induced vasoconstriction (Kaur et al., 2018). Then, hypercapnia triggers cerebral vasodilation, which induces an increase in MCAv. MCAv increases during hypercapnia to wash out CO_2 from the brain tissue to regulate and maintain cerebrospinal fluid pH (Xie et al., 2006). In hypobaric conditions (i.e., HH and HN), CVR showed a left shift of the midpoint, indicating a resetting to a lower $P_{ET}CO_2$ values (i.e., hypocapnia). On the contrary, NH induced smaller left shift compared to HH, likely due to a lesser hyperventilation. Consequently, our results indicate an effect of hypobaria per se on CBF, as we observed significant differences between NH versus HH regarding midpoint and the slope of the sigmoid curve.

The analysis of CO_2 sensitivity is based on the subjects' exposure to a range of arterial CO_2 going from hypocapnia to hypercapnia. In the present study, hypocapnia resulted from voluntary hyperventilation of the subjects (as instructed by the experimenters in the present study and in a previous study (Fan et al., 2016)). Then, the subjects breathed normally so that capnia went back to initial value, at which point the subjects were exposed to 5% CO_2 to create the hypercapnic exposure. With such methods, we had $P_{ET}CO_2$ values ranging from 15.5 ± 2.6 to 42.0 ± 2.8 mmHg (i.e., from hyperventilation to hypercapnia in 5,500 m HH and NN conditions, respectively) which is comparable to rebreathing methods although with slightly less progressive changes in the hypercapnic part (Ainslie & Duffin, 2009; Fan et al., 2016). However, the sigmoid behavior of the CO_2 response could clearly be seen, as expected and the fits were of good quality (Figure 1).

We also evaluated CVR in acute HN environment to determine the putative influence of hypobaria on CVR in normoxia. Some early studies have explored the effects of hypobaric

normoxic (HN), when exposed to chronic high altitude while breathing pure enriched oxygen gas mixture (Cerretelli, 1976; Marconi et al., 2004). These studies reported higher $\dot{V}O_{2max}$ value in HN than in NN and postulated that it might arise from a lower air density. Similarly, $\dot{V}E_{max}$ was reported to be higher and the time to exhaustion during incremental running on treadmill to be extended under HN compared to NN, showing an enhanced exercise performance in HN, when air density is reduced (Ogawa et al., 2019). In the present study, cerebrovascular reactivity to CO_2 was assessed under hypobaric conditions (HH and HN). Our results showed a left shift of midpoint in HH and HN compared to NN, indicating a specific hypobaric effect on CVR. The influence of barometric pressure on respiratory pattern (lower tidal volume and higher breathing frequency) was observed in hypoxia (HH vs. NH) (Savourey et al., 2003). In hypobaria, the putative increased physiological dead space and altered alveolo-capillary diffusion in HH compared to NH (Millet et al., 2012). The present results of $\dot{V}E$ (10.3 vs. 12.1 L/min in HN vs. NN) are in line with previous values in HN versus NN at rest (11.5 vs. 15.6 L/min) (Petrassi et al., 2018). The lower $P_{ET}CO_2$ in HN versus NN was observed in the three phases (baseline, hyperventilation, and hypercapnia) without any hyperventilation. First, one cannot rule out that the inhalation of hyperoxic gas mixture (F_IO_2 ~40%) needed in HN for normalizing P_IO_2 may have a direct (yet unclear) effect on ventilation and $P_{ET}CO_2$. Second, the increased dead space in hypobaria has an influence on $P_{ET}CO_2$-$PaCO_2$ gradient. When dead space is greater, $P_{ET}CO_2$-$PaCO_2$ gradient may be increased (Donnellan, 2011). The present data of the decoupling between $P_{ET}CO_2$ and $\dot{V}E$ between HN and NN (i.e., decreased $P_{ET}CO_2$ without increased $\dot{V}E$ in the present study at rest) was already observed (Ogawa et al., 2019) at maximal intensity (i.e., increased $\dot{V}E$ without decreased $P_{ET}CO_2$). This last observation suggests a complex interaction between hypobaria and hypoxia on ventilatory responses. The mechanisms remain unclear and deserve further investigation on these specific ventilatory responses (the present study focusing more on CVR).

4.2 | Relation between cerebrovascular reactivity and hypocapnia

A recent review on cerebrovascular reactivity discussed the importance of change in $PaCO_2$ as a mediator of cerebral microvascular hemodynamic function (Ogoh, 2019). It is mentioned that there is a decrease or increase in MCAv induced by cerebral constriction or dilation, when $PaCO_2$ is low or high (i.e., hypo- or hypercapnia, respectively) (Markwalder, Grolimund, Seiler, Roth, & Aaslid, 1984). In addition, it was shown that cerebral autoregulation also is enhanced or attenuated by hypocapnia or hypercapnia, respectively (Aaslid, Lindegaard, Sorteberg, & Nornes, 1989). More specifically, full restoration of blood flow to the pretest level was seen in hypocapnia (i.e., after 4.1 s), while the response was slower in normo- and hypercapnia (Aaslid et al., 1989). Thus, it is likely that changes in $PaCO_2$ may influence the myogenic tone of cerebral vasculature and affect the dynamic of cerebral autoregulation (Ogoh, 2019). However, it appeared that there is a close relationship between extracellular pH and the contractile response of cerebral arteries and arterioles, independently of PCO_2 (Kontos, Raper, & Patterson, 1977; Toda, Hatano, & Mori, 1989). In the present study, CVR was increased in HH conditions (i.e., greater sigmoid slope), when hypocapnia and increased minute ventilation was observed. During acute hypoxic exposure, respiratory alkalosis is observed as a $[HCO_3^-]$ reduction in cerebrospinal fluid, leading to a greater elevation in $[H^+]$ for a given increase in PCO_2 (Siesjö, 1972). Moreover, the sigmoid slope remained increased in acute high-altitude exposure when plotting MCAv against $[H^+]$ (Fan et al., 2016), suggesting that cerebrovascular reactivity to CO_2 was likely mediated by an increase in $[H^+]$ sensitivity (Fan et al., 2016). As $[H^+]$ was not measured during hypercapnic procedure in the present study, we have plotted the sigmoid slope against $P_{ET}CO_2$ only.

One may speculate that the increased CVR in acute hypobaric conditions (i.e., HH and HN) may be mediated by the respiratory alkalosis-induced hypocapnia. On the contrary, minute ventilation remained unchanged with similar CVR and no significant left shift in midpoint (i.e., no hypocapnia) in NH condition compared to NN. Therefore, our results indicate a hypobaric effect on cerebrovascular reactivity to CO_2 more pronounced between hypoxic than normoxic conditions (i.e., NH vs. HH and NN vs. HN, respectively).

4.3 | Alteration in cerebrovascular reserve affects the cerebral oxygen delivery in hypobaria

It has been previously shown that cerebrovascular reserve was impaired at high altitude when midpoint was reset to a lower resting arterial PCO_2 (Fan et al., 2016). Midpoint corresponds to the optimization point of a sigmoid curve between maximal vasoconstriction and vasodilation (Battisti-Charbonney et al., 2011). Previous study showed lowered resting arterial PCO_2 by around 12 mmHg on acute exposure to 5,260 m (Subudhi et al., 2014). In the present study, we observed an increase in cerebral oxygen delivery during hypercapnia compared to baseline in normobaric conditions (NN and NH) while cDO_2 remained similar to baseline values in hypobaric conditions (HN and HH): This suggests a lower vascular dilation capacity (i.e., lower MCAv increase) in hypobaria and suggests that

the alteration in cerebrovascular reserve due to hypoxia is higher in hypobaric than in normobaric conditions (Figure 4). Interestingly, the MCAv increase between hyperventilation and the end of hypercapnia (relative delta, %Δ) tended to be lower in 5,500 m HH (+50.9 ± 18.5%) and HN (+58.6 ± 20.6%) than in NN (+77.5 ± 9.5%, $p = .065$). This could explain the decreased cDO_2 in 5,500 m HH during hypercapnia. Our data suggest that the vasodilation reserve was diminished in hypobaria (i.e., smaller increase in MCAv from hyperventilation to hypercapnia (%Δ)). Our results indicate a decrement of cerebral blood flow regulation capacity in hypobaric conditions possibly impacting cDO_2. Our findings support a previous study that showed blunted vessel's ability to respond to change in CO_2 concomitant to hyperventilation-induced hypocapnia at high altitude (Fan et al., 2016). Such blunting effect could possibly impair cerebral autoregulation during acute or chronic high-altitude exposure, as previously demonstrated (Ainslie & Burgess, 2008; Iwasaki et al., 2011; Jansen et al., 1999; Subudhi et al., 2014). We suggest that vascular reserve to dilate may be blunted in hypobaria (HH vs. NH and HN vs. NN, Figure 3), either in hypoxic or normoxic conditions, since midpoint was left shifted. This is of interest since absolute values in cerebral oxygen delivery were similar during baseline and hyperventilation between all conditions. The fact that cDO_2 was increased during hypercapnia only in normobaric conditions (i.e., NN and NH) when compared to baseline values suggest that this hypobaric effect on cDO_2 regulation occurs only with hypercapnia. The reliability of the cDO_2 data is based primarily on three assumptions: (1) MCA diameter is not changing during hypocapnic and hypercapnic states, (2) MCAv represents global CBF, that is, anterior and posterior circulation can be equally represented by just the MCAv; and (3) capillary blood samples provide an accurate index [Hb]. Assumptions 2 and 3 might hold true but assumption 1 likely does not. The present study was designed to discriminate the effects of hypobaria on cerebrovascular reactivity to CO_2; however, some methodological considerations should be acknowledged when interpreting our findings. Transcranial Doppler ultrasound (TCD) was used to measure MCAv as an index of global CBF changes. This assumed that the MCA carries approximately 80% of the cerebral blood flow to the two hemispheres (Lindegaard et al., 1987); and that the changes in MCAv reflect changes in global CBF (Bishop, Powell, Rutt, & Browse, 1986; Serrador, Picot, Rutt, Shoemaker, & Bondar, 2000).

In addition, on the one hand, the changes in MCAv in response to CO_2 changes are comparable to the changes in internal carotid blood flow (Sato et al., 2012); and on the other hand, the diameter of the MCA does not change during the observed changes in arterial blood gases (Serrador et al., 2000) or with even stronger stimuli (Fan et al., 2014). In support, MCAv has been shown to reflect changes in CBF assessed with the direct Fick method, at least during initial exposure to high altitude (Milledge, 1979; Møller et al., 2002; Roy et al., 1968). Previous study reported that the MCA diameter remains relatively unchanged up to 5,300 m (Wilson et al., 2011). However, we have not measured the MCA diameter, and it may change (Coverdale, Gati, Opalevych, Perrotta, & Shoemaker, 2014) in the sense that MCAv may overestimate CBF in the hypocapnic and underestimate it in the hypercapnic states. Therefore, calculating CDO_2 from MCAv during those states may result in smaller differences than those occurring. Hence, potentially explaining why there was no difference in cDO_2 between conditions (Figure 4b).

Despite alteration in cerebrovascular reserve in HN, cDO_2 during hypercapnia in HN was not significantly different than in NN. When compared to NN, relative cDO_2 during hypercapnia was similar in NH but diminished in 5,500 m HH, suggesting a greater influence with hypobaria in hypoxia.

4.4 | Application in aviation physiology

In the present study, we aimed to be as specific as possible to flight conditions for pilots (i.e., to investigate cerebral responses to CO_2 as pilots breathing hyperoxic gases at high altitude). Pilots are daily exposed to hypobaric environment during flights either in normoxia (HN) or hypoxia (HH), in case of cabin decompression (Muehlemann, Holper, Wenzel, Wittkowski, & Wolf, 2013) or unpressurized cabins (Nishi, 2011). In addition, military crew may be exposed to hypobaric hypoxic environment during flights, but perform training in flight simulator (i.e., in NH condition). It is thus paramount to investigate how cerebral functions may be altered during acute exposure to various environments, such as NH, HH, and HN conditions.

4.5 | Limitations

A fixed inspired concentration of CO_2 was used in the present hypercapnic test, which does not translate to precise control of the actual vasoactive stimulus (i.e., the arterial partial pressure of CO_2) (Fisher, 2016). Moreover, when breathing a fixed fraction of CO_2, the gradient between $P_{ET}CO_2$ (which is measured) and $PaCO_2$ (the hemodynamic response determinant) changes, meaning that the representativeness of $P_{ET}CO_2$ for the stimulus at the arterial level are likely variable (Fisher, 2016). Control of alveolar ventilation through sequential gas delivery should be used in future studies (Fisher, Iscoe, & Duffin, 2016).

Of minor concern is that P_IO_2 was not perfectly matched between NN and HN (141 ± 1 vs. 133 ± 3 mmHg), as well as

between NH and HH (74 ± 1 vs. 70 ± 2 mmHg) conditions. However, these conditions can still be compared to each other. Based on equation [$P_IO_2 = F_IO_2*(P_B-47)$] (Conkin, 2016), a difference of 3–4 mmHg in P_IO_2 corresponds to approximately 15–20 mmHg of barometric pressure (i.e., 300–400 m of simulated altitude) if inspired oxygen pressure remains stable. During each trial session, barometric pressure in the hypobaric chamber was stabilized (fluctuation of 100–200 m). Meteorology records (by www.meteoSwiss.ch) confirmed a variation of 800 m of simulated altitude (between 5,100 m and 5,900 m) for a barometric pressure of 375 mmHg measured at the same location over a year period. Consequently, the difference of 3–5 mmHg of P_IO_2 between our experimental conditions in the hypobaric chamber is negligible and much lower than the natural meteorological variability.

5 | CONCLUSION

The present study was the first one to compare cerebrovascular CO_2 reactivity during acute exposure in various normobaric/hypobaric and normoxic/hypoxic conditions. The left shift in hypobaric versus normobaric conditions for a similar P_IO_2 (i.e., significant in hypoxia for HH vs. NH and a trend in normoxia for HN vs. NN) demonstrates a specific effect of hypobaria on CVR. In hypobaric conditions, CVR showed a left shift of the midpoint, indicating a resetting to a lower $P_{ET}CO_2$ values. On the contrary, NH induced smaller left shift compared to HH, likely due to a lesser hyperventilation and possibly unaffected $P_{ET}CO_2$-$PaCO_2$ gradient due to normobaric environment. Our results suggest that vascular reserve to dilate may be blunted in hypobaria (i.e., HH vs. NH and HN vs. NN), either in hypoxic or normoxic conditions, since midpoint was left shifted. This blunt effect in hypobaria could impair cerebral oxygen delivery.

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CONFLICT OF INTEREST

The authors declare no conflict of interest and have no financial relationship to disclose.

AUTHORS' CONTRIBUTIONS

MRA, NB, AK, DB, and GPM were part of the conception, protocol design. MRA conducted the experiments, was responsible for data acquisition, and wrote the manuscript. MRA, NB, and GPM interpreted the data. MRA and NB conducted the analysis. NB and GPM revised critically the manuscript and gave advises for corrections to MRA. MRA, NB, AK, DB, and GPM gave their final approval of this version to be published.

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Article 4: - Electroencephalography beta power increase without change in microstates during acute hypobaric hypoxia exposures

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Electroencephalography beta power increase without change in microstates during acute hypobaric hypoxia exposures

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Running title: EEG changes in hypobaric hypoxia

Abstract

Introduction: The electrical activity of the brain is sensitive to its oxygen furniture. The present study aimed to evaluate the effect of hypoxia on brain electrical activity through electroencephalography (EEG) measurements and microstates, which correspond to the synchronized activation of different neuronal configurations.

Methods: EEG was recorded in twelve healthy pilot trainees (10 men and 2 women, age 26±4 years; height 175±8 cm; weight 68±8 kg) in normobaric normoxia (NN) and two altitude levels in hypobaric hypoxia (HH, P_B: 523.8±6.9 and 381.7±9.2 mmHg for 3000 m and 5500 m respectively). Continuous EEG was using a 21-channels EEG cap that was connected to a portable EEG system (Trackit, Lifelines, USA). Datasets collected in each condition were then used to estimate the optimal set of topographies (i.e., maps illustrating the microstates) that best explain the input EEG signal. Some physiological parameters, such as heart rate (HR) and pulse oxygen saturation (SpO_2) were also recorded. It was hypothesized that lower SpO_2 would lead to an alteration of the EEG signal in acute HH.

Results: At 3000 m, no significant change in power values was observed after Bonferonni correction. Beta synchronization (amplitude increase) was greater in acute HH at 5500 m. This strong beta power increase was observed when SpO_2 was low (≈73 %). Results regarding the microstates revealed no significant difference between NN and the two altitudes in HH. There was no correlation between physiological parameters and EEG activity.

Discussion: The present results imply a minimal impact of hypoxia at 3000 m, but induced a "freezing" state of the overall brain activity at 5500 m in acute hypobaric hypoxia, which could be associated to mental fatigue. The present study adds new insights regarding how the cerebral activity is modulated in acute hypoxic environments. The absence of correlation between physiological responses and EEG changes underlines the complexity of the neuronal activity regulation in hypoxic environment.

Introduction

When the brain is exposed to hypoxia, one adaptive response is an increased cerebral blood flow (CBF) in order to limit oxygen deprivation (Wilson et al., 2011) and potential irreversible functional and cellular damages in case of a prolonged misbalance between O_2 supply and energy needs (Krnjević, 1999). Hypoxia is observable in three different circumstances; when the oxygen supply to the blood is insufficient in case of hypoxic exposure, anaemia or ischemia (Plum and Posner, 1982). Since neuroimaging studies have shown associations between the brain metabolic response and its oscillatory activity (Bazanova and Vernon, 2014), investigating the cerebral adaptations to acute oxygen deprivation by means of multi-channels electroencephalography (EEG) recordings comes at the advantage of its temporal resolution and the possibility to run various spatiotemporal analyses at the scalp level (e.g. frequency domain, topographic variations for example).

It is also known that the electrical activity of the brain is sensitive to its oxygen supply, time exposure and altitude level (Ozaki et al., 1995; Goodall et al., 2014; Zhao et al., 2016). The very first studies on this topic have shown alterations of EEG signal during sessions of acute cerebral hypoxia (Berger, 1931; Gibbs et al., 1935; Walter, 1969).

More recent studies have investigated the influence of acute hypoxic exposure on brain's electrical activity, when exposed to normobaric hypoxia (NH) (Rebuck et al., 1976; Schellart and Reits, 2001; Burykh, 2005; Rice et al., 2019a, 2019b) or hypobaric hypoxia (HH) (Kraaier et al., 1988; Ozaki et al., 1995; Papadelis et al., 2007). In the former condition (NH), frontal and temporal EEGs' increased in delta- and theta-range, whereas average level of the phase shift decreased in beta-range when using 8% oxygen content gas mixture (Burykh, 2005). All frequency bands (alpha, beta, gamma, and theta) showed a power decrease for all channels in acute NH (25000 ft, 7620 m) (Rice et al., 2019a). Alpha activity deviated strongly

in NH with eyes closed, to greater extent during first 20 minutes of exposure (Schellart and Reits, 2001).

Researches conducted to investigate HH condition revealed non-similar, but also inconsistent, patterns of results. For example, HH induced a decrease in alpha activity and a non-significant decrease in beta activity (Kraaier et al., 1988). Ozaki and colleagues showed that first stages of HH exposure (from 3000m to 4000m) was not characterized by significant modulations of alpha activity, whereas further elevation above 5000 m led to an increase of alpha power and a significant enhancement in theta activity (Ozaki et al., 1995). This pattern of results has been described as EEG slowing (Ernsting, 1963; Kraaier et al., 1988; Ozaki et al., 1995) (Goodall et al., 2014) that have been related to a reduced neuronal activity (Papadelis et al., 2007). Interestingly, EEG slowing occurred when pulse oxygen saturation (SpO_2) decreased below 75% (Goodall et al., 2014). On the contrary, no EEG change was observed when SpO_2 remained above 75% (Rebuck et al., 1976). Accordingly, it has been stated that the central nervous system is functionally impaired from an altitude level above 4500 m (Luks et al., 2021) where the hypoxia is expected to induce deleterious effect on the excitatory and inhibitory mechanisms involved in synaptic transmission (Krnjević, 1999). However, the meaning of oscillation changes according to the different frequency bands and the altitude level is far from being elucidated. Therefore, one aim of the study was to evaluate the effects of altitude severity (below and above 4500 m where) on EEG power changes during awake restful state in simulated acute hypobaric hypoxia condition. To reduce the risk of type I error (increase the false positive), we have deliberately chosen conservative statistics methods (randomization tests on power maps and Bonferroni corrections) without focusing on any predetermined regions of interest or specific electrodes.

