HYPOXIC VENTILATORY CHEMOSENSITIVITY, AND CEREBRAL AND MUSCLE DEOXYGENATION RESPONSES TO EXERCISE IN ACUTE HYPOXIA BEFORE AND AFTER A 2-MONTH CLIMBING EXPEDITION ON MT. EVEREST

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ABSTRACT

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With increasing altitude, the O_2 availability to the body is decreased which stimulates ventilation. The increase in ventilation improves maintenance of arterial oxygenation and thus O_2 delivery to the tissues. However, it simultaneously leads to decreased arterial CO_2 pressure (i.e., hypocapnia), which causes cerebral vasoconstriction and may attenuate cerebral oxygenation. The effect of prolonged acclimatization on cerebral and muscle tissue oxygenation is not well known. The purpose of the present study was to examine the extent of cerebral and muscle tissue deoxygenation in acute hypoxia before and after acclimatization, and their association with hypoxic ventilatory response.

Nine experienced male climbers (age 37 ± 6 years, $\dot{V}_{O_2}$max 55.0 ± 6.7 ml·kg^{-1}·min^{-1}) performed walking exercise on a treadmill with constant load (speed 5.5 km·h^{-1}, grade 3.8°, theoretical $\dot{V}_{O_2}$ 22.75 ml·kg^{-1}·min^{-1}) before (PRE) and after (POST) expedition aiming to summit Mt. Everest (8848 m). After rest and baseline walking in normoxia, partial pressure of inspired O_2 was reduced in 3-min steps to simulate altitudes of camps during the ascent to Mt. Everest. Partial pressure of end tidal CO_2 was allowed to vary naturally, i.e. procedure was poikilocapnic. The test was terminated at latest when SpO_2 fell to 62 %. Local cerebral (frontal cortex), active (m. vastus lateralis) and inactive muscle (m. biceps brachii) oxygenation was measured with near-infrared spectroscopy (NIRS). Acute hypoxic ventilatory chemosensitivity (AHVR) was determined by linear regression slope of $\dot{V}_E$ vs. SpO_2 % (L·min^{-1}·%$^{-1}$). Deoxyhemoglobin concentration (Δ[HHb]) and tissue saturation index (TSI) recordings described tissue deoxygenation status reflecting the mismatch between regional O_2 delivery and utilization.

In comparison to PRE, AHVR was greater in POST (1.25 ± 0.33 vs. 1.63 ± 0.38 L·min^{-1}·%$^{-1}$, $P < 0.05$). Cerebral oxygenation decreased in both tests with increasing hypoxia (↑Δ[HHb], ↓TSI) but less in POST. Leg Δ[HHb] increased and TSI decreased with increasing hypoxia, with no differences between PRE and POST. In the arm, the reduction of Δ[HHb] was more rabid than in the leg in both tests. No association was found between AHVR and cerebral deoxygenation, but high AHVR was related to rapid increase in leg Δ[HHb] ($r=0.73$, $P < 0.05$) in PRE.

In conclusion, AHVR increases and cerebral oxygenation is improved following acclimatization. Differences in cerebral oxygenation responses were not explained by differences in ventilatory chemosensitivity. Local muscle oxygenation remained similar before and after acclimatization. Different effect of prolonged acclimatization on cerebral and muscle tissue oxygenation responses may suggest different adaptation strategies of the tissues unfolding in the muscle already in acute hypoxia and in the cerebral tissue along with altitude acclimatization.

Key words: hypoxia, acclimatization, ventilation, near infrared spectroscopy, oxygenation, climbers, exercise

TIIVISTELMÄ

Mutanen, Niina 2011 Hypoksinen ventilatorinen kemosensitiivisyys ja aivo- ja lihaskudoksen happeutuminen rasituksessa akuutissa hypoksiassa ennen ja jälkeen kahden kuukauden Mt. Everestille kiipeämisen. Liikuntafysiologian Pro gradu -tutkielma. Liikuntabiologian laitos, Jyväskylän yliopisto, 89 s.

Korkeuden kasvaessa hapen osapaine sisäänhengitysilmassa ja valtimoveressä laskee, mikä kiihdyttää hengitystä. Tämä edesauttaa valtimoveren O_2 -osapaineen ylläpitoa ja siten hapen toimitusta kudoksille. Ventilaation kasvu johtaa myös valtimoveren CO_2 -osapaineen laskuun, mikä supistaa aivovaltimoita ja saattaa heikentää aivojen happeutumista. Tällä hetkellä tietämys pitkäaikaisen korkeaan ilmanalaan sopeutumisen vaikutuksista aivo- ja lihaskudoksen happeutumiseen on vähäistä. Tämän tutkimuksen tarkoituksena oli tutkia aivo- ja lihaskudoksen happeutumista ennen ja jälkeen kroonisen hypoksian sekä happeutumisvasteiden yhteyttä akuuttiin hypoksiseen ventilatoriseen kemosensitiivisyyteen (AHVR).

Yhdeksän kokenutta miesvuorikiipeilijää (37 ± 6 v, $\dot{V}_{O_2}$max 55,0 ± 6,7 ml·kg^{-1}·min^{-1}) suoritti juoksumatolla kävelytestin tasaisella kuormituksella (nopeus 5 km·h^{-1}, kulma 3,8°, teoreettinen $\dot{V}_{O_2}$ 22,75 ml·kg^{-1}·min^{-1}) ennen (PRE) ja jälkeen (POST) Mt. Everestille (8848 m) pyrkimisen. Levon sekä normoksisen kävelyn jälkeen hengitysilman happipitoisuutta pienennettiin kolmen minuutin välein siten, että poikilokapninen (CO_2:a ei kontrolloitu) hypoksinen altistus vastasi kiipeilijöiden leirikorkeuksia Mt. Everestillä. Testi keskeytettiin viimeistään valtimoveren O_2-saturaation (SpO_2%) laskiessa alle 62 %. Kudosten happeutuminen mitattiin lähi-infrapuna spektroskopialla (NIRS) aivoista (otsalohko), aktiivisesta (m. vastus lateralis) ja inaktiivisesta (m. biceps brachii) lihaksesta. AHVR määritettiin ventilaation nousuna SpO_2:n laskun suhteen (L·min^{-1}·%$^{-1}$) lineaarisella regressioyhtälöllä. NIRS -muuttujista deoksihemoglobiini (Δ[HHb]) ja kudoksen happeutumisindeksi (TSI) kuvaavat tasapainoa kudoksen hapenkuljetuksen ja käytön välillä.

Tuloksissa PRE -testiin verrattuna AHVR oli suurempi POST -testissä (1,25 ± 0,33 vs. 1,63 ± 0,38 L ·min^{-1}·%$^{-1}$, $P < 0,05$). Aivojen happeutuminen heikkeni kasvavan hypoksian myötä molemmissa testeissä (↑Δ[HHb], ↓TSI), mutta heikkeneminen oli pienempää POST -testissä. Jalan Δ[HHb] nousi ja TSI laski kasvavan hypoksian myötä ja vasteissa ei ollut eroja testien välillä. Käden Δ[HHb] muutosnopeus oli jalkaa suurempi sekä PRE- että POST -testissä. Yhteyttä AHVR:n ja aivojen happeutumisen välillä ei löydetty, mutta voimakas AHVR oli yhteydessä nopeaan jalan Δ[HHb]:n nousuun (r=0,73, $P < 0,05$) PRE -testissä.

Johtopäätöksenä on, että AHVR kasvaa ja aivojen happeutuminen paranee akklimatisaation seurauksena. Erot yksilöllisissä hengitysvasteissa eivät selitä eroja aivojen happeutumisessa. Erot kudosten happeutumisvasteiden muutoksissa saattavat viitata erilaisiin sopeutumismekanismeihin, jotka turvaavat aktiivisen lihaksen riittävän happeutumisen akuutissa hypoksiassa, mutta parantavat aivojen happeutumista vasta akklimatisaation seurauksena.

Avainsanat: hypoksia, akklimatisaatio, ventilaatio, lähi-infrapuna spektroskopia, happeutuminen, vuorikiipeily, kiipeilijät, rasitus

ABBREVIATIONS

AHVR	acute hypoxic ventilatory chemosensitivity
CaO_2	arterial oxygen content
CBF	cerebral blood flow
COX	Cerebral oxygenation
Fb	breathing frequency
Hb	hemoglobin
HHb	deoxyhemoglobin
HR	heart rate
HVD	hypoxic ventilatory decline
MCAv	middle cerebral artery velocity, an index of CBF
MOX	muscle oxygenation
NIRS	near-infrared spectroscopy
O_2Hb	oxyhemoglobin
$PaCO_2$	arterial partial pressure of carbon dioxide
PCO_2	partial pressure of carbon dioxide
PaO_2	arterial partial pressure of oxygen
$PETCO_2$	end-tidal partial pressure of carbon dioxide
PiO_2	partial pressure of inspired oxygen
PN_2	partial pressure of nitrogen
PO_2	partial pressure of oxygen
$\dot{Q}$	cardiac output
SpO_2	arterial oxygen saturation
tHb	total hemoglobin (O_2Hb + HHb)
TSI	tissue saturation index
$\dot{V}_E$	ventilation
$\dot{V}_{O_2}$	oxygen consumption
$\dot{V}_{O_2}max$	maximal oxygen uptake
VT	tidal volume

CONTENTS

1 INTRODUCTION

Travelling or climbing in mountainous regions has become more and more popular among travellers (Karinen et al. 2008). With increasing altitude, the barometric and hence the partial pressure of inspired oxygen (PiO_2) decrease. Abnormally low oxygen availability to the body, defined as hypoxia, is the greatest adaptive challenge to the body along ascent to high altitude. (Hornbein & Schoene 2001a, 199.) Hypoxia requires adaptation of multiple systems to secure sufficient oxygen supply to tissues and organs (Bartsch & Saltin 2008). Adequate adaptation is essential for successful mountaineering, in which hypoxic challenge is combined with exercise and increased oxygen requirements must be met despite decreased oxygen driving pressure (Grover et al. 1986). If the adaptation process fails, life-threatening conditions may develop. Adaptation process depends not only on ascent rate but also on characteristics of the climber. (Karinen et al. 2010.)

Upon exposure to hypoxia, ventilation is rapidly increased (Easton et al. 1986), the magnitude of which is greatly amplified with any physical exertion (West 2000). This initial increase, called acute hypoxic ventilatory chemosentitivity (AHVR) (Zhang & Robbins 2000), is caused by stimulation of peripheral chemoreceptors by the decreased arterial O_2 pressure (PaO_2) (Weil & Zwillich 1976). The magnitude of AHVR depends on the individual ventilatory sensitivity to hypoxia (Moore at al. 1984). With chronic hypoxia, ventilatory sensitivity to hypoxia further increases and elevates PaO_2 and arterial O_2 saturation (SpO_2) (Hupperets et al. 2004). Very high AHVR has been observed in experienced climbers (Schoene 1982; Masuyama et al. 1986) although there are studies in which successful climbers are characterized by a lower ventilatory sensitivity than less successful climbers (Bernardi at al. 2006.)

In addition to improved arterial blood oxygenation, a strong AHVR also leads to decreased arterial CO_2 pressure ($PaCO_2$), hypocapnia, which causes cerebral vasoconstriction and reduces cerebral blood flow (CBF) (Ainslie & Poulin 2004; Ainslie et al. 2008). It has been theorized that subjects with high AHVR are more likely to have poor cerebral oxygenation

(COX) due to hypocapnia-induced decrease in CBF that more than offsets the increase in SpO_2 (Hornbein et al. 1989). Indeed, a strong association has been observed between AHVR and reduced COX during exercise in acute hypoxia (Peltonen at al. 2009). However, the effect of chronic hypoxia on this interaction is not known.

Uninterrupted supply of blood flow and oxygenation is a prequisite for proper brain function at rest and during exercise. Brain function is impaired when average COX is reduced more than 10 % (reviewed by Secher et al. 2008). Previous studies have reported slowed performance on more complex tasks of cognitive and motor function already at moderate altitude. While at altitude, individuals with high AHVR seem to have more neurobehavioral impairment. (Hornbein 2001b.) It has been suggested that cerebral hypoxia may limit exercise performance, particularly in severe hypoxia. Several studies have found that increasing PiO_2 can improve COX and prolong exercise time. However, in contrast to COX, muscle oxygenation (MOX) seems to be only modestly improved likely indicating better balance between O_2 delivery and consumption despite increasing work rates. (Amann et al. 2006; Subudhi et al. 2008.) This might predict tissue-specific changes in oxygenation after prolonged hypoxia too, when acclimatization has returned the arterial oxygen content (CaO_2) to or even above sea level values.

The purpose of the present study was to examine acute responses to constant hypoxic exercise in experienced climbers before and after acclimatization to chronic hypoxia at the Mount Everest. For execution of progressively increasing poikilocapnic hypoxia, a method was developed to simulate increasing environmental hypoxia, while the end tidal partial pressure of CO_2 ($PETCO_2$) was allowed to decrease naturally with increasing hypoxia. Of special interest was the extent of cerebral and muscle tissue deoxygenation in acute hypoxia before and after acclimatization, and their association with hypoxic ventilatory response.

2 OXYGEN DELIVERY AND UTILIZATION

The main purpose of ventilation is to exchange gases: O_2 moves from the air into the cells and CO_2 moves to the opposite direction. The respiratory and cardiovascular systems work in concert to provide an efficient delivery system of gases to and from the tissues. Respiration includes four steps: ventilation, diffusion, transport of gases via the blood and peripheral gas exchange. (Guyton & Hall 2006, 471.) During exercise, the O_2 demand increases in direct proportion to muscle work, challenging the O_2 transport system to its limits at maximal exercise (reviewed by Calbet & Lundby 2009).

2.1 Pulmonary ventilation

Pulmonary ventilation means the inflow and outflow of air between the atmosphere and the lung alveoli (Guyton & Hall 2006, 471). The airways lead inspired air to the gas-exchanging regions of the lung, the alveoli (West 2008, 2). The essential importance of pulmonary ventilation is to continually refresh the air in the gas exchange areas of the lung, which is surrounded by the blood-gas interface and pulmonary capillary blood.

Ventilation increases with increasing exercise intensity and can reach very high levels. A well trained athlete with a maximal O_2 consumption ($\dot{V}_{O_2}max$) of 4 liters/min can have a pulmonary ventilation of 120 liters/min at maximal exercise, about 15-fold the resting state value. (West 2008, 135.) Ventilation increases linearly during light-to-moderate exercise when plotted against oxygen consumption. At more intense exercise ventilation increases more rapidly and disproportionately in relation to O_2 consumption. This results from lactic acid production and its buffering that stimulates ventilation. (McArdle et al. 2001, 293; West 2008, 135.)

During normal quiet breathing the amount of energy required for pulmonary ventilation is only 3–5 % of the total energy expenditure. However, during heavy exercise the energy requirement can increase as much as 50-fold. Thus, at high ventilation levels the ability to

provide enough energy for the ventilatory processes can become a performance limiting factor. (Guyton & Hall 2006, 475.)

Partial pressures of respired gases. Oxygen makes up 20.93 % of the atmosphere at any altitude. At the sea level the barometric pressure is 760 mmHg and partial pressure of oxygen (PO_2) is 159 mmHg (0.2093 · 760 mmHg). When air is inhaled it is warmed to the body temperature of 37 ºC and saturated with water vapour (partial pressure of 47 mmHg at any altitude). Thus, the PO_2 of inspired humidified air is 149 mmHg (0.2093 · (760 - 47 mmHg)). Carbon dioxide exerts a small partial pressure of only 0.23 mmHg (0.0003 · 760 mmHg) and the humidification has a minor effect on it because of carbon dioxide's negligible contribution to inspired air. Nitrogen makes up 78 % of ambient air, but it is neither used nor produced in metabolic processes. (McArdle et al. 2001, 271–273.) Thus, it is not considered in this work.

Alveolar air composition differs substantially from the incoming, moist ambient air. Partial pressure of O_2 in the alveolar air is about 104 mmHg due to continuous oxygen removal by pulmonary blood and only partial replacement of alveolar air with each breath (West 2008, 56–57.) Similarly, PCO_2 of alveolar air is 40 mmHg. Carbon dioxide is constantly diffusing from the pulmonary blood into the alveoli and being removed from there by ventilation. (Guyton & Hall 2006, 495.) The volume of alveolar air replaced by fresh atmospheric air per each breath is only one seventh of the total volume. The inspired new air is mixed with the air in the alveoli, while some of the alveolar gas is exhaled to the environment. The slow replacement maintains the alveolar and blood gas concentrations somewhat stable. This stabilizes the respiratory control mechanism and helps to prevent excessive changes in tissue oxygenation and tissue CO_2 concentration. (Guyton & Hall 2006, 494.)

The alveolar partial pressures of both O_2 and CO_2 are determined by the level of alveolar ventilation and the rate of transfer of the gases through the respiratory membrane governed by the metabolic activity of the tissues (Guyton & Hall 2006, 499). Alveolar gas composition is kept relatively constant even during strenuous exercise by coupling pulmonary ventilation tightly to increased metabolic needs (McArdle et al. 2001, 275).

2.2 Diffusion in the lung

Gas exchange between the alveoli and the pulmonary blood and arterial blood and tissues occurs by passive diffusion along partial pressure gradients (i.e. gas partial pressure difference). The pressure gradient between two sides of the tissue sheets, like respiratory membrane, drives gases from higher partial pressure to an area of lower partial pressure. Figure 1 illustrates the pressure gradients at rest favoring gas transfer in different areas of the body.

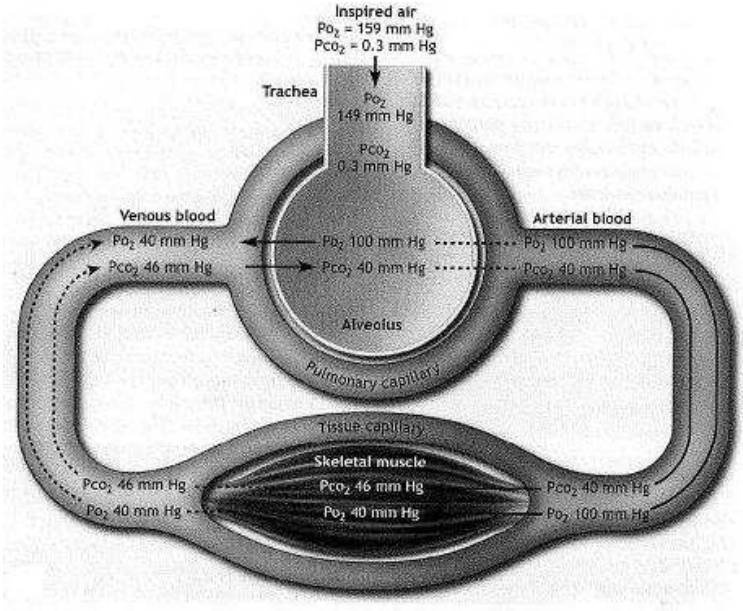

FIGURE 1. Partial pressure of O_2 and CO_2 of ambient, tracheal and alveolar air and these gas pressures in venous and arterial blood and muscle tissue. Both gases diffuse downhill along gradients of decreasing pressure. (McArdle et al. 2001, 274.)

Gas exchange in the lungs takes place at the respiratory membrane where gas is brought to one side of the membrane by pulmonary ventilation and to the other side by pulmonary capillary blood (West 2008, 2; Guyton & Hall 2006, 496). Gas exchange serves two major functions: it replenishes the blood's O_2 supply and removes CO_2 out of the blood (Wilmore & Costill 2004, 274). Once gases have diffused through the respiratory membrane oxygenated blood returns to the heart and is pumped further to systemic circulation to deliver oxygen to the peripheral tissues.

During high-intensity exercise the pulmonary blood flow increases fourfold to sevenfold increasing diffusing capacity. This results from recruitment and distension of pulmonary capillaries and also from improved ventilation-perfusion ratio in the upper parts of the lung. The increase in diffusing capacity is normally at least threefold, in endurance trained athletes even higher (Guyton & Hall 2006, 486–503, 1062). With increasing blood flow also the time that blood remains in the pulmonary capillary is shortened, even more than 50 % (Guyton & Hall 2006, 502). Because of safety margins blood is still almost saturated with oxygen when leaving the lungs. At rest, the red cell moves through the capillary in 0.75 seconds and the arterial PO_2 nearly equals that of alveolar gas by the time red cell is only one third of its way along the capillary. (West 2008, 29; Guyton & Hall 2006, 503).

2.3 Transportation of respiratory gases

In addition to adequate alveolar ventilation and pulmonary diffusion, the transport of O_2 and CO_2 between the lungs and the tissues depends on blood flow and capacity of blood to transport respiratory gases (Ganong 1999, 635). The transportation of respiratory gases is described in this chapter.

