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EFFECT OF HYPOXIA ON CEREBRAL BLOOD FLOW REGULATION DURING REST AND EXERCISE: ROLE OF CEREBRAL OXYGEN DELIVERY ON PERFORMANCE

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Abstract

English version

Adequate supply of oxygen to the brain is critical for maintaining normal brain function. Severe hypoxia, such as that experienced during high altitude ascent, presents a unique challenge to brain oxygen (O_2) supply. During high-intensity exercise, hyperventilation-induced hypocapnia leads to cerebral vasoconstriction, followed by reductions in cerebral blood flow (CBF), oxygen delivery (DO_2), and tissue oxygenation. This reduced O_2 supply to the brain could potentially account for the reduced performance typically observed during exercise in severe hypoxic conditions. The aims of this thesis were to document the effect of acute and chronic exposure to hypoxia on CBF control, and to determine the role of cerebral DO_2 and tissue oxygenation in limiting performance during exercise in severe hypoxia.

We assessed CBF, arterial O_2 content (CaO_2), haemoglobin concentration ([Hb]), partial pressure of arterial O_2 (PaO_2), cerebrovascular CO_2 reactivity, ventilatory response to CO_2, cerebral autoregulation (CA), and estimated cerebral DO_2 (CBF $\times$ CaO_2) at sea level (SL), upon ascent to 5,260 m (ALT1), and following 16 days of acclimatisation to 5,260 m (ALT16). We found an increase in CBF despite an elevated cerebrovascular CO_2 reactivity at ALT1, which coincided with a reduced CA. Meanwhile, PaO_2 was greatly decreased despite increased ventilatory drive at ALT1, resulting in a concomitant decrease in CaO_2. At ALT16, CBF decreased towards SL values, while cerebrovascular CO_2 reactivity and ventilatory drive were further elevated. Acclimatisation increased PaO_2, [Hb], and therefore CaO_2 at ALT16, but these changes did not improve CA compared to ALT1. No differences were observed in cerebral DO_2 across SL, ALT1, and ALT16. Our findings demonstrate that cerebral DO_2 is maintained during both acute and chronic exposure to 5,260 m, due to the reciprocal changes in CBF and CaO_2.

We measured middle cerebral artery velocity (MCAv: index of CBF), cerebral DO_2, ventilation ($\dot{V}E$), and performance during incremental cycling to exhaustion and 15km time trial cycling in both normoxia and severe hypoxia (11% O_2, normobaric), with and without added CO_2 to the inspirate (CO_2 breathing). We found MCAv was higher during exercise in severe hypoxia compared in normoxia, while cerebral tissue oxygenation and DO_2 were reduced. CO_2 breathing was effective in preventing the development of hyperventilation-induced hypocapnia during intense exercise in both normoxia and hypoxia. As a result, we were able to increase both MCAv and cerebral DO_2 during exercise in hypoxia with our CO_2 breathing setup. However, we concomitantly increased $\dot{V}E$ and PaO_2 (and presumably respiratory work) due to the increased hypercapnic stimuli with CO_2 breathing, which subsequently contributed to the cerebral DO_2 increase during hypoxic exercise. While we effectively restored cerebral DO_2 during exercise in hypoxia to normoxic values with CO_2 breathing, we did not observe any improvement in cerebral tissue oxygenation or exercise performance. Accordingly, our findings do not support the role of reduced cerebral DO_2 in limiting exercise performance in severe hypoxia.

French version

Un apport adéquat en oxygène au niveau du cerveau est primordial pour le maintien des fonctions cérébrales normales. L'hypoxie sévère, telle qu'expérimentée au cours d'ascensions en haute altitude, présente un défi unique pour l'apport cérébral en oxygène (O_2). Lors d'exercices à haute intensité, l'hypocapnie induite par l'hyperventilation entraîne une vasoconstriction cérébrale suivie par une réduction du flux sanguin cérébral (CBF), de l'apport en oxygène (DO_2), ainsi que de l'oxygénation tissulaire. Cette réduction de l'apport en O_2 au cerveau pourrait potentiellement être responsable de la diminution de performance observée au cours d'exercices en condition d'hypoxie sévère. Les buts de cette thèse étaient de documenter l'effet de l'exposition aiguë et chronique à l'hypoxie sur le contrôle du CBF, ainsi que de déterminer le rôle du DO_2 cérébral et de l'oxygénation tissulaire comme facteurs limitant la performance lors d'exercices en hypoxie sévère.

Nous avons mesuré CBF, le contenu artériel en oxygène (CaO_2), la concentration en hémoglobine ([Hb]), la pression partielle artérielle en O_2 (PaO_2), la réactivité cérébrovasculaire au CO_2, la réponse ventilatoire au CO_2, et l'autorégulation cérébrale sanguine (CA), et estimé DO_2 cérébral (CBF x CaO_2), au niveau de la mer (SL), au premier jour à 5.260 m (ALT1) et après seize jours d'acclimatation à 5.260 m (ALT16). Nous avons trouvé des augmentations du CBF et de la réactivité cérébrovasculaire au CO_2 après une ascension à 5.260 m. Ces augmentations coïncidaient avec une réduction de l'autorégulation cérébrale. Simultanément, la PaO_2 était grandement réduite, malgré l'augmentation de la ventilation ($\dot{V}E$), résultant en une diminution de la CaO_2. Après seize jours d'acclimatation à 5.260 m, le CBF revenait autour des valeurs observées au niveau de la mer, alors que la réactivité cérébrovasculaire au CO_2 et la $\dot{V}E$ augmentaient par rapport à ALT1. L'acclimatation augmentait la PaO_2, la concentration en hémoglobine, et donc la CaO_2, mais n'améliorait pas l'autorégulation cérébrale, comparé à ALT1. Aucune différence n'était observée au niveau du DO_2 cérébral entre SL, ALT1 et ALT16. Nos résultats montrent que le DO_2 cérébral est

maintenu constant lors d'expositions aiguë et chronique à 5.260m, ce qui s'explique par la réciprocité des variations du CBF et de la CaO_2.

Nous avons mesuré la vitesse d'écoulement du sang dans l'artère cérébrale moyenne (MCAv : un indice du CBF), le DO_2 cérébral, la $\dot{V}E$ et la performance lors d'exercice incrémentaux jusqu'à épuisement sur cycloergomètre, ainsi que des contre-la-montres de 15 km en normoxie et en hypoxie sévère (11% O_2, normobarique) ; avec ajout ou non de CO_2 dans le mélange gazeux inspiré. Nous avons trouvé que MCAv était plus haute pendant l'exercice hypoxique, comparé à la normoxie alors que le DO_2 cérébral était réduit. L'ajout de CO_2 dans le gaz inspiré était efficace pour prévenir l'hypocapnie induite par l'hyperventilation, qui se développe à l'exercice intense, à la fois en normoxie et en hypoxie. Nous avons pu augmenter MCAv et le DO_2 cérébral pendant l'exercice hypoxique, grâce à l'ajout de CO_2. Cependant, nous avons augmenté la $\dot{V}E$ et la PaO_2 (et probablement le travail respiratoire) à cause de l'augmentation du stimulus hypercapnique. Alors que nous avons, grâce à l'ajout de CO_2, efficacement restauré le DO_2 cérébral au cours de l'exercice en hypoxie à des valeurs obtenues en normoxie, nous n'avons observé aucune amélioration dans l'oxygénation du tissu cérébral ou de la performance. En conséquence, nos résultats ne soutiennent pas le rôle d'un DO_2 cérébral réduit comme facteur limitant de la performance en hypoxie sévère.

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Abbreviations

AMS	Acute mountain sickness
CA	Cerebral autoregulation
CaO_2	Arterial oxygen content
CBF	Cerebral blood flow
CMR	Cerebral metabolic rate
$CMRO_2$	Cerebral metabolic rate of O_2
CVCi	Cerebrovascular conductance index
DO_2	Oxygen delivery
$FICO_2$	Fraction of inspired CO_2
FIO_2	Fraction of inspired O_2
$[H^+]$	Hydrogen ion concentration
Hct	Haematocrit
[Hb]	Haemoglobin concentration
[HHb]	Deoxygenated haemoglobin concentration
HVR	Hypoxic ventilatory response
ICA	Internal carotid artery
MAP	Mean arterial blood pressure
MCA	Middle cerebral artery
MCAv	Middle cerebral artery blood velocity
$[O_2Hb]$	Oxygenated haemoglobin concentration
$PaCO_2$	Partial pressure of arterial CO_2
PaO_2	Partial pressure of arterial O_2
$PETCO_2$	Partial pressure of end-tidal CO_2
$PETO_2$	Partial pressure of end-tidal O_2

PO_2	Partial pressure of O_2
PCO_2	Partial pressure of CO_2
RC	Respiratory compensation threshold
RPE	Rate of perceived exhaustion
SaO_2	Arterial saturation of haemoglobin with O_2
TT	Time trial
VA	Vertebral artery
$\dot{V}_E$	Minute ventilation

Introduction

The human brain accounts for ~2% of the total body mass, yet due to its extremely high metabolic requirements, it receives ~15% of cardiac output and utilises ~20% of the total body oxygen (O_2) consumption at rest (Kety & Schmidt, 1948b; Lassen, 1959; Wade & Bishop, 1962). Because of this high metabolic demand, the brain is relatively intolerant to hypoxic insults. Adequate supply of O_2 is critical in maintaining normal brain function, and irreversible neuronal damage can occur if the supply of O_2 is compromised for more than a few minutes (Panickar & Anderson, 2011). Disruption of cerebral blood flow (CBF), as seen during ischaemic stroke, can lead to a cascade of adverse consequences such as metabolic impairment, energy failure, free radical production, excitotoxicity, altered calcium ion (Ca^{2+}) homeostasis, and activation of proteases (Wexler, 1970; Choi, 1988; Martins *et al.*, 1988; Panickar & Norenberg, 2005). Severe disruption in the CBF or O_2 supply can lead to increases in oxidative stress and mitochondrial damage, resulting in necrosis and apoptosis-mediated neuronal cell death (Sims & Muyderman, 2010).

High altitude ascent is an endeavour carried out by various groups such as mountaineers, extreme sports practitioners (e.g., paragliders and skydivers), porters, trekkers, pilgrims, military personnel, rescue workers, miners and scientists alike. Ascending to high altitude often leads to the development of acute altitude sickness, which can further develop into life-threatening illnesses like high altitude cerebral oedema (Basnyat & Murdoch, 2003). More dramatically, reductions in cerebral oxygen delivery (DO_2) associated with rapid ascents to extreme altitudes (>8000 m) can result in the loss of consciousness within seconds (Luft *et al.*, 1951; Luft & Noell, 1956) and death within minutes (Bert, 1943). However, with staged acclimatisation at progressively higher altitudes, cerebral DO_2 can be maintained well enough for humans to reach the summit of Mount Everest (8,848 m) without supplementary O_2. This extraordinary feat is made possible due to various intrinsic mechanisms that are in place to closely match brain DO_2 to metabolic requirements, and

highlights the critical role of cerebral DO_2 in our abilities to cope with extreme hypoxic conditions. How cerebral DO_2 is regulated during both acute and chronic exposures to high altitude, and by which mechanisms, remain largely unresolved. The first part of this thesis deals with the regulation of CBF upon ascent to high altitude and following a period of acclimatisation. In this thesis, the term *moderate hypoxia* will be used to refer to a level of hypoxia equivalent to altitudes between ~1,500-2,500 m, *severe hypoxia* will be used to refer to a level of hypoxia equivalent to altitudes between ~4,500-6,000 m, and the term *extreme hypoxia* will be used to refer to a level of hypoxia equivalent to altitudes above ~6,000 m.

It is well established that ascent to high altitude is detrimental to one's aerobic capacity [see (Calbet *et al.*, 2002; Calbet *et al.*, 2003; Amann & Kayser, 2009; Calbet & Lundby, 2009) for reviews]. However, despite more than a century of research on the detrimental effects of hypoxia on exercise performance, the underlying mechanisms remain poorly understood. While the cessation of maximal exercise, or the reduction of exercise intensity at the onset of fatigue, implies reduced motor recruitment by the central nervous system, the mechanisms responsible for this muscular de-recruitment remain elusive. During exercise in normoxia and moderate hypoxia, muscle afferents appear to play an important role in limiting exercise performance (Amann, 2006; Amann *et al.*, 2006; Amann & Dempsey, 2008; Amann *et al.*, 2011). Meanwhile, studies indicate that cerebral tissue deoxygenation may play a pivotal role in impairing aerobic capacity during exercise in severe hypoxic conditions (Kjaer *et al.*, 1999; Amann *et al.*, 2006; Amann *et al.*, 2007b; Subudhi *et al.*, 2007b; Rasmussen *et al.*, 2010b; Vogiatzis *et al.*, 2011). However, it remains unclear whether improving cerebral DO_2 and tissue oxygenation would improve performance during exercise in severe hypoxia.

Mass cerebral DO_2 is the product of CBF and arterial O_2 content (CaO_2). CBF is controlled by vascular smooth muscle tone in the arterioles, which are under the influences of chemical, physical, neural, and metabolic factors [see (Edvinsson & Krause, 2002; Attwell *et al.*, 2010) for reviews], while CaO_2 is determined by partial pressure of arterial O_2 (PaO_2), arterial haemoglobin O_2 saturation (SaO_2), and haemoglobin concentration ([Hb]). During severe hypoxia, resting CBF increases to protect the brain against reduced PaO_2 (Cohen *et al.*, 1967). This CBF response depends on the relative degree of prevailing hypoxia (dilating) and associated hyperventilation-induced hypocapnia (constricting), these being determined by the altitude, the individual's ventilatory response to hypoxia, and acclimatisation state (Dempsey *et al.*, 1974; Forster *et al.*, 1974; Dempsey & Forster, 1982). Given the scope that is covered by this thesis, a critical reappraisal of the current relevant literature is needed. Specifically, this encompasses the effects of acute and chronic hypoxia on the chemical, physical, neural, and metabolic control of the cerebral blood vessels, and exercise performance limitation in severe hypoxia.

Cerebral blood flow

The entire capillary network of the brain is covered by astrocyte extensions, surrounding the endothelial cells, which constitute the blood-brain barrier (Secher *et al.*, 2008; Alvarez *et al.*, 2013). The O_2 diffusion distance plays a vital role during changes in local neural activation and associated changes in local cerebral O_2 consumption. Given that there is no functional or anatomical capillary recruitment in the brain (Göbel *et al.*, 1989), an increase in CBF is required to elevate the O_2 pressure gradient (Secher *et al.*, 2008). Further, since all of the brain capillaries are assumed to be perfused during rest (Buxton & Frank, 1997), any changes in CBF would be due to changes in capillary blood flow velocity mediated by alterations in cerebrovascular resistance [see (Faraci & Heistad, 1990; Edvinsson & Krause, 2002) for reviews]. In the face of severe hypoxia, apart from

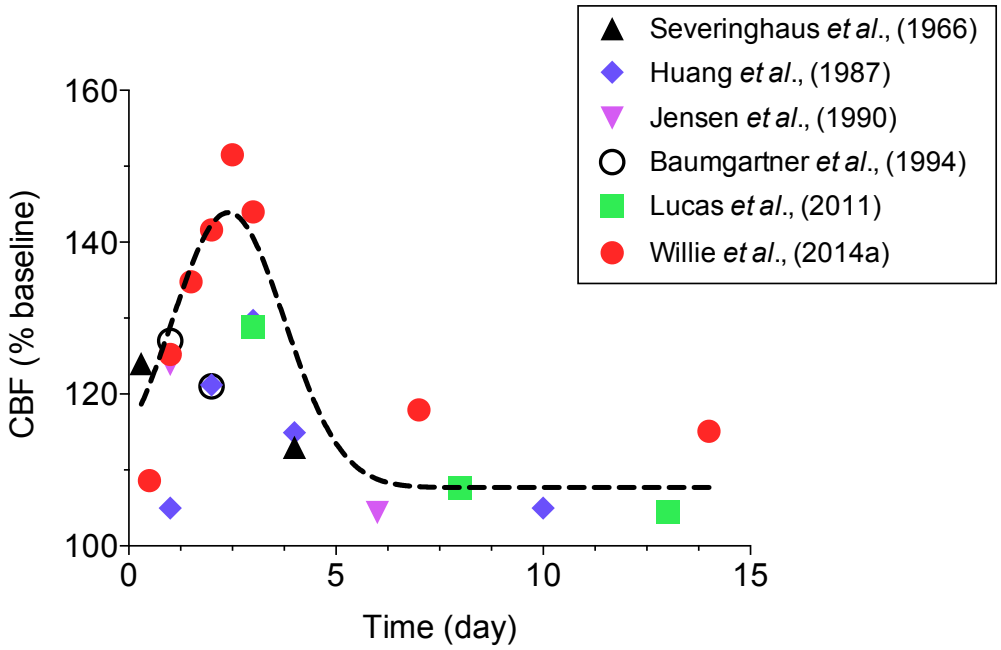

Figure 1. Changes in resting cerebral blood flow (CBF) with acute and chronic exposure to high altitude. Initial exposure to high altitude (6-8 h) elevates CBF by 20-57%, which subsequently returned gradually towards sea-level values after ~5 days of acclimatisation. Modified from Willie *et al*., (2014b).

increasing flow, the cerebral O_2 extraction fraction is also elevated in order to meet cerebral metabolic rate for O_2 ($CMRO_2$) (Kety & Schmidt, 1948a).

During hypoxic exposure, the pattern of the CBF change during both acute and chronic hypoxia can be summarised as the *net effect* of hypoxia-induced vasodilation and hypocapnia-induced vasoconstriction in the cerebral blood vessels (Xu & Lamanna, 2006; Brugniaux *et al.*, 2007). During initial exposure to severe hypoxia, the vasodilatory drive in the cerebral blood vessels typically exceeds that of the constrictor drive, thereby elevating CBF (Figure 1) (Severinghaus *et al.*, 1966; Willie *et al.*, 2014a). During acclimatisation, carbon dioxide (CO_2) is 'blown off' with increased ventilation ($\dot{V}E$), which lowers the partial pressure of arterial CO_2 ($PaCO_2$) and elevates PaO_2 (Brugniaux *et al.*, 2007), resulting in greater vasoconstrictor drive and restoring of CBF to pre-exposure values (Figure 1) (Severinghaus *et al.*, 1966). Therefore, resting CBF at high altitude is determined by the severity of hypoxia during acute exposure, which is subsequently modulated by the magnitude of increase in ventilatory drive and the associated changes in PaO_2 and $PaCO_2$ during acclimatisation.

Cerebrovascular CO_2 reactivity

Changes in $PaCO_2$ and associated arterial hydrogen ion concentration ($[H^+]$) are potent stimuli for altering cerebrovascular resistance and, thus, CBF (Lassen, 1959; Edvinsson & Krause, 2002). A rise in $PaCO_2$ (and subsequent respiratory acidosis) leads to vasodilation in the cerebral vessels, elevating CBF. Conversely, a drop in $PaCO_2$ (and subsequent respiratory alkalosis) leads to cerebral vasoconstriction, lowering CBF. Despite extensive research, the exact mechanisms by which $PaCO_2/[H^+]$ alter cerebrovascular vessel resistance remain poorly understood. *In vivo* and *in vitro* studies have provided data implicating endogenous mediators in CO_2-induced changes in cerebrovascular tone, such as nitric oxide, C-natriuretic peptide, prostanoids, and endothelin-1

(Eriksson *et al.*, 1983; Kobayashi *et al.*, 1994; Henze *et al.*, 2007; Peebles *et al.*, 2008). These endogenous mediators have been attributed to the neighbouring endothelial and neuronal cells, and act via second messenger and potassium ion channel activation/hyperpolarising pathways to alter intracellular Ca^{2+} concentration in the vascular smooth muscle, thus eliciting contraction or relaxation (Edvinsson & Krause, 2002).

The CBF responsiveness to CO_2, termed cerebrovascular CO_2 reactivity, provides a useful non-invasive index of cerebrovascular function (Faraci & Heistad, 1990; Ainslie & Ogoh, 2009). In healthy humans, one mmHg rise in $PaCO_2$ elicits 2-8% increase in global CBF as determined by transcranial Doppler ultrasound (Peebles *et al.*, 2007; Ainslie & Burgess, 2008; Fan *et al.*, 2010a; Battisti-Charbonney *et al.*, 2011), vascular Doppler ultrasound (Sato *et al.*, 2012; Willie *et al.*, 2012), Fick's principle (Kety & Schmidt, 1946, 1948a; Peebles *et al.*, 2008), and magnetic resonance imaging (MRI) (Noth *et al.*, 2008). Cerebrovascular CO_2 reactivity appears to play a crucial role in determining basal CBF during both acute and chronic exposures to high altitude (Lucas *et al.*, 2011). To date, only a handful of studies have investigated the effect of acclimatisation to high altitude on cerebrovascular CO_2 reactivity (Table 1). Moreover, interpretation of their findings is difficult as the majority of those studies examined the effect of high altitude ascent on cerebrovascular CO_2 reactivity at only one time point (Fan *et al.*, 2010a; Fan *et al.*, 2012a), without controlling for confounding factors such as ascent profile, acclimatisation status or smoking (Jansen *et al.*, 1999), or prior exposures to higher altitudes (Ainslie & Burgess, 2008). Meanwhile others have assessed cerebrovascular function in artificial normobaric hypoxia (Poulin *et al.*, 2002; Kolb *et al.*, 2004).

Data from Fan *et al.*, (2010a; 2012a), obtained from the same group of subjects at different stages of altitude acclimatisation, indicate that cerebrovascular CO_2 reactivity (assessed using a modified

rebreathing technique) increases with prolonged exposure to high altitude. In these same subjects, Lucas *et al*., (2011) found cerebrovascular CO_2 reactivity (using a steady-state technique) to be lower following 14 days at 5,050 m. However, since Lucas *et al*., (2011) did not carefully control the partial pressure of end-tidal O_2 ($P_{ET}O_2$) at high altitude, the cerebrovascular CO_2 reactivities reported in that study reflected CBF changes from *hypoxic hypocapnia* (room air breathing) to relative *hypercapnic hyperoxia* (93% O_2 and 7% CO_2). Accordingly, the findings by Lucas *et al*., (2011) do not represent true cerebrovascular CO_2 reactivity *per se*. In other cohort studies, Rupp *et al*., (2014) and Villien *et al.*, (2013) found cerebrovascular CO_2 reactivity was reduced following 5 days at 4,350 m, using a steady-state *hypoxic* hypercapnia method ($P_{ET}O_2$: ~55 mmHg). Given the differences in background O_2 levels and other methodological discrepancies (i.e., steady-state vs. rebreathing method) between these studies, it is not surprising that the current literature shows little agreement on the effect of high altitude exposure on cerebrovascular CO_2 reactivity. For example, studies have found cerebrovascular CO_2 reactivity to increase (Fan *et al.*, 2010a), decrease (Villien *et al.*, 2013; Rupp *et al.*, 2014), or remain unchanged (Jansen *et al.*, 1999) upon early exposure to high altitude, whilst acclimatisation either increases (Fan *et al.*, 2012a) or decreases it (Lucas *et al.*, 2011; Villien *et al.*, 2013; Rupp *et al.*, 2014). Thus, the effect of high altitude ascent and subsequent acclimatisation on cerebrovascular CO_2 reactivity remains unclear.

Table 1. Summary of cerebrovascular CO_2 reactivity studies at high altitude.

	Participants	Altitude	Duration	Method of assessment	Cerebrovascular CO_2 reactivity	Comments
Jansen *et al*., (1999)	43 subjects	4,243 m	Not controlled	CO_2 inhalation (0.6-1 l/min) and hyperventilation. Concentration unknown	↔ CO_2 inhalation ↑ hyperventilation	No measurements of end-tidal or arterial PCO_2. Reactivity estimated from cutaneous PCO_2. Not controlled for smoking.
Ainslie & Burgess (2008)	5 subjects	3,840 m	Unspecified	Modified rebreathing & hypoxic rebreathing	↓ modified rebreathing ↑ hypoxic rebreathing	Measurements taken following 9-day ascent to >5,000 m. Compared to values obtained at 1,400m.
Poulin *et a*l., (2002)	10 subjects	~2,800 m	48 h	Steady-state hyperoxic hypercapnia	↑	Simulated altitude in a normobaric hypoxic chamber.
Fan *et al*., (2010)	17 subjects	5,050 m	2-4 days	Modified rebreathing	↑	After 7 days trekking to 5,050 m.
Lucas *et al*., (2011)	12-17 subjects	5,050 m	2-4, 7-9, 12-15 days	Hyperventilation and steady-state hyperoxic hypocapnia	↑ hyperventilation ↓ hyperoxic hypercapnia	Background O_2 level not controlled for.
Fan *et al*., (2012)	12 subjects	5,050 m	5-11 days	Modified rebreathing	↑	After 7 days trekking to 5,050 m.
Villien *et al*., (2013)	11 subjects	4,350 m	6 days	Steady-state hypoxic hypercapnia	↓	Rapid ascent with helicopter. Background hypoxia used.
Rupp *et al*., (2014)	11 subjects	4,350 m	5 days	Steady-state hypoxic hypercapnia and normoxic hypocapnia	↔ normoxic hypocapnia ↓ hypoxic hypercapnia	Rapid ascent with helicopter. Background hypoxia used.

Cerebral autoregulation

As potent as CO_2/[H^+] are on altering cerebrovascular tone, a decrease in cerebrovascular resistance alone cannot increase CBF without sufficient perfusion pressure to maintain the pressure gradient. Depending on the severity of change, reductions in mean arterial blood pressure (MAP) have been shown to either attenuate or abolish cerebrovascular CO_2 reactivity in both dogs and humans (Harper & Glass, 1965; Ainslie *et al.*, 2012). Therefore, it seems that the chemical component of CBF regulation is intricately linked to perfusion pressure (Carrera *et al.*, 2009). Accordingly, the perfusion pressure-blood flow relationship in the brain is critical to our understanding of cerebrovascular function changes at high altitude.

Cerebral autoregulation (CA) is a term traditionally used to describe intrinsic, myogenic cellular phenomena and neurogenic mechanisms (Paulson *et al.*, 1990; Edvinsson & Krause, 2002), which are responsible for adjusting cerebrovascular resistance to maintain CBF constant within a wide range of perfusion pressures (~60-150 mmHg, Figure 2) (Lassen, 1959). Early pioneering work by Bayliss *et al*., (1895) demonstrated that cerebral circulation passively follows changes in arterial pressure in anaesthetised dogs. Accordingly, they concluded that a rise in MAP increases CBF; conversely, a fall in arterial pressure lowers cerebral perfusion. In humans, a reduction of CA was observed during CO_2 breathing (5%) and has been linked to the cerebrovascular CO_2 reactivity (Carrera *et al.*, 2009). This observation led the authors to speculate that hypercapnia-induced vasodilation might reduce the dynamic range of autoregulation mechanically by lowering the vasodilatory reserve, thus limiting the ability of the arterioles to further dilate in response to changes in blood pressure (i.e., the pressure-flow relationship becomes more linear and steeper). Findings by Carrera *et al*., (2009) give support to the notion that, despite being long considered to be independent of each other, CA and CO_2 reactivity might in fact share the same vascular reserve.

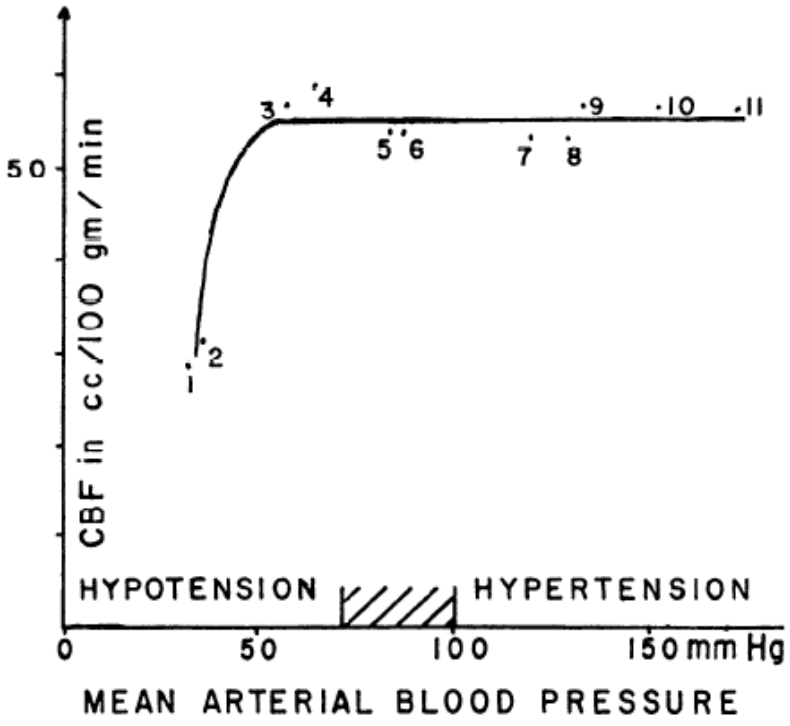

Figure 2. Cerebral blood flow (CBF) and mean arterial blood pressure. Mean values of 11 groups of subjects reported in 7 studies. 1 and 2, drug-induced severe hypotension; 3 and 4, drug-induced moderate hypotension; 5 and 6, normal pregnant women and normal young men; 7, drug-induced hypertension; 8, hypertensive toxaemic pregnancy; 9 and 10, essential hypertension. From Lassen (1959).

Impairments in CA have been reported in both permanent residents and newcomers to high altitude (Jansen *et al.*, 2000; Jansen *et al.*, 2007; Ainslie *et al.*, 2008), and appear to persist despite 1–30 days of high altitude exposure (Levine *et al.*, 1999; Jansen *et al.*, 2000; Ainslie *et al.*, 2008; Iwasaki *et al.*, 2011; Ainslie *et al.*, 2012). Jansen *et al*., (2007) reported a linear relationship between the severity of hypoxic stress and the degree of CA loss in high altitude residents (i.e., greater loss of CA at higher altitudes), indicating that CA is influenced by the level of hypoxia and does not improve with chronic hypoxic exposure. Meanwhile, other CA studies have either omitted measurements upon arrival at high altitude (Levine *et al.*, 1999; Cochand *et al.*, 2011; Iwasaki *et al.*, 2011), or followed slow ascent profiles that allowed for partial acclimatisation prior to initial measurements (Jansen *et al.*, 2000; Ainslie *et al.*, 2012). To date, no longitudinal studies have been carried out to fully characterise the CA changes across both acute and chronic high altitude exposures.

Cerebral metabolism

The brain is not homogeneous, it is composed of a variety of tissues and discrete structures that often function independently or even inversely with respect to one another (Clarke & Sokoloff, 1999). There is little reason to expect that the perfusion and metabolic rates between these distinct brain regions would be similar. At the regional level, changes in CBF are closely coupled with glucose utilisation and O_2 consumption in the brain (Raichle *et al.*, 1976; Raichle, 1998; Clarke & Sokoloff, 1999; Raichle *et al.*, 2001; Vaishnavi *et al.*, 2010). A change in metabolic activity associated with regional activation is arguably the main determinant of regional CBF under physiological conditions (see section *'Neurovascular coupling'* below). However, since regional changes in cerebral metabolic activities are difficult to quantify, the majority of the literature focuses on global cerebral metabolic changes in hypoxia.

Using the Kety-Schmidt technique, Møller *et al*., (2002) found resting cerebral O_2 and glucose metabolism were unchanged, while resting cerebral metabolic rate of lactate increased slightly following 3 weeks at 5,260 m. In that study, they observed a net cerebral lactate efflux at rest, while O_2-glucose index, lactate-glucose index, and lactate-O_2 index (indices of oxidative metabolism) tended to be reduced at 5,260 m compared to sea level. Similarly, Overgaard *et al*., (2012) reported no changes in resting $CMRO_2$ in severe hypoxia (FIO_2: 0.10), while net cerebral lactate release was elevated by ~46% after 10 min of exposure, accounting for ~2% of the energy production in the brain. More recently, Ainslie *et al*., (2014) found global cerebral metabolic rates for O_2, glucose, and lactate to be unaltered during progressive hypoxia (PaO_2: ~35-60 mmHg), while global cerebral glucose delivery and net lactate efflux were both elevated. Their data showed a trend for the ratio of O_2 to glucose uptake by the brain (index of oxidative glucose metabolism) to be decreased with increasing hypoxaemia, while the O_2 and glucose extraction fractions remained unchanged down to 70% SaO_2. Taken together, it appears that hypoxia increases the lactate release in the resting brain, which is suggestive of increased non-oxidative metabolism (i.e., anaerobic glycogenolysis and glycolysis). Nevertheless, energy production and cell metabolism appear to remain predominately reliant on oxidative metabolism under hypoxic conditions—presumably due to the brain's ability to maintain cerebral DO_2 in the face of severe hypoxia (see section '*Cerebral oxygen delivery*' below). However, the contribution of minor substrates such as glutamate and ketones for energy supply to the hypoxic brain remains unknown.

Neurovascular coupling

The close coupling of regional CBF to local substrate utilisation—termed neurovascular coupling—is the product of the anatomical and metabolic relationship of the neurovascular unit, which is comprised of neurons, glial cells, and the cerebral arterioles and capillaries (Willie *et al.*, 2014b). Due to this coupling, the cerebral arteriovenous O_2 difference at the whole-brain level varies

inversely with global CBF during hypercapnia (CO_2 inhalation) and hypocapnia (hyperventilation), while $CMRO_2$ remains constant (Kety & Schmidt, 1946, 1948a). Further, CBF is regulated to ensure appropriate glucose and O_2 delivery to the brain in order to meet $CMRO_2$, which can be calculated using the Fick's principle (Strangfeld *et al.*, 1969):

$$CMRO_2 = CBF \times (a - v)$$

Where CMR is the cerebral metabolic rate in μmol/min/g tissue, CBF is cerebral blood flow in mL/min/g, and (a – v) is the difference between arterial (a) and cerebral venous (v) substrate concentration in μmol/mL.

However, despite this 'close coupling' of blood flow and metabolism in the brain, functional MRI studies have found increases in regional CBF usually outstrip $CMRO_2$ and adenosine 5' triphosphate (ATP; the currency of energy) consumption by 2-6 fold during sustained neuronal activation (Fox & Raichle, 1986; Davis *et al.*, 1998; Hoge *et al.*, 1999; Kim *et al.*, 1999; Lin *et al.*, 2010; Vafaee *et al.*, 2012). Further, this coupling between CBF and cerebral metabolism can be uncoupled using pharmacological means. Specifically, a muscarinic receptor antagonist (Villringer & Dirnagl, 1995) and a neuronal nitric oxide synthase inhibitor (Cholet *et al.*, 1996) have been shown to abolish the expected CBF increase during somatosensory stimulation, despite elevated neuronal (and therefore metabolic) activity. Conversely, activation of acetylcholine-containing fibre projection to the cerebral cortex during nucleus basalis stimulation, has been shown to increase cortical blood flow without concurrently increasing local glucose metabolism (Vaucher *et al.*, 1997). These findings of CBF and cerebral metabolism uncoupling give support to possible 'in-parallel' regulatory mechanisms (Magistretti & Pellerin, 1999), whereby synaptic activation and subsequent glutamate release leads to: 1) neuronal nitric oxide formation, which contributes to the local activity-dependent CBF increase (Attwell *et al.*, 2010); and 2) glutamate reuptake by the surrounding astrocytes, which stimulates astrocytic glucose metabolism and lactate production

(Magistretti, 1997). With these observations, Magistretti and Pellerin (1999) concluded that while CBF and metabolism are linked under physiological conditions, this relationship could be uncoupled by selective pharmacological manipulations or during certain pathophysiological conditions.

Arterial oxygen content

The ability to maintain physiological homeostasis in the face of hypoxic stress is vastly advantageous, particularly for individuals challenged by changes in barometric pressure and associated arterial hypoxaemia (Beall, 2007). Humans have a number of compensatory mechanisms to hypoxia, thus enabling communities to live at high altitude. These mechanisms range from fast-onset responses [within minutes; e.g., elevation of minute ventilation ($\dot{V}_E$, fast-component) and sympathetic activity (Dempsey & Forster, 1982; Xing *et al.*, 2014)], to slower responses [days and weeks; e.g., increases in $\dot{V}_E$ (slow-component), haematocrit (Hct), and brain capillary density (Dempsey & Forster, 1982; Ainslie & Ogoh, 2009)]. One of the most distinct and rapid physiological compensatory responses to hypoxia is the elevation in $\dot{V}_E$, termed hypoxic ventilatory response (HVR, Figure 3). The HVR is initiated within minutes of exposure to hypoxia and increases progressively over time before stabilising after ~2 weeks at high altitude (Dempsey & Forster, 1982; Sato *et al.*, 1992). This response is primarily mediated by the peripheral chemoreceptors (Heymans & Bouckaert, 1930; Forster *et al.*, 1981; Lahiri *et al.*, 1981; Lahiri *et al.*, 2006), located in the glomus cells of the carotid bodies, at the bifurcation of the common carotid arteries (Eyzaguirre & Zapata, 1984). These peripheral chemoreceptors are responsible for eliciting the HVR when resting PaO_2 drops below 60 mmHg (Dempsey & Forster, 1982). Carotid body denervation via unilateral and bilateral carotid body resection attenuates or completely abolishes HVR in humans (Wade *et al.*, 1970; Lugliani *et al.*, 1971; Prabhakar & Peng, 2004).

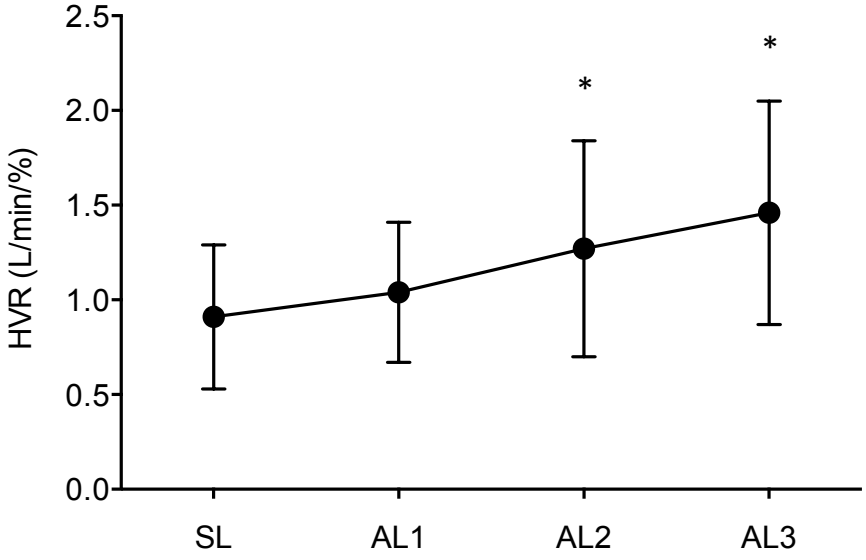

Figure 3. Effect of time at altitude on hypoxic ventilatory response (HVR; mean ± SD). AL1, AL2, and AL3, 30 ± 18, 76 ± 19, and 115 ± 10h, respectively, at 3,810 m altitude. * Different from sea level (SL; $P<0.05$). Modified from Sato *et al*., (1992).

Importantly, increased ventilatory drive and Hct during chronic high altitude exposure improves SaO_2 and normalises resting CaO_2 to sea level values, thus partly alleviating arterial hypoxaemia in spite of lowered PaO_2.

Given the importance of O_2 supply in maintaining normal brain function, it is not surprising that the O_2 carrying capacity of the blood (i.e., Hct and [Hb]) and the level of O_2 in the blood (i.e., PaO_2 and SaO_2) are important determinants of CBF. For example, CBF varies inversely with Hct in many species under both acute (i.e., blood transfusion, acute anaemia) and chronic (i.e., erythropoiesis) experimental conditions (Jones *et al.*, 1981; Brown *et al.*, 1985; Hudak *et al.*, 1986; Ulatowski *et al.*, 1998). A handful of studies have found reducing CaO_2 elevates CBF during acute and chronic anaemia (Hare, 2004; Gottesman *et al.*, 2012), haemodilution (Brown *et al.*, 1985; Hudak *et al.*, 1986; Todd *et al.*, 1994; Ulatowski *et al.*, 1998), and carbon monoxide inhalation (Paulson *et al.*, 1973). Conversely, elevating Hct lowers CBF in haemodialysis patients (Metry *et al.*, 1999). Meanwhile, Rebel *et al.*, (2001) found CBF progressively increases with decreasing Hct in conscious rats.

A critical consideration in the regulation of cerebral DO_2 is whether it is the arterial-tissue PO_2 gradient driving diffusion (i.e., PaO_2), or the blood O_2 concentration (i.e., CaO_2), which plays a more important role in CBF control in hypoxia. An elegant study by Jones *et al.*, (1981) demonstrated that, by selectively altering Hct and PaO_2 in unanaesthetised new-born lambs, the relationship between CBF and PaO_2 depended on Hct and *vice versa*; in contrast, the relationship of CBF to CaO_2 was independent of both Hct and PaO_2. From these findings, they concluded that the changes in CaO_2 are accompanied by reciprocal changes in CBF in order to maintain constant cerebral DO_2 (Figure 4). Similarly, Roach *et al.*, (1999) found cardiac output and limb blood flow

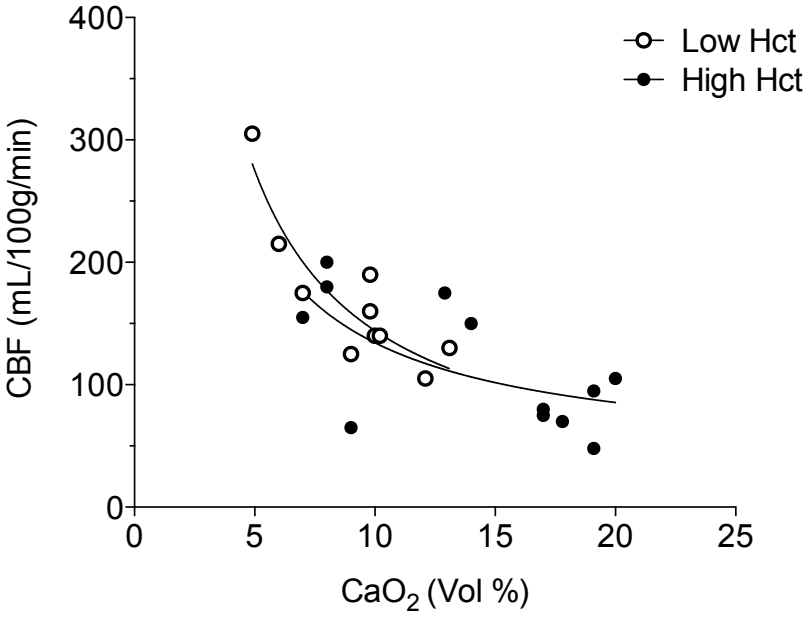

Figure 4. Relationship between CBF and CaO_2. High haematocrit: CBF = $1045.85/CaO_2 + 33.99$ (R=0.65); Low haematocrit: CBF = $1191.67/CaO_2 + 25.86$ (R=0.88). Modified from Jones *et al*., (1981).

during knee extension was elevated when CaO_2 was reduced with isovolemic haemodilution, while lowering PaO_2 with hypoxia had no effect. They concluded that O_2 delivery, rather than capillary PO_2, is the main regulatory goal of vasodilation in the peripheral vasculature. Furthermore, the authors speculated that the regulatory mechanisms of CaO_2 on blood flow could be due to red blood cell ATP release (Ellsworth *et al.*, 1995), O_2-sensitive arachidonic acid metabolite release (Harder *et al.*, 1996), or a Hb specific effect on scavenging of nitric oxide to elicit vasodilation in the face of anaemia (Stamler *et al.*, 1997). These findings indicate that under both physiological and pathological conditions, CaO_2 rather than PaO_2 determines the CBF response during hypoxic conditions. This is of particular importance when considering the role of CaO_2 on CBF regulation during acute and chronic exposure to extreme altitudes.

Cerebral oxygen delivery

Despite the reduction in environmental PO_2 at high altitude and subsequent initial decrease in CaO_2, cerebral DO_2 appears to be well maintained (Wolff, 2000; Wolff *et al.*, 2002). Based on a re-analysis of the data collected by Severinghaus *et al*., (1966), Wolff (2000) found cerebral DO_2 was maintained during the first 8 h of high altitude exposure (3,810 m), largely due to an increase in CBF associated with early hypoxic exposure. Furthermore, despite a progressive decline in CBF during prolonged high altitude exposure (5 days), cerebral DO_2 was maintained due to the increases in PaO_2 and [Hb] (therefore CaO_2) associated with acclimatisation (Wolff, 2000; Wolff *et al.*, 2002). Grocott *et al*., (2009) examined the changes in CaO_2 and [Hb] in climbers during extreme altitude ascent (8,400 m; PaO_2: ~30 mmHg). In that study, they found that the increase in [Hb] associated with high altitude acclimatisation was sufficient to compensate for the drop in SaO_2, such that resting CaO_2 was maintained until 7,100 m (Figure 5). To date, no studies have examined how the CBF and CaO_2 interact at a fixed altitude during acute exposure and following acclimatisation to high altitude.

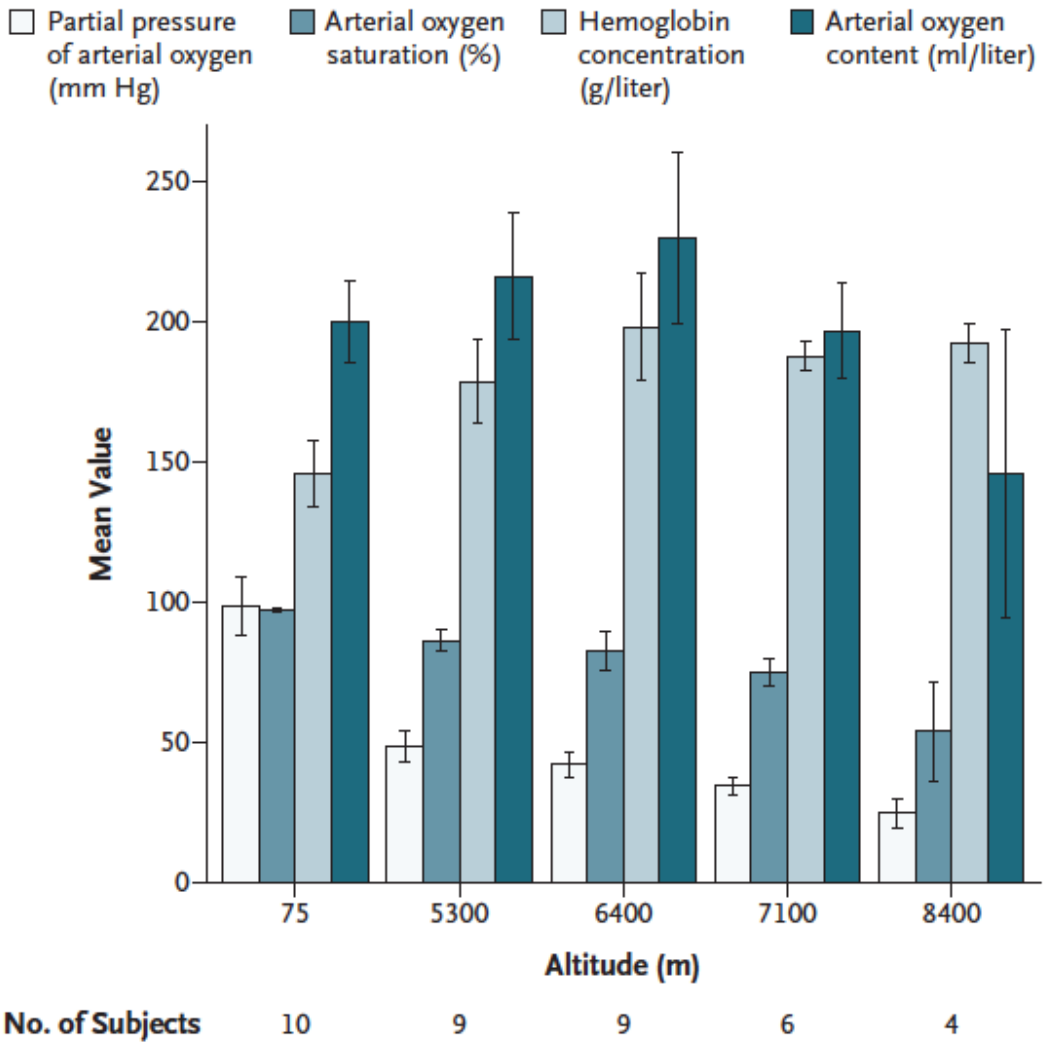

Figure 5. Changes in mean PaO_2, SaO_2, [Hb], and CaO_2 on Mount Everest. From Grocott *et al*., (2009).

Limitation of exercise performance

Hypoxia limits O_2 transport from the air to the muscle mitochondria, thereby compromising the body's capacity to perform work (Calbet & Lundby, 2009). Aerobic exercise capacity is a vital determinant of an individual's ability to thrive at high altitude (Curran *et al.*, 1998), and exposure to high altitude poses problems for many physical endeavours. Despite its importance, the underpinning mechanisms of reduced aerobic power and exercise performance impairment in severe hypoxia (SaO_2 < 75 %) are still not well understood. While the lack of O_2 is the obvious cause, the exact mechanisms by which hypoxia initiates an earlier disengagement from an exercise challenge remain unclear.

The brain is responsible for deciding whether to slow down or to stop exercise (Kayser, 2003). While various factors such as sensory feedback from the working limbs (Amann *et al.*, 2008; Amann *et al.*, 2011), respiratory muscle work (Amann *et al.*, 2007a), circulating metabolites (Karlsson *et al.*, 1975; Hogan & Welch, 1984), CaO_2 (Amann, 2006; Amann *et al.*, 2006), the body's glycogen stores (Bergstrom *et al.*, 1967; Hermansen *et al.*, 1967), and thermal comfort (Nybo & Nielsen, 2001; Nybo *et al.*, 2002), all contributing towards the development of fatigue [see (Amann & Calbet, 2008; Dempsey *et al.*, 2008; Nybo, 2008; Amann & Kayser, 2009; Ortenblad *et al.*, 2013) for reviews], the cessation of exercise is ultimately initiated by the brain (Kayser, 2003). From this perspective, the end-point of increasing fatigue (i.e., exhaustion) is of central nervous system origin (Secher *et al.*, 2008).