The spontaneous electrical brain activity can also be described as a set of scalp topographies (i.e. “Microstates”) that correspond to periodically recurring patterns in the spatial distribution of the electric field. Theses microstates correspond to synchronised activation of different neuronal configurations (Brunet et al. 2010) and reflect the functional states of neurocognitive networks (Michel and Koenig, 2018). More generally, EEG microstates could represent the “electrophysiological correlate of a process of global, ‘conscious’ integration at the brain scale level” (Michel and Koenig, 2018). Studies have shown that four to seven dominant microstates explain more than 75 % of the EEG signal, each remaining stable about 100 ms and abruptly switch to another stable configuration (Koenig et al., 2002; Lehmann and Michel, 2011). Interestingly, even if the majority of the studies use 64 channels and more, Khanna and colleagues have shown that the EEG microstates features showed high test-retest reliability with 19 and 8 electrodes (Khanna et al., 2014). Each map has been spatially correlated with a specific distributed brain network (Britz et al., 2010). Thus, map A has been associated with the visual resting state network (RSN), map B with the auditory RSN, map C with the salience RSN, and map D with the attentional RSN (Britz et al., 2010). Microstates have been proven to convey information about sleep stages and vigilance state (Brodbeck et al., 2012; Bréchet et al., 2020), different attentional and cognitive processes (Milz et al., 2016; Seitzman et al., 2017) and the effect of acute physical exercise (Spring et al., 2017, 2018). For instance, Spring and collaborators (2017) found an increase of mean duration and time coverage of map C following a submaximal 30 min cycling exercise. The authors postulated that alteration of this specific microstate might be related to an afferent signaling pathway via projections to the salience RSN (Spring et al 2017). In the study of Seitzman and collaborators (2017), several features of the microstate D were modulated in a serial subtraction task as compared to the resting condition which was associated with the greater participation of the dorsal attention system.

Given that hypoxia (and more globally altitude) has an impact on arousal and cognitive performance, one might wonder if brain response to hypobaric hypoxia condition at rest can be captured by EEG microstates analyses. Therefore, the present study also aims to explore the effect of acute hypobaric hypoxic exposures on the electrocortical brain dynamic by means of EEG microstates analyses, which has not been already done yet, to our knowledge. In addition to the analyses of the EEG signal, we investigated changes in physiological measurements, such as heart rate, pulse oxygen saturation, cerebral blood flow velocity and oxygenation. Then, we evaluated perceived sleepiness state of young healthy participants exposed to acute hypobaric hypoxia. We hypothesized that a lower SpO_2 would lead to a slowing of the EEG signal in hypobaric hypoxia. Secondly, the increase of altitude level would affect EEG signal to a greater extend and potentially alter microstates features.

Methods

Ethical Approval

This study was performed according to the Declaration of Helsinki and was approved by the Swiss Ethic Committee of Zürich (Swissethics, BASEC ID: 2017-00752). This clinical trial can be found on ClinicalTrials.gov (ID: NCT03303118). All participants were informed about all procedures of this study and gave their written informed consent before participating to this study.

Subject Recruitment and Screening

Twelve healthy pilot trainees (10 men and 2 women, age 26±4 years; height 175±8 cm; weight 68±8 kg) participated voluntarily in this study. None of the participant had experienced hypoxic exposure before enrolment in the present study and/or altitude exposure in the days before the test visits. A physician screened the participants during a familiarization

visit to ensure that they were healthy and did not report any medical or altitude related issues. Moreover, none of the participants was on medication during the present study. After obtaining written informed consent, participants were enrolled and took part to the test visit.

Study design

This study was conducted at the Aeromedical Center (AeMC), medical center of the Swiss Air Force, in Dübendorf in Switzerland. Participants came for a test visit and underwent experimental trials at sea level in normobaric normoxia (NN, Dübendorf altitude level of 440 m, P_B: 725.7±5.8 mmHg) and hypobaric hypoxia (HH, P_B: 523.8±6.9 and 381.7±9.2 mmHg for 3000 m and 5500 m respectively). Equipment was first installed on each participant before starting a pre-test in NN. Then, participants were exposed to the two altitude levels in a randomized order using the hypobaric chamber of the Swiss Air Force. Each altitude exposure was interspersed with a 30-min period in NN. Twenty-four hours before the test visit, participants were asked to avoid physical exercise, heavy meal and alcohol or caffeine consumption. Participants remained seated at rest during the entire experimental procedures, which consisted of: (1) Five min of altitude acclimatization; (2) Capillary blood gas sample; (3) Seven min seated at rest with eyes closed for electroencephalography recordings; (4) A 4-min cognitive test (Mathias R. Aebi et al., 2020); and (5) Finally, the participants assessed a hypercapnic modified breathing test to evaluate their cerebrovascular reactivity to CO_2 (Aebi et al., 2020a).

Electroencephalography

Continuous EEG was recorded at a sampling rate of 200 Hz with a 21-channels EEG cap (Waveguard connect, eemagine, Germany) mounted according to the International 10-20 recommendations. Electrodes were connected to a portable EEG system (Trackit, Lifelines,

USA). The impedance (<8 kΩ) was checked before each EEG data collections. Offline analyses were performed with the Cartool software developed by Denis Brunet (Brunet et al., 2011). Raw signals were band-pass filtered between 1 and 40 Hz (notch filter was set at 50Hz) to exclude unwanted slow wave activities generated by sweating and skin potentials but also to avoid muscular artifacts and non-cortical electrical sources. By using a custom MATLAB code (MathWorks Inc.), an infomax-based Independent Component Analysis (ICA) was applied to remove eye-blinks and cardiac artefacts based on the topography, the waveform, and the time course of the ICA component (Jung et al. 2000). Bad electrodes (1 to 3 electrodes; not included in the ICA) were interpolated using a 3-D spherical spline and the EEG signal at each electrode was recomputed to the common average reference. Finally, for each participant and each altitude level, we used four minutes of EEG datasets for the subsequent analyses of resting states.

Power Analysis

The pre-processed datasets were segmented into epochs of 10 seconds and submitted to a Fast Fourier Transform analysis (Hanning window with a 25% overlap; frequency resolution = 0.10 Hz). The absolute power (μV^2) was computed at each electrode and for the delta (1-3 Hz), theta (4-7 Hz), alpha (8-12 Hz) and beta (14-25 Hz) frequency bands. Power maps in each frequency band were generated for each participant and each condition.

Microstates Analysis

The microstate analysis followed a conventional procedure applied in previous studies (Khanna et al., 2014; Tomescu et al., 2014; Spring et al., 2017, 2018). The pre-processed datasets collected in both NN periods, 3000 m HH and 5500 m HH were used to estimate the optimal set of topographies that best explain the input EEG signal. K-means clustering

algorithm was applied to the EEG datasets by using the global field power (GFP) peaks to maximize the signal to noise ratio and focus on periods of stable neuronal synchronization (Pascual-Marqui et al., 1995; Britz et al., 2010; Tomescu et al., 2014; Michel and Koenig, 2018). The polarity of the maps was ignored in this clustering procedure. After applying the clustering method at the individual level and for each condition first, a second step was run using the best individual clusters in each condition to obtain the optimal number of representative microstates at global level. Based on a combination of seven independent optimization criteria (Metacriterion; see supplementary material in (Custo et al., 2017)), CARTOOL© automatically generated the optimal number of topographies for the group, the template used for the back-fitting step. During the back-fitting process, each topography at each time point of the individual pre-processed EEG recording is allocated to one map based on their spatial correlation. Temporal smoothing parameters [window half size = 5, strength (Besag Factor) = 10] ensured that the noise during low GFP did not interrupt the temporal segment of stable topography (Brunet et al., 2011). In addition, segment lower than 5 time-frames (25 ms) were ignored. This back-fitting process allows to compute for each microstate and each participant the mean continuous period of time a given microstate remain stable whenever it appears (mean duration), the average number of times per second a map occurs (frequency of occurrence), the relative percentage of time covered by one map (time coverage), the global explained variance (GEV) for each map. Prior to statistical analysis, absolute delta scores between the preceding control condition, NN, and the conditions of altitude (3000 m HH or 5500 m HH) were computed for each EEG microstate parameter.

Heart rate and pulse oxygen saturation

Heart rate (HR) was monitored during the entire experimental procedure using a heart rate monitor (Polar RS800CX, FI-90440 Kempele, Finland). Pulse oxygen saturation (SpO_2, %)

was measured at the left earlobe using an oximeter (3100 pulse oximeter, Nonin, Plymouth, MN) and acquired at 0.5 Hz. Mean HR and SpO_2 was calculated for the last minute of EEG recording in each condition.

Cerebral blood flow velocity

Cerebral blood flow velocity was measured in the left middle cerebral artery (MCAv) using a 2-MHz pulsed Transcranial Doppler ultrasound system (Spencer technology, Redmond, WA 98052-2559 USA). The Doppler ultrasound probe was fixed to an adjustable headband and positioned over the left temporal window. Data acquired at 1000 Hz (MP150, Biopac Systems inc, Goleta, CA 93117, USA) and transcript to Acknowledge software for data recording. The signal was acquired at depths ranging from 43 to 54 mm as described in a previous study (Fan et al., 2015). Signal quality was optimized and shown on a screen in M-mode to ensure signal quality visualization and similar probe placement during the entire test session.

Cerebral oxygen delivery

Cerebral oxygen delivery (cDO_2) was calculated based on MCAv and estimated arterial oxygen content (CaO_2) with known equation ($cDO_2=MCAv_{mean}\times CaO_2$). CaO_2 can be estimated with haemoglobin concentration ([Hb]) and pulse oxygen saturation (SpO_2) values using the following equation ($CaO_2=[Hb]\times 1.36\times SpO_2/100$). [Hb] was measured with a capillary blood-gas analysing system (OPTI CCA-TS, OPTI Medical Systems, Roswell, GA, USA) after 5 min of exposure at each altitude level. Mean cDO_2 was calculated during the last minute of EEG recording in each condition.

241 **Cerebral oxygenation**

242 Cerebral tissue oxygenation index (TOI, %) was recorded during the entire trial using Near-
243 infrared spectrometry (NIRS) technology (NIRO-200-NX, Hamamatsu Photonics, Japan) and
244 acquired at 500 Hz. Forehead was previously cleaned with disinfectant ethyl for limiting skin
245 impedance. Two detection and emission probes were connected to the NIRS monitor and
246 firmly stuck on the forehead horizontally close to each other, as high as possible on the border
247 of the EEG cap, with double-sided adhesive tape.

248

249 **Sleepiness rating**

250 At each altitude, participants indicated their subjective sleepiness using the 9-point KSS
251 (Akerstedt and Gillberg, 1990) was used: 1=very alert, 3=alert, 5=neither alert nor sleepy,
252 7=sleepy (but not fighting sleep), 9=very sleepy (fighting sleep). A previous study showed
253 high validity in measuring sleepiness with EEG (Kaida et al., 2006). In the present study, a
254 native speaker of the research team translated the original KSS into German. KSS score was
255 collected directly after the EEG measurement in NN and at each altitude level in HH.

256

257 **Statistical Analyses**

258 One-way repeated measures ANOVA were assessed for all parameters (HR, SpO_2, MCAv,
259 cDO_2, TOI and KSS absolute values) to evaluate significance between conditions (NN, 3000
260 m and 5500 m in HH). CARTOOL© was utilized for the statistics with the power maps. to
261 compare the resting conditions, we ran paired-randomisation tests (5000 permutations)
262 without and with correction for multiple comparisons (Bonferroni test). We firstly compared
263 the two NN conditions and the statistics did not reveal any significant differences in any
264 frequency bands. By consequence, we used the power map extracted for the first 30-min
265 period in NN as the baseline to compare with the power maps of the two HH conditions. For

each microstate parameter (delta score), a 2 X 6 repeated measure analyses of variance (ANOVA) was performed with Conditions (3000 m HH, 5500 m HH) and Microstate Map (A, B, C, D, E, F) as within-subject factors. Greenhouse-Geisser correction has been applied if the sphericity assumption was not valid. Tukey post-hoc tests were applied for significant interaction between Condition and Map. Jamovi software was used for the statistical analyses (2018, version 0.9). The significant threshold was set at $p<0.05$.

Results

EEG Power Analysis

In the delta band, a marginal significant effect is observed for only one electrode in the 5500 m HH condition (5% of the electrodes) that is not confirmed after the Bonferroni corrections. In the theta band, the statistics showed a significantly power increase for three electrodes (16% of the electrodes) at 5500 m HH compared to NN. Bonferroni corrections did not confirm the effect again. The statistical analyses did not reveal any significant change in alpha power. In the beta bands, the randomisation tests revealed significant changes from baseline to 3000 m (31% of the electrodes; $p<0.05$) and 5500 m HH (58% of the electrodes) compared to NN when no correction is applied. After Bonferroni corrections, only the 5500 m HH still revealed a significant increase of beta power (31% of the electrodes). The data indicate that the power values in the beta band increase in the highest altitude.

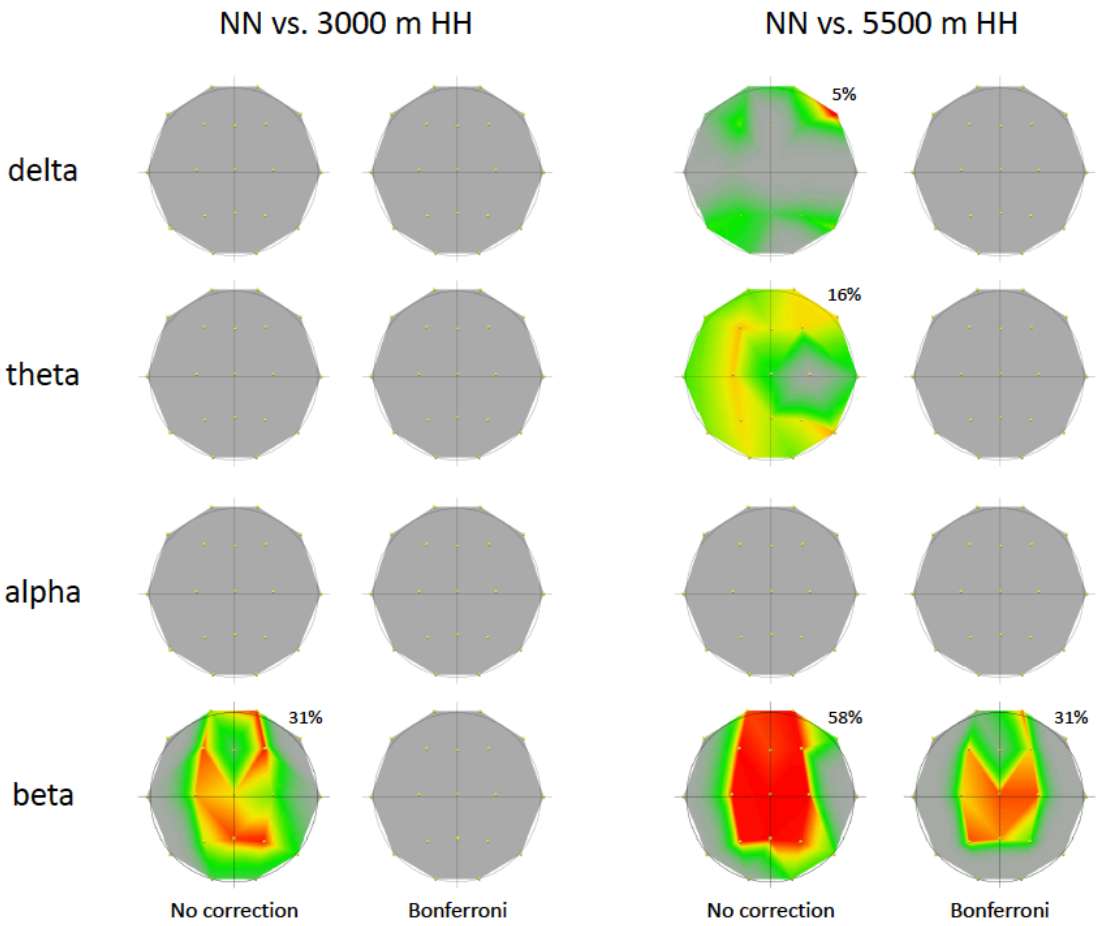

Figure 1: Statistical maps (a Delaunay triangulation is applied) showing the electrodes on the scalp that significantly differ ($p<0.05$) from each condition of HH and the NN control condition for the delta (1-3 Hz), theta (4-7 Hz), alpha (8-13 Hz) and beta (13.5-30 Hz) frequency bands. NN = normobaric normoxia; HH = hypobaric hypoxic (for altitude levels of 3000 m and 5500 m). The statistics were applied with (Bonferroni) and without (No correction) corrections for multiple comparisons. The percentage of electrodes that are significantly different is mentioned on the upper left side of each map.

EEG Microstates Parameters

The meta-criterion revealed six microstates explaining 86% of the global variance (**Figure 2**). The first four clusters of the six microstates identified in the present study are similar to the 4 maps (labeled A, B, C and D) previously described in the literature and explain 71% of the

EEG signal at rest (Koenig et al., 1999, 2002; Lehmann et al., 2005; Britz et al., 2010; Tomescu et al., 2014; Spring et al., 2017, 2018). The other two clusters – labeled E and F – resemble to additional topographies recently reported in the study of Custo and collaborators (2017) and explain 15% of the global variance.

For the delta GEV and delta time coverage, the repeated measures ANOVA did not reveal any main effects nor interactions between the two conditions of altitude and the six maps. However, the statistical analyses revealed significant interaction for the delta mean duration, $F(5, 45) = 2.50$; $p= 0.044$, which disappear once the Greehouse-Geisser correction has been applied ($p=0.140$). Tukey post-hoc test revealed that the significant but uncorrected interaction is explained by a decrease of mean duration at 5500 m HH as compared to baseline for the map C only (Figure 3). The same holds for the delta of frequency of occurrence, with a significant interaction between condition of altitude and maps, $F(5, 45) = 2.0$; $p= 0.045$ that disappears after Greehouse-Geisser correction ($p=0.103$). For the map C, the data reveal that the frequency of occurrence slightly increase at 5500 mm HH as compared to the baseline.

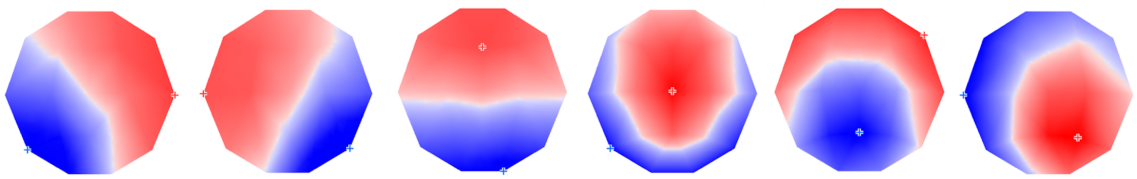

Figure 2: The six microstates with their respective global explained variance (GEV). The first four maps obtained across condition were labeled in map A, B, C, D according to previous studies. The two other maps E and F correspond to additional topographies obtained in Custo et al (2017) (maps F and G).

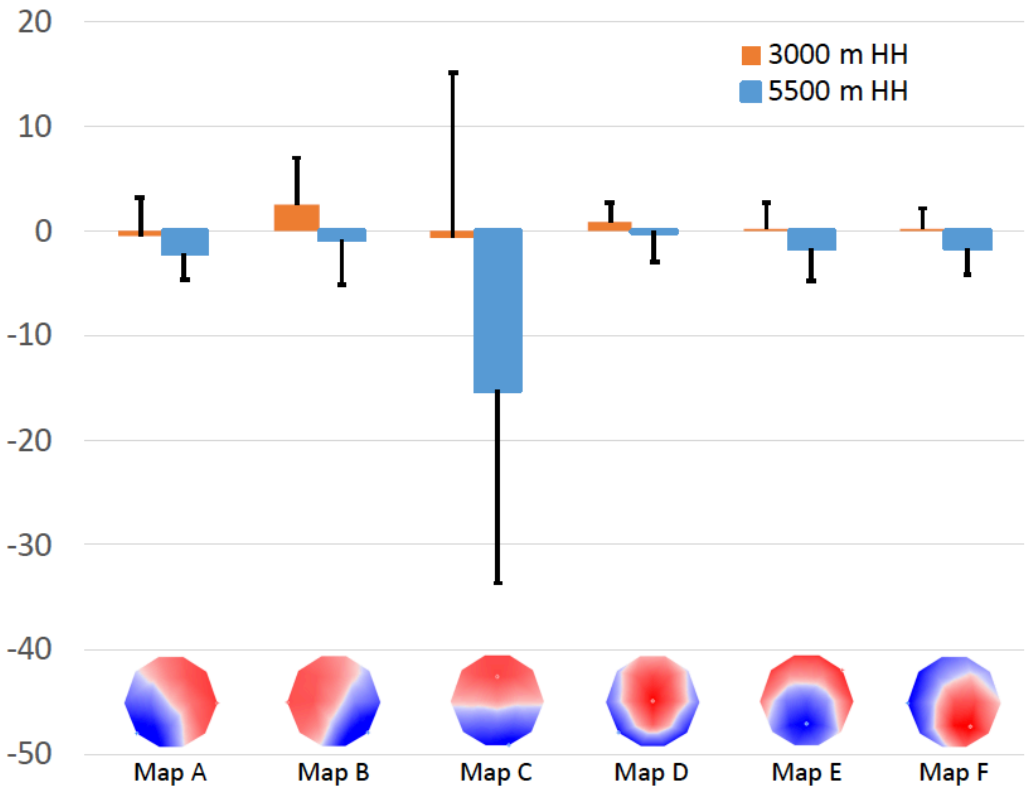

Figure 3: Mean and standard deviation of the delta scores (in ms) for the mean duration according to the two altitude levels and the six microstates. Negative delta score indicates a decrease of mean duration of the map in the HH condition as compared to the NN condition. If the mean duration does not change whatever the level of altitude, the mean duration of the map C decrease at 5500 m HH but this significant effect is no longer observed after the Greehouse-Geisser correction has been applied.