2.3.1 Oxygen

The amount of O_2 in the blood depends on the amount of dissolved O_2, the amount of hemoglobin (Hb) and the affinity of Hb for O_2 (Ganong 1999, 635). 3 % of the oxygen is carried in the blood dissolved in blood plasma and about 97 % in combination with Hb in the red blood cells. The amount of dissolved O_2 in normal arterial blood is a linear function of the PO_2 (0.3 ml O_2 · 100 ml with PO_2 of 100 mmHg). Normal arterial blood contains about 15 gm of Hb · 100 ml^{-1} and each gram of Hb can bind 1.34 ml O_2. Therefore, 100 ml of blood contains 20.1 ml of O_2 combined with Hb in ideal conditions, when Hb is 100 % saturated. However, Hb is normally 97 % saturated due to physiologic shunt and binds 19.4 ml of O_2 per 100 ml of blood. Physiologic shunt results from addition of venous blood to oxygenated pulmonary blood. This shunted blood from the bronchial and coronary circulation has supplied tissues and enters the arterial system without going through the gas ex-

change areas, thus decreasing the oxygen content of the blood returning the heart. (West 2008, 59; Guyton & Hall 2006, 505–506.)

The primary function of Hb is to combine reversibly with oxygen in the pulmonary capillaries, where PO_2 is high, and release this oxygen in the peripheral tissue capillaries, where PO_2 is low (Guyton & Hall 2006, 424–425). During normal conditions hemoglobin saturation is reduced to 75 % on passing through the tissue capillaries. Thus, the amount of O_2 extracted on the way from lungs to tissues is ~25% or 5 ml of O_2 per 100 ml blood. During strenuous exercise, the amount of O_2 released to tissues can increase more than threefold. Under these conditions, 15 ml of O_2 is delivered to the tissues by each 100 ml of blood and SpO_2 is decreased to about 25 %. (Guyton & Hall 2006, 506–507.) Even though Hb plays a major role in O_2 transport, dissolved O_2 serves important functions. The random movement of dissolved oxygen molecules makes up the PO_2 of the plasma and tissue fluids and thus determines loading of Hb (oxygenation) in the lungs and its unloading (deoxygenation) at the tissues. Arterial O_2 pressure also regulates breathing, especially at high altitudes where ambient PO_2 is lowered. (McArdle et al. 2001, 275.)

Hemoglobin O_2 dissociation curve is shown in Figure 2. The curve demonstrates percentage saturation of hemoglobin with O_2 at various PO_2 values. The curve has a sigmoid shape that is physiologically beneficial. The flat upper portion means that even if the alveolar PO_2 falls to as low as 60 mmHg the oxygen loading and thus the Hb saturation (SpO_2 [%]) are affected only slightly. This is of great importance when ascending to high altitudes, where ambient PO_2 falls with increasing altitude. The steep lower portion of the dissociation curve ensures that only a small drop in capillary PO_2 is needed for release of large amounts of oxygen into tissue cells. Thus dissociation curve maintain relatively constant tissue PO_2 even when alveolar PO_2 and rate of tissue metabolism vary. (West 2008, 78.)

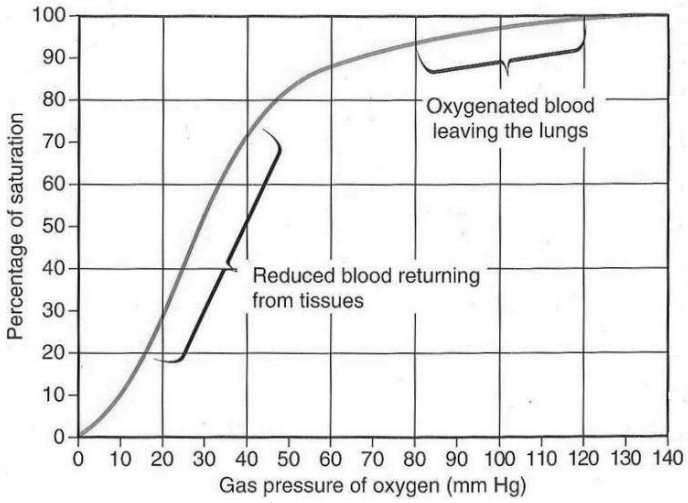

FIGURE 2. Hemoglobin O_2 dissociation curve. (Modified from Guyton & Hall 2006, 506).

Hemoglobin O_2 dissociation curve moves to the right by increased PCO_2, temperature, acidity and 2,3-diphosphoglycerate (2,3-DPG). This reduces the affinity of Hb for O_2 and increases delivery of O_2 to tissues. Most of the effects of PCO_2, which is known as Bohr effect, is mediated by hydrogen ion concentration and thus acidification. These changes are important factors improving oxygenation of exercising muscles. Opposite changes shift the curve to the left. (West 2008, 78–79; Guyton & Hall 2006, 507–508.)

2.3.2 Carbon dioxide

Carbon dioxide is transported in the blood in three forms: as bicarbonate, in combination with proteins as carbamino compounds and dissolved. Bicarbonate is the most important form of CO_2 transportation as it accounts for about 60 % of the transport. The second mean of transport is combination of CO_2 with blood proteins, mainly with Hb. This proportion of transport is approximately 30 %. About 10 % of the total CO_2 amount is transported to the lung in dissolved state. (West 2008, 81–82.) Although the amount of dissolved CO_2 is small, it is of great importance for ventilatory control, for which the background level of CO_2 provides the chemical basis (McArdle et al. 2001, 275).

2.4 Tissue oxygenation

Tissue oxygenation status is determined by arterial oxygen content (CaO_2), hematocrit and blood flow and generally by the balance between O_2 utilization and delivery. Increased demand for O_2 is met by an increase in the blood flow and O_2 extraction from oxyhemoglobin (O_2Hb). (Wittenberg & Wittenberg 1989.) Tissue blood flow is tightly coupled to the metabolic rate of the particular tissue (Guyton & Hall 2006, 195) and generally determined by vascular conductance and arterial blood pressure (Levick 2003, 266).

An increase in tissue metabolism, e.g. as a result of muscle contraction (Rowell 2004) or increase in brain neuronal activity during exercise (Ogoh & Aislie 2009), leads to an increase in tissue blood flow (hyperemia) (Rowell 2004; Ogoh & Ainslie 2009). The blood flow to each tissue is controlled by local (intrinsic) and general (extrinsic) control mechanisms. Extrinsic control includes sympathetic nervous system and humoral mechanisms, while intrinsic control mechanisms originate from within blood vessels (e.g., myogenic and endothelial factors) and from the surrounding tissue. The tissue mechanisms are linked to tissue metabolism or other biochemical pathways (e.g. histamine and bradykinin). The balance between local control mechanisms and extrinsic factors determines the vascular tone and thus the blood flow within the tissue. (Rowell 1986, 14–25.)

The exchange of water, nutrients, respiratory gases and waste products between blood and tissue occurs at the level of microcirculation in capillaries (Figure 3) (Guyton & Hall 2006, 183). After the blood unloads O_2 and loads CO_2 in tissue capillaries it is returned to the heart and delivered again to the lungs. Near-infrared spectroscopy (NIRS) allows non-invasive evaluation of relative changes in the oxygenation at the level of microcirculation (Figure 3) at rest and during exercise (Ferrari et al. 1997). The method is discussed in Chapter 6.5.

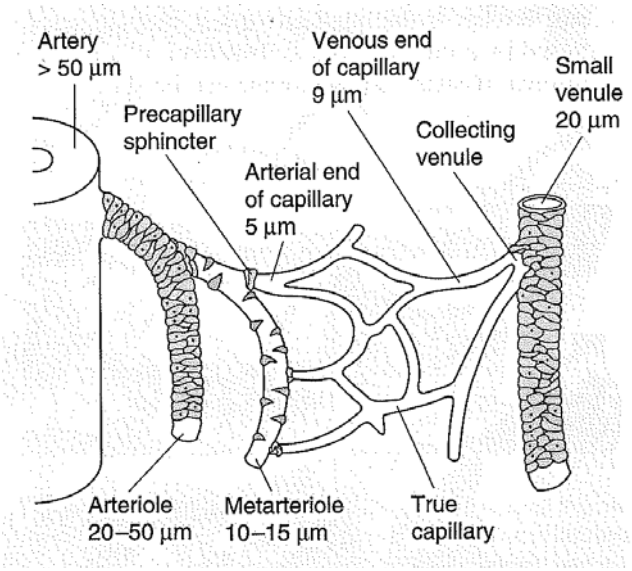

FIGURE 3. Structure of the microcirculation. Arterioles give rise to metarterioles (= terminal arterioles), which give rise to capillaries. The capillaries drain via collecting venous to venules. The arterioles are highly muscular, the metarterioles are encircled by smooth muscles at intermittent points and muscular precapillary sphincters are located at the openings of capillaries. Venules contain weaker muscular coat than arteries. (Ganong 1999, 551.)

2.4.1 Cerebral tissue

In an adult, normal blood flow through the brain averages 50 to 65 milliliters of blood per 100 grams of brain tissue per minute. This amounts for the entire brain 750 to 900 ml/min or 15 % of the resting cardiac output even though the brain constitutes only 2 % of the total body mass. Most of the flow is directed to grey matter and its O_2 consumption accounts nearly 20 % of the total O_2 consumption at rest. In addition to high blood flow, this is enabled by O_2 extraction of ~35 %. (Guyton & Hall 2006, 761–762; Levick 2003, 265.)

Because of dependence on aerobic metabolism grey matter needs an uninterrupted delivery of O_2 to maintain normal neuronal activity and thus is highly sensitive to hypoxia (Guyton & Hall 2006, 761–762; Levick 2003, 265). Unconsciousness occurs within a few seconds after cerebral ischemia and brain function is impaired when average cerebral oxygenation is reduced more than 10 % (reviewed by Secher et al. 2008). In addition to need for continu-

ous global flow, alterations in local blood flow occur due to changes in cerebral neuronal activity and metabolism. Cerebral neuronal activity increases the local metabolic rate in the segments of the brain involved in a given task. The cerebral circulation adjusts to the altering metabolic demands by increasing the blood flow regionally. (Lassen 1959.) For example dynamic voluntary movement is associated with cortical activation and results in blood flow increase in the supplementary motor area and in the primary sensorimotor area (Orgogozo & Larsen 1979).

The cerebral vasculature has some structural peculiarities different from other peripheral vasculature. The arterioles are short and thin walled. As a result, the role of cerebral microcirculation in vascular resistance is smaller than elsewhere in the periphery and large cerebral arteries account for an unusually high proportion of the vascular resistance. The low arteriolar resistance ensures the high basal flow through the brain. The amount of capillaries in the grey matter is very high, about 3000–4000 per mm^2 cross-section, equal to myocardium. This means diffusion distance of ≤ 10 μm. (Levick 2003, 265–269.) Short diffusion distance is highly important during brain activation, because within the brain, in contrast to skeletal muscle, there is no capillary recruitment. The absence of capillary recruitment emphasizes the role of increased regional cerebral blood flow to maintain cerebral perfusion (reviewed by Secher et al. 2008.) Reductions in CBF can be compensated for by a modest increase in O_2 extraction. However, a relatively high O_2 gradient is needed to maintain optimal cerebral function. Thus, the maximal extraction fraction is much lower for the brain than for the muscle. (Nybo & Rasmussen 2007.)

Cerebral blood flow is regulated mainly by $PaCO_2$ and PaO_2 (reviewed by Ainslie & Duffin 2009). Increased $PaCO_2$, hypercapnia, causes rapid vasodilation and increase in cerebral blood flow. This is shown to be associated with endothelial NO formation and fall in vascular pH by dissociation of carbonic acid. (Peebles et al. 2008.) The washout of the excess CO_2 is of importance because increased H^+ concentration depresses neuronal activity (Guyton & Hall 2006, 761; Levick 2003, 266). In contrast, decreased $PaCO_2$, hypocapnia, leads to vasoconstriction (Peebles et al. 2008). If the $PaCO_2$ (normal value 40 mmHg) is lowered to 15 mmHg by hyperventilation, the cerebral blood flow is reduced by about 50 % due to

vasoconstriction (Levick 2003, 266). Both increased and decreased PaO_2 have an influence on cerebral blood flow (reviewed by Ogoh & Ainslie 2009). The influence of hypoxia on cerebral perfusion is discussed in Chapter 4.1.4.1.

Cerebral perfusion is maintained within a narrow range despite changes in mean arterial pressure between 60 and 150 mmHg. This is brought about by cerebral autoregulation. If blood pressure should fall, cerebral resistance vessels dilate and thus maintain cerebral blood flow. Below 50 mmHg autoregulation mechanisms fail and cerebral blood flow becomes severely decreased. Another mechanism to avoid cerebral underperfusion is the brain's ability to control the systemic arterial pressure through baroreflex affecting rest of circulation except coronary circulation. (Guyton & Hall 2006, 762; Levick 2003, 266).

During dynamic exercise at mild to moderate intensity up to ~60 % $\dot{V}_{O_2}max$, global cerebral blood flow increases with parallel increase in task specific regional CBF. At high intensity exercise leading to exhaustion, CBF decreases toward baseline and is no longer matched to neuronal activity and metabolism. These findings indicate that other factors than neuronal activity and metabolism regulate CBF during exercise. Cerebral autoregulation and $PaCO_2$ have been suggested to play a key role in CBF regulation during exercise. In addition, a variety of other factors like cardiac output ($\dot{Q}$), the arterial baroreflex, and respiratory chemoreflex control might take part in regulation. (Reviewed by Ogoh & Ainslie 2009.)

Cerebral oxygenation is maintained or increased during light- to moderate-intensity exercise. At exercise intensities above ~60 % $\dot{V}_{O_2}max$, the COX begins to decline continuously until maximal exercise is attained (Figure 4). (Bhambhani et al. 2007; Subudhi et al. 2007; Subudhi et al. 2008; Subudhi et al. 2009; Peltonen et al. 2009.) The decline in COX is associated with hyperventilation-induced hypocapnia and diminished cerebral blood flow (Imray et al. 2005).

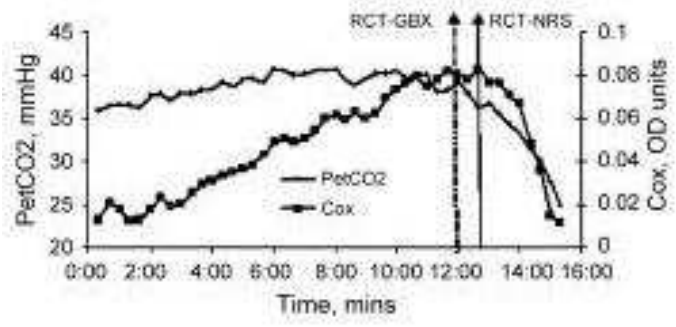

FIGURE 4. Cerebral oxygenation (Cox = here $\Delta[O_2Hb]$) response during stepwise incremental exercise test until volitional fatigue. Here the decline in Cox occurs near respiratory compensation threshold which was determined as a decline in $PETCO_2$. (Bhambhani et al. 2007.)

2.4.2 Muscle tissue

Skeletal muscle blood flow is increased almost linearly in proportion to metabolic rate (Levick 2003, 258). In the resting state, metabolic activity of the muscle is low and the blood flow through muscles is correspondingly low, only 4 ml of blood per 100 grams of muscle tissue per minute. The total blood flow through inactive muscles accounts 15 to 20 % (750–1000 ml/min) of the cardiac output, although muscles constitute between 30 and 40 % of the total body mass (Guyton & Hall 2006, 195-196; Levick 2003, 304). During strenuous exercise, muscular metabolic activity can increase more than 60-fold (Guyton & Hall 2006, 195) and the blood flow more than 20 times, rising to as high as 80 ml/min/100g of muscle (or 19 000 ml/min), which accounts > 80 % of cardiac output (Levick 2003, 304).

In muscle the distance between open capillaries at rest is about 50 µm (West 2008, 88). The increased blood flow through active muscle is due almost entirely to an increase in vascular conductance and to lesser extent to small rise in arterial pressure (Levick 2003, 258). During exercise, metabolism increases and additional capillaries open up in active muscles reducing the diffusion path to the mitochondria and increasing the diffusion area (Guyton & Hall 2006, 503; West 2008, 88). The capillary surface area can be increased two to threefold (Guyton & Hall 2006, 246). In addition to capillary recruitment, vasodilation of arterioles and metarterioles increases flow though active muscle tissue. These changes greatly increase the rate of O_2 transport between blood and muscle fibres. (West 2008, 88; Levick

2003, 304.) The O_2 diffusion to muscle fibers depends on the gradient between the capillary and mitochondrial PO_2, which is close to 0 mmHg at maximal exercise (reviewed by Calbet & Lundby 2009).

During exercise, activation of sympathetic nervous system occurs throughout the body, which redistributes blood flow and maintains systemic arterial pressure. This leads to constriction of the peripheral resistance vessels, i.e. arterioles, except for the arterioles in the active muscles. Actively metabolizing cells surrounding arterioles release vasoactive substances that cause vasodilation by blunting sympathetic vasoconstriction (Rowell 1986, 25.) This phenomenon is called functional sympatholysis (Thaning et al. 2011). The local mechanisms including a fall in tissue PO_2, a rise in tissue PCO_2 and in interstitial osmolarity, accumulation of K^+ and other vasodilator metabolites like adenosine and nitric oxide play an important role in hyperemia (Levick 2003, 258; Guyton & Hall 2006, 196). Despite extensive research, no single regulator factor has been found to be responsible for exercise-induced hyperemia (Heinonen 2010). Sympathetic-mediated vasoconstriction in non-exercising muscles (Bevegard & Shepherd 1966) maintains the total blood pressure that would otherwise decrease during exercise using large muscle groups (Levick 2003, 305). In addition to muscle hyperemia, the amount of O_2 extracted from blood increases with increasing exercise intensity. In resting muscle, the O_2 extraction is 25−30 % and can increase during exercise up to three-fold to a level of 80−90 %. (Levick 2003, 258.)

Muscle oxygenation pattern during incremental exercise to voluntary fatigue has been well documented (Belardinelli et al. 1995; Bhambhani et al. 2001). At the onset of exercise, there is an initial increase in muscle oxygenation reflecting the activation of muscle pump (Shoemaker & Hughson 1999). With increasing work rate, oxygenation decreases progressively (Figure 5). Different oxygenation patterns are observed at intensities above lactate (ventilatory) thresholds. Some authors have reported more steep rate of deoxygenation (Belardinelli et al. 1995; Grassi et al. 1999), while other have observed slowed or continuous linear decrease at these intensities (Subudhi et al. 2007; Peltonen et al. 2009). Near $\dot{V}_{O_2}$max muscle deoxygenation slows and either plateaus (Subudhi et al. 2007; Subudhi et al. 2008) or approaches minimal value (Peltonen et al. 2009). During active recovery period,

there is a rapid increase in muscle oxygenation to a level usually higher than resting baseline value. Maximal oxygenation is reached during first minutes of recovery. (Belardinelli et al. 1995; Bhambhani et al. 2001).

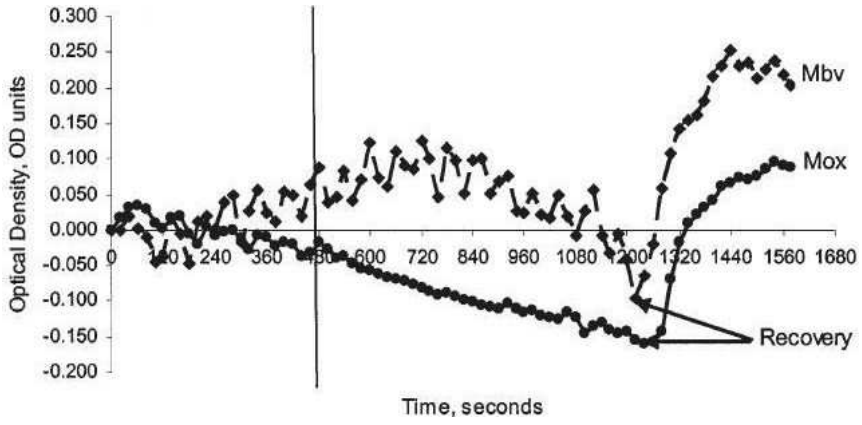

FIGURE 5. Muscle oxygenation (Mox = here $\triangle[O_2Hb]$) response during stepwise incremental cycle exercise test until volitional fatigue. Here the exaggerated reduction in Mox begins near the ventilatory threshold identified by the V-slope method. Mbv = muscle blood volume. (Bhambhani 2004.)

3 VENTILATORY CONTROL

The level of ventilation is tightly regulated. Even during heavy exercise, the rate of alveolar ventilation is matched to metabolic needs of the body, maintaining the levels of PaO_2 and $PaCO_2$ relatively normal. The rhythmic pattern of breathing is generated by the respiratory center, located in the brainstem that sends nerve impulses to respiratory muscles causing ventilation. The basic function of respiratory center is adjusted by various chemoreceptors that monitor variations in blood chemistry. (Guyton & Hall 2006, 515–516.) Parallel to the chemical control of breathing, a number of nonchemical factors can affect respiration in certain situations (Table 1).

TABLE 1. Stimuli affecting the respiratory center (Ganong 1999, 642).