Aerobic exercise capacity is highly sensitive to changes in SaO_2 (Amann & Kayser, 2009), and small reductions associated with exercise-induced arterial hypoxaemia impair exercise capacity in normoxia [see (Dempsey & Wagner, 1999) for review]. This detrimental effect of arterial hypoxaemia on aerobic capacity is amplified during exercise in hypoxia (Kayser *et al.*, 1994;

Amann & Calbet, 2008). As Amann & Kayser (2009) noted, this impairment of aerobic capacity during both acute and chronic exposure to high altitude has been well documented in both moderately and highly trained individuals (Dill *et al.*, 1966; Klausen *et al.*, 1966; Dill *et al.*, 1967; Maher *et al.*, 1974; Calbet *et al.*, 2003; Lundby *et al.*, 2004; Lundby *et al.*, 2006). There is a performance drop of ~1% for every 100 m ascended from 1,500 m above sea level (Buskirk *et al.*, 1967; Pugh, 1967). Further, it appears that people with higher aerobic fitness at sea level are more adversely affected in hypoxia (Martin & O'Kroy, 1993; Gore *et al.*, 1996; Ferretti *et al.*, 1997; Calbet & Lundby, 2009), due to greater reductions in SaO_2, CaO_2, and therefore mass O_2 transport (Ekblom *et al.*, 1975; Chapman *et al.*, 1999; Calbet & Lundby, 2009). This reduction in mass O_2 transport ultimately leads to an early onset of muscle fatigue and exhaustion compared to equivalent exercise at lower altitudes (Amann & Kayser, 2009).

Peripheral fatigue in hypoxia

Under normoxic conditions, the major causes of fatigue development during muscle contraction have been attributed to muscle contractile apparatus failure and impairment of excitation-contraction coupling mechanisms associated with excess accumulation of metabolic by-products and impaired sarcoplasmic reticulum Ca^{2+} handling (Merton, 1954; Dawson *et al.*, 1978; Fitch & McComas, 1985; MacIntosh *et al.*, 2012; Ortenblad *et al.*, 2013). Accordingly, it was hypothesised that greater metabolic disturbance associated with reduced CaO_2, at the level of the muscle fibre, could be responsible for the reduced exercise performance observed in severe hypoxia (Bylund-Fellenius *et al.*, 1981; Idstrom *et al.*, 1986). However, evidence from operation Everest II, a chamber simulation of a Mount Everest ascent, does not support the idea that the development of fatigue during dynamic exercise involving a large muscle mass in severe hypoxia is of muscular origin. Data from this chamber study showed that metabolic enzyme activity, oxidative capacity, and energy stores in the vastus lateralis to be largely preserved at high altitude (Green *et al.*, 1989),

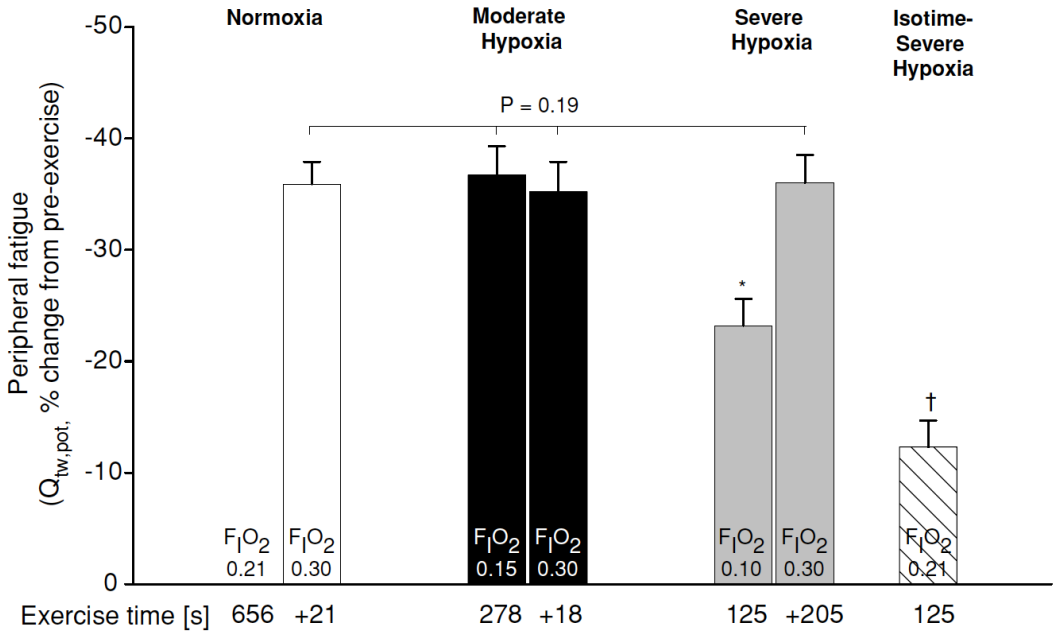

Figure 6. Peripheral quadriceps fatigue (Qtw,pot, potentiated twitch force elicited by magnetic nerve stimulation) assessed 2 min following various trials with different values for FIO_2. * Different from FIO_2: 0.15 and 0.30 ($P<0.05$); † Different from FIO_2: 0.10, 0.15 and 0.30 ($P<0.05$). From Amann *et al*., (2007b).

while electrical stimulation elicited full ankle dorsiflexion under extreme hypoxic conditions (Garner *et al.*, 1990). Since biochemical, electromyographic, and mechanical signs of muscle fatigue at exhaustion are lower compared to normoxia, Verges *et al*., (2012) summarised that the main cause of impaired whole body exercise performance in severe hypoxia is unlikely due to muscle metabolic fatigue. Instead, it was proposed by Kayser *et al*., (1994) that the central nervous system might play a primary role in limiting performance under severe hypoxic conditions. Supporting this contention, Amann *et al*., (2007b) found smaller reductions in potentiated quadriceps twitch force—index of peripheral fatigue—at the end of constant-load cycling to exhaustion (~80% normoxic power output) in severe hypoxia (FIO_2: 0.10), compared to normoxia and moderate hypoxia (FIO_2: 0.15) (Figure 6). From this, they postulated that peripheral muscle fatigue is the main determinant of central motor drive and exercise performance in normoxia and moderate hypoxia (Amann *et al.*, 2006; Romer *et al.*, 2007), while a hypoxia-sensitive central component might play a greater role during high intensity, exhaustive exercise in severe hypoxia.

Following exhaustive constant-load cycling (~50% normoxic power output) in acute hypoxia (FIO_2: 0.105), Amann *et al*., (2013) found both potentiated quadriceps twitch force and maximal voluntary contraction force were reduced compared to pre-exercise values (Figure 7). In contrast, they found no sign of peripheral fatigue following an identical bout of exercise in normoxia. Since inspiratory muscle work was higher and CaO_2 was lower during hypoxic exercise compared to normoxia, the authors attributed the development of peripheral fatigue to reduced muscle DO_2 and increased respiratory work associated with hypoxic exercise (Figures 7 & 8). Increasing respiratory work, either by voluntarily increasing the tidal integral of transdiaphragmatic pressure or by hypoxia-induced hyperventilation, exacerbates diaphragm fatigue during exercise (Babcock *et al.*, 1995; Vogiatzis *et al.*, 2008). Diaphragm fatigue (and other respiratory muscle fatigue) augments

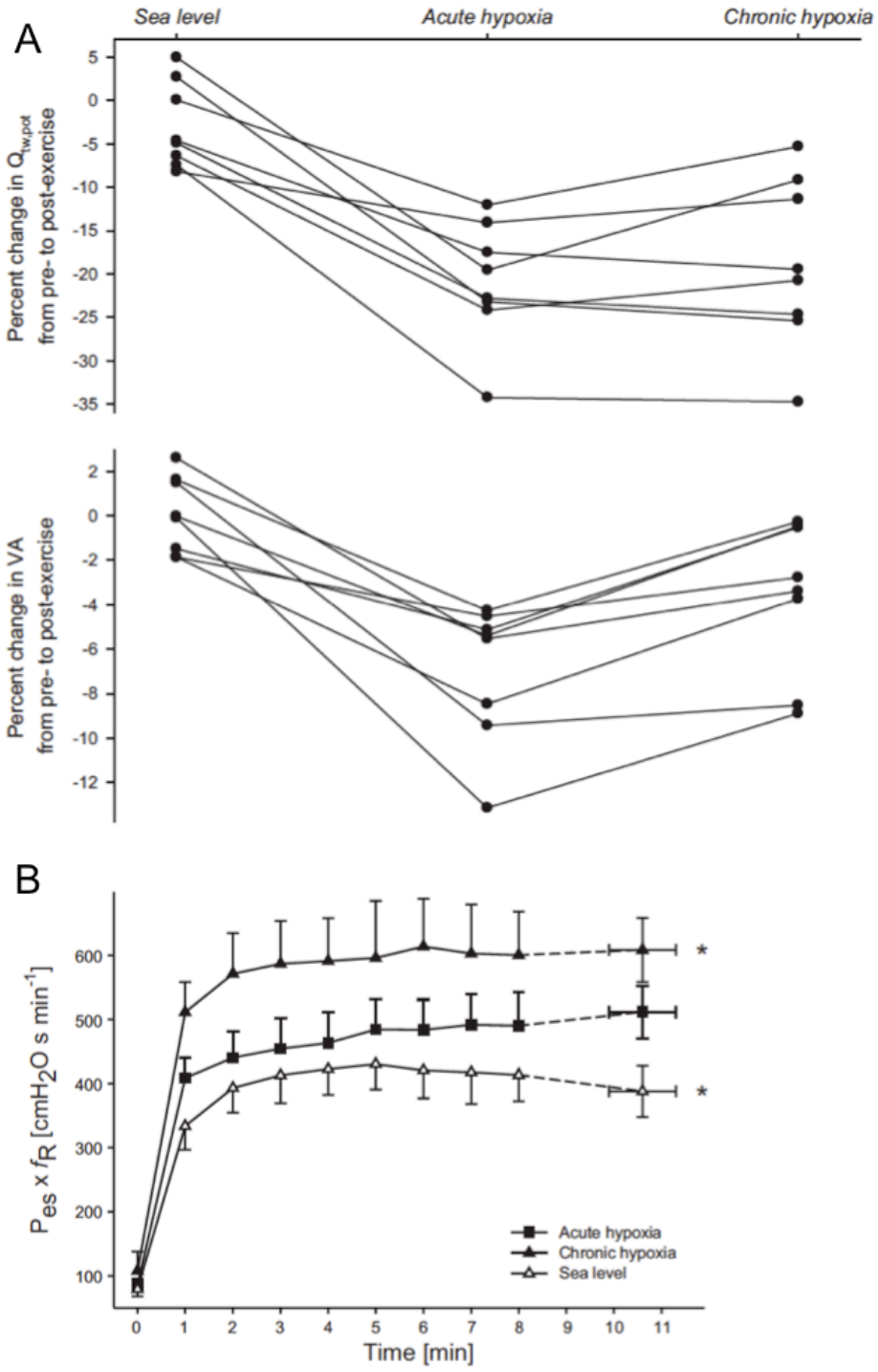

Figure 7. Panel A: Individual data illustrating the effects of constant-load cycling exercise (138 ± 14 W; 10.6 ± 0.7 min) on electrically elicited potentiated quadriceps twitch force (Qtw,pot; top) and voluntary muscle activation (VA; bottom). Panel B: Inspiratory muscle pressure-time production [esophageal pressure (P_{es}) × respiratory frequency (f_R)] during identical constant-load cycling exercise. Sea level (CaO_2: 19.3 ± 0.7 ml O_2/dl) and in acute hypoxia (17.3 ± 9.5 ml O_2/dl) and chronic hypoxia (20.3 ± 1.3 ml O_2/dl). * Different from acute hypoxia ($P<0.05$). From Amann *et al*., (2013).

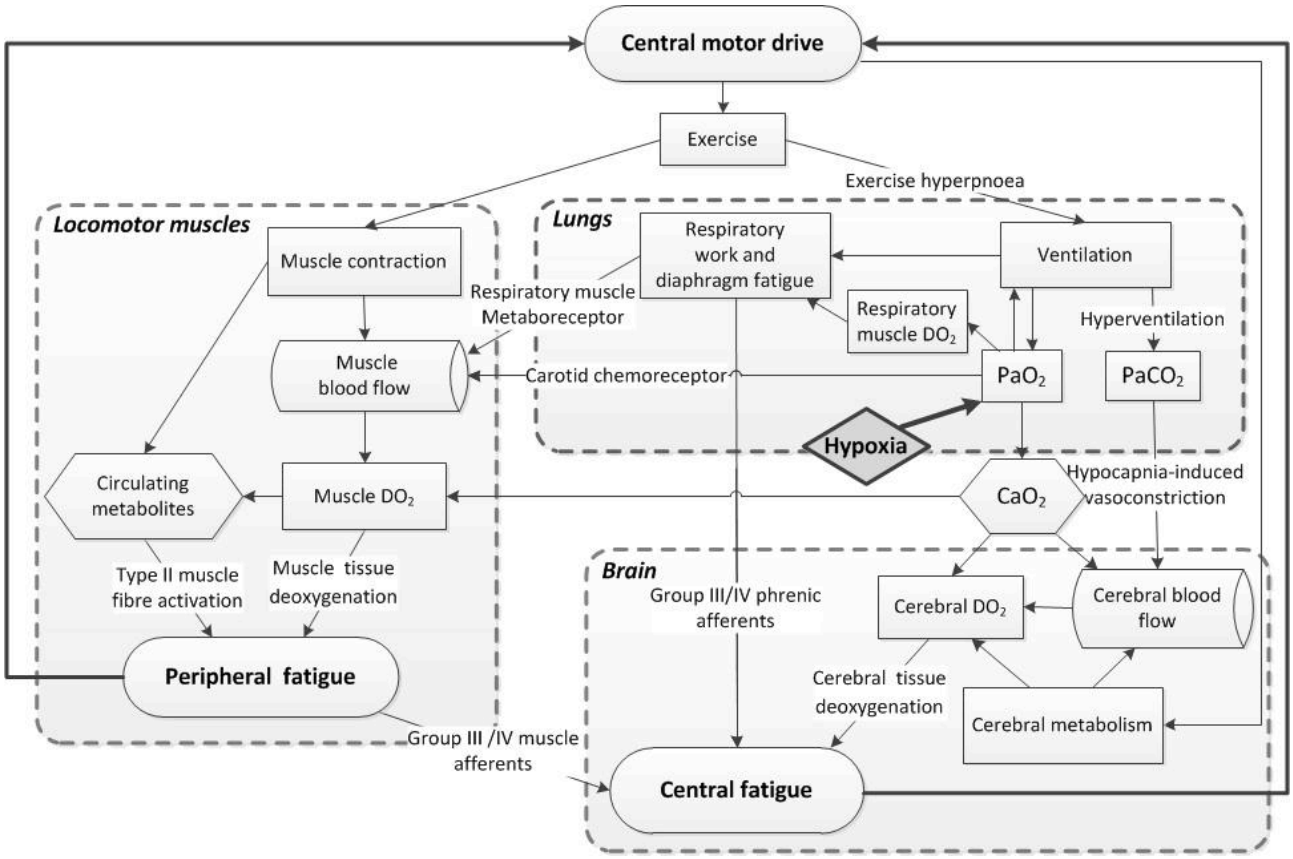

Figure 8. Schematic representation of the effect of hypoxia on central motor drive. *Lungs:* Hypoxia lowers PaO_2, thus elevating ventilation and lowering CaO_2 and respiratory muscle DO_2 (Amann *et al.*, 2007a). The combination of increased ventilation and reduced respiratory muscle DO_2 accelerates the development of respiratory muscle fatigue, resulting in activation of respiratory muscle metaboreceptors and group III/IV phrenic afferents (Dempsey *et al.*, 2006). *Locomotor muscles:* Increases in both carotid chemoreceptor activity and respiratory metaboreceptor activity, elicit vasoconstriction of the locomotor vasculature, thereby lowering limb blood flow (Dempsey *et al.*, 2006). The reduced limb blood flow, combined with reduced CaO_2 in hypoxia, lowers locomotor muscle DO_2, augments the accumulation of metabolites, and accelerates the development of peripheral fatigue (Amann & Calbet, 2008). Consequently, the increase in peripheral fatigue modulates central motor drive via group III/IV muscle afferents (Amann *et al.*, 2006). *Brain:* Increases in central motor drive associated with exercise elevate regional neuronal activity, and therefore metabolic requirements (Raichle *et al.*, 1976). These in turn elicit local vasodilation via neurovascular coupling, thereby increasing local blood flow and DO_2 (Jorgensen *et al.*, 1992a; Jorgensen *et al.*, 1992b). The increased ventilatory drive associated with hypoxia exacerbates hypocapnia and associated cerebral vasoconstriction. This cerebral vasoconstriction is partly alleviated by hypoxaemia-mediated vasodilation and cortical activation-mediated vasodilation associated with exercise. The resulting reduction in cerebral blood flow, coupled with reduced CaO_2, lowers cerebral DO_2 and tissue oxygenation (Subudhi *et al.*, 2007b). The subsequent reduction cerebral tissue oxygenation, combined with increased group III/IV phrenic and muscle afferents discharge, exacerbates the development of central fatigue and lowers central motor drive during exercise in hypoxia (Amann & Dempsey, 2008; Amann *et al.*, 2013).

metabolite accumulation and activates unmyelinated group IV phrenic afferents (Dempsey *et al.*, 2006); these in turn increase sympathetic nerve activity (St Croix *et al.*, 2000) and activate respiratory muscle metaboreceptors (Hussain *et al.*, 1991; Sheel *et al.*, 2001; Sheel *et al.*, 2002). Elevations in sympathetic nerve activity and respiratory muscle metaboreceptor activation elicit constriction of the limb muscle vasculature via a supraspinal reflex (Dempsey *et al.*, 2006), which redistributes cardiac output from the locomotor muscles to the respiratory muscles (the 'blood steal' effect) (Harms *et al.*, 1997; Harms *et al.*, 1998). As a result, increasing respiratory work during exercise causes earlier respiratory muscle fatigue onset and lowers locomotor muscle blood flow, thereby increasing the severity of locomotor muscle fatigue and perceptions of leg discomfort (Romer *et al.*, 2006; Taylor & Romer, 2008). These adverse effects of increased respiratory work on muscle DO_2, muscle fatigue, and perceptions of leg discomfort are amplified by hypoxia (Amann *et al.*, 2007a; Verges *et al.*, 2010). Collectively, these findings demonstrate that muscle DO_2 can be impaired via increased respiratory work and the associated metaboreflex-mediated reduction in locomotor limb blood flow during exercise in hypoxia (Figure 8).

A reduction in muscle DO_2 in hypoxia leads to a mismatch between O_2 requirement and supply (Amann & Calbet, 2008). As a consequence, for a given absolute workload, the relative exercise intensity in hypoxia is *greater* than in normoxia (Amann & Calbet, 2008). This increase in relative exercise intensity, associated with reduced muscle DO_2, elevates type II muscle fibre recruitment (Merletti *et al.*, 1990), and lowers the recruitment of fatigue-resistant, O_2-dependent type I muscle fibres during muscle contractions (Dousset *et al.*, 2001). Consequently, more type II fibres are recruited during hypoxic exercise, which accelerates the accumulation of metabolite (e.g., H^+ and phosphates) (Edwards, 1981; Hogan *et al.*, 1999; Amann *et al.*, 2006). Metabolite accumulation disrupts sarcoplasmic reticulum Ca^{2+} release and uptake pathways (Allen *et al.*, 1995; Dutka *et al.*, 2005), and inhibits the contractile apparatus (Godt & Nosek, 1989; Stienen *et al.*, 1990; Fryer *et al.*,

1995). An elevated metabolite accumulation in hypoxia can lead to early onset of excitation–contraction coupling failure within the muscle fibre (i.e., muscle fatigue) (Amann & Calbet, 2008). The development of locomotor muscle fatigue—termed peripheral fatigue—plays a vital role in exercise performance by modulating central motor drive in a dose-dependent manner via group III/IV muscle afferent feedback (Figure 8) (Gandevia, 2001; Amann & Dempsey, 2008; Amann *et al.*, 2010; Amann, 2011, 2012).

Central fatigue in hypoxia

It has been proposed that hypoxia of the central nervous system may lead to a reduced central motor command, which ultimately limits exercise performance under severe hypoxic conditions (Kayser *et al.*, 1994; Amann *et al.*, 2007b; Amann & Calbet, 2008; Amann & Kayser, 2009). It was thought that reduced cerebral DO_2 and subsequent cerebral tissue deoxygenation might be a major limiting factor of exercise performance in hypoxia (Kjaer *et al.*, 1999; Amann *et al.*, 2006; Amann *et al.*, 2007b; Subudhi *et al.*, 2007b; Rasmussen *et al.*, 2010b; Vogiatzis *et al.*, 2011). The pioneering work by Kayser *et al.*, (1994) was the first to demonstrate that exhaustive exercise at 5,050 m could be prolonged with a rapid increase in FIO_2 (FIO_2: 1.0) at the point of maximal exertion, in acclimatised subjects during constant-load cycling to exhaustion. This observation has since been repeated during both constant-load exercise to exhaustion (Amann *et al.*, 2007b) and incremental cycling (Subudhi *et al.*, 2007b; Koglin & Kayser, 2013). These later studies further demonstrated that rapid switching to hyperoxia (FIO_2: 0.30-0.60) improved performance during exercise in acute and chronic hypoxia, while no improvement could be found by increasing FIO_2 at exhaustion in normoxia (Figures 9 & 10). Since the effect of the FIO_2 increase was too quick to reverse the metabolic factors associated with peripheral fatigue, Kayser *et al.*, (1994) and others (Amann *et al.*, 2007b; Subudhi *et al.*, 2007b; Koglin & Kayser, 2013) concluded that the improvement in exercise performance was due to cerebral tissue reoxygenation. However, as noted by Subudhi *et al.*,

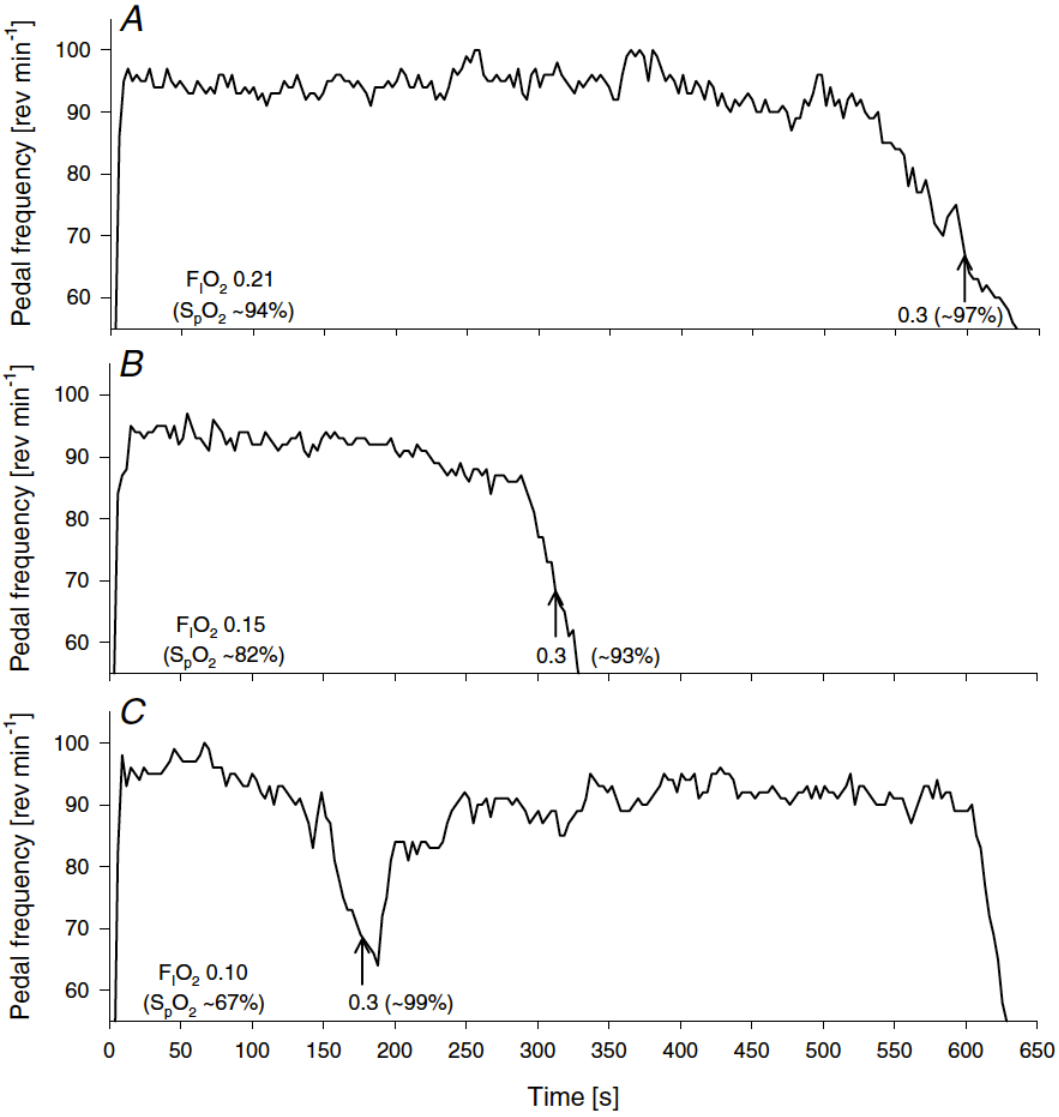

Figure 9. Representative example showing the effects of a rapid increase in F_IO_2 at exhaustion on pedal frequency and endurance time to exhaustion. The arrow indicates the point at which the inspirate was switched from normoxia (A; F_IO_2: 0.21), moderate hypoxia (B, F_IO_2: 0.15), or severe hypoxia (C; F_IO_2: 0.10) to hyperoxia (F_IO_2: 0.30). From Amann *et al*., (2007b).

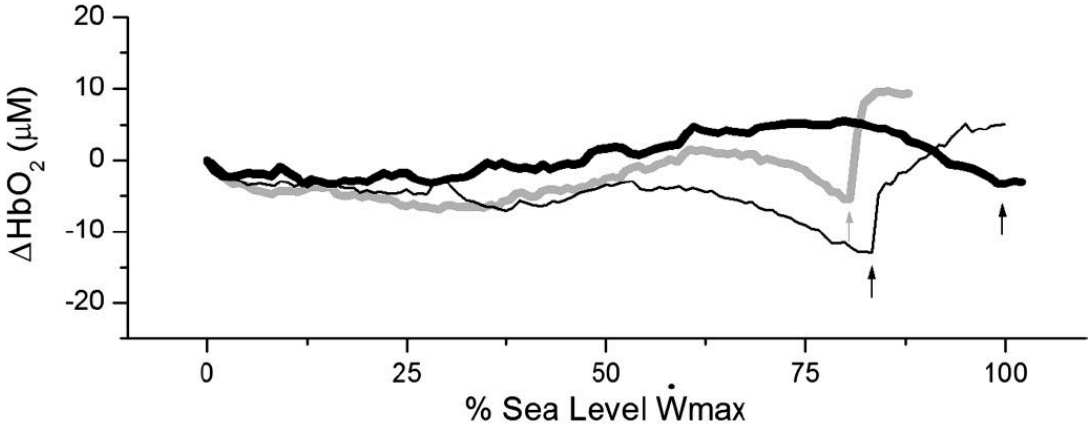

Figure 10. Representative changes in cerebral oxyhaemoglobin (HbO_2) from a single subject performing incremental exercise to maximal exertion at sea level (thick solid line), acute hypoxia (thick shaded line), and chronic hypoxia (thin solid line). Arrows mark the gas switch to 60% inspired oxygen. The subject could not continue exercise after the gas switch at sea level; however, during the hypoxic trials, the gas switch improved cerebral oxygenation and maximal exercise performance by 9% and 20% in acute hypoxia and chronic hypoxia, respectively. From Subudhi *et al*., (2007b).

(2011b), these conclusions based on rapid O_2 switching studies are limited by the fact that the improvement of arterial oxygenation is systemic, and thus not localised only to the brain.

During high-intensity exercise, hyperventilation-induced hypocapnia leads to cerebral vasoconstriction, which counteracts the hypoxia-induced vasodilation, thereby lowering CBF, cerebral DO_2 and cerebral oxygenation (Figure 8) (Herholz *et al.*, 1987; Thomas *et al.*, 1989; Jorgensen *et al.*, 1992b; Madsen *et al.*, 1993; Subudhi *et al.*, 2007a). An enhanced ventilatory drive associated with hypoxia exacerbates the hyperventilation-induced hypocapnia during heavy exercise, and further lowers CBF and cerebral oxygenation. Evidence implicating cerebral deoxygenation on performance comes from the observation that cerebral deoxygenation precedes the development of central fatigue during exercise, which coincides with both reduced cortical motor output and increased cerebral metabolism (Secher *et al.*, 2008; Subudhi *et al.*, 2009a; Rasmussen *et al.*, 2010a). Under severe hypoxic conditions, several studies have reported a relationship between performance and cerebral deoxygenation during various exercise modes such as repeated sprints (Smith & Billaut, 2010), incremental exercise (Peltonen *et al.*, 2009; Subudhi *et al.*, 2009a; Vogiatzis *et al.*, 2011), and static maximal muscle contraction to exhaustion (Rasmussen *et al.*, 2007; Rupp & Perrey, 2009; Goodall *et al.*, 2010). Similarly, exacerbation of the exercise-induced cerebral deoxygenation with non-selective beta-blockade reduces maximal exercise performance under normoxic conditions (Seifert *et al.*, 2009). From these findings, it was hypothesised that reduced cerebral DO_2 and associated cerebral tissue deoxygenation may be a limiting factor of performance during exercise in severe hypoxia (Nybo & Rasmussen, 2007; Amann & Dempsey, 2008; Amann & Kayser, 2009). In support of this notion, Goodall *et al*., (2014) found reduced cortical voluntary activation (i.e., index of supraspinal fatigue) and prefrontal tissue oxygenation (compared to baseline values) following constant-load cycling in acute hypoxia (FIO_2: 0.105), while no reductions were observed in normoxia (Figure 11). Interestingly, these

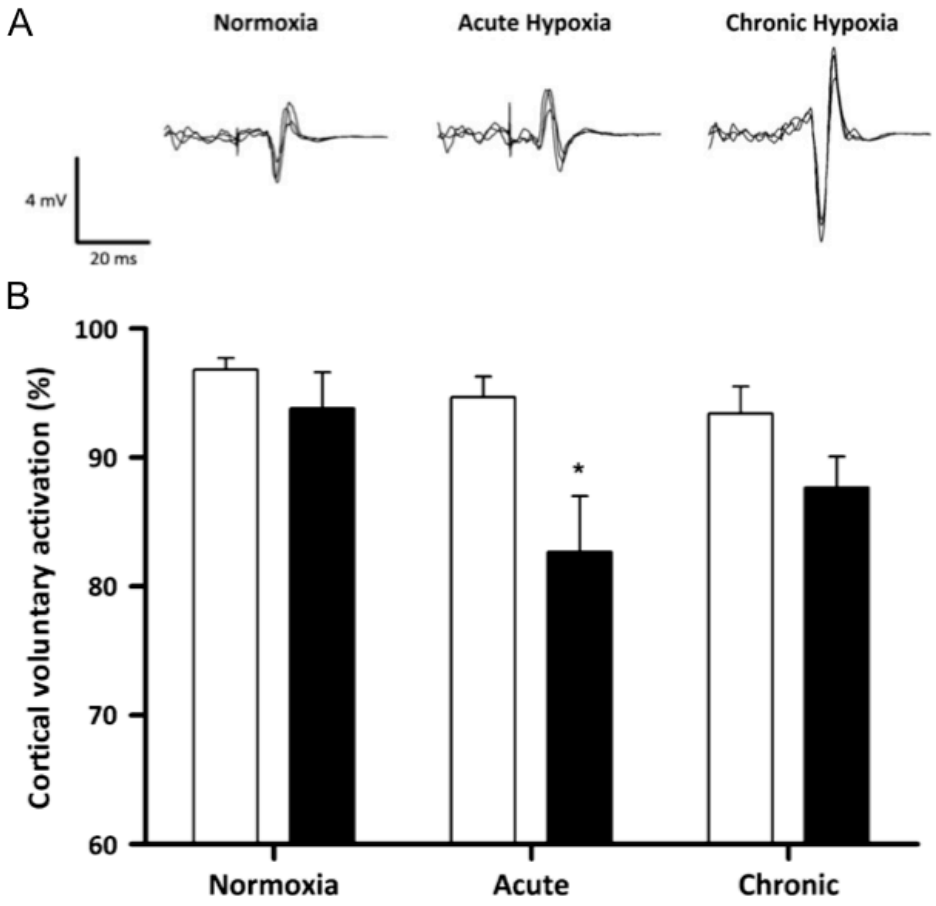

Figure 11. Panel A: Representative motor-evoked potentials evoked during knee extensor contractions at 50% maximal voluntary contraction before exercise in each condition. Note the increase in motor-evoked potential amplitude (corticospinal excitability) after acclimatisation. Panel B: Cortical voluntary activation measured before (open bars) and immediately after (<2.5 min; closed bars) constant-load exercise (131 W) in normoxia, acute hypoxia and chronic hypoxia. * Different between pre- vs. post-exercise ($P<0.05$). From Goodall *et al*., (2014).

reductions in post-exercise cortical voluntary activation and prefrontal tissue oxygenation in acute hypoxia were abolished after 16 days of acclimatising to 5,260 m, while corticospinal excitability was increased twofold compared to pre-exercise values (Figure 11). These findings led the authors to conclude that: i) exhaustive exercise in acute hypoxia is associated with the development of supraspinal fatigue—termed central fatigue—which is attenuated after a period of high altitude acclimatisation, and ii) that the observed reduction in central fatigue with acclimatisation was likely due to enhanced corticospinal excitability associated with increased cerebral DO_2 and tissue oxygenation.

Recently, studies have attempted to investigate the role of cerebral tissue oxygenation on aerobic capacity by selectively elevating CBF (therefore cerebral DO_2) with CO_2 breathing during incremental cycling to exhaustion in severe hypoxia (Subudhi *et al.*, 2011b; Siebenmann *et al.*, 2013). Subudhi *et al*., (2011b) reported impaired exercise capacity at 1,600 m and 4,875 m (hypobaric chamber) where they clamped the subjects' partial pressure of end-tidal CO_2 ($P_{ET}CO_2$) either at: 1) 50 mmHg throughout incremental exercise or 2): 40 mmHg from ~75% maximal power output until exhaustion (Figure 12). They found $\dot{V}_E$ to be greatly elevated (by ~50 L/min) with $P_{ET}CO_2$ clamping during submaximal exercise intensities (37 and 75% of maximal hypoxic power output), compared to room air breathing. Accordingly, they attributed the reduced performance with $P_{ET}CO_2$ clamping to earlier functional limitation by the respiratory system with CO_2 breathing. Siebenmann *et al*., (2013) completed those observations by investigating, at a more moderate altitude (3,454 m), the impact of clamping $P_{ET}CO_2$ at 40 mmHg on aerobic capacity. These studies found clamping $P_{ET}CO_2$ increased MCAv (and presumably cerebral DO_2) and attenuated the decrease in cerebral oxygenation, but slightly decreased maximal power output without affecting maximal O_2 uptake. However, the subjects studied were either residents of moderate altitude (Subudhi *et al.*, 2011b) or low altitude residents following a day spent at high altitude (Siebenmann

et al., 2013). These two conditions are known to enhance cerebrovascular and ventilatory responsiveness to CO_2 (Fan *et al.*, 2010a; Fan *et al.*, 2012a), and thus the acute effect elevating CBF (therefore cerebral DO_2) during exercise in severe hypoxia in an altitude-naïve population remained unclear. Given the importance of aerobic capacity on our ability to thrive in extreme environments, the potential role of reduced cerebral DO_2 and subsequent cerebral tissue deoxygenation in limiting performance in hypoxia warrants further investigation.

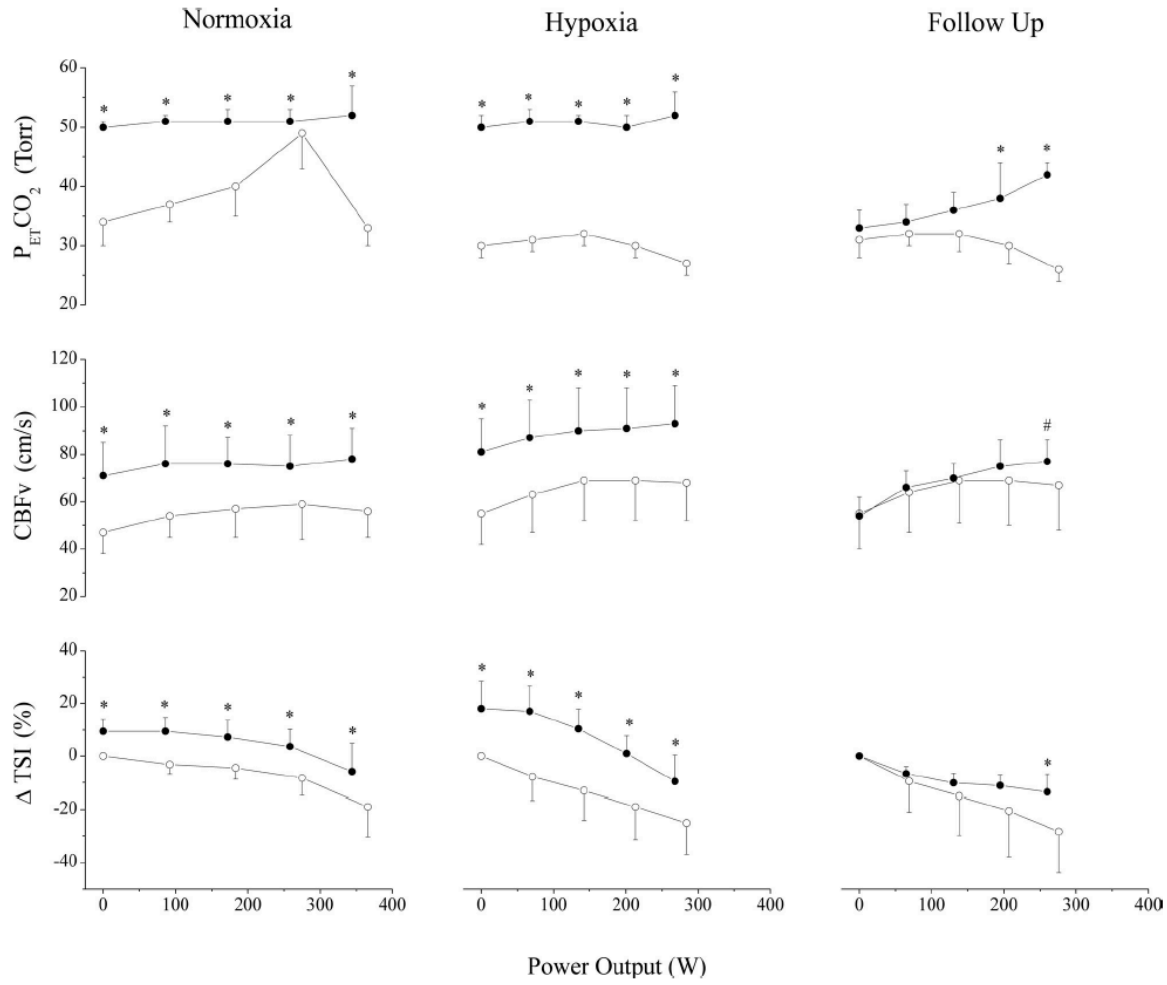

Figure 12. Control (○) and clamp (●) experiments in normoxia, hypoxia, and follow-up. Mean ± SD at 0 W, 25, 50, 75, and 100% of maximal power output. $P_{ET}CO_2$ was clamped at 50 mmHg in normoxia and hypoxia throughout exercise. In follow-up trials, $P_{ET}CO_2$ was clamped at 40 mmHg from 75% to 100% maximal power output. Clamping increased cerebral blood flow velocity (CBFv) and cerebral oxygenation, but decreased maximal power output. ΔTSI, change in tissue saturation index. * Different from control ($P<0.05$); # Non-significant trend ($P<0.10$). From Subudhi *et al*., (2011).

Aim

The primary aim of this thesis work was to quantify the changes in CBF control upon exposure to high altitude and following a period of acclimatisation. The secondary aim of this thesis work was to determine the role of cerebral DO_2 and tissue oxygenation for the limits of endurance performance during exercise in severe hypoxia in an altitude-naïve population.

Hypotheses

Two novel hypotheses were tested:

1) The changes in CBF and CaO_2 at high altitude are matched as to maintain cerebral DO_2 constant during initial exposure and following acclimatisation. This would be due, in part, to changes in cerebrovascular CO_2 reactivity and CA.

2) In altitude-naïve subjects, CO_2 breathing during whole-body exercise in severe hypoxia selectively increases CBF and cerebral DO_2, thereby improving cerebral tissue oxygenation and exercise performance.

Summary of results

Article one

Subudhi AW, **Fan JL**, Evero O, Bourdillon N, Kayser B, Julian CG, Lovering AT & Roach RC. (2014). AltitudeOmics: Effect of ascent and acclimatization to 5,260 m on regional cerebral oxygen delivery. *Experimental Biology* **99,** 772-781.

We assessed changes in blood flow in the internal carotid artery (ICA) and vertebral artery (VA) (as indexes of global and regional CBF), cerebral DO_2, and CaO_2 in 21 lowlanders (9 females), at sea level (SL), during initial exposure to 5,260m (ALT1), after 16 days of acclimatisation (ALT16), and upon re-exposure to altitude following either 7 (POST7) or 21 days (POST21) at low altitude (1,525m). Global CBF increased ~70% upon arrival at ALT1 ($P<0.05$) and returned to SL values at ALT16 as a result of changes in cerebral vascular resistance. A reciprocal pattern in CaO_2 maintained global cerebral DO_2 across acclimatisation. During acute exposure to high altitude at ALT1, DO_2 to the posterior cerebral circulation (via VA) was increased by ~25% at ALT1 ($P<0.05$), while no difference was observed in DO_2 via the ICA ($P>0.05$).

Contribution: Contributed to the conception and design of the experiment, carried out data collection, and assisted in the interpretation of data and manuscript revision.

Article two

Fan JL, Subudhi AW, Evero O, Bourdillon N, Kayser B, Lovering AT & Roach RC. (2014). AltitudeOmics: Enhanced cerebrovascular reactivity and ventilatory response to CO_2 with high altitude acclimatisation and re-exposure. *Journal of Applied Physiology (1985)* **116,** 911-918.

We assessed changes in steady-state responses of middle cerebral artery velocity (MCAv), cerebrovascular conductance index (CVCi), MAP, and $\dot{V}E$ to varied levels of CO_2 in 21 lowlanders (9 females), at sea level (SL), during initial exposure to 5,260m (ALT1), after 16 days of acclimatisation (ALT16), and upon re-exposure to altitude following either 7 (POST7) or 21 days (POST21) at low altitude (1,525m). In addition, we measured resting pH and bicarbonate concentration at these time points. In the non-acclimatised state (ALT1), MCAv and $\dot{V}E$ responses to CO_2 were elevated compared to SL (by 79 ± 75% and 14.8 ± 12.3 L/min, respectively, $P<0.05$). Acclimatisation at ALT16 further elevated both MCAv and $\dot{V}E$ responses to CO_2 compared to ALT1 (by 89 ± 70% and 48.3 ± 32.0 L/min, respectively, $P<0.05$). Likewise, both CVCi-CO_2 and MAP-CO_2 slopes were elevated at ALT1 (by 82 ± 79% and 256 ± 265%, respectively, $P<0.05$), while acclimatisation at ALT16 further elevated MAP-CO_2 slope by 164 ± 1370%, $P<0.05$) but not CVCi-CO_2 slope ($P>0.05$). The acclimatisation gained for $\dot{V}E$ responses to CO_2 at ALT16 was retained by 38% upon re-exposure to altitude at POST7 ($P<0.05$ vs. ALT1), while no retention was observed for the MCAv responses ($P>0.05$). We found good agreement between steady-state and modified rebreathing estimates of MCAv and $\dot{V}E$ responses to CO_2 across all time points ($P<0.05$, pooled data). Furthermore, we found resting bicarbonate concentration correlated with steady-state MCAv-CO_2 slope ($R=-0.771$) and $\dot{V}E$ at 40 mmHg ($R=-0.723$, $P<0.05$ for both).

Contribution: Contributed to the conception and design of the experiment, carried out data collection, and led the analysis, interpretation, writing of the manuscript and the editorial process.

Article three

Subudhi AW, **Fan JL**, Evero O, Bourdillon N, Kayser B, Julian CG, Lovering AT, Ronney B & Roach RC. (2014). AltitudeOmics: Cerebral autoregulation during ascent, acclimatization, and re-exposure to high altitude and its relation with acute mountain sickness. *Journal of Applied Physiology (1985)* **116,** 724-729.

We assessed symptoms of acute mountain sickness (AMS) and dynamic CA during room air breathing in 21 lowlanders (9 females) at sea level (SL), during initial exposure to 5,260m (ALT1), after 16 days of acclimatisation (ALT16), and upon re-exposure to altitude following either 7 (POST7) or 21 days (POST21) at low altitude (1,525m). CA was impaired upon arrival at ALT1 ($P<0.05$) and did not change with acclimatisation at ALT16 or upon re-exposure at POST7. CA was not associated with AMS scores (all $R<0.50$, $P>0.05$).

Contribution: Contributed to the conception and design of the experiment, carried out data collection, and assisted in the interpretation of data and manuscript revision.

Article four

Fan JL & Kayser B. (2014). Repeated pre-syncope from increased inspired CO_2 in a background of severe hypoxia: a case report. *High Altitude Medicine and Biology* **15,** 70-77.

We describe a case of experimentally-induced pre-syncope in a healthy young man when exposed to increased inspired CO_2 in a background of normobaric hypoxia (FIO_2: 0.11). Acute severe hypoxia was tolerated, but adding CO_2 to the inspirate caused pre-syncope symptoms accompanied by hypotension and large reductions in both mean and diastolic middle cerebral artery velocity, while systolic flow velocity was maintained. The mismatch of cerebral perfusion pressure and vascular tone caused unique retrograde CBF at the end of systole and a reduction in cerebral tissue oxygenation.

Contribution: Carried out data collection, and led the analysis, interpretation, writing of the manuscript and the editorial process.

Article five

Fan JL & Kayser B. (2014). Effect of adding CO_2 to hypoxic inspired gas on cerebral blood flow velocity and breathing during incremental exercise. *PLoS One* **8,** e81130.

We assessed the effect of CO_2 breathing and normobaric hypoxia (FIO_2: 0.11) on CBF and ventilatory response to exercise during incremental cycling to exhaustion in 10 healthy male subjects. During exercise in normoxia, augmenting the fraction of inspired CO_2 ($FICO_2$) elevated MCAv throughout exercise and lowered both respiratory compensation threshold (RC) onset and $\dot{V}E$ slope below RC ($P<0.05$). In hypoxia, MCAv and $\dot{V}E$ slope below RC during exercise were elevated, while the onset of RC occurred at lower exercise intensity ($P<0.05$). CO_2 breathing in hypoxia increased $\dot{V}E$ at RC ($P<0.05$) but no difference was observed in RC onset, MCAv, or $\dot{V}E$ slope below RC ($P>0.05$). The $\dot{V}E$ slope above RC was unchanged with either hypoxia or CO_2 breathing ($P>0.05$). We found CO_2 breathing increased CBF during sub-maximal exercise in normoxia, but not in hypoxia, indicating that the 'normal' cerebrovascular response to hypercapnia is blunted during exercise in hypoxia, possibly due to an exhaustion of cerebral vasodilatory reserve.

Contribution: Carried out data collection, and led the analysis, interpretation, writing of the manuscript and the editorial process.

Article six

Fan JL, Bourdillon N & Kayser B. (2014). Effect of end-tidal CO_2 clamping on cerebrovascular function, oxygenation, and performance during 15-km time trial cycling in severe normobaric hypoxia: the role of cerebral O_2 delivery. *Physiological Reports* **1,** 1-15.

We assessed the effect of clamping end-tidal PCO_2 on CBF, cerebral tissue oxygenation, DO_2, and exercise performance during 15 km time trial (TT) cycling in normoxia and normobaric hypoxia (FIO_2: 0.11) in 10 healthy male subjects. During exercise, hypoxia elevated MCAv and lowered cerebral concentration of oxyhaemoglobin ($[O_2Hb]$), cerebral DO_2 and muscle concentration of $[O_2Hb]$ ($P<0.05$). CO_2 clamping elevated $PETCO_2$ and MCAv during exercise in both normoxic and hypoxic conditions ($P<0.05$), but had no effect on either cerebral and muscle concentration of $[O_2Hb]$ ($P>0.05$). Nevertheless, CO_2 clamping elevated cerebral DO_2 during TT in both normoxic and hypoxic conditions ($P<0.05$). CO_2 clamping restored cerebral DO_2 to normoxic values during TT in hypoxia and tended to have a greater effect on TT performance in hypoxia compared to normoxia ($P>0.05$). Despite this, post hoc analysis revealed no effect of CO_2 clamping on TT performance either in normoxia or in hypoxia ($P>0.05$).

Contribution: Carried out data collection, and led the analysis, interpretation, writing of the manuscript and the editorial process.

Article seven

Fan JL, Leiggener C, Rey F & Kayser B. (2012). Effect of inspired CO_2 on the ventilatory response to high intensity exercise. *Respiratory Physiology and Neurobiology* **180,** 283-288.

We assessed the effect of CO_2 breathing on ventilatory response to exercise during incremental cycling to exhaustion in 10 healthy male subjects in normoxia. During high-intensity exercise, CO_2 breathing elevated $P_{ET}CO_2$ during incremental cycling ($P<0.05$), while $P_{ET}O_2$ remained unchanged ($P>0.05$). During high intensity exercise, CO_2 breathing elevated tidal volume at 80, 90 and 100% of maximal O_2 consumption ($P<0.05$), while no differences were observed in $\dot{V}_E$, breathing frequency, CO_2 production, O_2 consumption or power output ($P>0.05$). CO_2 breathing lowered both RC and $\dot{V}_E$ slope below RC during incremental cycling ($P<0.05$). No difference was observed in the $\dot{V}_E$ slope above RC between CO_2 breathing and control ($P>0.05$).