Physiological and cerebral responses to hypobaric hypoxia

HR showed a gradual increase with increase of altitude level in 3000 m HH (80 ± 9 bpm, $p=0.041$) and 5500 m HH (94 ± 12 bpm, $p<0.001$) when compared to NN (73 ± 6 bpm, Table 2). On the contrary, SpO_2 was lower in HH (92.5 ± 3.4 and 73.0 ± 6.5 %, at 3000 m and 5500 m respectively, $p<0.001$) than NN (99.3 ± 0.7 %).

MCAv increased in 5500 m HH only when compared to NN ($p=0.023$). Moreover, cDO_2 was ≈12% and ≈15% lower in 5500 m than 3000 m HH ($p=0.019$) and NN ($p=0.004$), respectively. Cerebral TOI (%) also decreased with altitude level increase in HH (Table 1). Finally, perceived sleepiness (KSS score) remained similar between NN and 3000 m HH, but was significantly higher in 5500 m HH (+2 points, $p<0.001$). All physiological and cerebral values are represented in Table 2.

Discussion

The present study evaluated the hypoxic severity on brain's electrical activity, physiological and cerebral responses when exposed to acute hypobaric hypoxia. First, there were weak to no EEG changes at altitude level of 3000 m, whereas 5500 m significantly increased beta and theta power with a persistent significant increase of beta power after corrections for multiple comparisons. Secondly, microstates analysis revealed no significant change in both altitudes, except a small decrease of the mean duration and frequency of occurrence for the map C at 5500m HH that does not remain after correction for sphericity. Physiological changes showed a gradual HR elevation and SpO_2 reduction with altitude increase. The hypoxemic states resulted in MCAv increase at 5500 m in order to limit oxygen deprivation to the brain. However, cDO_2 and cerebral TOI were lower in 5500 m when compared to 3000 m and NN. Overall, the present results suggest a quasi-null influence of acute exposure in HH at 3000 m. However, greater physiological response and cerebral regulations were observed at 5500 m in HH with beta-related EEG modulations and subjective mental fatigue.

EEG changes in acute hypobaric hypoxia

Hypoxia is observable in three different cases; when the oxygen supply to the blood is insufficient in case of hypoxic exposure, anaemia or ischemia (Plum and Posner, 1982). In the

present study, exposures to acute hypobaric hypoxia were assessed. Hypobaric hypoxia can be performed either naturally (i.e., altitude exposure in the mountains) or simulated in a hypobaric chamber by decreasing the ambient barometric pressure. This second method, used in the present study, is considered as a safe experimental model for the study of hypoxia and allow precise standardization of the hypoxic exposure (Kraaier et al., 1988). Previous studies have investigated the influence of hypoxia on brain's electrical activity, when exposed to normobaric hypoxia (Rebuck et al., 1976; Schellart and Reits, 2001; Burykh, 2005; Rice et al., 2019a, 2019b) or hypobaric hypoxia (Kraaier et al., 1988; Ozaki et al., 1995; Papadelis et al., 2007).

The neuronal activity is sensitive to brain's oxygen supply (Ozaki et al., 1995; Goodall et al., 2014). Previously, neuronal activity during hypobaric hypoxic exposure was characterized by selective suppression of alpha EEG activity at 3000 m, whereas further elevation in altitude over 5000 m resulted in strong suppression of alpha activity in the posterior brain's areas and significant increase of theta activity in the anterior areas (Ozaki et al., 1995). In the present study, we used a conservative statistics method to avoid false positive. Consequently, only beta power changes reach the significant threshold at 5500 m which is in line with previous findings (Ozaki et al., 1995). Moreover, it was previously shown that a decrease in SpO_2 <75% resulted in impairments in neuronal activity and slowing of EEG (Goodall et al., 2014). Slowing of the EEG signal was also reported in hypobaric hypoxia (Ernsting, 1963; Kraaier et al., 1988; Ozaki et al., 1995). More recently, EEG slowing was related with an increased power of theta and alpha bands (Papadelis et al., 2007). Moreover, no change in EEG were observed when SpO_2 remained higher than 75% (Rebuck et al., 1976). Our results showed a very weak impact on EEG at 3000 m when SpO_2 decreased to ≈80%. However, There was a strong beta power increase with significant lower SpO_2 at 5500 m (≈73 %), which is in line

392 with previous studies suggesting greater alteration of EEG with SpO_2 lower than 75%
393 (Rebuck et al., 1976; Goodall et al., 2014).
394 Beta activity represents a marker of cortical arousal (Spiegelhalder et al., 2012), and has been
395 studied in various populations in normoxic conditions. As an example, beta and theta power
396 increase was related to mental fatigue in healthy drivers (Craig et al., 2012). More precisely,
397 when a person fatigues, the brain loses capacity and slows its activity, which lead to increased
398 beta activity in order to maintain vigilance (Craig et al., 2012). In primary insomnia patients,
399 beta power increase was related to drowsiness (Spiegelhalder et al., 2012). Moreover, EEG
400 beta power was also increased in primary insomnia patients when compared to good sleepers
401 (Freedman, 1986; Buysse et al., 2008). In the present study, beta power increased in acute
402 hypoxia at 5500 m when higher values in KSS score (i.e., greater perceived sleepiness) were
403 observed. KSS score was higher when mental fatigue was induced following long-term
404 cognitive work (Liu et al., 2010). Thus, the present results suggest that a greater KSS score
405 (perceived sleepiness) may be related to mental fatigue, when a beta power increase was
406 observed in participants exposed to acute hypobaric hypoxia at 5500 m. At a more
407 mechanistic level, modulations in beta oscillations are known to be related to GABA-ergic
408 neurons (primary inhibitory neurotranmitter) activity. Recently, it has been stated that any
409 changes during spontaneous EEG beta oscillatory activity could be attributed to
410 neurotransmitter alteration induced by variation in oxygen availability (Zhao et al 2016). As it
411 has been claimed that, in case of severe hypoxia, that an increase of GABA-ergic activity
412 might play a protective role by suppressing cellular excitability and avoiding electrical brain
413 disorders (Hossein-Javaheri and Buck, 2020), we can speculate that the significant increased
414 beta power observed in our data at 5500 m could be a manifestation of the preventive GABA
415 receptors activity.

The present study may be of clinical relevance as it highlights EEG changes during cerebral hypoxia at different altitude level exposure (i.e., different hypoxic severity). Cerebral hypoxia is observed in patients hospitalized with acute respiratory failure, because of inadequate oxygen delivery to the brain (Bernard et al., 1994). As an example, quantitative electroencephalography measures can assist intensive care units to evaluate cerebral hypoxia severity in patients (Papadelis et al., 2006). Cerebral hypoxia is suggested as a potential predictor of the optimal time-point to disconnect the patient from the ventilator according to their neurological outcome (Papadelis et al., 2006). Research on such patients is difficult to conduct, as it may be associated with life-threatening complications and do not allow precise hypoxic levels standardization (Papadelis et al., 2007). While more studies on healthy participants are needed at different levels of hypoxia, the present study adds more insights on EEG changes when exposed to various simulated levels of acute hypobaric hypoxia.

Microstates in hypoxia

The EEG microstates analyses conducted in this research confirmed that it is possible to identify the classical maps found in the majority of the researches with 19 electrodes (Khanna et al., 2014). Our results showed that 6 maps explained 85% of our EEG dataset. The concept of "microstate of cognition" consists of an electrophysiological observation of a given structure of the overall scalp electric field (i.e., topography), supported by Lehmann and colleagues' (Lehmann et al., 1987). Microstates, measured with multichannel EEG, remains stable for periods of approximately 100 milliseconds and then switches to a new state (Lehmann et al., 1987). It was suggested by Lehmann's team, that these broadband EEG "microstates" represent the "atoms of thought", corresponding to the edifice of human cognition (Lehmann, 1990; Michel and Koenig, 2018). The (electrophysiological) neural networks generating the resting state scalp topographies was recently estimated and recorded

441 with 256-channel EEG (Custo et al., 2017). To our knowledge, this direct approach to
442 estimate the EEG resting state topographies has been investigated by few research teams
443 (Pascual-Marqui et al., 1995; Milz et al., 2016; Custo et al., 2017).
444 The present study investigated brain's electrical changes and microstates in young healthy
445 participants exposed to acute hypobaric hypoxia, which is novel in this research field. Our
446 results showed no significant influence of acute hypoxic exposures on brain's dynamic of
447 functional networks (i.e., microstates) in hypobaria at altitude levels of 3000 m and 5500 m,
448 when compared to control condition in NN.
449
450 **Physiological responses in acute hypobaric hypoxia**
451 In hypoxia, HR increases to maintain systemic oxygen delivery (Siebenmann and Lundby,
452 2015). Moreover, cerebral blood flow increases to maintain cerebral oxygen furniture
453 (Brugniaux et al., 2007; Ainslie and Subudhi, 2014). It is well known that MCA vasodilates in
454 hypoxia in order to regulation cerebral oxygen delivery (Wilson et al., 2011; Imray et al.,
455 2014; Mikhail Kellawan et al., 2017). In the present study, HR increased gradually with
456 altitude level increase in acute HH. MCAv increased significantly in 5500 m HH, but cDO_2
457 showed lower absolute values at this altitude, when compared to 3000 m HH and NN. MCA
458 diameter was not measured, which is one limitation of the present study. Nevertheless, it has
459 been shown that MCAv is highly correlated with CBF (Brauer et al., 1998). We therefore
460 speculate that MCAv increase in the present study induced an elevation in CBF in 5500 m HH
461 in order to limit cerebral oxygen deprivation. None of the physiological variables was
462 correlated with EEG changes, which underlines the complexity of the neuronal activity
463 regulation in hypoxic environment.
464
465

Conclusion

Acute 5500 m HH exposure led to greater beta synchronization (amplitude increase). In hypoxia, pulse oxygen saturation reduction induced an elevation in heart rate and middle cerebral artery blood velocity, in order to limit cerebral oxygen deprivation. Cerebral oxygen delivery was maintained in 3000 m HH, but decreased in 5500 m HH. The reduction in cerebral tissue oxygenation index, suggest a hypoxic state of the brain at high-altitude. Overall, the present results suggest a moderate impact of hypoxia at 3000 m, but a kind of idling or "freezing" state of the global brain activity at 5500 m, when exposed to acute hypobaric hypoxia. The present study proposes more insights how the cerebral activity is modulated in acute hypoxic environments. The co-absence of modulations at the level of EEG microstates features and the alpha power reinforce the link between alpha oscillations and the dynamic of EEG microstates across time (Milz et al., 2016).

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Author Contributions

MRA, GPM, NB, DB and JB were part of the conception of the protocol. MRA conducted the experiments, was responsible for data acquisition and wrote the manuscript with the support

of JB. MRA and JB conducted the analysis and interpreted the results. NB, GPM, DB and JB revised critically the manuscript and gave advises for corrections to MRA. MRA, GPM, NB, DB and JB gave their final approval of this version to be published.

Conflict of interest

The authors declare no conflict of interest and have no financial relationship to disclose.

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679
680 **Table 1.** Absolute values are Mean±SD (n=12). Physiological and cerebral responses; heart
681 rate (HR), pulse oxygen saturation (SpO_2), middle cerebral artery blood velocity (MCAv),
682 cerebral oxygen delivery (cDO_2), cerebral tissue oxygenation index (TOI), and Karolinska
683 sleepiness scale (KSS) in: Normobaric normoxia (NN); Hypobaric hypoxia (HH) at altitude
684 levels of 3000 m and 5500 m.
685

	NN mean	3000 m HH	5500 m HH	P values
HR (bpm)	73 ± 6	80 ± 9 *	94 ± 12 *** ###	*NN-3000 m: P=0.041*
SpO_2 (%)	99.3 ± 0.7	92.5 ± 3.4 ***	73.0 ± 6.5 *** ###	
MCAv (cm/s)	47.5 ± 7.7	48.7 ± 10.7	54.9 ± 13.0 * (#)	*NN-5500 m: P=0.023* *3000 m-5500 m: P=0.060*
cDO_2 (n.u.)	1017 ± 163	987 ± 226	864 ± 135 ** #	*NN-5500 m: P=0.004* *3000 m-5500 m: P=0.019*
TOI (%)	82.3 ± 0.9	77.9 ± 5.7 *	68.6 ± 6.0 *** ###	*NN-3000 m: P=0.049*
KSS	2.8 ± 0.9	3.0 ± 0.9	5.0 ± 1.4 *** ###	

686
687 * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ for difference with NN
688 (#) $p=0.06$, # $p<0.05$, ## $p<0.01$ and ### $p<0.001$ for difference with 3000 HH.

Article 5 – Cognitive impairment during combined normobaric vs. hypobaric and normoxic vs. hypoxic acute exposure

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Cognitive Impairment During Combined Normobaric vs. Hypobaric and Normoxic vs. Hypoxic Acute Exposure

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INTRODUCTION: Exposure to hypoxia has a deleterious effect on cognitive function; however, the putative effect of hypobaria remains unclear. The present study aimed to evaluate cognitive performance in pilot trainees who were exposed to acute normobaric (NH) and hypobaric hypoxia (HH). Of relevance for military pilots, we also aimed to assess cognitive performance in hypobaric normoxia (HN).

METHODS: A total of 16 healthy pilot trainees were exposed to 4 randomized conditions (i.e., normobaric normoxia, NN, altitude level of 440 m; HH at 5500 m; NH, altitude simulation of 5500 m; and HN). Subjects performed a cognitive assessment (KLT-R test). Cerebral oxygen delivery (cDO_2) was estimated based middle cerebral artery blood flow velocity (MCAv) and pulse oxygen saturation (S_pO_2) monitored during cognitive assessment.

RESULTS: Percentage of errors increased in NH (14.3 ± 9.1%) and HH (12.9 ± 6.4%) when compared to NN (6.5 ± 4.1%) and HN (6.0 ± 4.0%). Number of calculations accomplished was lower only in HH than in NN and HN. When compared to NN, cDO_2 decreased in NH and HH.

DISCUSSION: Cognitive performance was decreased similarly in acute NH and HH. The cDO_2 reduction in NH and HH implies insufficient MCAv increase to ensure cognitive performance maintenance. The present study suggests negligible hypobaric influence on cognitive performance in hypoxia and normoxia.

KEYWORDS: cognition, acute exposure, hypobaria, hypoxia.

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Military personnel, pilots, and mountaineers are often exposed to acute moderate or severe hypoxia. In hypoxia, arterial oxygen partial pressure (P_aO_2) is reduced.[27] Decreased oxygen availability at moderate and high-altitude [around 1500–7500 m (4921–24,606 ft)] has been shown to induce cognitive function impairments in human individuals.[1,9] In a narrative review, Taylor et al. demonstrated that cognitive function tended to be altered in acute hypoxia.[38] Another review on clinical neuropsychological parameters suggested a tendency for acute hypoxia to induce decrement in P300 latency and amplitude, with short-term memory impairment noticeable above 6000 m (19685 ft).[40] When evaluating cognitive function, tasks are usually categorized as either "simple" or "complex,"[29] including memory (working, spatial, and verbal), attention, and executive function.[15] Taylor et al. presented a simplistic task categorization.[38] For instance, tasks including short-term memory and simple arithmetic are considered "simple cognitive tasks," whereas arithmetic efficiency and working-memory tasks are "complex cognitive tasks."[38] In the literature, because of inter- and intraindividual variations, the hypoxic effect on complex tasks remains unclear. The present study aimed to evaluate arithmetic efficiency, including working-memory, defined as the ability to keep and process short-term information long enough to sustain attention to perform a cognitive task,[36] when acutely exposed to different combinations of hypoxic and hypobaric conditions.

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Altitude exposure can be simulated with the use of a hypobaric chamber by reducing the ambient barometric pressure (P_B) (i.e., hypobaric hypoxia, HH) or by decreasing the inspired oxygen fraction (F_IO_2) without changing P_B (i.e., normobaric hypoxia, NH). Various studies have reported cognitive performance impairment during acute exposure in HH[3,4,37] or in NH.[9,28] Recently, a review suggested that low P_aO_2 (30–60 mmHg) was the key predictor of cognitive performance impairment, independently of the type of hypoxic exposure (i.e., NH or HH).[20] More precisely, it was suggested that increased cerebral blood flow is unable to compensate for the lack of oxygen sufficiently enough for cognitive performance maintenance when P_aO_2 level is low (<60 mmHg).[20] Moreover, Ochi et al. reported a negative correlation between arterial oxygen saturation and executive function impairment during gradual simulated altitudes in normobaric hypoxia.[26] Nevertheless, hypoxic conditions with similar inspired oxygen pressure (P_IO_2) are not considered equivalent (i.e., normobaric and hypobaric hypoxia),[7] even if this point remained in debate.[21,30] For the last two decades, there are increased evidences that HH is a more severe environmental condition,[22] leading to larger hypoxemia.[32] Moreover, symptoms seem also qualitatively different in HH,[10] with increased acute mountain sickness in HH than NH.[31] Therefore, cognitive performance and symptoms may vary between NH and HH acute exposures. To our knowledge, there are very few studies that have investigated cognitive performance in NH vs. HH. Long ago, a study showed similar decrease in visual attention at an altitude of 3450 m (11,319 ft) in NH and HH when compared to sea level.[33] McMorris et al. suggested that NH may be associated with greater reductions in cognitive function; however, their findings regarding the use of normobaric vs. hypobaric hypoxic conditions were inconclusive.[20] Therefore, more studies comparing the effect of NH and HH on cognition are needed. The first aim of the present study was in fact to compare the effects of acute NH and HH exposure on cognitive performance and symptoms in pilot trainees.

The present study also aimed to better evaluate the specific effect of hypobaria, independently of hypoxia, on cognitive performance. By using a hypobaric normoxic (HN) condition, which allows comparing similar normoxic conditions with different P_B, one may therefore isolate the hypobaric effect from the hypoxic one. The HN consists of a low P_B environment combined with enriched oxygen gas mixture to obtain a comparable P_IO_2 than in normoxic normoxia (NN). Supplemental oxygen administration (35%) improved cognitive performance at 4300 m (14,108 ft; for two tests out of nine) on the first day of exposure in male soldiers.[8] Nevertheless, the effects of hypobaria in normoxia on cognitive performance remain unexplored.

The assessment of cognitive performance in hypobaric normoxia and hypoxia is, therefore, of interest in the context of both aviation [pilots exposed to hypobaria in the cockpit using supplemental oxygen (HN)] or workers at high terrestrial altitude with supplemental oxygen, for example, in dormitories (HN) vs. high-altitude residents/mountaineers/workers without supplemental oxygen (HH). More precisely, pilots during flights at high altitude may be exposed to hypobaria in unpressurized cabin aircraft,[25] in case of sudden cabin depressurization during commercial flights,[23] or in military aircraft while breathing a hyperoxic gas mixture (i.e., HN). In the present study, we aimed to evaluate the putative effect of hypobaria during acute exposure between conditions with comparable P_IO_2 (NH vs. HH and NN vs. HN) on cognitive performance. We first hypothesized that increased altitude level in HH would gradually decrease cognitive performance. Hypoxic conditions (NH and HH) would induce cognitive performance impairment, with possibly larger alteration in HH than in NH. Finally, we hypothesized that cognitive performance in HN would be similar to NN.

METHODS

Subjects

Participating voluntarily in this study were 16 healthy pilot trainees (13 men and 3 women, age 26 ± 4 yr; height 177 ± 7 cm; weight 71 ± 9 kg). None of the subjects had experienced hypoxic exposure before enrollment in the present study and/or altitude exposure in the days before the test visits. A physician screened the subjects during a familiarization visit to ensure that they were healthy and did not report any medical or altitude-related issues. Moreover, none of the subjects was on medication during the present study.

This study was performed according to the Declaration of Helsinki and was approved by the Swiss Ethics Committee of Zürich (Swissethics, BASEC ID: 2017-00,752). This clinical trial can be found on ClinicalTrials.gov (ID: NCT03303118). All subjects were informed about all procedures of this study and gave their written informed consent before participating in this study.

Equipment

The "Konzentrations Leistungs Test-Revidierte Fassung" (KLT-R) is a concentration-performance test on paper with the use of a pencil which evaluates both quantity and quality of the capacity of concentration.[12] The whole KLT-R test consists of 9 blocks, each including 20 separate arithmetic tasks. In the present study, subjects performed only two blocks in each condition. After exactly 2 min, the subjects have to progress to the second block whatever the progress. In the present study, the signals to start, continue, and finish the test were provided by the experimenter using a timer to allow precise intervals (total test duration of 4 min). In order to avoid any learning effects, subjects were given two blocks in a randomized order using different but complementary versions of the KLT-R in each condition. Before enrollment in the present study, subjects were first drilled with KLT-R during a familiarization visit.

Heart rate (HR, bpm) was monitored during the entire experimental procedure using a heart rate monitor (Polar RS800CX, FI-90,440, Kempele, Finland). Pulse oxygen saturation (S_pO_2, %) was monitored at the left earlobe using an oximeter (3100 pulse oximeter, Nonin, Plymouth, MN) and acquired

at 0.5 Hz. A subset of these data has been previously published in a parallel article on cerebrovascular hypercapnic responses,[2] but the analyses were not performed over the same periods and the number of subjects was lower ($N = 9$). Mean HR and S_pO_2 were calculated during the last minute of cognitive assessment in each condition.