Chemical control
CO_2 (via CSF and brain interstitial fluid H^+ concentration)
O_2, H^+ (via carotid and aortic bodies)
Nonchemical control
Vagal afferents from receptors in the airways and lungs
Afferents from the pons, hypothalamus, and limbic system
Afferents from proprioceptors
Afferents from baroreceptors: arterial, atrial, ventricular, pulmonary

3.1 Respiratory centers

The automatic control of ventilation is brought about by respiratory center that is composed of collection of neurons, located in the pons and medulla. Three main groups of neuron collections are recognized. 1) Dorsal respiratory group is located in the dorsal portion of the medulla and it plays a key role in the inspiration and thus in the basic rhythm of respiration. 2) Ventral respiratory group, located in the ventral area of the medulla, is associated mainly with expiration. At rest, expiration is passive and the expiratory area is almost totally inactive. During heavy exercise when high levels of ventilation are needed, ventral respiratory neurons are activated and provide extra respiratory drive, especially through abdominal

muscles activation. 3) Pneumotaxic center is found in the upper pons, and it regulates the inspiration volume and respiratory rate by inhibiting inspiration. (West 2008, 124–125; Guyton & Hall 2006, 514–515.)

3.2 Chemical control of breathing

The chemical regulation of ventilation is based on responses to changes in arterial O_2, CO_2 and H^+ and it is brought about by chemoreceptors (Ganong 1999, 642). A chemoreceptor is a sensory receptor that detects changes in the concentrations of chemicals in its environment (Guyton & Hall 2006, 572), transducing and conveying the information to respiratory centers to help regulate homeostasis. There are central chemoreceptors in the brain and peripheral chemoreceptors located in the carotid and aortic bodies. (Ganong 1999, 642.)

3.2.1 Central chemoreceptors

Central chemoreceptors are located just beneath the ventral surface of the medulla and are anatomically separate from the respiratory centers. Central chemoreceptors detect changes in H^+ concentration of cerebrospinal fluid, including extracellular fluid, and excite the respiratory center. An increase in H^+ concentration stimulates ventilation, while a decrease inhibits it. However, the blood-brain barrier is relatively impermeable to H^+ ions in the blood, whereas blood CO_2 penetrates easily through the barrier. Blood CO_2 stimulates the respiratory center through its indirect effect on central chemoreceptors. As blood CO_2 rises, CO_2 diffuses through blood-brain barrier reacting with water of the tissues. As a result, carbonic acid forms and dissociates into bicarbonate ions and H^+, and the latter one stimulates the chemoreceptors. Changes in blood PO_2 have no effect on central chemoreceptors. (West 2008, 126–127; Guyton & Hall 2006, 516–517).

3.2.2 Peripheral chemoreceptors

There are peripheral chemoreceptors located in the carotid bodies at the bifurcation of the common carotid arteries and aortic bodies along the arch of the aorta (Figure 6). They de-

tect changes primarily in O_2, but also in CO_2 and carotid body in pH, but to a lesser degree than for O_2. Most of the chemoreceptors are in the carotid bodies which are of greatest importance in humans. Carotid bodies are composed of two types of glomus cells. Type I cells contain a variety of neurotransmitters, including acetylcholine and dopamine. These cells are located close to afferent carotid sinus nerve endings. The carotid bodies also contain type II cells, of which function is unknown. The blood flow of carotid bodies is greatest per unit of tissue in the human body meaning that chemoreceptors are exposed continuously to arterial blood. The mechanism of the carotid bodies has not been identified yet, but presumably glomus cells are the sites of chemoreception and release neurotransmitters in response to changes in arterial PO_2, PCO_2 and pH. This leads to depolarization of carotid sinus nerve afferent terminals and increased action potential traffic to the respiratory center stimulating ventilation. (Powell 2007; West 2008, 128–129; Guyton & Hall 2006, 518.)

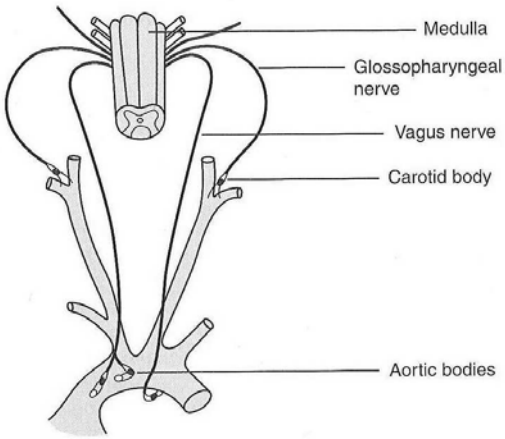

FIGURE 6. Peripheral chemoreceptors in the carotid and aortic bodies (Guyton & Hall 2006, 518).

3.3 Control during exercise and hypoxia

Under normal conditions, arterial CO_2 is the major controller of ventilation. The link between CO_2 and ventilation is so strong that the arterial PCO_2 is held within 3 mmHg even during exercise. However, under hypoxic conditions like at high altitude, arterial PO_2 falls below normal. The stimuli to increase ventilation come then from the peripheral chemore-

ceptors that respond to the decreased O_2 concentration (see Chapter 4.1.1). (West 2008, 129–131.)

Despite extensive research on ventilatory control during exercise, it is still a topic of considerable uncertainty. However, there is strong consensus that control mechanisms include elements of both feedback (central and carotid chemosensory) and feedforward systems (central command and muscle reflex). (Ward 2007.) Measurements of $PaCO_2$ and PaO_2 (which are the main quantities directly regulated by ventilation) show little changes during moderate exercise, although large increases in ventilation are observed already during light to moderate exercise. At more strenuous work levels, $PaCO_2$ often falls and PaO_2 increases. (West 2008, 131, 141.) Thus, it is probable that feedback mechanisms do not play a major role in ventilatory control during exercise (Ursino & Magosso 2004). Feedforward contributions consist of neurogenic and humoral part. Potassium (K^+), produced by exercising muscles, has been suggested to regulate ventilation through its direct effect on arterial chemoreceptor firing, thus elevating ventilation via its peripheral drive. Because this substance is not directly modified by ventilation, it is considered as feedforward in type. Other possible feedforward mechanisms are central neurogenesis (i.e. impulses from motor cortex are transmitted simultaneously into the respiratory center and to the exercising muscles) and peripheral neurogenesis (i.e. impulses from joint and muscle receptors of moving limbs might participate in stimulation of ventilation). (Ursino & Magosso 2004; Guyton & Hall 2006, 520; West 2008, 135.)

4 PHYSIOLOGICAL RESPONSES TO ALTITUDE

Altitude exposure and acclimatization have been of great interest since the first ascent of Everest in 1953 (Hornbein & Schoene 2001a, *iii*). The greatest adaptive challenge at high altitude is hypoxia, abnormally low oxygen availability to the body. With increasing altitude the barometric and hence the PiO_2 decrease. (Hornbein & Schoene 2001a, 199.) This results in less complete oxygenation of blood within the lung, a challenge amplified during exercise, when increased oxygen requirements must be met despite decreased oxygen driving pressure (Grover et al. 1986).

Low pressure itself is not deleterious unless the decompression is rapid (Ward et al. 1989, 27). On the summit of Mount Everest (Mt. Everest), the barometric and oxygen pressures are approximately one-third of sea level values. The barometric pressure at 8848 m was first measured directly in 1981 during the American Medical Research Expedition and measurements indicated a value of 253 mmHg. (West et al. 1983.) Sudden exposure to extreme hypoxia similar to that at the summit of Mt. Everest leads to unconsciousness within minutes and if prolonged, to death (West & Wagner 1980).

The whole O_2 transport chain is affected by lowered PO_2. Both acute and chronic exposures to hypoxia provoke multiple systems (i.e. pulmonary, cardiovascular) in the body to adjust to secure sufficient oxygen supply to tissues and organs (Hornbein & Schoene 2001a, 140; Bartsch & Saltin 2008). Acute responses refer to immediate effects occurring from seconds to hours upon hypoxic exposure (Ward et al. 1989, 67). Chronic hypoxia is related to prolonged exposures and occurrence of numerous adaptive responses over periods of hours to months to improve tolerance to high altitude. Chronic adaptation to altitude is defined as acclimatization. (Ward et al. 1989, 69.)

The magnitude and the speed of the responses are altitude dependent, each system having their own threshold altitude. Despite generalities, current evidence indicates substantial inter-individual variability in responsiveness to high altitude that may facilitate or hinder the

acclimatization process. (Reviewed by Bartsch & Saltin 2008.) It is beyond the scope of this study to describe in detail the time scale of each adaptive changes happening at altitude between acute hypoxia and two months. Rather, we focus on chosen changes seen during constant, moderate exercise in acute hypoxia before and after sustained 2-month stay at altitude. A summary of the effects of different altitudes and acclimatization on key variables of oxygen transport chain is shown in the Figure 7.

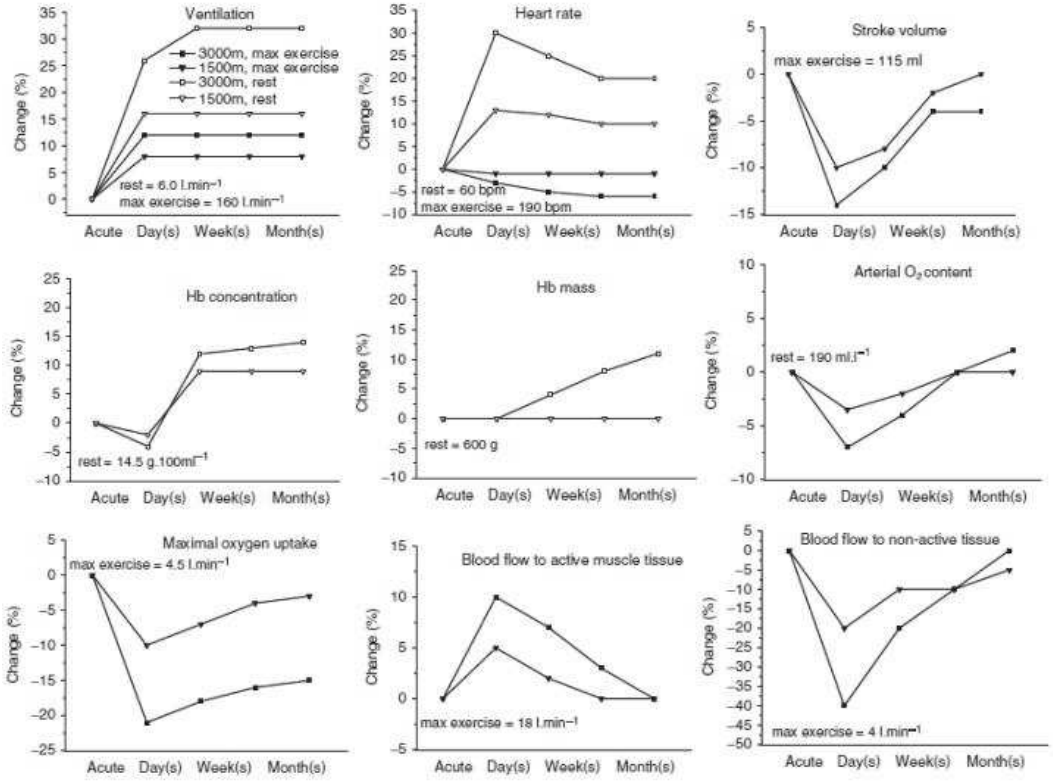

FIGURE 7. Physiological responses at rest and/or during maximal exercise at two altitudes (1500–2000 and 3000–3500m above sea level). The x-axis denotes the time scale and the y-axis denotes relative changes, using sea level as baseline. (Modified from Bartsch & Saltin 2008.)

4.1 Acute hypoxia

Upon exposure to hypoxia, there is an attempt to maintain sufficient O_2 delivery ($\dot{Q} \cdot CaO_2$) to tissues despite lowered PiO_2. Cardiac output is increased upon exposure to hypoxia.

(Vogel & Harris 1967.) In addition, hypoxia-induced hyperventilation and left-shift of hemoglobin O_2 dissociation curve are critical to improve blood oxygenation at 3000 m and higher, although the role of $\dot{Q}$ in preserving oxygen delivery to tissues is relatively greater when CaO_2 is significantly decreased. In acute hypoxia, convective O_2 transport is preserved up to ~4500 m during submaximal exercise, after which proper tissue function might be compromised. (Reviewed by Calbet & Lundby 2009; Levick 2003, 317.)

4.1.1 Acute ventilatory response to hypoxia

Ventilation ($\dot{V}_E$) rises rapidly at the onset of hypoxia at rest (Easton et al. 1986). This initial response is called acute hypoxic ventilatory response (AHVR) (Zhang & Robbins 2000). Acute hypoxic ventilatory response results from stimulation of the peripheral chemoreceptors, primarily the carotic bodies, by the decreased PaO_2 (Weil & Zwillich 1976). Increased ventilatory chemosensitivity results in increased PaO_2 and SpO_2 (Levick 2003, 317). However, it also leads to concomitant decrease in arterial PCO_2, which may counter the hypoxic drive to breath. The magnitude of hypocapnia-induced ventilatory inhibition depends on individual ventilatory responsiveness to CO_2. (Moore et al. 1984.) The state of reduced $PaCO_2$ in the blood is called hypocapnia and it is often caused by deep or rapid breathing, known as hyperventilation (Weil & Zwillich 1976). Increasing level of hypoxia has been shown to provoke a larger hypoxic ventilatory response (Pandit & Robbins 1991).

When hypoxia is sustained, $\dot{V}_E$ declines somewhat over the subsequent 20–30 minutes. This phenomenon is called hypoxic ventilatory decline (HVD). The magnitude of this decrease in $\dot{V}_E$ (hypoxic ventilatory decline, HVD) is related to the size of the acute response. (Easton et al. 1986.) The accurate mechanisms behind HVD remain controversial. Peripheral chemoreceptor desensitization (Dahan et al. 1996) and build-up of inhibitory and/or decline in excitatory neurochemicals with net inhibitory influences on respiratory neurons have been suggested (Long at al. 1994). To summarize, initial ventilatory response to hypoxia is biphasic (Easton et al. 1986). Within several hours of sustained hypoxia, AHVR begins to increase progressively until reaching a plateau after days or weeks (Sheel et al. 2010).

Diminished ventilatory chemosensitivity has been observed in athletes with high aerobic exercise capacity in comparison with normal controls (Byrne-Quinn et al. 1971; Schoene 1982). However, discrepant results were reported by Sheel et al. (2006) and Levine et al. (1992), who found no relationship between AHVR and maximal aerobic capacity. Experienced climbers have been reported to show vigorous respiratory responses to hypoxia (Schoene 1982; Masuyama et al. 1986). Based on these findings, a very high AHVR has been regarded as prerequisite for reaching extreme altitudes without supplemental oxygen. Recently Bernardi et al. (2006) brought new insight to the topic by describing in more detail differences between subgroups of elite climbers. It was reported that successful climbers reaching extreme high altitudes (Mt. Everest and K2, 8848 and 8611 m, respectively) without supplemental oxygen were characterized by a lower ventilatory sensitivity, as compared to those who did not succeed to summit or needed supplemental oxygen. It was concluded that instead of very high hypoxic ventilatory response, moderate AHVR combined to a high ventilatory efficiency would be key requirement to climb in extreme hypoxia. This was speculated to provide more ventilatory reserve allowing sustainable ventilation near the summit. Of interest was the notion, that the differences between climbers became apparent only during acclimatization, but not at sea level, suggesting differences in adaptation strategies. (Bernardi et al. 2006.)

4.1.1.1 Methodology

A variety of methodologies are used for assessment of AHVR including multi-step decreases (Ainslie et al. 2003), progressive, ramp-like decreases (Moore et al 1984; van Klaveren & Demedts 1998; Foster et al. 2005), single step decreases (Steinback & Poulin 2007; Pandit & Robbins 1991) and single breath decreases in SpO_2, PaO_2 or fraction of inspired oxygen (FiO_2). Also methods assessing ventilatory responses to hypercapnia (rise in arterial PCO_2) have widely been used (Hirshman et al. 1975; Kronenberg et al. 1972; van Klaveren & Demedts 1998).

Most investigators have measured responses under isocapnic conditions, in which $PETCO_2$ is held constant by adding CO_2 to the inspired air (Weil & Zwillich 1976). Isocapnic hy-

poxia is regarded as pure stimulus to increase ventilation because it is independent of inhibiting effects of hyperventilation induced hypocapnia (Moore et al. 1984). However, results obtained under isocapnic conditions are not representative of the high altitude environment. At high altitude, hyperventilation causes hypocapnia, and poikilocapnic measurements resemble these conditions by allowing $PETCO_2$ to decrease natural way. Thus, in poikilocapnic hypoxic procedures no CO_2 is added to the inspired air (Moore et al. 1984).

It is known that the variability of AHVR between subjects is wide. Coefficient of variation of 24 ± 5 % for AHVR within individuals has been reported (Moore et al. 1984). Considerable between-day variability has also been reported ranging from 17 % (Ainslie et al. 2005) to 26 % (Zhang & Robbins 2000).

4.1.1.2 Determination of acute hypoxic ventilatory response

Reduction in PaO_2 is the stimulus to ventilation during hypoxia rather than CaO_2 or SpO_2. The relationship of ventilation to PO_2 is hyperbolic (Figure 8). However, the relationship between $\dot{V}_E$ and SpO_2 is linear as shown in Figure 8. (Weil & Zwillich 1976.) Traditional way to quantify AHVR is to divide the increase in ventilation by the fall in SpO_2 so that values for ventilatory sensitivities per unit desaturation are obtained (Steinback & Poulin 2007). Curvilinear relationships between PaO_2 and $\dot{V}_E$ can also be solved by hyperbolic function to give a reflection of the hypoxic responsiveness (Weil & Zwillich 1976). This method is used less due to its complexity and the fact that detectable changes in ventilation require considerable low PaO_2 values.

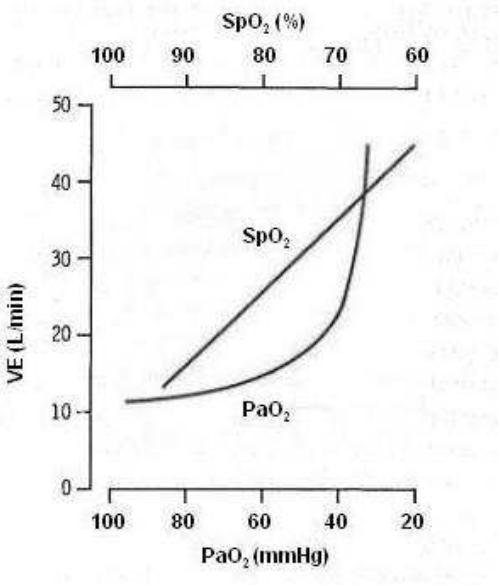

FIGURE 8. Relationship between ventilation and PaO_2 / SpO_2 (modified from Ward et al. 1989, 86).

4.1.1.3 Acute hypoxic ventilatory response during exercise

During exercise in acute hypoxia at a given absolute intensity, ventilatory response is exaggerated compared to normoxia as reflected by higher $\dot{V}_E/\dot{V}_{O2}$ ratio (reviewed Calbet & Lundby 2009). Exercise increases AHVR compared to the resting values (Weil et al. 1972; Pandit & Robbins 1991; Sato et al. 1996; Ainslie et al. 2007) and responsiveness increases with increasing exercise intensity (Weil et al. 1972). It has also been shown that inter-subject variability in AHVR is amplified during exercise so that high responders to hypoxia at rest show even higher responses during exercise (Sato et al. 1996). According to findings of Dahan et al. (1996) and Pandit & Robbins (1991) exercise reduces or even abolishes HVD.

4.1.2 Cardiovascular responses

Immediately after exposure to hypoxia the resting $\dot{Q}$ increases (Vogel & Harris 1967). This is a compensatory response that preserves oxygen delivery to tissues despite decreased CaO_2. In the study of Danish lowlanders, CaO_2 at rest at sea level was 180 ± 7 $ml \cdot L^{-1}$ and

during acute hypoxia at sea level (corresponding to an altitude of 5300 m) 166 ± 9 $ml \cdot L^{-1}$ (Calbet et al. 2003a). Thus, normal resting O_2 consumption (~ 3.5 $ml \cdot kg^{-1} \cdot min^{-1}$) is maintained. The increase in $\dot{Q}$ is brought about by increased heart rate (HR) while stroke volume remains unchanged. (Reviewed by Naeije 2010; Vogel & Harris 1967.) The increased HR results from withdrawal of vagal inhibition of sinus node (Levick 2003, 318). After a few days of hypoxic exposure, $\dot{Q}$ returns to normal or slightly below in conjunction with decreasing stroke volume, whereas HR remains elevated (Vogel & Harris 1967; Klausen 1966). Reduced stroke volume results primarily from smaller venous return secondary to the smaller blood volume produced by hemoconcentration. That in turn increases the oxygen-carrying capacity of the blood even before the onset of polycythemia, the increase in the red blood cell mass. (Grover et al. 1986.)