Contribution: Carried out data collection, and led the analysis, interpretation, writing of the manuscript and the editorial process.

Discussion

This thesis set out to test two major hypotheses. First, that the changes in CBF and CaO_2 at high altitude would be sufficient to maintain constant cerebral DO_2 during initial exposure and following acclimatisation, with these changes possibly mediated by changes in cerebrovascular function. Secondly, that the prevention of hypocapnia with CO_2 breathing selectively increases CBF and cerebral DO_2, thereby improving cerebral tissue oxygenation and exercise performance during heavy exercise in severe hypoxia.

In agreement with our first hypothesis, we found cerebral DO_2 to be maintained during both acute and chronic exposure to high altitude. This was primarily mediated by reciprocal changes in global CBF and CaO_2. Our findings also showed enhanced cerebrovascular CO_2 reactivity during acute exposure to high altitude, which was further increased with acclimatisation. In agreement with previous studies, we found CA to be impaired upon ascent to high altitude, which persisted after acclimatisation despite improvements in PaO_2 and CaO_2. Since the changes in cerebrovascular CO_2 reactivity correlated with the changes in arterial blood acid-base balance across all time points, we speculate that the alterations in cerebrovascular function at high altitude might be linked to a common mechanism of the arterial buffering capacity.

In contrast to our second hypothesis, we were unable to improve exercise performance by preventing the normal hyperventilation-induced hypocapnia and associated cerebral deoxygenation during cycling in severe hypoxia. Despite a greater increase in $P_{ET}CO_2$ with our CO_2 breathing setup during hypoxic exercise, we observed only modest elevations in CBF compared to during normoxic exercise. We attributed this to an impaired cerebrovascular CO_2 reactivity during exercise due to an exhausted vasodilatory reserve associated with hypoxia. Nevertheless, we restored cerebral DO_2 to normoxic values during hypoxic exercise with our setup due to an increase in $\dot{V}_E$

(and therefore PaO_2 associated with hypercapnia) coupled with a modest increase in CBF. Since these effects did not lead to improved cerebral tissue oxygenation or exercise performance, our findings do not support the role of hyperventilation-induced hypocapnia and associated cerebral deoxygenation in limiting performance during whole-body exercise in severe hypoxia.

Regulation of cerebral blood flow during high altitude exposure

The effect of high altitude exposure on resting CBF has been documented using the Kety-Schmidt method (Severinghaus *et al.*, 1966; Milledge & Sorensen, 1972; Jensen *et al.*, 1990; Møller *et al.*, 2002), vascular Doppler ultrasound (Huang *et al.*, 1987; Willie *et al.*, 2014a), and transcranial Doppler ultrasound (Baumgartner *et al.*, 1994; Thomas *et al.*, 2008). In his pioneering work, Severinghaus *et al*., (1966) found CBF to be increased by 24% after 6-12 h at 3,810 m, which later declined to 13% above sea-level values following 3-5 days of exposure. Subsequent studies have added detail to this initial observation, by showing that the increase in CBF peaks after ~1-2 days at high altitude, before returning to sea-level values following 1-3 weeks of acclimatisation (Milledge & Sorensen, 1972; Huang *et al.*, 1987; Jensen *et al.*, 1990; Baumgartner *et al.*, 1994; Møller *et al.*, 2002; Willie *et al.*, 2014a). Using vascular Doppler ultrasound, we measured ICA and VA blood flow during acute and chronic high altitude exposures, from which we were able to calculate global CBF (Subudhi *et al.*, 2014b). Our data extends those previous studies by demonstrating that the decline in CBF with acclimatisation to high altitude is offset by a reciprocal elevation in CaO_2, thus maintaining global cerebral DO_2 (Figure 13). Accordingly, our findings demonstrate that the intrinsic mechanisms, which regulate CBF reciprocally to the changes in CaO_2, are well suited to maintain global cerebral DO_2 across acclimatisation at 5,260 m. This reciprocal relationship, whether evolved or serendipitous, is advantageous for survival in these extreme conditions as it mitigates negative consequences of cerebral hypoxaemia.

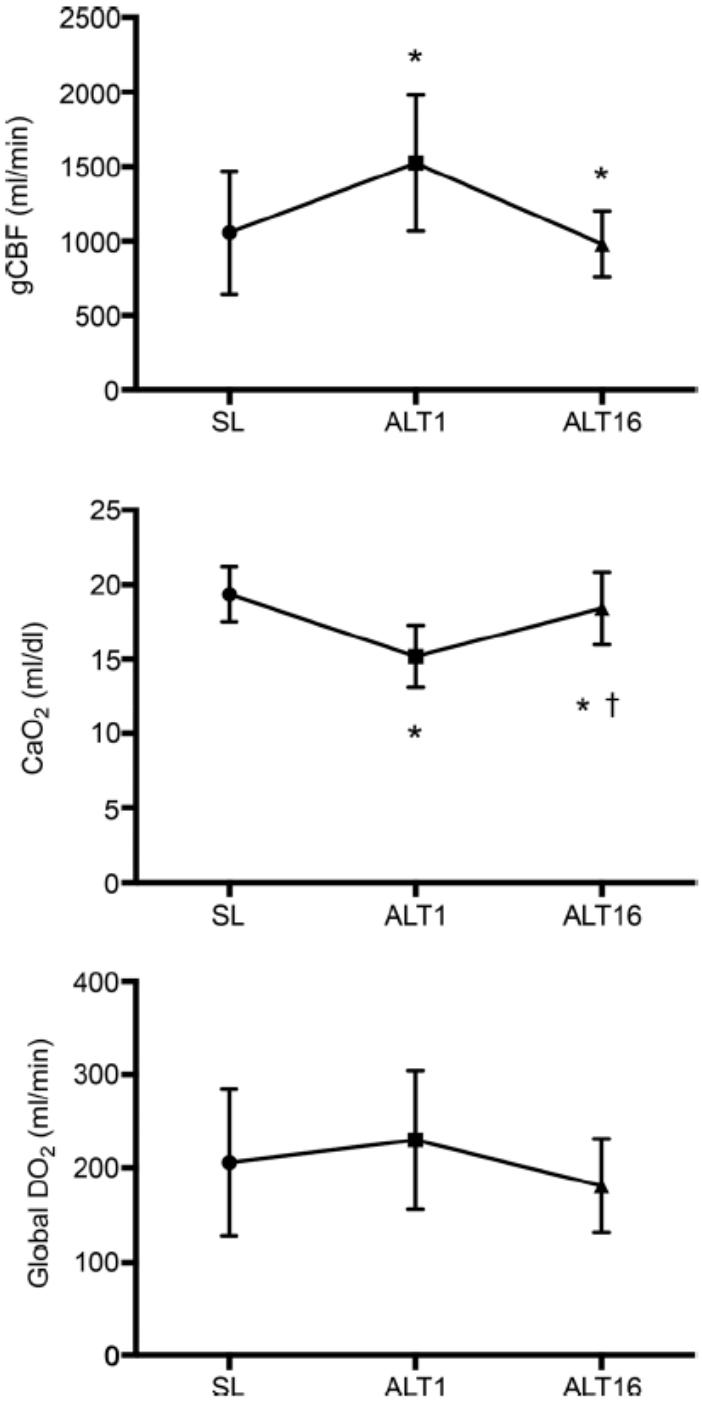

Figure 13. Reciprocal changes in global cerebral blood flow (gCBF) and CaO_2 maintained global cerebral DO_2 throughout the study. Values expressed as mean ± SD. * Different from SL ($P<0.05$); † Different from ALT1 ($P<0.05$). From Subudhi *et al*., (2014b).

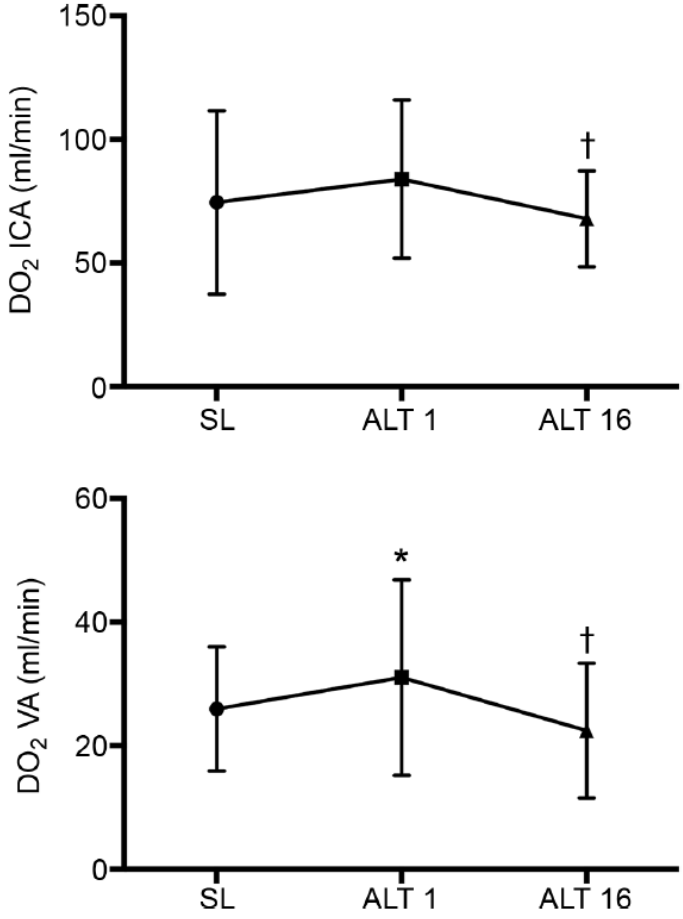

Figure 14. Regional DO_2 delivery in the vertebral but not in the internal carotid artery upon arrival to high altitude (ATL1). Values expressed as mean ± SD. Regional DO_2 in reduced with acclimatisation, but not below SL values. * Different from SL ($P<0.05$); † Different from ALT1 ($P<0.05$). From Subudhi *et al*., (2014b).

We found regional differences in CBF responses to acute hypoxia (therefore cerebral DO_2), with higher VA blood flow upon ascent to 5,260 m (Figure 14). As a result, brain DO_2 via the VA was elevated upon ascent to high altitude, while ICA DO_2 was unchanged (Figure 14). This observation supports findings by Ogoh *et al*., (2013), which showed a 10% increase in VA blood flow during acute hypoxic exposure (FIO_2: 0.12, PIO_2: 86 mmHg), while ICA blood flow was slightly reduced due to the constricting effect of relative hypocapnia associated with hypoxia. Similarly, Willie *et al*., (2012) found VA diameter increased by 8% during severe hypoxic exposure (PaO_2: 35 mmHg), while hypocapnia *per se* ($PETCO_2$: 15, 20, and 30 mmHg) had no effect. They also found ICA diameter to be highly sensitive to changes in $PaCO_2$, but not to hypoxia. In agreement, Sato *et al*., (2012) found the VA blood flow response to hypocapnia to be relatively blunted compared to the ICA blood flow response. Together with our observations, these findings are suggestive of a high sensitivity of the vertebral vascular bed (and thus VA blood flow) to hypoxia but not to hypocapnia. During exposure to hypoxia and associated hypocapnia, such selectivity of VA blood flow responses to these blood gas changes may serve to maintain sufficient O_2 supply to posterior brain regions, given these posterior areas are involved in vital cerebral functions associated with homeostasis.

Cerebrovascular CO_2 reactivity at high altitude

During acute and chronic high altitude exposure, the control of CBF is the net result of hypoxia-induced vasodilation, offsetting the vasoconstriction associated with hyperventilation-induced hypocapnia (Brugniaux *et al.*, 2007). With acclimatisation to high altitude, the progressive increase in ventilatory drive lowers $PaCO_2$ and elevates PaO_2. These arterial blood gas changes alleviate hypoxia and exacerbate hyperventilation-induced hypocapnia, thus shifting the balance of the cerebrovascular tone in favour of vasoconstriction. It is this hyperventilation-induced hypocapnia that is responsible for restoring CBF to sea-level values following initial exposure to high altitude.

Accordingly, the cerebrovascular CO_2 reactivity plays a pivotal role in regulating CBF at high altitude. Fan *et al*., (2010a; 2012a) found the cerebrovascular CO_2 reactivity to be elevated after 1-3 days and ~7 days at 5,050 m in subjects whom spent 8 days trekking to this altitude (and thus were partially acclimatised). Our data extends those observations by showing cerebrovascular CO_2 reactivity was increased during initial exposure to high altitude at ALT1 (<8 hours), in a larger cohort rapidly ascending from 1,600 m to 5,260 m (~3 hours) (Figure 15). We found cerebrovascular CO_2 reactivity to be further elevated following 16 days of acclimatisation to high altitude. Our findings, together with the reports by Fan *et al*., (2010a; 2012a), indicate that irrespective of the rate of ascent, cerebrovascular CO_2 reactivity is elevated upon initial exposure to high altitude, and further elevated with acclimatisation.

An important consideration when examining the changes in cerebrovascular CO_2 reactivity is the method of assessment. Two methods are commonly used for assessing ventilatory CO_2 sensitivity and cerebrovascular CO_2 reactivity; the modified Read's rebreathing and the steady-state method. The original Read's method was first described by Read (1967) and was subsequently modified by Duffin & McAvoy (1988), which consisted of a period of voluntary hyperventilation followed by rebreathing from a 6-L bag filled with 6-7% CO_2 in O_2. Meanwhile, in the steady-state method F_ICO_2 is manipulated to increase P_ETCO_2 in a stepwise manner to a new level, and held constant at this new higher P_ETCO_2 until $\dot{V}_E$ reaches a new steady state. At sea level, the steady-state method results in higher estimates of cerebrovascular CO_2 reactivity (Pandit *et al.*, 2001, 2003, 2007) and lower estimates of ventilatory CO_2 sensitivity (Tenney *et al.*, 1963; Berkenbosch *et al.*, 1989; Jacobi *et al.*, 1989; Fan *et al.*, 2010b) compared to the modified rebreathing test. This discrepancy between the two methods has been attributed to the presence of a PCO_2 gradient (between alveolar, arterial, and cerebrospinal fluid compartments) during the steady-state method, which is supposedly abolished or minimised during rebreathing (Berkenbosch *et al.*, 1989). Meanwhile, elevated basal

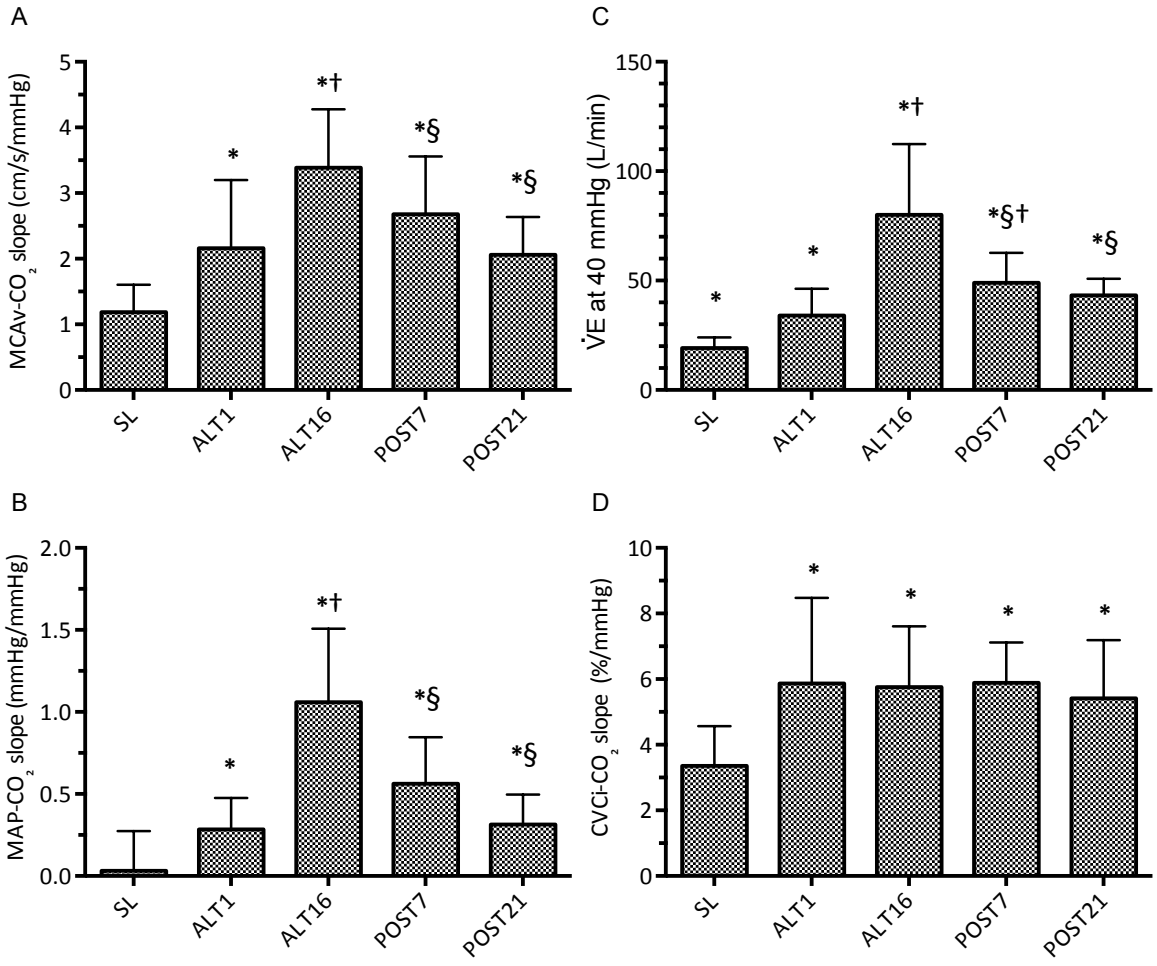

Figure 15. Changes in steady-state estimates of cerebrovascular, cardiovascular, and ventilatory responsiveness to CO_2 with acclimatisation and reexposure to 5,260 m. Values expressed as mean ± SD. * Different from sea-level (SL; $P<0.05$); † Different from arrival at altitude (ALT1; $P>0.05$); § Different from acclimatisation (ALT16; $P<0.05$). From Fan *et al*., (2014).

$\dot{V}_E$ and subsequent underestimation of the ventilatory CO_2 sensitivity has been proposed as another possible explanation for lower steady-state estimates (Mohan *et al.*, 1999). In our work, we found close agreement between steady-state and rebreathing estimates of CBF and ventilatory response to CO_2 at sea level and high altitude (Figure 16). Specifically, the trend of changes in steady-state and rebreathing estimates of cerebrovascular CO_2 reactivity and ventilatory CO_2 sensitivity were identical upon ascent and following acclimatisation to 5,260 m. These findings support the use of both the steady-state method and the modified rebreathing method for the assessment of cerebrovascular and ventilatory changes with high altitude ascent.

In contrast to our results, Lucas *et al*., (2011) found steady-state cerebrovascular CO_2 reactivity to be increased initially, and lowered subsequently with acclimatisation. However, since they did not control $P_{ET}O_2$, the cerebrovascular CO_2 reactivities reported by Lucas *et al*., (2011) at 5,050 m reflected MCAv changes from *hypoxic hypocapnia* (room air breathing: $P_{ET}O_2$ ~44-48 mmHg, and $P_{ET}CO_2$: ~26-22 mmHg) to *hypercapnic hyperoxia* ($P_{ET}O_2$ >310 mmHg, and $P_{ET}CO_2$ ~30 mmHg), and thus do not represent isolated reactivities to CO_2. Accordingly, the discrepancies between our finding and those by Lucas *et al*., (2011) can be accounted for by the better control of changes in PaO_2 and $PaCO_2$ in our study. Indeed, we assessed the cerebrovascular CO_2 reactivity over similar arterial blood gas ranges (i.e., $PaCO_2$: ~20-40 mmHg, $P_{ET}O_2$: >200 mmHg) across all time points, thus making comparisons possible. More recently, Rupp *et al*., (2014) and Villien *et al*., (2013) found, using the same cohort, reduced cerebrovascular CO_2 reactivity during steady-state *hypoxic hypercapnia* following 5 days at 4,350 m. Since hypoxia has been shown to exacerbate the CBF response to CO_2 (Battisti-Charbonney *et al.*, 2011), the cerebrovascular CO_2 reactivities reported by Rupp *et al*., (2014) and Villien *et al*., (2013) were likely overestimating the 'true' sea-level values due to the background hypoxia used. Furthermore, the level of background hypoxia used by these

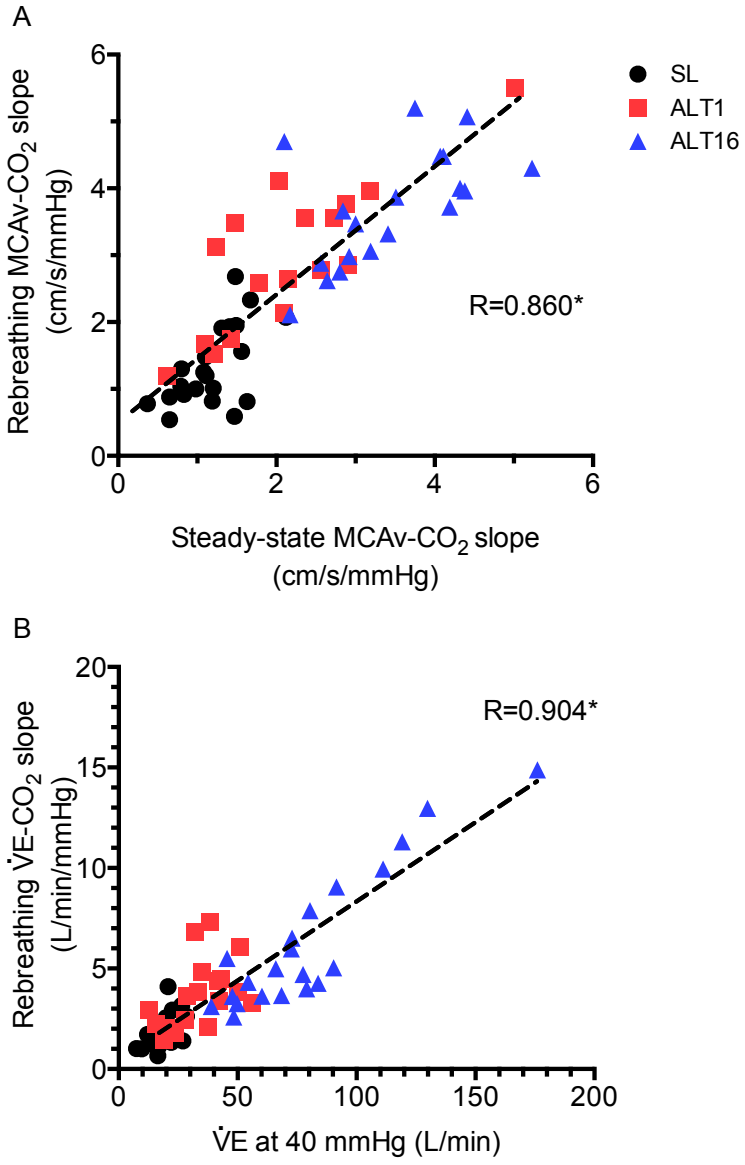

Figure 16. Comparison of steady-state and rebreathing estimates of cerebrovascular and ventilatory responsiveness to CO_2 with acclimatisation to 5,260 m. * Significant correlations ($P<0.05$). From Fan *et al*., (2014)

studies ($P_{ET}O_2$ ~55 mmHg) was actually slightly *hyperoxic* compared to room-air breathing at 4,350 m (reported resting $P_{ET}O_2$: ~47-50 mmHg), potentially leading to an underestimation of the cerebrovascular CO_2 reactivity values at high altitude. These findings highlight the importance of selecting appropriate ranges of end-tidal (and therefore arterial) gases when assessing cerebrovascular reactivity. Particular care should be taken when choosing the background O_2 level to ensure identical relative and absolute stimuli between time points, thus avoiding any potential confounding effect of hypoxia/hyperoxia on cerebrovascular CO_2 reactivity between testing sessions.

In contrast to cerebrovascular CO_2 reactivity, the CVCi-CO_2 reactivity was unaltered with acclimatisation at ALT16 (Figure 15). From this perspective, it seems that the major part of the increase in cerebrovascular CO_2 reactivity following acclimatisation to high altitude may be due to an enhanced blood pressure response to CO_2. In support, we found the MAP response to CO_2 to be enhanced upon acute exposure to 5,260 m and further elevated with acclimatisation (Figure 15). Under normal conditions, an increase in MAP does not lead to concurrent increases in CBF. This is due in part to the presence of CA, which serves to maintain CBF constant within a wide range of perfusion pressures. However, an impaired CA associated with high altitude exposure, coupled with enhanced MAP-CO_2 response, could account for the enhanced cerebrovascular CO_2 reactivity with acclimatisation, as observed in our study (see section '*Perfusion pressure and cerebrovascular CO_2 reactivity*' below).

Cerebral autoregulation at high altitude

It has long been postulated that an impaired CA may be involved in the pathogenesis of AMS, high altitude headache and cerebral oedema (Lassen & Harper, 1975; Hackett & Roach, 2001; Van Osta *et al.*, 2005; Bailey *et al.*, 2009; Cochand *et al.*, 2011). Previous work by Subudhi *et al.*, (2010;

2011a) and Ainslie *et al*., (2008) found CA impairment preceded the development of AMS during hypobaric hypoxia. However, these studies did not find a link between the drop in CA and the severity of AMS. In our study, rapid ascent to high altitude increased transfer function coherence and gain at ALT1, while phase shift and autoregulation scores were reduced (Figure 17), consistent with the classic definition of impaired CA. This impaired CA, already present after less than one day of travel from low to high elevation, confirms findings by Subudhi *et al*., (2010) of reduced CA parameters after 4 h in a hypobaric chamber. Our work fills an important gap in the literature between studies conducted in laboratories with hypoxic gas mixtures [where normobaric hypoxia was achieved in a matter of minutes (Iwasaki *et al.*, 2007; Bailey *et al.*, 2009; Subudhi *et al.*, 2009b; Querido *et al.*, 2013)], and studies of trekkers where several days of progressive ascent preceded initial high-altitude measurements (Jansen *et al.*, 2000; Van Osta *et al.*, 2005; Ainslie *et al.*, 2008; Ainslie *et al.*, 2012). With the exception of the work by Querido *et al*., (2013), an impaired CA at rest in acute hypoxia is a consistent finding in the literature, suggesting that neither the mode nor rate of ascent affect the general effect of hypoxia on CA.

We found no improvement in CA with acclimatisation to high altitude despite increased PaO_2 (Figure 17), which by itself is known to improve CA (Ainslie *et al.*, 2008; Subudhi *et al.*, 2010). Our longitudinal findings are consistent with numerous cross-sectional studies which have found impaired CA at various time points after arrival to high altitude (Jansen *et al.*, 2000; Van Osta *et al.*, 2005; Ainslie *et al.*, 2008; Cochand *et al.*, 2011; Ainslie *et al.*, 2012), as well as in permanent high-altitude residents (Jansen *et al.*, 2000; Jansen *et al.*, 2007). We found no correlations between the measures of CA and AMS symptom scores at ALT1 (Figure 18). This observation confirms work by Subudhi *et al*., (2010), which showed similar CA impairment in those subjects who developed AMS and those who did not, following 10 h of hypobaric hypoxic exposure. Accordingly, we conclude that the development of ‘impaired’ CA is a normal adaptive physiological response

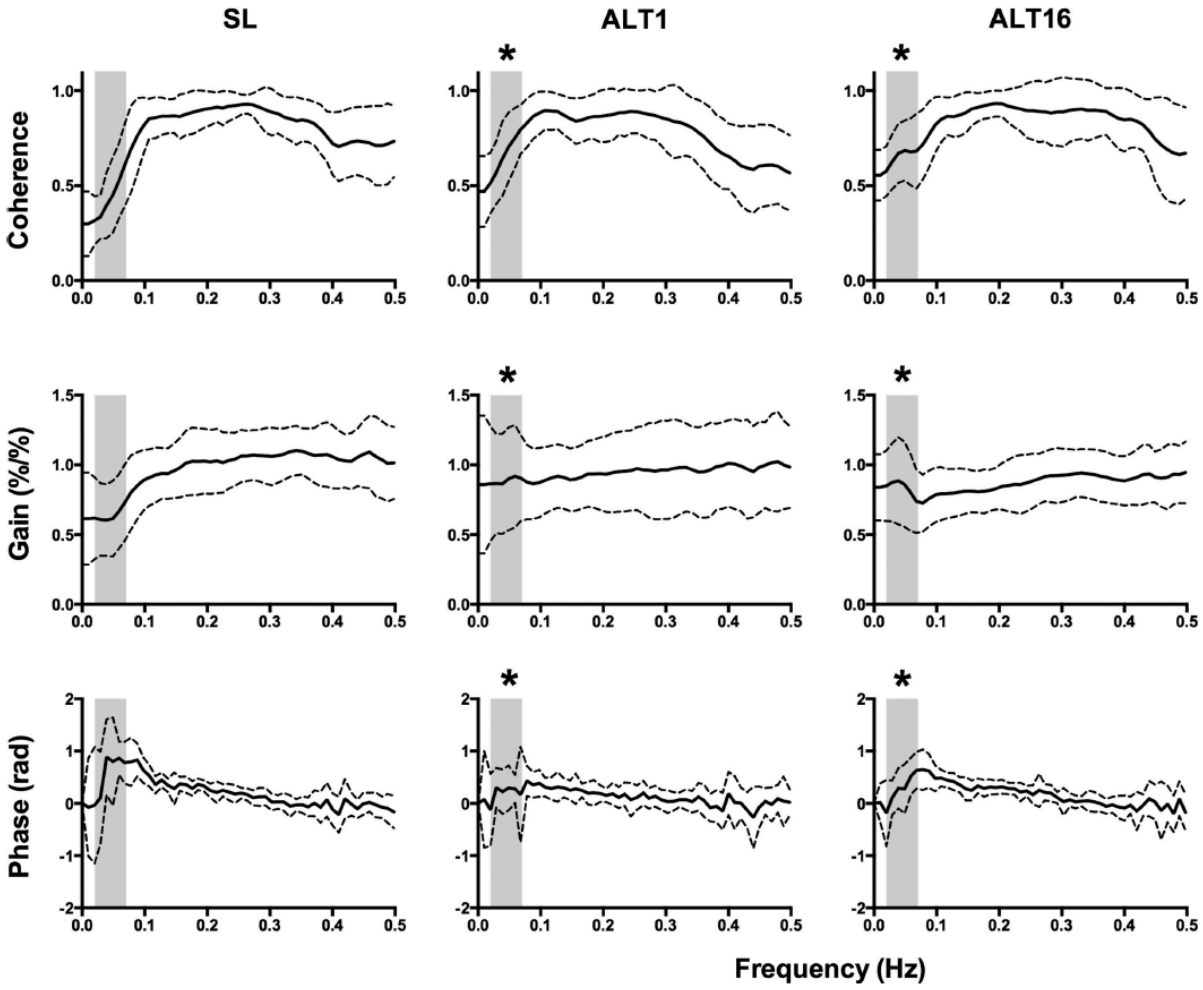

Figure 17. Arterial blood pressure to cerebral blood flow velocity transfer function metrics (mean ± SD from 0 to 0.5 Hz) at sea level (SL), upon arrival at 5,260 m (ALT1), after 16 days of acclimatisation (ALT16). Similar impairments in cerebral autoregulation (increased coherence and gain and decreased phase shift) from SL were seen in the very low frequency (0.02-0.07 Hz; shaded areas) at ALT1 and ALT16 ($P<0.05$). rad, radians. * Different from SL. From Subudhi *et al*., (2014a).

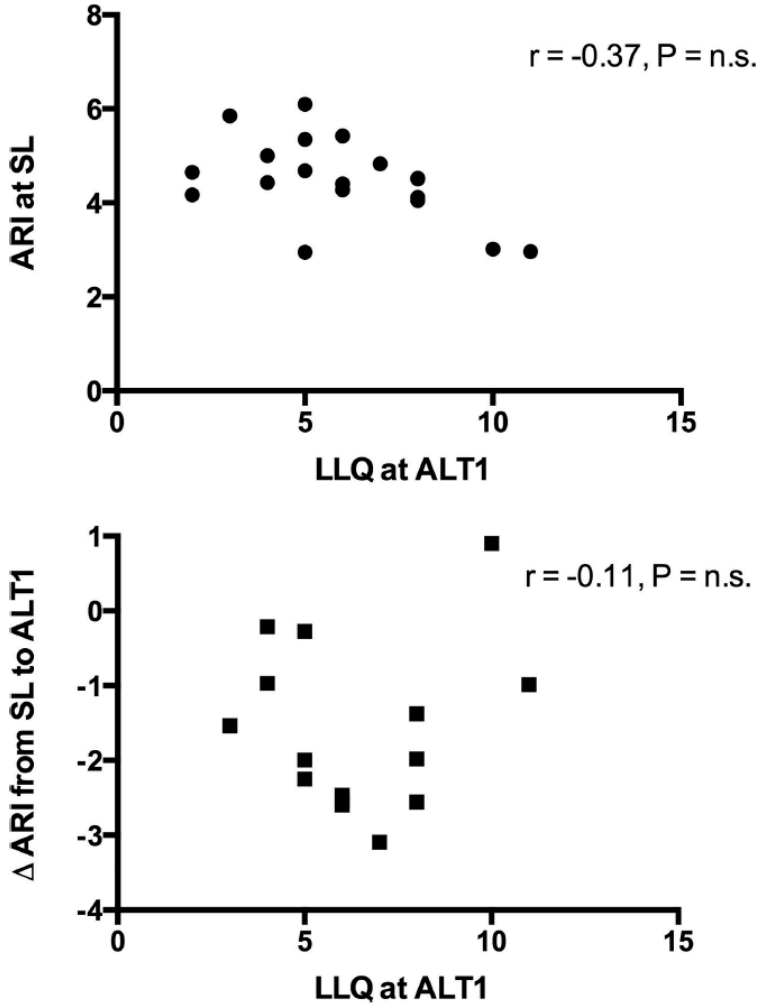

Figure 18 Scatter plots showing no relation (P>0.05) between autoregulation indices (ARI), measured at SL (top) and as the change (Δ) from SL to arrival at high altitude (ALT1; bottom), and acute mountain sickness symptoms' scores from the Lake Louise Questionnaire (LLQ) at ALT1. From Subudhi *et al*., (2014a).

to high altitude exposure, in the absence of pathology. However, since our findings are at odds with observations by other studies, which found some association between CA and AMS symptoms (Van Osta *et al.*, 2005; Bailey *et al.*, 2009), we cannot completely exclude the possibility that alterations in CA might partly contribute to the pathogenesis of AMS during acute exposure to high altitude.

Perfusion pressure and cerebrovascular CO_2 reactivity

In a case study, we reported dramatic reductions in MAP during CO_2 breathing in background hypoxia in a healthy young male subject (Fan & Kayser, 2014). This severe and acute hypotension associated with hypercapnia in background hypoxia led to a dramatic reduction in CBF, severe prefrontal deoxygenation, and subsequent development of pre-syncope symptoms (Figures 19 & 20). To the best of our knowledge, this is the first report documenting reduced CBF during a pre-syncope response triggered by CO_2 enriched inspired gas in a background of hypoxia. The observation during the second episode of a drop in frontal cortex oxygenation suggests that the pre-syncope syndrome was accompanied by reduced cerebral oxygen availability (Figure 20). We found dramatic reductions in mean and diastolic MCAv during and immediately following CO_2 breathing in hypoxia (Figure 19). Meanwhile, the reduction in cerebral [O_2Hb] followed closely the changes in diastolic MCAv (Figure 20), suggesting that diastolic perfusion pressure was the main determinant of cerebral DO_2. We attribute the observed reduction in CBF and subsequent cerebral tissue desaturation to the direct effect of severe hypotension *per se*.

Our findings are in agreement with an early study by Harper and Glass (1965), who demonstrated that reducing MAP to 100 mmHg blunts the cerebrovascular CO_2 reactivity ($PaCO_2$ = 80 mmHg) by 50% in anaesthetised dogs, while severe hypotension (MAP = 50 mmHg) completely abolished the CBF response to hypercapnia. Similarly, Ainslie *et al.*, (2012) found cerebrovascular CO_2

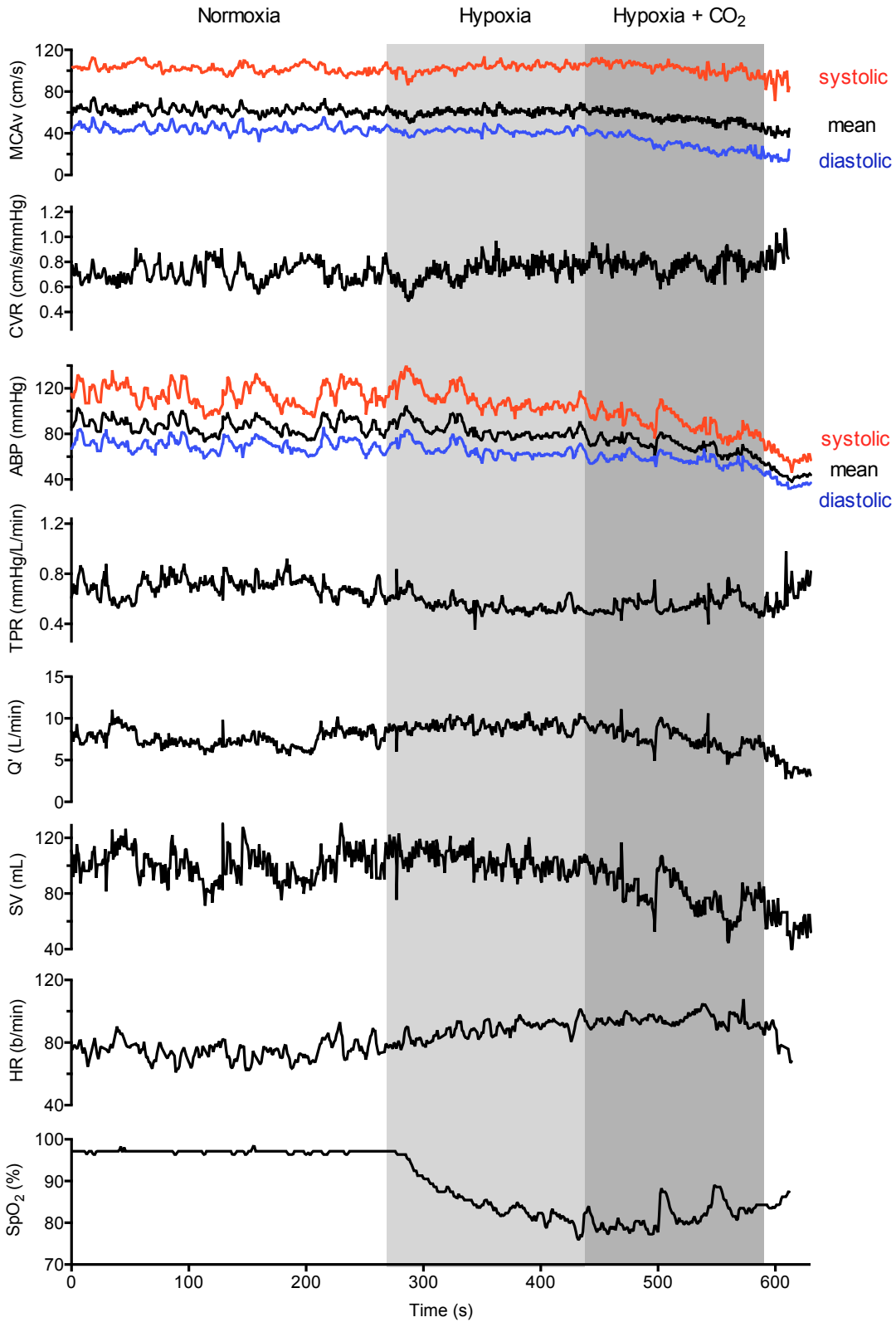

Figure 19. Cerebrovascular and cardiovascular variables during visit one. Cerebral vascular resistance (CVR), arterial blood pressure (ABP), total peripheral resistance (TPR), cardiac output (Q'), and stroke volume (SV). During exposure to hypoxia + CO_2, despite increases in HR, mean, systolic and diastolic ABP progressively decrease due to decreases in Q' and SV. From Fan & Kayser (2014).

reactivity to be reduced due to a blunted MAP-CO_2 response following α and β sympathetic blockade at sea level and following ascent to high altitude. Collectively, it appears that blood pressure plays an important role in the CBF response to CO_2.

What are potential mechanisms for such an effect of CO_2 breathing in a background of hypoxia? In normoxic conditions, hypercapnia has been shown to elicit a vasodilatory effect on the peripheral vasculature in humans (Lennox & Gibbs, 1932; Kontos, 1971; Kontos *et al.*, 1972; Gastaldo *et al.*, 1974; Ainslie *et al.*, 2005). Hypercapnia (10% CO_2) causes an immediate, but transient hypotension, which is corrected within 30-40 s from increased sympathetic activity, in anesthetised, sino-aortic denervated and vagotomised rats (Takakura *et al.*, 2011). Since inhibition of the retro-trapezoid nucleus lowers sympathetic nerve activity and attenuated mean MAP recovery at the end of the hypercapnic exposure (Takakura *et al.*, 2011), those authors concluded that the compensatory increase in sympathetic nerve activity is partly dependent on retro-trapezoid nucleus activation during hypercapnic exposure. Similarly, pontine noradrenergic neurons, astrocytes, neurons of the nucleus solitary tract and wake-ON orexinergic neurons, have all been shown to be hypercapnia/pH-sensitive and linked to sympathetic tone, contributing to peripheral vascular tone during hypercapnic exposure (Dean *et al.*, 1989; Dun *et al.*, 2000; Johnson *et al.*, 2008; Allen & Barres, 2009). In humans, profound sympathoinhibition has been reported during exposure to combined hypoxia and hypercapnia, resulting in vasovagal syncope (Halliwill, 2003). Our finding of a reduced ratio between low-to-high frequencies of heart rate variability (index of sympathetic activity) is in agreement with that finding (Fan & Kayser, 2014). Furthermore, since our subject's MAP was well maintained during hypercapnic exposure in normoxia, we speculate that, the output of the brain regions responsible for the compensatory sympathetic response to hypercapnia might be attenuated during exposure to hypoxia.

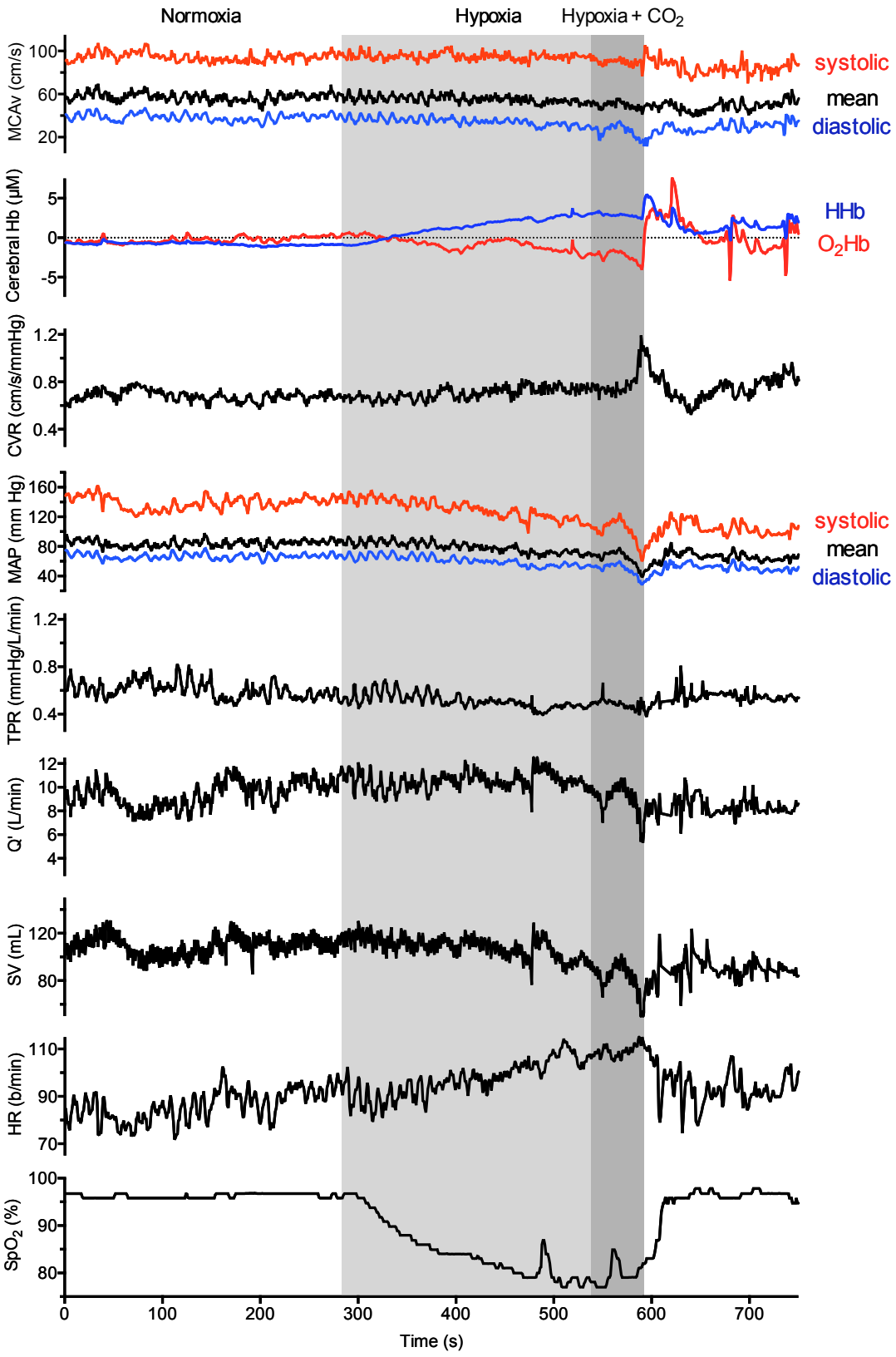

Figure 20. Cerebrovascular and cardiovascular variables during visit two. Cerebral vascular resistance (CVR), arterial blood pressure (ABP), total peripheral resistance (TPR), cardiac output (Q'), and stroke volume (SV). From Fan & Kayser (2014).

In a recent review, Willie *et al*., (2014b) speculated that CA and cerebrovascular CO_2 reactivity might be linked, utilising the same vascular reserve. Along the same line, Carrera *et al*., (2009) reported an inverse relationship between the reduction in CA parameters with CO_2 breathing (5% CO_2) and cerebrovascular CO_2 reactivity. In our altitude studies, we found the increase in cerebrovascular CO_2 reactivity was primarily driven by enhanced MAP response to CO_2 with acclimatisation (Fan *et al.*, 2014), while CA was lowered during both acute and chronic exposures to high altitude (Subudhi *et al.*, 2014a). Since the CBF response to CO_2 appears to be closely linked to perfusion pressure (Harper & Glass, 1965; Ainslie *et al.*, 2012), it seems reasonable to postulate that the enhanced cerebrovascular CO_2 reactivity observed at high altitude might be due to the combined effect of an enhanced MAP response to CO_2 and a reduced CA, with the latter increasing the compliance of CBF to arterial blood pressure. Together, these findings give support to the role of MAP on CBF responses to changes in $PaCO_2$, thus highlighting the dependence of cerebrovascular CO_2 reactivity on perfusion pressure.

CO_2 and cerebral blood flow regulation during exercise

CO_2 is a potent vasodilator of the cerebral vessels causing global CBF to increase by 2-4% for a given mmHg rise in $PaCO_2$ (Fortune *et al.*, 1992; Sato *et al.*, 2012; Willie *et al.*, 2012), while it elicits relatively small changes in limb blood flow of the peripheral vasculature (Lennox & Gibbs, 1932; Ainslie *et al.*, 2005). Hypercapnia is a useful tool to investigate the role of cerebral DO_2 and tissue oxygenation on exercise performance as it allows us to selectively increase CBF during exercise in severe hypoxia. In our studies, we examined the effect of preventing hyperventilation-induced hypocapnia (by adding CO_2 to inspirate) on CBF, prefrontal oxygenation, cerebral DO_2, aerobic capacity, and exercise performance during incremental-intensity (Fan & Kayser, 2013) and TT cycling (Fan *et al.*, 2013) in normoxia and severe hypoxia (FIO_2 = 0.11, equivalent of 5,000 m

elevation). Since CO_2 increases $\dot{V}E$ during both rest and exercise, we also examined the effect of CO_2 breathing on exercise hyperpnoea during incremental cycling in normoxia (Fan *et al.*, 2012b).

During incremental cycling in normoxia, we found MCAv followed the decline in $PETCO_2$ at moderate-to-high intensity of exercise (>60% of maximal O_2 consumption), which we were able to prevent with CO_2 breathing (Figure 21). In contrast, we found MCAv to be greatly elevated during incremental cycling in hypoxia compare to normoxic conditions, despite a lower $PETCO_2$ throughout exercise (Figure 21). We observed a 'plateau' of CBF at the higher, near-maximal, exercise intensities, suggestive of a possible presence of some hypocapnia-induced vasoconstriction. Surprisingly, we were unable to elevate MCAv with CO_2 breathing during incremental exercise in severe hypoxia. During TT cycling, we were able to further elevate CBF by clamping $PETCO_2$ with CO_2 breathing in normoxia (Figure 22). However, similar to our findings during incremental cycling, we found CBF to be elevated in hypoxia compared to normoxic control, while clamping $PETCO_2$ elicited only small increases in MCAv during TT cycling in hypoxia. Our data demonstrates that during both incremental and TT cycling exercise, hypoxia *per se* greatly elevates CBF, while adding CO_2 to the inspirate to prevent the normal drop in $PETCO_2$ elevates CBF in normoxia, but by a lesser extent in severe hypoxia.