Middle cerebral artery velocity (MCAv) and cerebral oxygen delivery (cDO_2) were measured as described previously.[2] Mean MCAv and cDO_2 were calculated in each condition during the last minute of the cognitive assessment.

At the end of every condition and of every washout period in NN, subjects were asked to report any kind of symptoms they had experienced during the past condition. Acute mountain sickness was not measured in the present study. We asked the subjects to report their symptoms by answering a questionnaire in order to have more qualitative data regarding personal feeling during each condition. Subjects did not report persisting symptoms from a previous condition/exposure at the end of each NN period. Subjects attested being symptom-free before starting the next condition. Moreover, 1-factor RM-ANOVA showed no physiological changes across NN conditions for S_pO_2 [F(degree of freedom = 4) = 1.61; $P = 0.190$] and MCAv [$F(4) = 0.137$; $P = 0.968$]. Moreover, there was no significant difference across all NN conditions regarding cognitive performance since percentage of error (Err%) and number of errors during KLT also remained similar along NN conditions [$F(4) = 1.07$; $P = 0.379$ and $F(4) = 1.24$; $P = 0.307$, respectively]. Therefore, these results suggest that subjects had fully recovered after each condition and that there was minimal learning effect for the KLT test.

Procedure

This study was conducted at the Aeromedical Center of the Swiss Air Force. Subjects came for a test visit and underwent experimental trials near sea level [Dübendorf, 440 m (1444 ft), P_B: 727 ± 4 mmHg) and in hypobaric and/or hypoxic conditions. After material installation, subjects underwent a pretest in normobaric normoxia. Then, in a randomized order, all subjects ($N = 16$) undertook four experimental conditions of 30 min [NN as a control condition, HH at 5500 m (18,045 ft), NH to simulate 5500 m of altitude, and HN] in a hypobaric chamber interspersed with three washout periods of 30 min in NN for a total session duration of 5 h. Subjects undertook KLT-R after 5 min of acclimatization followed by 7 min of electroencephalography recording (i.e., from T+12 to T+16 min). After completing the KLT-R and in order to evaluate sleepiness, subjects had to rate their subjective sleepiness state on the 9-point scale using the Karolinska Sleepiness Scale (KSS). Subjects were asked to avoid physical exercise, heavy meals, and alcohol or caffeine consumption 24 h before the test visit.

In order to evaluate putative hypobaric effect between normoxic and hypoxic conditions, P_IO_2 between NN vs. HN (141 ± 1 vs. 133 ± 3 mmHg) and NH vs. HH (74 ± 1 vs. 70 ± 2 mmHg) were compared by adjusting P_B in the hypobaric chamber or F_IO_2 based on a known equation [$P_IO_2 = (P_B - 47) \times F_IO_2$], when 47 mmHg corresponds to water vapor pressure at 37°C.[7] Subjects breathed ≈11% and ≈40% O_2 gas mixture (0.03% CO_2) concentration for NH and HN, respectively, while P_B remained similar between NH and NN, but was similarly decreased in HN and HH. In order to achieve the NH condition, the hypobaric chamber was closed, but was not depressurized while subjects were switched to another gas cylinder containing 11% oxygen to simulate normobaric hypoxia. Regarding the experimental conditions, the altitude indicator (i.e., altimeter) in the hypobaric chamber was hidden and changes in pressure were unknown by the subjects. Moreover, gas concentrations in the mask were also unknown by the subjects.

Statistical Analysis

One-way repeated measures ANOVA were assessed for all parameters (KLT parameters, HR, S_pO_2, MCAv, cDO_2, and KSS absolute values) to evaluate significance between conditions using statistical software (Jamovi project 2018, version 0.9, https://www.jamovi.org). Pearson or Spearman correlations were calculated between absolute or relative differences with NN in physiological responses and cognitive parameters, respectively. Significant difference was set for $P < 0.05$.

RESULTS

Cognitive and physiological parameters in NN, HN, NH, and HH are displayed in **Table I**. Number of calculations assessed was lower only in HH when compared to NN [$F(3) = 5.35$; $P = 0.018$] and HN ($P = 0.011$). The number of right answers was decreased to the same extent in NH and HH when compared to normoxic conditions [NN and HN, $F(3) = 17.1$; $P < 0.001$]. %Err increased in the two hypoxic conditions when compared to normoxic conditions. Δ%Err was not significantly correlated with ΔS_pO_2 in NH ($r = -0.484$, $P = 0.097$). There was no significant difference between NN vs. HN and NH vs. HH regarding cognitive performance.

S_pO_2 decreased in NH and HH compared to normoxic conditions, with significant lower values in HH than NH [$F(2.1) = 102$; $P = 0.008$]. HH induced higher HR value than NH [$F(3) = 11.2$; $P = 0.026$]. MCAv was greater in HH only than in all other conditions. ΔMCAv was significantly correlated with ΔS_pO_2 in HH ($r = -0.741$, $P = 0.008$). Moreover, absolute MCAv was correlated with cDO_2 in HH ($r = 0.698$, $P = 0.012$) and NH ($r = 0.589$, $P = 0.044$). Estimated cDO_2 was significantly lower in NH [$F(3) = 3.4$; $P = 0.033$] and HH ($P = 0.016$) than in NN. Nevertheless, there was no significant correlation between KLT-R parameters and MCAv, cDO_2, or S_pO_2.

All symptoms for each condition are reported in **Fig. 1**. Interestingly, some symptoms were more represented in hypoxic conditions (NH and HH), such as: dizziness, tiredness, and calculation difficulties. Subjects reported being dizzy, having postural alterations, cold hands, and nausea only in NH and HH. Globally, subjects reported more symptoms in HH than NH (46 vs. 25 observations in HH vs. NH, respectively). Finally, two subjects had red eyes in the hypobaric conditions (HH and

Table I. KLT-R Parameters and Physiological Data During Cognitive Assessment.

	NN	HN	NH	HH	STATISTICS
Calculations (nb)	20.9 ± 5.8	21.1 ± 5.9	19.7 ± 4.2	18.9 ± 5.9*‡	$F(3) = 5.35$ $P = 0.003$
Right (nb)	19.5 ± 5.5	19.9 ± 5.6	16.9 ± 4.5***‡‡‡	16.7 ± 6.0***‡‡‡	$F(3) = 17.1$ $P < 0.001$
Errors (nb)	1.4 ± 0.9	1.3 ± 0.9	2.7 ± 1.8*‡	2.2 ± 0.9	$F(3) = 5.25$ $P = 0.004$
Errors (%)	6.5 ± 4.1	6.0 ± 4.0	14.3 ± 9.1**‡‡	12.9 ± 6.4**‡	$F(3) = 8$ $P < 0.001$
Physiological responses during KLT					
S_pO_2 (%)	99.7 ± 0.4	98.5 ± 2.2	83.5 ± 5.6***‡‡‡	78.9 ± 5.8***‡‡‡§§	$F(2.1) = 102$ $P < 0.001$
HR (bpm)	77.2 ± 9.6	79.5 ± 7.6	87.2 ± 11.8***	94.8 ± 11.7***‡‡‡§	$F(3) = 11.2$ $P < 0.001$
MCAv ($cm \cdot s^{-1}$)	48.3 ± 8.1	48.5 ± 9.5	52.1 ± 10.0	55.5 ± 11.6***‡‡‡§	$F(3) = 12.3$ $P < 0.001$
cDO_2 (n.u.)	1007 ± 166	983 ± 170	904 ± 208*	827 ± 154*	$F(3) = 3.4$ $P = 0.03$
KSS score	2.97 ± 0.86	3.43 ± 0.95	4.50 ± 1.41***‡	4.83 ± 2.08***‡‡	$F(3) = 10.4$ $P < 0.001$

S_pO_2: pulse arterial oxygen saturation; HR: heart rate; MCAv: middle cerebral artery cerebral blood flow velocity; cDO_2: estimated cerebral oxygen delivery; NN: normobaric normoxia; HN: hypobaric normoxia; NH: normobaric hypoxia; and HH: hypobaric hypoxia. Data are mean ± SD ($N = 16$).
* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ for difference with NN; ‡ $P < 0.05$, ‡‡ $P < 0.01$, and ‡‡‡ $P < 0.001$ for difference with HN; § $P < 0.05$ and §§ $P < 0.01$ for difference with NH.

HN). Regarding subjective sleepiness of the subjects, KSS score was higher in NH and HH when compared to NN [$F(3) = 10.4$; $P < 0.001$] and HN ($P = 0.022$ and $P = 0.006$ for NH and HH, respectively). KSS score remained similar between NN and HN ($P = 0.664$) and NN conditions [$F(3) = 0.808$; $P = 0.497$].

DISCUSSION

The main aim of the present study was to evaluate the putative effect of hypobaria during acute exposure in normoxia and hypoxia on cognitive performance. NH and HH conditions had a deleterious effect on cognitive performance. However, cognitive performance was maintained in HN when compared to NN. Overall, these results confirm the deleterious effect of hypoxia and add new insights regarding the negligible influence of hypobaria on cognitive performance.

In the present study, cognitive performance was deteriorated in acute HH, whereas MCAv was increased. This is in line with previous studies that have shown a deleterious effect of hypoxia on cognitive function in humans.[9,24] As individuals ascend to altitude above 5000 m (16,404 ft), cognitive impairments to, for example, working memory, have been observed.[6,17] Moreover, working memory was reduced in pilots exposed to acute HH at a simulated altitude level of 10,000 m (32,808 ft).[17] It was also suggested in a recent review that cognitive performance tends to become

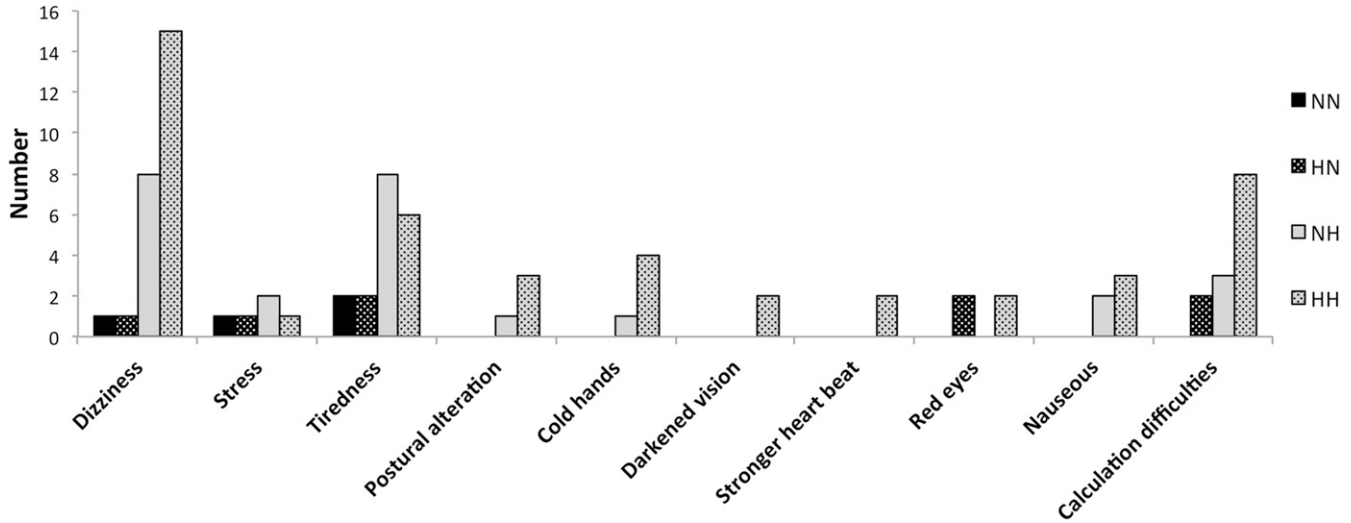

Fig. 1. Representation of the types of symptoms (X-axis) and number of symptoms reported by the subjects (Y-axis) for each condition: normobaric normoxia (NN), hypobaric normoxia (HN), normobaric hypoxia (NH), and hypobaric hypoxia (HH).

more impaired with increasing altitude, but with large interindividual variation among studies.[18] Ochi et al. reported a negative correlation between arterial oxygen saturation and executive function impairment during gradual simulated altitudes in NH.[26] However, ΔS_pO_2 was not significantly correlated with Δ%Err in NH in the present study.

The present study aimed to evaluate the putative hypobaric effect on cognitive performance in acute NH and HH. Both hypoxic conditions decreased cognitive performance to the same extent. McMorris et al. suggested that NH may be associated with greater reductions in cognitive function than HH.[20] However, NH induced comparable %Err than HH in the present study. Nevertheless, number of accomplished calculations decreased only in HH compared to NN. This suggests a slower speed in HH to assess the arithmetic task (i.e., lower arithmetic efficiency). Time to completion was greater at 5334 m (17,500 ft) and more than doubled at 7620 m (25,000 ft) in HH when compared to sea level,[3] which is in line with our results (i.e., decreased calculation numbers in HH). The mechanisms which explain how acute hypoxia negatively affects cognitive function are not completely understood, although it is likely a combination of factors, which may include neuronal damage[5] and fatigue.[40] Moreover, some physiological changes occur in the brain in HH, which can impair working memory tasks.[16] Surprisingly, we did not observe any correlations between changes in physiological responses to hypoxia (i.e., S_pO_2, MCAv, cDO_2) and cognitive performance.

To our knowledge, the present study is among the first studies to evaluate cognitive performance during acute exposure in NH vs. HH at high altitude. Overall, our results showed cognitive impairments in acute NH and HH when compared to NN, but with some slight differences (i.e., decreased speed in HH and higher number of mistakes in NH only).

The physiological differences between HH and NH (decreased S_pO_2 and increased heart rate in HH) are in line with several studies recently published.[21,22] In hypoxemia (i.e., decreased S_pO_2), the vasomotor tone enhances vasodilatation and consequently increases cerebral blood flow. In the present study, MCAv increased in NH and HH when S_pO_2 decreased in order to elevate cDO_2, which confirms cerebral vasodilation (i.e., in the MCA) in acute hypoxia to limit cDO_2 decrease.[13,41] However, the MCAv elevation in HH was insufficient to maintain cDO_2, resulting in putative cognitive performance reduction, whereas MCAv in NH remained similar to that in NN.

The present study aimed also to evaluate cognitive performance in acute HN in order to isolate the specific effect of hypobaria in normoxic condition. Supplementary oxygen is known as a logical aid, which may counterbalance the negative side effects of hypobaric hypoxia on cognitive function, although literature on this topic scarcely exists.[38] One previous study showed cognitive performance improvement for two tests (out of a test battery of nine cognitive tests) at 4300 m (14,108 ft) in HH while breathing a supplemental oxygen gas mixture (35%).[8] The present results showed similar cognitive performance in HN and NN. One may speculate that the maintenance of S_pO_2 and cDO_2 in HN permitted the subjects to remain effective during cognitive task assessment.

In the present study, the subjects reported the symptoms they had experienced during each condition. The second aim of the present study was to collect qualitative data in order to evaluate the individual sensitivity and subjects' feelings when exposed to various acute hypoxic and hypobaric conditions. Interestingly, subjects reported more symptoms in HH than NH. Some symptoms seem representative of hypoxic exposure, such as dizziness, tiredness, postural alteration, cold hands, and nausea. Nevertheless, a few symptoms were reported only in HH (i.e., darkened vision, feeling of a stronger heartbeat), which may be related to hypobaria. Our observations are in line with previous studies in which acute mountain sickness differed qualitatively between NH and HH and was greater in HH than NH,[10,11,31] suggesting that NH and HH may be not completely interchangeable.[11]

The present study suggests that cognitive performance decreased in NH and HH to the same extent. However, the symptoms qualitatively differed between NH and HH. Military pilots often train in a flight simulator in NH. A recent study showed cognitive and flight performance impairment during training in normobaric hypoxia.[39] However, as previously recommended,[14,34,35] it remains paramount to regularly assess hypoxia awareness training, to teach military and civilian pilots to recognize their individual symptoms, in hypobaric hypoxia. Moreover, further research investigating the hypobaric normoxic environment are needed, as such circumstance may occur in flights during cabin depressurization at high altitude while breathing a hyperoxic gas mixture.

One may expect the differences in physiological parameters observed between conditions would be related to the differences in cognitive performance. However, no correlation was reported and this might be because the differences were not large enough or that the study was insufficiently powered. P_IO_2 was not perfectly matched between NN and HN or between NH and HH, corresponding to a slight difference of 400–500 m (1312–1640 ft) of altitude. This is less than the "natural" variation of "simulated altitude" due to the meteorological variability and, therefore, we argue that our results remain of practical significance. Finally, serial testing in a single day introduces significant confounders that need to be addressed, as cognitive impairment remains degraded for at least 2 h after acute hypoxia.[28] Moreover, the present study does not allow direct translation to prolonged exposure.[19]

In conclusion, the present study confirmed the detrimental effect of hypoxia on cognitive performance. Both normobaric and hypobaric hypoxia negatively affected cognitive performance with some slight differences, although the present results showed no additional deleterious effect of hypobaria on cognitive performance in hypoxia. However, symptoms seemed qualitatively different and more exaggerated in hypobaric than normobaric hypoxia. Finally, cognitive performance was unaffected in hypobaric normoxia when compared to normobaric normoxia, suggesting a negligible influence of hypobaria on cognitive performance in a normoxic environment.

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Article 6 – Hypobaric effect in acute hypoxia on physiological responses, cerebral and muscular oxygenation during submaximal cycling exercise

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1 **Hypobaric effect in acute hypoxia on physiological responses during exercise**

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16

Abstract

Introduction: This study aimed to evaluate the putative effect of hypobaria on physiological responses during a submaximal cycling exercise in normoxic and hypoxic conditions.

Methods: Eighteen healthy pilot trainees (26±3 years old, 177±10 cm, 70±11 kg) performed a 6-min moderate-intensity (1 W/kg) cycling exercise in four randomized conditions: normobaric normoxia (NN), hypobaric normoxia (HN), normobaric hypoxia (NH) and hypobaric hypoxia (HH). Inspired oxygen pressure was matched between normoxic (NN vs. HN, 141.2±0.8 vs. 141.5±1.5 mmHg) and hypoxic (NH vs. HH, 75.7±0.4 vs. 74.3±1.0 mmHg) conditions. Gas exchanges, pulse oxygen saturation (SpO_2), heart rate (HR), middle cerebral artery velocity (MCAv), cerebral and muscular oxygenation (NIRS) were recorded at rest and during cycling exercise.

Results: During exercise, minute ventilation was greater in HH than in NH ($p=0.024$), NN and HN ($p<0.001$). NH and HH induced higher HR and lower SpO_2 than the normoxic ones ($p<0.01$). Moreover, HR was greater ($p=0.002$) and SpO_2 lower ($p<0.001$) in HH than in NH. MCAv was higher in HH (56.8±6.2 cm/s) than in NN (48.1±6.3 cm/s, $p=0.01$) and HN (47.9±6.5 cm/s, $p=0.011$). Tissue oxygenation index in the *vastus lateralis* was lower in NH (63 ± 6%) and HH (59 ± 6%) when compared to NN (68 ± 5%, $p<0.001$) with HH lower ($p<0.001$) than NH.

Conclusion: Respiratory and physiological responses at exercise appeared to be more pronounced in hypobaric hypoxia than in normobaric hypoxia, which confirm a putative additive effect of hypobaria on exercise severity in hypoxia. Moreover, the present results imply an additive hypobaric influence on muscle deoxygenation during hypoxic exercise. Contradictory, the effect of hypobaria seems negligible in normoxia since no significant differences were observed between NN and HN.

Key words: Hypoxia; hypobaria; exercise; ventilation; oxygenation.

Introduction

In hypoxia, limitation in exercise performance has been attributed to a lowered arterial O_2 partial pressure (PaO_2), reducing O_2 delivery to tissues with critical consequences on muscle metabolism and contraction (1, 2). Maintaining O_2 delivery in the cerebral areas (cDO_2) is also of importance since hypoxia decreases prefrontal oxygenation leading to cognitive performance impairment, despite a cerebral blood flow (CBF) increase (3).

Hypoxia can be simulated either by decreasing inspired oxygen fraction (F_IO_2; normobaric hypoxia, NH) or reducing the barometric pressure (P_B) in mountainous environment or by using a hypobaric chamber (hypobaric hypoxia, HH). It is known that all responses to hypoxia are caused by the PO_2 decrease, but NH is no longer considered as a surrogate of HH (4) since this later condition induces a more severe hypoxic stimulus (i.e., lower oxygen saturation, SpO_2) (5–7). Difference between these two hypoxic conditions may arise from different ventilatory patterns, alveolar gas disequilibrium and hypoxic ventilatory responses. For proper application in altitude and aviation medicine, awareness and consideration of these differences between NH and HH is of importance (8). Nowadays, the underlying mechanisms remain unclear (7). To isolate a putative effect of hypobaria not only in hypoxia but also in normoxia, it is of interest to compare normobaric normoxic (NN) and hypobaric normoxic (HN) conditions. In HN condition, barometric pressure is reduced combined with higher F_IO_2 so that the P_IO_2 is equivalent to NN value. This condition is particularly valuable in aviation for pilots breathing supplemental oxygen in a depressurized cabin. By comparing NN, NH, HN and HH it is therefore possible to isolate the putative additive or combined effects of both hypoxia and hypobaria.