Unlike during exercise in normoxia where SpO_2 in maintained well up to maximal exercise SpO_2 falls in acute hypoxia with increasing exercise intensity (Fukuda et al. 2010; Peltonen 2001). Thus, $\dot{Q}$ plays even bigger role in providing adequate O_2 delivery (Calbet et al. 2009). During submaximal exercise at a given absolute intensity, $\dot{Q}$ and HR are increased in comparison with sea level values (Fukuda et al. 2010; Peltonen 2001), maintaining systemic O_2 delivery at the same level as in normoxia (Calbet et al. 2009). Stroke volume remains unchanged from sea level values (Calbet et al. 2003a). Blood pressure response to dynamic whole body exercise remains similar or is slightly reduced compared to normoxia (Calbet et al. 2009).

Hypoxia induces vasoconstriction of pulmonary arteries leading to pulmonary hypertension. Resting mean pulmonary artery pressure is almost doubled to about ~30 mmHg improving apical perfusion and the ventilation-perfusion ratio. If pulmonary hypertension is prolonged, the work of the right ventricle is increased and right heart failure might occur. (Levick 2003, 318.) Pulmonary hypertension increases in relation to the altitude level and the degree of exercise (Penaloza & Arias-Stella 2007).

4.1.3 Saturation

Oxygen saturation begins to falls significantly at PO_2 values below 60 mmHg, corresponding to altitude of 3000 m and higher, where the downslope of the hemoglobin O_2 dissociation curve is at steepest (Levick 2003, 317). Hypoxia-induced hyperventilation causes hypocapnia and alkalosis that initiates left-shift of oxyhemoglobin dissociation curve, thus improving arterial O_2 saturation (Sheel et al. 2006). Calbet et al (2003a) estimated that, due to hyperventilation-induced alkalosis, SpO_2 was ~8 % higher during maximal exercise in acute hypoxia than it would have been with pH similar to that in normoxia.

4.1.4 Tissue oxygenation

During constant exercise in acute hypoxia, tissue oxygenation may become insufficient unless tissue blood flow is increased enough to counterbalance the reduced CaO_2. With reduced CaO_2, tissue O_2 delivery must be met by increased tissue blood flow and O_2 extraction. If the O_2 supply to the brain or respiratory muscles is insufficient to maintain their metabolic rate, premature fatigue may develop. (Reviewed by Amann & Calbet 2008.)

4.1.4.1 Cerebral tissue

Both PaO_2 and $PaCO_2$ are reduced on acute exposure to hypoxia and during exercise at altitude, and have opposite effects on cerebral blood flow. Hypoxia, reflected in a fall in PaO_2 below hypoxic vasodilatory threshold (< 40 mmHg; normal value ~100 mmHg) produces immediate cerebral vasodilation (Gupta et al. 1997). Systemic hypoxia simultaneously stimulates ventilation via peripheral chemoreceptor activation leading to lowering of $PaCO_2$ and thus to counteracting cerebral vasoconstriction and hypoperfusion (Levick 2003, 266). The net effect of these two opposing mechanisms affecting cerebral blood flow and oxygenation is dependent on individual balance between AHVR and cerebral CO_2 reactivity, an “index” of the ability of the cerebrovascular bed to dilate or constrict in response to changes in $PaCO_2$ (reviewed by Secher et al. 2008). This balance is expected to change during acclimatization (reviewed by Ainslie & Ogoh 2010).

Upon initial exposure to hypoxia, CBF is increased as a result of greater relative degree of hypoxia compared with hypocapnia. Cerebral blood flow reaches its peak value in 2–3 days and return toward sea level values in 1–3 weeks, coincident with ventilatory acclimatization. Over the period of ventilatory acclimatization, PaO_2 increases and $PaCO_2$ decreases and the latter becomes more powerful determinant of CBF (Lucas et al. 2011). Individuals with brisk ventilatory response have higher PaO_2 and reduced $PaCO_2$. Thus individuals with high AHVR are likely to have lower CBF. (Reviewed by Ainslie & Ogoh 2010.)

Cerebral oxygenation is diminished during incremental exercise in acute hypoxia. Subudhi et al. (2007, 2008) found different deoxygenation pattern in hypoxia than in normoxia as indicated by increased deoxyhemoglobin (HHb) and decreased O_2Hb. In hypoxia, cerebral oxygenation decreased consistently across all work rates during cycling exercise, in contrast to normoxia, where decrease was seen after 75 % of peak power. In addition, changes in cerebral oxygenation were larger during hypoxia at all relative and absolute work rates. (Subudhi et al. 2007, Subudhi et al. 2008.) Peltonen et al. (2009) reported greater cerebral deoxygenation in hypoxia compared with normoxia during cycling exercise. In hypoxia, HHb was higher at any given work rate and decrease in cerebral tissue saturation index (TSI) started earlier than in normoxia.

Despite maintenance or increase in cerebral blood flow, COX is decreased during exercise in acute hypoxia. Ainslie et al. (2007) found that middle cerebral artery blood flow velocity (MCAv), a reliable and valid index of CBF (Secher et al. 2008), was maintained at normoxic level during hypoxic exercise, despite greater degree of hypocapnia during submaximal exercise in hypoxia. However, maintained MCAv was inadequate to meet the cerebral O_2 demand, as seen by reduced COX. (Ainslie et al. 2007.) Likewise, others have reported similar results (Subudhi et al. 2009; Peltonen et al. 2009; Imray et al. 2005). Exercise might modify the interaction between PaO_2 and $PaCO_2$ in the regulation of CBF, because similar or increased CBF is seen during hypoxic exercise, but not at rest, compared to normoxia (Ainslie et al. 2007.). This might results from hypoxia-induced changes in cerebral autoregulation, sympathetic nerve activity, and/or changes in the sensitivity of the cerebrovascular bed to hypoxia and hypocapnia (reviewed by Ogoh & Ainslie 2009).

4.1.4.2 Muscle tissue

Studies concerning muscle oxygenation in acute hypoxia have produced equivocal results. Peltonen et al. (2009) did not detect any differences in the extent of muscle deoxygenation between hypoxia and normoxia, while Subudhi et al. (2007, 2008) reported greater muscle deoxygenation at all relative and absolute work rates under hypoxic conditions. All studies included similar very heavy intensity ramp incremental cycling exercise and subjects did not differ considerably in their characteristics. However, the studies differed in the severity of hypoxia (PiO_2 118 ± 5 mmHg, PiO_2 ~75 mmHg, and PiO_2 86 mmHg in Peltonen et al. 2009, Subudhi et al. 2007 and Subudhi et al. 2008, respectively).

Muscle O_2 delivery (muscle blood flow · CaO_2) during submaximal exercise is maintained in acute hypoxia at similar level than in normoxia. In addition to increased $\dot{Q}$, this is brought about by increased skeletal muscle blood flow. (Koskolou et al. 1997; Roach et al. 1999.) In contrast to this common finding, Calbet et al. (2003a) observed a reduction in systemic and leg O_2 delivery (22 % and 25 %, respectively) during submaximal constant intensity exercise in acute hypoxia, despite 21 % and 25 % increases in $\dot{Q}$ and leg blood flow, respectively. Thus, O_2 delivery did not match O_2 demand, which was compensated by increased anaerobic energy production. The discrepancy in the observations was likely due to more severe hypoxia induced in the study of Calbet et al (2003), eliciting PaO_2 of 31 mmHg. Distribution of $\dot{Q}$ between exercising muscles and other body tissues was similar to normoxia. (Calbet et al. 2003a.)

4.2 Chronic exposure to hypoxia

Pulmonary O_2 uptake remains similar during moderate exercise in acute hypoxia before and after 9–10 wk acclimatization to 5260 m. After altitude acclimatization, however, a marked increase in systemic O_2 delivery is seen when compared to acute hypoxia due to the acclimatization-induced elevation in blood [Hb]. (Calbet et al. 2003b.)

4.2.1 Ventilatory acclimatization

Acclimatization to chronic hypoxia involves a steady increase in AHVR (Sato et al. 1992). At a given degree of hypoxia, increase in ventilation is greater after chronic hypoxia compared with acute hypoxia. This ventilatory acclimatization is likely due to further increase in carotid body activity (Powell 2007). Studies aiming to determine the maximum degree of chemoreceptor sensitization have failed to detect signs of blunted AHVR after continuous stay at 3800 m for 12 days (Sato et al. 1994) or 8 weeks (Hupperets et al. 2004). According to these findings, ventilatory chemosensitivity determined at rest increases significantly at a given altitude compared to sea level value, and remains elevated for up to two months.

4.2.2 Cardiovascular acclimatization

With altitude acclimatization, pulmonary gas exchange during submaximal exercise is improved compared to acute hypoxia, as indicated by reduced alveolar-arterial O_2 difference. Different underlying mechanisms are likely to account for this: 1) increased PAO_2, 2) improvement in lung diffusing capacity, mainly due to higher [Hb], and 3) more time available for diffusion equilibration between the alveoli and the pulmonary capillaries because of lower $\dot{Q}$ at a given submaximal work rate. (Calbet et al. 2003b; Lundby et al. 2004.) Calbet et al. (2003a) calculated that in acute hypoxia about 60 % of the reduction in CaO_2 during maximal exercise was explained by the impairment of pulmonary gas exchange, the rest by the lowered PiO_2.

Following acclimatization, arterial oxygen content is increased to or even above sea level values. In the study of Danish lowlanders, CaO_2 at rest at sea level was 180 ± 7 $ml \cdot L^{-1}$ and after 9–10 wk acclimatization to 5260 m 196 ± 11 $ml \cdot L^{-1}$ (Calbet et al. 2003b). This is brought about by increased hemoglobin concentration (36 % in Calbet et al. 2003b) after weeks of hypoxic exposure that is sufficient to offset the decrease in SpO_2 caused by the reduced PiO_2 (Lundby et al. 2004). Despite lowered $\dot{Q}$ (15 %) after acclimatization, systemic O_2 delivery was higher than in acute hypoxia during submaximal exercise. Mean ar-

terial pressure was slightly increased after acclimatization in comparison with acute hypoxia. (Calbet et al. 2003b.)

4.2.3 Saturation

After acclimatization, the oxyhemoglobin dissociation curve is moved to the same position than observed at sea level before hypoxic exposure, and more right when compared to acute hypoxia (Calbet et al. 2003b). This can be seen in increased P_{50} value (Calbet et al. 2003b), describing the PO_2 for 50 % O_2 saturation (West 2008, 78–79). Because of this, hemoglobin affinity for O_2 is impaired after acclimatization compared to acute hypoxia, and O_2 unloading in the tissues is improved (West 2008, 78–79). Calbet et al (2003b) showed that increase in P_{50} after acclimatization counterbalanced the respiratory alkalosis-induced left-shift of hemoglobin O_2 dissociation curve as ~10 % lower SpO_2 was observed after acclimatization, despite similar pH and blood temperature responses during submaximal exercise in acute and chronic hypoxia. Consequently, SpO_2 had only a minor effect on improved arterial O_2 content during exercise after acclimatization. In addition, higher P_{50} seemed not to facilitate muscle O_2 extraction in the actively working muscles. (Calbet et al. 2003b.) With acclimatization, more 2,3-DPG is produced by the red cells causing right-shift of the dissociation curve. This improves O_2 unloading in the tissues. (Guyton & Hall 2006, 508.)

4.2.4 Tissue acclimatization

The increase in CaO_2 following acclimatization is counterbalanced by a reduction in leg blood flow such that O_2 delivery to the working muscles is maintained (Roach et al. 1999; Calbet et al. 2003b). In the brain, multiple factors are regulating the cerebral blood flow (reviewed by Ainslie & Ogoh 2010) and slightly improved O_2 delivery has been suggested after short term acclimatization (Imray et al. 2005).

4.2.4.1 Cerebral tissue

Only few studies have investigated the effect of acclimatization on cerebral oxygenation during exercise. Subudhi et al. (2008) studied the effect of short time acclimatization on cerebral and muscle (See Ch. 4.2.5.2) tissue deoxygenation (indicated by increased HHb and decreased O_2Hb) during incremental cycling exercise. The subjects were tested at sea level, in acute hypoxia and in chronic hypoxia. The test of acute hypoxia was performed at sea level in hypobaric chamber (PiO_2 86 mmHg) after 20 min of resting exposure. The second test of chronic hypoxia was performed under environmental hypoxia after 5 days of acclimatization to moderate altitude (2200 m, PiO_2 115 mmHg) and 24 h of exposure to high altitude (4300 m, PiO_2 86 mmHg). Cerebral oxygenation followed similar pattern to that seen in acute hypoxia, i.e. COX decreased throughout exercise until W_{max}. The extent of deoxygenation was greater than at sea level at each absolute work rate and greater than in acute hypoxia at 175 W. When oxygenation was expressed relative to sea level W_{max}, deoxygenation showed greater response at W_{max} in chronic hypoxia relative to acute hypoxia. (Subudhi et al. 2008.)

Imray et al. (2005) studied effect of cycling exercise on cerebral perfusion at different altitudes. Measurements took place at 3610 m, at 4750 m and at 5260 m, 24–36 h after arrival in each altitude. Results obtained from two highest altitudes were considered to represent partial acclimatization, because they were performed 4–7 days after arrival at 3610 m. At all altitudes, COX was decreased with increasing altitude and exercise intensity. The decline followed similar pattern at 3610 m and at 4750 m. At 5260 m, the decline in COX was slightly less than expected that was speculated to result from small altitude difference and some acclimatization between the tests. At 5260 m, fell in COX occurred during submaximal exercise despite increase in MCAv. (Imray et al. 2005.)

4.2.4.2 Muscle tissue

After six days acclimatization at 2200–4300 m, muscle oxygenation showed similar response than in acute hypoxia, i.e. oxygenation was reduced progressively from the start to

cessation of exercise without plateauing near maximal exercise. Muscle $\Delta[O_2Hb]$ was smaller and Δ[HHb] greater than at sea level at each absolute work rate, but not different from acute hypoxia. When oxygenation was expressed relative to sea level W_{max}, no differences were seen between the three conditions at any work rate. (Subudhi et al. 2008.)

Martin et al. (2009) investigated a group of mountaineers at sea level before the expedition and on different altitudes and time points during acclimatization to 5300 m. As a subgroup, climbers reaching the summit of Mt. Everest (8848 m) were studied. When responses after prolonged chronic hypoxia (69–71 days) were compared to short time acclimatization responses (4–6 days, 3500 m), no differences in the extent of deoxygenation (indicated as decrease in TSI) or oxygenation profiles were seen. In comparison with sea level, the absolute values of MOX were about 15 % lower throughout the exercise protocol. The change of MOX from unloaded cycling to maximal exercise and rate of desaturation were smaller in climbers than in those who remained at 5300 m. (Martin et al. 2009.)

During submaximal exercise in chronic hypoxia, higher O_2 delivery to working muscles was found after 9–10 wk acclimatization in comparison with acute hypoxia. Leg blood flow was slightly, though not significantly, reduced. Distribution of $\dot{Q}$ between exercising muscles and tissues other than the active muscles was similar in acute and chronic hypoxia. (Calbet et al. 2003b.)

4.2.5 Exercise performance

As $\dot{V}_{O_2}max$ is determined by maximum O_2 transport ($\dot{V}_{O_2}max = \dot{Q} \cdot CaO_2$), it will be limited at high altitude with less oxygen available. For each 1000 m increase, $\dot{V}_{O_2}max$ decreases 7–9 %. (Fulco et al. 1998.) However, mountaineering does not involve maximal exercise as it has been shown to occur at $\dot{V}_{O_2}$ of 50–75 % of $\dot{V}_{O_2}max$ (Pugh 1958). Submaximal exercise performance (time-to exhaustion), is also impaired at altitude and improved with acclimatization (Fulco et al. 1998; Schuler et al. 2007). A strong association has been found between the altitude-associated increase (%) in [Hb] and time to exhaustion ($r^2 = 0.971$; $P < 0.05$) (Schuler et al. 2007).

5 PURPOSE OF THE STUDY, RESEARCH PROBLEMS AND HYPOTHESES

The purpose of the present study was to examine acute responses to constant exercise in experienced climbers under progressively increasing poikilocapnic hypoxia. For adjustment of poikilocapnic hypoxic exposure, a method was developed to simulate increasing environmental hypoxia. The ascending hypoxic steps were produced by altering PO_2 and PN_2 of inspired gas mixture, while keeping the total pressure normobaric. Of special interest was the extent of cerebral and muscle tissue oxygenation in acute hypoxia before and after acclimatization, and their relationship with ventilatory chemosensitivity.

Measurements were performed during exercise to effectively stimulate cardiorespiratory responses and mimic the work load while ascending at real altitude. Moderate, constant exercise intensity was chosen to induce increased ventilation by progressive hypoxia alone, i.e. by keeping the exercise intensity below respiratory compensation point (Takano 2000). Highly selected subjects uniquely enabled execution of exercise protocol under hypoxia ranging from mild to severe hypoxia, as well as examination of subjects after acclimatization to environmental hypoxia. The research problems and their hypothesis are presented as follows:

1. Does the protocol suit to simultaneous measurement of acute hypoxic ventilatory chemosensitivity, and cerebral and muscle oxygenation during constant exercise in progressive poikilocapnic acute hypoxia?

Acute hypoxic ventilatory response has previously been determined during constant exercise under isocapnic, progressive hypoxia (Sato et al. 1996) and during ramp incremental exercise in poikilocapnic hypoxia (Peltonen et al. 2009). Acute hypoxic ventilatory chemosensitivity and oxygenation responses have been measured simultaneously at rest under progressive isocapnic hypoxia (Peltonen et al. 2007; Kolb et al. 2004) and during ramp incremental exercise in poikilocapnic hypoxia (Peltonen et al. 2009).

It is hypothesized that the protocol will be suitable to measure both AHVR and cerebral and muscle oxygenation responses to progressive poikilocapnic hypoxia during constant exercise.

2. Does 2-month chronic hypoxia affect AHVR, and cerebral and muscle tissue oxygenation responses to constant exercise in acute hypoxia?

It is hypothesized that

a) increased HVR will be seen after acclimatization (Hupperets et al. 2004; Sato et al. 1994) and the increase in HVR still persists after return to sea level (Steinacker et al. 1996).
b) muscle oxygenation will be maintained after acclimatization (Subudhi et al. 2008; Martin et al. 2009).
c) cerebral oxygenation will be maintained or slightly decreased with acclimatization (Subudhi et al. 2008; Imray et al. 2005).

3. Does AHVR explain differences in cerebral and muscle tissue oxygenation responses?

It is hypothesized that

a) if AHVR differs among subjects, hyperventilation induced hypocapnia will lead to decreased cerebral oxygenation in subjects with high AHVR (Peltonen et al. 2009) in both tests.
b) no association will be found between AHVR and muscle tissue oxygenation in either tests (Peltonen et al. 2009).

6 METHODS

6.1 Subjects

Nine healthy males (height 179 ± 7 cm, body mass 80.4 ± 8.6 kg, age 37 ± 6 years, body mass index (BMI) 25.1 ± 2.57, $\dot{V}_{O_2}$max 55.0 ± 6.7 ml·kg^{-1}·min^{-1}) took part in the study after providing informed written consent. This study was approved by the institutional research board and the University of Helsinki ethics committee, and it was conducted according to the Declaration of Helsinki. Subjects were trained elite climbers and members of Airborne Ranger Club of Finland taking part in expedition aiming to summit Mount Everest. Medically screening including standard 12-lead ECG at rest and flow-volume spirometry (Medikro Spiro 2000, Medikro, Kuopio, Finland) were performed before the study. The subjects were also examined by a physician to finalize preparticipation screening. None of the subjects had history of cardiovascular, respiratory, or musculoskeletal diseases, and all were free of medication that would affect cardiorespiratory response to exercise during acute hypoxia. Maximal O_2 uptake values were obtained from incremental step tests performed before pre measurements.

6.2 Experimental design

The experimental protocol was performed twice with each subject, on average 12 d before (range 6–20 d) and 13 d (range 9–15 d) after the expedition. Measurements were performed at sea level at ambient temperature and humidity for both pre and post tests. At the beginning and in the end of the expedition, the climbers spent 5 and 6 days, respectively, in Kathmandu at an altitude of 1300 m. The climbers ascended first to the base camp of Mount Everest (5300 m) and reached progressively higher camps at various altitudes. Finally, four out of nine subjects reached the Everest summit (8848 m) with oxygen supplementation, after 59 days. Each climber reached at least the altitude of 7100 m. The time course and altitude ascent profile is summarized in Figure 9. Expedition day 1 was defined

as the day of arrival in Kathmandu. The expedition lasted 72 days in total, including two month stay at an altitude above 2500 m from which 49 days above 5300 m.