While controversy remains, there is a large body of literature suggesting that nitric oxide, prostanoids and C-natriuretic peptide are involved in the cerebral vasodilatory responses to both hypercapnia and hypoxia through a number of intermediate interacting/co-activating pathways (Ainslie & Ogoh, 2009). We speculate that if one stimulus, such as hypoxia, is of sufficient magnitude to exhaust the cerebral vessels' capacity to dilate, thereby nearing maximal diameter of the vessels, then any further stimuli would have little additional effect on decreasing cerebrovascular resistance (i.e., dilating vessel diameter). In such instances, changes in CBF would

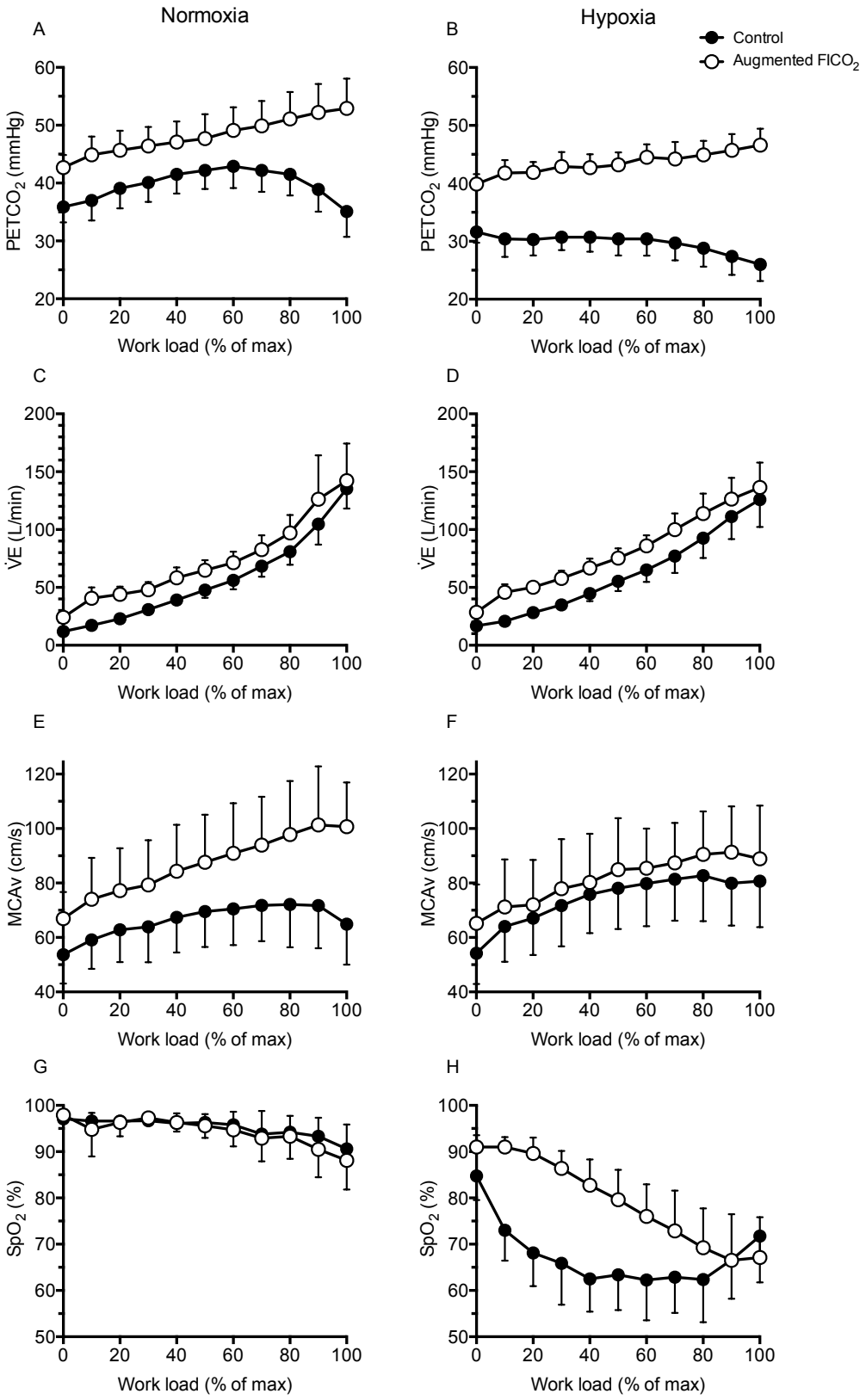

Figure 21. Effect of hypoxia and augmented $FICO_2$ on group respiratory, cerebrovascular variables and SpO_2 during incremental cycling to exhaustion. Left panels: group data in normoxia (mean ± SD); right panels group data in hypoxia. From Fan & Kayser (2013).

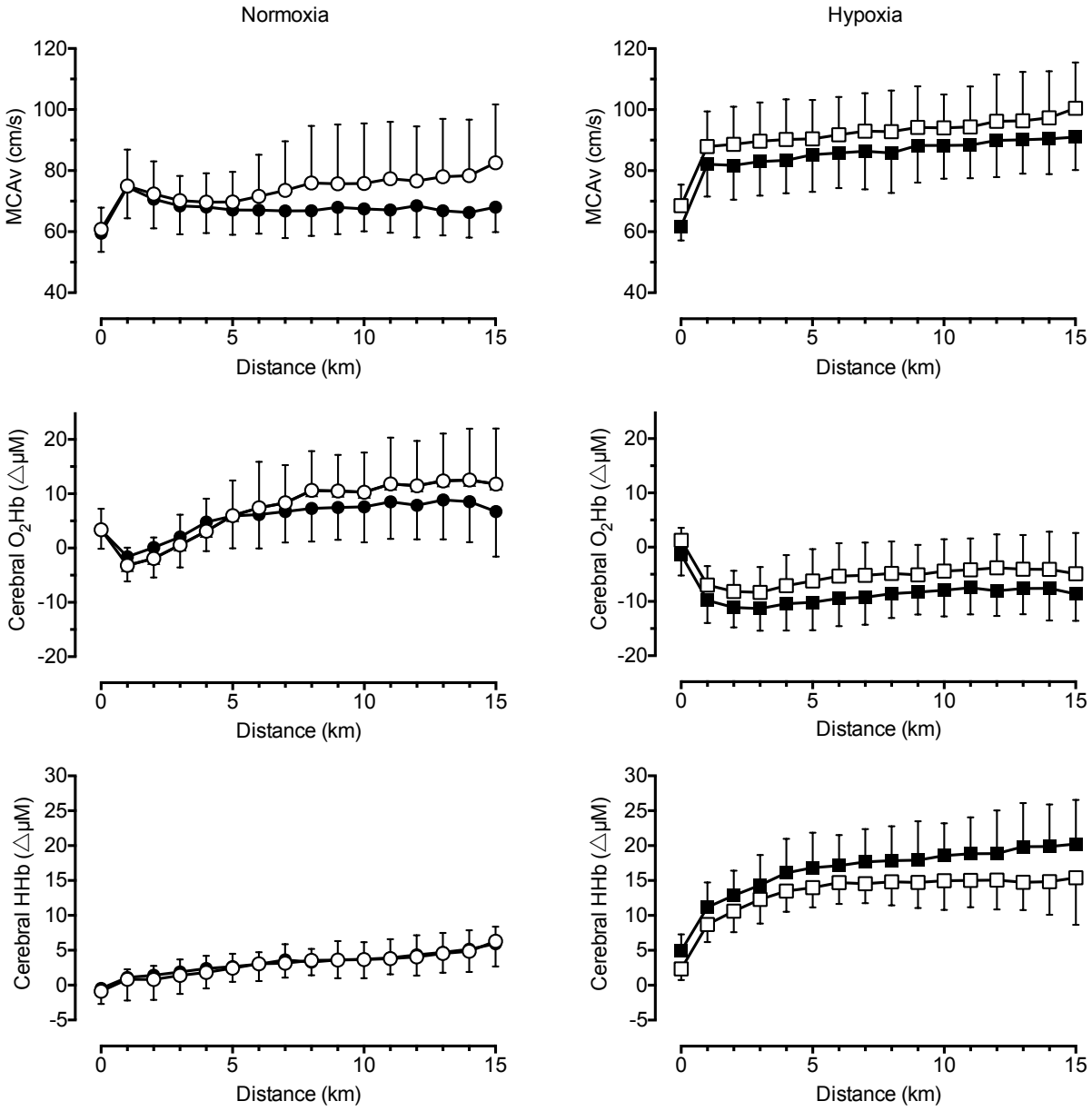

Figure 22. Effect of hypoxia and CO_2 clamping on cerebral variables during 15-km time trial cycling. Cerebral O_2Hb and HHb are expressed as delta changes from normoxia (room air breathing). Left panels, group data in normoxia (mean ± SD); right panels group data in hypoxia. ●, normoxia control; ○ normoxia CO_2 clamp; ■, hypoxia control; □, hypoxia CO_2 clamp. From Fan *et al*., (2013).

follow changes in perfusion pressure rather than arterial blood gases, as alluded to by Battisti-Charbonney *et al*., (2011). Mardimae *et al*., (2012) demonstrated a synergistic role of CO_2 and hypoxia on the control of CBF at rest whereas our results favour a negative effect during exercise, whereby the presence of severe hypoxia attenuates the effect of CO_2 on cerebrovascular resistance—presumably by exhausting the vascular dilatory reserves. In agreement, our data indicates that during both incremental and TT cycling in severe hypoxia, the role of CO_2 in the regulation of cerebrovascular tone appears to be diminished, at least in an unacclimatised population. However, we cannot exclude the possibility that there might be regional differences in the cerebrovascular response to hypoxia and CO_2, which could display additive or synergistic relationships. Indeed, regional differences have been observed in vascular ultrasound studies whereby the VA blood flow response was found to be higher during hypoxia and lower during both hypocapnia and incremental cycling, compared to either ICA or MCA blood flow responses (Sato *et al.*, 2011; Sato *et al.*, 2012; Willie *et al.*, 2012). Furthermore, the precise effects of combining hypoxia and CO_2 on cerebral metabolism are still unknown. Given the limited literature on this topic, further studies on the effect of hypoxia, CO_2, and acclimatisation status on the regulation of cerebrovascular tone and cerebral DO_2 during exercise at high altitude are certainly warranted.

Our findings contradict earlier studies in which CBF was significantly elevated by clamping $P_{ET}CO_2$ with added CO_2 during high intensity exercise at 4,875 m [simulated; (Subudhi *et al.*, 2011b)] and 3,454 m (Siebenmann *et al.*, 2013). Since these studies were conducted in subjects living at 1,600 m and in sojourners following one night at high altitude respectively, while the subjects in our studies were low altitude residents (~400 m) acutely exposed to normobaric hypoxia (~4 min), it is possible that these discrepant findings are due to the differences in acclimatisation status between the studies. For example, both acute and chronic high altitude exposures have been shown to enhance both the CBF and ventilatory responsiveness to CO_2 (Mathew *et al.*, 1983; Fan *et*

al., 2010a; Fan *et al.*, 2012a). This adaptive enhancement of cerebrovascular CO_2 reactivity at high altitude would certainly account for the greater elevations in CBF with $P_{ET}CO_2$ clamping during incremental exercise in hypoxia, as reported by Subudhi *et al*., (2011b) and Siebenmann *et al*., (2013). Furthermore, an enhanced ventilatory CO_2 sensitivity would also explain the large increases in $\dot{V}_E$ (~50 L/min) reported by Subudhi *et al*., (2011b) with $P_{ET}CO_2$ clamping during hypoxic exercise.

Enhanced cortical activation during exercise in hypoxia?

Functional activation during exercise leads to cerebral hyperperfusion and an associated increase in cerebral tissue oxygenation (Ide *et al.*, 1999). Since cerebral function deteriorates when cerebral tissue oxygenation drops (Van Lieshout *et al.*, 2003), Quistorff *et al*., (2008) speculated that the cerebral hyperperfusion might serve as an important precaution for brain function deterioration during exercise. Therefore, the large elevation in CBF observed during exercise in severe hypoxia is likely a protective response, serving to prevent excessive cerebral tissue deoxygenation in the face of prevailing hypoxaemia. Alternatively, this progressive increase CBF during TT cycling in severe hypoxia, despite relatively stable CaO_2 and SaO_2, could be due to greater sensorimotor cortex activation associated with prolonged exercise in hypoxia. Both blood flow and metabolic rate corresponding to the cortical representation of the sensory input are increased during hand movement (Raichle *et al.*, 1976). Likewise, early findings showed CBF redistribution to sensorimotor cerebral cortex, spinal cord and cerebellum in exercising dogs (Gross *et al.*, 1980). Since axillary afferent blockade abolishes the increase in blood flow to the sensorimotor and premotor cortices during static handgrip (Friedman *et al.*, 1991) and dynamic hand contraction (Friedman *et al.*, 1992), these findings indicate that an increase in cortical activation of sensorimotor areas associated with neural input from the working limbs is critical for exercise-induced increases in CBF. In our studies, we found higher rates of perceived effort (RPE) for a

given power output during both incremental and TT cycling in hypoxia when compared to normoxia (Fan *et al*., 2013; Fan & Kayser 2013), thus confirming the report of a direct effect of hypoxia on effort perception during exercise in severe hypoxia (Amann & Dempsey, 2008). We postulate that increased sensorimotor cortex activation—as indicated by higher RPE—coupled with a compensatory cerebral vasodilation in the face of severe hypoxaemia, could at least partially account for the elevated CBF observed during exercise in severe hypoxia.

As Rasmussen *et al*., (2010a) pointed out, since the brain relies largely on oxidative metabolism, a minimal mitochondrial O_2 tension ($P_{mito}O_2$) is essential in sustaining adequate cerebral metabolism for normal homeostatic function of the brain. Since they observed good correlations between RPE with estimated $P_{mito}O_2$ and cerebral O_2-carbohydrate index during cycling exercise in normoxia (44%, 81%, and 100% of maximum workload) and hypoxia (44% of maximum workload), they concluded that the RPE ratings during 20 min steady-state cycling reflect the state of cerebral $P_{mito}O_2$, regardless of exercise intensity or O_2 provision. While this conclusion supports our own speculation of a link between RPE and increased cortical activation during exercise in severe hypoxia (Fan *et al.*, 2013), we must acknowledge that RPE is a subjective measure that is dependent upon both sensory information and cortical output (Rasmussen *et al.*, 2010a). Caution should be taken when interpreting these correlations.

Cerebral metabolism during hypoxic exercise

During steady-state cycling in acute severe hypoxia (FIO_2: 0.10), Rasmussen *et al*., (2010a) found both cerebral DO_2 and $CMRO_2$ were reduced compared to normoxic conditions. Overgaard *et al*., (2012) examined cerebral lactate turnover during high intensity exercise in hypoxia (70% maximal power output) and found a sevenfold increase in cerebral lactate release, as well as elevated cerebral lactate uptake and release kinetics, while cerebral glucose uptake tended to be elevated. They also

found a 10% reduction in cerebral DO_2 during heavy exercise in hypoxia, while cerebral arteriovenous differences in glucose and pyruvate concentration were unchanged. From these observations they concluded that there is an increase in cerebral lactate oxidation during exercise in hypoxia, accounting for up to 33% of total cerebral substrate use. While this increase in cerebral lactate uptake and oxidation does not appear to be specific to exercise in hypoxia *per se* (Volianitis *et al.*, 2008), rather as a mechanism of lactate clearance in the face of elevated lactate production, such increase in lactate oxidation would spare glucose utilisation in the brain during exercise in hypoxia (Quistorff *et al.*, 2008). Since the degree of hypoxia (~10% O_2) and the intensity of exercise were similar (~140 W versus ~119 W) between our TT study and that by Overgaard *et al*., (2012), it seems reasonable to assume that cerebral lactate uptake and oxidation were also enhanced during hypoxic exercise in our subjects.

In acclimatised subjects, Møller *et al*., (2002) found cerebral DO_2 and cerebral oxidative metabolism to be preserved during moderate exercise at 5,260 m. They attributed this preservation to a compensatory increase in cerebral O_2 extraction during exercise at high altitude. Similar to acute exposure to hypoxia, they found evidence of increased cerebral lactate uptake during exercise following acclimatisation to high altitude. The observed increase in cerebral lactate release by both Overgaard *et al*., (2012) and Møller *et al*., (2002) could be interpreted as indirect evidence for increased non-oxidative metabolism during exercise in hypoxia. Since the rate of glycogenolysis relative to glucose utilisation has been shown to reach levels as high as ~290% during anoxia and ischaemia compared to normoxia (Lowry *et al.*, 1964; Gross & Ferrendelli, 1980; Harik *et al.*, 1982), it has been suggested that glycogenolysis might serve as a means to help preserve neuronal function during metabolic crisis (Dienel & Cruz, 2006). Further, it is conceivable that hypoxia also enhances non-oxidative metabolism during sustained motor cortex activation in order to reduce $CMRO_2$ (Vafaee *et al.*, 2012). Whilst speculative, an increase in cerebral non-oxidative metabolism

would spare O_2 utilisation in the face of reduced brain O_2 supply, and thus account for the reduced $CMRO_2$ observed by Rasmussen *et al*., (2010a) during exercise in acute severe hypoxia.

Cerebral oxygenation and performance

In two of our studies (Fan *et al*., 2013; Fan & Kayser 2013), increased respiratory drive associated with CO_2 breathing elevated $P_{ET}O_2$ and O_2 saturation, which in turn tended to elevate CaO_2. However, since we did not measure blood gases or prefrontal oxygenation during the incremental cycling to exhaustion, we will focus our discussion on the 15km TT study. During TT cycling in hypoxia, the modest elevations in both MCAv and CaO_2 with CO_2 breathing normalised the cerebral DO_2 to normoxic values (Fan *et al.*, 2013). This finding is further supported by reduced cerebral [HHb] and increased cerebral concentration of delta Hb (Figure 22). Accordingly, we contend that cerebral DO_2 was restored to normoxic levels during the TT in hypoxia using our CO_2 breathing setup. Our data corroborate with those of Subudhi *et al*., (2011), which showed significant increases in cerebral tissue oxygenation with $P_{ET}CO_2$ clamping during incremental exercise in hypoxia. We also found a tendency for muscle oxygenation to be elevated with CO_2 breathing in hypoxia, presumably due to a slightly higher SaO_2 (Figure 23). Since the restoration of cerebral DO_2 with CO_2 breathing in our study was not accompanied by improvements in exercise time, motor drive, or mean power output, our data does not support the role of reduced cerebral DO_2 and associated cerebral tissue deoxygenation in limiting performance in hypoxia. It should be acknowledged that changes in global cerebral DO_2 might not be representative of local changes in arterial and/or capillary PO_2 in the brain. Therefore it is possible that we were unable to restore DO_2 to the critical brain regions involved in motor recruitment with our CO_2 breathing setup.

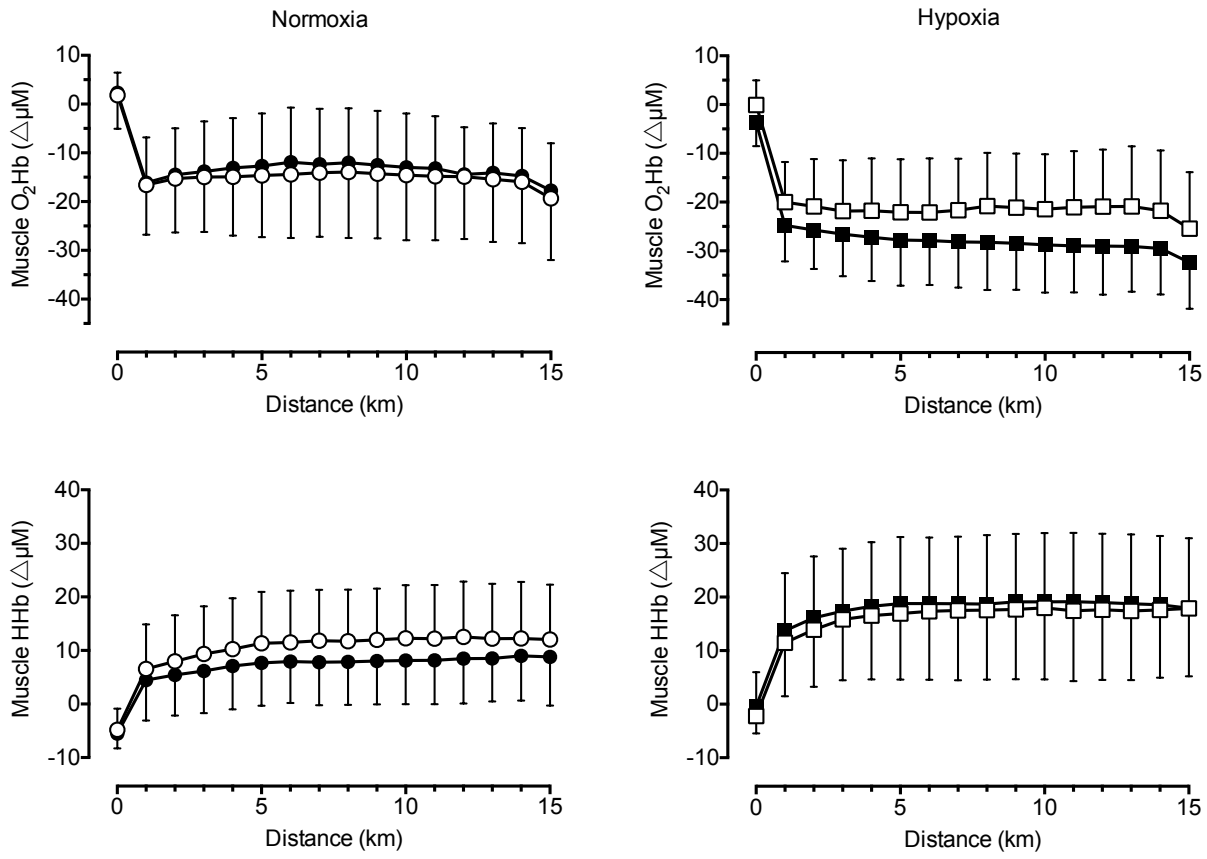

Figure 23. Effect of hypoxia and CO_2 clamping on muscle oxygenation during 15-km time trial cycling. Muscle O_2Hb and HHb are expressed as delta changes from normoxia (room air breathing). Left panels, group data in normoxia (mean ± SD); right panels group data in hypoxia. ●, normoxia control; ○ normoxia CO_2 clamp; **■,** hypoxia control; □, hypoxia CO_2 clamp. From Fan *et al*., (2013).

We cannot exclude the possibility that CO_2 breathing during heavy exercise might also introduce other confounding factors, which could override the potential benefits of improved cerebral DO_2. For example, Subudhi *et al*., (2011b) attributed the reduced exercise capacity with $P_{ET}CO_2$ clamping in hypoxia in their study to increased respiratory muscle work, and associated 'steal effect' of blood flow from the working limbs, as well as an elevated RPE related to their experimental setup. These factors could have out-weighed the benefits of elevated cerebral DO_2. They found $\dot{V}_E$ to be elevated by ~50 L/min with $P_{ET}CO_2$ clamping during incremental cycling in hypoxia. Likewise, we found $\dot{V}_E$ to be elevated by ~20 L/min with CO_2 breathing during incremental and TT cycling in hypoxia (Figures 21 & 24), while the rate of RPE increase was also higher with CO_2 breathing. As mentioned before, increased respiratory work augments peripheral fatigue during exercise and perception of leg discomfort. Accordingly, the detrimental effects of enhanced ventilatory drive and respiratory work associated with CO_2 breathing could *outweigh* any potential benefits of improved cerebral DO_2 and tissue saturation during exercise in hypoxia in our studies.

Siebenmann *et al*., (2013) further argued that CO_2 breathing may exacerbate the metabolic acidosis associated with heavy exercise, shifting the oxyhaemoglobin curve rightward and lowering SaO_2, thus limiting maximal O_2 consumption. In agreement, we observed a tendency for maximal exercise capacity to be impaired with CO_2 breathing in hypoxia (Fan & Kayser, 2013). However, contrary to Siebenmann *et al*., (2013), we found O_2 saturation to be higher with CO_2 breathing in hypoxia throughout submaximal exercise intensities (Figure 21), which does not support the notion of a limited pulmonary O_2 uptake associated with a right-ward shift in the oxyhaemoglobin curve.

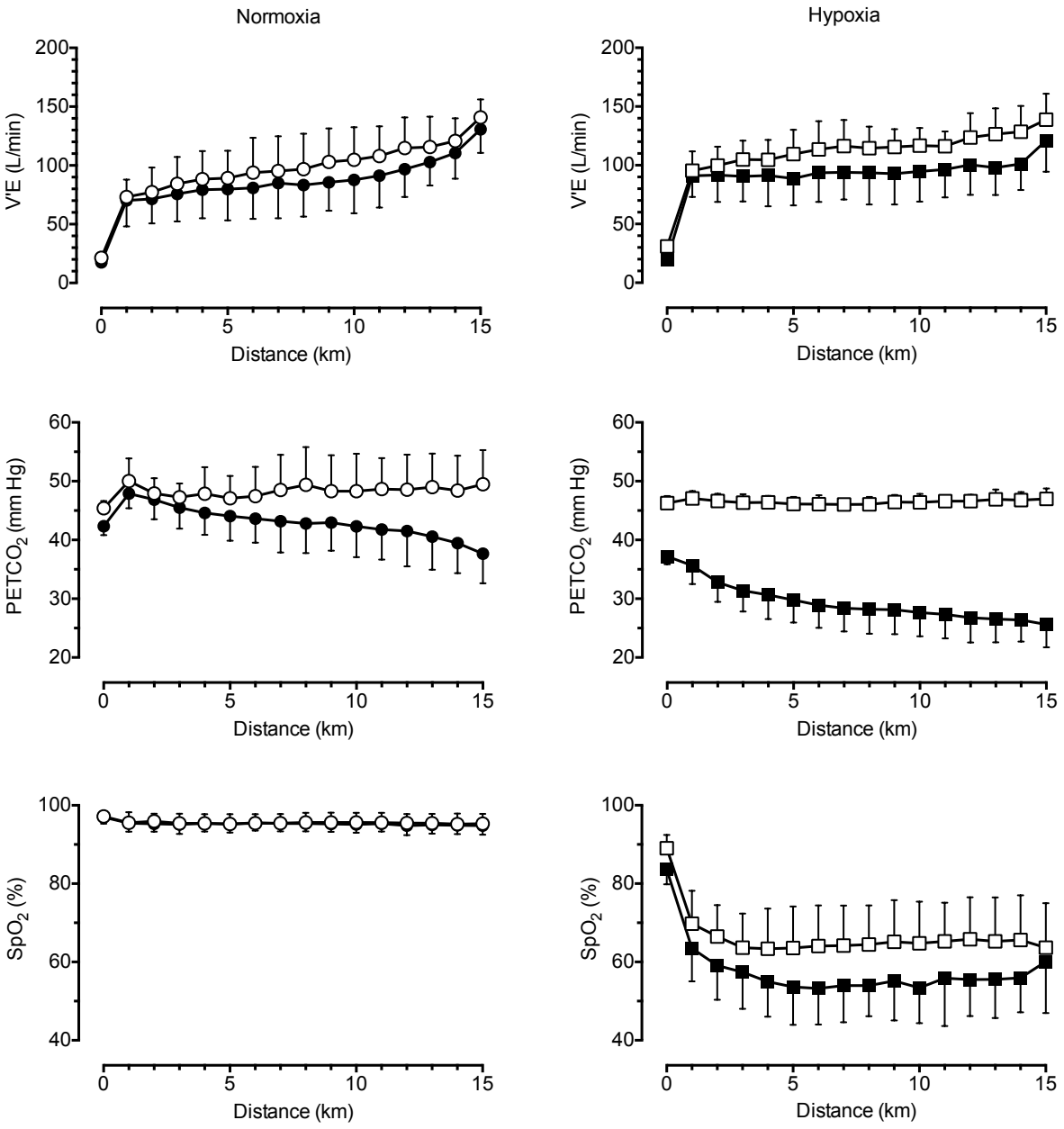

Figure 24. Effect of hypoxia and CO_2 clamping on respiratory variables during 15-km time trial cycling. Left panels, group data in normoxia (mean ± SD); right panels group data in hypoxia. ●, normoxia control; ○ normoxia CO_2 clamp; ■**,** hypoxia control; □, hypoxia CO_2 clamp. From Fan *et al*., (2013).

Chemoreception of CO_2 and exercise hyperpnoea

The regulation of exercise hyperpnoea has been extensively studied during the past 100 years [see (Mateika & Duffin, 1995; Forster, 2000; Ward, 2000; Péronnet & Aguilaniu, 2006; Poon *et al.*, 2007; Forster *et al.*, 2012) for reviews]. Nevertheless, the role of chemoreception in the regulation of exercise hyperpnoea, especially during heavy exercise, remains controversial (Babb *et al.*, 2010; Hopker *et al.*, 2011). To date, only a handful of studies have examined the effect on exercise hyperpnoea of chemoreceptor stimulation with CO_2 breathing alone (Clark *et al.*, 1980; Babb, 1997; Babb *et al.*, 2003; Olin *et al.*, 2012) or the combined effect of CO_2 breathing with hypoxia (Asmussen & Nielsen, 1957). With the exception of Olin *et al*., (2011) who only reported maximal $\dot{V}E$, these studies primarily focused on the effect of CO_2 breathing on exercise hyperpnoea during low and moderate intensity steady-state exercise. In young sedentary subjects, increased $FICO_2$ (3% CO_2) has been shown to augment the ventilatory response to exercise (i.e., *greater* rise in $\dot{V}E$) below RC (Babb, 1997). Since maximal $\dot{V}E$ was higher with hypercapnia compared to room-air breathing, the author concluded that during near-maximal and maximal exercise in hypercapnia, the respiratory system was not limited by the expiratory airflow.

In our work, CO_2 breathing did not alter $\dot{V}E$ during high intensity exercise in normoxia or hypoxia (Fan *et al*., 2012; Fan & Kayser 2013). Findings from these two studies revealed that CO_2 breathing in normoxia blunts the $\dot{V}E$ response to incremental exercise (i.e., a lower rise in $\dot{V}E$) at lower exercise intensities, below RC. Since absolute $\dot{V}E$ was similar at RC between CO_2 breathing and control conditions despite occurring at a lower power output with CO_2 breathing, we attribute this 'blunting' of the ventilatory response to exercise to an elevated $\dot{V}E$ at rest with CO_2 breathing. During moderate-to-high intensity exercise in normoxia (i.e., above RC), we observed no difference between room-air and CO_2 breathing (Figure 25). Hypoxia alone enhanced the $\dot{V}E$ rise during exercise below RC, and caused RC onset to occur at a lower power output, while hypoxia and CO_2

breathing further lowered the power output of RC onset and elevated $\dot{V}_E$ at RC (Figure 25). Interestingly hypoxia, either alone or in combination with CO_2 breathing, had no effect on the $\dot{V}_E$ rise during moderate-to-high intensity exercise. Our data agrees with those by Wasserman *et al*., (2011), which showed a dissociation between arterial $[H^+]$ and $\dot{V}_E$ during exercise above RC. We thus confirm that both hypoxia and hypercapnia can modulate the rate of ventilatory response to increasing exercise below RC, presumably through chemoreceptor stimulation. However, these chemoreflexes appears to become blunted once the exercise intensity is above RC.

Limitations

An important methodological consideration when interpreting our findings is the use of transcranial Doppler ultrasound to measure MCAv as a representative index of global CBF changes during rest and dynamic whole-body exercise in hypoxic conditions. This is based on the assumption that: i) the MCA accounts for approximately 80% of the blood flow to the respective hemispheres (Lindegaard *et al.*, 1987); ii) changes in MCAv reflex changes in global CBF (Bishop *et al.*, 1986; Serrador *et al.*, 2000); iii) the changes in MCAv in response to $PaCO_2$ changes and during incremental exercise are comparable to those changes observed in the ICA blood flow (Sato *et al.*, 2011; Sato *et al.*, 2012); and iii) the diameter of the MCA remains constant during changes in arterial blood gases (~25-40 mmHg) and blood pressure (Serrador *et al.*, 2000). In support, MCAv has been shown to follow CBF changes during initial exposure to high altitude, when compared with the direct Fick's method (Roy *et al.*, 1968; Milledge & Sorensen, 1972; Møller *et al.*, 2002).

The possibility of large conducting arteries being involved in CBF regulation was first proposed by Faraci *et al*., (1987), who observed changes in feline basilar artery diameter in response to changes

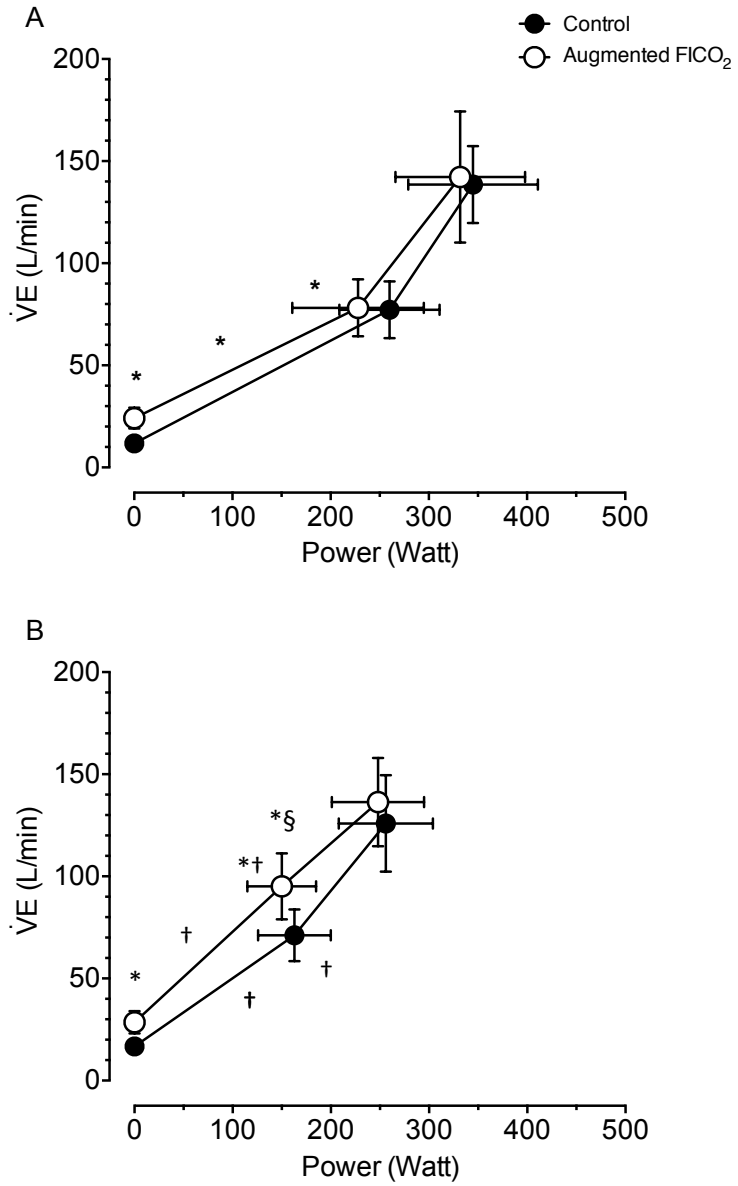

Figure 25. Effect of hypoxia and augmented $FICO_2$ on respiratory compensation threshold and ventilatory response to exercise during incremental cycling to exhaustion. A: group data in normoxia (mean ± SD). B: group data in hypoxia. * Different from control ($P<0.05$); † different from normoxia ($P<0.05$); § trend for non-significant difference ($P<0.10$). From Fan & Kayser (2013).

in arterial CO_2 tension. Accordingly, they concluded that resistances of the large arteries account for a third of total vascular resistance in the brain stem. Using transcranial colour-coded Doppler, Wilson *et al*., (2011) found that the diameter of MCA changes depending on the altitude (i.e., 5.30 mm at 75 m, 5.51 mm at 3,500 m, 5.23 mm at 5,300 m and 9.34 mm at 7,950 m). The data from Wilson *et al*., (2011) demonstrated that the MCA diameter remained relatively unchanged up to 5,300 m. However, it should be noted that the MCA diameters measured with transcranial colour-coded Doppler in that study were 80-90% greater than the values obtained using MRI in the same subjects. Meanwhile, in contrast to findings by Sato *et al*., (2012), Willie *et al*., (2012) found lower cerebrovascular CO_2 reactivity in the MCAv response compared to ICA blood flow. Those authors concluded that this underestimation of cerebrovascular CO_2 reactivity was due to dilatation of the MCA in hypercapnia. Further, they reported a ~17% change in ICA diameter between extreme levels of hypocapnia and hypercapnia ($PaCO_2$: 16-62 mmHg). More recent studies, using transcranial colour coded Doppler, found MCA diameter to increase (by 0.6-1.0 mm) during acute and chronic exposure to 5,050 m (Imray *et al.*, 2014; Willie *et al.*, 2014a). Taken together, there appears to be mounting evidence supporting the role of large conduit arteries in CBF regulation during changes arterial blood gases.

Poiseuille's law states that flow in a circular vessel is proportional to the fourth power of the radius. Therefore small changes in MCA diameter would lead to relatively large changes in the estimates of CBF obtained using transcranial Doppler ultrasound (Imray *et al.*, 2014). Any MCA dilation with hypoxia, CO_2 breathing, or exercise would result in an underestimation of the true CBF changes assessed with MCAv in our studies. Since we observed increases in CBF during prolonged exercise in hypoxia and with CO_2 breathing (Fan *et al*., 2012; Fan *et al*., 2013; Fan & Kayser 2013), such underestimation of CBF would change the size of the effects observed in our studies, but would not affect our main conclusions.

We used left prefrontal [O_2Hb] and [HHb] measured by near-infrared spectroscopy (NIRS) as indicators of global cerebral tissue oxygenation, which is a simplification, even though good agreements have been found between NIRS and functional MRI measurements of cerebral tissue oxygenation levels over various cortices (Kleinschmidt *et al.*, 1996; Strangman *et al.*, 2002). NIRS is also subject to bias from skin blood flow variations, since the light traverses the skin and the bone layers before entering the tissue of interest. According to Tew *et al*., (2010), such changes due to skin effects can be considered negligible compared to the changes in the brain tissue.

Another limitation of this thesis is that we elevated end-tidal rather than arterial PCO_2 during incremental cycling to exhaustion. As the end-tidal–arterial PCO_2 gradient varies with exercise (Jones *et al.*, 1979; Liu *et al.*, 1995), it is possible that we did not elevate $PaCO_2$ to the desired levels. In one of our studies (Fan *et al.*, 2013), we measured capillary blood samples as a surrogate of arterial measurements, and were able to elevate and maintain capillary PCO_2 within 1 mmHg throughout exercise under both normoxic and hypoxic conditions. As Mollard *et al*., (2010) found good correlations between pre-warmed capillary earlobe samples and radial artery measurements of PaO_2 ($R^2 = 0.99$), $PaCO_2$ ($R^2 = 0.86$), and SaO_2 ($R^2 = 0.99$) during rest, submaximal, and near maximal exercise intensities, we are confident that our protocol was effective in elevating $PaCO_2$. Furthermore, using our setup, Siebenmann *et al*., (2013) were successful in elevating and maintaining both end-tidal and arterial PCO_2 at around 40 mmHg during incremental exercise in hypoxia. We therefore believe it is likely that we were able to sufficiently elevate and maintain $PaCO_2$ using our CO_2.

Further Perspectives

This thesis attempted to investigate the role of reduced cerebral DO_2 and tissue deoxygenation associated with hyperventilation-induced hypocapnia on exercise performance in severe hypoxic conditions (Fan *et al*., 2012; Fan *et al*., 2013; Fan & Kayser 2013). To examine this, we prevented the development of hypocapnia with CO_2 breathing during incremental-intensity and TT cycling in hypoxia in an attempt to selectively increase blood flow (and thus O_2 supply) to the brain without altering systemic O_2 levels. However, the increased ventilatory drive associated with CO_2 breathing elevated both respiratory work and PaO_2, which might have confounded our findings. An alternative way to answer our question would be to examine the effects of selectively altering CBF and cerebral DO_2 using pharmacological means. For example, indomethacin is a reversible cyclooxygenase inhibitor, which has been shown to impair the cerebral vessels' ability to dilate in response to hypercapnia and hypoxia via inhibiting prostaglandin synthesis (Parfenova *et al.*, 1994). Studies have found that indomethacin lowers resting CBF (by 25-30%) and attenuates cerebrovascular CO_2 reactivity under normoxic and hypoxic conditions (Eriksson *et al.*, 1983; Markus *et al.*, 1994; St Lawrence *et al.*, 2002; Fan *et al.*, 2010a), without affecting cerebral metabolism (Hohimer *et al.*, 1985; Kraaier *et al.*, 1992) or systemic vascular functions (Taylor *et al.*, 2014). Indomethacin could potentially serve as an ideal tool for selectively reducing CBF and thus to investigate the effect of reduced cerebral DO_2 on performance during exercise in hypoxia.

Despite our best attempt to quantify the changes in cerebral DO_2 and cerebral tissue oxygenation during hypoxic exercise, a number of important questions remain unresolved. For example, a clinical study in patients with traumatic brain injuries alluded to the notion that brain tissue PO_2 might be determined by variables related to cerebral O_2 diffusion, rather than cerebral DO_2 and metabolism (Rosenthal *et al.*, 2008). Whether this is true in healthy populations and how we might measure brain tissue O_2 tension in healthy (and intact) exercising humans presents a significant

technical issue. Perhaps animal models could be employed to provide direct measures of brain tissue O_2 tension changes during exercise in severe hypoxia. However, given the inter-species differences in physiological responses to exercise (Forster *et al.*, 2012), the applicability of findings from animal models of exercise in hypoxia to human performance remains unclear.

As Dienel (2013) aptly pointed out, regional heterogeneity and compartmentalisation of function and metabolism is one of the hallmark characteristics of the brain. However, the majority of the brain and exercise literature has focused on the brain as a whole. The effect of hypoxia on the brain during whole-body exercise on specific brain regions remains largely unknown. Existing techniques such as functional MRI, positron emission tomography, electroencephalography, and multi-site NIRS could potentially be employed in this area of research. In theory, they would enable the assessment of region-specific cortical and other brain areas' activities. Despite the potential advantages of these techniques, their practical applications in exercise testing have not been well explored, presumably due to the technological and practical limitations as well as the availability and financial costs of these systems. Nevertheless, assessment of neuronal activity could be undertaken with electroencephalography and NIRS, in order to map out the specific regional changes in cortical activity (and therefore metabolism) during dynamic whole-body exercise in severe hypoxia. Findings from these studies would provide the groundwork for future investigations. Quantifying these cortical activity changes during hypoxic exercise would help us identify the specific regions of interest, perhaps even help locate the 'origin' of the central fatigue in the brain. However, we cannot exclude the possibility that further advancement of neural imagining techniques for exercise testing is needed before any breakthrough can be achieved.

Other aspects that have been largely overlooked in this field are the psychological and cognitive effects of hypoxia during exercise (Ando *et al.*, 2013) It is conceivable that hypoxia could alter

brain processes involved in motivation, both directly and indirectly [e.g., via increased perception of dyspnoea (De Peuter *et al.*, 2004; Weinberger & Abu-Hasan, 2009)], which could account for some of the detriments in performance. There is no denying that motivation plays a large part in sporting performances. Implementing psychological measurements will improve understanding of the effect of hypoxia on psychological and cognitive functions, thus providing further insight to the mechanisms behind the performance detriments observed during exercise in hypoxia.

Conclusions

We found cerebral DO_2 to be well maintained during acute exposure to high altitude and following acclimatisation, which was mediated by reciprocal changes in CBF and CaO_2. Our findings showed an increase in cerebrovascular CO_2 reactivity during acute high altitude exposure, which was further elevated with acclimatisation. Since the increase in cerebrovascular CO_2 reactivity was abolished once we normalised for arterial blood pressure changes, we attribute this to an enhanced systemic pressure response with acclimatisation. Our data also showed impaired dynamic CA during exposure to high altitude, which persisted following acclimatisation. Such 'impairment' in dynamic CA would result in a greater change in CBF for any given change in perfusion pressure. Therefore, is it conceivable that a reduced CA might facilitate cerebral DO_2 in the face of severe arterial hypoxaemia during acute and chronic high altitude exposures. Further, since this reduction in CA does not appear to be linked with symptoms of acute mountain sickness, we speculate that these changes in CA at high altitude are intrinsic consequences of hypoxia in the absence of pathology.

During exercise in normoxia and normobaric hypoxia, we attempted to selectively elevate CBF, thus elevating cerebral DO_2 and cerebral tissue oxygenation with CO_2 breathing during exercise. We observed only moderate elevations in CBF with CO_2 breathing during both incremental-intensity and TT cycling exercise in severe hypoxia, which we attributed to an exhausted

cerebrovascular dilatory reserve. We were nevertheless able to increase CaO_2 with CO_2 breathing, via increased ventilatory drive. Accordingly, we were able to improve cerebral DO_2 during TT cycling in hypoxia, restoring it to normoxic values. Since the restoration of cerebral DO_2 was not accompanied by improvements in either prefrontal oxygenation or exercise performance, our findings do not appear to support the role of cerebral DO_2 in limiting performance during exercise in severe hypoxia.

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Articles

Article one

Subudhi AW, **Fan JL**, Evero O, Bourdillon N, Kayser B, Julian CG, Lovering AT & Roach RC. (2014). AltitudeOmics: Effect of ascent and acclimatization to 5,260 m on regional cerebral oxygen delivery. *Experimental Biology* **99,** 772-781.

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Research Paper

AltitudeOmics: effect of ascent and acclimatization to 5260 m on regional cerebral oxygen delivery

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New Findings

- **What is the central question of this study?**
  Hypoxia associated with ascent to high altitude may threaten cerebral oxygen delivery. We sought to determine whether there are regional changes in the distribution of cerebral blood flow that might favour oxygen delivery to areas associated with basic homeostatic functions to promote survival in this extreme environment.
- **What is the main finding and its importance?**
  We show evidence of a 'brain-sparing' effect during acute exposure to high altitude, in which there is a slight increase in relative oxygen delivery to the posterior cerebral circulation. This may serve to support basic regulatory functions associated with the brainstem and hypothalamus.

Cerebral hypoxaemia associated with rapid ascent to high altitude can be life threatening; yet, with proper acclimatization, cerebral function can be maintained well enough for humans to thrive. We investigated adjustments in global and regional cerebral oxygen delivery (D_{O_2}) as 21 healthy volunteers rapidly ascended and acclimatized to 5260 m. Ultrasound indices of cerebral blood flow in internal carotid and vertebral arteries were measured at sea level, upon arrival at 5260 m (ALT1; atmospheric pressure 409 mmHg) and after 16 days of acclimatization (ALT16). Cerebral D_{O_2} was calculated as the product of arterial oxygen content and flow in each respective artery and summed to estimate global cerebral blood flow. Vascular resistances were calculated as the quotient of mean arterial pressure and respective flows. Global cerebral blood flow increased by ∼70% upon arrival at ALT1 ($P < 0.001$) and returned to sea-level values at ALT16 as a result of changes in cerebral vascular resistance. A reciprocal pattern in arterial oxygen content maintained global cerebral D_{O_2} throughout acclimatization, although D_{O_2} to the posterior cerebral circulation was increased by ∼25% at ALT1 ($P = 0.032$). We conclude that cerebral D_{O_2} is well maintained upon acute exposure and acclimatization to hypoxia, particularly in the posterior and inferior regions of the brain associated with vital homeostatic functions. This tight regulation of cerebral D_{O_2} was achieved through integrated adjustments in local vascular resistances to alter cerebral perfusion during both acute and chronic exposure to hypoxia.

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Experimental Physiology

Introduction

Although the brain represents only about 2% of body weight, it is a highly metabolic tissue that receives ∼15% of cardiac output and accounts for ∼20% of total body oxygen consumption at rest (Wade & Bishop, 1962). Maintenance of cerebral oxygen delivery (D_{O_2}) is essential for vital cerebral functions associated with homeostasis. In the face of severe hypoxaemia, such as experienced during rapid ascent to extreme altitudes (>8000 m), reduction in cerebral D_{O_2} results in loss of consciousness within seconds (Luft *et al.* 1951; Luft & Noell, 1956) and death within minutes (Bert, 1943). However, with staged acclimatization to progressively higher elevations, cerebral D_{O_2} can be maintained well enough for humans to reach the summit of Mount Everest (8848 m) without supplemental oxygen. The mechanisms responsible for this remarkable plasticity in cerebral D_{O_2} are complex and not completely understood.

Cerebral D_{O_2} is the product of cerebral blood flow (CBF) and arterial oxygen content (C_{aO_2}). It is well established that CBF rises upon acute exposure to high altitude and returns to near sea-level values with acclimatization (Severinghaus *et al.* 1966; Huang *et al.* 1987; Jensen *et al.* 1990), while C_{aO_2} decreases in acute hypoxia and returns to sea-level values with acclimatization. These opposing CBF and C_{aO_2} responses to altitude appear to offset one another and maintain cerebral D_{O_2} throughout acclimatization (Severinghaus *et al.* 1966; Wolff *et al.* 2002). The pattern of CBF change in response to hypoxia has been attributed to the relative balance of hypoxic vasodilatation and hypocapnic vasoconstriction in the brain (Xu & Lamanna, 2006; Brugniaux *et al.* 2007). During acute, severe hypoxia, vasodilatation typically exceeds vasoconstriction, resulting in greater CBF (Mardimae *et al.* 2012; Willie *et al.* 2012). With acclimatization, increased ventilatory drive reduces the arterial partial pressure of CO_2 (P_{aCO_2}) and improves the arterial partial pressure of O_2 (P_{aO_2}), tipping the balance in favour of vasoconstriction and restoring CBF to pre-exposure values. Changes in the P_{aO_2}/P_{aCO_2} ratio have been shown to account for ∼40% of the variation in global CBF over acclimatization (Lucas *et al.* 2011), with other biochemical (e.g. pH, HCO_3^-, nitric oxide) and haematological factors (e.g. haemoglobin, haematocrit, blood viscosity) presumably accounting for the rest of the response (Todd *et al.* 1994; Tomiyama *et al.* 1999; Severinghaus, 2001) to maintain global cerebral D_{O_2}.