66 In both acute and chronic exposure to hypoxia, it is known for long that the aerobic
67 performance is impaired (Pugh, 1967). More recently, this decrement compared to normoxia
68 was shown lower in normobaric than hypobaric hypoxia (9). During submaximal exercise,
69 however some compensatory mechanisms (i.e., increased heart rate and cardiac output; higher
70 skeletal muscle blood flow) occur in order to counterbalance the decreased arterial oxygen
71 concentration (CaO_2) and to maintain oxygen delivery (10). In addition to the cardiovascular
72 and convective factors, in severe hypoxia (arterial O_2 saturation <70–75%), cerebral
73 deoxygenation *per se* plays a role on impaired performance and reduced motor drive during
74 moderate-intensity exercise (11).
75
76 Near-infrared spectrometry (NIRS) is a non-invasive technique to measure changes in oxy-,
77 deoxy- and total haemoglobin concentration (O_2Hb, HHb and tHb, respectively; changes in
78 Δμmol). NIRS has shown very high reliability regarding muscle oxygen consumption during
79 exercise at low- to moderate-intensity (12). As expected, hypoxic condition impacts both
80 muscle and cerebral oxygenation; i.e. when cycling at 60–70% of normoxic maximal O_2
81 uptake for five minutes, HHb concentration increased when exposed to NH at an altitude level
82 of 3000 m (13). Moreover, cerebral, but not muscle, tissue showed larger deoxygenation
83 when resting in acute hypoxia (13, 14). In addition, muscle tissue oxygenation index (TOI)
84 remained equivalent between normoxia and hypoxia at rest, whereas cerebral TOI was
85 significantly lower in hypoxia (15). To our knowledge, however, it is unknown if hypobaria
86 modifies the hypoxia-induced decrease in muscle and cerebral oxygenation.
87
88 The primary goal of the present study was therefore to investigate the putative additive effect
89 of hypobaria, either in normoxia or in hypoxia, on the physiological responses during
90 moderate-intensity exercise. By comparing normoxic and hypoxic conditions with equivalent

inspired oxygen pressure (P_IO_2), the present study investigated the effect of hypobaria on physiological responses and oxygenation changes in the cerebral and muscular areas at rest and during submaximal cycling exercise. The present results are of interest for athletes or pilots regularly exposed to hypoxia. We hypothesized that (i) exercising in hypobaric hypoxia would lead to greater physiological compensatory cardiovascular mechanisms in order to limit the oxygen deprivation in the cerebral and muscular compartments; (ii) hypobaria would induce an additive detrimental effect in hypoxia regarding cerebral and muscle deoxygenation during a moderate-intensity cycling exercise at 5000 m; and (iii) conversely, hypobaria would play a negligible role in normoxia at rest and during exercise.

Methods

Ethics

This study was performed according to the Declaration of Helsinki and was approved by the Swiss Ethic Committee of Zürich (Swissethics, BASEC ID: 2018–00006). This clinical trial can be found on ClinicalTrials.gov (ID: NCT03439202). All participants were informed about all procedures of this study and gave their written informed consent before participation.

Participant recruitment and screening

Eighteen healthy pilot trainees (14 men and 4 women, age 26 ± 3 years; height 177 ± 9 cm; weight 70 ± 11 kg) participated voluntarily in this study. A physician screened the participants during the familiarization visit to ensure they were healthy and did not report any medical or altitude-related issues. None of the participants was on medication during this study. Participants attested not being exposed to altitude in the days preceding the test visit nor to hypoxia before enrolment in the present study. Twenty-four hours before test visit,

participants were asked to avoid physical exercise and consuming a heavy meal, alcohol and caffeine.

Study design

This study was conducted at the Aeromedical Center of the Swiss Air Force, Dübendorf, Switzerland. Participants came for one test visit and were exposed in a random order to four environmental conditions: In normobaric normoxia (NN, Dübendorf, 440 m, P_B 723±4 mmHg), hypobaric normoxia (HN, F_IO_2 39.4 %, P_B 406±4 mmHg), normobaric hypoxia (NH, F_IO_2 11.2 %, P_B 723±4) and hypobaric hypoxia (HH, F_IO_2 20.9 %, P_B 403±5 mmHg). Each condition was interspersed with by 30-min rest period in NN. Twenty-four hours before all visits, participants were asked to avoid physical exercise, heavy meal and alcohol or caffeine consumption. Participants remained at rest, seated on a cycle ergometer for 3 minutes and cycled for 6 minutes (1W/kg at averaged 80 rpm) on a cycle ergometer (eBike II basic, GE medical systems, Germany). Participants gave their rating of perceived exertion (RPE, using BORG scale) at the end of exercise.

Condition comparison

In order to isolate the effect of hypobaria, inspired oxygen pressure (P_IO_2) was matched in NN vs. HN (141.2±0.8 vs. 141.5±1.5 mm Hg) and in NH vs. HH (75.7±0.4 vs. 74.3±1.0 mm Hg). Matching was achieved by adjusting the barometric pressure (P_B) in the hypobaric chamber or the F_IO_2 using known equation: ($P_IO_2=(P_B-47)\times F_IO_2$), when the water vapour pressure at 37°c is 47 mmHg (4). Participants breathed a mixed gas containing 11.2 % or 39.4 % O_2 concentration (0.03% CO_2) for NH and HN, respectively. The barometric pressure was similar in NN and NH, whilst it was decreased similarly in HN and HH.

Ventilation

Ventilatory data were measured using a gas analyser (K5, Cosmed, Roma, Italy), which was calibrated outside of the hypobaric chamber before test visit. Flow volume was calibrated using a 3L syringe. Zero CO_2 calibration was performed with a scrubber. Gas concentration calibration was performed using a certified gas bottle (16% O_2 and 5% CO_2, Cosmed, Italy). Ventilatory responses were recorded breath-by-breath and then exported with dedicated software for later analysis (OMNIA, Cosmed, Roma, Italy). Means were calculated for the last minute of baseline (at rest) and last minute of exercise periods.

Heart rate and pulse oxygen saturation

Heart rate (HR, bpm) was monitored during the entire experimental procedure using a heart rate monitor (Polar RS800CX, FI-90440 Kempele, Finland). Pulse oxygen saturation (SpO_2, %) was monitored at the left earlobe using an oximeter (3100 pulse oximeter, Nonin, Plymouth, MN) and acquired at 0.5 Hz. Mean HR and SpO_2 were calculated during the last minute of baseline and exercise periods.

Cerebral blood flow velocity and oxygen delivery

Middle cerebral artery velocity (MCAv) was recorded and cerebral oxygen delivery (cDO_2) was estimated as described previously (16). The MCAv signal was acquired from the left middle cerebral artery at depths ranging from 43 to 54 mm. Mean MCAv and cDO_2 were calculated in each condition during the last minute of baseline and exercise periods.

Near-infrared spectroscopy (NIRS) Monitoring

Changes in oxyhemoglobin (O_2Hb) de-oxyhemoglobin (HHb), tissue oxygenation index (TOI) and total hemoglobin (tHb) concentrations were monitored using a NIRO-200NX

(Hamamatsu Photonics, Hamamatsu City, Japan). A first probe was placed on the participants' forehead horizontally on the left side. A second probe was placed on the left *vastus lateralis* (VL) muscle at one third of the distance from the patella to the greater trochanter of the femur. Data were collected every second (1 Hz) during entire test protocol. The TOI was automatically calculated by the NIRS device based on following equation: TOI (%) = (O_2Hb/tHb) × 100, which refers to the O_2Hb proportion in the brain tissue at a depth of 1 cm below the brain and muscle's (*vastus lateralis*) surfaces. Based on NIRO-200NX settings recommendations, pathlengths of 17.8 cm and 14 cm were chosen for brain and calf's regions, respectively. Mean cerebral and calf TOI values were calculated for the last minute of baseline and exercise periods. The NIRO-200NX measures the concentration changes for O_2Hb, HHb and tHb in real time. Changes in these parameters from baseline to exercise end period were calculated.

Statistical Analysis

Repeated measures ANOVA were assessed with absolute values for condition comparison. Greenhouse-Geisser sphericity correction was applied when Mauchly's test statistic showed significance ($p<0.05$). Then, Tukey *post hoc* test was performed for condition comparison when appropriate. Repeated measures ANOVA (non-parametric, Friedman) were performed in case of violation of normality (i.e., delta values: for changes from NN and difference between baseline and exercise periods). Statistical analysis was assessed using Jamovi software (Jamovi project 2018, version 0.9). Significant difference was set for $p<0.05$.

Results

Ventilatory responses

During the resting period, V̇E was similar between the four conditions. However, during exercise, V̇E was higher in HH than in NN and HN ($p < 0.001$, **Figure 1**), as well as in NH ($p < 0.024$). VT and Rf significantly increased during exercise compared to rest in all conditions, and did not differ between conditions during exercise (**Figure 1**). Results for V̇E, VT and Rf, are detailed in **Table 1**. In addition, partial pressure of end tidal CO_2 ($P_{et}CO_2$) was lower ($p<0.001$) in HH (23±1 mmHg) and HN (23±3 mmHg) and when compared to NN and NH (36±4 and 33±4 mmHg, respectively). As expected, end-tidal pressure in O_2 ($P_{et}O_2$) was lower in hypoxia (NH and HH) when compared to in NN and HN, but with an additive effect of hypobaria (HN <NN<; 92±13 vs. 101±4 mmHg; $p < 0.001$ and HH<NH; 41±2 vs. 50±7 mmHg; $p < 0.001$).

***Add Figure 1 around here ***

Physiological responses

All physiological data are displayed in **Table 2**.

HR was significantly higher at rest in HH than in NN ($p=0.024$). During exercise, HR was greater in hypoxic conditions than in normoxic ones, as well as in HH than in NH ($p=0.002$). SpO_2 was similar between NN and HN at rest and during cycling exercise but was higher than in the two hypoxic conditions ($p<0.001$). Moreover, HH showed lower SpO_2 values than in NH at rest ($p=0.027$) and during exercise (-10%, $p<0.001$).

MCAv was elevated at rest in hypoxia and increased during exercise (+8 cm/s) in HH only when compared to NN ($p=0.01$) and HN ($p=0.011$). Estimated cDO_2 at rest was lower in HH than in NN only ($p=0.031$). Moreover, cDO_2 did not show significant differences between conditions during exercise. Finally, RPE was greater in NH and HH ($p<0.001$) than in NN and HN (Table 2).

214

215 **Cerebral and muscular oxygenation**

216 Cerebral and muscular TOI were similar between normoxic conditions (NN and HN) at rest
217 (**Table 3**) and exercise (**Table 4**). At rest, TOI in VL was similar between the two hypoxic
218 conditions and NN but was lower than in HN. During exercise, TOI in VL was lower in
219 hypoxia when compared to normoxic conditions as well as in HH vs. NH ($p<0.001$).
220 Moreover, change in [O_2Hb] was lower ($p=0.002$) and [HHb] greater ($p<0.001$) in HH
221 compared to all other conditions in the VL. There was no significant difference in [tHb]
222 between conditions.

223

224 **DISCUSSION**

225 The present study investigated the effect of hypobaria on cerebral and muscular oxygenation
226 at rest and during moderate-intensity exercise in both normoxic and hypoxic conditions. The
227 influence of hypobaria in hypoxia confirmed the already known greater ventilatory stimulus
228 (i.e., increased minute ventilation) and blood oxygen desaturation. Of importance is the novel
229 finding of an additive effect of hypobaria *per se* observable only in hypoxia, at rest in the
230 cerebral areas, and during exercise in both cerebral and muscular compartments.

231

232 **Cardiorespiratory responses: greater stimulus of hypobaria in hypoxia**

233 In the present study, minute ventilation was similar between conditions at rest but was greater
234 in HH versus NH at 5000 m during exercise, implying a slight additive influence of hypobaria
235 on minute ventilation when exposed to acute severe hypoxia. Although unclear since minute
236 ventilation was shown as similar between NH and HH (17, 18), the hypobaric effect *per se*
237 on ventilation has already been shown (19). Moreover, hypobaria affected pulmonary
238 resistance through pressure gradient changes (20). In hypobaric normoxia, the O_2 pressure

gradient between the pulmonary alveolus and the gas is similar to sea level values, but the CO_2 pressure gradient is greater than in normobaria (i.e., NN and NH), which may cause a decrease in $P_{et}CO_2$ as observed in the present study. $P_{et}CO_2$ values were equivalent between HN and HH but were lower than in normobaric conditions. Hypocapnic state likely induced pulmonary vasoconstriction, which may have affected the O_2 diffusion capacity from the alveolar compartment to the blood in hypobaric normoxia. In hypobaric hypoxia, both the pulmonary hypoxic vasoconstriction and the reduction in O_2 gradient from the alveoli to the pulmonary circulation may decrease arterial O_2 content, as indirectly shown by the lower S_pO_2. These differences may be related to a larger physiological dead-space with hypobaria (5) or a greater hypoxic pulmonary vasoconstriction that could result in changes of alveolar dead space and altered ventilation–perfusion ratio (19). During exercise, the hypocapnia-induced cerebral vasoconstriction seems partially compensated by the hypoxemia- and exercise-mediated vasodilation.

Of interest is that HR was around 10 bpm higher in HH than in NH suggesting greater cardiorespiratory responses at exercise with hypobaria in acute severe hypoxia (5000 m). The principal factor of performance impairment between HH and NH seemed to be the altered convection in HH at rest, as well as during exercise (21), and the present results are in line with previous studies (5, 22, 23) or a review (24). Potential mechanisms greater intravascular bubble formation and ventilation/perfusion disparity, greater alveolar dead space as well as changes in alveolar fluid permeability and chemosensitivity in HH when compared to NH (24, 25). On the contrary, hypobaria did not have any additional influence on S_pO_2 in normoxia, which could explain equivalent cardiorespiratory responses between both normoxic conditions (NN and HN).

Cerebral blood flow and oxygenation

When resting in acute hypoxia (from minutes to hours), cerebral vasodilatation (i.e., increase of MCA diameter) occurs to limit the cDO_2 decrease (26–28), which leads to a rise in cerebral oxygen delivery by 0.5-2.5% per 1% decreasing SaO_2 (29–31). Simultaneously, oxygen arterial content is reduced leading to ventilatory drive stimulation (32). Hypoxia-induced hyperventilation and concomitant hypocapnia result in a vasoconstrictor stimulus, resulting in little change in cerebral blood flow (33, 34). However, despite hypocapnia, vasodilation typically prevails as consistent increase in CBF is observed at altitude (35). There are several studies demonstrating the compensatory elevation in CBF upon acute exposure to isocapnic hypoxia to maintain cDO_2 [for review see (36)]. Thus, cDO_2 in acute hypoxia is related to cerebral vasodilation, which compensates the hypocapnic vasoconstriction induced by chemoreflex-driven ventilation (37). In the present study, MCAv significantly increased while estimated cDO_2 was slightly lower in HH than NN at rest. However, MCAv and cDO_2 showed comparable values between NH and NN.

During exercise, however, it remains unclear how hypobaria influences ventilation and CBF regulation when exposed to acute hypoxia. Cerebral oxygenation has been reported to decrease during submaximal exercise while MCAV was increased after prolonged exposure to high altitude (38). In the present study, estimated cDO_2 remained comparable between conditions, but with a significant increase in MCAv in HH only. However, on limitation is that MCA diameter has not been measured and is assumed constant. Nevertheless, NIRS measurements showed significant decrease in cerebral oxygenation in hypoxic conditions at rest and during submaximal exercise. In the present study, there was a greater decrease in $[O_2Hb]$ and greater increase in [HHb] in HH versus NH that implies a greater cerebral deoxygenation. Moreover, cerebral TOI significantly decrease in hypoxic conditions, and to greater extent in HH versus NH, confirming the higher severity of the hypoxic stimulus in the HH condition.

289

290 **Muscular oxygenation**

291 Muscle oxygenation parameters assessed by NIRS is known to reflect the metabolic changes
292 that occur at the muscle level (39). In the present study, muscle TOI did not differ between
293 control and hypoxic conditions at rest, but significantly decreased in NH and HH during
294 exercise. Interestingly, muscle TOI was reduced to greater extent in HH vs NH, which implies
295 – once again - an additive influence of hypobaria on muscle deoxygenation in hypoxia. In
296 previous studies, cerebral, but not muscle, tissue showed greater deoxygenation during acute
297 hypoxia at rest (13, 14). Moreover, Muscle TOI remained equivalent between normoxia and
298 hypoxia after a 15-min rest period, whereas SpO_2 and cerebral TOI significantly decreased in
299 hypoxia (15). The present results thus confirmed previous findings (13–15) and showed that
300 cerebral oxygenation significantly decreased in hypoxia during rest and exercise, whereas
301 muscle oxygenation remained comparable between normoxia and hypoxia at rest. However,
302 the present study adds novelty by showing an additive influence of hypobaria on muscle
303 deoxygenation (i.e., decreased muscle TOI and [O_2Hb], with increased [HHb]) at exercise)
304 when exposed to acute hypoxia. We hypothesize that this latter difference may be related to a
305 larger hypoxemia (lower SpO_2) and slight greater ventilatory response, which lead to a more
306 pronounced hypocapnia in HH altering the oxygen diffusion. These differences were not
307 observed between NN and HN, implying that the influence of hypobaria on muscle
308 oxygenation is negligible in normoxia.

309

310 **Conclusion**

311 To our knowledge, this study was the first to evaluate the effect of hypobaria in both
312 normoxic and hypoxic conditions on ventilatory, physiological and oxygenation parameters.
313 The present results demonstrate a specific effect of hypobaria *per se* on cerebral and muscular

oxygenation at exercise that were observed in hypoxia and not in normoxia, and was attributed primarily to a larger desaturation and a greater ventilatory response. The latter induced larger hypocapnia, which may have triggered larger pulmonary vasoconstriction affecting O_2 diffusion from the alveolar area to the blood. This finding is of interest for altitude physiology and aviation since athletes and military pilots regularly train either in normobaric or hypobaric hypoxia. To conclude, hypobaria decreased to greater extent cerebral and muscular oxygenation during submaximal cycling exercise in hypoxia, whereas it had negligible influence in normoxia.

Acknowledgments

The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by ACSM.

Figure legend

Figure 1

Respiratory frequency (Rf), tidal volume (VT) and minute ventilation ($\dot{V}E$) in normobaric normoxia (NN), hypobaric normoxia (HN), normobaric hypoxia (NH) and hypobaric hypoxia (HH), during rest (baseline, BSL) and exercise.

* $p < 0.05$ for difference with NN, # $p < 0.05$ for difference with HN, † $p < 0.05$ for difference with NH, +++ $p < 0.001$ for difference with BSL values in all conditions.

336

337 **Disclosure**

338 MRA, NB, DB and GPM have no conflicts of interest, sources of funding, or financial ties to
339 disclose and no current or past relationship with companies or manufacturers who could
340 benefit from the results of the present study. MRA is an employee of armasuisse. NB is an
341 employee of be.care SA. The remaining authors declare that the research was conducted in the
342 absence of any commercial or financial relationships that could be construed as a potential
343 conflict of interest.

344

345 **Author contribution**

346 MRA, NB and GPM designed the study. MRA collected the data. MRA and NB analysed the
347 data. MRA wrote the article. GPM reviewed the article. All the authors approved the final
348 version of the manuscript and declare no conflict of interest.

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		NN	HN	NH	HH
V̇E (L/min, btps)	**Baseline**	12.5 ± 2.3	13.3 ± 3.1	12.4 ± 2.6	14.6 ± 2.4
	Exercise	33.9 ± 6.3	33.9 ± 6.4	37.7 ± 7.9	46.9 ± 7.6 *** ### †
Rf (bpm)	**Baseline**	16.3 ± 4.0	17.1 ± 4.4	17.4 ± 5.1	17.0 ± 3.7
	Exercise	21.7 ± 5.3	22.8 ± 5.8	23.0 ± 5.3	25.1 ± 5.8
VT (bpts)	**Baseline**	0.81 ± 0.19	0.80 ± 0.32	0.77 ± 0.29	0.94 ± 0.15
	Exercise	1.61 ± 0.35	1.57 ± 0.48	1.75 ± 0.50	1.92 ± 0.42

Table 1: Ventilatory parameters at rest (baseline) and exercise. Minute ventilation (V̇E), respiratory frequency (Rf) and tidal volume (VT). Normobaric normoxia (NN); hypobaric normoxia (HN); normobaric hypoxia (NH); hypobaric hypoxia (HH). ***p<0.001 for difference with NN; ### p<0.001 for difference with HN; † p<0.001 for difference with NH.