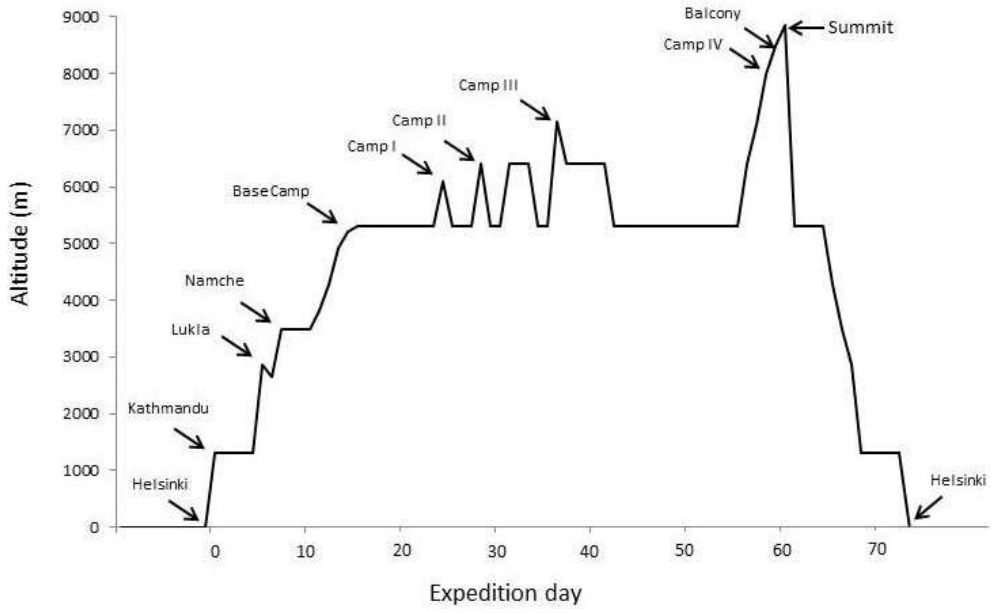

FIGURE 9. Average ascent profile.

6.3 Experimental protocol

The subjects arrived to the laboratory three to four hours after a meal and after 24 h without alcohol ingestion and physical exercise for at least 12 h. The experiment consisted of walking on a treadmill with constant load (speed 5.5 $km \cdot h^{-1}$, grade 3.8°, theoretical $\dot{V}_{O_2}$ 22.75 $ml \cdot kg^{-1} \cdot min^{-1}$) while the subjects were exposed to increasing hypoxic stimulus. The actual hypoxic exposure was preceded by 3-min of familiarization at rest while subject stand relaxed and breathed normoxic room air through a mask. Then subject began 3-min baseline walking in normoxia, after which hypoxic breathing was started. The hypoxic stimulus was varied by reducing the inspired O_2 concentration at predetermined 3-min steps to simulate altitudes of camps during an ascent to Mt. Everest. The targeted altitudes were chosen to correspond to camp locations along the climbing route. The total pressure of gas mixture was kept normobaric by increasing N_2 concentration simultaneously. The time course of the acute hypoxic exposure is shown in Table 2.

The duration of each hypoxic step (3 min) was chosen to ensure enough time for adjustment of gas mixture and for measured variables to settle to a new level of PiO_2. A total duration of the hypoxic exposure (< 20 min) was chosen to avoid possible hypoxic ventilatory decline during longer exposures (Howard & Robbins 1995).

TABLE 2. Hypoxic protocol.

Time (min)	Target altitude (m)	Target PiO_2 (mmHg)
0 (rest)	0	159
3	0	159
6	2400	120
9	3500	105
12	4800	90
15	5200	86
18	5800	80
21	6400	75
24	7100	69
27	8300	60

Hypoxic protocol was executed with a flow meter designed in cooperation with Woikoski Inc. The apparatus consisted of gas bottles (O_2 and N_2), adjustment device for O_2 and N_2 gas flow, mixing chamber for inspiratory gases and a tube to conduct gases from mixing chamber to a three-way valve. The three-way valve directed either normoxic room air or apparatus delivering O_2/N_2 mixtures to the inspiratory port of nonrebreathing valve. The adjustments of inspiratory O_2 and N_2 gas flow were made by comparing the measured fractional gas concentration of inspired O_2 and N_2 with the desired values and adjusting the gas mixture manually as needed.

To ensure the well-being of the subject, exercise was terminated immediately when the subject was willing to stop, subject started to exhibit impaired coordination of walking or latest when SpO_2 fell to 62 %. The hypoxic exposure was monitored by a physician. Immediately after the termination of the test, the subjects were given 100 % O_2 via respiratory mask to speed up the recovery of SpO_2 to the normal level.

6.4 Measurement of cardiorespiratory responses

Heart rate was continuously recorded using ECG and SpO_2 was measured by a pulse oximetry (Nonin 9600, Nonin Medical, Inc., Plymouth, USA) on the right forefinger tip. Ventilation and alveolar gas exchange including end-tidal partial pressures for O_2 and CO_2 ($PETO_2$ and $PETCO_2$, respectively) were measured breath-by-breath throughout the test. The inspiratory and expiratory flow and volumes were monitored by low-deadspace low-resistance turbine (Triple V, Jaeger Mijnhardt, Bunnik, The Netherlands) connected to a mask (Hans Rudolph Inc., Kansas City MO USA). The turbine was calibrated before each test by a 3.00-L syringe (Hans Rudolph Inc., Kansas City, MO, USA). Inspired and expired gases were sampled continuously at the mouth and concentrations of O_2, CO_2, N_2 and argon (Ar) were analyzed by mass spectrometry (AMIS 2000, Innovision, Odense, Denmark) after calibration with precision analyzed gas mixtures. The raw data were transferred to a computer where gas delays were determined for each breath to align concentrations with volume data, and to build a profile of each breath. Breath-by-breath alveolar gas exchange was calculated with the AMIS algorithms which are slightly modified from the original algorithms of Beaver et al. (1981), and interpolated to give second by second values. In this study $PETCO_2$ was used as an index of $PaCO_2$ (Nielsen et al. 2001; Ogoh et al. 2005) instead of direct $PaCO_2$ measurement.

6.5 Regional cerebral and muscle oxygenation

Regional cerebral (frontal cortex (FC)) and muscle (m. vastus lateralis (VL) and m. biceps brachii (BB)) tissue oxygenation profiles were monitored non-invasively by near-infrared-spectroscopy (NIRS). The used NIRS device (Oxymon Mk III Near Infrared Spectrophotometer, Artinis Medical Systems, Zetten, the Netherlands) enabled continuous monitoring of relative concentration changes in oxy- ($\Delta[O_2Hb]$), deoxy- ($\Delta[HHb]$), and total ($\Delta[tHb]$) hemoglobin. The tissue saturation index (TSI, % = $[O_2Hb]/[Hb_{tot}]$) was calculated from the light attenuation slope along the distance from the three emitting points as detected by the sensor in the receiving optode.

The cerebral probe was located over the right frontal cortex, mid-optode position on average ~3 cm above the right eyebrow and ~4.5 cm lateral from the middle line of the forehead. This site over prefrontal cortex is assumed to be involved in the higher aspects of motor control and the planning of voluntary movement. (Sahyoun et al. 2004.) Muscle optodes were placed on m. VL of the right leg and on m. BB of the right arm. M. VL was chosen to represent active muscle as it is a powerful knee-extensor, which is activated at several phases of gait cycle (Murray et al. 1984). The optode in m. VL was placed ~14 cm above the upper edge of patella and ~4 cm lateral from middle line in parallel with the long axis of the muscle. M. BB has been shown to be mainly inactive during cycling (Ogata et al. 2007). This was thought to be the case also during walking in the present study. In m. BB, the optode was placed ~10 cm above the elbow joint and at middle line.

The interoptode distances were 40 mm for both muscles and 45 mm for FC but they were changed, if needed, when checking the quality of signals before starting the measurements. For both cerebral and muscle measurements, the optodes with three transmitters and one receiver were housed in an optically dense plastic holder and attached firmly on the skin using two-sided and one-sided tape. The FC optode was additionally fixed with a headband. Before fixing optodes hair, if existed, was shaved and skin was wiped. The exact place of all optodes was marked and measured individually during both tests. The accurate measurement of optode location in PRE allowed successful reproduction of the probe placement in POST.

The theory of NIRS is described in detail elsewhere (Ferrari et al. 2004). Briefly, NIRS measurement is based on the modified Beer-Lambert law to quantify the attenuation of light, accounting for scattering through biological tissues. The method is based on the differential absorption properties of chromophores in the near infrared region (650–1100 nm). (Ferrari et al. 1997.) Light in near-infrared spectrum penetrates skin, fat and bone and is absorbed in underlying tissues predominantly by oxy- and deoxyhemoglobin (O_2Hb, HHb), O_2Hb being absorbed at 850 nm and HHb at 760 nm. Using the modified Beer-Lambert law changes in O_2Hb and HHb concentration can be calculated over time. NIRS is suited to evaluation of oxygenation at multiple locations, such as at brain and muscle tissue. In addi-

tion, it has widely been used during dynamic exercise (Bhambhani et al. 2001; Bhambhani et al. 2007; DeLorey et al. 2003).

The used NIRS device was calibrated before each test according to manufacturer's guidelines. One bundle of optical fibers carried the NIR light produced by laser diodes to the tissues while a second bundle returned the transmitted light from the tissue to a photon detector in the spectrometer. The light source was provided by two different wavelength laser diodes (765 and 860 nm). The values used for the differential pathlength factor (DPF) were 5.51 for m. VL and 4.16 for m. BB. Cerebral DPF was calculated as DPF = 4.99 + 0.067 x $Age^{0.814}$. This formula derived from data of Duncan et al. (1996) is valid for measurements on brain tissue for 17–50 year old human subjects. NIRS data was recorded with a sampling frequency of 50 Hz and signal was transferred to a computer and stored for further analysis. In addition, NIRS data were averaged to give values in 1 s intervals, and time-aligned with gas exchange data.

The Δ[HHb] (Ferrari et al. 1997; Grassi et al. 2003) and TSI (calculated as [O_2Hb / (HHb + O_2Hb)]) (Peltonen et al. 2009) recordings are regarded as reliable estimators of changes in tissue deoxygenation status reflecting the mismatch between regional O_2 delivery and utilization. The sum of Δ[O_2Hb] and Δ[HHb] is a measure for the total blood volume (Δ[tHb]) in the tissue and indicates the change in localized blood volume (Van Beekvelt et al. 2001).

6.6 Index for hypoxic ventilatory chemosensitivity (AHVR)

Index for acute hypoxic ventilatory chemosensitivity (AHVR) was obtained by calculating the linear regression slope of $\dot{V}_E$ versus SpO_2 ($L \cdot min^{-1} \cdot \%^{-1}$) both from the beginning of baseline walking (at sea level) up to a standard load (4800 m above sea level) and to peak exercise (baseline walking – peak exercise). Thus, ventilation was plotted as a function of SpO_2 on second-by-second basis and the slope of the regression line, $\Delta \dot{V}_E / \Delta SpO_2$, was defined as AHVR. This method to determine AHVR during exercise has previously been used (e.g. Sato et al. 1996; Ainslie et al. 2008; Peltonen et al. 2009).

Likewise, the Δ[HHb] versus SpO_2 ($\mu M \cdot \%^{-1}$) and TSI versus SpO_2 ($\% \cdot \%^{-1}$) slopes were calculated for cerebral and muscle tissues, representing the change in NIRS-parameters per unit decline in SpO_2, i.e. the rate of deoxygenation (Peltonen et al. 2009). When performing NIRS analyses, the values obtained during exercise were compared with the values of baseline walking. Walking was chosen instead of rest because at the onset of exercise muscle pump expels blood from muscles towards the heart which is expected to explain the rapid temporary changes on NIRS measurements (DeLorey et al. 2003). Thus, the starting level (zero-level) of NIRS measurements was rest, but instead of using rest as a reference point, the level at 3-min baseline walking was chosen for this purpose.

6.7 Statistical analysis

The mean values of the last 30 s at rest and of each hypoxic step were chosen for statistical analysis. To compare data before and after chronic hypoxia, paired t-test was used. Variables were statistically compared up to 4800 m, as well as at peak exercise. All subjects were able to exercise at 4800 m at least 1 min in the pre test. Individual peak value was calculated as a mean of the last 30 s of the last hypoxic step, at which the subject was able to exercise at least 1 min. Variables plotted against SpO_2 or $PETCO_2$ could not be compared statistically due to different x-values in PRE and POST but are presented to illustrate the physiological phenomena. Relationships between key variables were tested by Pearson product correlation. All statistical analyses were performed using SPSS (v18.0, SPSS). Results are expressed as means ± SD unless otherwise indicated. A *P*-value of < 0.05 was considered statistically significant.

7 RESULTS

In the test before chronic hypoxia (PRE), all subjects completed walking at 3500 m and were able to exercise at 4800 m at least 1 min. In the test after chronic hypoxia (POST), all subjects completed exercise at 5200 m and were able to walk at 5800 m at least 1.5 minutes. Simulated altitudes matched well with targeted altitudes, and statistically significant differences in PiO_2 were not seen between PRE and POST at any given level of hypoxia (Figure 10). The mean realized peak altitude was in PRE 5300 m (range 4750–6450 m) and in POST 6050 m (range 4800–6750 m). The progressive hypoxia was tolerated in PRE for 11:32 ± 3:57 (min:sec) and in POST 16:30 ± 2:09 with increased time in 8 of 9 subjects ($P < 0.01$).

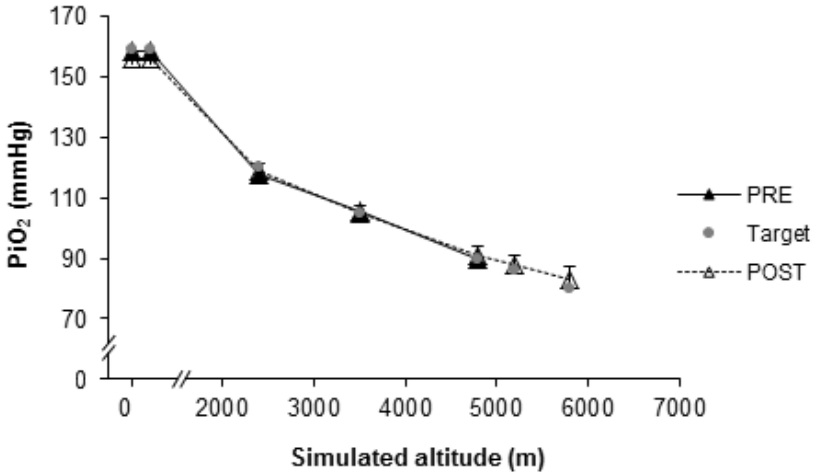

FIGURE 10. Mean (± SD) realized hypoxic stimulus during the tests. For PRE, symbols represent values from rest and baseline walking up to 4800 m, and for POST from rest and baseline walking up to 5800 m. In order to improve the clarity of the figure, the x-value of baseline walking is artificially raised to 200 m.

7.1 Cardiorespiratory responses

The mean responses of cardiorespiratory variables before and after chronic hypoxia are presented in Figures 11, 12 and 13. In Figure 14, the group mean responses of chosen cardiorespiratory variables are plotted against SpO_2. In POST, SpO_2 was higher at 4800 m with

no other differences observed between PRE and POST (Figure 11). Arterial O_2 saturation decreased to 65 ± 4 % and 64 ± 3 % during PRE and POST, respectively. In POST, HR was lower at 4800 m with no other differences seen between PRE and POST (Figure 12A). Oxygen consumption ($\dot{V}_{O2}$) remained relatively constant throughout exercise, and did not differ between PRE and POST at any given hypoxic step (Figure 12B).

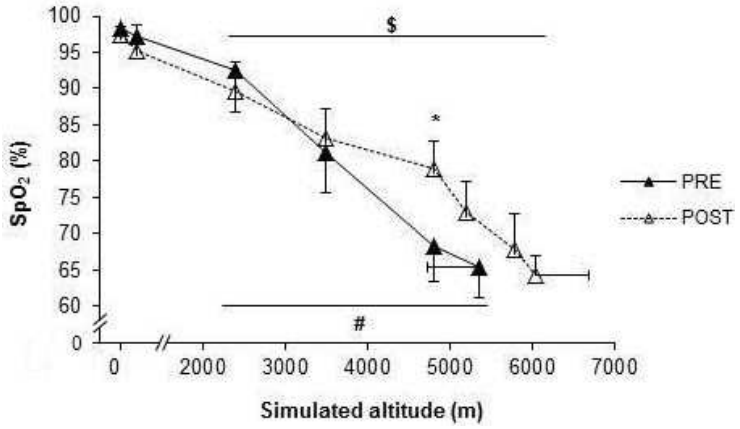

FIGURE 11. Mean (± SD) SpO_2 (%) during progressive hypoxia. For PRE symbols represent values from rest and baseline walking up to 4800 m, and for POST from rest and baseline walking up to 5800 m. Last value is the peak value in both PRE and POST curves. * Significantly different from PRE ($P < 0.05$). # Significantly different from baseline walking in PRE ($P < 0.05$). $ Significantly different from baseline walking in POST ($P < 0.05$). In order to improve the clarity of the figure, the x-value of baseline walking is artificially raised to 200 m.

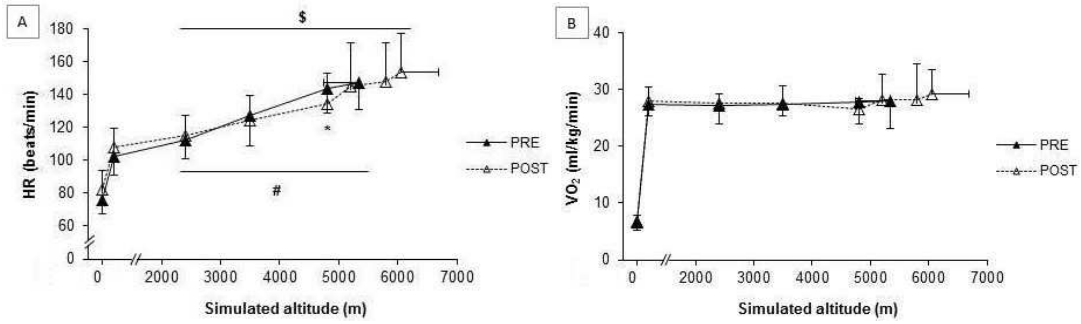

FIGURE 12. Mean (± SD) (A) HR and (B) $\dot{V}_{O2}$ relative to body weight ($ml \cdot kg^{-1} \cdot min^{-1}$) at rest, during baseline walking and progressive hypoxia. * Significantly different from PRE ($P < 0.05$). # Significantly different from baseline walking in PRE ($P < 0.05$). $ Significantly different from baseline walking in POST ($P < 0.05$). See details from Fig. 11.

Ventilation was higher in POST than in PRE during baseline walking, at 3500 m and at peak exercise (Figure 13A). Breathing frequency (Fb) showed similar response than $\dot{V}_E$ being higher in POST than in PRE during whole exercise except at 4800 m (Figure 13B). When plotted against SpO_2, $\dot{V}_E$ and Fb were higher in POST than in PRE at a given level of SpO_2 (Figures 14A and 14B, respectively). There were no differences in tidal volume (VT) between PRE and POST at any given hypoxic step (Figure 13C). In POST, $PETCO_2$ was lower than in PRE at rest and throughout the hypoxic protocol (Figure 13D). At a given level of SpO_2, $PETCO_2$ was higher in POST than in PRE (Figure 14D).

7.2 Hypoxic ventilatory chemosensitivity

The slopes for acute hypoxic ventilatory chemosensitivity (AHVR) from baseline walking to 4800 m were 1.25 ± 0.33 and 1.63 ± 0.38 $L \cdot min^{-1} \cdot \%^{-1}$ in PRE and POST, respectively, with significant increase after chronic hypoxia ($P < 0.05$) (Figure 15A). Acute hypoxic ventilatory responses from baseline walking to peak performance were 1.29 ± 0.34 and 1.40 ± 0.33 $L \cdot min^{-1} \cdot \%^{-1}$ in in PRE and POST, respectively, with no differences between the tests (Figure 15B). The response was greater in POST than in PRE in 8 of 9 subjects when measured from baseline walking to 4800 m, and in 6 of 9 subjects when measured from baseline walking to peak exercise.