Recent data demonstrate that acute normobaric hypoxia (i.e. breathing hypoxic gas) affects the regional distribution of CBF within the brain. Data from positron emission tomography (PET) studies show greater perfusion of the brainstem, hypothalamus, thalamus and cerebellum during acute hypoxia, with (Binks *et al.* 2008) or without controlled levels of P_{aCO_2} (Buck *et al.* 1998). Regional differences in cerebrovascular reactivity to O_2 and CO_2 have been postulated to control the distribution of CBF. Vascular Doppler studies of the major tributary vessels of the brain suggest that a greater percentage of blood flow may be directed towards the posterior cerebral circulation, including the brainstem, in response to controlled levels of hypoxia and hypocapnia (Sato *et al.* 2012). From a teleological perspective, this could help preserve vital homeostatic functions at the expense of higher cognitive processing; however, it is unclear whether regional distribution of CBF is affected in a similar manner in hypobaric hypoxia (i.e. high altitude) or if it changes with acclimatization, because not all studies report significant regional differences (Huang *et al.* 1987; Willie *et al.* 2012, 2013).

Despite the importance of O_2 supply for cerebral function, longitudinal studies of cerebral D_{O_2} at high altitude are sparse. In a secondary analysis of data from original study by Severinghaus *et al.* (1966) of CBF at high altitude, global cerebral D_{O_2} in four subjects appeared stable and in excess of oxygen demand after 6–12 h and 3–5 days, respectively, of exposure to 3810 m (Severinghaus, 2001; Wolff *et al.* 2002). Using similar methodology (Kety–Schmidt technique), no differences were found in global cerebral D_{O_2} measured after 5 weeks at 5260 m and return to sea level (Møller *et al.* 2002). Unfortunately, these studies were based on a limited number of observations, which makes it difficult to detect small differences, if they existed (type II error), and used methodology that can only measure global cerebral D_{O_2}. A more recent magnetic resonance imaging (MRI) study with a larger sample size reported a tendency towards elevation of cerebral D_{O_2} after subjects returned from 2 days at 3800 m (Smith *et al.* 2013), but no measurements of regional cerebral D_{O_2} were made. Based the limited data to date, it is uncertain whether global or regional cerebral D_{O_2} varies over time at high altitude.

In this study, we used vascular Doppler technology in conjunction with arterial blood sampling to allow us to quantify global and regional changes in CBF and cerebral D_{O_2} in the field as healthy people rapidly ascended and acclimatized to high altitude (5260 m). We tested the hypothesis that upon acute exposure cerebral D_{O_2} would be maintained to regions of the brain associated with homeostasis at the expense of other tissues, but that these changes would normalize with acclimatization.

Methods

Subject recruitment and screening

This study was conducted as part of the AltitudeOmics project, for which a detailed description of the protocol is published elsewhere (Subudhi *et al.* 2014). Briefly, following institutional ethics approval from

the Universities of Colorado and Oregon and the US Department of Defense Human Research Protection Office, young, healthy, sea-level residents were recruited from the greater Eugene, OR area (elevation 128 m). Potential subjects were screened to exclude anyone who was born or had lived at altitudes >1500 m for longer than 1 year or had travelled to altitudes >1000 m in the past 3 months. After obtaining written informed consent, physical examinations and the Army Physical Fitness Test (push ups, sit ups and 3.2 km run) were performed to verify health and fitness status.

Study overview

To evaluate effects of altitude acclimatization on cerebrovascular haemodynamics, subjects were studied on three occasions, as follows: (i) at sea level (SL, 130 m); (ii) upon acute exposure to 5260 m (ALT1); and (iii) after 16 days of acclimatization (ALT16). Specifically, ∼4 weeks following SL measurements in Eugene, OR, subjects were flown to La Paz, Bolivia. They spent two nights at low altitude (Coroico, Bolivia; 1525 m) before being driven to the Chacaltaya Research Station at 5260 m while breathing supplemental oxygen. Acute responses to high altitude were assessed 2–4 h after arrival and cessation of supplemental oxygen (ALT1). Subjects acclimatized to altitudes ranging from 3800 to 5260 m over the next 15 days, with a majority of the time (75%) spent at 5250 m. Measurements were repeated on ALT16.

Instrumentation

Subjects were studied in an upright, seated position with feet on the floor. Arterial blood pressure was monitored via a fluid-filled pressure transducer (Utah Medical, Salt Lake City, UT, USA) positioned at heart level and attached to a 22 gauge catheter in a radial artery. Blood flow velocity in the left middle cerebral artery ($MCA_{velocity}$) was measured by transcranial Doppler (2 MHz probe, Spencer Technologies, Seattle, WA, USA; affixed to a custom-made headset) at depths ranging from 43 to 54 mm. Signal quality was optimized, and an M-mode screen shot was recorded to facilitate subsequent probe placements. Arterial saturation was measured on the right side of the forehead by pulse oximetry (Nellcor N-200, Mansfield, MA, USA). Limb lead electrodes were used to measure ECG (ADInstruments BioAmp, Colorado Springs, CO, USA and Sonosite Micromaxx, Bothell, WA, USA). Metabolic variables, including expired ventilation and gas concentrations were assessed via breath-by-breath (Medgraphics PFX, St Paul, MN, USA and Vacumed UVM, Ventura, CA, USA) and mixing chamber systems (Oxigraf O_2cap, Mountain View, CA, USA), calibrated with the same 3 l syringe and known concentrations of O_2 and CO_2 prior to each test. Additionally, core temperature was monitored by telemetry pill (CorTemp HQInc., Palmetto, FL, USA). Analog data were sampled and recorded at 200 Hz (ADinstruments Powerlab 16/30, Colorado Springs, CO, USA).

Cerebral blood flow

After verification of signal quality, resting data were recorded for 10 min while subjects breathed room air. At 6 min, 2 ml of arterial blood was drawn anaerobically for blood gas analysis (described in section 'Cerebral oxygen delivery'). During the last 4 min of the resting period, the diameter and blood flow velocities in the left internal carotid (ICA; 1.5 cm distal to the carotid bifurcation) and vertebral arteries (VA; between spinous processes of C4 and C5) were recorded over a minimum of five cardiac cycles by a registered diagnostic sonographer (SonoSite Micromaxx L25 probe, Bothell, WA, USA). Briefly, vessel diameter from a longitudinal view was identified and measured with digital callipers in synchronization with the ECG trace to identify systole and diastole. Velocity was measured in the centre of the vessel with an insonation angle <60 deg and a sample volume maximized for vessel diameter. The peak velocity trace across cardiac cycles was used for calculation of mean velocity (time-averaged peak) and volumetric flow. This procedure was used to verify accurate tracing of the spectral envelop during data collection and results in higher values than the time-averaged mean method (Schöning *et al.* 1994). All data were downloaded in DICOM format for verification of measurements offline (Sante DICOM Editor, Athens, Greece).

Regional blood flow (in millilitres per minute) in the ICA and VA (ICA_{flow} and VA_{flow}) was determined using standard, validated ultrasound techniques (Hoskins, 2008), where:

$$\text{flow} = \pi \times (\text{diameter in centimetres}/2)^2 \times \text{time averaged peak velocity in centimetres per second} \times 60\,\text{s}$$

Average coefficients of variation determined from three repeated measurements of ICA and VA flow measurements in seven subjects at SL were 4.0 ± 2.6 and 4.0 ± 2.1%, respectively.

Global CBF (gCBF) was estimated assuming symmetrical bilateral flow in the major tributary arteries of the brain (Ogoh *et al.* 2013; Willie *et al.* 2013) as follows:

$$\text{gCBF} = (ICA_{flow} + VA_{flow}) \times 2$$

Regional and global measurements of CBF were also expressed relative to estimates of cardiac output (%$\dot{Q}$) derived from simultaneous intra-arterial blood pressure

Table 1. Cardiopulmonary and haematological values

Variable	Units	SL	ALT1	ALT16
Ventilation	l min^{-1}	12.05 ± 2.50 (21)	11.93 ± 2.92 (17)	14.88 ± 2.65 (21)∗†
Arterial P_{O_2}	mmHg	102.2 ± 5.5 (21)	36.1 ± 2.8 (18)∗	45.3 ± 3.2 (20)∗†
Arterial P_{CO_2}	mmHg	38.1 ± 4.4 (21)	26.5 ± 3.1 (18)∗	20.9 ± 2.5 (20)∗†
Arterial O_2 saturation	%	98 ± 1 (21)	76 ± 6 (18)∗	82 ± 3 (20)∗†
Haemoglobin concentration	g dl^{-1}	13.9 ± 1.4 (21)	14.2 ± 1.5 (18)∗	16.0 ± 2.0 (20)∗†
Arterial O_2 content	ml dl^{-1}	19.4 ± 1.9 (21)	15.2 ± 2.1 (18)∗	18.4 ± 2.4 (20)∗†
Heart rate	beats min^{-1}	76 ± 12 (21)	90 ± 16 (16)∗	96 ± 13 (20)∗
Stroke volume	ml	91 ± 27 (21)	85 ± 20 (16)	83 ± 21 (20)
Mean arterial blood pressure	mmHg	79 ± 8 (21)	76 ± 13 (16)	80 ± 10 (20)

Values are given as means ± SD (*n*). ∗Different at sea level (SL), and on the 1st and 16th days at 5260 m (ALT1, ALT16, respectively). †Different from ALT1.

traces (Bogert *et al.* 2010). Cerebral vascular resistance index (CVRi) was calculated as follows:

$$\text{CVRi} = \text{mean ABP}/\text{flow}$$

Cerebral oxygen delivery

Arterial blood was immediately analysed for P_{aO_2}, P_{aCO_2} (Siemens RAPIDLab 248, Erlangen, Germany), haemoglobin concentration ([Hb]), arterial oxygen saturation (S_{aO_2}; Radiometer OSM3, Copenhagen, Denmark) and haematocrit (M24 Centrifuge, LW Scientific, Lawrenceville, GA, USA). Blood gases were temperature corrected (Kelman & Nunn, 1966; Severinghaus, 1966). The C_{aO_2} (vol%) was calculated as follows:

$$C_{aO_2} = 1.39 \times [\text{Hb}] \times S_{aO_2} + P_{aO_2} \times 0.003$$

Regional and global cerebral D_{O_2} were calculated as the products of C_{aO_2} and ICA_{flow}, VA_{flow} and gCBF.

Data analysis

After verification of normality, mixed repeated-measures ANOVAs were used to analyse the interaction of time by sex for each variable of interest ($\alpha = 0.05$). Subsequent estimation-maximization and multiple-imputation (five trials) analyses verified negligible effects of missing values (SPSS 20, IBM, Chicago, IL, USA). Student's paired *t* tests (without imputation of missing values) were used for *post hoc* comparisons with the Holm procedure to control for type I error. *A priori* power calculations ($\alpha = 0.05$, $\beta = 0.20$) were integrated into the study design to limit type II error. Pearson product–moment correlations were used to describe shared variance between variables. Data are presented as means ± SD.

Based on the hypothesis that increased CBF may play a role in the pathogenesis of acute mountain sickness (AMS; Jensen *et al.* 1990; Baumgartner *et al.* 1994, 1999), a secondary analysis was performed to evaluate potential relationships (Spearman correlations) between changes in CBF and D_{O_2} with the severity of Lake Louise Questionnaire symptom scores reported in these subjects on ALT1 (Subudhi *et al.* 2014). Student's paired *t* tests were used to evaluate differences in CBF and D_{O_2} between those with severe AMS (Lake Louise Questionnaire symptoms scores ≥ 6, including headache) and those remaining healthy.

Results

Subject characteristics

Detailed baseline characteristics of the 21 subjects (12 men and nine women; 21 ± 1 years old) participating in AltitudeOmics are presented elsewhere (Subudhi *et al.* In Review). Men exhibited higher [Hb], C_{aO_2} and D_{O_2} than females over the course of the study (all $P < 0.05$), but as no interactions in CBF or D_{O_2} were detected throughout acclimatization, combined data are presented below.

Cerebral blood flow and oxygen delivery

Acute exposure to 5260 m (atmospheric pressure 408 ± 1 mmHg) decreased P_{aO_2}, S_{aO_2} and C_{aO_2} by 66.1 ± 5.4 mmHg, 22 ± 6% and 4.1 ± 1.2 ml dl^{-1}, respectively (all $P < 0.001$; Table 1). This severe degree of hypoxia increased heart rate by 14 ± 11 beats min^{-1} ($P < 0.001$) without affecting mean arterial blood pressure ($P = 0.380$). Cerebral blood flow increased by 74 ± 81% in the ICA ($P = 0.018$), 59 ± 54% in the VA ($P = 0.001$) and 69 ± 57% globally ($P = 0.003$). Respective CVRi values fell (all $P < 0.001$; Table 2), allowing a larger percentage of cardiac output to perfuse the brain ($P = 0.010$). Increased ICA_{flow} was characterized by increased ICA velocity ($P = 0.004$) without a change in diameter ($P = 0.068$), while increased VA_{flow} was explained by an increase in VA diameter ($P = 0.005$) without a change in velocity ($P = 0.120$). The $\text{MCA}_{\text{velocity}}$ was unchanged ($P = 0.953$). Increased gCBF offset the decrease in C_{aO_2} to maintain global cerebral D_{O_2} (Fig. 1), although a small increase in VA D_{O_2} was observed ($P = 0.039$; Fig. 2). Observed changes

Table 2. Cerebrovascular values

Variable	Units	SL	ALT1	ALT16
ICA diameter	cm	0.51 ± 0.08 (21)	0.54 ± 0.07 (16)	0.50 ± 0.07 (20)†
ICA velocity	cm s^{-1}	29.8 ± 8.2 (21)	38.9 ± 8.1 (16)*	32.1 ± 5.4 (20)†
ICA flow	ml min^{-1}	384 ± 197 (21)	556 ± 203 (16)*	379 ± 97(20)†
ICA CVRi	mmHg ml^{-1} min^{-1}	0.25 ± 0.12 (21)	0.16 ± 0.09 (16)*	0.23 ± 0.07 (19)†
VA diameter	cm	0.36 ± 0.06 (20)	0.41 ± 0.06 (16)*	0.36 ± 0.06 (19)†
VA velocity	cm s^{-1}	21.4 ± 4.4 (20)	24.4 ± 6.4 (16)	19.3 ± 7.1 (19)†
VA flow	ml min^{-1}	133 ± 47 (20)	206 ± 98 (16)*	122 ± 55 (19)†
VA CVRi	mmHg ml^{-1} min^{-1}	0.66 ± 0.24 (20)	0.46 ± 0.28 (16)*	0.84 ± 0.58 (19)†
gCBF	ml min^{-1}	1057 ± 413 (20)	1524 ± 456 (16)*	981 ± 223 (19)†
gCBF CVRi	mmHg ml^{-1} min^{-1}	0.09 ± 0.03 (20)	0.05 ± 0.02 (16)*	0.08 ± 0.02 (19)†
D_{O_2} ICA	ml min^{-1}	75 ± 37 (21)	84 ± 32 (16)	68 ± 19 (19)†
D_{O_2} VA	ml min^{-1}	26 ± 10 (20)	31 ± 16 (16)*	22 ± 11 (19)†
D_{O_2} gCBF	ml min^{-1}	206 ± 79 (20)	230 ± 74 (16)	181 ± 51 (19)†
MCA velocity	cm s^{-1}	59.5 ± 10.3 (21)	61.1 ± 13.3 (17)	57.7 ± 7.1 (21)
MCA CVRi	mmHg cm^{-1} s^{-1}	1.36 ± 0.25 (21)	1.28 ± 0.32 (17)	1.41 ± 0.24 (20)
ICA%$\dot{Q}$	%	5.4 ± 2.7 (21)	7.6 ± 2.7 (15)*	4.8 ± 1.4 (18)†
VA%$\dot{Q}$	%	1.9 ± 0.8 (20)	2.6 ± 1.1 (15)*	1.5 ± 0.7 (18)†
gCBF%$\dot{Q}$	%	15.0 ± 5.8 (20)	20.4 ± 6.2 (15)*	12.6 ± 3.4 (18)†

Values are given as means ± SD (*n*). *Different at sea level (SL), and on the 1st and 16th days at 5260 m (ALT1, ALT16, respectively). †Different from ALT1. Abbreviations: ICA, internal carotid artery; CVRi, cerebrovascular resistance index; VA, vertebral artery; gCBF, global cerebral blood flow; D_{O2}, oxygen delivery; MCA, middle cerebral artery; and Q, cardiac output.

in measures of regional and global CBF and D_{O_2} were not correlated with Lake Louise Questionnaire symptom scores of AMS ($r = -0.07$ to -0.23, $P = 0.38$–0.78), nor were they different between those reporting severe AMS and those remaining healthy ($P = 0.57$–0.97).

Following acclimatization, a 32 ± 36% rise in ventilation was accompanied by a 5.5 ± 2.7 mmHg decrease in P_{aCO_2} and 9.2 ± 4.1 mmHg increase in P_{aO_2} (ALT1 *versus* ALT16; all $P < 0.001$). The values of S_{aO_2} and [Hb] rose by 6 ± 5% and 1.8 ± 0.9 g dl^{-1}, respectively, improving C_{aO_2} by 3.1 ± 1.2 ml dl^{-1} (all $P < 0.001$; Table 1). Arterial blood pressure was unaffected by acclimatization (ALT1 *versus* ALT16; $P = 0.211$). The ICA_{flow}, VA_{flow} and gCBF returned to SL values (SL *versus* ALT16; $P = 0.810$, 0.977 and 0.620, respectively; Table 2). Respective CVRi values increased as both ICA and VA diameters decreased from ALT1 to ALT16 (all $P < 0.020$) and restored the relative distribution of cardiac output back to SL values (SL *versus* ALT16; $P = 0.121$). Cerebral D_{O_2} fell from ALT1 to ALT16 (ICA D_{O_2} $P = 0.028$, VA D_{O_2} $P = 0.020$ and global D_{O_2} $P = 0.011$) as the reductions in CBF outweighed the increase in C_{aO_2} (Fig. 1); however, neither global nor regional cerebral D_{O_2} values fell below that measured at SL (all $P > 0.420$; Figs 1 and 2).

Discussion

This is the first study to assess regional cerebral oxygen delivery in the field over a period of acclimatization to high altitude. Our findings confirm that global cerebral D_{O_2} was preserved across acclimatization through a changing balance between CBF and C_{aO_2}, but there was a slight increase in relative D_{O_2} to the posterior cerebral circulation during acute exposure. Although changes in CBF and D_{O_2} were not associated with the incidence or severity of AMS, regional regulation of CBF may serve to support vital homeostatic cerebral functions in hypoxia.

Preservation of cerebral oxygen delivery

The increase in CBF upon arrival at high altitude and decrease back to sea-level values with acclimatization was opposed by changes in C_{aO_2} (Fig. 1). These responses preserved cerebral D_{O_2} close to sea-level values and affirm that components of C_{aO_2} (P_{aO_2}, S_{aO_2} and [Hb]) outweigh the influence of P_{aCO_2} in regulating CBF in severe hypoxia. Increased CBF upon arrival at high altitude resulted from reduced cerebral vascular resistance rather than increased blood pressure (Tables 1 and 2). Although a reduction in cerebral vascular resistance is commonly attributed to dilatation of pial and parenchymal arterioles in the brain (Fog, 1938), we observed an increased diameter of larger tributary arteries, supporting a global vascular response to this degree of hypoxia (Heistad *et al.* 1978; Faraci & Heistad, 1990; Willie *et al.* 2012). The mechanisms governing hypoxic vasodilatation are complex, involving local (e.g. astrocyte regulation, nitric oxide) and diffuse mechanisms (e.g. central chemoreception, autonomic nervous system), but all stem from a reduction in P_{aO_2} (Severinghaus, 2001; Xu & Lamanna, 2006). When P_{aO_2} is above 60 mmHg, little vasodilatation is evident (Mardimae *et al.* 2012; Willie *et al.* 2012). Below this threshold, the degree of vasodilatation increases exponentially and outweighs the degree of hypocapnic vasoconstriction

(Mardimae *et al.* 2012; Willie *et al.* 2012); presumably, to provide greater blood flow in a time of need. While the correlation between changes in gCBF and C_{aO_2} was not significant, the change in C_{aO_2} from SL to ALT1 was similar among all subjects and may not have afforded an appropriate range of values to detect the relationship that has previously been shown with progressive haemodilution (Korosue & Heros, 1992). Qualitatively, the ∼70% increase in gCBF was within the expected range during acute hypocapnic hypoxia (Severinghaus, 1966, 2001; Jensen *et al.* 1990; Brugniaux *et al.* 2007) and proportional to the ∼60% reduction in P_{aO_2} that was responsible for the reduction in C_{aO_2}. This reciprocal relationship, whether evolved or serendipitous, is advantageous for survival in these extreme conditions because it mitigates the negative consequences of cerebral hypoxaemia.

Although increased CBF has been suggested to play a role in the pathogenesis of AMS (Baumgartner *et al.* 1994), our results were more similar to those refuting the hypothesis (Jensen *et al.* 1990; Baumgartner *et al.* 1999). Regional and global CBF and D_{O_2} measurements were not correlated with AMS symptom scores and did not differentiate between those with severe AMS and those who remained healthy after rapid ascent to high altitude. Nonetheless, our data should be interpreted with caution because it is possible that increased CBF contributes to the development of AMS when other, yet to be described, factors are present.

Increased P_{aO_2} and decreased P_{aCO_2} after 16 days at high altitude are hallmarks of ventilatory acclimatization that are addressed elsewhere (Fan *et al.* 2014). As a result, P_{aO_2}-mediated vasodilatation was reduced and P_{aCO_2}-mediated vasoconstriction was increased, thereby lowering CBF. Assuming a cerebral O_2 reactivity of 3% CBF $\%S_{aO_2}^{-1}$ and a CO_2 reactivity of 4% CBF (mmHg $CO_2)^{-2}$ from a previous duplex ultrasound study (Willie *et al.* 2012), we could account for the entire decrease in gCBF across acclimatization. Specifically, the 5% increase in S_{aO_2} could be expected to reduce CBF by ∼15% and the 5.5 mmHg decrease in P_{aCO_2} could be

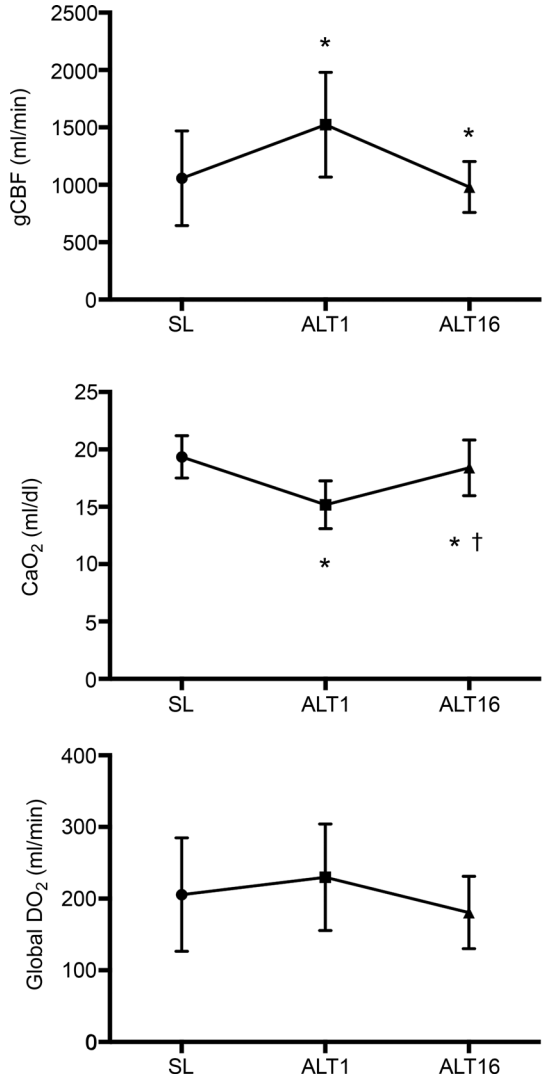

Figure 1. Reciprocal changes in global cerebral blood flow (gCBF) and arterial oxygen content (C_{aO_2}) maintained global cerebral oxygen delivery (D_{O_2}) throughout the study
∗Different from sea level (SL). †Different from arrival at altitude (ALT1). Abbreviation: ALT16, 16th day at 5260 m.

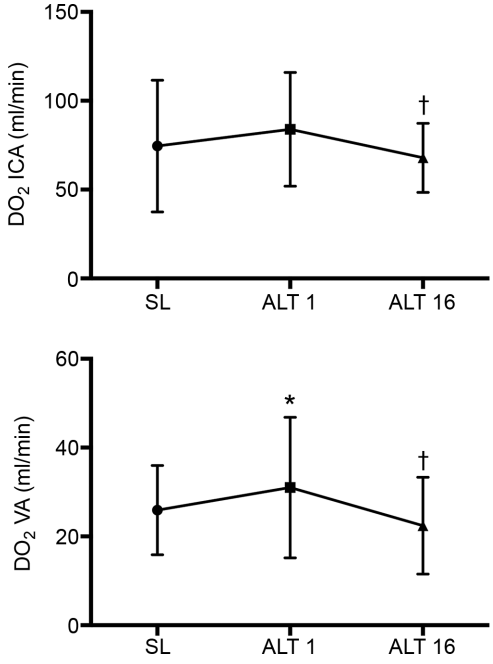

Figure 2. Regional oxygen delivery (D_{O_2}) increases in the vertebral artery (VA) but not in the internal carotid artery (ICA) on arrival at altitude (ALT1)
Regional D_{O_2} is reduced with acclimatization, but not below sea-level (SL) values. ∗Different from SL. †Different from ALT1.

expected to reduce CBF by ∼22%, thus accounting for the 36% decrease in gCBF we observed from ALT1 to ALT16 (Table 2). We acknowledge that increased cerebrovascular CO_2 reactivity with acclimatization in our subjects (Fan *et al.* 2014) may account for an even greater proportion of the net effect on CBF at ALT16. Also, the relative influence of other haematological factors, such as increased haematocrit and blood viscosity (Sorensen *et al.* 1974; Todd *et al.* 1994; Tomiyama *et al.* 1999) from erythropoiesis and plasma volume contraction, may have contributed to the reduction of CBF throughout acclimatization (data to be presented elsewhere). Yet our data suggest that the inherent vascular reactivities to O_2 and CO_2 are sufficient to maintain tight control over cerebral D_{O_2} in hypoxia. Consistent delivery of oxygen may help to offset the decreased P_{O_2} gradient (plasma to mitochondria) and support the cerebral metabolic demand for oxygen at this altitude (Severinghaus *et al.* 1966; Møller *et al.* 2002) to preserve cerebral function. Together, our data demonstrate that integrated mechanisms controlling cerebral blood flow are well suited to preserve global cerebral oxygen delivery at 5260 m.

Regional cerebral oxygen delivery

We observed a small increase in D_{O_2} through the posterior cerebral circulation upon arrival at high altitude (Table 2) that dissipated with acclimatization. The acute increase in D_{O_2} was characterized by an increase in VA diameter and supports recent findings of greater VA (*versus* ICA) vasoreactivity during acute hypoxia (Willie *et al.* 2012; Ogoh *et al.* 2013). Of note, Ogoh *et al.* (2013), showed that acute hypoxia (∼15 min) increased VA, but not ICA, blood flow. Given that the areas perfused by the VA include the brainstem and posterior aspects of the thalamus and hypothalamus, increased blood flow and D_{O_2} to these regions during acute hypoxia (Buck *et al.* 1998; Binks *et al.* 2008) may be seen as necessary to maintain vital homeostatic functions (Sheldon *et al.* 1979; Bilger & Nehlig, 1993). As increased cardiorespiratory drive with acclimatization was not associated with a continued elevation of VA D_{O_2}, we speculate that the increased VA D_{O_2} during acute hypoxia was protective, to defend against a potential threat to oxygen supply, rather than merely to support neuronal metabolic activity associated with heightened autonomic activity (i.e. neurovascular coupling). Although such hypothetical explanations for regional differences in the regulation of CBF and D_{O_2} are intriguing, our results must be interpreted with caution because measured differences were small and are not consistently reported in the literature (Huang *et al.* 1987; Willie *et al.* 2013). Future studies with more focal measurements of D_{O_2} (e.g. PET and MRI) and neuronal activity in key regulatory regions of the brain, as well as measurements of neurovascular coupling (as an index of neuronal plasticity) during acute and prolonged hypoxia are needed to yield further insight into this question.

Brain sparing

Reduced cerebral vascular resistance associated with vasodilatation upon arrival at altitude can explain the proportional increase in CBF and greater allocation of cardiac output. This effect could be magnified if there is net constriction in other vascular beds at rest. Previous studies have shown that superior mesenteric and renal artery blood flow decrease in acute hypoxia and could allow for greater perfusion of the brain (Greene & Roach, 2004). With acclimatization, cerebral vascular resistance and blood flow returned to sea-level values. These results are similar to fetal 'brain-sparing' effects (Campbell *et al.* 1967; Peeters *et al.* 1979; Sheldon *et al.* 1979) that are presumed to preserve vital homeostasis during hypoxia *in utero* (Pearce, 2006; Salihagić-Kadić *et al.* 2006). Similar effects have also been shown in newborn dogs (Cavazzuti & Duffy, 1982), piglets (Goplerud *et al.* 1989) and premature infants (Daven *et al.* 1983). The largest response to hypoxia tends to occur in the brainstem during the early postnatal period and decreases with age (Bilger & Nehlig, 1993). We are the first to demonstrate that such a brain-sparing reaction exists in healthy human adults exposed to acute hypoxia and recedes with acclimatization. Preferential distribution of cardiac output to the brain upon acute altitude exposure may represent a conserved mechanism that protects against hypoxic brain damage in mammals, particularly in brain regions associated with basic cardiovascular and respiratory control during periods of acute hypoxia. Measurements of regional cerebral metabolism are needed to determine whether brain sparing effectively matches D_{O_2}, or if the increase in CBF represents a protective form of overcompensation.

Limitations

Our rapid ascent profile in combination with supplemental oxygen during transport from low to high altitude was designed to induce an abrupt change in P_{aO_2}, similar to that which can be achieved in laboratory studies with hypoxic gas or hypobaric chambers. As such, our results must be interpreted in this context and thus may be expected to be different from other field studies that have followed more traditional progressive ascents (Huang *et al.* 1987; Jensen *et al.* 1990; Baumgartner *et al.* 1994; Willie *et al.* 2013).

We used duplex sonography primarily because it is a non-invasive technique that can be used in field settings. This technique yields volumetric measurements, in terms

of millilitres per minute, which, based on first principles, can be multiplied by C_{aO_2} to yield D_{O_2}. Our low coefficients of variation were in line with a previous study showing similarity between duplex sonography and both PET and xenon inhalation methods of measuring gCBF (Schöning & Scheel, 1996). Nevertheless, we acknowledge that all these techniques are limited by the lack of an absolute standard for validating CBF. Our gCBF measurements were based on unilateral, left-sided measurements of the ICA and VA, which are the main arteries perfusing the brain. While left VA flow has been reported to be ∼20% higher than the right (Schöning *et al.* 1994), this was not expected to have an effect on global measurements because ICA flow represents the majority of gCBF (Schöning & Scheel, 1996). Yet, unilateral VA measurements may have influenced our finding of increased VA D_{O_2}. Future studies are needed to determine whether brain-sparing effects are attenuated when independent measurements of left and right VA flow are summed.

Given that the ICA feeds the MCA, we expected that changes in ICA flow would be reflected in $MCA_{velocity}$. This was not the case; ICA flow increased by ∼70% while $MCA_{velocity}$ was unchanged throughout the study. A similar discrepancy between ICA flow and $MCA_{velocity}$ has been described previously by Willie *et al.* (2012) and argued to support dilatation of the MCA in hypoxia (Wilson *et al.* 2011). We calculated that a 12% increase in MCA diameter could explain the measured discrepancy between ICA_{flow} and $MCA_{velocity}$. This exact degree of vasodilatation has recently been demonstrated at high altitude with a colour-coded ultrasound technique (Willie *et al.* 2013), yet because additional studies are needed to clarify artery-specific responses to hypoxia and validate MCA-diameter measurement techniques, we chose to refrain from further interpretation of $MCA_{velocity}$.

Summary and implications

Overall, our findings highlight the integrative nature of responses that preserve oxygen delivery to the brain at high altitude. Regional cerebral vasoreactivity to O_2 and CO_2 may favour oxygen delivery to posterior and inferior regions of the brain during acute hypoxia to sustain vital cerebral functions associated with homeostasis. Whether these mechanisms evolved to promote survival in conditions provoking cerebral hypoxia is not clear at present, but further research in this area may yield important insights into human tolerance and adaptation to chronic states of hypoxaemia.

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Additional Information

Competing interests

None declared.

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Article two

Fan JL, Subudhi AW, Evero O, Bourdillon N, Kayser B, Lovering AT, Roach RC. (2014). AltitudeOmics: Enhanced cerebrovascular reactivity and ventilatory response to CO_2 with high altitude acclimatisation and re-exposure. *Journal of Applied Physiology (1985)* **116,** 911-918.

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HIGHLIGHTED TOPIC | *Hypoxia*

AltitudeOmics: enhanced cerebrovascular reactivity and ventilatory response to CO_2 with high-altitude acclimatization and reexposure

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[1]*Institute of Sports Sciences, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland;* [2]*Lemanic Neuroscience Doctoral School, University of Lausanne, Lausanne, Switzerland;* [3]*Altitude Research Center, Department of Emergency Medicine, University of Colorado Denver, Aurora, Colorado;* [4]*Department of Biology; University of Colorado, Colorado Springs, Colorado; and* [5]*Department of Human Physiology, University of Oregon, Eugene, Oregon*

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Fan JL, Subudhi AW, Evero O, Bourdillon N, Kayser B, Lovering AT, Roach RC. AltitudeOmics: enhanced cerebrovascular reactivity and ventilatory response to CO_2 with high-altitude acclimatization and reexposure. *J Appl Physiol* 116: 911–918, 2014. First published December 19, 2013; doi:10.1152/japplphysiol.00704.2013.—The present study is the first to examine the effect of high-altitude acclimatization and reexposure on the responses of cerebral blood flow and ventilation to CO_2. We also compared the steady-state estimates of these parameters during acclimatization with the modified rebreathing method. We assessed changes in steady-state responses of middle cerebral artery velocity (MCAv), cerebrovascular conductance index (CVCi), and ventilation ($\dot{V}_E$) to varied levels of CO_2 in 21 lowlanders (9 women; 21 ± 1 years of age) at sea level (SL), during initial exposure to 5,260 m (ALT1), after 16 days of acclimatization (ALT16), and upon reexposure to altitude following either 7 (POST7) or 21 days (POST21) at low altitude (1,525 m). In the nonacclimatized state (ALT1), MCAv and $\dot{V}_E$ responses to CO_2 were elevated compared with those at SL (by 79 ± 75% and 14.8 ± 12.3 l/min, respectively; $P = 0.004$ and $P = 0.011$). Acclimatization at ALT16 further elevated both MCAv and $\dot{V}_E$ responses to CO_2 compared with ALT1 (by 89 ± 70% and 48.3 ± 32.0 l/min, respectively; $P < 0.001$). The acclimatization gained for $\dot{V}_E$ responses to CO_2 at ALT16 was retained by 38% upon reexposure to altitude at POST7 ($P = 0.004$ vs. ALT1), whereas no retention was observed for the MCAv responses ($P > 0.05$). We found good agreement between steady-state and modified rebreathing estimates of MCAv and $\dot{V}_E$ responses to CO_2 across all three time points ($P < 0.001$, pooled data). Regardless of the method of assessment, altitude acclimatization elevates both the cerebrovascular and ventilatory responsiveness to CO_2. Our data further demonstrate that this enhanced ventilatory CO_2 response is partly retained after 7 days at low altitude.

cerebral blood flow; cerebral CO_2 reactivity; rebreathing; altitude acclimatization

THE ABILITY TO MAINTAIN ADEQUATE oxygen transport to the brain by cerebral blood flow (CBF) in hypoxic environments is vital. The CBF responsiveness to CO_2, termed cerebrovascular CO_2 reactivity, provides a useful, noninvasive index of cerebrovascular function (3, 19). To date, only a handful of studies have investigated the effect of acclimatization to high altitude on cerebrovascular CO_2 reactivity (1, 16, 17, 24, 30, 49). It is difficult to interpret the findings from these studies due to the timing of measurements at high altitude (1, 16, 17, 24, 25), the confounding effects of previous high altitude exposure (1), artificial normobaric hypoxia (28, 46), and the method used to assess reactivity (24, 30, 49). Data obtained by Fan et al. (16, 17) on subjects at different stages of altitude acclimatization suggest that cerebrovascular CO_2 reactivity is elevated with prolonged exposure to high altitude when using a modified rebreathing technique. In contrast, Lucas et al. (30) reported, using a steady-state technique (poikilocapnic hypoxia), reduced cerebrovascular CO_2 reactivity in the same subjects assessed at the end of a 14-day stay at 5,050 m. More recently, Rupp et al. (49) reported a reduced cerebrovascular CO_2 reactivity during steady-state hypoxic hypercapnia following 5 days at 4,350 m. Thus the effect of altitude acclimatization on cerebrovascular CO_2 reactivity remains unclear.

In addition, it is unknown whether and for how long changes in cerebrovascular CO_2 reactivity from acclimatization persist after descent. Repetitive 7-mo exposures to high altitude were reported to improve arterial O_2 saturation (Sa_{O_2}), lower resting heart rate (HR), and decrease susceptibility to acute mountain sickness (AMS) upon subsequent reexposures (59). Remarkably, these prior exposure adaptations persisted despite a 5-mo deacclimatization period. The specific effect of high-altitude reexposure on cerebrovascular and ventilatory responsiveness to CO_2 has yet to be examined.

Changes in cerebrovascular CO_2 reactivity with high-altitude acclimatization depend on the method of assessment. At sea level, the steady-state method results in higher cerebrovascular CO_2 reactivity (40–42) and lower ventilatory CO_2 sensitivity (6, 18, 23, 55) compared with the modified rebreathing test. These differences have been attributed to the presence of a P_{CO_2} gradient (between alveolar, arterial, and cerebrospinal fluid compartments) during the steady-state method, which is supposedly abolished or minimized during rebreathing (6). Meanwhile, elevated basal $\dot{V}_E$ and subsequent underestimation of the ventilatory CO_2 sensitivity has been proposed as one possible explanation for lower steady-state estimates (34). No studies have directly compared the steady-state and modified rebreathing test estimates of cerebrovascular and ventilatory CO_2 responsiveness following ascent or acclimatization to high altitude.

The purpose of the present study was therefore twofold: first, we wished to assess the effect of altitude exposure on cerebro-

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vascular and ventilatory responsiveness to CO_2 in acute conditions after acclimatization and upon reexposure to high altitude after a period spent at low altitude; second, we wished to compare the steady-state and modified rebreathing methods for assessing the ventilatory and cerebrovascular responsiveness to CO_2 at high altitude.

METHODS

Subject Recruitment and Screening

This study was conducted as part of the AltitudeOmics project. Following institutional ethics approval, young (19–23 years old), healthy, sea-level residents were recruited from the greater Eugene, Oregon, area (elevation 130 m). Potential subjects were screened to exclude anyone who was born or had lived at altitudes >1,500 m for more than 1 year or had traveled to altitudes >1,000 m in the past 3 mo. A detailed description of subject recruitment procedures, including inclusion and exclusion criteria, has been presented elsewhere (54).

Ethical Approval

The study was performed according to the Declaration of Helsinki and was approved by the institutional review boards of the University of Colorado and the University of Oregon, and by the Human Research Protection Office of the U.S. Department of Defense. All participants were informed regarding the procedures of this study, and written informed consents were obtained prior to participation.

Experimental Design

After familiarization with the experimental procedures outlined below (*visit 1*), the subjects underwent experimental trials near sea level (SL) (130 m; barometric pressure 749 mmHg) and three times at high altitude (5,260 m, Mt. Chacaltaya, Bolivia; barometric pressure 406 mmHg) on the 1st and 16th days at high altitude (ALT1 and ALT16, respectively), and again after either 7 (POST7; $n = 14$) or 21 (POST21; $n = 7$) days at low altitude (1,525 m; barometric pressure 639 mmHg). An overview of the entire experimental design and protocol has been described in detail elsewhere (54).

Experimental Protocol

For each subject, all ALT measurements were carried out around the same time of day to minimize any confounding effect of circadian rhythm. Measurements were taken upon arrival at ALT1 to minimize the influence of AMS. Likewise, no symptoms of AMS were observed at ALT16 or POST7.

For this study, following 10–15 min of quiet rest in a seated position, each experimental testing session consisted of *1*) instrumentation, *2*) 10 min in room air at baseline, and *3*) cerebrovascular CO_2 reactivity tests. The cerebrovascular CO_2 reactivity tests consisted of *1*) 10 min with end-tidal P_{CO_2} ($P_{ET_{CO_2}}$) clamped at 40 mmHg; *2*) 3 min of voluntary hyperventilation to lower $P_{ET_{CO_2}}$ to ~20 mmHg; *3*) the modified rebreathing test (details below); and *4*) 3 min with $P_{ET_{CO_2}}$ clamped at 50 mmHg. The entire cerebrovascular CO_2 reactivity protocol was carried out in a background of hyperoxia (end-tidal P_{O_2} [$P_{ET_{O_2}}$] >250 mmHg).

Experimental Setup

Throughout the protocol, the subjects sat upright and breathed through a mouthpiece attached to a two-way, nonrebreathing valve (Hans-Rudolph 2700, Hans-Rudolph, Shawnee, KS). The breathing circuit allowed switching from room air to either an end-tidal clamping system or a rebreathing system. The end-tidal clamping setup used in the present study is a modified version of the system previously described by Olin et al. (39). The setup allowed stabilizing $P_{ET_{CO_2}}$ at 40 and 50 mmHg. Throughout the end-tidal P_{CO_2} clamping, we maintained $P_{ET_{O_2}}$ at >250 mmHg by titrating 50% or 100% O_2 into the inspiratory reservoir at SL and ALT, respectively.

Modified Rebreathing Method

The modified rebreathing method is well established for assessing both ventilatory and cerebrovascular CO_2 reactivities (14, 16, 34, 41). By using hyperoxia ($P_{ET_{O_2}}$ >250 mmHg) the test minimizes the output of peripheral chemoreceptors (11, 21), and the ventilatory response to the modified rebreathing method can thus be interpreted as the ventilatory CO_2 sensitivity primarily from the central chemoreflex. The details of the modified rebreathing method have been previously described in Fan al. (16, 17). The rebreathing bag was filled with gas to achieve inspired P_{CO_2} and P_{O_2} of 0 mmHg and 300 mmHg, respectively, at each altitude. Subjects were instructed to hyperventilate for 3 min (*part 2*) to lower and then maintain $P_{ET_{CO_2}}$ at 20 mmHg at both sea level and 5,260 m (in background $P_{ET_{O_2}}$ >250 mmHg). Subjects were then switched to the rebreathing bag, and following two initial deep breaths to mix the gas from the bag with that in the respiratory system, they were instructed to breathe ad libitum (*part 3*). The rebreathing tests were terminated when $P_{ET_{CO_2}}$ reached 50 mmHg, $P_{ET_{O_2}}$ dropped below 200 mmHg, or the subject reached the end of his or her hypercapnic tolerance.

Measurements

Cerebrovascular variables. Middle cerebral artery velocity (MCAv, an index of cerebral blood flow) was measured in the left middle cerebral artery using a 2-MHz pulsed Doppler ultrasound system (ST3; Spencer Technology, Seattle, WA). The Doppler ultrasound probe was positioned over the left temporal window and held in place with an adjustable plastic headband (Marc 600 Headframe; Spencer Technology). The signal was acquired at depths ranging from 43 to 54 mm. Signal quality was optimized, and an M-mode screen shot was recorded to facilitate subsequent probe placements. Peripheral saturation was measured on the right side of the forehead by pulse oximetry (N-200; Nellcor, Hayward, CA).

Cardiovascular variables. Beat-to-beat mean arterial blood pressure (MAP) was measured from an arterial catheter inserted in a radial artery, and connected to a calibrated, fluid-filled, disposable pressure transducer positioned at the level of the heart (DELTRAN II; Utah Medical, Salt Lake City, UT). HR was determined using three-lead electrocardiography systems (ADInstruments BioAmp & Micromaxx; SonoSite, Bothell, WA). Cerebrovascular conductance index (CVCi) was calculated using the equation CVCi = MCAv/MAP and normalized to values obtained at a $P_{ET_{CO_2}}$ of 20 mmHg, and expressed as percentage change.

Respiratory variables. $\dot{V}_E$ was measured using a pneumotachograph (Universal Ventilation Meter; Vacu·Med, Ventura, CA; Ultima series; Medgraphics CPX, Minneapolis, MN) and expressed in units adjusted to body temperature and pressure, saturated (BTPS). $P_{ET_{O_2}}$ and $P_{ET_{CO_2}}$ were measured using fast-responding gas analyzers (O_2Cap Oxygen analyzer; Oxigraf, Mountain View, CA). The pneumotachograph was calibrated using a 3-liter syringe (Hans-Rudolph 5530) and the gas analyzers were calibrated using gas mixtures of known concentrations of O_2 and CO_2 prior to each testing session.

Arterial blood gas variables. An arterial catheter (20–22 gauge) was placed into a radial artery and blood samples (2 ml) were taken over approximately five cardiac cycle periods. Core body temperature was telemetrically recorded from an ingested pill (CorTemp; HQInc, Palmetto, FL). All samples were analyzed immediately for arterial pH, P_{O_2} (Pa_{O_2}), P_{CO_2} (Pa_{CO_2}) (Rapidlab 248; Siemens Healthcare Diagnostics, Munich, Germany), hemoglobin concentration, and O_2 saturation (Sa_{O_2}) (Radiometer OSM3; Radiometer Medical ApS, Copenhagen, Denmark). The blood gas values were analyzed in triplicate and temperature-corrected (26, 53). Arterial bicarbonate concentration

($[HCO_3^-]$) was subsequently calculated using the Henderson-Hasselbalch equation.

Data Acquisition

All analog data were sampled and recorded at 200 Hz on a personal computer for off-line analysis (Powerlab 16/30; ADInstruments, Bella Vista, Australia).

Data Analysis

Steady-state responses. Because the subjects could not tolerate $P_{ET_{CO_2}}$ clamping at 50 mmHg at ALT16, the steady-state MCAv-CO_2, MAP-CO_2, and CVCi-CO_2 slopes were estimated from the difference in mean MCAv, MAP, and CVCi at the end of 20 and 40 mmHg $P_{ET_{CO_2}}$ clamping (20-s averages) and plotted against the change in Pa_{CO_2} between these two conditions across all time points (SL, ALT1, ALT16, POST7, and POST21). The absolute value of $\dot{V}_E$ at clamp 40 mmHg was used as an estimate of steady-state $\dot{V}_E$ responsiveness to CO_2, because voluntary hyperventilation was necessary to reduce $P_{ET_{CO_2}}$ to 20 mmHg.

Modified rebreathing. The rebreathing data were first reduced to 1-s averages across the entire rebreathing period. The $\dot{V}_E$-CO_2 slopes were analyzed using a specially designed program (Analyse $\dot{V}_E$ Rebreathing programme rev11; University of Toronto, Toronto, ON, Canada) as previously described (15, 16, 34). The MCAv-CO_2 slopes were analyzed using a commercially available graphing program (Prism 5.0d; GraphPad Software, San Diego, CA), whereby segmental linear regression (least squares fit) was used to estimate the MCAv-CO_2 slope during the modified rebreathing. For comparison, we plotted the MCAv-CO_2 slopes using a sigmoid curve as described by Battisti-Charbonney et al. (4) using the Prism program. To minimize the sum of squares for nonlinear regression (Levenberg-Marquardt algorithm) we used the equation $MCAv = a + (b/\{1 + \exp[-(P_{ET_{CO_2}} - c)/d]\})$, where MCAv is the dependent variable in cm/s, $P_{ET_{CO_2}}$ is the independent variable in mmHg, a is the minimum MCAv determined from the mean MCAv of the hypocapnic (hyperventilation) region, b is the maximum MCAv value, c is the midpoint value of MCAv, and d is the range of the linear portion of the sigmoid (inverse reflection of the slope of the linear portion).

We found good agreement in the MCAv-CO_2 slope obtained from these two models ($R^2 = 0.71$). However, due to the range of $P_{ET_{CO_2}}$ used in this study, segmental linear regression generally provided better fit across all conditions, whereas the sigmoidal curve model was the preferred model for only 12 out of 58 trials. As such, only the MCAv-CO_2 slopes obtained using the segmental linear model are presented.

Statistical Analysis

Due to logistical impacts on planning and transportation, not all subjects were able to participate in all high-altitude studies. See the Figs. 1–3 and Table 1 for complete sample size reporting for each procedure. Most data are reported as the improvement over the time of acclimatization (change from ALT1 to ALT16) and as the amount of that improvement that was retained after time at low altitude, calculated as % retention = (POST7 or POST21 − ALT1)/(ALT16 − ALT1)·100 (5). The effects of altitude acclimatization and reexposure (between SL, ALT1, ALT16, POST7, and POST21) on the steady-state MCAv-CO_2 slope, CVCi-CO_2 slope, and $\dot{V}_E$ at 40 mmHg were analyzed using a mixed-model linear regression (IBM SPSS Statistics version 21; IBM, Armonk, NY). To assess the effects of altitude acclimatization (between SL, ALT1, and ALT16) on the rebreathing estimates of MCAv-CO_2 and $\dot{V}_E$-CO_2 slopes, we used mixed-model linear regression analysis (diagonal repeated covariance assumed). The interactions between variables of interest were assessed using correlational (Pearson) analysis (IBM SPSS Statistics version 21). Data are shown as mean ± SD. Results were considered significant at $\alpha < 0.05$. Trends were consider at the $\alpha < 0.10$ level. A priori power calculations ($\alpha = 0.05$, $\beta = 0.20$) were used to determine sample size and limit type II error.