		NN	HN	NH	HH
HR (bpm)	**Baseline**	72.0 ± 13.7	73.6 ± 14.5	77.4 ± 13.7	86.5 ± 17.7 *
	Exercise	105.7 ± 15.6	108.3 ± 16.4	118.8 ± 15.4 *** ##	131.2 ± 16.9 *** ### ††
SpO_2 (%)	**Baseline**	99.4 ± 0.5	98.3 ± 2.1	83.5 ± 6.0 *** ###	74.7 ± 5.1 *** ### †
	Exercise	99.2 ± 0.9	97.6 ± 1.9	80.8 ± 4.2 *** ###	69.2 ± 5.7 *** ### †††
MCAv (cm/s)	**Baseline**	42.7 ± 5.8	41.5 ± 5.2	46.1 ± 5.6 (*) ##	48.4 ± 7.1 * ##
	Exercise	48.1 ± 6.3	47.9 ± 6.5	51.5 ± 7.2	56.8 ± 6.2 ** #
cDO_2 (n.u.)	**Baseline**	903.7 ± 136.2	856.4 ± 123.2	803.7 ± 128.5	808.3 ± 153.0 * (#)
	Exercise	1011.4 ± 154.9	976.5 ± 168.4	901.2 ± 157.1	870.6 ± 126.5
RPE	**Exercise**	8.1 ± 1.3	9.1 ± 1.3	11.3 ± 2.2 *** ###	11.8 ± 2.3 *** ###

Table 2: Data are mean ± SD during baseline and exercise periods. Heart rate (HR), pulse oxygen saturation (SpO_2), middle cerebral artery velocity (MCAv), estimated cerebral oxygen delivery (cDO_2) and BORG scale values. Normobaric normoxia (NN); hypobaric normoxia (HN); normobaric hypoxia (NH); hypobaric hypoxia (HH). *p<0.05, **p<0.01 and ***p<0.001 for difference with NN; # p<0.05, ## p<0.01 and ### p<0.001 for difference with HN; † p<0.05, †† p<0.01 ††† p<0.001 for difference with NH.

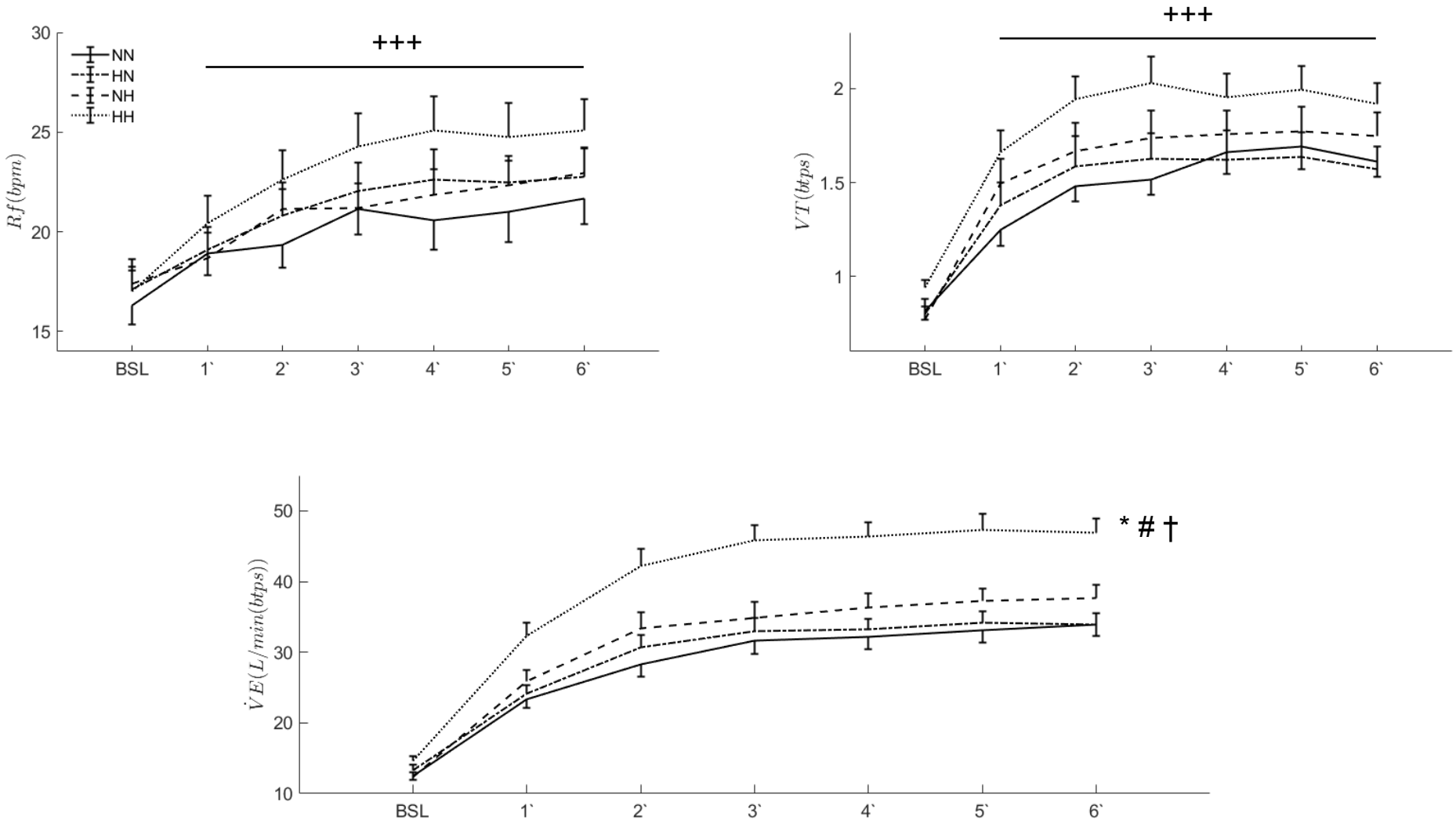
	Rest	NN	HN	NH	HH
Prefrontal	[**O_2Hb**] (µm)	5.512 ± 5.629	0.092 ± 4.036 **	-2.549 ± 7.779 *** #	-10.034 ± 7.404 *** ### †††
	[**HHb**] (µm)	2.938 ± 1.906	-0.363 ± 2.513 ***	13.056 ± 5.694 *** ###	11.675 ± 5.491 *** ### †
	TOI (%)	73.28 ± 4.75	72.66 ± 4.26	65.14 ± 5.25 *** ###	61.65 ± 5.07 *** ### †††
	[**tHb**] (µm)	1.166 ± 0.181	1.188 ± 0.266	1.339 ± 0.516	1.177 ± 0.216
Muscular (VL)	[**O_2Hb**] (µm)	0.718 ± 2.747	-0.319 ± 3.945	0.088 ± 2.644	-4.571 ± 3.603 *** # †††
	[**HHb**] (µm)	5.466 ± 4.163	-1.482 ± 4.775 ***	7.459 ± 5.725 * ###	4.393 ± 4.346 ### †††
	TOI (%)	65.97 ± 4.19	67.24 ± 4.97	64.70 ± 3.03 ##	64.87 ± 3.21 ##
	[**tHb**] (µm)	1.073 ± 0.071	1.075 ± 0.043	1.085 ± 0.070	1.085 ± 0.060

Table 3: Data are mean ± SD during resting period. Change in near-infrared spectrometry (NIRS) parameters: Concentration's changes in Oxy-Δ[O_2Hb], deoxy- Δ[HHb] and total haemoglobin Δ[tHb] and tissue oxygenation index (TOI) in cerebral and muscular (vastus lateralis, VL) regions. Repeated-measures Friedman (non-parametric) ANOVA was assessed for condition comparison. Normobaric normoxia (NN); hypobaric normoxia (HN); normobaric hypoxia (NH); hypobaric hypoxia (HH).*p<0.05, **p<0.01 and ***p<0.001 for difference with NN; # p<0.05, ## p<0.01 and ### p<0.001 for difference with HN; † p<0.05, †† p<0.01 ††† p<0.001 for difference with NH

	Exercise	NN	HN	NH	HH
Prefrontal	[**O_2Hb**] (µm)	4.693 ± 5.361	0.145 ± 4.591 ***	-2.908 ± 6.072 ***	-12.324 ± 8.186 *** ### †††
	[**HHb**] (µm)	2.562 ± 2.197	-0.167 ± 3.587 ***	12.322 ± 5.188 *** ###	15.244 ± 4.940 *** ### †††
	TOI (%)	72.86 ± 4.81	72.20 ± 4.75	64.77 ± 5.73 *** ###	57.93 ± 5.05 *** ### †††
	[**tHb**] (µm)	1.149 ± 0.191	1.166 ± 0.227	1.330 ± 0.440 * #	1.205 ± 0.212
Muscular (VL)	[**O_2Hb**] (µm)	-0.876 ± 3.132	-3.582 ± 3.883 **	-3.409 ± 3.766 *	-11.746 ± 4.295 *** ### †††
	[**HHb**] (µm)	-0.237 ± 5.224	-3.807 ± 6.623 ***	5.759 ± 6.163 *** ###	5.786 ± 6.354 *** ### †
	TOI (%)	67.85 ± 4.79	66.69 ± 6.33	62.96 ± 6.26 *** ###	59.08 ± 6.11 *** ### †††
	[**tHb**] (µm)	1.025 ± 0.062	1.040 ± 0.061	1.064 ± 0.064 *** #	1.064 ± 0.057 *** #

Table 4: Data are mean ± SD during exercise period. Change in near-infrared spectrometry (NIRS) parameters: Concentration's changes in Oxy-Δ[O_2Hb], deoxy- Δ[HHb] and total haemoglobin Δ[tHb] and tissue oxygenation index (TOI) in cerebral and muscular (vastus lateralis, VL) regions. Repeated-measures Friedman (non-parametric) ANOVA was assessed for condition comparison. Normobaric normoxia (NN); hypobaric normoxia (HN); normobaric hypoxia (NH); hypobaric hypoxia (HH). *p<0.05, **p<0.01 and ***p<0.001 for difference with NN; # p<0.05, ## p<0.01 and ### p<0.001 for difference with HN; † p<0.05, †† p<0.01 ††† p<0.001 for difference with NH.

Figure 1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Appendices

Appendix A: Cardiovascular and cerebral responses during a vasovagal reaction without syncope (article 7)

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CASE REPORT
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Cardiovascular and Cerebral Responses During a Vasovagal Reaction Without Syncope

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This clinical case report presents synchronous physiological data from an individual in whom a spontaneous vasovagal reaction occurred without syncope. The physiological data are presented for three main phases: Baseline (0–200 s), vasovagal reaction (200–600 s), and recovery period (600–1200 s). The first physiological changes occurred at around 200 s, with a decrease in blood pressure, peak in heart rate and vastus lateralis tissue oxygenation, and a drop in alpha power. The vasovagal reaction was associated with a progressive decrease in blood pressure, heart rate and cerebral oxygenation, whilst the mean middle cerebral artery blood flow velocity and blood oxygen saturation remained unchanged. Heart rate variability parameters indicated significant parasympathetic activation with a decrease in sympathetic tone and increased baroreflex sensitivity. The total blood volume and tissue oxygenation index (TOI) dropped in the brain but slightly increased in the vastus lateralis, suggesting cerebral hypoperfusion with blood volume pooling in the lower body part. Cerebral hypoperfusion during the vasovagal reaction was associated with electroencephalography (EEG) flattening (i.e., decreased power in beta and theta activity) followed by an EEG high-amplitude "slow" phase (i.e., increased power in theta activity). The subject developed signs and symptoms of pre-syncope with EEG flattening and slowing during prolonged periods of symptomatic hypotension, but did not lose consciousness.

Keywords: vasovagal mechanism, pre-syncope symptoms, hypotension and bradycardia, cerebral hypoperfusion, EEG flattening and slowing

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INTRODUCTION

Vasovagal reactions include arterial vasodilation and bradycardia as mechanisms that may precipitate a syncopal response (Lewis, 1932); defined as a transient loss of consciousness caused by cerebral hypoperfusion followed by spontaneous recovery (Freeman et al., 2011). Syncope is a common cause of transient loss of consciousness among children and adults (Ganzeboom et al., 2003). Vaso-vagal events are usually triggered by parasympathetic over-activation associated with a reduced sympathetic response (Gunnar Wallin and Sundlöf, 1982), which in turn causes a reduction in cardiac output, hypotension and cerebral hypoperfusion (Wieling et al., 2016). Arterial blood pressure decreases below the auto-regulatory threshold (Van Lieshout et al., 2003) and cannot be compensated for by the delayed

normal auto-regulatory reflex-loop blood pressure adjustment of the baroreflex (Chapleau, 2003), leading to cerebral hypoperfusion (Lund et al., 2017). Furthermore, parasympathetic activation corresponds to an increase in heart rate variability and is related with slow alpha power on the electroencephalography (EEG), mainly in the frontal area (Takahashi et al., 2005). Individuals with anxiety often exhibit a desynchronization in alpha frequency during attentional tasks (Ward et al., 2018). The EEG signal shows EEG slowing during vasovagal reaction without and with syncope (loss of consciousness). Hypotension without syncope is associated with EEG slowing (Heyer et al., 2016) and may be associated with prolonged hypotension (Heyer, 2019). EEG slowing corresponds with a shift from high to low frequencies. Previous reports on vasovagal syncope describe two types of central nervous system activity: a reduction in brain-wave amplitude during hypotension (Ammirati et al., 1998), with EEG signal flattening during cerebral hypoperfusion (Dijk et al., 2014). The usual symptoms of vasovagal reactions are warmth, nausea, altered concentration and visual disturbance (Jardine et al., 2018). Despite the numerous mechanisms involved, there is a scarcity of synchronous physiological data measured during spontaneous vasovagal reactions. This case study reports continuous physiological changes with simultaneous EEG results during a spontaneous and unexpected vasovagal reaction without loss of consciousness, which occurred in a young individual participating in a clinical trial.

PARTICIPANT CHARACTERISTICS

The 20-year-old subject was a tall, thin (178 cm, 52.3 kg, BMI 16.5*)* male flight attendant with no significant medical history. The subject was participating in a study investigating cerebral responses to low intensity cycling, in a small room of 22 m^3 in which he may have felt confined. No other participants (n = 20) reported discomfort during the clinical trial. The initial electrocardiogram (performed supine and at rest before study enrolment) was normal (PR: 110 ms, PQ: 140 ms, QRS: 96 ms, QT: 386 ms and QTc: 410 ms, no ST/T changes). The vasovagal reaction occurred unexpectedly at rest directly after the start of recordings. The subject remained conscious during vasovagal reaction but showed decreased postural tone and auditory impairment. The study he was participating in was approved by the ethical committee of Zürich, Switzerland (2018-00006). Written informed consent was obtained before study enrolment and for the publication of this case report. This clinical trial is accessible on ClinicalTrials.gov (NCT03439202).

MATERIALS AND METHODS

Electroencephalography activity signals were recorded from 19 bipolar EEG channels and sampled at 200 Hz. Pre-processing was carried out with custom-written MATLAB code (MathWorks Inc.) and the EEGLAB analysis tools (Delorme and Makeig, 2004). The data set was filtered between 0.5 and 70 Hz using a zero-phase Butterworth filter (Notch-filter was set to 50 Hz) before an independent component analysis was used to remove blink artifacts. The signals were re-referenced to a common average. A time-frequency analysis based on a continuous complex Morlet's wavelet transformation of the signal (between 1 and 45 Hz with 0.5-Hz steps) was carried out. Time-frequency power values were converted to decibel units (dB) and a baseline was calculated as the mean value of the first 60 s of the signal subtracted from the time-frequency power of the whole data. Power values in theta (4–7 Hz), alpha (8–13 Hz), beta (13.5–30 Hz), and gamma (30.5–45 Hz) frequency bands were averaged in periods of 1 min for each electrode.

Continuous blood pressure was measured at the middle and index finger of the left hand using a double pneumatic cuff (NIBP100D, BIOPAC Systems, Inc., Goleta, CA, United States) and acquired at 500 Hz. Baroreflex sensitivity was calculated using the sequence method (Parati et al., 1988) by (1) extracting systolic blood pressures (SBP) and inter-beat intervals (IBI) from the BP trace and (2) identifying at least three consecutive beats in which an increase (or decrease) of at least 1 mmHg in SBP is followed by an increase (or decrease) of at least 5 ms in IBI. For each of these SBP-IBI sequences, the slope of the regression line was calculated when correlation coefficient was $\geq$0.85. BRS was the average of all slopes for each phase.

Heart rate and R-R intervals were recorded using a heart rate monitor (Polar RS800CX, FI-90440 Kempele, Finland). Ectopic beats in the R-R series were compensated for by interpolation of means to obtain normal-to-normal intervals. Mean heart rate and root mean square of the successive differences (RMSSD) were computed from the normal-to-normal intervals and spectral power in the high-frequency band (HF, 0.15–0.40 Hz) was computed using the Welch method after resampling normal-to-normal intervals at 4 Hz. All signal processing was performed using MATLAB® 2015a (MathWorks, 160 Natick, MA, United States). Middle cerebral artery velocity was measured in the left middle cerebral artery trough left temporal window using a transcranial doppler (Spencer technology, Redmond, WA 98052-2559 United States) and acquired at 500 Hz. Finger arterial oxygen saturation was monitored using a finger oximeter (Wristox 3150 with 8000SM-WO Sensor, Nonin, Plymouth, MN, United States) and acquired at 0.5 Hz. Cerebral and muscular oxygenation was measured using near-infrared spectroscopy technology (NIRO-200-NX, Hamamatsu Photonics, Japan) and acquired at 1 Hz. Two detection and emission probes were located on the forehead and on the vastus lateralis in order to measure tissue oxygenation index (TOI), defined as the ratio of oxy-hemoglobin to change of total hemoglobin concentration, and relative concentration of total hemoglobin (tHb, normalized tissue hemoglobin index) in cerebral and muscular areas.

RESULTS

The participant remained seated for 45 min during device installation and experimental trial preparation. He demonstrated a normal health state at baseline (0–200 s in **Figures 1–3**). The first physiological changes were observed at about 200 s with a progressive decrease in blood pressure, a drop in alpha

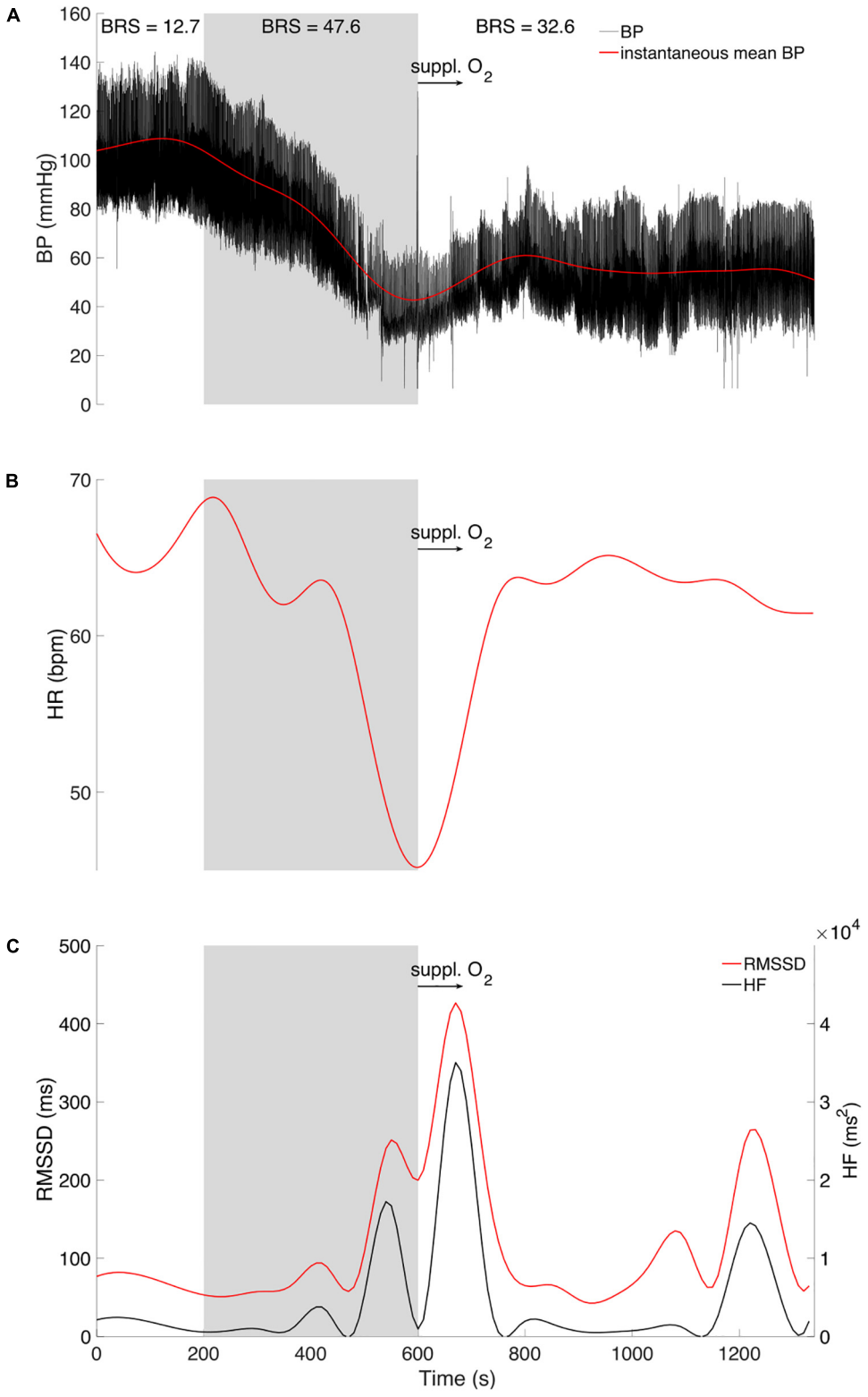

FIGURE 1 | Original tracing of instantaneous **(A)** arterial blood pressure (BP, black trace) and mean value (red trace), **(B)** Heart rate (HR) in red tracing, and **(C)** Root mean square of the successive differences (RMSSD, red trace) and high frequency power (HF, black trace). Displayed for three phases: Baseline (0–200 s), vasovagal reaction (gray area, 200–600 s), and recovery period (600–1200 s) with brief 100% medical oxygen administration (black arrow, 600–700 s). Baroreflex sensitivity (BRS) is presented for each of the three phases.