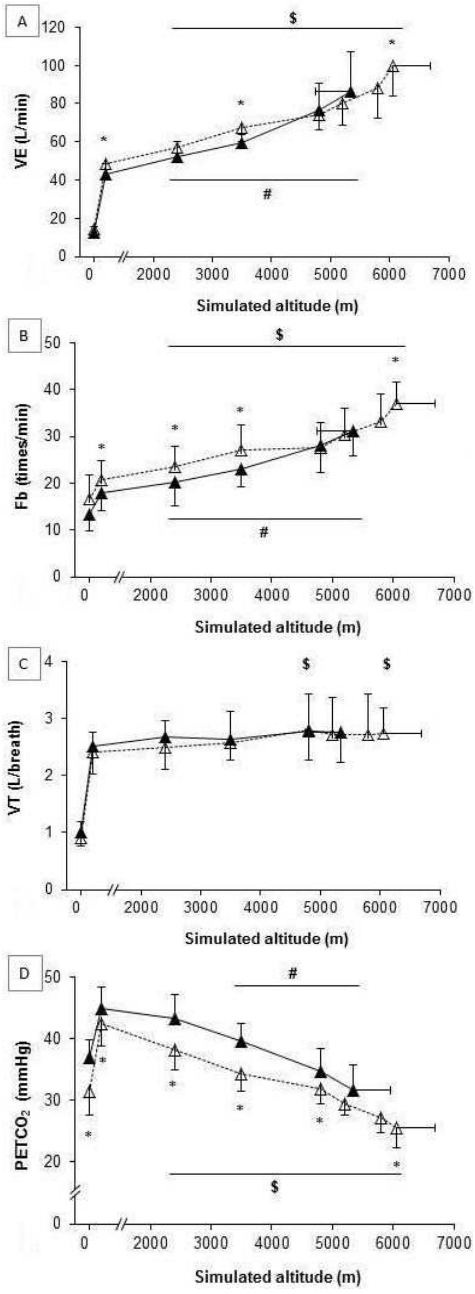

FIGURE 13. Mean (± SD) A) VE, B) Fb, C) VT and D) $PETCO_2$ at rest, during baseline walking and progressive hypoxia. ▲ PRE, △ POST. * Significantly different from PRE ($P < 0.05$). # Significantly different from baseline walking in PRE ($P < 0.05$). $ Significantly different from baseline walking in POST ($P < 0.05$). See details from Fig. 11.

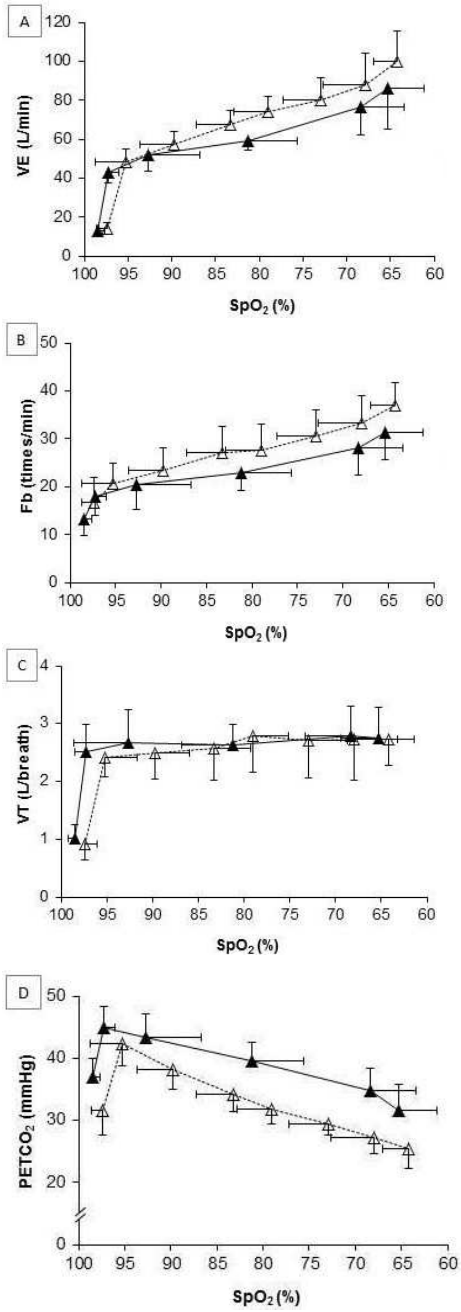

FIGURE 14. Mean (± SD) A) VE, B) Fb, C) VT and D) $PETCO_2$ plotted against SpO_2 (%) at rest, during baseline walking and progressive hypoxia. ▲ PRE, △ POST. Last value is the peak value in both PRE and POST curves.

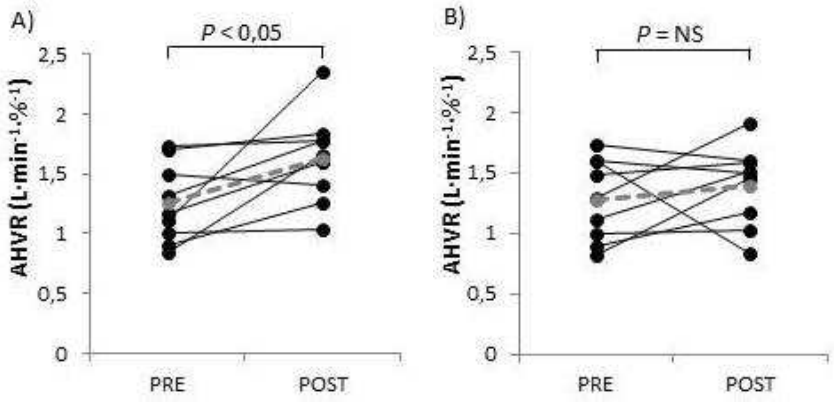

FIGURE 15. Acute hypoxic ventilatory responses (AHVR) in PRE and POST measured A) from baseline walking to 4800 m and B) from baseline walking to peak exercise. NS = non-significant. Individual AHVR values (solid circles, ●), average AHVR values (grey circles, ●).

7.3 Regional tissue oxygenation responses

Regional cerebral, and active and inactive muscle oxygenation profiles for a representative subject are presented in Figures 16, 17 and 18 and group mean responses in Figures 19 (cerebral), 23 (active muscle) and 26 (inactive muscle). In Figures 20, 24 and 27, the group mean responses for NIRS variables are plotted against SpO_2. Changes in cerebral and active and inactive muscle tissue NIRS values from baseline walking to peak exercise, i.e. delta values, are presented in Table 3. Tissue specific oxygenation responses are described in more detail in the following chapters.

TABLE 3. Mean (±SD) regional oxygenation responses of whole exercise in different tissues before and after chronic hypoxia.

	Cerebral		Active muscle		Inactive muscle	
	PRE	POST	PRE	POST	PRE	POST
Δ_tTSI (%)	-23.7 ± 3.3	-24.0 ± 4.1	-14.8 ± 6.6	-22.9 ± 7.7*	-18.1 ± 12.9	-30.0 ± 10.0*
Δ_t[HHb] (µM)	11.2 ± 3.0	14.3 ± 4.3†	9.6 ± 4.7	15.4 ± 4.6†	10.9 ± 7.2	16.8 ± 5.3
Δ_t[O_2Hb] (µM)	-8.0 ± 1.4	-9.9 ± 1.3†	-3.3 ± 3.3	-4.9 ± 3.4	-6.5 ± 5.0	-11.5 ± 3.1†
Δ_t[tHb] (µM)	3.2 ± 3.7	4.4 ± 4.2	6.2 ± 3.2	10.5 ± 2.1†	4.4 ± 5.3	5.3 ± 4.1

Δ_tTSI/HHb/O_2Hb/tHb = Change in tissue saturation index / deoxyhemoglobin / oxyhemoglobin / total hemoglobin concentration from baseline walking to peak exercise. * Significantly different from PRE ($P < 0.05$). † Significantly different from PRE ($P < 0.01$).

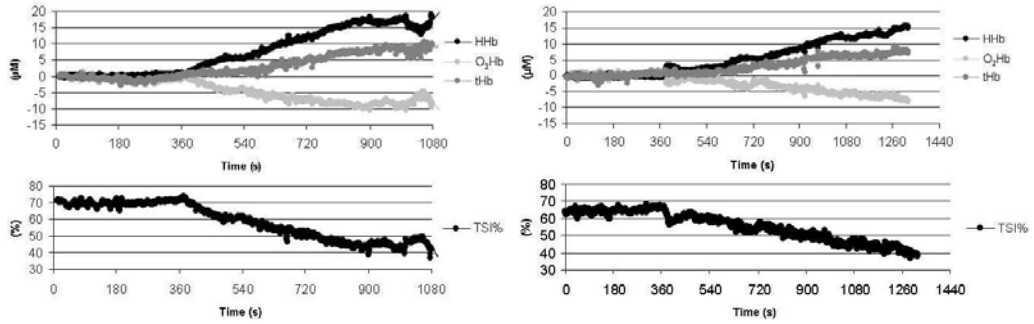

FIGURE 16. Cerebral tissue NIRS recordings vs. time (s) for a representative subject before (left panels) and after (right panels) chronic hypoxia. Three concentration signals (y-axis, [μM]) are presented in the pictures above: black = Δ[HHb], grey= Δ[O_2Hb], dark grey = Δ[tHb]. Cerebral TSI (y-axis, [%]) is presented in the lower pictures.

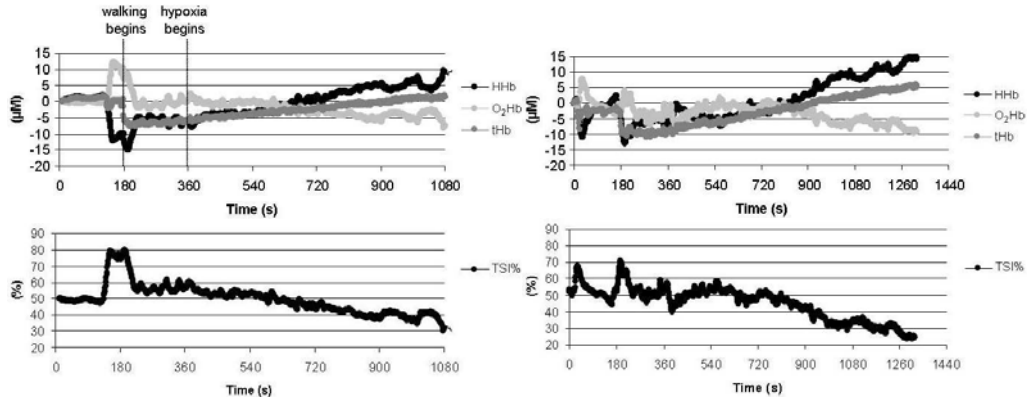

FIGURE 17. Active leg muscle NIRS recordings vs. time (s) for a representative subject before (left panels) and after (right panels) chronic hypoxia. See details from Fig. 16.

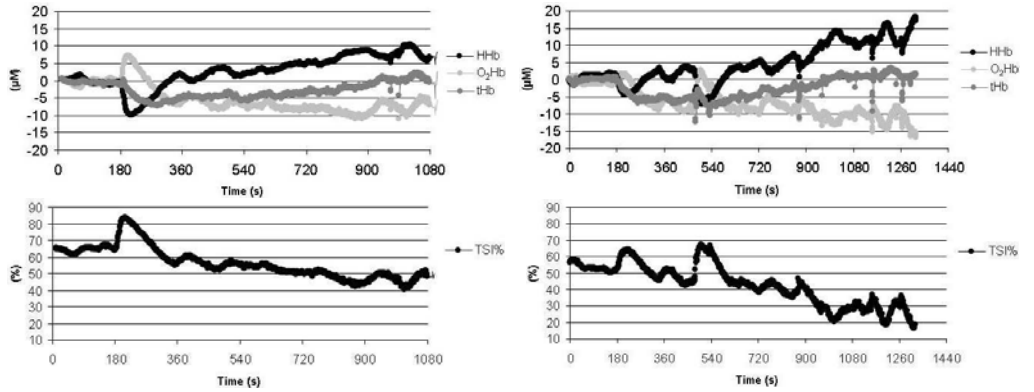

FIGURE 18. Inactive arm muscle NIRS recordings vs. time (s) for a representative subject before (left panels) and after (right panels) chronic hypoxia. See details from Fig. 16.

7.3.1 Cerebral tissue (frontal cortex) oxygenation

Decrease of cerebral TSI relative to normoxic baseline walking was significant at all hypoxic steps in both PRE and POST (Figure 19A). There were no differences between PRE and POST at any given hypoxic step (Figure 19A) or in delta values (Table 3). When plotted against SpO_2, cerebral TSI was greater in POST than in PRE at a given level of SpO_2 between SpO_2 of 93 and 82 % (Figure 20A). There was no association between cerebral TSI slope and AHVR (Figure 21A). Previously determined $\dot{V}_{O_2}max$ ($ml \cdot kg^{-1} \cdot min^{-1}$) was associated with cerebral TSI slope in PRE ($r = 0.77$, $P < 0.05$). At a given level of $PETCO_2$, cerebral TSI was lower in PRE than POST (Figure 22).

Cerebral Δ[HHb] increased in both tests. The increase relative to baseline walking was significant in both PRE and POST at all hypoxic steps. In POST, Δ[HHb] was lower than in PRE at 3500 m and 4800 m, and higher than in PRE at peak exercise (Figure 19B). The increase in Δ[HHb] from baseline walking to peak exercise was significantly greater in POST (Table 3). At a given level of SpO_2, Δ[HHb] was lower in POST than in PRE between SpO_2 of 93 and 82 % (Figure 20B). There was no association between cerebral [HHb] slope and AHVR (Figure 21B).

Cerebral Δ[O_2Hb] decreased consistently throughout the exercise in both PRE and POST (Figure 19C). In POST, Δ[O_2Hb] was significantly lower than in PRE at peak exercise (Figure 19C). The decrease in Δ[O_2Hb] from baseline walking to peak exercise was significantly greater in POST (Table 3).

Cerebral Δ[tHb] showed a slight increase in both tests. In PRE, the increase was significant from 4800 m onwards. In POST, Δ[tHb] remained near baseline walking level up to 5200 m and was significantly higher than baseline value from 5800 m onwards. (Figure 19D.) There were no differences between PRE and POST at any given hypoxic step or in delta values (Figures 19D and Table 3). When plotted against SpO_2, cerebral [tHb] was smaller in POST than in PRE at a given level of SpO_2 between SpO_2 of 93 and 73 % (Figure 20D).

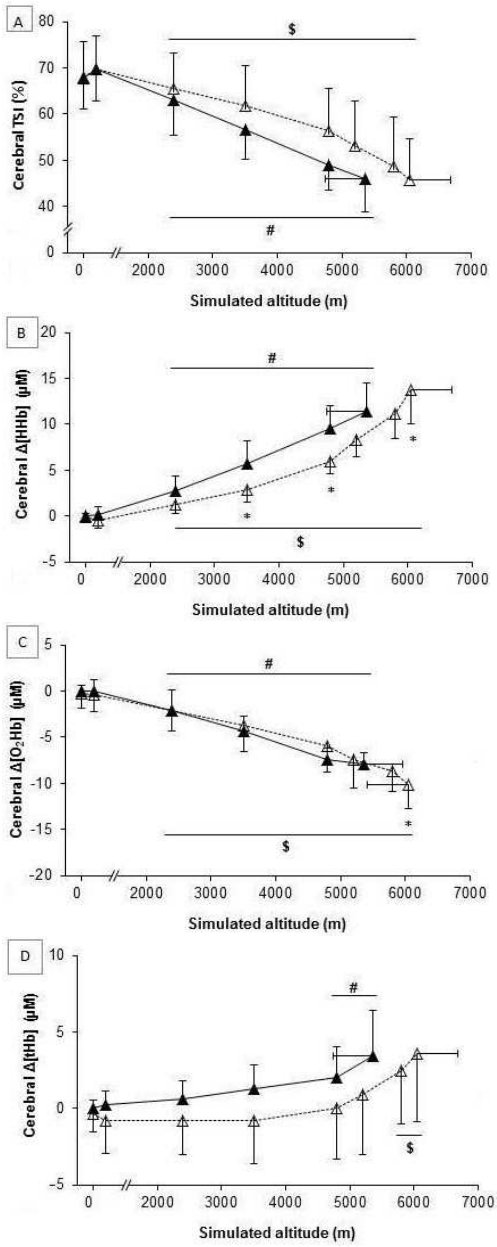

FIGURE 19. Cerebral mean (± SD) A) TSI, B) Δ[HHb], C) Δ[O_2Hb] and D) Δ[tHb] at rest, during baseline walking and progressive hypoxia. ▲ PRE, △ POST. * Significantly different from PRE ($P < 0.05$). # Significantly different from baseline walking in PRE ($P < 0.05$). $ Significantly different from baseline walking in POST ($P < 0.05$). See details from Fig. 11.

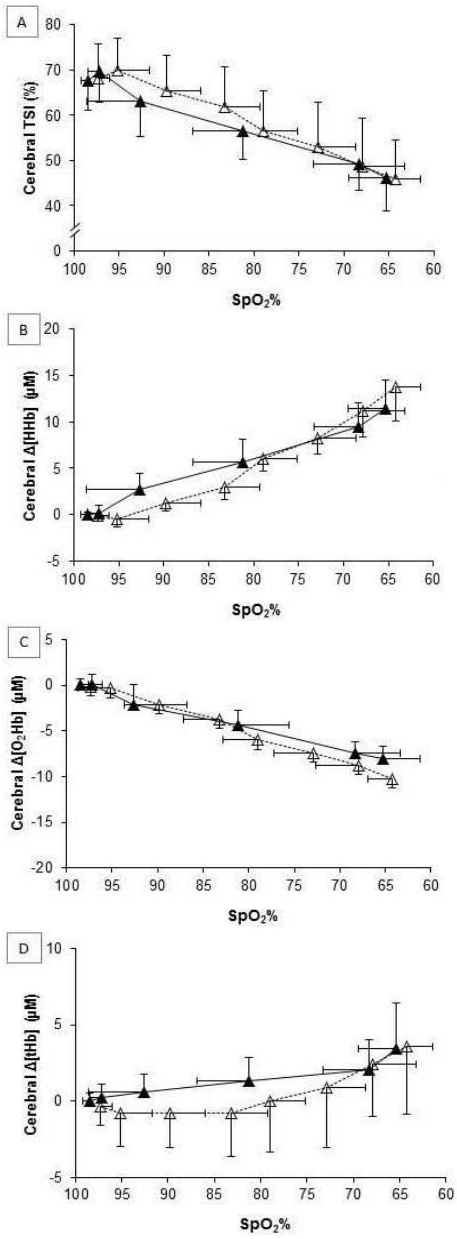

FIGURE 20. Cerebral mean (± SD) A) TSI, B) Δ[HHb], C) Δ[O_2Hb] and D) Δ[tHb] plotted against SpO_2 (%) at rest, during baseline walking and progressive hypoxia. ▲ PRE, △ POST. Last value is the peak value in both PRE and POST curves.

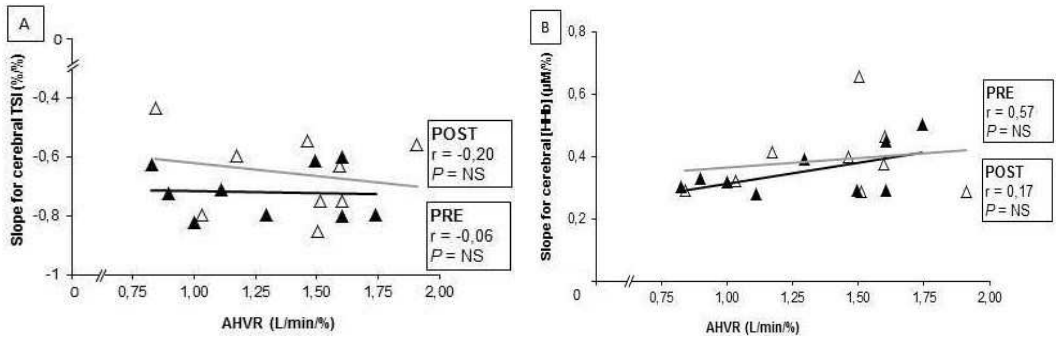

FIGURE 21. Scatter plot of A) cerebral TSI slope and AHVR, and B) cerebral [HHb] slope and AHVR before and after chronic hypoxia. ▲ PRE, △ POST, — PRE, — POST, NS = non-significant.

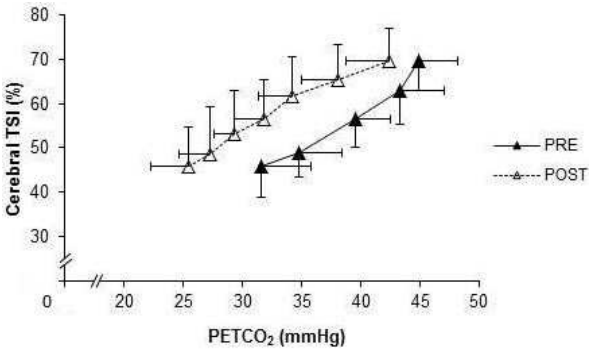

FIGURE 22. Cerebral TSI plotted against $PETCO_2$ before and after chronic hypoxia. For PRE symbols represent values from baseline walking up to 4800 m and peak and for POST from baseline walking up to 5800 m and peak. Values are means ± SD.

7.3.2 Active muscle (m. vastus lateralis) oxygenation

Active muscle tissue (m. vastus lateralis) TSI decreased in both PRE and POST. The decrease in TSI relative to baseline walking was significant in PRE at all hypoxic steps. In POST, TSI remained at baseline level during first two hypoxic steps and started to decrease from 4800 m on (Figure 23A). In POST, TSI was significantly lower during baseline walking and at peak exercise (Figure 23A). The TSI slope, i.e. the rate of deoxygenation, (-0.63 and -0.46 $\%\cdot\%^{-1}$, respectively) and the decrease in TSI from baseline walking to peak exercise were significantly greater in POST than in PRE (Table 3). At a given level of SpO_2, leg TSI was lower in POST than in PRE between SpO_2 of 81 and 65 % (i.e. after first hypoxic

steps) (Figure 24A). There was no association between leg TSI slope and AHVR (Figure 25A).