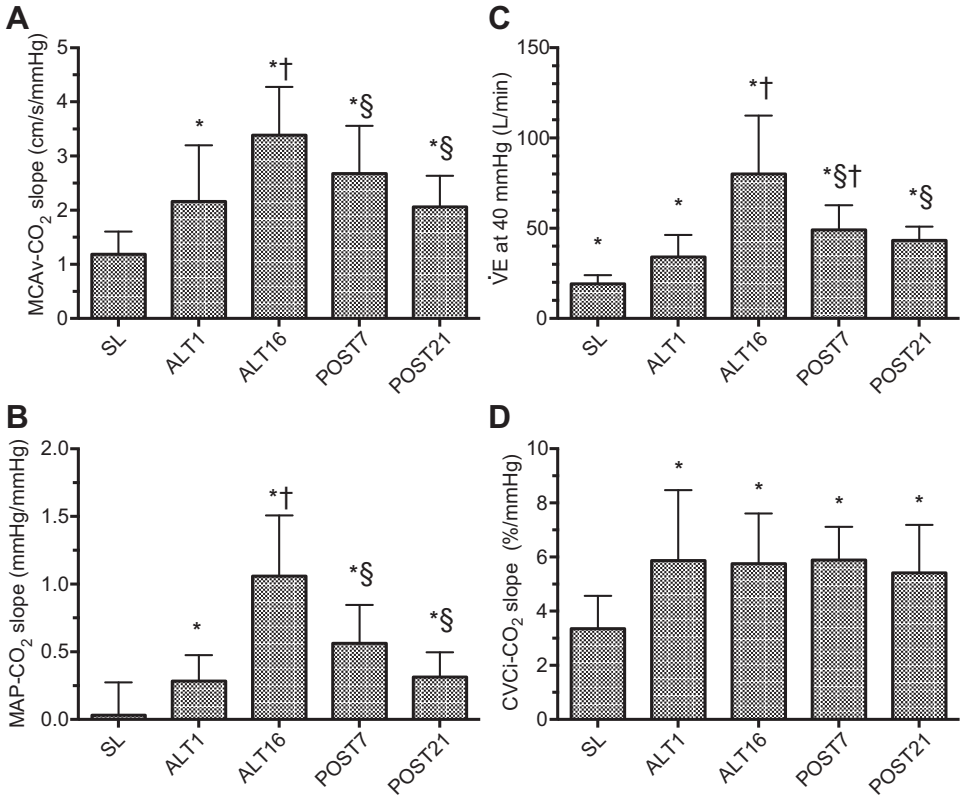

Fig. 1. Changes in steady-state estimates of cerebrovascular, cardiovascular, and ventilatory responsiveness to CO_2 with acclimatization and reexposure to 5,260 m. Values are mean ± SD. *Different from SL ($P < 0.05$); †different from ALT1 ($P < 0.05$), §different from ALT16 ($P < 0.05$).

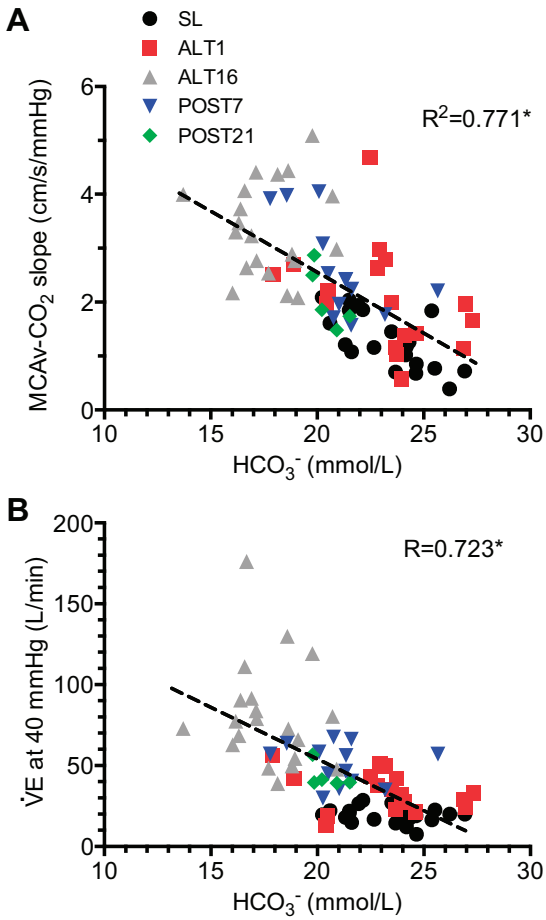

Fig. 2. Relationship between standard basic excess and steady-state cerebrovascular, ventilatory, and cardiovascular responsiveness to CO_2 with acclimatization to altitude. *Significant correlations ($P < 0.05$).

RESULTS

Detailed baseline characteristics of the 21 (9 women; age 21 ± 1 years) subjects participating in AltitudeOmics are presented elsewhere (54). All 21 subjects completed the protocol at SL. Due to logistical issues, 4 of 21 subjects were unable to complete the entire experimental protocol at ALT1. Upon reexposure to altitude, 14 of 14 subjects completed the protocol at POST7, and 5 of 7 completed the protocol at POST21. No comparison was carried out between ALT1 and POST21 due to the low number of subjects.

Resting Variables

The resting variables across acclimatization and reexposure have already been reported in detail elsewhere (54) and will not be reproduced in this paper.

Steady-State Method

Acclimatization. Compared with SL, the steady-state MCAv-CO_2 slope was elevated at ALT1 (by 79 ± 70%; $P < 0.001$), and was further elevated at ALT16 (by 89 ± 70% vs. ALT1; $P = 0.001$) (Table 1). Similarly, the steady-state MAP-CO_2 slope was elevated at ALT1 (by 0.24 ± 0.23 mmHg/mmHg; $P = 0.013$) and further elevated at ALT16 (by 0.80 ± 0.46 mmHg/mmHg vs. ALT1; $P < 0.001$). The steady-state CVCi-CO_2 slope was elevated at ALT1 (by 82 ± 79%; $P < 0.001$), and remained higher at ALT16 (by 93 ± 81%; $P < 0.001$ vs. SL, no difference with ALT1). $\dot{V}_E$ at 40 mmHg was elevated at ALT1 compared with SL (by 14.8 ± 12.3 l/min; $P = 0.011$), and further elevated at ALT16 (by 48.3 ± 32.0 l/min vs. ALT1; $P < 0.001$).

Reexposure. Upon reexposure to altitude, it appears that the acclimatization gained in the steady-state MCAv-CO_2 slope was not retained at POST7 ($P = 0.145$ vs. ALT1). Compared with ALT16, the steady-state MCAv-CO_2 slope was lowered at both POST7 and POST21 ($P = 0.029$ and $P = 0.003$, respectively), but nevertheless remained higher compared with SL ($P < 0.001$ and $P = 0.024$, respectively). Similarly, 49% of the acclimatization gained in the MAP-CO_2 slope was retained at POST7. Specifically, the MAP-CO_2 slope remained higher at POST7 compared with ALT1 ($P = 0.005$). Compared with ALT16, the MAP-CO_2 slope was lowered at both POST7 and POST21 ($P < 0.001$ for both). Nevertheless, the MAP-CO_2 slope was higher at POST7 and POST21 compared with SL ($P < 0.001$ and $P = 0.020$, respectively). In contrast, no difference was observed in the CVCi-CO_2 slope at POST7 compared with ALT1 or ALT16 ($P = 0.980$ and $P = 0.804$, respectively), but it remained higher compared with SL ($P < 0.001$). Likewise, the CVCi-CO_2 slope tended to remain higher

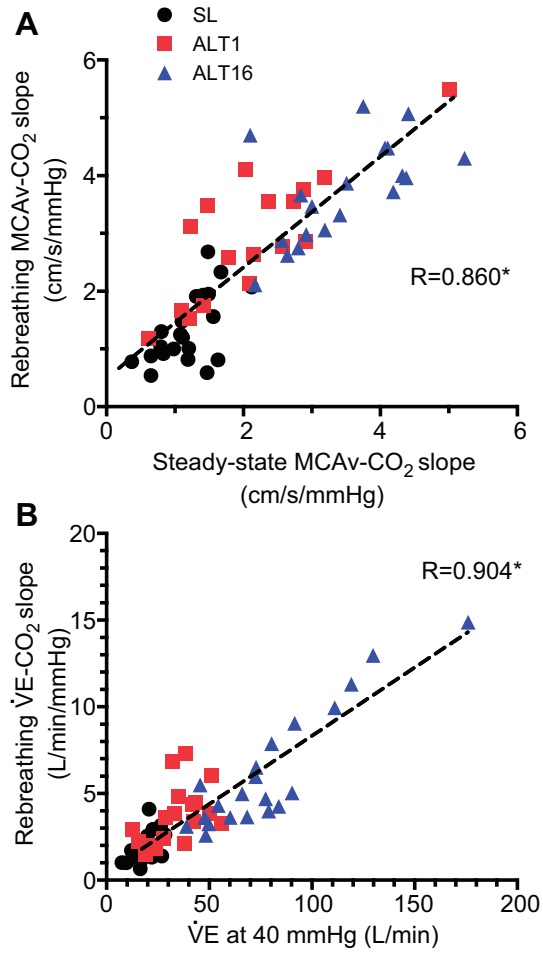

Fig. 3. Comparison of steady-state and rebreathing estimates of cerebrovascular and ventilatory responsiveness of CO_2 with acclimatization to 5,260 m. *Significant correlations ($P < 0.05$).

Table 1. *Cerebrovascular and ventilatory reactivity parameters during the steady-state and modified rebreathing*

	SL (n = 21)	ALT1 (n = 17)	ALT16 (n = 20)	POST7 (n = 14)	POST21 (n = 5)
Steady-state					
MCAv-Pa_{CO_2} slope (cm·s^{-1}·mmHg^{-1})	1.19 ± 0.42	2.16 ± 1.05*	3.39 ± 0.89*†	2.68 ± 0.88*‡	2.06 ± 0.57*‡
CVCi-Pa_{CO_2} slope (%/mmHg)	3.35 ± 1.21	5.87 ± 2.60*	5.75 ± 1.85*	5.89 ± 1.23*	5.41 ± 1.78*
MAP-Pa_{CO_2} slope (l/min)	0.03 ± 0.24	0.28 ± 0.19*	1.06 ± 0.45*†	0.56 ± 0.29*‡	0.32 ± 0.18*‡
$\dot{V}_E$ at 40 mmHg (l/min)	19.15 ± 4.89	34.06 ± 12.23*	80.05 ± 32.32*†	49.03 ± 13.68*†‡	43.25 ± 7.56*‡
Modified rebreathing					
MCAv-$P_{ET_{CO_2}}$ slope (cm·s^{-1}·mmHg^{-1})	1.34 ± 0.60	2.95 ± 1.11*	3.67 ± 0.87*†	—	—
$\dot{V}_E$-CO_2 slope (l·min^{-1}·mmHg^{-1})	1.90 ± 0.81	3.49 ± 1.51*	6.28 ± 3.56*†	—	—
$\dot{V}_E$ recruitment threshold (mmHg)	38.7 ± 3.4	33.7 ± 3.7*	29.2 ± 2.1*†	—	—

All values are mean ± SD. SL, sea level; ALT1, day 1 at high altitude; ALT16, day 16 at high altitude; POST7, reexposure following 7 days at low altitude; POST21, reexposure following 21 days at low altitude. *Different from SL ($P < 0.05$); †different from ALT1 ($P < 0.05$); ‡different from ALT16 ($P < 0.05$).

at POST21 compared with SL ($P = 0.058$) but was not different from ALT16 ($P = 0.715$).

Upon reexposure, the effect of acclimatization on $\dot{V}_E$ at 40 mmHg was retained by 38% at POST7 ($P = 0.004$ vs. ALT1). Compared with ALT16, $\dot{V}_E$ at 40 mmHg was lower at POST7 and POST21 ($P = 0.001$ and $P < 0.001$, respectively), but these values remained higher compared with SL ($P < 0.001$ and $P = 0.001$, respectively).

Modified Rebreathing Method

Similar to the steady-state method, the rebreathing MCAv-CO_2 slope was elevated at ALT1 (by 137 ± 117%; $P < 0.001$), and further elevated at ALT16 (by 35 ± 33% vs. ALT1; $P = 0.040$) (Table 1). The rebreathing $\dot{V}_E$-CO_2 slope was elevated at ALT1 compared with SL (by 1.61 ± 1.14 l·min^{-1}·mmHg^{-1}; $P = 0.038$), and further elevated at ALT16 (by 2.86 ± 2.61 l·min^{-1}·mmHg^{-1} vs. ALT1; $P = 0.004$). The ventilatory recruitment threshold was lowered at ALT1 (by 4.4 ± 4.0 mmHg; $P < 0.001$ vs. SL) and further lowered at ALT16 (by 4.4 ± 3.2 mmHg vs. ALT1; $P < 0.001$).

Acid-Base Buffering Capacity Correlations

Based on previous findings (16), we performed correlations between the pooled steady-state data with [HCO_3^-] and found that resting [HCO_3^-] correlated with the steady-state MCAv-CO_2 slope ($R = -0.771$) and $\dot{V}_E$ at 40 mmHg ($R = -0.723$; $P < 0.001$ for both) (Fig. 2).

Steady-State vs. Modified Rebreathing

We observed correlations between the steady-state and rebreathing MCAv-CO_2 slope at SL ($R = 0.609$; $P = 0.003$), ALT1 ($R = 0.817$; $P < 0.001$), and ALT16 ($R = 0.596$; $P = 0.007$), whereas the pooled MCAv-CO_2 slopes (combined SL, ALT1, and ALT16) between the two methods also correlated well ($R = 0.860$; $P < 0.001$) (Fig. 3). Likewise, there were significant correlations between $\dot{V}_E$ at 40 mmHg and the rebreathing $\dot{V}_E$-CO_2 slope at SL ($R = 0.476$; $P = 0.029$), ALT1 ($R = 0.506$; $P = 0.038$), and ALT16 ($R = 0.927$; $P < 0.001$), whereas the pooled ventilatory data across all time points also correlated ($R = 0.904$; $P < 0.001$).

DISCUSSION

The present study is the first to assess the effect of altitude acclimatization and reexposure on cerebrovascular CO_2 reactivity using both the steady-state and modified rebreathing methods. We demonstrate that cerebrovascular CO_2 reactivity was elevated immediately upon arrival at 5,260 m and is further elevated following 16 days of acclimatization regardless of the method of assessment. In addition, we found that cerebrovascular and ventilatory responsiveness to CO_2 remains elevated upon reexposure to altitude, despite 7 or 21 days at low altitude. Because these changes in cerebrovascular and ventilatory responsiveness to CO_2 correlated with the changes in resting arterial [HCO_3^-] across all time points, we speculate that these changes might be partly due to an altered pH buffering capacity associated with exposure to high altitude. Our data thus demonstrate that the changes in cerebrovascular and ventilatory control gained due to altitude acclimatization over a period of 16 days are partially preserved upon subsequent exposure to altitude, at least for up to a period of 3 wk spent at low altitude.

Effects of Acclimatization on Cerebrovascular CO_2 Reactivity

Our findings extend those from Fan et al. (16, 17) by demonstrating that the MCAv-CO_2 slope is elevated upon arrival at 5,260 m and further elevated following 16 days of acclimatization (Fig. 1*A*). Importantly, previous studies by Fan et al. (16, 17) assessed MCAv-CO_2 slope in subjects who spent 8 days ascending to 5,050 m, whereas the subjects in the present study ascended rapidly to altitude (~3 h), thus making direct comparison difficult. Our findings contradict those of Lucas et al. (30), who found that the MCAv-CO_2 slope was initially elevated at 5,050 m, but had returned toward sea level values following 2 wk at 5,050 m. However, because $P_{ET_{O_2}}$ was not controlled, the MCAv-CO_2 slopes reported by Lucas et al. (30) reflect MCAv changes from polkilocapnic hypoxia (room air breathing at 5,050 m: $P_{ET_{O_2}}$ ~48 mmHg and $P_{ET_{CO_2}}$ 26–22 mmHg) to hypercapnic hyperoxia ($P_{ET_{O_2}}$ > 310 mmHg and $P_{ET_{CO_2}}$ ~30 mmHg), and thus do not represent isolated reactivity to CO_2. Rupp et al. (49) recently found the MCAv response to steady-state hypoxic hypercapnia ($P_{ET_{O_2}}$ = 55 mmHg) to be reduced following 5 days at 4,350 m. Therefore, discrepancies between findings by Rupp et al. (49) and those of the present study can be attributed the differences in $P_{ET_{O_2}}$ (55 mmHg vs. >200 mmHg), altitude (4,350 m vs. 5,260 m), and acclimatization state of the subjects (5 days vs. 16 days). The results from the present study demonstrate for the first time that cerebrovascular CO_2 reactivity per se is enhanced with acclimatization to high altitude when studied using a background level of hyperoxia. Furthermore, discrepancies between studies

highlight how methodological differences can yield vastly different results. Thus future studies are warranted to clarify the effect of hypoxic and hyperoxic background on assessing cerebrovascular functions at both sea level and following ascent to high altitude.

Altered Acid-Base Buffering Capacity?

During altitude acclimatization, there is a progressive and parallel reduction in arterial and cerebrospinal fluid (CSF) bicarbonate concentration, which serves to compensate for the changes in pH associated with hyperventilation-induced hypocapnia (12, 13, 20). These changes in acid-base buffering capacity, in both the arterial and CSF compartments, would lead to a greater rise in arterial and CSF $[H^+]$ for a given rise in Pa_{CO_2}. In support of this notion, lowering CSF bicarbonate concentration elevates the cerebrovascular CO_2 reactivity in an anesthetized dog model (27), whereas bicarbonate infusion increases cerebral perfusion pressure in patients with posttraumatic head injury (9), elevates cerebral blood volume in preterm infants (57), and lowers ventilation in healthy exercising humans at SL (44). As such, it has been suggested that the MCAv responses to CO_2 at high altitude are linked to changes in arterial acid-base balance (16, 25). In the present study, we observed concomitant increases in cerebrovascular and ventilatory responsiveness to CO_2 with acclimatization to high altitude and reexposure (Fig. 1), which occurred in parallel to the changes in $[HCO_3^-]$ (Fig. 2). While such correlations do not imply causality, the possible role for acid-base status changes on cerebrovascular and ventilatory responsiveness to CO_2 at high altitude remains to be further studied.

Interaction Between Cerebrovascular and Ventilatory Responsiveness to CO_2

Interaction between cerebrovascular CO_2 reactivity and central chemoreceptor activation was first alluded to by Heyman et al. (22) and has been subsequently expanded upon by others (10, 16–18, 38, 43, 60–62). It was postulated that changes in cerebrovascular CO_2 reactivity affect the stability of the ventilatory response to CO_2 by modulating the degree of H^+ washout at the level of the central chemoreceptor (38). Accordingly, a blunted cerebrovascular CO_2 reactivity would lead to less central H^+ washout and subsequently greater central chemoreceptor activation. Conversely, an enhanced cerebrovascular CO_2 reactivity would result in lower central $[H^+]$ and therefore lower ventilatory CO_2 sensitivity. In agreement with previous altitude studies (16, 17), we observed concomitant increases cerebrovascular and ventilatory responsiveness to CO_2 (Fig. 1). These findings seem to contradict the modulating role of cerebrovascular CO_2 reactivity on central chemoreceptor activation, possibly due to other overriding factors such as enhanced central chemosensitivity and changes in acid-base balance associated with ascent to high altitude. Future work is necessary to further unravel the interaction between the regulation of cerebral blood flow and ventilation.

Going Back Up

Despite the large body of literature regarding high-altitude acclimatization over the past century, the effect of prior exposure on physiological parameters during subsequent exposures is not well documented. Most attention has focused on the effect of a recent altitude exposure on the risk for AMS (7, 31, 45, 51) or the rate of ascent (56). However, the dose of previous altitude exposure and acclimatization were generally not controlled in these studies. Wu et al. (59) found a progressive reduction in the incidence of AMS, lower HR, and higher SpO_2 in lowland railroad workers over the course of several 7-mo exposures to high altitude interspersed with 5 mo spent at low altitude. Similarly, MacNutt et al. (32) found faster rate of ascent, lower AMS, and higher SpO_2 in trekkers with a recent altitude exposure compared with altitude-naive trekkers, despite a 7- to 30-day deacclimatization period. In the present study, we compared the cerebrovascular and ventilatory responsiveness to CO_2 with acclimatization and upon reexposure to 5,260 m following a period of either 7 or 21 days at low altitude. We found that 38% of the gain in ventilatory response to CO_2 over acclimatization was retained at POST7 (Fig. 1*C*), whereas essentially none of the gain in MCAv-CO_2 reactivity over acclimatization was retained at POST7 (Fig. 1*A*). Regardless of the underpinning mechanism(s), our findings suggest that the effect of previous altitude acclimatization over 16 days on the ventilatory response to CO_2 is partially retained after 7 days at low altitude, whereas it is reversed in the cerebrovascular response to CO_2. Our data extend findings by Muza et al. (36) showing that ventilatory acclimatization gained at 4,300 m is retained following 8 days spent at low altitude. Because we found the CVCi-CO_2 slope to be consistently elevated by 60–80% across all time points (Fig. 1*D*), whereas the changes in MAP-CO_2 slope closely follow the changes in MCAv-CO_2 slope (Fig. 1*B*), we speculate that the changes in MCAv-CO_2 slope at high altitude can be primarily accounted for by an enhanced sensitivity of the cerebral vessels to CO_2, whereas the remainder can be attributed to an enhanced perfusion pressure response.

Steady-State or Modified Rebreathing Method?

There has been much debate over the use of the steady-state or the modified rebreathing method for the assessment of cerebrovascular and ventilatory control, and attempts at consensus have produced no uniform agreement [(18, 40), also see (2, 14) for reviews]. The steady-state ventilatory responses to CO_2 were found to be either similar (34, 37, 40–42, 47) or lower (6, 18, 23, 55) compared with rebreathing estimates, whereas steady-state cerebrovascular CO_2 reactivity has been shown to be consistently higher than rebreathing values (18, 40–42). The present study demonstrates that the changes in cerebrovascular and ventilatory CO_2 responsiveness with altitude acclimatization were similar between the steady-state and the modified rebreathing method (Table 1), possibly due to tight control of arterial P_{CO_2} and P_{O_2} with our end-tidal clamping setup. Moreover, we observed strong correlations in these parameters between the two methods across all time points (Fig. 3). We therefore conclude that both methods can be used to assess the changes in cerebrovascular and ventilatory responses to CO_2 with high altitude exposure and acclimatization, provided that the level of CO_2 is comparable across all the conditions, under identical levels of background O_2.

Limitations

Although the present study provided the opportunity to assess the effects of acclimatization and reexposure to 5,260 m on cerebrovascular CO_2 reactivity, an important methodological consideration should be acknowledged when interpreting our findings. In the present study, transcranial Doppler ultrasound (TCD) was used to measure MCAv as an index of global CBF changes during initial exposure, acclimatization, and subsequent reexposure to 5,260 m. This is based on the assumption that *1*) the MCA carries approximately upward of 80% of the overall blood flow to the respective hemisphere (29); *2*) changes in MCAv reflect changes in global CBF (8, 52); *3*) the changes in MCAv in response to Pa_{CO_2} changes are comparable to the changes in internal carotid blood flow (50); and *4*) the diameter of the MCA does not change during the observed changes in arterial blood gases (52). In support, MCAv has been shown to reflect changes in CBF assessed with the direct Fick method, at least during initial exposure to high altitude (33, 35, 48).

Recent findings by Wilson et al. (58) indicate that the diameter of the MCA, as measured using TCD, varies depending on the altitude (e.g., 5.30 mm at 75 m, 5.51 mm at 3,500 m, 5.23 mm at 5,300 m, and 9.34 mm at 7,950 m). Importantly, the results reported by Wilson et al. (58) demonstrate that the MCA diameter remains relatively unchanged up to 5,300 m. It should be noted that the MCA diameters measured with TCD in that study were 80–90% greater than the values obtained using magnetic resonance imaging in the same subjects. Because our measurements were carried out in background hyperoxia ($P_{ET_{CO_2}}$ >300 mmHg), it seems unlikely that our cerebral blood velocity values would be confounded by any effect of hypoxia-induced vasodilation of the MCA. Further studies are needed to evaluate MCAv responses to CO_2 while holding $P_{ET_{O_2}}$ at consistent levels of hypoxia.

Conclusion

Findings from the present study clearly show that both cerebrovascular and ventilatory responsiveness to CO_2 is elevated upon arrival at high altitude and further elevated with acclimatization. We demonstrate for the first time that this effect of high-altitude acclimatization on the ventilatory response to CO_2 is partially retained after a period at low altitude, whereas prior acclimatization has no effect on the cerebrovascular response to CO_2. Our data suggest that the increased cerebrovascular CO_2 reactivity with acclimatization may be accounted for by the changes in acid-base balance in the blood and possibly the CSF compartment.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.-L.F., A.W.S., O.E., A.T.L., and R.C.R. conception and design of research; J.-L.F., A.W.S., O.E., and N.B. performed experiments; J.-L.F. and A.W.S. analyzed data; J.-L.F., A.W.S., N.B., B.K., and R.C.R. interpreted results of experiments; J.-L.F. prepared figures; J.-L.F. drafted manuscript; J.-L.F., A.W.S., O.E., B.K., A.T.L., and R.C.R. edited and revised manuscript; J.-L.F., A.W.S., O.E., N.B., B.K., A.T.L., and R.C.R. approved final version of manuscript.

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Article three

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AltitudeOmics: cerebral autoregulation during ascent, acclimatization, and re-exposure to high altitude and its relation with acute mountain sickness

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Subudhi AW, Fan JL, Evero O, Bourdillon N, Kayser B, Julian CG, Lovering AT, Panerai RB, Roach RC. AltitudeOmics: cerebral autoregulation during ascent, acclimatization, and re-exposure to high altitude and its relation with acute mountain sickness. *J Appl Physiol* 116: 724–729, 2014. First published December 26, 2013; doi:10.1152/japplphysiol.00880.2013.—Cerebral autoregulation (CA) acts to maintain brain blood flow despite fluctuations in perfusion pressure. Acute hypoxia is thought to impair CA, but it is unclear if CA is affected by acclimatization or related to the development of acute mountain sickness (AMS). We assessed changes in CA using transfer function analysis of spontaneous fluctuations in radial artery blood pressure (indwelling catheter) and resulting changes in middle cerebral artery blood flow velocity (transcranial Doppler) in 21 active individuals at sea level upon arrival at 5,260 m (ALT1), after 16 days of acclimatization (ALT16), and upon re-exposure to 5,260 m after 7 days at 1,525 m (POST7). The Lake Louise Questionnaire was used to evaluate AMS symptom severity. CA was impaired upon arrival at ALT1 ($P < 0.001$) and did not change with acclimatization at ALT16 or upon re-exposure at POST7. CA was not associated with AMS symptoms (all $R < 0.50$, $P > 0.05$). These findings suggest that alterations in CA are an intrinsic consequence of hypoxia and are not directly related to the occurrence or severity of AMS.

transcranial Doppler; cerebral blood flow; cerebral oxygenation; transfer function analysis; hypoxia

CEREBRAL AUTOREGULATION (CA) is a general term used to describe dynamic myogenic, neurologic, and metabolic responses that adjust cerebrovascular resistance to maintain relatively constant cerebral blood flow across a wide range of perfusion pressures (25). Dynamic CA is said to be impaired if fluctuations in mean arterial blood pressure (ABP) lead to concurrent fluctuations in mean cerebral blood flow. Impairments in CA are associated with cerebrovascular disorders (3, 24, 31), yet the relative importance of CA in the development and course of certain pathologies is unclear.

Our initial interest in CA stemmed from the hypothesis that impaired CA may be involved in the development of acute mountain sickness (AMS), high-altitude headache, and cerebral edema (5, 7, 9, 16, 37). Conversely, we showed that impairments in CA upon acute exposure to hypobaric hypoxia preceded, but were not associated with, the development of AMS (2, 33, 35). Furthermore, since several cross-sectional studies demonstrated that impairments in CA persist from 1 to 30 days of high-altitude exposure (1, 2, 11, 12, 17)—when AMS is not present—and are evident in healthy, permanent high-altitude residents (12, 13), it seems reasonable to suggest that a shift in CA may be an inherent and relatively benign consequence of hypoxemia.

To date, no longitudinal studies have characterized CA and tested its relation with AMS during acute and chronic high-altitude exposures. Previous studies have either omitted CA measurements upon arrival at high altitude (7, 11, 17) or followed slow ascent profiles that allow for partial acclimatization before initial measurements (1, 12, 39). In this study, we present novel data from sea-level (SL) residents who rapidly ascended to high altitude (5,260 m; ALT1), acclimatized for 16 days (ALT16), and were subsequently re-exposed to high altitude after spending 7 days at low altitude (1,525 m; POST7). Specifically, we tested the hypotheses that CA would be: *1*) impaired upon rapid ascent to high altitude, *2*) unaffected by 16 days of acclimatization, *3*) unaffected upon re-exposure to the same altitude, and *4*) unrelated to the occurrence or severity of AMS.

METHODS

Study overview. This study was conducted as part of the AltitudeOmics project. Briefly, institutional ethics approval was obtained from the Universities of Colorado and Oregon and the U.S. Department of Defense Human Research Protection Office. Young, healthy SL residents were recruited from the greater Eugene, Oregon, area (elevation 128 m) and screened to exclude anyone who was born or had lived at altitudes >1,500 m for more than 1 yr or had traveled to altitudes >1,000 m in the past 3 mo. After obtaining written consent, physical exams and the Army Physical Fitness Test (push-ups, sit-ups, and a 3.2-km run) were performed to verify health and fitness status. Approximately 4 wk following SL measurements in Eugene, Oregon, subjects were flown to La Paz, Bolivia. They spent two nights at low altitude (1,525 m in Coroico, Bolivia) before being driven to the Chacaltaya Research Station at 5,260 m, while breathing supplemental oxygen. Acute responses to high altitude were assessed ~4 h after arrival and cessation of supplemental oxygen (ALT1). Subjects acclimatized to altitudes ranging from 3,800 to 5,260 m over the next 15 days, with most of the time (75%) spent at 5,250 m. On the 16th day (ALT16), measurements were repeated at 5,260 m before subjects were driven down to Coroico for either 7 or 21 days. Subjects were driven back to the laboratory at 5,260 m for POST7 or POST21 re-exposure measurements.

This report focuses on novel data regarding resting CA, evaluated immediately before a series of cerebrovascular, respiratory, and exercise interventions, as outlined elsewhere (32). We have carefully

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avoided replication of data among reports, except where common variables were necessary to describe subjects' basic physiologic status at the time points of interest [e.g., heart rate (HR), blood pressure, arterial blood gases].

Subjects. We studied 21 subjects at SL (12 men and nine women; 21 ± 1 yr old). Because of logistical problems upon arrival in Bolivia, complete data sets were not obtained on the first seven subjects upon arrival at ALT1. Since the first seven subjects comprised the cohort studied at POST21, longitudinal assessments of CA were limited to the remaining 14 subjects who completed the study at POST7.

Physiology protocol. All subjects were familiarized with study procedures during a practice session at least 48 h before experimental testing at SL. Subjects followed standardized exercise and dietary regimens for 24 h before each measurement period. At each time point, a 22-gauge catheter was inserted into a radial artery at least 1 h before instrumentation. Subjects were seated in an upright position for 15 min, while sensors were placed to measure physiologic variables of interest. Limb lead electrodes were used to measure ECG (Bio Amp; ADInstruments, Colorado Springs, CO). ABP was monitored via a fluid-filled pressure transducer (Deltran II; Utah Medical Products, Midvale, UT) attached to the radial artery catheter. Core temperature was recorded telemetrically from an ingested pill (CorTemp; HQInc, Palmetto, FL). Cerebral blood flow velocity (CBFv) in the left middle cerebral artery (MCA) was measured by transcranial Doppler (2 MHz; Spencer Technologies, Seattle, WA) at depths ranging from 43 to 54 mm. Signal quality was optimized, and an M-mode screen shot was recorded to facilitate subsequent probe placements and insonation angles.

After verification of signal quality, resting data were recorded for 6 min, while subjects breathed room air to assess CA at each altitude. Continuous analog data [ABP, CBFv, ECG, oxygen (O_2), and carbon dioxide (CO_2)] were recorded at 200 Hz (PowerLab 16/30; ADInstruments) for offline analysis. Core temperature and arterial blood samples (2 ml) were taken during the last 30 s of measurement periods. Blood samples were taken from the radial artery catheter, and blood gases were analyzed for partial pressure of arterial CO_2 (Pa_{CO_2}) and partial pressure of arterial O_2 (Pa_{O_2}) in triplicate (RAPIDLab 248; Siemens, Erlangen, Germany) and corrected for body temperature (15, 29).

Acute mountain sickness. Self-reported sections of the Lake Louise Questionnaire (LLQ) were used to assess AMS on ALT1 and POST7 (~12 h after arrival). Moderate and severe AMS was defined as LLQ ≥ 3, and LLQ ≥ 6, including headache, respectively (27).

Data analysis. Transfer function analyses were used to assess dynamic CA, based on spontaneous fluctuations in the raw ABP and CBFv signals, as described previously (33, 34). Briefly, 6-min recordings of instantaneous ABP and CBFv were reduced to beat-by-beat averages, resampled at 5 Hz, and transformed from the time-to-frequency domain using fast Fourier transformations (512 points/segment with 40% overlap). The transfer function from mean ABP to CBFv was expressed in terms of coherence, gain, and phase shift in the very low frequency range (0.02–0.07 Hz), where dynamic CA is most active (21, 22), as well as in low (0.07–0.20 Hz) and high (0.20–0.35 Hz) frequency ranges. All data were used in subsequent statistical analyses. Reduction in phase shift was considered the primary criterion for impaired CA, because it signifies shorter delay in transmission of pressure (ABP) into flow (CBFv) or a reduction in the ability of the cerebrovascular system to buffer changes in ABP and maintain consistent blood flow. Yet, since increases in gain (increase in CBFv relative to a change in ABP) and coherence (linear correlation between ABP and CBFv) may also suggest CA impairment (8, 24, 41), all three transfer function metrics are reported. To address difficulties in interpreting possible permutations of these three variables, the inverse transfer function of the resulting gain and phase shift was used to express results in the time domain as a step function that could be fitted to one of 10 curves representing a single autoregulation index (ARI) score (36). An ARI score of zero indicates complete lack of autoregulation, and nine indicates perfect autoregulation.

Statistics. After calculating descriptive statistics (mean ± SD) and verifying normality (D'Agostino and Pearson tests), variables were analyzed by repeated-measures ANOVA to evaluate the effect of time on CA metrics with Fisher's least significant difference post hoc tests and the Holm procedure to correct for multiple comparisons ($\alpha = 0.05$).

Spearman ρ correlations were run to evaluate relations between CA metrics and the severity of LLQ symptom scores. Specifically, we tested the ability of CA assessments, measured at SL and upon arrival at ALT1, to predict ensuing symptoms of AMS (7). Also, because AMS classification is dichotomous (i.e., positive vs. negative), we used receiver-operating characteristic (ROC) analyses (14, 18) to evaluate the sensitivity (true positive rate) and specificity (true negative rate) of the ability of ARI scores to detect mild and severe AMS. The ROC area under the curve (AUC) statistic was used as an indicator of test accuracy. An AUC of 1.0 signifies a perfect test, with no chance of false-positive or false-negative results, whereas an AUC of 0.5 signifies a meaningless test, where the probability of identifying a true positive result is only 50%.

RESULTS

Effect of rapid ascent to high altitude. At SL, resting cardiovascular (HR, ABP, CBFv) and CA (coherence, gain, phase shift, and ARI scores) measurements were characteristic of young, healthy individuals with intact CA (Table 1 and Fig. 1). From SL to ALT1, Pa_{O_2} and Pa_{CO_2} decreased (65% and 26%, respectively; $P < 0.001$; Table 1). This degree of hypoxia increased HR ($P < 0.001$) but did not affect mean ABP or CBFv. Very low frequency power spectral density of ABP and

Table 1. *Resting data*

Variable		SL	ALT1	ALT16	POST7
Pa_{O_2}	mmHg	103 ± 5	36 ± 3*	45 ± 4*†	42 ± 4*†‡
Pa_{CO_2}	mmHg	37 ± 4	28 ± 2*	21 ± 3*†	24 ± 3*††
HR	beats/min	73 ± 9	90 ± 18*	95 ± 12*	85 ± 15*‡
ABP	mmHg	77 ± 6	76 ± 14	81 ± 10	76 ± 8
CBFv	cm/s	62 ± 9	63 ± 14	59 ± 7	57 ± 9
PSD ABP	$mmHg^2/Hz$	11 ± 13	9 ± 4	9 ± 5	6 ± 4
PSD CBFv	$(cm/s)^2/Hz$	13 ± 19	14 ± 16	10 ± 6	11 ± 8
Coherence		0.42 ± 0.12	0.64 ± 0.15*	0.70 ± 0.16*	0.55 ± 0.12*‡
Gain	%/%	0.64 ± 0.24	0.88 ± 0.35*	0.85 ± 0.25*	0.97 ± 0.33*
Phase shift	radians	0.48 ± 0.28	0.17 ± 0.21*	0.27 ± 0.09*	0.25 ± 0.19*
ARI		4.4 ± 1.0	2.8 ± 0.9*	2.8 ± 1.0*	3.3 ± 1.6*

*Different from sea level (SL); †different from arrival at 5,260 m (ALT1); ‡different from after 16 days of acclimatization (ALT16). $n = 14$; mean ± SD. POST7, re-exposure to 5,260 m after 7 days at 1,525 m; Pa_{O_2}, partial pressure of arterial oxygen; Pa_{CO_2}, partial pressure of arterial carbon dioxide; HR, heart rate; ABP, arterial blood pressure; CBFv, cerebral blood flow velocity; PSD, power spectral density; ARI, autoregulation index.

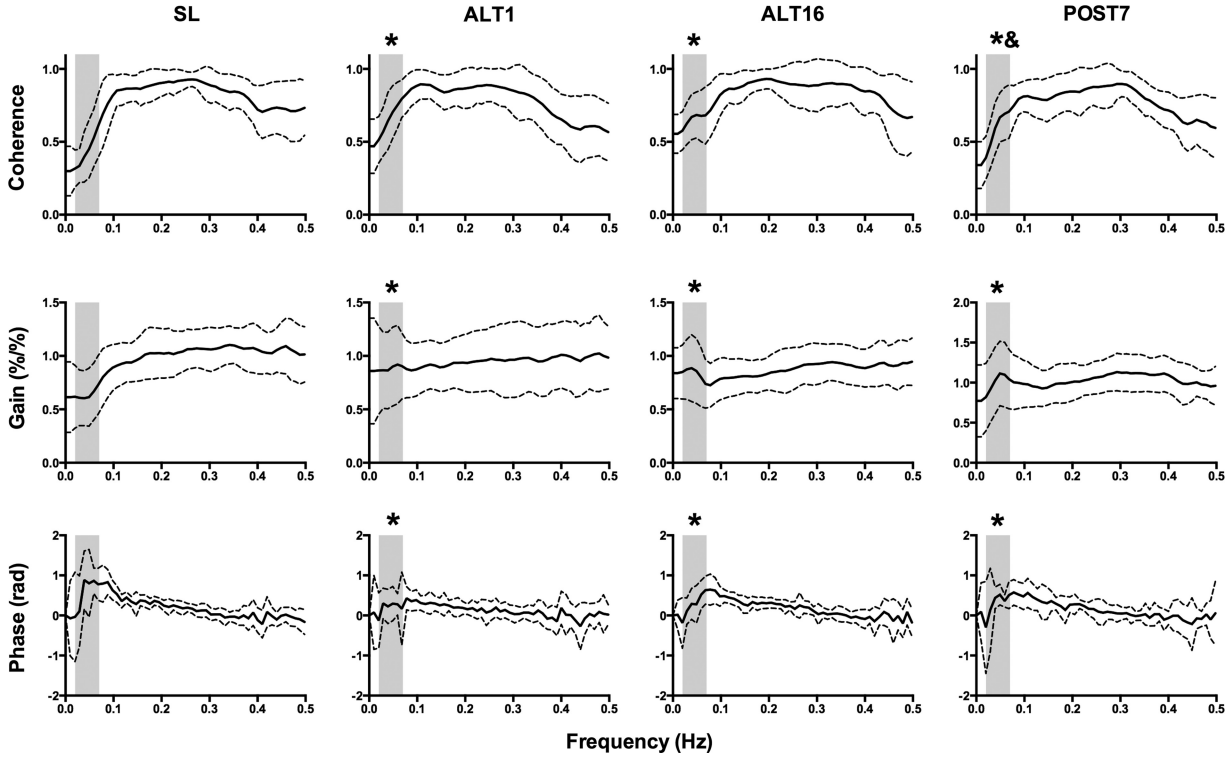

Fig. 1. Arterial blood pressure to cerebral blood flow velocity transfer function metrics (mean ± SD from 0 to 0.5 Hz) at sea level (SL), upon arrival at 5,260 m (ALT1), after 16 days of acclimatization (ALT16), and upon re-exposure to 5,260 m after 7 days at low altitude (POST7). Similar impairments in cerebral autoregulation (increased coherence and gain and decreased phase shift) from SL were seen in the very low frequency (0.02–0.07 Hz; shaded areas) at ALT1, ALT16, and POST7 ($P < 0.05$). rad, radians. *Different from SL; &different from ALT16.

CBFv was unaltered, but increases in transfer function coherence ($P < 0.001$) and decreases in phase shift ($P < 0.050$) and ARI score ($P < 0.001$) were consistent (in 13 of 14 subjects) with the definition of impaired CA at ALT1.

Effect of acclimatization to high altitude. Acclimatization increased resting Pa_{O_2} (27%) and decreased Pa_{CO_2} (22%) from ALT1 to ALT16 (both $P < 0.001$), without affecting HR, ABP, or CBFv. Measures of CA at ALT16 were unchanged from ALT1 and remained impaired relative to SL in the very low frequency range (all $P < 0.010$; Table 1 and Fig. 1).

Effect of re-exposure to high altitude. Resting Pa_{O_2} and Pa_{CO_2} at POST7 fell between ALT1 and ALT16 values (all $P <$ 0.050 vs. ALT1 and vs. ALT16), indicating that the degree of acclimatization achieved at ALT16 was partially maintained at POST7. Assessments of CA at POST7 were similar to those at ALT1 and ALT16 and remained impaired relative to SL in the very low frequency range ($P < 0.050$; Table 1 and Fig. 1).

Association between CA and AMS. Of the 21 subjects, 17 reported symptoms of at least moderate AMS at ALT1 (LLQ = 6.4 ± 2.2), 10 of who met the criteria for severe AMS (LLQ = 7.8 ± 1.7). Correlations among CA metrics preceding the development of AMS symptom were weak (all $r < 0.50$, $P >$ 0.050; Fig. 2). The ROC analysis revealed that ARI scores measured at SL were not sensitive or specific predictors of moderate (AUC = 0.54, $P = 0.788$) or severe (AUC = 0.69, $P = 0.139$) AMS. Additionally, the degree of impairment in CA (measured as the change in ARI from SL to ALT1) was not a sensitive or specific predictor of moderate (AUC = 0.53, $P =$ 0.881) or severe (AUC = 0.72, $P = 0.124$) AMS. None of the 14 subjects studied at POST7 reported symptoms of AMS; thus associations with CA could not be tested.

DISCUSSION

The key findings of this study were that CA, as assessed by transfer function analysis, is *1*) impaired upon rapid ascent to high altitude, *2*) unaffected by acclimatization or *3*) subsequent re-exposure to the same altitude, and *4*) not a sensitive or specific predictor of AMS. Based on our results, we question whether the so-called impairment in CA that persists at high altitude is characteristic of pathological insufficiency in cerebrovascular regulation (16) or alternatively, reflects a relatively benign relaxation in autoregulation.

Effect of high altitude on CA. This is the first longitudinal study of CA at high altitude—from rapid ascent through acclimatization and upon re-exposure after a short period at low altitude. We show that impairment of CA was a consistent characteristic across this high-altitude exposure profile.

Increased transfer function coherence and gain, along with reduced phase shift and ARI score upon rapid ascent, were all consistent with the classic definition of impaired CA (Table 1) and outside the normal range of expected variability (6), implying that changes in ABP were transmitted more readily into the cerebral circulation as changes in CBFv at high altitude. Our finding of impaired CA after <1 day of travel from low to high elevation is consistent with our previous findings after 4 h in a hypobaric chamber (35) and fills an important gap in the literature between studies conducted in laboratories with hypoxic gas mixtures, where normobaric

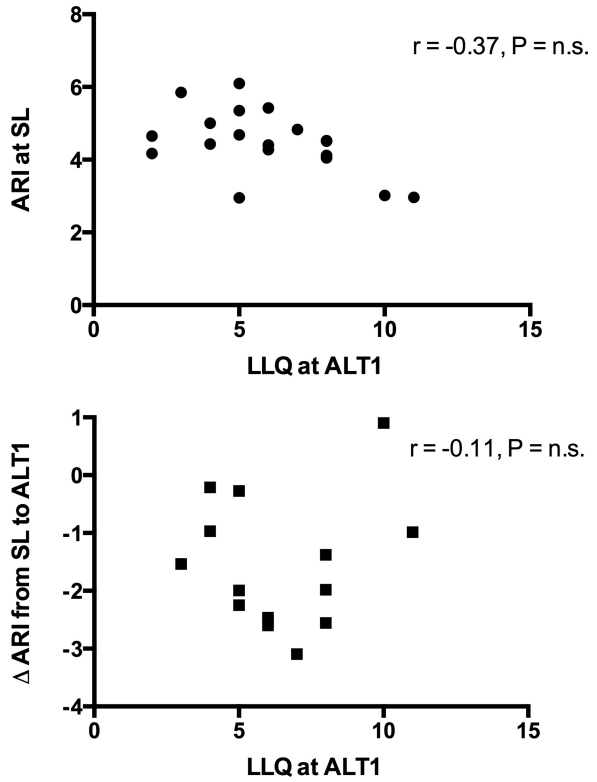

Fig. 2. Scatter plots showing no relation ($P > 0.05$) between autoregulation indices (ARI), measured at SL (*top*) and as the change (Δ) from SL to arrival at high altitude (ALT1; *bottom*), and acute mountain sickness symptoms' scores from the Lake Louise Questionnaire (LLQ) at ALT1.

hypoxia was achieved in a matter of minutes (5, 10, 26, 34), and studies of trekkers, where several days of progressive ascent preceded initial high-altitude measurements (1, 2, 12, 37). Impaired CA at rest in acute hypoxia is a consistent finding among all but one study (26), suggesting that neither the mode nor rate of ascent appears to affect the general assessment.

By evaluating CA upon initial exposure and after 16 days at high altitude, we were able to determine if changes in CA occur with acclimatization, as might be expected with increased Pa_{O_2} (2, 35), decreased Pa_{CO_2} (19, 23, 26), and further sympathoexcitation (1). On the contrary, we found no change in CA over the course of acclimatization (Table 1). Our longitudinal findings are consistent with other cross-sectional studies, demonstrating impaired CA at various time points after arrival at high altitude (1, 2, 7, 11, 12, 37) and in permanent high-altitude residents (12, 13). These results may indicate that assessments of CA are less sensitive to changes in Pa_{O_2} and Pa_{CO_2} near their respective extremes. Alternatively, a slight improvement in CA, due to increased Pa_{O_2} (2, 35), may have been masked if the opposing effects of Pa_{CO_2} (19, 23, 26) and/or sympathoexcitation (1) on CA were heightened over time at altitude. Further testing with manipulation of arterial gases and sympathetic activity is necessary to determine the relative influence of arterial gases and neural stimulation on CA at high altitude, yet impaired CA remains a consistent, functional consequence across time at high altitude.

As an additional test of the hypothesis that impaired CA is a consistent response to hypoxemia, we sent subjects down to low altitude for 7 days and re-evaluated their CA response after a second rapid ascent back to high altitude. Upon re-exposure, the measured impairment in CA was similar to that observed upon the first ascent (ALT1) and after acclimatization (ALT16). Together, these results demonstrate that impaired autoregulation was a consistent characteristic of hypoxemia across our study and imply that slow fluctuations in arterial pressure were dampened less effectively by the cerebral vasculature, regardless of the state of acclimatization. What remains to be determined is if such a tenuous pressure-flow relation may be potentially harmful.

Relation of CA to AMS. Impairment of CA has been suggested to play a role in the development of AMS by either permitting cerebral overperfusion and mechanical disruption of the blood brain barrier (i.e., vasogenic cerebral edema) when mean ABP is elevated or by cerebral underperfusion and exacerbation of cerebral hypoxia/ischemia when mean ABP is lowered (9, 16). In the present study, we found no correlation between measures of CA and subsequent AMS symptom scores (Fig. 2), which opposes the notion that lower CA predisposes people to AMS or conversely, that higher CA confers protection from AMS. Our additional ROC analyses of AMS status confirmed that ARI scores were neither sensitive nor specific indicators for the development of moderate or severe AMS upon arrival at high altitude. These findings are congruent with our previous report following the time course of changes in CA and AMS symptoms over the first 10 h of exposure to hypobaric hypoxia (35), where we found similar levels of CA impairments in subjects who eventually developed AMS or stayed healthy, but are at odds with other studies showing some association between CA and AMS symptoms (5, 37). Our data also counter a recent finding that SL assessments of CA predict ensuing severity of AMS (7).

Discrepancies among studies may be explained by the various methods used to assess CA (transfer function vs. leg cuff; see Limitations below), the questionnaires used to assess AMS (LLQ vs. Environmental Symptoms Questionnaire), and the statistical approach used to evaluate the relation between CA and AMS (correlation vs. ROC). We acknowledge that caution should be exercised when interpreting correlations with an ordinal-level variable, such as the LLQ score, because by definition, the scale has limited mathematical meaning. For example, a LLQ score of six does not imply that symptom severity is exactly twice that of a score of three. Due to the intrinsic level of measurement, we believe that LLQ scores are best restricted to dichotomous classification of positive or negative AMS status and thus place more emphasis on the negative results of our ROC analysis. We encourage others to consider this method of analysis for future AMS studies.