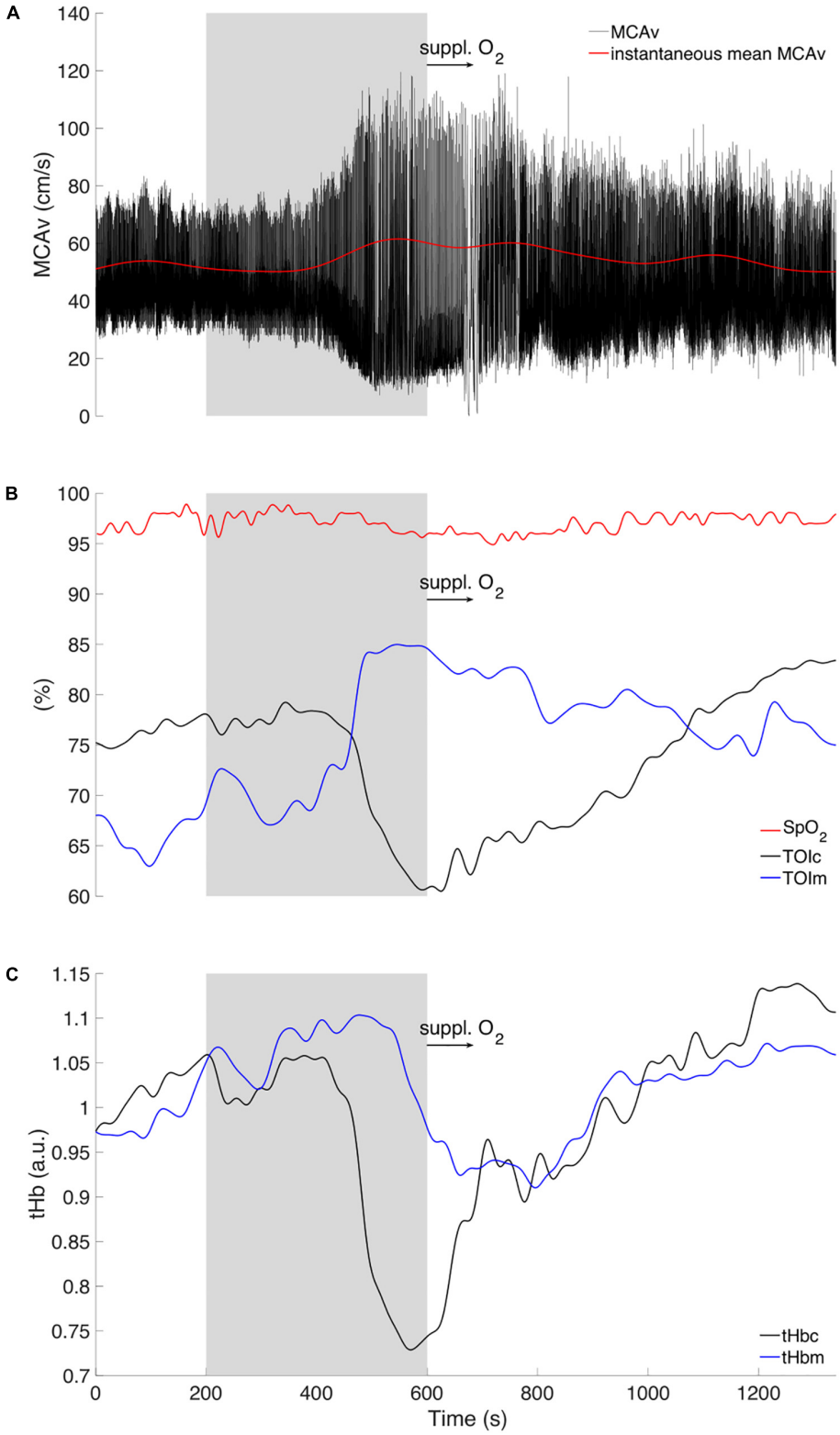

FIGURE 2 | Original tracing of instantaneous **(A)** middle cerebral artery velocity (MCAv, black trace) and mean value (red trace). **(B)** Pulse oxygen saturation (SpO_2) (red trace). Hemodynamic responses of cerebral tissue (TOI_c) and muscular (TOI_m) oxygenation index in black and blue tracings, respectively. **(C)** Tracings represent cerebral (tHb_c, black trace) and muscular (tHb_m, blue trace) total hemoglobin relative concentrations. Displayed for three phases: Baseline (0–200 s), vasovagal reaction (gray area, 200–600 s), and recovery period (600–1200 s) with brief 100% medical oxygen administration (black arrow, 600–700 s).

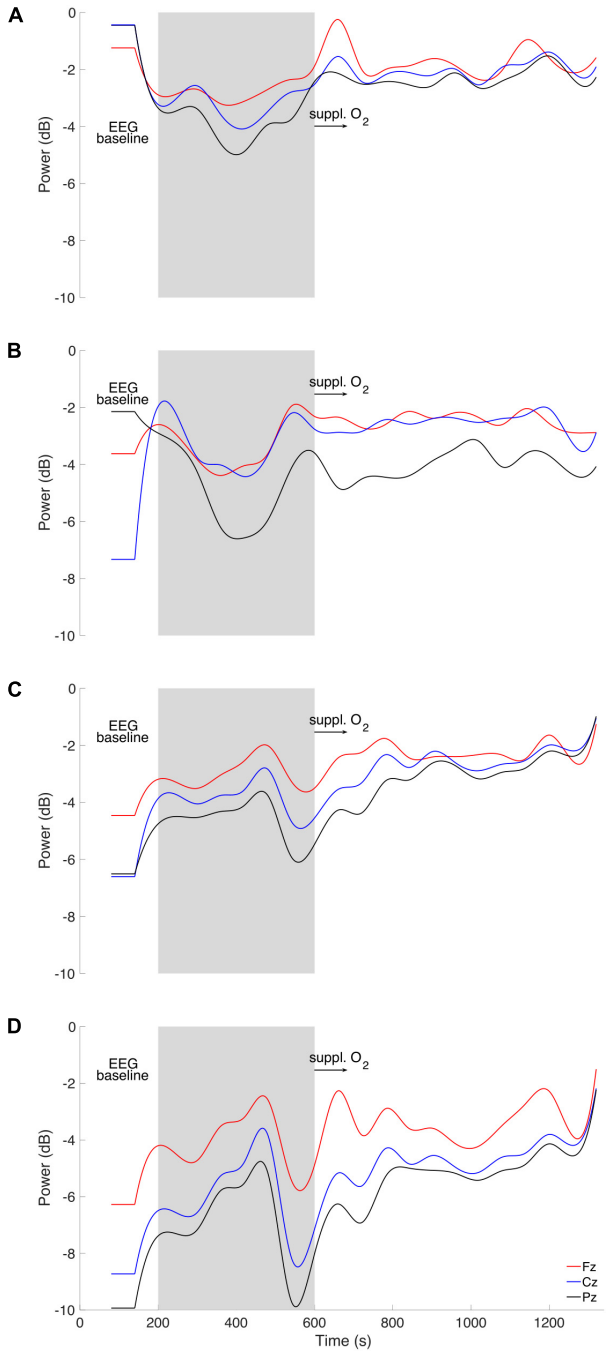

FIGURE 3 | Time course of EEG **(A)** theta, **(B)** alpha, **(C)** beta, and **(D)** gamma power (in decibel; dB) for frontal (Fz, red), central (Cz, blue) and posterior (Pz, black) electrodes are displayed for three phases: Baseline (0–200 s), vasovagal reaction (gray area, 200–600 s), and recovery period (600–1200 s) with brief 100% medical oxygen administration (black arrow, 600–700 s).

power as well as a peak in HR and vastus lateralis TOI. The participant started having postural tone alterations when the vasovagal reaction occurred, but he did not lose consciousness. He communicated discomfort, felt dizzy and nauseous and looked pale. His vision darkened with flashing in his eyes and his hearing and concentration were altered. His symptoms resolved slowly during the recovery period (600–1200 s), after brief 100% medical oxygen administration (600–700 s).

His blood pressure started to progressively decrease at around 200 s (**Figure 1A**) with a simultaneous peak in HR (i.e., +5 bpm, **Figure 1B**), vastus lateralis TOI (i.e., +10%, **Figure 2B**), and systematic variations in EEG (i.e., drop in alpha power, **Figures 3B**, **4**). After 200 s, the blood pressure showed classically reported behavior, with a progressive decrease (≈5 min), followed by steady low values (mean blood pressure ≈40 mmHg). Baroreflex sensitivity increased significantly during the vasovagal reaction. Bradycardia occurred simultaneously with the reduction in blood pressure. RMSSD and HF signals increased during, and shortly after the vasovagal reaction (**Figure 1C**). While peak systolic MCAv increased slightly, diastolic MCAv drastically decreased (<30 mmHg; **Figure 2A**), resulting in constant MCAv. Pulse oxygen saturation remained unchanged (**Figure 2B**). The cerebral oxygenation index decreased by ≈15% and total hemoglobin relative concentration by ≈25% (**Figures 2B,C**). However, the muscular oxygenation index increased by 20% in the vastus lateralis. Total hemoglobin in the frontal area decreased by 25%, while it remained stable in the vastus lateralis. EEG beta and gamma power decreased between 500 and 550 s, which corresponds to EEG flattening during cerebral hypoperfusion (Dijk et al., 2014; **Figures 3C,D**). EEG alpha power substantially decreased before the symptomatic vasovagal reaction (**Figure 3B**) in the frontal, central and posterior brain regions. This EEG switch from high to low frequencies corresponds to simultaneous EEG slowing (Heyer et al., 2016) and was associated with hypotension during pre-syncope. The EEG signal did not show any signs of epilepsy.

During the recovery period after brief 100% O_2 inhalation (600–700 s), the participant remained unwell (with pallor and gastrointestinal discomfort). His blood pressure gradually increased but to values lower than baseline (**Figure 1**), whilst his heart rate increased nearly back to normal RMSSD and HF decreased back to baseline values, despite a small peak ≈10 min after vasovagal reaction. The diastolic MCAv increased progressively back to baseline values, whilst the systolic MCAv remained higher than baseline (**Figure 2**). The cerebral TOI and total relative hemoglobin concentration increased gradually to baseline. EEG alpha power returned to normal in the frontal and central regions following the vasovagal reaction but remained low in the posterior regions (**Figure 3**). The recovery period was associated with EEG slowing with an increase only in power of theta frequency (**Figure 3A**). EEG beta and gamma power returned to baseline, corresponding to the end of the "flat" phase. The participant remained conscious at all times.

DISCUSSION

This case report presents continuous and synchronous hemodynamic and EEG changes during a spontaneous and unexpected vasovagal reaction. Cerebral hypoperfusion, de-oxygenation and EEG alpha power in the fronto-central and

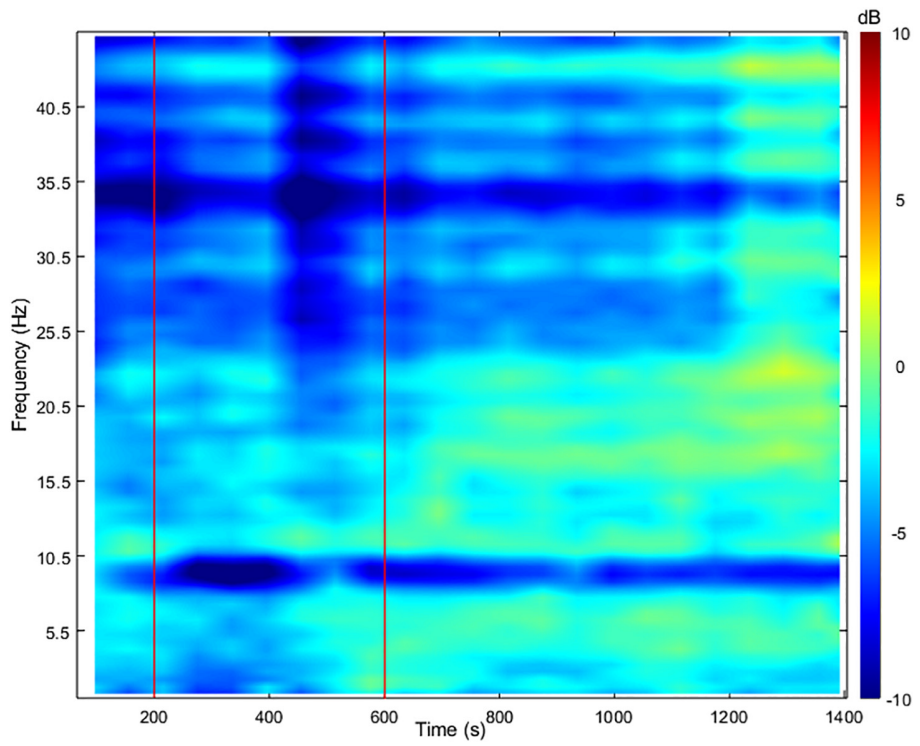

FIGURE 4 | Change in time-frequency power spectrum from baseline on the posterior (Pz) electrode. Two vertical red lines delimit the vasovagal reaction (200–600 s).

posterior brain regions were decreased. The vasovagal reaction was associated with EEG flattening (i.e., reduced power in beta and gamma activity) followed by a "slow" phase (i.e., increased power in theta activity). These continuous recordings give insight to the succession of events before, during and following vasovagal reaction without syncope.

The peaks in HR and TOI in the vastus lateralis, with associated blood pressure decrease and EEG systematic variations, suggest that the onset of the vasovagal reaction was at around 200 s after the start of the recordings. The decrease in blood pressure and HR are likely due to the parasympathetic over-activation (RMSSD and HF increase) known to trigger vasovagal reactions, which in turn decrease cardiac output (Jørgensen et al., 1993). Baroreflex sensitivity increased drastically during vasovagal reaction, indicating that the auto-regulatory loop was overwhelmed by the large and sudden drop in blood pressure probably induced by a systemic vasodilation. The EEG amplitude decrease is not delayed when compared to blood pressure, which favors a connection between cortical areas and the medulla. If cerebral hypoperfusion had altered the EEG, its traces would have changed after the MCAv signal was detected, which in our case would have been around 400 s, whilst EEG traces dipped at around 200 s. Moreover, the electrocortical depression (expressed by a drop of theta and alpha power amplitudes) has previously been related to cerebral hypoperfusion and bradycardia (Dijk et al., 2014). Therefore, the posterior alpha power decrease may be interpreted as cerebral reactivity to visual and attentional dysfunction as reported by the participant. Cerebral hypoperfusion probably occurred primarily because of decreased blood pressure. The resulting cerebral hypoperfusion is considered as a primary driver in a vasovagal reaction. Overall, our data suggest that the onset of the vasovagal reaction at around 200 s was associated with a peak in heart rate and a progressive drop in blood pressure with a simultaneous drop in alpha power (i.e., desynchronization).

The participant reported being anxious in the reduced environment where the measurements took place. In addition, the participant's equipment may have added to his anxiety. The start of the recordings may have triggered the vasovagal reaction and pre-syncope.

The EEG is among the first parameters to change and to recover at around 550 s, whilst blood pressure, heart rate, and MCAv remained abnormal. As a consequence, cardiac output and cerebral perfusion were reduced. In this case, cerebral hypoperfusion may be limited by the fact that the participant was seated (as opposed to standing) during the vasovagal reaction, which may explain why he did not lose consciousness. Despite limited orthostatic pressure, the parasympathetic over-activation (and sympathetic withdrawal in the muscles) still induced blood pooling in the lower limbs (Gunnar Wallin and Sundlöf, 1982), likely via systemic vasodilation (Lund et al., 2017). This can be seen from the NIRS traces, which showed marked decrease in the brain from 400 to 600 s and a light increase in the vastus lateralis over the same period. The data presented show the lasting effects of the vasovagal reaction with prolonged hypotension up to 6 min, during which SBP remained low

(<80 mmHg). Patients with prolonged hypotension have longer EEG slowing phase during vasovagal reaction without syncope (Heyer, 2019). Moreover, EEG slowing was related to cerebral hypoperfusion and bradycardia (Dijk et al., 2014) and denotes changes of synaptic function due to cerebral ischemia during vasovagal syncope (Hofmeijer and van Putten, 2011). One may speculate that fast EEG switching to baseline values may explain why our participant did not lose consciousness. Our participant experienced vasovagal mechanisms with pre-syncope symptoms, but without progressing to syncope.

CONCLUSION

We have presented simultaneous physiological recordings during spontaneous and unexpected vasovagal reaction without loss of consciousness in a young individual. The onset of the vasovagal reaction was associated with a peak in heart rate and progressive decrease in blood pressure, with a simultaneous drop in alpha power. Parasympathetic over-activation led to hypotension and cerebral hypoperfusion. His emotional status (anxiety and feeling of oppression) and concomitant prolonged hypotension and bradycardia with EEG flattening are in favor of a vasovagal mechanism without syncope. The recovery period was associated with EEG slowing and restoration of normal EEG pattern and arousal.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Zurich, Switzerland (2018-00006). The patient/participant provided his written consent to participate in this study.

AUTHOR CONTRIBUTIONS

MA, NB, GM, and DB conceived and designed the research. MA performed the experiments and drafted the manuscript. MA, NB, HM, and JB analyzed the data and prepared the figures. MA, NB, JB, GM, and DB interpreted the results of the experiments. MA, NB, EN, JB, GM, and DB edited and revised the manuscript. All authors approved the final version of manuscript.

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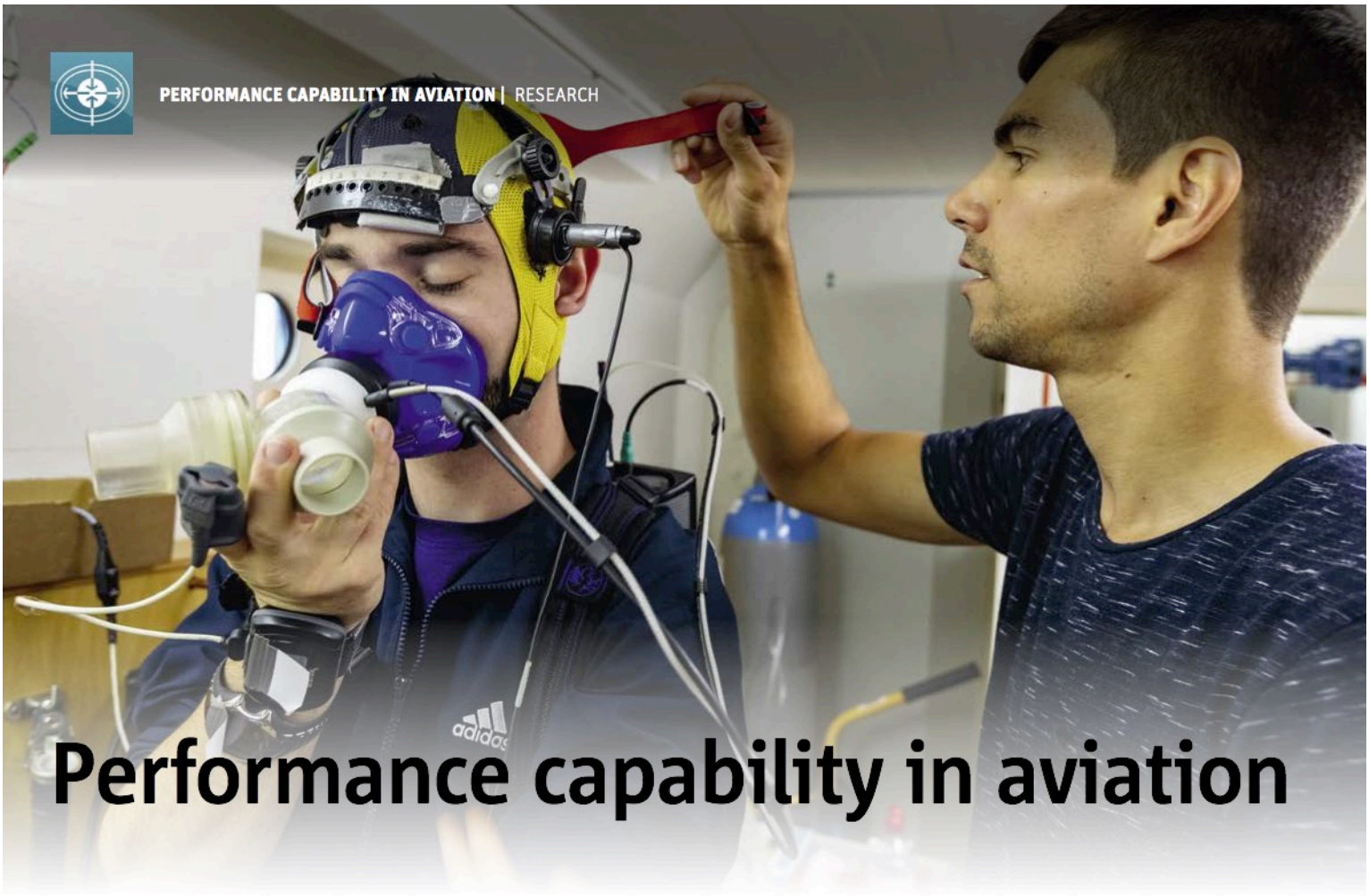
Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix B: Armasuisse: Performance capability in aviation

Article published in the journal of Armasuisse science and technology in Mai 2021.