Leg Δ[HHb] and Δ[tHb] increased in both tests. In PRE, the increase in Δ[HHb] and Δ[tHb] relative to baseline walking was significant at all hypoxic steps as well as in POST for Δ[tHb]. Δ[HHb] increased in POST from 3500 m on (Figures 23B and 23D). There were no differences in Δ[HHb] or in Δ[tHb] between PRE and POST at any given hypoxic step (Figures 23B and 23D). The increase in Δ[HHb] and Δ[tHb] from baseline walking to peak exercise was significantly greater in POST than in PRE (Table 3). The HHb slope was greater in POST than in PRE (0.43 and 0.28 $\mu M \cdot \%^{-1}$, respectively). When plotted against SpO_2, both Δ[HHb] and Δ[tHb] were greater in POST than in PRE at a given level of SpO_2 between SpO_2 of 81 and 65 % (Figures 24B and 24D). An association was observed between leg [HHb] slope and AHVR in PRE, but not in POST ($r = 0.73$, $P < 0.05$) (Figure 25B).

There was hardly any change in leg Δ[O_2Hb] during exercise. In PRE, Δ[O_2Hb] remained at baseline walking levels during first two hypoxic steps and started to decrease at 4800 m. In POST, Δ[O_2Hb] increased significantly during the first two hypoxic steps and decreased to significantly lower level than at baseline at peak exercise (Figure 23C). There were no differences in leg Δ[O_2Hb] between the PRE and POST at any given hypoxic step (Figure 23C and Table 3).

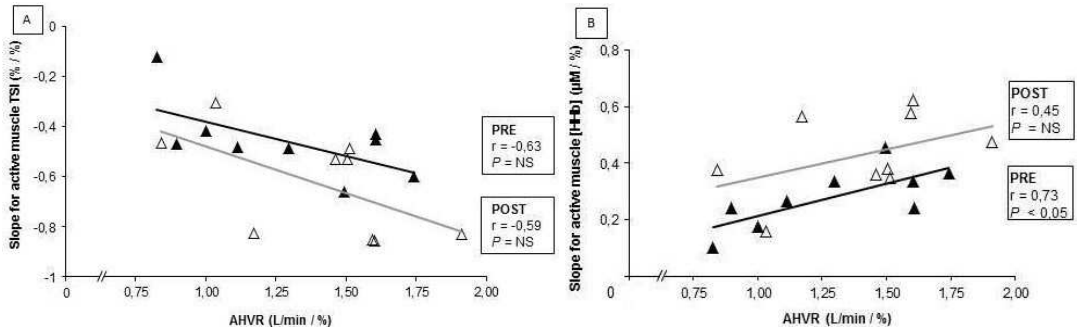

FIGURE 25. Scatter plot of A) leg TSI slope and AHVR, and B) leg [HHb] slope and AHVR before and after chronic hypoxia. ▲ PRE, △ POST, ━ PRE, ― POST, NS = non-significant.

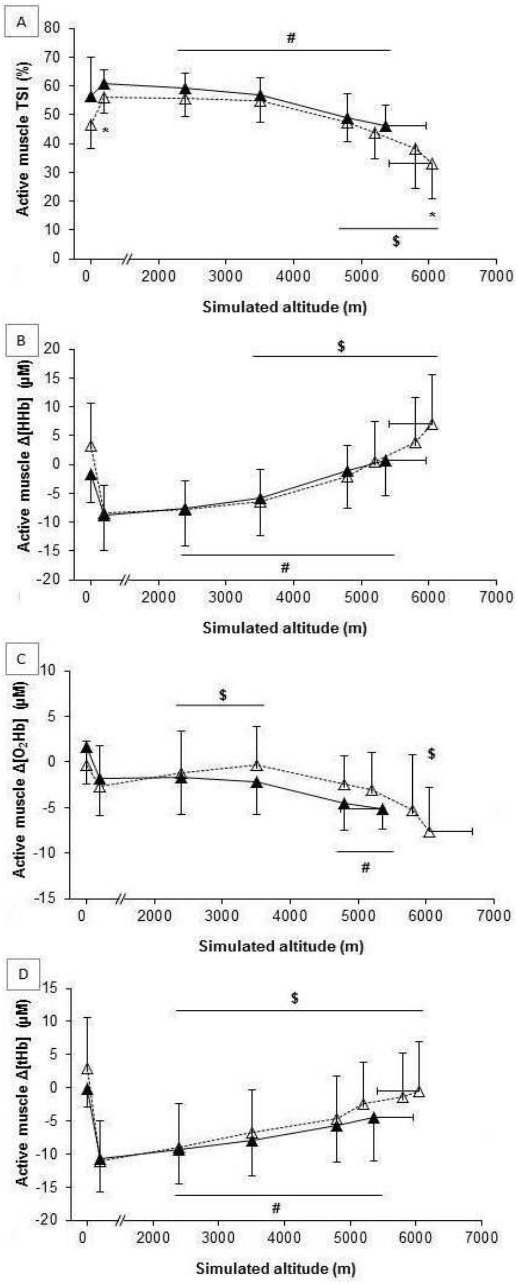

FIGURE 23. Leg mean (± SD) A) TSI, B) Δ[HHb], C) Δ[O_2Hb] and D) Δ[tHb] at rest, during baseline walking and progressive hypoxia. ▲ PRE, △ POST. * Significantly different from PRE ($P < 0.05$). # Significantly different from baseline walking in PRE ($P < 0.05$). $ Significantly different from baseline walking in POST ($P < 0.05$). See details from Fig. 11.

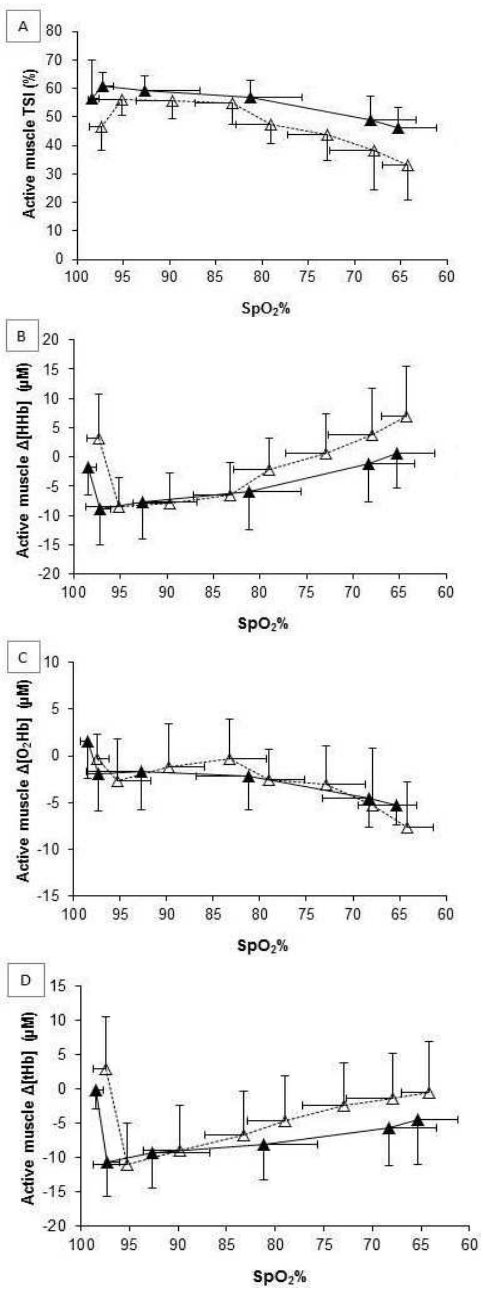

FIGURE 24. Leg mean (± SD) A) TSI, B) Δ[HHb], C) Δ[O_2Hb] and D) Δ[tHb] plotted against SpO_2 (%) at rest, during baseline walking and progressive hypoxia. ▲ PRE, △ POST. Last value is the peak value in both PRE and POST curves.

7.3.3 Inactive muscle (m. biceps brachii) oxygenation

Tissue saturation index of inactive arm muscle (m. biceps brachii) decreased in both tests. The decrease in TSI relative to baseline walking was evident from 3500 m and 4800 m onwards in PRE and POST, respectively (Figure 26A). Tissue saturation index was significantly lower in POST than in PRE at rest and at peak exercise (Figure 26A). The decrease in TSI from baseline walking to peak exercise was significantly greater in POST (Table 3). At a given level of SpO_2, arm TSI was lower in POST than in PRE between SpO_2 of 81 and 65 % (Figure 27A). There was no association between arm TSI slope and AHVR (Figure 28A).

Arm Δ[HHb] remained at baseline levels during the first two hypoxic steps and started to increase from 4800 m on in both PRE and POST (Figure 26B). In POST, Δ[HHb] was lower than in PRE at 4800 m (Figure 26B). When plotted against SpO_2, arm Δ[HHb] was smaller in POST than in PRE at a given level of SpO_2 between SpO_2 of 93 and 82 % (Figure 27B). No association was found between arm [HHb] slope and AHVR (Figure 28B).

Arm Δ[O_2Hb] showed a decrease at peak exercise and from 5200m on in PRE and POST, respectively (Figure 26C). There were no differences between PRE and POST at any given hypoxic step. The decrease in Δ[O_2Hb] from baseline walking to peak exercise was significantly greater in POST (Table 3). At a given level of SpO_2, Δ[O_2Hb] was lower in POST than in PRE between SpO_2 of 72 and 65 % (Figure 27C).

Arm Δ[tHb] increased slightly in both tests. In PRE, the increase was evident at peak exercise. In POST, Δ[tHb] first decreased significantly at 2400 m and increased from 5200 m on when compared to baseline walking value (Figure 26D). In POST, Δ[tHb] was significantly lower than in PRE during baseline walking and at 2400 m (Figure 26D).

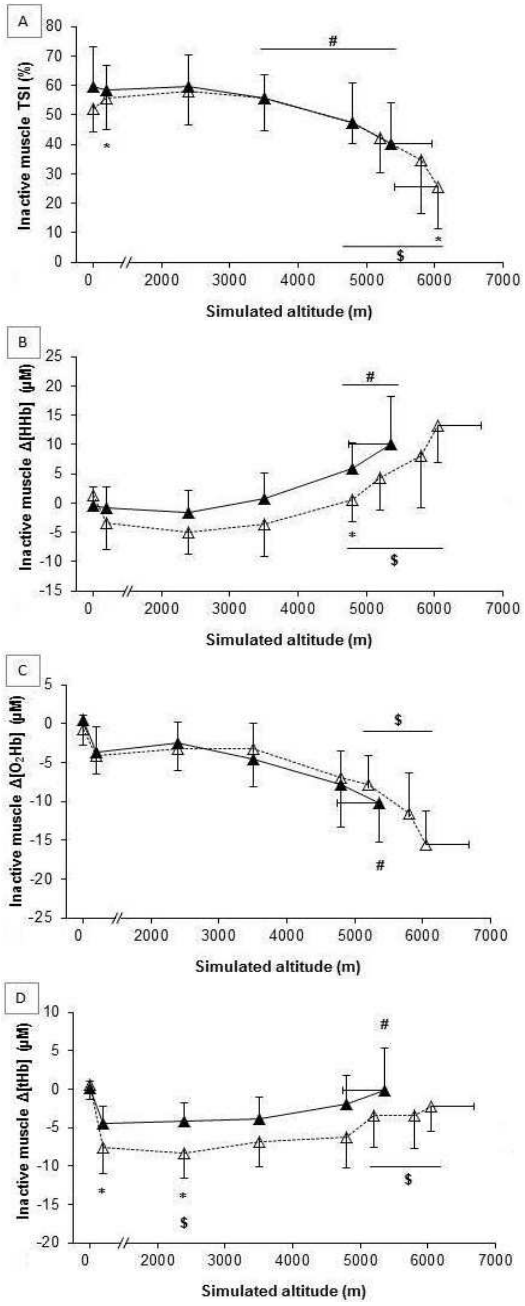

FIGURE 26. Arm mean (± SD) A) TSI, B) Δ[HHb], C) Δ[O_2Hb] and D) Δ[tHb] at rest, during baseline walking and progressive hypoxia. ▲ PRE, △ POST. * Significantly different from PRE ($P < 0.05$). # Significantly different from baseline walking in PRE ($P < 0.05$). $ Significantly different from baseline walking in POST ($P < 0.05$). See details from Fig. 11.

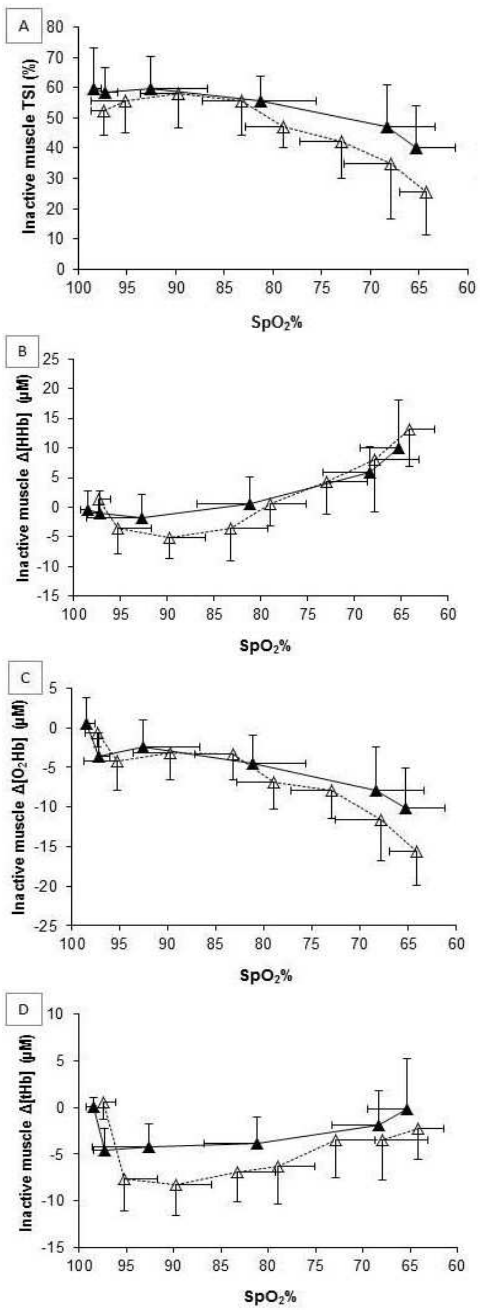

FIGURE 27. Arm mean (± SD) A) TSI, B) Δ[HHb], C) Δ[O_2Hb] and D) Δ[tHb] plotted against SpO_2 (%) at rest, during baseline walking and progressive hypoxia ▲ PRE, △ POST. Last value is the peak value in both PRE and POST curves.

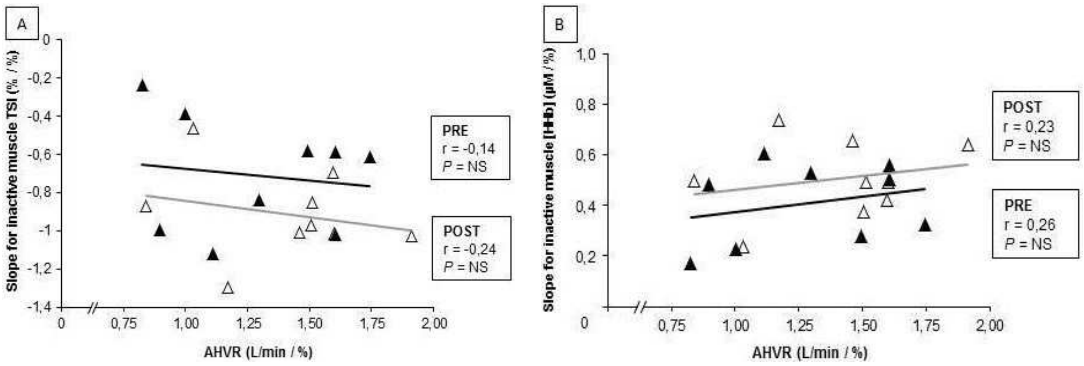

FIGURE 28. Scatter plot of A) arm TSI slope and AHVR, and B) arm [HHb] slope and AHVR before and after chronic hypoxia. ▲ PRE, △ POST, ━ PRE, ─ POST, NS = non-significant.

8 DISCUSSION

The purpose of the study was to examine if cerebral and skeletal muscle deoxygenation responses to exercise in acute hypoxia differed before and after acclimatization to chronic hypoxia, and whether the magnitude of these responses was associated with AHVR. In addition, it was aimed to develop a protocol that is suitable for simultaneous measurement of AHVR and tissue oxygenation during walking under progressively increasing poikilocapnic hypoxia, analogous to actual climbing to high altitude. To execute poikilocapnic progressive hypoxia, a method to modify PiO_2 was developed. There are no published studies at the present that describe simultaneous responses in AHVR, and cerebral and muscle tissue oxygenation during constant exercise under progressive poikilocapnic hypoxia before and after a typical climbing expedition to high altitude.

The main findings of this study were: 1) with a novel method to modify PiO_2, PiO_2 was successfully controlled over increasing levels of hypoxia, 2) the protocol was suitable for simultaneous measurement of acute hypoxic ventilatory chemosensitivity (AHVR) and tissue oxygenation, 3) AHVR increased following acclimatization, 4) cerebral oxygenation (COX) was improved after acclimatization (as indicated by smaller increase in Δ[HHb]) but muscle deoxygenation was similar before and after acclimatization and 5) no association was found between AHVR and cerebral deoxygenation.

8.1 Protocol to measure simultaneously AHVR and tissue oxygenation

The method to modify PiO_2 successfully enabled generation of wide range of hypoxic stimuli, as realized levels of PiO_2 were within ±3 mmHg of the targeted ones in both PRE and POST, with no differences between the tests at a given hypoxic step. However, the adjustment of PiO_2 and PN_2 was made manually and some fluctuation did occur in the hypoxic level.

The present protocol enabled simultaneous detection of ventilatory, and local cerebral and muscle tissue oxygenation responsiveness. To be effective, such a protocol requires several levels of hypoxia that must be long enough for both ventilatory and cerebrovascular response to unfold, but short enough to prevent development of hypoxic ventilatory decline (Kolb et al. 2004). A hypoxic step representing high altitude, i.e. 4800 m, was wanted to be included to the statistical comparison, although it was not completed by all subjects in PRE. Instead, all subjects exercised at least 1 min at 4800 m. As individual mean value was mainly obtained from the final 30 s of each hypoxic step, 30 s mean calculated from 60 s step might not represent unfolded ventilatory and cerebrovascular responses (Mou et al. 1995; Poulin et al. 2002). This was of concern only in PRE and in some peak-steps.

Total hypoxic period of less than 20 min was chosen to avoid possible HVD during longer hypoxic exposures (Howard & Robbins 1995). The realized hypoxic durations were 11:32 ± 3:57 and 16:30 ± 2:09 min:sec in PRE and POST, respectively. However, it is likely that some of the subjects developed HVD in POST, as in 6 out of 9 subjects AHVR calculated from baseline walking to peak performance was less than the value obtained from baseline walking to 4800 m (1.40 ± 0.33 and 1.63 ± 0.38 $L \cdot min^{-1} \cdot \%^{-1}$, respectively). In other words, in most subjects the ventilatory response to hypoxia was blunted during the last hypoxic steps in POST. In addition to longer test duration, greater level of hypoxia itself could have induced HVD in POST. Thus, possible "contamination" of the post test data with HVD could have influenced our findings. In the future, technical improvements are needed to speed up the adjustment of gas mixture to decrease the time spent at each level of hypoxia.

8.2 Cardiorespiratory responses

Constant and similar $\dot{V}_{O_2}$ was observed throughout exercise in both PRE and POST. This was expected since exercise load was kept constant, indicating unchanged metabolic cost over a wide range of hypoxic steps. However, when $\dot{V}_{O_2}$ at peak exercise was examined more closely at individual level, an association was found between $\dot{V}_{O_{2peak}}$ and AHVR ($r = 0.79$, $P < 0.05$) in POST so that subjects with high ventilatory chemosensitivity had higher oxygen consumption (range 22.3–36.5 $ml \cdot kg^{-1} \cdot min^{-1}$). Greater work of breathing seen at

high altitude compared with the same exercise at sea level might blunt the increase in PaO_2, being counterproductive. The O_2 cost of breathing during exercise at 5050 m was found to be 24–26 % $\dot{V}_{O_2}$max, whereas estimated sea level value is ~10–16% of $\dot{V}_{O_2}$max (reviewed by Sheel et al. 2010).