Overall, given the similarity in CA responses among individuals with a wide range of AMS scores, we do not believe that changes in CA cause AMS. This assertion is supported further by the complete lack of association between impaired CA at POST7 when no symptoms of AMS were reported and previous reports documenting impaired CA in healthy, high-altitude natives (12, 13). Nonetheless, we must acknowledge that the alteration in CA upon acute altitude exposure may set up a tenuous pressure-flow relation that could permit AMS to develop if other, yet-unidentified factors are present at the same time.

Since impairment of CA appears to be a consistent physiological response in hypoxic environments and unrelated to AMS status, it is tempting to speculate that the underlying change in the cerebral pressure-flow relation may actually promote successful acclimatization or adaptation to chronic states of hypoxemia (4). It is possible that impairment of CA could promote cerebral oxygen delivery in a time of need, since it allows greater cerebral perfusion for a given increase in ABP. This potentially beneficial consequence of impaired CA during hypoxemic stress might outweigh the relative risk of reduced cerebral perfusion if ABP were to drop. We therefore raise the possibility that the term "impaired CA" may be a misnomer, because it implies an association with pathology that has yet to be substantiated in acute or chronic hypoxemia. We suggest that "relaxation of CA" might be a more accurate term to describe changes in the cerebral pressure-flow relation from normoxia to hypoxia in the absence of pathology.

Limitations. One major limitation affecting the field is the lack of a gold-standard method to assess CA. We have chosen to evaluate rhythmical fluctuations in CA via transfer function analysis, primarily because we believe it captures the natural cerebral pressure-flow relation over time and thus has greater practical relevance over methods that induce larger, more abrupt changes in ABP, as with leg-cuff inflation/deflation, rapid tilting, or more sustained changes in ABP, such as with pharmaceutical interventions. Still, we acknowledge that transfer function analysis of resting data monitors relatively subtle fluctuations in ABP and CBFv, which if amplified, may not show impairment in CA (39). These factors may limit the generalizability of resting CA assessments and lead to an overstatement of the clinical relevance of the findings. Additionally, there are no universal standards for the parameter settings used in transfer function analysis or interpretation of subsequent results, which makes comparisons among studies problematic. Future work is needed to clarify differences in methods used to assess CA in hypoxemic states and evaluate if these changes are generalizable to clinical settings.

Most CA studies rely on transcranial Doppler measurements of flow velocity and assume that vessel diameter is unchanged; yet, there is evidence to suggest that this assumption may be invalid at extreme altitudes (39, 40). Dilation of the MCA at ALT1 may explain why MCA velocity did not follow the expected increase in CBF upon acute exposure to high altitude (30). We do not believe potential MCA dilation affected our interpretation, because the phase shift—our primary criterion for assessing changes in CA—measures the relative timing of oscillations in ABP and CBFv and thus is largely independent of absolute flow. However, since small changes in diameter can have profound effects on flow (flow $\sim$ radius^4), future studies must consider the use of continuous flow measurements, instead of velocity measurements, to assess CA accurately in hypoxia.

Finally, our measurements of CA were limited to the MCA and relied on pressure measurements taken in the radial artery. Since regional differences in cerebrovascular regulation have been reported recently (20, 28, 38), more specific measurements of regional pressure and flow are needed to characterize CA fully.

Conclusions. Our data demonstrate that the initial impairment of CA upon acute exposure to high altitude is invariant with acclimatization and re-exposure, suggesting that relaxation in the regulation of the cerebral pressure-flow relation is a characteristic response to hypoxia that is unaffected by the degree of acclimatization. Since changes in CA do not follow the progression and resolution of AMS, we question the clinical relevance of impaired CA at high altitude.

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DISCLOSURES

The authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

Author contributions: A.W.S., J-L.F., O.E., B.K., C.G.J., A.T.L., R.B.P., and R.C.R. conception and design of research; A.W.S., J-L.F., O.E., and N.B. performed experiments; A.W.S., O.E., and N.B. analyzed data; A.W.S., J-L.F., B.K., R.B.P., and R.C.R. interpreted results of experiments; A.W.S. prepared figures; A.W.S. drafted manuscript; A.W.S., J-L.F., O.E., N.B., B.K., C.G.J., A.T.L., R.B.P., and R.C.R. edited and revised manuscript; A.W.S., J-L.F., O.E., N.B., B.K., C.G.J., A.T.L., R.B.P., and R.C.R. approved final version of manuscript.

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Article four

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Repeated Pre-Syncope from Increased Inspired CO_2 in a Background of Severe Hypoxia

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Abstract

Fan, Jui-Lin, and Bengt Kayser. Repeated pre-syncope from increased inspired CO_2 in a background of severe hypoxia. *High Alt Med Biol.* 15:70–77, 2014.—We describe a case of experimentally induced pre-syncope in a healthy young man when exposed to increased inspired CO_2 in a background of hypoxia. Acute severe hypoxia ($FIO_2 = 0.10$) was tolerated, but adding CO_2 to the inspirate caused pre-syncope symptoms accompanied by hypotension and large reductions in both mean and diastolic middle cerebral artery velocity, while systolic flow velocity was maintained. The mismatch of cerebral perfusion pressure and vascular tone caused unique retrograde cerebral blood flow at the end of systole and a reduction in cerebral tissue oxygenation. We speculate that this occurrence of pre-syncope was due to hypoxia-induced inhibition of brain regions responsible for compensatory sympathetic activity to relative hypercapnia.

Key Words: vasovagal syncope, hypoxia, hypercapnia

Introduction

Syncope is defined as a transient loss of consciousness followed by rapid recovery, and it accounts for a significant portion of emergency department visits and hospital admissions (Kapoor, 1990; Kapoor and Brant, 1992; Van Lieshout et al., 2003). Vasovagal or reflex syncope is the most common type of syncope in both young and elderly subjects (Ganzeboom et al., 2003; Serletis et al., 2006; Tan and Parry, 2008; Van Lieshout et al., 2003) and is preceded by pre-syncope signs (epigastric discomfort, nausea, sweating, desire to change position), with a gradual decline in vascular tone, bradycardia, and hypotension (Kapoor, 2002; Van Lieshout et al., 2003). The pathophysiology of the hypotension/bradycardia reflex in vasovagal syncope is not completely understood, but changes in sympathetic tone are believed to be critical in the peripheral vasodilation preceding syncope (Kapoor, 1990; Kapoor and Brant, 1992; Van Lieshout et al., 2003; Wang et al., 1996). While the hypotension underscores the role of cardiovascular regulation in the development of vasovagal syncope, the subsequent loss of consciousness is ultimately the result of cerebral hypo-perfusion (Ganzeboom et al., 2003; Serletis et al., 2006; Tan and Parry, 2008; Van Lieshout et al., 2003). As such, it is of interest to monitor the response of cerebral blood flow (CBF) while observing such events.

Syncope is well documented during acute exposure to hypoxia (Nicholas et al., 1992; Sagawa et al., 1997; Westendorp et al., 1997; Kapoor, 2002; Roche et al., 2002; Van Lieshout et al., 2003; Halliwill and Minson, 2004), while high altitude acclimatization appears to be rather protective against syncope at high altitude (Thomas et al., 2010). In this case report, we present novel data from two trials of acute exposure to severe hypoxia in combination with relative hypercapnia. We observed pre-syncope symptoms accompanied by hypotension, cerebral hypo-perfusion, and cerebral tissue desaturation in a 21-year old male, when inspired CO_2 tension was increased in a background of hypoxia equivalent to an altitude of 5000 m.

Methods

Subject details

The subject was one of a group of volunteers participating in a study designed to examine the effect of inspired CO_2 on exercise capacity in hypoxia. He was a 21-year-old healthy nonsmoking physically active male (BMI = 23.2, $V_{O_2}max$ = 45 mL/kg/min), with no known medical conditions, not taking any regular medication, and no history of syncope. He abstained from caffeine for 12 h and from heavy exercise and alcohol for 24 h prior to the experiments. The experiments

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were carried out under consistent laboratory conditions (temperature 22.3 ± 0.5°C, humidity 27 ± 7%, barometric pressure 725 ± 4 mmHg). Two medical practitioners were present during the experimental sessions. The study was approved by the Research Ethical Committee of the University Hospitals of Geneva and conformed to the standards set by the *Declaration of Helsinki*.

Consent

Written informed consent was obtained from the subject for publication of this case report.

Measurements

The following variables were recorded continuously: bilateral systolic, diastolic and mean middle cerebral artery velocity (MCAv, transcranial Doppler ultrasound, ST3, Spencer Technology, Seattle, USA; monitoring probe (2 MHz) power: 50%, depth: 57 mm); heart rate (HR), cardiac output (Q'), stroke volume (SV), total peripheral resistance (TPR), beat-to-beat systolic, diastolic and mean arterial blood pressure (ABP, finger plethysmography, Finometer-MIDI, Finapress Medical Systems, Amsterdam, Netherlands); peripheral O_2 saturation (Spo_2; Rad-7, Masimo, Irvine, USA); breath-by-breath ventilation (V'E), partial pressure of end-tidal O_2 ($PETO_2$) and CO_2 ($PETCO_2$) (Medgraphics CPX, Loma Linda, USA). Cerebrovascular resistance (CVR) was estimated by dividing mean MCAv by mean ABP. Beat-to-beat heart rate variability was analyzed with dedicated software (Kubios, version 2.1, freely available at http://kubios.uef.fi). We analyzed the HR data separately during the normoxia, hypoxia, and CO_2 + hypoxia epochs, and when symptoms were maximal, just after coming off the mouthpiece. Because of the nonstationary characteristics of the data, we removed trend components prior to analysis using the smoothing priors algorithm with a lambda of 500, as recommended by the software manufacturer. The data were analyzed in the frequency domain using a fast Fourier transformation and a Welch window of 256 with a 50% window overlap. We report, as a rough index of sympathetic versus parasympathetic activity, the LF/HF (ratio between low frequency (0.04–0.15 Hz) and high frequency (0.15–0.4 Hz) power, unit less).

Experimental protocol

The original protocol foresaw, with the subject seated on a cycle-ergometer, 4 min of normoxia baseline measurements, 2–3 min of hypoxia, followed by 4 min of increased inspired CO_2, to bring $PETCO_2$ back to normoxic resting levels, before starting an incremental exercise protocol.

Throughout the experimental sessions, the subject wore a nose-clip and breathed through a mouthpiece attached to a low resistance one-way non-rebreathing valve (Hans-Rudolph 2700, Kansas City, USA). The hypoxia and added CO_2 to the inspirate were achieved using a modified gas mixing system (Altitrainer, SMTec, Nyon, Switzerland), described in detail by Fan et al., (2013). For this experiment FIO_2 was set at either 0.21 or 0.10 (in Geneva the equivalent of an altitude of about 5000 m) during normoxia and hypoxia conditions, respectively. Under feedback of on-screen $PETCO_2$ (Datex infrared CO_2 analyzer, Helsinki, Finland), $FICO_2$ was continuously adapted as to bring $PETCO_2$ to its target value of 40–45 mmHg. The subject was unaware of the rationale of the study and was not informed to what gas mixture he was breathing.

Results

The subject attended the laboratory on four occasions. On the first two visits, he successfully completed incremental maximal cycling tests (30 w/min ramp) in conditions of normoxia and normoxia + CO_2 without any adverse events. On the third occasion, the subject was seated on the cycle ergometer in preparation of the next test, breathing room-air for 4 min. He was then switched to hypoxia for 3 min, followed by increased inspired CO_2 in hypoxia. After 134 sec, pre-syncope symptoms prompted the subject to come off the mouthpiece and request the session to be aborted. The subject reported symptoms of dizziness, blurry vision, and nausea. We invited the subject to come back to the laboratory on another occasion, this time adding the measurement of oxy-(O_2Hb) and deoxygenated hemoglobin (HHb) over the left frontal cortex with near-infrared spectroscopy (index of brain tissue oxygenation; NIRS, Oxymon Mk-II, Artinis, Zetten, Netherlands), expressed as absolute changes from baseline room-air breathing. The experiments were performed at the same time of the day. On the second occasion, the subject was left on hypoxia for more than 4 min before switching to increased inspired CO_2 to exclude that he was not simply developing a vasovagal reaction to hypoxia. The subject indicated that he was fine, after which he was switched to added CO_2. Following 55 sec of breathing CO_2-enriched hypoxic gas, the subject indicated that pre-syncope symptoms were developing again and came off the mouthpiece, requesting the experiment to be aborted. We then excluded the subject from further participation.

Table 1 shows the values for the measured and derived variables during the different epochs of the two episodes of hypoxic + CO_2, as well as the data from the normoxic + CO_2 visit for reference (means over 20 sec at the end of each epoch). Hypoxia alone lowered $PETO_2$, $PETCO_2$, and SPO_2 during the two episodes by 51–58 mmHg, 3–4 mmHg, and 18%–19 %, respectively (Fig. 1 and 2). At the same time, hypoxia lowered mean ABP, systolic ABP, diastolic ABP, TPR, and HR by 12–20 mmHg, 15–35 mmHg, 11–17 mmHg, 23%–18%, and 12–9 b/min, while V'E, mean, systolic and diastolic MCAv (mean of left and right MCAv), Q', and SV remained relatively unchanged with hypoxia (Fig. 1 and 2). At the end of the period of added CO_2 in hypoxia, when pre-syncope developed, mean, systolic and diastolic ABP were further lowered by 9–18 mmHg, 14–30 mmHg, and 3–15 mmHg, respectively (Fig. 2). SV dropped by 27–26 mL with inspired CO_2 in hypoxia compared to normoxia, while HR remained relatively unchanged compared to hypoxia only (Figs. 1 and 2). Mean and diastolic MCAv had now dropped by 15%–20% and 51%–55%, respectively, compared to normoxia, while both systolic MCAv and CVR remained relatively unchanged (Figs. 1 and 2). During breathing CO_2 enriched gas in hypoxia, we observed a reduced cardiac cycle time, which was predominantly mediated by a reduced time in diastole (Fig. 3). Inspired CO_2 elevated V'E and SPO_2 by 8.1 L/min and 4%, respectively (Table 1). When pre-syncope symptoms were strongest (when the subject came off the mouthpiece), there was bradycardia and hypotension (Figs. 1 and 2). We observed a distinct triphasic pattern in the MCAv

Table 1. Means (over 20 sec) Over Measured and Derived Variables at the End of Each Epoch During the Two Hypoxic + CO_2 Episodes and a Reference Episode (normoxia + CO_2)

	Reference		*Visit 1*				*Visit 2*			
	Normoxia	*Normoxia + CO_2*	*Normoxia*	*Hypoxia*	*Hypoxia + CO_2*	*Post*	*Normoxia*	*Hypoxia*	*Hypoxia + CO_2*	*Post*
Ventilatory										
P_{ETO_2} (mm Hg)	98	115	92	41	46	o.m.	101	43	45	o.m.
P_{ETCO_2} (mm Hg)	37	43	39	36	42	o.m.	41	37	38	o.m.
V'E (L/min)	14	25	15	15	23	o.m.	15	17	24	o.m.
Spo_2 (%)	98	98	97	80	84	85	96	78	80	96
Cerebrovascular										
Mean MCAv (cm/s)	57	65	61	61	52	46	62	53	49	55
Systolic MCAv (cm/s)	91	101	99	108	102	108	103	97	117	88
Diastolic MCAv (cm/s)	40	47	45	40	22	15	43	29	19	35
CVR (mmHg/cm/s)	0.65	0.74	0.72	0.83	0.81	1.21	0.69	0.75	0.83	0.81
Cerebral O_2Hb (μmol)	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	0.24	−2.10	−4.07	.066
Cerebral HHb (μmol)	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	−0.88	2.92	2.30	2.40
Cardiovascular										
Mean ABP (mm Hg)	88	87	85	73	64	41	88	69	51	66
Systolic ABP (mm Hg)	121	123	114	99	85	55	144	110	80	106
Diastolic ABP (mm Hg)	70	70	67	56	53	34	69	53	38	51
TRP (mm Hg/L/min)	0.68	0.63	0.65	0.50	0.51	0.67	0.56	0.46	0.45	0.52
Q' (L/min)	7.8	8.6	7.6	9.1	6.7	3.7	10.5	10.5	9.2	8.6
SV (mL)	106	108	101	97	74	51	110	99	84	90
HR (beat/min)	74	80	81	93	91	72	95	104	113	94
HRV index LF/HF			4.6	4.7	0.9	1.2	6.3	2.2	2.7	1.3

n.m.: not measured; o.m.: off mouth piece.

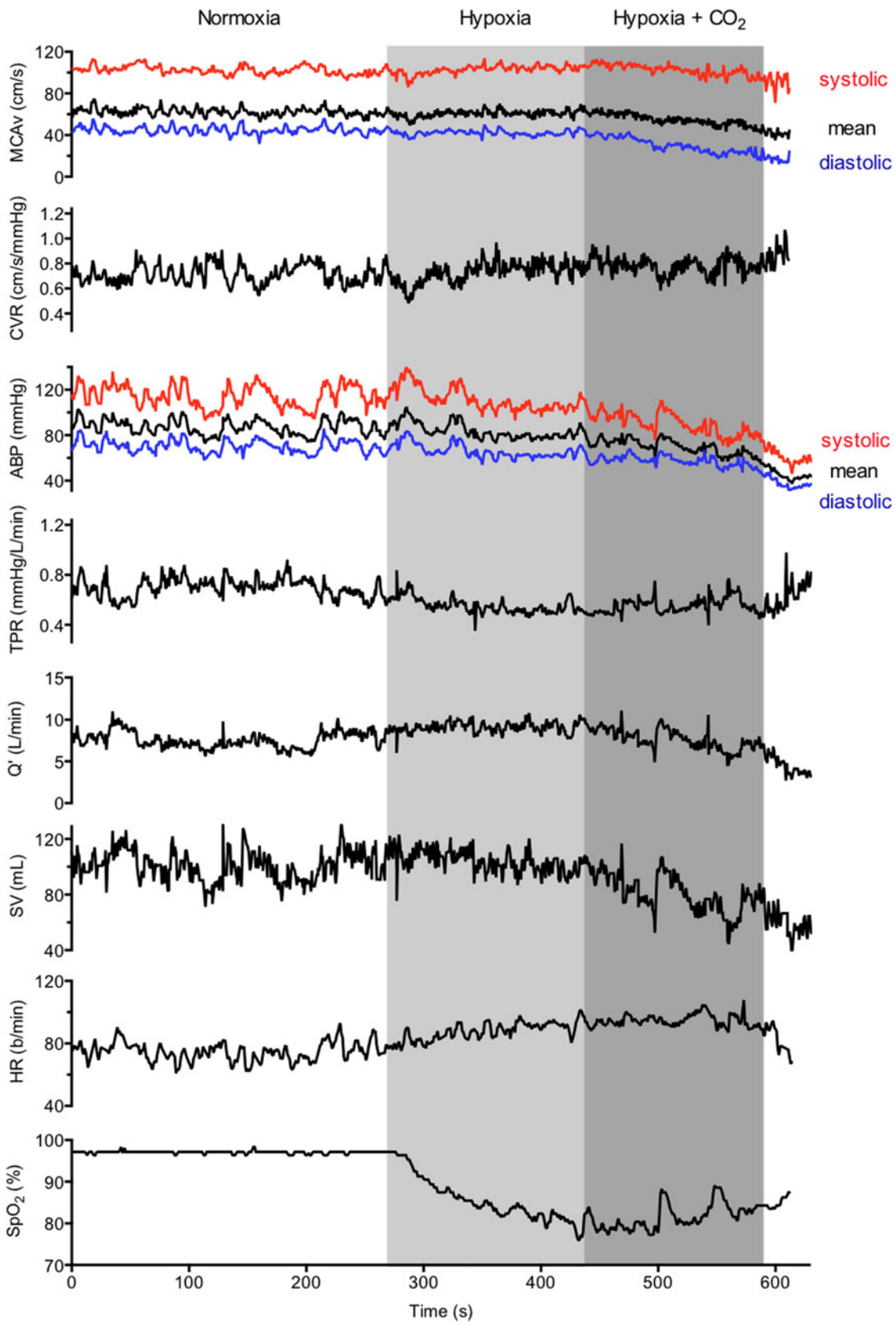

FIG. 1. Cerebrovacsular and cardiovascular variables during visit one. Middle cerebral artery velocity (MCAv), cerebral vascular resistance (CVR), arterial blood pressure (ABP), total peripheral resistance (TPR), cardiac output (Q'Q), stroke volume (SV), heart rate (HR), and peripheral O_2 saturation (Spo_2). During exposure to hypoxia + CO_2 despite increases in HR, mean, systolic and diastolic ABP progressively decrease due to decreases in Q' and SV. This reduction in ABP causes declines in mean and diastolic MCAv, while systolic MCAv is maintained. The mean, systolic, and diastolic ABP continued to decline following the hypoxia + CO_2 exposure, despite the subject coming off the mouthpiece.

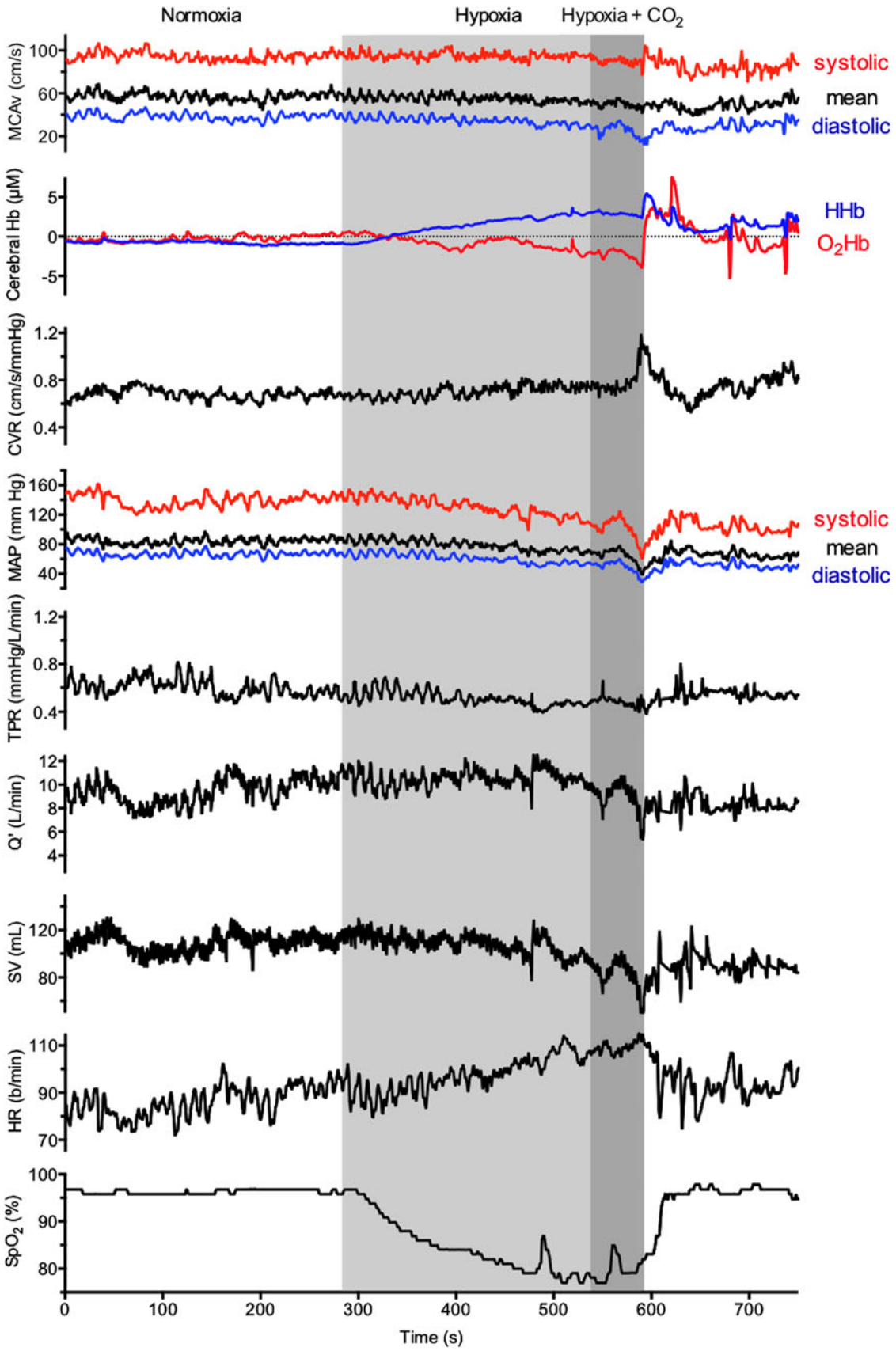

FIG. 2. Cerebrovascular and cardiovascular variables during visit two. Middle cerebral artery velocity (MCAv), cerebral hemoglobin concentration (Hb), cerebral vascular resistance (CVR), arterial blood pressure (ABP), total peripheral resistance (TPR), cardiac output (Q'), stroke volume (SV), heart rate (HR), and peripheral O_2 saturation (SpO_2).

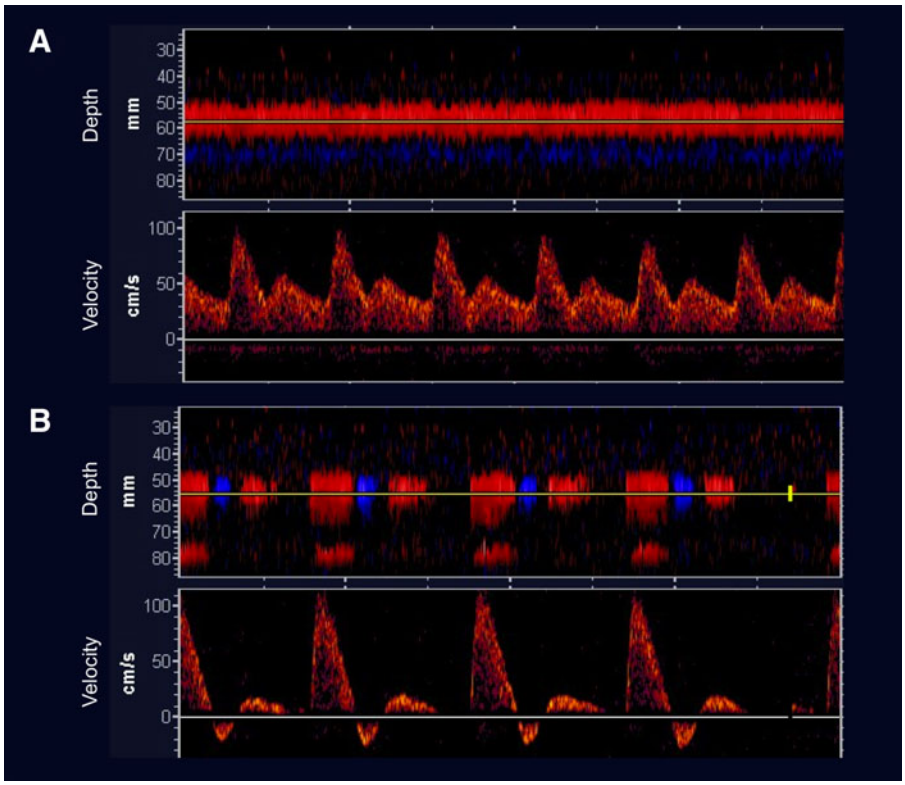

FIG. 3. Middle cerebral artery velocity traces during hypoxia **(A)** and immediately following hypoxia + CO_2 exposure **(B)**. The velocity trace immediately following hypoxia + CO_2 exhibits a distinct triphasic flow pattern, characterized by large flow during systole, followed by retrograde flow at the end of systole and small flow during diastole. The *white horizontal line* denotes zero flow.

traces, which resulted in slight retrograde blood flow at the end of systole (Fig. 3). During the first visit, the HRV index LF/HF was 4.6 during normoxia, 4.7 during hypoxia, 0.9 during hypoxia + CO_2, and 1.2 after the subject came off the mouthpiece. During the second visit, LF/HF was 6.3 during normoxia, 2.2 during hypoxia, 2.7 during hypoxia + CO_2, and 1.3 after the subject came off the mouthpiece.

Discussion

We report the sudden development of pre-syncope symptoms in a healthy 21-year old male during acute exposure to inspired CO_2 in a background of severe hypoxia. The pre-syncope symptoms were accompanied by bradycardia, hypotension, cerebral hypo-perfusion, and cerebral tissue deoxygenation. The observed changes in vital signs, HRV index LF/HF, and development of symptoms seems coherent with a vasovagal reaction.

The mechanisms involved in acute hypoxia syncope are complex. The increased susceptibility to syncope in hypoxia has been attributed to a reduced baroreflex sensitivity (Nicholas, 1992; Sagawa et al., 1997; Roche et al., 2002; Klemenc and Golja, 2011), which is further compounded by greater cardiac output decline and withdrawal of sympathetic vasoconstrictive tone during orthostatic stress in hypoxia, resulting in greater hypotension (Wang et al., 1996; Van Lieshout et al., 2003). Westendorp et al., (1997) proposed that an enhanced vasodilatory response to epinephrine during hypoxia (Blauw et al., 1995) would lead to greater sympathetic withdrawal as well as elevated parasympathetic activity. They further proposed that an increase in atrial natriuretic peptide during hypoxemia might attenuate the carotid baroreflex-mediated cardiac acceleration and inhibit sympathetic nervous activity, thus further increasing the risk of syncope in hypoxia.

To the best of our knowledge, this is the first report documenting reduced CBF during a pre-syncope response triggered by CO_2-enriched inspired gas in a background of hypoxia. The observation during the second episode of a drop in frontal cortex oxygenation suggests that the pre-syncope syndrome was accompanied by reduced cerebral oxygen availability (Fig. 2). We found dramatic reductions in mean and diastolic MCAv during and immediately following hypoxia + CO_2 exposure (Fig. 1). Meanwhile, the reduction in cerebral O_2Hb followed closely to the changes in diastolic MCAv (Fig. 2), suggesting that diastolic perfusion pressure is the main determinant of cerebral O_2 delivery. Vasovagal syncope typically involves both central and peripheral mechanisms. However, since severe and uncompensated hypotension, below the threshold of cerebral autoregulation, leads to cerebral hypo-perfusion (Skinhoj and Strandgaard, 1973; Chillon and Baumbach, 1997), while hypotension *per se* blunts the CBF responsiveness to CO_2 during both normoxic and hypoxic conditions (Harper and Glass, 1965; Ainslie et al., 2012), we attribute the observed reduction in CBF and subsequent cerebral tissue deoxygenation to the direct effect of severe hypotension *per se*.

According to Van Lieshout et al., (2003), when a pre-syncope progresses, it is the fall in mean ABP below the

lower limit of cerebral autoregulation that reduces cerebral perfusion and oxygenation, leading to unconsciousness. In the present case, we observed dramatic reductions in mean ABP with increased inspired CO_2 in background hypoxia, which persisted for some time following the exposure (Fig. 1). In addition to this drop in ABP, we observed a distinct triphasic flow pattern in the CBF velocity waveform, characterized by large flow during systole, followed by a distinct retrograde flow at the end of systole, and very little flow during diastole (Fig. 3). Since CO_2 is a potent vasodilator of the cerebral vasculature, we reasoned that the reduction in mean ABP associated with increased inspired CO_2 would lead to a *greater* mismatch of cerebral perfusion pressure and vascular tone (i.e., impairment of cerebral autoregulation). This mismatch of cerebral perfusion pressure and vascular tone could potentially account for the retrograde flow observed in our subject.

What are potential mechanisms for such an effect of increased inspired CO_2 in a background of hypoxia? In normoxic conditions, hypercapnia has been shown to elicit a vasodilatory effect on the peripheral vasculature in humans (Lennox and Gibbs, 1932; Kontos, 1971; Kontos et al., 1972; Gastaldo et al., 1974; Ainslie et al., 2005). Hypercapnia (10% CO_2) causes an immediate, but transient hypotension, which is corrected within 30–40 sec from increased sympathetic activity, in anesthetized, sino-aortic denervated and vagotomized rats (Takakura et al., 2011). Since inhibition of the retro-trapezoid nucleus (RTN) lowers sympathetic nerve activity and attenuates mean ABP recovery at the end of the hypercapnic exposure (Takakura et al., 2011), those authors concluded that the compensatory increase in sympathetic nerve activity is partly dependent on RTN activation during hypercapnic exposure. Similarly, pontine noradrenergic neurons, astrocytes, neurons of the nucleus solitary tract, and wake-ON orexinergic neurons, have all been shown to be hypercapnia/pH-sensitive and linked to sympathetic tone, contributing to peripheral vascular tone during hypercapnic exposure (Dean et al., 1989; Dun et al., 2000; Johnson et al., 2008; Allen and Barres, 2009). In humans, profound sympatho-inhibition has been reported during exposure to combined hypoxia and hypercapnia, resulting in vasovagal syncope (Halliwill, 2003). Our data of reduced LF/HF (Table 1) are in agreement with that finding. Furthermore, since our subject's ABP was well maintained during hypercapnic exposure in normoxia (Table 1), we speculate that the output of the brain regions responsible for compensatory sympathetic response to hypercapnia might be attenuated during exposure to hypoxia.

An alternative explanation is a blunted peripheral chemoreflex response to severe hypoxia, which might increase an individual's susceptibility to hypoxia-induced syncope due to greater central hypoxic depression associated with greater arterial desaturation. We observed Spo_2 of 78–80% at the end of a 3–4 min exposure to hypoxia (Table 1), at the lower end of the 77%–91% range typically observed in our laboratory using this setup (Fan et al., 2013; Fan and Kayser, 2013). Since cerebral O_2Hb was relatively well maintained during hypoxia and decreased only at the onset of hypotension during hypoxia + CO_2 (Fig. 2), it seems unlikely that a blunted hypoxic ventilatory response and associated arterial (and presumably cerebral) desaturation was responsible of the pre-syncope observed in our case report. Nevertheless, due to a lack of a more prolonged hypoxic control trial, we cannot fully exclude the possibility that hypoxia alone was responsible for the development of pre-syncope observed in this report.

Implications

The present case report, together with previous findings, highlights the increased risk of syncope for climbers and trekkers upon rapid ascent to high altitude. Accordingly, particular care should be taken during postural changes, whereby impaired compensatory mechanisms, coupled with reduced arterial and cerebral tissue oxygenation associated with hypoxic exposure, would lead to greater risk of hypotension and subsequently greater cerebral hypo-perfusion/tissue deoxygenation during orthostatic challenges.

Conclusions

We present a case of repeated pre-syncope during exposure to relative hypercapnia in a background of severe hypoxia. The development of hypotension suggests an impaired compensatory sympathetic response to an increase in $Paco_2$, leading to reduced venous return, decreased stroke volume, and a drop in cardiac output. The resultant mismatch of perfusion pressure and cerebrovascular tone caused a distinctive triphasic flow pattern in the brain, characterized by a unique retrograde flow at the end of systole. Since the subject exhibited no adverse response to CO_2 during normoxic conditions, we speculate that sympatho-inhibition and subsequent loss of systemic vascular tone was responsible for the development of pre-syncope.

Acknowledgments

B.K. contributed to the conception and design of the experiment, the interpretation of the data, and the writing of the manuscript. J-L.F. carried out data collection, and led the analysis, interpretation, and writing of the manuscript. Both authors approved the final version of this manuscript.

Author Disclosure Statement

The authors do not declare any competing financial interests.

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The Effect of Adding CO_2 to Hypoxic Inspired Gas on Cerebral Blood Flow Velocity and Breathing during Incremental Exercise

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Abstract

Hypoxia increases the ventilatory response to exercise, which leads to hyperventilation-induced hypocapnia and subsequent reduction in cerebral blood flow (CBF). We studied the effects of adding CO_2 to a hypoxic inspired gas on CBF during heavy exercise in an altitude naïve population. We hypothesized that augmented inspired CO_2 and hypoxia would exert synergistic effects on increasing CBF during exercise, which would improve exercise capacity compared to hypocapnic hypoxia. We also examined the responsiveness of CO_2 and O_2 chemoreception on the regulation ventilation ($\dot{V}$E) during incremental exercise. We measured middle cerebral artery velocity (MCAv; index of CBF), $\dot{V}$E, end-tidal PCO_2, respiratory compensation threshold (RC) and ventilatory response to exercise ($\dot{V}$E slope) in ten healthy men during incremental cycling to exhaustion in normoxia and hypoxia ($FIO_2 = 0.10$) with and without augmenting the fraction of inspired CO_2 ($FICO_2$). During exercise in normoxia, augmenting $FICO_2$ elevated MCAv throughout exercise and lowered both RC onset and $\dot{V}$E slope below RC ($P<0.05$). In hypoxia, MCAv and $\dot{V}$E slope below RC during exercise were elevated, while the onset of RC occurred at lower exercise intensity ($P<0.05$). Augmenting $FICO_2$ in hypoxia increased $\dot{V}$E at RC ($P<0.05$) but no difference was observed in RC onset, MCAv, or $\dot{V}$E slope below RC ($P>0.05$). The $\dot{V}$E slope above RC was unchanged with either hypoxia or augmented $FICO_2$ ($P>0.05$). We found augmenting $FICO_2$ increased CBF during sub-maximal exercise in normoxia, but not in hypoxia, indicating that the 'normal' cerebrovascular response to hypercapnia is blunted during exercise in hypoxia, possibly due to an exhaustion of cerebral vasodilatory reserve. This finding may explain the lack of improvement of exercise capacity in hypoxia with augmented CO_2. Our data further indicate that, during exercise below RC, chemoreception is responsive, while above RC the ventilatory response to CO_2 is blunted.

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Introduction

At high intensities of exercise in normoxia, above the respiratory compensation threshold (RC), hyperventilation-induced hypocapnia constricts the cerebral vessels, thereby reducing cerebral blood flow (CBF) and lowering cerebral oxygen delivery [1–3]. In conditions of hypoxia, in spite of hypoxic vasodilation, the hypocapnia-induced limitation of CBF at high exercise intensities is further aggravated due to a greater ventilatory drive. Such a limitation of CBF might lead to inadequate cerebral O_2 delivery, triggering early motor drive withdrawal, thus limiting exercise performance in hypoxia [4,5]. In support, several studies found a relationship between performance and cerebral deoxygenation in hypoxia using various exercise modes such as repeated sprints [6], incremental exercise [7,8], and static maximal muscle contraction to exhaustion [9–12]. Similarly, under normoxic conditions, exacerbation of exercise induced cerebral deoxygenation, by administering a non-selective beta-blocker, is accompanied by impaired maximal exercise performance [13]. This has led to the hypothesis that an exaggerated hyperventilation-induced hypocapnia and subsequent reduction in CBF during heavy exercise might account, in part, for the impaired performance in hypoxia.

Recently, studies have assessed the effect of preventing the normal drop in end-tidal PCO_2 on CBF and exercise performance in normoxia [14] and hypoxia [15,16]. Subudhi et al., [15] reported impaired exercise capacity at 1,600 m and 4,875 m (hypobaric chamber) when they clamped end-tidal PCO_2 ($PETCO_2$) either: 1) at 50 mmHg throughout incremental exercise or 2): at 40 mmHg from ~75% maximal work rate until exhaustion. Siebenmann et al., [16] completed those observations by investigating, at a more moderate altitude (3,454 m), the impact of clamping $PETCO_2$ at 40 mmHg, on performance. Both of these studies found clamping $PETCO_2$ increased MCAv and attenuated the decrease in cerebral oxygenation, but slightly decreased peak power output without affecting maximal oxygen uptake. Subudhi et al., [15] found ventilation ($\dot{V}$E) was elevated (by ~50 L/min) during submaximal exercise intensities (37 and 75% of maximal hypoxic work rate) with $PETCO_2$ clamping,

compared to without added CO_2. As such, they attributed the reduced performance with CO_2 clamping to earlier functional limitation by the respiratory system associated with CO_2 breathing. Since these studies were carried out in subjects living at moderate altitude [15] and in low altitude residents following a day spent at high altitude[16], respectively, both conditions accompanied by enhanced cerebrovascular and ventilatory responsiveness to CO_2 [17,18], the acute effect of augmented $FICO_2$ and hypoxia on CBF during exercise in an altitude-naïve population remained unclear.

For climbing 8,000 m peaks, a strong ventilatory response at altitude is believed to be a factor for success [19], but increases the risk of brain damage because of excessive hyperventilation-induced cerebral hypoxemia [20]. By contrast, Bernardi et al., [21] found that acclimatized elite climbers with a smaller ventilatory response to hypoxia at 5,200 m were more successful climbing to extreme altitude (Everest and K2), perhaps due to a greater ventilatory reserve (difference between maximal voluntary ventilation and effective minute ventilation) at these altitudes. Therefore, the effect of augmenting $FICO_2$ on ventilation during exercise is of interest, particularly in the presence of enhanced ventilatory drive associated with hypoxia.

We tested the hypotheses that, in non-altitude acclimatized subjects, increasing $PETCO_2$ during incremental exercise in normobaric hypoxia would: i) further augment CBF during exercise in hypoxia and improve exercise capacity; and ii) exacerbate the increase in ventilation at sub-maximal exercise intensities but not at maximal effort.

Methods

Ethics

The study was approved by the Research Ethical Committee of the University Hospitals of Geneva and conformed to the standards set by the Declaration of Helsinki. All subjects were informed regarding the procedures of this study, and informed signed consent was given prior to participation.

Subjects

Ten healthy male subjects with a mean age of 24 ± 3 yr (mean $\pm$ SD), a body mass index of 22.7 ± 2.4 kg/m^2 and $\dot{V}O_2$max of 58.3 ± 10.5 ml/min/kg participated in this study. Subjects were non-smokers, had no previous history of cardiovascular, cerebrovascular, or respiratory disease and were not taking any medication. All the subjects were residents of Geneva, Switzerland ($\sim$400 m) and partook in regular exercise (3–6 h per week); none were acclimatized to altitude (i.e., they had not travelled to altitudes $>$1,000 m in the past 2 months).

Experimental design

The subjects visited the laboratory on five occasions. After a full familiarization with the experimental procedures outlined below (visit one), subjects underwent four experimental exercise trials in a randomized, single-blind and balanced fashion (to prevent order effects), with a 3-day washout period between each experimental session, consisting of the following conditions: i) control normoxia (ambient air, 389 m); ii) augmented $FICO_2$ in normoxia; iii) control hypoxia (normobaric simulated altitude, 5,000 m); and iv) augmented $FICO_2$ in hypoxia. Before each experimental session, the subjects were asked to abstain from caffeine for 12 h and heavy exercise, and from alcohol for 24 h. Each experimental testing session comprised 20 min instrumentation followed by a 4 min resting baseline collection with the subject breathing room-air and seated on an electronically braked cycle ergometer (Ergoselect 100, Ergoline GmbH, Bitz, Germany). During the hypoxia and augmented $FICO_2$ trials, the subjects were then switched to a gas mixing system (described below), breathing the respective gas mixtures for an additional 4 min resting baseline (condition baseline) prior to the incremental cycling test.

Exercise test

The subjects were instructed to begin cycling at 0 watts at a pedaling rate of 70 rpm. The work rate was increased by 0.5 watts every second (30 W/min) thereafter until the subject reached voluntary exhaustion. Throughout each experimental session, the subjects wore a nose-clip and breathed through a mouthpiece attached to a low resistance one-way non-rebreathing valve (Hans-Rudolph 2700, Kansas City, USA). The hypoxia and augmented $FICO_2$ were achieved using a modified gas mixing system (Altitrainer, SMTec, Nyon, Switzerland), which has been previously described in detail [14]. In brief, additional CO_2 was bled into the inspired gas mixture under constant feedback of on-screen $PETCO_2$ and $FICO_2$ was constantly adapted as to keep $PETCO_2$ to a target value of approximately 45 mmHg during both normoxia and hypoxia. The FIO_2 was held constant at either 0.21 (normoxia, ambient air) or 0.10 (hypoxia). The subjects breathed through the same circuit in all four conditions and were kept unaware to what gas mixture they were breathing and were naive to the rationale of the study. For each subject the experiments were carried out at the same time of day under consistent laboratory conditions (temperature $22.3\pm0.5^{\circ}$C, humidity $27\pm7\%$, barometric pressure 725 ± 4 mmHg).

Measurements

Respiratory variables. Gas exchange was monitored on a breath-by-breath basis (Medgraphics CPX, Loma Linda, USA) measuring flow at the mouth with a Pitot tube and the fractions of inspired and expired O_2 and CO_2 with fast responding gas analyzers (infrared and paramagnetic) integrated in the system. Ventilation ($\dot{V}E$) was derived from the flow signal and expressed in body temperature and pressure saturated (BTPS) and L/min, respectively. The partial pressure of end-tidal O_2 ($PETO_2$), CO_2 ($PETCO_2$), O_2 consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) were calculated by the gas analysis system. Prior to each experimental session the system was calibrated using a 3-L syringe (M9474, Medikro Oy, Finland) and precision gas mixtures of known O_2 and CO_2 concentrations.

Cerebrovascular and cardiovascular variables. Bilateral middle cerebral artery blood flow velocities (MCAv: as index of CBF) were measured in the middle cerebral arteries using a 2-MHz pulsed Doppler ultrasound system (ST3, Spencer technology, Seattle, USA). The Doppler ultrasound probes were positioned over the temporal windows and held firmly in place with an adjustable headband (Marc 600 Headframe, Spencer technology, Seattle, USA). The signals were at depths ranging from 43 to 54 mm. Signal quality was optimized and an M-mode screen shot was recorded to facilitate subsequent probe placements. In our hands, day-to-day reproducibility of MCAv has a coefficient of variation of $<10\%$. The bilateral MCAv were averaged to represent an index of global CBF during rest and exercise. Heart rate was measured using a thoracic belt and recorded directly onto a PC via a wireless receiver pod (PC-POD, Suunto, Vantaa, Finland). Peripheral O_2 saturation (SpO_2) was measured from the right ear lobe using pulse oximetry (Satlite, Datex, Helsinki, Finland, and ML320 Oximeter Pod, ADInstruments, Bella Vista, Australia). Blood pressure was measured with

an automated arm sphygmomanometer (Ergoselect 100, Ergoline GmbH, Bitz, Germany).

Perceived exertion. During exercise, the subjects were asked to score their perceived exercise exertion on the 0–10 Borg scale every minute [22].

Data and statistical analysis

Respiratory compensation threshold and ventilatory response to exercise. The respiratory compensation threshold (RC) was obtained using the v-slope method previously described by Beaver et al., [23]. The assessment of the ventilatory response to exercise (i.e., the slope of $\dot{V}E$ rise during exercise: $\dot{V}E$-slope to power output) has been previously described [24,25]. In brief, breath-by-breath $\dot{V}E$ points were plotted against mechanical power output and a least squares regression was used to determine the $\dot{V}E$-slope to exercise above and below RC.

Statistical analysis. Resting values were obtained by averaging the data obtained in the last two minutes of the four minutes resting periods just prior to exercise. For the incremental exercise, we took the last 20 sec of every 10% increase in exercise intensity until exhaustion (100%). We then averaged all of the 20 sec segments of the data to obtain a *single mean* value for each variable during all four experimental conditions. The effects of hypoxia and augmenting $FICO_2$ on cardiorespiratory and cerebrovascular responses at rest were assessed using two-way repeated-measures ANOVA with α-level of $P<0.05$ (IBM SPSS Statistics version 21.0, IBM Corporation, Armonk, USA). Likewise, two-way repeated measure ANOVA was used to assess the effects of hypoxia and augmenting $FICO_2$ on these variables during incremental exercise. For significant interactions between hypoxia and augmenting $FICO_2$, four pairwise comparisons (Bonferroni corrected) were performed to isolate the effect of hypoxia and augmenting $FICO_2$ on the dependent measures within subjects with a α-level of 0.0125, indicated where appropriate with the superscript ^{B}. Trends were considered when $P<0.10$. All data are reported as means ± SD.

Results

All ten subjects completed the experimental protocol. Due to poor gas exchange traces, one subject had to repeat the augmented $FICO_2$ in hypoxia trial. Two subjects had to repeat their control normoxia trials, as they initially did not maintain a pedaling rate of 70 rpm during high intensity exercise. One subject reported respiratory dyspnea during exercise in augmented $FICO_2$, in hypoxia, while another reported a mild headache lasting 20 min following the control hypoxia trial. No other subjects reported any side effects such as headache or dyspnea following the experiments. Due to poor blood pressure signals during exercise, we were unable to carry out repeated-measures ANOVA analysis on the MAP data during exercise.

Resting variables (Table 1)

MCAv: Hypoxia had no effect on resting MCAv (hypoxia: $P=0.790$ vs. normoxia), while augmenting $FICO_2$ elevated MCAv by 25±21% during both normoxic and hypoxic conditions (CO_2: $P=0.001$, interaction: $P=0.681$).

Ventilatory variables. Resting $PETCO_2$ was lower with hypoxia (hypoxia: $P=0.001$), while it was higher with augmented $FICO_2$ (CO_2: $P<0.001$, interaction: $P=0.224$). There was an interaction between the effects of hypoxia and augmenting $FICO_2$ on resting $PETO_2$ (interaction: $P=0.004$). Post-hoc analysis shows that $PETO_2$ was lowered with hypoxia during both control and augmented $FICO_2$ conditions ($P<0.001^B$ vs. normoxia for both), while augmenting $FICO_2$ elevated resting $PETCO_2$ by a greater extent in normoxia (by 20±5 mm Hg, $P<0.001^B$ vs. control normoxia) compared to in hypoxia (by 10±4 mm Hg, $P<0.001^B$ vs. control hypoxia). Resting $\dot{V}E$ was elevated by hypoxia ($P=0.008$ vs. normoxia) and augmented $FICO_2$ (CO_2: $P<0.001$, interaction: $P=0.848$). There was an interaction between the effect of hypoxia and augmenting $FICO_2$ on $\dot{V}CO_2$ (interaction: $P=0.003$). Post-hoc t-tests showed that hypoxia elevated $\dot{V}CO_2$ during control ($P<0.001^B$ vs. control normoxia) but not during augmented $FICO_2$ ($P=0.832^B$ vs. augmented $FICO_2$ in normoxia). Accordingly, $\dot{V}CO_2$ appeared to be lower with augmented $FICO_2$ in hypoxia ($P<0.001^B$ vs. control hypoxia) but not in normoxia ($P=0.131^B$ vs. control normoxia). No differences were observed in $\dot{V}O_2$ with either hypoxia or augmented $FICO_2$ (main effects: $P=0.217$ & $P=0.127$, respectively, interaction: $P=0.575$).