Performance capability in aviation

Fighter pilots are under a high degree of stress during their missions. Oxygen deficiency can lead to dangerous situations. The Aeromedical Centre (AMC) of the Swiss Air Force has developed a procedure with armasuisse Science and Technology for minimising the risks of oxygen deficiency in flight. An interview with Mathias Aebi, PhD student in high altitude physiology at the AMC in collaboration with the Institute of Sport Sciences (ISSUL) at the University of Lausanne.

Interview with Mathias Aebi, conducted by Dr Philip Noser

Using the hypobaric chamber, it is possible to simulate conditions at different heights, in particular oxygen deficiency, which can be dangerous for pilots during their missions.

Mathias Aebi, what makes it particularly interesting for you to do research on behalf of armasuisse at the AMC for the Air Force?
The Air Force has the only hypobaric chamber in Switzerland. Using this, it is possible to simulate conditions at different heights, in particular oxygen deficiency, which can be dangerous for pilots during their missions. As an example: The chamber simulates an ascent to an altitude of 5,500 meters in just 1.5 minutes. This imitates a rapid decompression, i.e. a barometric pressure decrease.

What questions do you investigate at the AMC?
I am interested in the effects of oxygen deficiency, called hypoxia, and low pressure, known in medical terminology as hypobaria, on different human physiological reactions and cognitive functions that can occur during a flight.

How do you proceed with your experiments?
The air pressure found at mountain altitudes corresponds to hypobaria. It is lower than on the flatlands – the higher you go, the "thinner" the air. We simulate this oxygen deficiency at various altitudes in the hypobaric chamber by lowering the barometric pressure. Due to this decrease in atmospheric pressure, a person can absorb less oxygen from the lungs into the bloodstream.

In normobaria, on the other hand, oxygen deficiency occurs with normal air pressure, as can be found on the plains. Oxygen deficiency is caused by the decrease in oxygen concentration. We are investigating the physical reactions triggered in people by the the loss of atmospheric air pressure for the same simulated height. For example, in the hypobaric chamber, we take measurements at a simulated

Mathias Aebi fitted the measuring devices on Tim Merriam's head before the experimental test on the bicycle.

altitude of 5,500 meters with hypobaria or by inhaling air with only 11% oxygen (instead of the normal level of just under 21%) with normobaria.

Pilots are not usually exposed to hypoxia, which is why we are also interested in the possible effects of low pressure, called hypobaria, with normal oxygen content, also known as normoxia. So we also compare a status in normobaria normoxia (NN-) at a height of around 440 metres above sea level with 20.9% oxygen as well as a status in hypobaria normoxia (HN-), which corresponds to the status in the cockpit of an aircraft. At the AMC we also try to assess the potential impact of a drop in barometric pressure with normoxia.

We are investigating the physical reactions triggered in people by the loss of atmospheric air pressure for the same simulated height.

THE AEROMEDICAL CENTRE OF THE SWISS AIR FORCE AMC

The Aeromedical Centre in Duebendorf is the Swiss centre of expertise for medical and psychological assessment of men and women with regard to safety and performance in military and civil aviation, transport and management. Its task is to evaluate the aptitude and health of professional air force pilot candidates. Through its strict selection procedures and intensive support for the pilots, it aims to ensure that missions are accomplished in the challenging environment of military aviation and to make flying safer. Apart from pilots, the AMC's customer groups include other specialists such as para scouts, drone operators, systems operators and general staff candidates. The AMC conducts applied research in collaboration with armasuisse, including that of Mathias Aebi.

The AMC comprises the sections of aviation medicine and aviation psychology and the AMC director's staff. A team of some 35 specialists includes aviation physicians, psychologists and medical experts, sports scientists and physiotherapists. The AMC is one of the Air Force's decisive mission-relevant terrains, and is recognised as an aeromedical centre in accordance with the regulations of the European Aviation Safety Agency (EASA) and the Federal Aviation Authority (FAA).

Tim Merriam during the concentration test.

Test subject Tim Merriam during a simulated ascent to 5,500 meters with 100% oxygen through the mask - under medical supervision by Andres Kunz (left, Director and Physician at the AMC).

Mathias Aebi gives Tim Merriam final instructions.

It is important for pilots to know the possible signs and consequences of oxygen deficiency in these two situations.

What effect does this have on the test subjects?
In order for pilots to be able to recognise and correct normobaria in time, we train Swiss jet pilots on a flight simulator in Payerne. On the other hand, to identify the signs of hypobaria, they are exposed to it once during their pilot training under the supervision of medical staff at the AMC in the special hypobaric chamber. It is important for pilots to know the possible signs and consequences of oxygen deficiency in these two situations. They need to know how the body and the cognitive functions can regulate themselves during oxygen deficiency with and without a barometric pressure decrease.

What did you measure exactly?
Volunteer students from the Zurich University of Applied Sciences in Winterthur took part in the study. We assessed subjects' concentration capacity and the symptoms that occurred during the various oxygen and pressure conditions. These tests took place during mildly intensive activities on a bicycle in the hypobaric chamber. From a physiological point of view, we measured the following body responses, amongst other things: blood oxygen saturation, heart rate, oxygen supply to the brain, blood velocity in the brain, electrical activity of the brain, gas exchange, cerebral vasoreactivity to changes in CO_2 concentration, and blood pressure regulation.

What are your findings so far?
Our results show the various consequences of hypoxia and hypobaria on the physiological reactions of people in a resting state. The physiological reactions during exertion such as riding a bicycle appear to be more strongly pronounced with HH than with NH, where lower oxygen saturation and higher heart rate occurs at the same intensity.

The results show that oxygen deficiency at low air pressure can have more severe consequences than with normobaria. This confirms that hypobaria amplifies the effect

Dr Denis Bron (left) and Mathias Aebi (right). Mathias Aebi received an award at the DGL-RM Conference in Berlin (2019).

The Air Force hypobaric chamber in Dübendorf.

of oxygen deficiency. It is therefore essential for pilots to be aware of their symptoms and physiological reactions to normobaric and hypobaric hypoxia, if these occur during a mission.

What surprised you the most?
It was the variety of symptoms which a person can exhibit if they are exposed to acute hypoxia without prior acclimatisation. It is impossible to predict how a pilot will or can react if symptoms of oxygen deficiency occur. Some of them will feel ill or very tired, while others will feel slightly or even very euphoric. It is particularly important to make the pilots aware of the various different symptoms of oxygen deficiency that can occur in people in different situations, so they can identify them quickly if they occur in flight.

Who uses your findings?
First and foremost we perform applied research. This extends our knowledge on the effects of hypoxia and hypobaria to the physiological regulation of humans when flying. The results of the experiments in the hypobaric chamber serve the purposes of the Swiss Air Force and the aircraft pilots. Thanks to the research and results of the AMC, the Armed Forces can continuously adjust the training of their military pilots and prepare them for potential difficulties and risks.

Where are your research results used?
I present the results at international scientific conferences, for example, at the Annual Scientific Meeting of the Aerospace Medical Association in the USA. I also received an award from the Aerospace Medicine Student and Resident Organisation Scientific Committee for young scientists for the research on oxygen deficiency. In addition, there are publications in various international scientific journals where we present our results to the scientific community for validation and appraisal.

The AMC cooperation with armasuisse will continue in future. We are planning exciting projects, such as assessing team performance in challenging socio-technical systems. The findings from this research are to be transferred and implemented in practice. These may also be incorporated into new or adapted army regulations.

DR PHILIP NOSER
Head of Aviation Psychology at the AMC

Philip Noser's activities include suitability and aptitude tests for candidates, training and supervising pilots and the other customer groups of the AMC, as well as crisis intervention and support for affected people and relatives following an accident.

FINDINGS OF THE STUDY
- The symptoms of oxygen deficiency at low air pressure, known as HH conditions in medical terminology, appear to be more severe and diverse than with oxygen deficiency at normal air pressure, called NH conditions. With some subjects we had to terminate the HH condition earlier due to attacks of nausea and low blood oxygen saturation (around 60% compared with 99% in normobaria).
- Ability to concentrate is maintained at 3,000 meters above sea level under HH conditions. However, this decreases at 5,500 meters under both NH and HH conditions. A direct relationship can be identified between blood oxygen content and the ability to concentrate.
- The oxygen supply to the brain is reduced with hypoxia and appears to be lower under HH conditions than under NH conditions.
- At 5,500 meters, subjects displayed greater fatigue than at 3,000 meters. Brain waves from electroencephalography confirm this intellectual fatigue.
- Under HN conditions, ability to concentrate capacity and physiological parameters are only slightly changed compared to NN conditions.

MATHIAS AEBI
Mathias Aebi completed his studies as a sports instructor and scientist at the University of Lausanne (UNIL) in the department of the Institute of Sports Sciences (ISSUL). His master's thesis looked at the physiology of movement in a hypoxic environment, in other words, moving when there is little oxygen. The results of this thesis motivated the experts at the AMC to launch a collaboration between the AMC and the University of Lausanne UNIL. armasuisse Science and Technology as the authority responsible for defence research at the DDPS, created the commercial framework conditions for this to happen. As a PhD student at the AMC, Mathias Aebi was able to test several different subjects in a hypobaric chamber for their reactions to oxygen deficiency.
Mathias Aebi is working on his dissertation with Professor Grégoire Millet (University of Lausanne) and his supervisor Dr Denis Bron (Head of Aviation Medicine at the FAI).

Appendix C: La recherche prend de l'altitude – Uniscope journal

Article published in the journal Uniscope in September 2018

le magazine du campus de l'UNIL | le savoir vivant |

l'uniscope

Des études de haut vol

Comment le corps humain réagit-il au manque d'oxygène et aux basses pressions? Doctorant à l'Institut des sciences du sport, Mathias Aebi mène des recherches sur ces questions à Dübendorf (ZH), dans les installations des Forces aériennes suisses. (p. 14)

N° 636 / 3 – 30 septembre 2018

UNIL | Université de Lausanne

La recherche prend de l'altitude

Doctorant à l'UNIL, Mathias Aebi étudie les effets du manque d'oxygène et des basses pressions sur l'organisme. Une collaboration fructueuse avec les Forces aériennes suisses lui permet de mener sa recherche, aussi bien appliquée que fondamentale.

David Spring Texte
Fabrice Ducrest Photos

«Drei, zwei, eins, los!» Au signal, bardé d'appareils de mesure, Tim Merriam commence à pédaler sur un vélo d'appartement. Etudiant en médecine de dernière année à l'Université de Zurich, ce pilote civil est observé par Mathias Aebi, doctorant à l'Institut des sciences du sport (ISSUL) de l'UNIL. Cette scène se déroule dans une petite pièce encombrée dotée de portes métalliques, dont les murs épais sont percés de hublots.

En basse pression et sans apport d'oxygène, Tim Merriam *(à g.)* se repose dans l'obscurité pour ne pas troubler les mesures prises par l'électroencéphalogramme. Mathias Aebi, au fond, surveille l'expérience.

Nous sommes dans les sous-sols de l'Institut de médecine aéronautique (FAI), à Dübendorf (ZH). Cette entité, qui dépend des Forces aériennes suisses, abrite le seul caisson hypobare du pays. En réduisant la quantité d'air dans ce dernier, des pompes vrombissantes y font baisser la pression barométrique, ce qui simule les conditions qui règnent en altitude.

En uniforme, Andres Kunz entre dans le caisson. Directeur du FAI, ce colonel jovial est également médecin. Sa présence est rendue nécessaire par la phase de tests à venir. À l'exception de Tim Merriam, chacun s'équipe d'un casque gris doté d'un masque à oxygène et d'un micro. Les pompes démarrent: un grand coup de frais, un nuage de condensation. Les oreilles craquent et s'habituent au changement de pression. En moins d'une minute, soit trois fois plus vite qu'un avion de ligne, nous nous retrouvons comme à 5000 mètres d'altitude. Sous l'œil attentif de Mathias Aebi, pendant l'heure suivante, Tim Merriam effectue une série d'exercices. Dans l'air raréfié, il effectue six minutes de vélo à basse intensité en portant un masque qui mesure les échanges gazeux, ainsi que six minutes d'un test cognitif basé sur des calculs arithmétiques. Ces deux séquences sont entrecoupées de moments de calme, dans le noir, afin de récolter sans perturbation les données fournies par l'électroencéphalographe posé sur sa tête.

De nombreuses autres informations, comme la vitesse du sang dans l'artère moyenne cérébrale, la pression artérielle, la fréquence cardiaque ou la saturation en oxygène mesurée au doigt, par exemple, filent vers un ordinateur portable au travers d'un grand nombre de câbles. Les expériences sont minutées de manière précise.

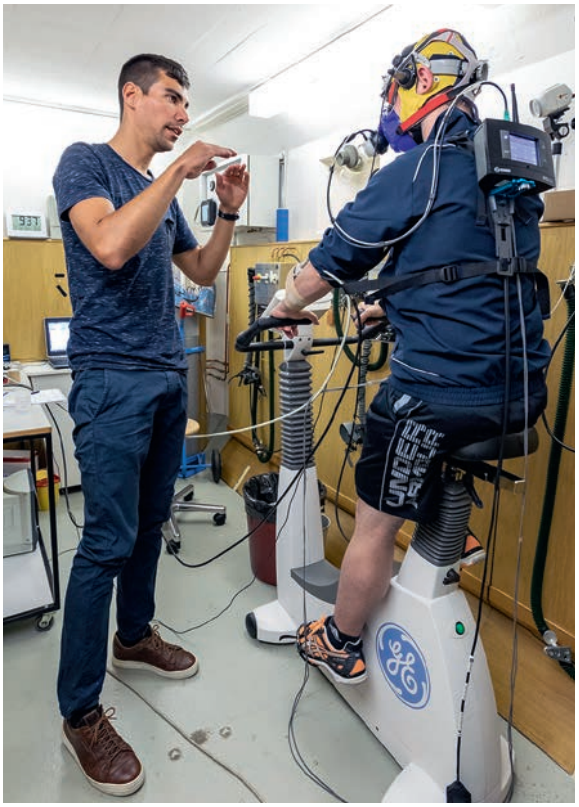
Dans le cadre de sa thèse, Mathias Aebi s'intéresse aux réponses physiologiques de l'hypoxie (quand l'organisme est sous-oxygéné) et de l'hypobarie. Lors de sa journée au FAI, Tim Merriam traverse ainsi trois «conditions» d'environ une heure chacune, entrecoupées de pauses.

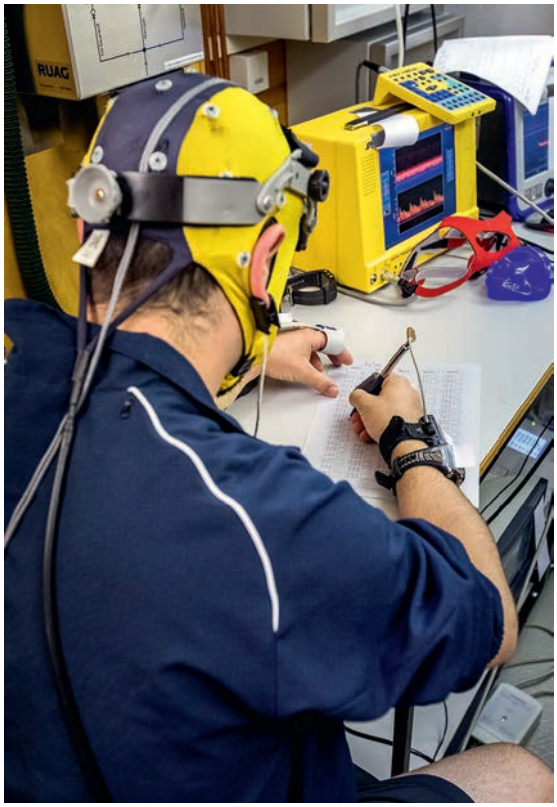
Dans la première, la plus rude, l'hypoxie est induite par la baisse de la pression barométrique. Même si l'air dans le caisson contient 20,9 % d'oxygène, soit la proportion normale de l'atmosphère terrestre, la plus faible pression diminue le transfert du gaz vital dans le sang. Dans la deuxième condition, muni d'un masque, Tim Merriam respire un mélange gazeux qui ne contient que 11 % d'oxygène. Par contre, la pression reste normale («normobarie»). Enfin, à nouveau expédié à 5000 mètres, l'étudiant en médecine est alimenté avec de l'air très chargé en O_2, pour contrebalancer l'hypoxie. Bravement, il répète sa série d'exercices et de moments de repos, pendant lesquels il somnole parfois.

Afin de nourrir sa recherche en données, Mathias Aebi mène des tests identiques sur vingt «sujets». Il n'a aucun mal à trouver des volontaires, dont la majorité sont de jeunes pilotes non professionnels. «Ils sont très intéressés à expérimenter l'hypoxie, afin d'en reconnaître les symptômes si cela devait leur arriver en vol», note le chercheur. Les effets varient d'une personne à l'autre. Certains s'endorment, d'autres deviennent euphoriques.

Formation des pilotes

«Malgré la pressurisation, l'hypoxie demeure un sujet important dans le domaine de l'aviation. C'est d'autant plus vrai pour les militaires, dont le corps est soumis à de fortes accélérations», relève Andres Kunz. Ainsi, le FAI forme tous les professionnels de l'armée appelés à prendre l'air, comme les éclaireurs-parachutistes ou les pilotes. Leurs collègues civils de Swiss reçoivent également une instruction. Les installations de Dübendorf permettent de pousser les organismes





(En haut à g.) Chercheur à l'UNIL, Mathias Aebi récolte de nombreuses données physiologiques sur son sujet. Ici, une prise de sang.

(En haut à dr.) Le seul caisson hypobare de Suisse, dans les sous-sols de l'Institut de médecine aéronautique (Dübendorf).

(À g.) Mathias Aebi donne des instructions à Tim Merriam, pilote dans le civil. Bardé d'instruments, ce dernier va effectuer 6 minutes de vélo à basse intensité.

(À dr.) Plusieurs fois lors de la journée, Tim Merriam va faire un test cognitif basé sur des calculs arithmétiques.

près de l'évanouissement et de simuler des décompressions explosives.

Le FAI a déjà mené des travaux sur l'hypoxie, notamment en s'intéressant à l'influence positive du CO_2 sur la tolérance au manque d'oxygène. « Nous possédons beaucoup d'expérience pratique, en particulier lorsqu'il s'agit de s'approcher des limites physiologiques, explique le médecin. Mais notre lien avec l'UNIL permet d'aller plus loin et apporte de nouvelles connaissances. » Mélange de recherches appliquée et fondamentale, la collaboration entre les institutions fonctionne très bien : Mathias Aebi a été engagé par l'institut pour sa thèse, sous la direction de Grégoire Millet, professeur de physiologie de l'exercice à l'ISSUL. Ce dernier signale que les travaux préliminaires de son doctorant lui ont permis de remporter le deuxième prix d'un congrès international de physiologie de l'aviation à Dallas, en mai dernier. Dix-septième « sujet » au programme, Tim Merriam considère sa journée comme une « bonne expérience », malgré sa fatigue. Pour Mathias Aebi, la récolte de données, « une quantité d'informations gigantesque », touche à sa fin. Ce grand sportif, triathlète à ses heures, va maintenant s'attaquer à leur traitement. Une tâche qui va sûrement lui demander un supplément d'oxygène.

Merci au personnel de la base aérienne de Dübendorf pour la mise à disposition du Pilatus PC-7 de la couverture.

Découvrez la recherche de Mathias Aebi en vidéo sur

youtube.com/uniltv
› L'actu en vidéo

Appendix D: Les effets de l'hypoxie sur le corps et le cerveau des pilotes militaires

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Radio program published on 11 June 2019, CQFD, La Radio Télévision Suisse (RTS).

Interview conducted by Stéphane Délétroz.

19:30 Télécharger

Les effets de l'hypoxie sur le corps et le cerveau des pilotes militaires

Stéphane Délétroz vous emmène à Dübendorf, dans les sous-sols de l'Institut de médecine aéronautique. Les effets de l'altitude et du manque d'oxygène sur les pilotes militaires y sont testés dans le seul caisson hypobare du pays.

Avec Mathias Aebi, doctorant à l'Institut des sciences du sport de l'Université de Lausanne, et Denis Bron, médecin chef de l'Institut de médecine aéronautique de Dübendorf.

Source:
https://www.rts.ch/la-1ere/programmes/cqfd/10466031-les-effets-de-lhypoxie-sur-le-corps-et-le-cerveau-des-pilotes-militaires-11-06-2019.html?mediaShare=1