A drop in SpO_2 with increasing hypoxia occurred in both PRE and POST despite substantial hyperventilation. However, SpO_2 was better maintained at high altitude in POST as seen in significantly higher SpO_2 at 4800 m (79 and 68 % in PRE and POST, respectively), being in agreement with existing knowledge (Calbet et al. 2003b). This might refer to improved ability to maintain blood oxygenation under hypoxic exposure equivalent to steep portion of hemoglobin O_2 dissociation curve. At peak exercise, similar SpO_2 was attained in both PRE and POST, at a significantly higher mean altitude in POST. Similar HR was observed at a given hypoxic step up to 3500 m in both tests, whereas 6 % lower HR was seen at 4800 m in POST. Similarly, Calbet et al. (2003b) reported a 15–20 % decrease in HR during submaximal exercise at 5260 m after 9–10 wk stay at the same altitude.

Following chronic hypoxia, $\dot{V}_E$ was higher during baseline walking, at 3500 m and at peak exercise. Calbet et al. (2003b) found unchanged $\dot{V}_E$ during submaximal exercise after acclimatization when compared to acute hypoxia. During the tests, subjects were exposed to one level of hypoxia (ambient hypoxia at 5260 m and equivalent normobaric hypoxia in the laboratory at sea level). (Calbet et al. 2003b.) In the present study, the increase in $\dot{V}_E$ was caused by an increase in Fb only in both PRE and POST, with nearly no contribution of VT. The finding is in contrast to previous studies where increase in $\dot{V}_E$ was mediated by VT only during light exercise under acute poikilocapnic progressive hypoxia (Sato et al. 1996) and during acute poikilocapnic single step hypoxia at rest (Steinback & Poulin 2007). However, after prolonged hypoxia $\dot{V}_E$ has been shown to increase primarily by an increase in Fb (Bender et al. 1987). In the present study, lower $PETCO_2$ was observed in POST at rest and throughout the test when compared to PRE, although similar $\dot{V}_E$ was seen at some hypoxic steps. Similar observation was made by Calbet et al. (2003b).

8.3 Hypoxic ventilatory chemosensitivity

Ventilatory chemosensitivity increased after acclimatization to high altitude. This is in accordance with previous studies (Sato et al. 1994; Hupperets et al. 2004; Bernardi et al. 2006). It is known that there is substantial day-to-day variability in AHVR (Ainslie et al. 2005; Kolb et al. 2004; Zhang & Robbins 2000), potentially hiding between subject differences in AHVR (Moore et al. 1984). In the present study, AHVR was assessed during exercise to better separate individual responses, as both the magnitude of AHVR (Sato et al. 1996; Ainslie et al. 2007) and inter-subject variability are amplified during exercise compared with rest (Sato et al. 1996).

The magnitude of AHVR differs from other studies assessing the ventilatory chemosensitivity during exercise in acute hypoxia. In comparison to present AHVR responses from baseline walking to 4800 m (1.25 ± 0.33 and 1.63 ± 0.38 $L \cdot min^{-1} \cdot \%^{-1}$, in PRE and POST, respectively), others have reported greater (6.02 ± 3.8 $L \cdot min^{-1} \cdot \%^{-1}$; Peltonen et al. 2009), fairly similar (1.5 ± 0.4 $L \cdot min^{-1} \cdot \%^{-1}$; Ainslie et al. 2007) and smaller (0.45 ±0.12 $L \cdot min^{-1} \cdot \%^{-1}$, Sato et al. 1996) responses. Differences are likely due to substantial variation in pattern and magnitude of hypoxic stimulus and exercise form and protocol because the greater the magnitude of hypoxic exposure (Pandit & Robbins 1991) and the exercise intensity (Weil et al. 1972), the greater the AHVR. In the study of Peltonen et al. (2009), incremental cycling exercise (30 W/min ramp) was performed under moderate hypoxia ($PETO_2$ 71 mmHg). The duration of hypoxic exposure was in average ~14 min. (Peltonen et al. 2009.) Ainslie et al. (2007) examined AHVR during cycling in a upright position at 60–70 % of $\dot{V}_{O_2}max$ while FiO_2 was reduced to 14 % for 4–5 min ($PETO_2$ 74 mmHg) (Ainslie et al. 2007). Sato et al. (1996) used 6-min constant light cycling exercise (12.5 W) in a supine position under isocapnic progressive hypoxia. The PiO_2 was at first increased to obtain $PETO_2$ over 150 mmHg and then progressively reduced over 6 min to a level of $PETO_2$ ~40 mmHg. (Sato et al. 1996.) All aforementioned studies elicited SpO_2 of ~80 %. It is known that isocapnic procedure induces greater AHVR response than poikilocapnia. The AHVR protocols differ substantially between studies, making meaningful comparisons challenging or even impossible. At the moment, no consensus has been found regarding a standard

method capable of comparing test results between subjects and within subjects under different conditions. The variation in methodology might have an influence on other physiological responses to hypoxia, like cerebral blood flow, too. (Duffin 2007.)

Despite time delay between the stay at altitude and the post measurements in the laboratory, an increase in HVR was observed in the present study, as seen previously 2 days after return from a sojourn of 6 days at 3810 m altitude (Sato et al. 1994) and 11 days after return from Himalaya basecamp (56 days, 30 days at 4900 m or higher) (Steinacker et al. 1996). The time that ventilatory sensitivity remains elevated after return to sea level might be related to the duration of the stay and the altitude (Steinacker et al. 1996).

8.4 Regional tissue oxygenation responses

Differential control mechanisms of perfusion and oxygenation tissue seem to exist in cerebral and muscle tissue, as has previously been reported both in acute hypoxia and immediately after chronic hypoxia. Acute hypoxia was shown to provoke greater cerebral deoxygenation both at rest and during exercise compared to normoxic conditions, while muscle oxygenation was maintained at normoxic levels (Peltonen et al. 2009). After short acclimatization, maintained (Subudhi et al. 2008) or improved (Ainslie et al. 2008) muscle oxygenation was seen during submaximal hypoxic exercise, while cerebral oxygenation was further decreased. In the present study, the effect of prolonged acclimatization on cerebral and muscle tissue oxygenation responses was different. At a given altitude, improved oxygenation was seen in cerebral tissue, while muscle oxygenation was similar in both tests. This might suggest different adaptation strategies of the tissues, so that muscle tissue oxygenation status is better maintained in acute hypoxia, while the beneficial changes in cerebral oxygenation become evident only after acclimatization.

8.4.1 Cerebral tissue oxygenation

Cerebral oxygenation was slightly improved after acclimatization, as indicated by smaller increase in Δ[HHb] at 3500 m and 4800 m. However, no differences were seen in TSI be-

tween PRE and POST at any given hypoxic step. The improvement in cerebral oxygenation after chronic hypoxia indicates some adaptations at the level of the brain to hypoxia during exercise. Although not measured in the present study, this might be due to improved oxygen delivery (Imray et al. 2005) or alternatively decreased cerebral oxygen consumption (Hochachka et al. 1994).

The number of studies describing CBF and oxygen delivery after acclimatization is limited. Cerebral oxygen delivery is decreased ~20 % during light exercise in hypoxia (FiO_2 0.10), compared to identical workload exercise in normoxia. Simultaneous decrease in cerebral oxygen metabolism was observed (Rasmussen et al. 2010). In contrast, Vogiatzis et al. (2011) reported unchanged frontal cortex O_2 delivery during light to moderate exercise in hypoxia equivalent to ~4000 m (FiO_2 0.12). The discrepancy might be due to different protocols employed in the studies (continuous and discontinuous protocols, respectively) (Vogiatzis et al. 2011). Alternatively, the magnitude of the hypoxic exposure might elicit differences seen in O_2 delivery.

In severe acute hypoxia, maintenance of cerebral oxygen delivery is critically dependent on the ability to increase CBF (reviewed by Secher et al. 2008). Following altitude acclimatization, CaO_2 is increased to or above sea level values, mainly because of increased [Hb]. Thus, systemic O_2 delivery is increased during submaximal exercise following acclimatization, compared to acute hypoxia. (Calbet et al. 2003b.) Similarly, a trend toward improved brain O_2 delivery at 5260 m was observed after partial acclimatization (Imray et al. 2005). Less of increase in cerebral deoxygenation seen in the present study after acclimatization might result from improved O_2 delivery seen at more severe hypoxic steps, indicating decreased mismatch between O_2 delivery and utilization in the cerebral microcirculation. However, further research is required to confirm this hypothesis.

The effect of altitude acclimatization on cerebral oxidative metabolism is conflicting. Both unchanged (Moller et al. 2002) and decreased (Hochachka et al. 1994) oxidative metabolism has been reported after prolonged acclimatization to high altitude. These inconsistencies are likely due to methodological differences. Moller et al. (2002) showed that 5 wk ac-

climatization above 3800 m had no effect on global CBF or cerebral oxidative metabolism during exercise. Furthermore, they found no change in CBF during exercise compared with rest (Moller et al. 2002), contrary to present view (reviewed by Ogoh & Ainslie 2009). Moller et al (2002) used Kety-Schmit method to measure CBF and compared global CBF during upright exercise with supine rest. Different postures likely resulted in underestimated CBF response to exercise (reviewed by Ogoh & Ainslie 2009), because jugular vein is collapsed in the upright position (Dawson et al. 2004). The cerebral metabolic rate of oxygen was calculated by the Fick principle, utilizing CBF as part of the equation. This complicates interpretation of the results.

Hochachka et al. (1994 and 1999) reported decreases in regional cerebral glucose metabolism after acclimatization (63 days at 3200–4400 m and 63 days at 2300–6200 m, respectively) in comparison to sea-level as measured by fluorodeoxyglucose and positron emission tomography. In the latter study, brain's metabolic patterns changed significantly after the prolonged high-altitude exposure so that five regions showed decreases and two regions increases in brain metabolism. The metabolic reductions were most pronounced in regions classically associated with higher cortical functions (e.g., frontal cortex) and least pronounced in primitive brain structures (e.g., cerebellum). It was proposed that acclimatization leads to relative hypometabolism of the brain that minimizes O_2 limitation impacts (Hochachka et al. 1994), by utilizing region-specific fine tuning of brain metabolism (Hochachka et al. 1999). Although speculative, the coordination of basic biological functions such as locomotion might be favored over the higher neurological function normally ascribed to the frontal cortex (Hochachka et al. 1999). Measurements were performed at rest in normoxia only. (Hochachka et al. 1994). However, region-specific changes in brain metabolism were still detectable shortly after return from chronic hypoxia. It cannot be excluded that less of a decrease in frontal cortex oxygenation in POST in the present study was due to reduced metabolism and thus lower O_2 demand. In that case, however, decrease would have been likely evident throughout the POST test, both in normoxia and hypoxia.

The present data concerning CBF, dynamic cerebral autoregulation or cerebral CO_2 reactivity during exercise after acclimatization is limited to duration of 12 days. Ainslie et al. re-

ported (2007, 2008) attenuated dynamic cerebral autoregulation and cerebral CO_2 reactivity during moderate-intensity exercise in acute hypoxia when compared to normoxia. However, decreased MCAv after chronic hypoxia (12 days at 1560 m) was likely due to greater hypocapnia, rather than a compromise in dynamic cerebral autoregulation or cerebral CO_2 reactivity. The decrease in MCAv was associated with reduced cerebral oxygenation ($r = 0.54$; $P < 0.05$) after acclimatization, but not at baseline. (Ainslie et al. 2008.) Similarly, Subudhi et al. (2008) reported decreased MCAv and COX during moderate intensity exercise (~75 % W_{max} in both tests) after acclimatization (6 days, at 2200–4300 m). In contrast to a finding of Ainslie et al (2008), reduced cerebrovascular CO_2 reactivity was observed after chronic hypoxia. It was proposed that decreased cerebrovascular CO_2 reactivity compensated for the hypocapnia-induced reduction in cerebral blood flow during the ventilatory acclimatization period. (Subudhi et al. 2008.)

When cerebral circulation becomes less sensitive to hypocapnia, the vasodilator influence of the hypoxia seems to override the hypocapnic-induced vasoconstriction (Ainslie et al. 2007). Lower reactivity to hypocapnia might be a protective mechanism to prevent cerebral ischaemia during transient reductions in $PaCO_2$ like during exercise and potentially maintains cognitive function during exposure to high altitude (Fan et al. 2010). However, capillary overperfusion might be involved in development of altitude illness. The mechanisms behind decreased cerebrovascular reactivity to CO_2 during exercise in acute and chronic hypoxia are not clear. Hypoxic-induced vascular remodeling and elevated sympathetic nerve activity have been suggested. (Ainslie et al. 2007.) In the present study, cerebral CO_2 reactivity was not measured. When cerebral TSI was plotted against $PETCO_2$, cerebral TSI was higher in POST at a given level of $PETCO_2$ compared to PRE. Although merely a hypothesis, this might indicate weaker effect of hypocapnia on cerebral arteries following acclimatization and thus improved oxygenation.

8.4.2 Active muscle oxygenation

During walking in increasing hypoxia, muscle deoxygenation increases. In the present study, the extent of deoxygenation of the VL muscle was similar in acute hypoxia before

and after acclimatization at a given hypoxic step, consistent with the findings of Subudhi et al. (2008) and Martin et al. (2009). During submaximal exercise in acute hypoxia, a mild reduction of CaO_2 is counterbalanced by an increase in leg blood flow to maintain steady levels of leg O_2 delivery (reviewed by Calbet & Lundby 2009). With altitude acclimatization, an increase in [Hb] allows normalization of the elevated muscle blood flow response in acute hypoxia during submaximal single-leg knee-extension exercise, being similar as in normoxia (Rådegran 2008). Exercise blood flow response is related to CaO_2 under hypoxic conditions (Rådegran 2008; Koskolou et al. 1997). Muscle O_2 utilization has been shown to be similar during submaximal exercise in acute hypoxia after altitude acclimatization (9–10 wk at 5 260 m) and at sea level (FiO_2 = 0.105, PiO_2 ~75 mmHg) (Calbet et al. 2003b).

8.4.3 Inactive muscle oxygenation

This is the first study to elucidate both inactive and active muscle tissue oxygenation during walking in acute hypoxia before and after acclimatization. The hemodynamics and oxygenation of inactive arm muscles during leg cycling in normoxia has previously been studied by Ogata et al. (2007). Decrease in inactive muscle oxygenation has been attributed to the decreased blood flow. This results potentially from increased sympathetic nerve activity that causes vasoconstriction and consequently reduces blood flow to the inactive muscles. Exercising muscles produce metabolites such as hydrogen ion, potassium and adenosine that counteract sympathetic vasoconstriction. (Ogata et al. 2002; Ogata et al. 2007.) In the present study, the deoxygenation of inactive muscle was greater than that of active muscle as indicated by greater rate of TSI reduction in both PRE and POST (data not shown). This result suggests that although both active and inactive muscle oxygenation decreased during moderate exercise in acute hypoxia, the decrease was suppressed in the exercising muscle at inactive muscle's expense.

8.5 Association between hypoxic ventilatory chemosensitivity and tissue deoxygenation

It was shown recently that linkage between ventilatory responsiveness and local cerebral deoxygenation might be better predicted during exercise than at rest (Peltonen et al. 2009). Contrary to that study, where subjects with high ventilatory chemosensitivity were found to have rapid rate of reduction in cerebral tissue oxygenation index during exercise in poikilocapnic hypoxia, no association was found in the present study between ventilatory chemosensitivity and cerebral deoxygenation. Although the protocol of the present study was able to separate individual AHVR responses, hyperventilation induced hypocapnia is not the sole regulator of CBF and oxygenation (reviewed by Secher et al. 2008). Other factors such as cerebral autoregulation, sympathetic nerve activity and cerebrovascular bed reactivity to hypoxia also take part in the CBF regulation (reviewed by Ainslie & Ogoh 2010). Furthermore, in addition to CBF, cerebral metabolism, arterial saturation and hematocrit influence NIRS parameters (reviewed by Ainslie & Ogoh 2010).

The influence of $PaCO_2$ on the brain is stronger, when compared with the muscle (Rasmussen et al. 2007; Ainslie et al. 2008; Ogata et al. 2007). Thus, the observation that ventilatory chemosensitivity was associated with steep increase in Δ[HHb] in PRE is likely not mediated by $PaCO_2$. It was recently shown that metaboreceptors located in the interstitial tissue of locomotor muscles contribute to the ventilatory response to exercise in the chronic heart failure population (Olson et al. 2010). This was thought to be triggered by neural traffic from the types III and IV afferent nerve endings originating in skeletal muscle, which are stimulated by the metabolic by-products such as lactate and hydrogen ions. (Olson et al. 2010.) Although the role of metaboreceptors in ventilatory control was shown to be minor in healthy controls during submaximal exercise in normoxia (Olson et al. 2010), it might contribute to the ventilatory regulation under severe hypoxia.

In chronic heart failure patients, increased metaboreceptor activation and ventilatory response to exercise is related to increased reliance on anaerobic metabolism (Olson et al. 2010). During exercise in severe hypoxia, insufficient O_2 delivery to the working muscles

is similarly counteracted in normal subjects by activated anaerobic energy pathways (Calbet et al. 2003a). Thus, metaboreceptor activation could potentially explain the association found between leg deoxygenation and AHVR in PRE.

8.6 Methodological considerations

Near infrared spectroscopy allows monitoring of oxygenation changes in multiple tissues, but is limited to the area of interrogation and by the penetration depth of the beam (2–2.5 cm deep in the present study). In the brain this suffices illumination of the cortical grey matter (Subudhi et al. 2009). Cerebral NIRS has been shown to track changes in jugular venous bulb saturation in healthy volunteers under progressive hypoxia. Bias (mean value between the differences of the methods) of -3.1% (Shah et al. 2000) and 5.2% (Kim et al. 2000) and precision of 12.1% (Shah et al. 2000) and 10.7% (Kim et al. 2000) were reported between the methods. It was concluded that regional cerebral saturation obtained by NIRS may serve as a reliable indicator of changes in brain oxygenation induced by hypoxemia (Shah et al. 2000; Kim et al. 2000). With respect to muscle tissue, NIRS data have been shown to reflect closely the muscle metabolic rate, as determined by magnetic resonance spectroscopy-derived PCr changes (a proxy for muscle O_2 consumption), but correlated poorly with the metabolic rate determined from blood flow and (a-v)O2 measurements (Boushel et al. 2001). Although NIRS results are affected by several factors, like the equipment (Yoshitani et al. 2002), the optode positioning and (Kishi et al. 2003) the algorithm used (Owen-Reece et al. 1999), they are suitable to tracking trends instead of providing fully accurate results (Henson et al. 1998).

The absolute value of TSI is a surrogate measure of venous saturation (Greisen 2006). Tissue saturation index is easily understandable and continuous measure of the balance between tissue oxygen delivery and utilization (Tisdall et al. 2009), potentially suited to comparison of values between individuals and different NIRS instruments. This is not possible with relative variables (O_2Hb and HHb). However, the accuracy of TSI as an index of tissue deoxygenation has been questioned by several groups (Rupp & Perrey 2008; Subudhi et al. 2009; Vogiatzis et al. 2011). The measure is the weighted average of arterial, capillary

and venous blood oxygenation, and cannot be easily validated. Optically homogeneous tissue is a prerequisite for this measurement, which is unlikely to be strictly the case, at least in the brain. (Greisen 2006.) Thus, it was difficult to find earlier studies to compare the present results of cerebral TSI with.

The cerebral blood velocity was not measured in the present study. It is acknowledged that information about the cerebral O_2 supply and cerebrovascular responsiveness would have been important to define the interactions between ventilatory chemosensitivity, cerebrovascular responsiveness and cerebral tissue oxygenation. The end-tidal value was taken to reflect the PCO_2 in arterial blood ($PaCO_2$), as has been done by others (Nielsen et al. 2001; Ogoh et al. 2005).

For logistical reasons, the POST measurement was performed in average 13 days after descending from altitude. This time between altitude exposure and POST testing probably produced some deacclimatization that affected the results. It was recently shown that cerebral and muscle oxygenation responses observed during exercise in acute hypoxia immediately after acclimatization (12 days at 1560 m) were returned to preexposure values following the 12-day recovery, when monitored during similar hypoxic test (Ainslie et al. 2008). Although the extent and duration of the hypoxic exposure of that study is not comparable to the present study, some variables may have experienced partial recovery by the POST test.

8.7 Conclusions

The approach used in this study may provide useful data to inform our understanding of adaptation to chronic hypoxia during climbing. The current findings suggested that the developed protocol was suitable to measure both AHVR and cerebral and muscle oxygenation responses to constant exercise under progressive poikilocapnic hypoxia executed by the novel method to modify PiO_2. Increased ventilatory chemosensitivity (an increase in $\dot{V}_E$ vs. a decrease in SpO_2, $L \cdot min^{-1} \cdot \%^{-1}$) and improved cerebral oxygenation were observed after acclimatization. However, differences in cerebral oxygenation responses were not explained by differences in ventilatory chemosensitivity. Local muscle oxygenation remained

similar before and after acclimatization. Different effect of prolonged acclimatization on cerebral and muscle tissue oxygenation responses suggests distinct adaptation strategies of the tissues unfolding in the muscle already in acute hypoxia and in the cerebral tissue along altitude acclimatization.

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