Cardiovascular variables. Hypoxia elevated resting HR (hypoxia: $P<0.001$ vs. normoxia); there was a slight but non-significant tendency for augmenting $FICO_2$ to attenuate this increase in HR (CO_2: $P=0.886$, interaction: $P=0.059$). The effect of hypoxia and augmented $FICO_2$ on resting SpO_2 interacted with each other (interaction: $P=0.004$). Specifically, post-hoc analysis found SpO_2 to be lower with hypoxia during both control and augmented $FICO_2$ conditions ($P<0.001^B$ vs. normoxia for both). Meanwhile, augmenting $FICO_2$ selectively elevated resting SpO_2 in hypoxia ($P<0.001^B$ vs. control hypoxia), but not in normoxia ($P=0.615^B$ vs. control normoxia). No changes were observed in resting MAP with either hypoxia or augmenting $FICO_2$ (main effects: $P=0.809$ and $P=0.123$, respectively, interaction: $P=0.370$).

Exercise

Performance. Hypoxia lowered maximal exercise capacity by 26% (251±15 W vs. 338±21 W, hypoxia vs. normoxia, hypoxia: $P=0.001$), whereas there was a tendency for maximal exercise capacity to be lowered with augmented $FICO_2$ (CO_2: $P=0.091$, interaction: $P=0.397$). Specifically, maximal exercise capacity tended to be lower by 4% in normoxia (332±66 W vs. 345±66 W, augmented $FICO_2$ vs. control, $P=0.052^B$), while no trend was observed in hypoxia (248±48 W vs. 256±48 W, $P=0.252^B$).

Ventilatory variables (Fig. 1A, 1B & 2A–D). During incremental exercise, the effect of augmenting $FICO_2$ on $PETCO_2$ was greater in hypoxia compared to normoxia (interaction: $P<0.001$). Post-hoc analysis revealed that throughout exercise, hypoxia lowered $PETCO_2$ during control by a greater extent than during augmented $FICO_2$ (10±3 mmHg vs. 5±3 mmHg, $P<0.001^B$ vs. normoxia for both). Meanwhile, augmenting $FICO_2$ elevated $PETCO_2$ by a lesser extent in normoxia compared to in hypoxia (8±3 mmHg vs. 14±3 mmHg, $P<0.001^B$ vs. control for both). In contrast, hypoxia blunted the effect of augmenting $FICO_2$ on $PETO_2$ (interaction: $P<0.001$). Post-hoc comparisons found hypoxia lowered $PETO_2$ by 49±2 mmHg during control and by 54±4 mmHg during augmented $FICO_2$ conditions ($P<0.001^B$ vs. normoxia for both). Furthermore, augmenting $FICO_2$ increased $PETO_2$ by 15±3 mmHg in normoxia and by 10±3 mmHg in hypoxia ($P<0.001^B$ vs. control for both).

During incremental exercise, both hypoxia and augmenting $FICO_2$ elevated $\dot{V}E$ (main effect: $P<0.001$ for both, interaction: $P=0.188$). At exhaustion, $\dot{V}E$ reached 74±12% of estimated maximal voluntary ventilation (MVV) in normoxia and 69±11% in hypoxia [26].

Table 1. Effect of hypoxia and augmented $FICO_2$ on resting respiratory, cerebrovascular and cardiovascular variables.

	Normoxia				Hypoxia			
	Control		Augmented $FICO_2$		Control		Augmented $FICO_2$	
Ventilatory								
$PETCO_2$ (mmHg)	36	± 3	43	± 2*	32	± 2†	40	± 2*†
$PETO_2$ (mmHg)	96	± 4	116	± 4*	45	± 3†	55	± 3*†
$\dot{V}E$ (L/min)	11.6	± 1.8	24.1	± 5.1*	16.5	± 2.4†	28.5	± 5.5*†
$\dot{V}O_2$ (ml/min)	350	± 64	394	± 63	346	± 91	375	± 75
$\dot{V}CO_2$ (ml/min)	320	± 67	285	± 51	444	± 85†	282	± 66*
Cerebrovascular								
MCAv (cm/s)	54	± 10	67	± 10*	54	± 12	66	± 16*
Cardiovascular								
HR (b/min)	70	± 7	74	± 13	88	± 7	84	± 7*
MAP (mmHg)	90	± 7	99	± 11	90	± 14	100	± 9
SpO_2 (%)	97	± 1	98	± 1	85	± 5†	91	± 3*†

Values are mean ± SD.
*different from control ($P<0.05$);
†different from normoxia ($P<0.05$).
N = 10 for all variables except VO_2 and VCO_2 where N = 9.
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MCAv (Fig. 1C, 2E & 2F). During incremental exercise, there was a significant interaction between the effect of hypoxia and augmenting $FICO_2$ (interaction: $P = 0.010$). Post-hoc analysis revealed that hypoxia elevated MCAv during control ($P = 0.002^B$ vs. control normoxia), but not during augmented $FICO_2$ condition ($P = 0.429^B$ vs. augmented $FICO_2$ in normoxia). Meanwhile, augmenting $FICO_2$ selectively elevated MCAv during incremental exercise in normoxia ($P<0.001^B$ vs. control normoxia), but not in hypoxia ($P = 0.145^B$ vs. control hypoxia).

Oxygen saturation (Fig. 1D, 2G & 2H). The effect of augmenting $FICO_2$ on SpO_2 during exercise was enhanced in hypoxia (interaction: $P<0.001$). As such, post-hoc analysis showed that hypoxia lowered SpO_2 by 26±6% during control condition ($P<0.001^B$ vs. control normoxia) and by 15±6% during augmented $FICO_2$ condition ($P<0.001^B$ vs. augmented $FICO_2$ in normoxia). Meanwhile, augmenting $FICO_2$ elevated SpO_2 by 12±5% in hypoxia ($P<0.001^B$ vs. control hypoxia), but not in normoxia ($P = 0.615^B$ vs. control normoxia).

Metabolism (Fig. 1E, 1F & 3A–D). Hypoxia lowered both $\dot{V}O_2$ and $\dot{V}CO_2$ throughout exercise (hypoxia: $P<0.001$ vs. normoxia for both), while augmented $FICO_2$ had no effect on these variables (CO_2: $P = 0.628$ & $P = 0.544$, respectively, interaction: $P = 0.954$ & $P = 0.304$).

Cardiovascular variables (Fig. 1G, 3E & 3F). During exercise, the effect of hypoxia on HR was offset by the effect of augmenting $FICO_2$ (interaction: $P = 0.003$). Accordingly, post-hoc t-tests found HR to be higher with hypoxia during control ($P = 0.001^B$ vs. control normoxia), but not during the augmented $FICO_2$ ($P = 0.360^B$ vs. augmented $FICO_2$ in normoxia). There were trends for augmenting $FICO_2$ to elevate HR during exercise in normoxia ($P = 0.075^B$ vs. control normoxia) and lower HR in hypoxia ($P = 0.019^B$ vs. control hypoxia).

Rate of perceived exertion (Fig. 1H, 3G & 3H). There was an interaction between effects of hypoxia and augmenting $FICO_2$ on RPE (interaction: $P = 0.045$). As a result, a post-hoc t-tests found a tendency for RPE to be elevated with augmented $FICO_2$ in normoxia ($P = 0.038^B$ vs. control normoxia), but not in hypoxia ($P = 0.598^B$ vs. control hypoxia). Hypoxia had no effect on RPE during control or augmenting $FICO_2$ ($P = 0.735^B$ & $P = 0.180^B$ vs. normoxia, respectively).

RC and $\dot{V}E$-slope to exercise (Fig. 4 & 5). During exercise, the onset of RC occurred at lower power output in hypoxia compared to in normoxia (by 86±36 W, Hypoxia: $P<0.001$). Likewise, the onset of RC occurred at lower power output with augmenting $FICO_2$ compared to control conditions (CO_2: $P = 0.015$; interaction: $P = 0.176$). Meanwhile, there was an interaction between the effect of hypoxia and augmenting $FICO_2$ on $\dot{V}E$ at RC (interaction: $P = 0.021$). Post-hoc analysis revealed that hypoxia tended to elevate $\dot{V}E$ at RC under the augmented $FICO_2$ condition ($P = 0.027^B$ vs. augmented $FICO_2$ in normoxia), but not during the control condition ($P = 0.112^B$ vs. control normoxia). Augmenting $FICO_2$ selectively increased $\dot{V}E$ at RC in hypoxia (by 23.95±21.70 L/min, $P = 0.007^B$ vs. control hypoxia), but not in normoxia ($P = 0.847^B$ vs. control normoxia). The effect of hypoxia and augmenting $FICO_2$ interacted with each other on the $\dot{V}E$-slope to exercise (the slope of $\dot{V}E$ rise against power) below RC (interaction: $P = 0.043$). As such, hypoxia elevated the $\dot{V}E$ slope to exercise below RC during both control (by 30±13%, $P<0.001^B$ vs. control normoxia) and augmented $FICO_2$ condition (by 93±78%, $P = 0.001^B$ vs. augmented $FICO_2$ in normoxia). Augmenting $FICO_2$ lowered the $\dot{V}E$ slope below RC during normoxia (by 27±14%, $P = 0.001^B$ vs. control normoxia), but not during hypoxia ($P = 0.786^B$ vs. control hypoxia). In contrast, no differences were observed in the $\dot{V}E$ slope to exercise above RC with either hypoxia or augmenting $FICO_2$ (hypoxia: $P = 0.256$, CO_2: $P = 0.155$, interaction: $P = 0.585$).

Discussion

Augmenting $FICO_2$ elevated CBF during incremental exercise in normoxia, but, unexpectedly, it did not during hypoxia. Furthermore, we found no improvement in exercise capacity with

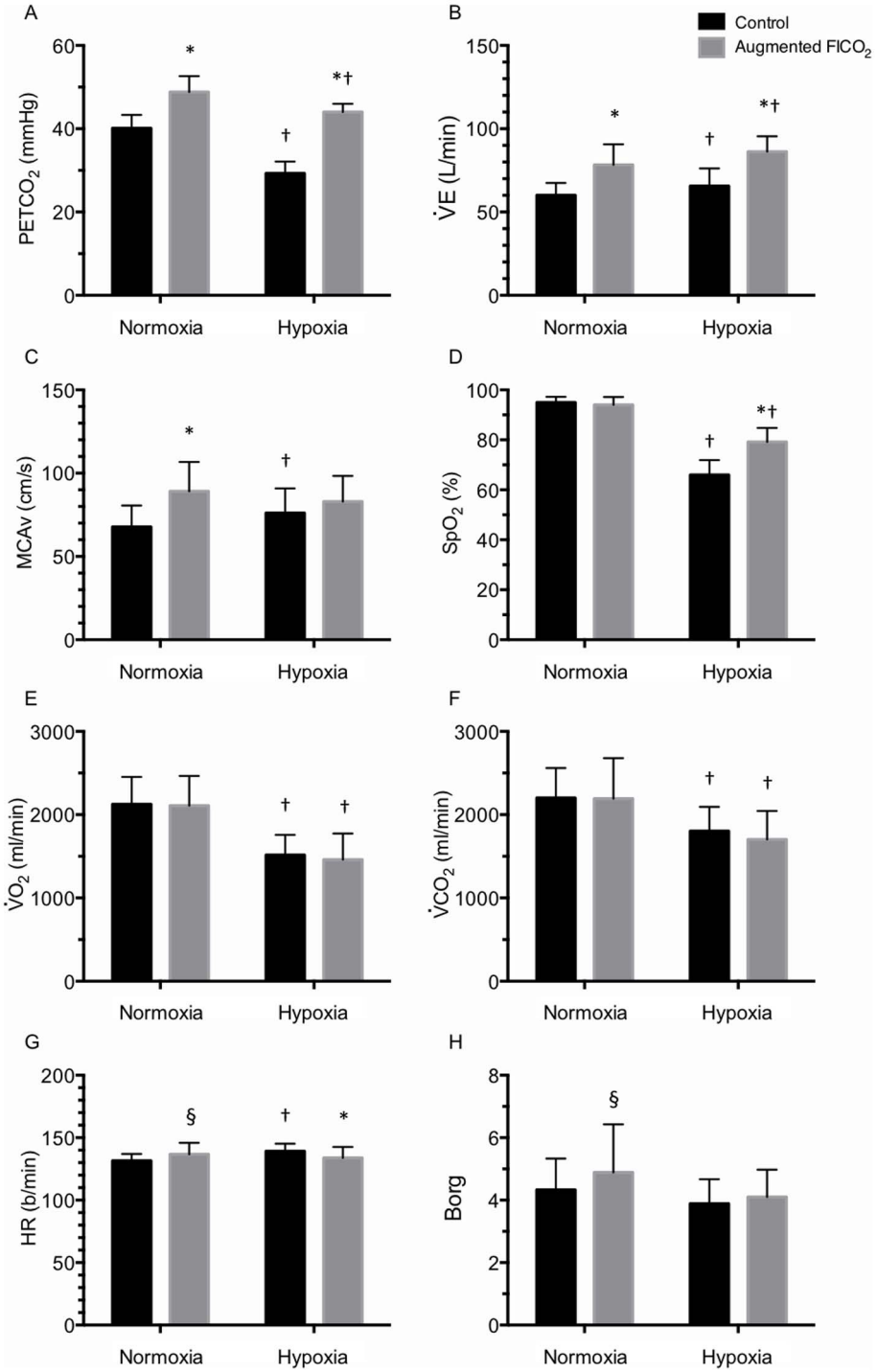

Figure 1. Effect of hypoxia and augmented $FICO_2$ on mean respiratory, cerebrovascular, metabolic and cardiac variables and perceived effort of exertion during incremental cycling to exhaustion. Values expressed as mean ± SD. * different from control ($P<0.05$); † different from normoxia ($P<0.05$); § trend for a difference ($P<0.10$).
doi:10.1371/journal.pone.0081130.g001

augmented $FICO_2$ during either normoxia or hypoxia. While the roles of hypercapnia and hypoxia in the regulation of CBF during rest have been extensively studied, their effects on cerebrovascular control during exercise are less well documented [see [27] for review]. During exercise, studies have found enhanced CBF response to hypercapnia [28], while the CBF response to hypocapnia remained unchanged [29]. During incremental exercise, we observed higher MCAv during both control hypoxia and augmented $FICO_2$ in normoxia (Fig. 1C). However, in contrast to the findings by Subudhi et al., [15], we observed no further increase in MCAv with augmented $FICO_2$ in hypoxia. The discrepancies between these findings could potentially be accounted for by the differences in the experimental setup and/or the level of hypercapnia achieved. In the study by Subudhi et al., [15],

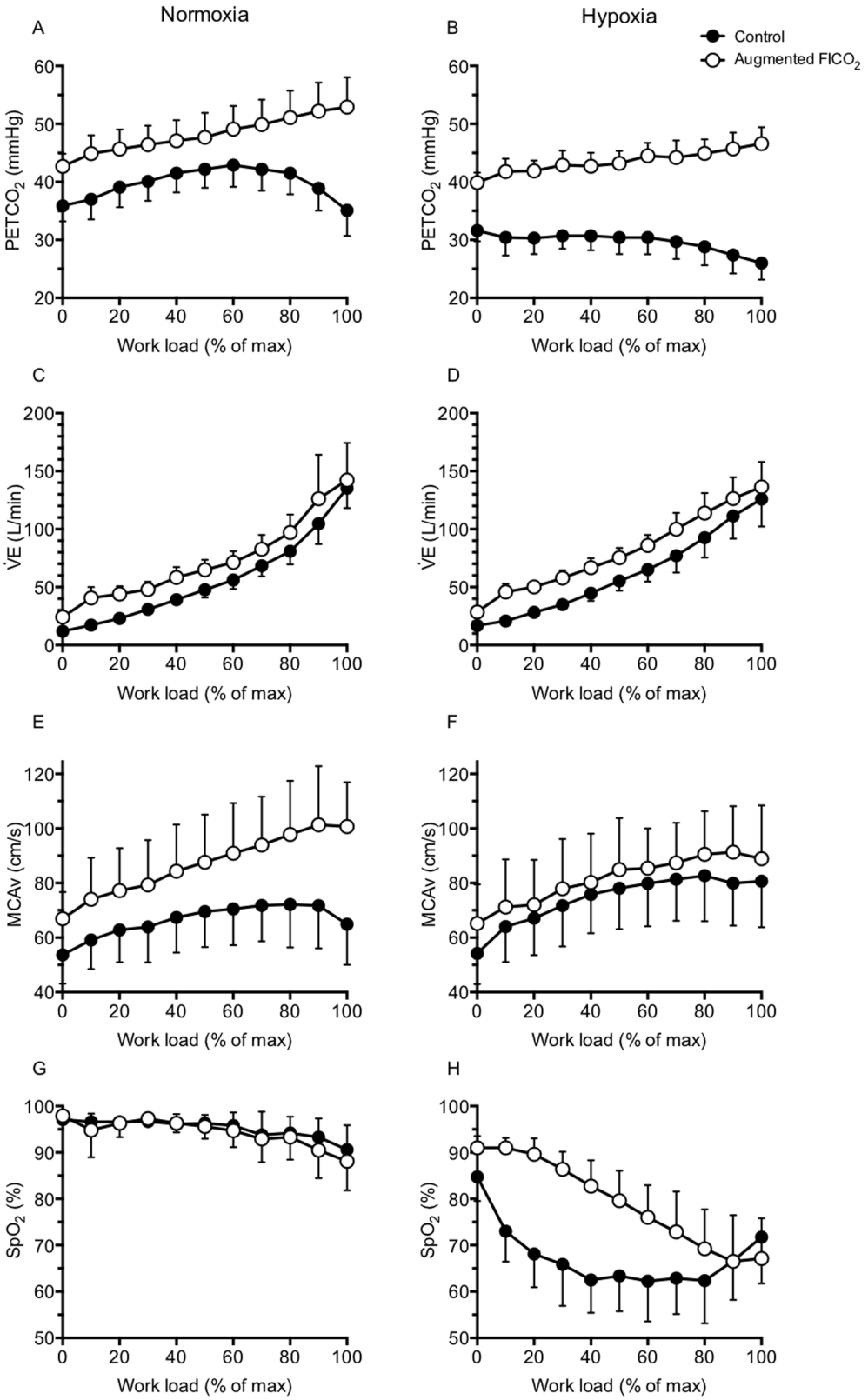

Figure 2. Effect of hypoxia and augmented $FICO_2$ on group respiratory, cerebrovascular variables and peripheral O_2 saturation during incremental cycling to exhaustion. Left panels: group data in normoxia (mean ± SD); right panels: group data in hypoxia. Note: these graphs are only intended for visualizing the changes in physiological parameters during incremental cycling. Statistical analyses were carried out using the average variable during the exercise session (see Figure 1).
doi:10.1371/journal.pone.0081130.g002

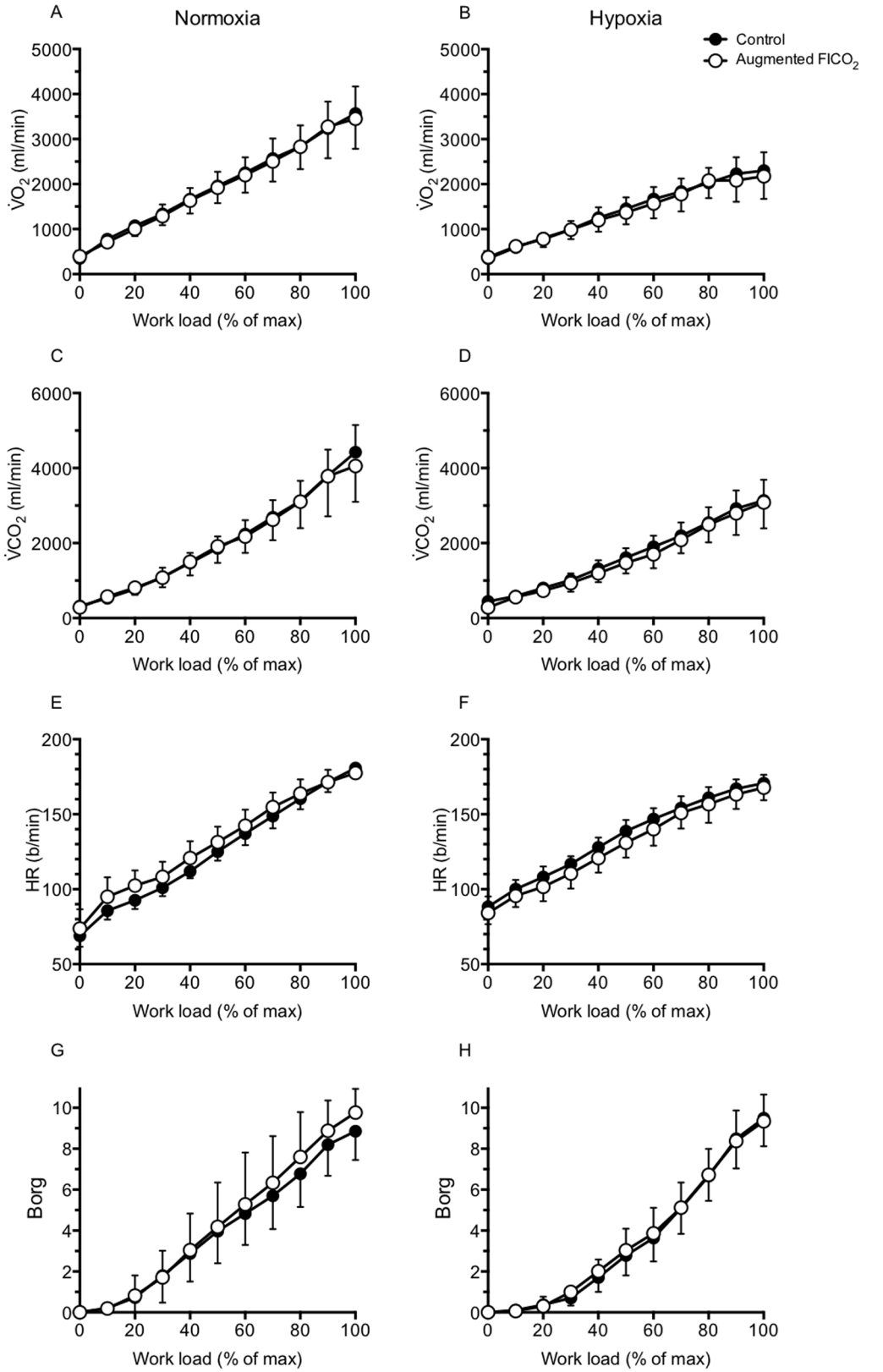

Figure 3. Effect of hypoxia and augmented $FICO_2$ on metabolic variables, heart rate, and perceived effort of exertion during incremental cycling to exhaustion. Left panels: group data in normoxia (mean ± SD); right panels: group data in hypoxia. Note: these graphs are only intended for visualizing the changes in physiological parameters during incremental cycling. Statistical analyses were carried out using the average variable during the exercise session (see Figure 1).
doi:10.1371/journal.pone.0081130.g003

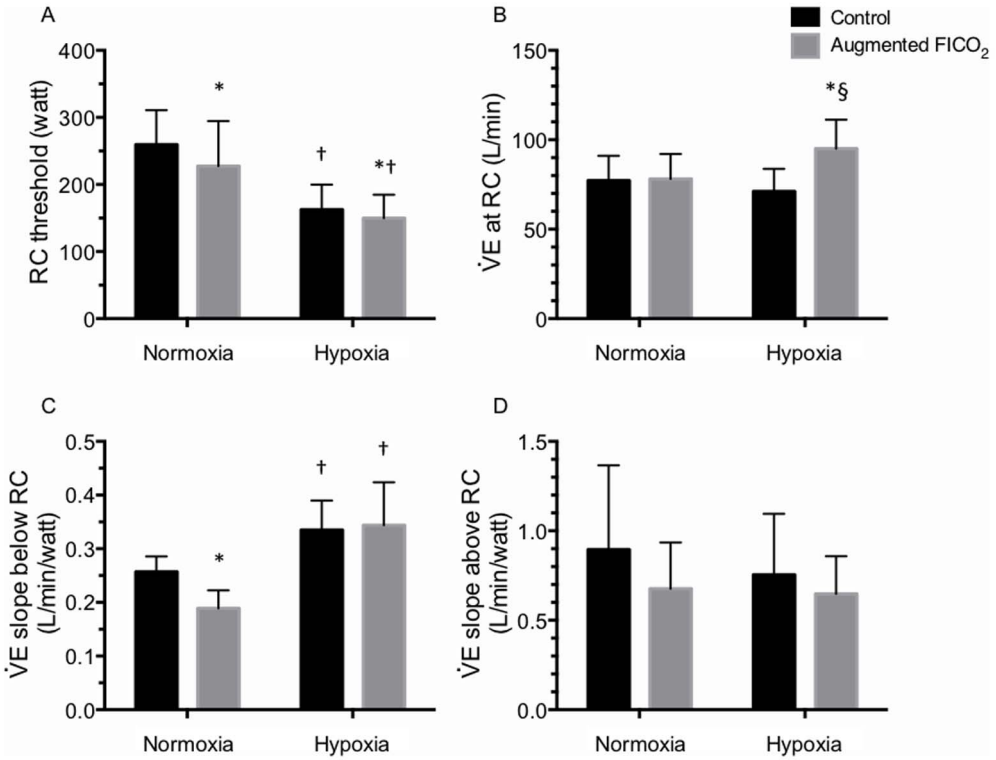

Figure 4. Effect of hypoxia and augmented $FICO_2$ on respiratory compensation threshold and ventilatory response to exercise during incremental cycling to exhaustion. Values expressed as mean ± SD. * different from control ($P<0.05$); † different from normoxia ($P<0.05$); § trend for a difference ($P<0.10$).
doi:10.1371/journal.pone.0081130.g004

$PETCO_2$ was clamped at 50 mmHg throughout exercise, while our subjects' $PETCO_2$ were on average, ~46 and ~43 mmHg in normoxia and hypoxia respectively (Fig. 1A), reaching ~53 and ~47 mmHg at maximal exercise intensity (Fig. 2A & 2B). Meanwhile, using our Altitrainer setup, Siebenmann et al., [16] were able to increase MCAv during incremental exercise in moderate hypobaric hypoxia (3,454 m) by clamping $PETCO_2$ at 40 mmHg in altitude sojourners. We deem it unlikely that differences in experimental setup are sufficient to account for these discrepant findings between the three studies. An alternative explanation is the potential influence of (partial) altitude acclimatization [30]. Subudhi et al., [15] examined the effect of CO_2 clamping on competitive cyclists living and training at 1,650 m, therefore already acclimatized to exercise at moderate altitude. Likewise, the subjects in Siebenmann et al., [16] had spent one night at altitude. In contrast, the subjects in our study had not been exposed to altitude >1000 m in the 2 months prior the study. Since acclimatization to altitude augments the cerebrovascular responsiveness to CO_2 [17], the increase in CBF observed by both Subudhi et al., [15] and Siebenmann et al., [16] may therefore be due to a partial state of acclimatization to altitude.

Cerebral O_2 delivery and performance in hypoxia

Contrary to our first hypothesis, augmenting $FICO_2$ during incremental exercise in normobaric hypoxia failed to improve aerobic exercise capacity. This finding corroborates recent findings in milder (hypobaric) hypoxic conditions [15,16]. The relationship between cerebral deoxygenation and exercise performance in normoxia [13] and hypoxia [6–12] is therefore unlikely to be causally related to the loss of aerobic capacity in hypoxia. However, it cannot be excluded that augmenting $FICO_2$ introduces limits that overrule the potential benefit of improved cerebral CO_2 delivery. Siebenmann et al., [16] argued that inspired CO_2 may exacerbate the metabolic acidosis associated with heavy exercise, shifting the oxyhemoglobin curve rightward, decreasing SaO_2, thus limiting peak $\dot{V}O_2$. In agreement, our results show a reduction in average heart rate in hypoxia (Fig. 1G) and a tendency for maximal exercise capacity to be actually impaired with augmented $FICO_2$ ($P = 0.091$), as also reported elsewhere [15,16]. In agreement with Subudhi et al., [15], we found the participants' RPE to increase more rapidly with augmented $FICO_2$ in normoxia (Fig. 3G), in parallel with the increase in ventilation (Fig. 2C). But contrary to Siebenmann et al., [16], our findings of higher SpO_2 with augmented $FICO_2$ in hypoxia throughout submaximal exercise intensities, and similar SpO_2 at exhaustion (Fig. 2H), which exclude a right-ward shift in the oxyhemoglobin curve would have limited pulmonary oxygen uptake.

Limits to cerebral vasodilation

While controversy remains, there is a large body of literature suggesting that nitric oxide, prostanoids and C-natriuretic peptide are involved in the cerebral vasodilatory responses to both hypercapnia and hypoxia through a number of intermediate interacting/co-activating pathways [31]. We speculate that if one chemical stimulus, such as hypoxia, is of sufficient magnitude to exhaust the cerebral vessels' capacity to vasodilate, thereby reaching maximal diameter of the vessels, then any additional stimuli would have no further effect on cerebrovascular resistance. Our data indicate that the CBF response to hypoxia is enhanced during exercise compared to rest, while adding CO_2 to the inspired gas during exercise only elevates CBF during normoxia,

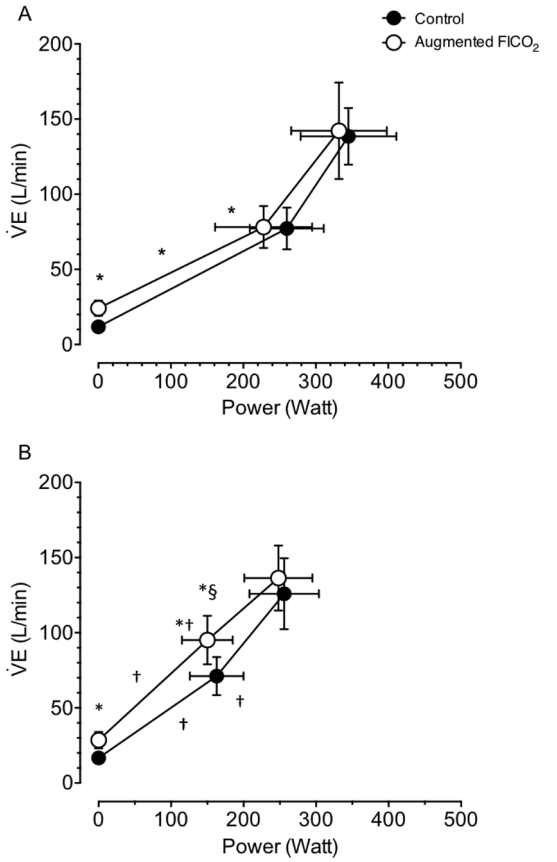

Figure 5. Effect of hypoxia and augmented $FICO_2$ on respiratory compensation threshold and ventilatory response to exercise during incremental cycling to exhaustion. A: group data in normoxia (mean ± SD); B: group data in hypoxia. * different from control ($P<0.05$); † different from normoxia ($P<0.05$); § trend for a difference ($P<0.10$).
doi:10.1371/journal.pone.0081130.g005

but not during severe hypoxia (Fig. 1C). The 'normal' cerebrovascular reactivity to hypercapnia thus appears to be abolished during exercise in severe hypoxia (Fig. 2F). In support, we found no difference between the MCAv values between augmented $FICO_2$ in normoxia and control hypoxia ($P = 0.193$). Such exhaustion of the cerebral vessels' vasodilatory reserve would account for the lack of change in MCAv with augmented $FICO_2$ during sub-maximal exercise in hypoxia. Mardimae et al., [32] demonstrated a synergistic role of CO_2 and hypoxia on the control of CBF at rest whereas our results would favor a negative effect, whereby the presence of severe hypoxia appears to attenuate the effect of hypercapnia on the cerebral vessels. Our data indicates that during incremental exercise in hypoxia, the role of hypercapnia in the regulation of cerebrovascular tone appears to be diminished – at least in an unacclimatized population. However, we cannot exclude the possibility that there might be regional differences in the cerebral vessel response to hypoxia and hypercapnia, which could display additive or synergistic interactions between the two chemical stimuli. Furthermore, the effect of combining hypoxia and hypercapnia on cerebral metabolism is unknown. Given the limited literature on this topic, further studies on the effect of hypoxia, hypercapnia and acclimatization status on regulation of cerebrovascular tone and therefore cerebral O_2 delivery during exercise in altitude is certainly warranted.

Chemoreception and exercise hyperpnea

The regulation of exercise hyperpnea has been extensively studied during the past 100 years [see [33–37] for reviews]. Nevertheless, the role of chemoreception in the regulation of exercise hyperpnea, especially during heavy exercise remains controversial [38,39]. To date, only a handful of studies have examined the effect of chemoreceptor stimulation with CO_2 alone [3,14,25,40,41] or the combined effects of hypoxia and hypercapnia on exercise hyperpnea [42]. Our second hypothesis stated that increasing $PETCO_2$ and decreasing $PETO_2$ would stimulate the chemoreceptors and lead to an increase in the ventilatory response to exercise. We found that hypoxia and augmented $FICO_2$ had an additive effect on exercise hyperpnea below the RC threshold (Fig. 4C). As reported before in conditions of normoxia [14], adding CO_2 to the inspirate during normoxic and hypoxic conditions indeed increased ventilation at rest and during incremental exercise (Table 1 & Fig. 5). However, at higher intensities, above the RC threshold, this effect progressively lessened, in spite of a progressive increase in $PETCO_2$, again similarly to what we found before in normoxia [14]. Hypoxia *per se* augmented the ventilatory response to incremental exercise (the rate of ventilation increase during exercise) below the RC threshold, while augmented $FICO_2$ attenuated this rate of increase (Fig. 5). Hypoxia shifted the RC threshold to lower exercise intensities, which is further exacerbated with augmented $FICO_2$ (Fig. 4A & 5B). No differences were observed in the slope of the ventilatory response to exercise above the RC threshold between any of the conditions (Fig. 4D). We thus confirm that below the RC threshold both hypoxia and hypercapnia can modulate the rate of ventilatory response to increasing exercise, presumably through chemoreceptor stimulation. However, once above the ventilatory compensation threshold, the chemoreflex ventilatory responses are blunted.

Siebenmann et al., [16] suggested that, during heavy exercise in hypoxia, the hyperventilation-induced hypocapnia might attenuate ventilatory drive and limit the ventilatory response to exercise. They further speculated that the mechanical constraints associated with high intensity exercise ventilation may be reduced in hypobaric hypoxia, while at the same time hypocapnia would be more pronounced, so that a blunting effect of hypocapnia on $\dot{V}E$ would persist or become more pronounced. Since we performed our experiments in normobaric conditions our results were not influenced by differences in air density, neglecting the small increases in N_2 and CO_2 in exchange of O_2 in our inspirates. In our young and active, but not-athletic subjects, mechanical limitation would seem unlikely since maximal ventilation only reached ~70% of estimated MVV with augmented $FICO_2$ both in normoxia and hypoxia.

Methodological considerations

Although the present study provided the opportunity to examine the effects of augmented $FICO_2$ on CBF response and exercise capacity during incremental exercise in hypoxia, an important limitation of the present study, as of many other studies, is the assumption that MCAv represents global CBF changes. Sato et al., [43] recently found that blood flow in the middle cerebral and internal carotid arteries declined from moderate (60% $\dot{V}O_2max$) to high intensity exercise (80% $\dot{V}O_2max$), whilst blood flow in the vertebral artery continued to increase at the higher intensities. This led Vogiatzis et al., [11] to suggest that vertebral artery blood flow may compensate for reductions of MCA blood flow during near-maximal or maximal exercise. However, since the relative contribution of the internal carotid artery (and

therefore MCA) to global CBF remains relatively unchanged (~65%) at high intensity exercise [43], we contend that MCAv is a reasonable index of changes in global CBF during heavy exercise. Further consideration when interpreting our MCAv data is the potential influence of hypoxia and hypercapnia on the MCA diameter. The cross-sectional area of the MCA was shown to remain relatively unchanged within a wide range of changes in $PETCO_2$ [44–47] and during exposure to hypoxia similar to that used in the present study (5,300 m) [48]. However, since no studies have examined the effect of exercise on MCA diameter, nor when superimposed with hypoxia and hypercapnia, we cannot exclude the possibility MCA diameter might have increased in our study, leading to an underestimation of the effect of augmented $FICO_2$ or hypoxia on CBF. In addition, since we did not measure cerebral tissue oxygenation, it is possible that we were unable to elevated cerebral O_2 delivery with our experimental setup. However, in the present study, SpO_2 was elevated with augmented $FICO_2$ throughout exercise in hypoxia (Fig. 1D), while MCAv was comparable between the control and augmented $FICO_2$ conditions (Fig. 1C). We would therefore expect cerebral O_2 delivery, which is the product of arterial O_2 saturation and CBF, to be elevated with augmented $FICO_2$ in hypoxia, leading to increased cerebral tissue oxygenation.

Another limitation of the present study is that we increased end-tidal rather than arterial PCO_2 during exercise, while it is known that the end-tidal-arterial PCO_2 gradient [49] varies with exercise [50,51]. Using our setup, Siebenmann et al., [16] were successful in clamping both end-tidal and arterial PCO_2 at around 40 mmHg during incremental exercise in hypoxia. Therefore, we believe it is likely that we were able to sufficiently elevate $PaCO_2$.

Finally, in the present study, we used a linear ramp protocol to assess the effect of augmented $FICO_2$ on the ventilatory response during incremental exercise. It should be acknowledged that during incremental exercise, lower peak $\dot{V}O_2$ and work output is obtained during ramp vs. step protocols [52], while the steepness of the ramp (i.e., 10 W/min, 30 W/min or 50 W/min), pedaling frequency and baseline workload can influence the onset of ventilatory threshold, peak $\dot{V}O_2$ and the $\dot{V}O_2$-workload relationship [53,54]. Therefore, our conclusions on the ventilatory response to exercise should only be limited to the specific ramp protocol (30 W/min) used in the present study. Furthermore, since all the aforementioned studies [15,16] only looked at aerobic capacity using incremental exercise tests until voluntary exhaustion, which have limited ecological validity [55], it remains to be investigated if increasing MCAv by augmenting $FICO_2$ during steady-state sub-maximal endurance exercise such as a time trial could improve performance in hypoxia, as time trial exercise provide a more accurate simulation of physiological responses during actual competitions [56] and correlate well with actual race performance [57].

Conclusions

We report two novel findings. Firstly, augmenting $FICO_2$ increases cerebral blood flow during sub-maximal exercise in normoxia, but not in hypoxia. This finding indicates that the 'normal' cerebrovascular response to hypercapnia is impaired during exercise in hypoxia, possibly due to an exhaustion of cerebral vasodilatory reserve. Secondly, above the ventilatory recruitment threshold, both in normoxia and hypoxia, the chemoreflex ventilatory responses are blunted.

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Author Contributions

Conceived and designed the experiments: BK. Performed the experiments: JF BK. Analyzed the data: JF. Wrote the paper: JF BK.

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Article six

Fan JL, Bourdillon N & Kayser B. (2014). Effect of end-tidal CO_2 clamping on cerebrovascular function, oxygenation, and performance during 15-km time trial cycling in severe normobaric hypoxia: the role of cerebral O_2 delivery. *Physiological Reports* **1,** 1-15.

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ORIGINAL RESEARCH

Effect of end-tidal CO_2 clamping on cerebrovascular function, oxygenation, and performance during 15-km time trial cycling in severe normobaric hypoxia: the role of cerebral O_2 delivery

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Abstract

During heavy exercise, hyperventilation-induced hypocapnia leads to cerebral vasoconstriction, resulting in a reduction in cerebral blood flow (CBF). A reduction in CBF would impair cerebral O_2 delivery and potentially account for reduced exercise performance in hypoxia. We tested the hypothesis that end-tidal P_{CO_2} ($PETCO_2$) clamping in hypoxic exercise would prevent the hypocapnia-induced reduction in CBF during heavy exercise, thus improving exercise performance. We measured $PETCO_2$, middle cerebral artery velocity (MCAv; index of CBF), prefrontal cerebral cortex oxygenation (cerebral O_2Hb; index of cerebral oxygenation), cerebral O_2 delivery (DO_2), and leg muscle oxygenation (muscle O_2Hb) in 10 healthy men (age 27 ± 7 years; VO_2max 63.3 ± 6.6 mL/kg/min; mean ± SD) during simulated 15-km time trial cycling (TT) in normoxia and hypoxia (FIO_2 = 0.10) with and without CO_2 clamping. During exercise, hypoxia elevated MCAv and lowered cerebral O_2Hb, cerebral DO_2, and muscle O_2Hb ($P < 0.001$). CO_2 clamping elevated $PETCO_2$ and MCAv during exercise in both normoxic and hypoxic conditions ($P < 0.001$ and $P = 0.024$), but had no effect on either cerebral and muscle O_2Hb ($P = 0.118$ and $P = 0.124$). Nevertheless, CO_2 clamping elevated cerebral DO_2 during TT in both normoxic and hypoxic conditions ($P < 0.001$). CO_2 clamping restored cerebral DO_2 to normoxic values during TT in hypoxia and tended to have a greater effect on TT performance in hypoxia compared to normoxia ($P = 0.097$). However, post hoc analysis revealed no effect of CO_2 clamping on TT performance either in normoxia ($P = 0.588$) or in hypoxia ($P = 0.108$). Our findings confirm that the hyperventilation-induced hypocapnia and the subsequent drop in cerebral oxygenation are unlikely to be the cause of the reduced endurance exercise performance in hypoxia.

Introduction

Altitude hypoxia represents a formidable environmental challenge to the human organism. Hypoxia limits oxygen transport from the air to the muscle mitochondria, which compromises aerobic capacity (Calbet and Lundby 2009). Exercise capacity is a vital determinant of a population's ability to thrive at high altitude (Curran et al. 1998), and permanent residence above 5000 m poses problems for mining and other human endeavor. Despite more than a century of research on the detrimental effects of hypoxia on exercise performance, the exact underlying mechanisms remain poorly understood [see (Amann and Kayser 2009; Verges et al. 2012) for reviews].

Even though the cessation of maximal exercise, or the reduction in exercise intensity, when fatiguing, imply

reduced motor recruitment by the central nervous system, the mechanisms that lead to the derecruitment of active muscle remain elusive. During normoxia and moderate hypoxia, leg muscle afferents appear to play an important role (Amann 2006; Amann and Dempsey 2007; Amann et al. 2011), whereas cerebral tissue oxygenation may play a more pivotal role under severe hypoxic conditions (Kjaer et al. 1999; Amann et al. 2006; Amann and Dempsey 2007; Subudhi et al. 2007a, 2009; Rasmussen et al. 2010; Vogiatzis et al. 2011). At rest, the brain protects itself against hypoxia by increasing cerebral blood flow (CBF) to compensate for the decreased arterial oxygen tension (Cohen et al. 1967). But during high-intensity exercise, hyperventilation-induced hypocapnia leads to cerebral vasoconstriction, which counteracts the hypoxia-induced vasodilation, thereby lowering CBF and cerebral oxygenation (Jorgensen et al. 1992a; Madsen et al. 1993; Ide et al. 1999; Subudhi et al. 2007b). As hypoxia per se enhances ventilatory drive, this would further exacerbate the hyperventilation-induced hypocapnia during heavy exercise and further lower CBF and cerebral oxygenation. It was proposed that this reduction in cerebral oxygenation may account for the reduced performance during heavy exercise in severe hypoxia (Amann and Dempsey 2007; Nybo and Rasmussen 2007; Amann and Kayser 2009; Subudhi et al. 2009; Rasmussen et al. 2010). In support, reduced cerebral oxygenation has been shown to coincide with both reduced cortical motor output (Subudhi et al. 2009) and increased cerebral metabolism (Rasmussen et al. 2010).

Contrary to this hypothesis, Subudhi et al. (2011) recently found exercise capacity to be *reduced* when they prevented the normal hyperventilation-induced hypocapnia with end-tidal CO_2 clamping during incremental cycling in hypobaric hypoxia (equivalent of 4875 m), despite elevated CBF and improved cerebral oxygenation. They attributed this finding to limiting factors such as increased respiratory muscle work and associated "steal" of blood flow, and an elevated perceived effort related to their experimental setup, which may have outweighed the benefits of elevated cerebral O_2 delivery. Siebenmann et al. (2012) completed those observations by investigating, at a more moderate altitude (3454 m), the impact of clamping $PETCO_2$ at 40 mmHg, on performance. Clamping increased middle cerebral artery velocity (MCAv), attenuated the decrease in cerebral oxygenation, but slightly decreased peak power output while it did not affect maximal oxygen uptake. Siebenmann et al. (2012) concluded that although hypocapnia decreases CBF and cerebral oxygenation, this does not limit maximum aerobic exercise capacity. In contrast, using a similar approach but in nonacclimatized subjects during incremental exercise to capacity in more severe hypoxia (equivalent of 5000 m), we were unable to improve CBF with inspired CO_2, despite a greater degree of hypercapnia (Fan and Kayser 2013). We attributed these discrepant findings to the different acclimatization states of the subjects in the different studies (389 m vs. 1600 m resident) and the type of hypoxia used (normobaric vs. hypobaric). However, all the aforementioned studies only looked at aerobic capacity using incremental exercise tests until voluntary exhaustion, which have limited ecological validity (Currell and Jeukendrup 2008). It thus remains unclear whether preventing a reduction in CBF and cerebral oxygenation associated with hyperventilation-induced hypocapnia would improve performance during prolonged, submaximal exercise paradigms such as time trial cycling, which are more representative of usual human exercise behavior.

In this study, we therefore examined the effect of clamping partial pressure of end-tidal CO_2 ($PETCO_2$) on CBF and performance during a 15-km time trial cycling in severe normobaric hypoxia, as time trial protocols provide a more accurate simulation of physiological responses during actual competitions (Foster et al. 1993) and has been shown to correlate well with actual race performance (Palmer et al. 1996). We tested the hypothesis that by preventing hyperventilation-induced hypocapnia, we would increase CBF and cerebral oxygen delivery without altering muscle tissue oxygenation during high-intensity exercise, improving exercise performance during 15-km time trial cycling at a simulated altitude of 5000 m in nonacclimatized low-altitude residents.

Methods

Participants

A power calculation was employed using a difference worth detecting of a 5% improvement in 15-km time trial cycling performance based upon previous findings by Jeukendrup et al. (2008), along with unpublished data from our laboratory. A sample size of nine control volunteers was sufficient to give a power of 0.80 and an α of 0.05. Ten healthy males with a mean age of 27 ± 7 year (mean $\pm$ SD), a body mass index of 22.3 ± 1.3 kg/m^2, VO_2max of 63.3 ± 6.6 mL/min/kg, and maximal power output of 385 ± 30 W participated in this study. Participants were nonsmokers, had no previous history of cardiovascular, cerebrovascular, or respiratory disease and were not taking any medication. All participants were informed regarding the procedures of this study, and informed consent was given prior to participation. All the participants were residents of Geneva, Switzerland (<500 m), and were trained cyclists. The study was approved by the Research Ethical Committee of the University Hospitals of Geneva and conformed to the standards set by the *Declaration of Helsinki*.

Experimental design

The participants visited the laboratory on five occasions. Following full familiarization, which included a VO_2max test to assess the participant's aerobic fitness (visit one), the participants underwent four experimental trials (randomized, balanced order, and in single-blind fashion) which consisted of the following conditions: (i) control normoxia (389 m); (ii) normoxia with CO_2 clamping; (iii) control normobaric hypoxia (FIO_2 = 0.10; simulated altitude of 5000 m); and (iv) normobaric hypoxia with CO_2 clamping. Before each experimental session, the participants were asked to abstain from caffeine for 12 h, and heavy exercise and alcohol for 24 h. Each experimental testing session comprised of 20-min instrumentation with the participants breathing room air while seated on a bicycle fitted to a Computrainer Pro Model trainer, calibrated according to the manufacturer's instructions (RacerMate, Seattle, WA). While breathing normal room air, 4-min baseline data were collected (room air baseline), the participants then performed a 5-min self-selected warmup exercise (heart rate <120 bpm). They were then switched to breathe from a modified gas mixing system (Altitrainer, SMTec, Nyon, Switzerland) followed by an additional 4-min resting baseline collection (condition baseline). The participants then did a 15-km time trial cycling as fast as possible. They were free to shift gears during the time trials, and constant feedback regarding the distance covered, but not speed, was provided on a computer screen (Computrainer™ 3D software version 3.0; Racermate). To prevent excessive thermal stress during the time trial exercise, two ventilators were placed ~60 cm in front of the participants and wind velocity was adjusted according to cycling speed. Throughout each experimental session, the participants wore a nose clip and breathed through a mouthpiece attached to a low-resistance one-way nonrebreathing valve (Hans-Rudolph 2700, Kansas City, MO). The simulated normobaric hypoxia and CO_2 clamping were achieved using the gas mixing system, which was attached to the inspiratory valve of the mouthpiece using a piece of large-bore low-resistance tubing. The device consists of a reservoir in which air and experimental gases are mixed and from which the participant inspires. This setup enables bleeding additional CO_2 into the inspired gas mixture, thereby increasing the fraction of inspired CO_2 ($FICO_2$) while keeping the fraction of inspired O_2 (FIO_2) constant at either 0.21 (as in ambient air) or 0.10 (in Geneva the equivalent of an altitude of ~5000 m) during normoxia and hypoxia conditions, respectively. $PETCO_2$ clamping was achieved under feedback of on-screen $PETCO_2$ (LabChart 7.2; ADInstruments, Colorado Springs, CO), continuously adapting $FICO_2$ keeping $PETCO_2$ to its target value (45 mmHg). The participants breathed through the same circuit in all four conditions and were unaware to what gas mixture they were breathing and naive to the exact rationale of the study. For each participant, the experiments were carried out at the same time of day under consistent laboratory conditions (temperature 21 ± 1°C, humidity 32 ± 5%, barometric pressure 726 ± 6 mmHg).

Measurements

Respiratory variables

Gas exchange was monitored on a breath-by-breath basis (Medgraphics CPX, Loma Linda, CA) measuring flow at the mouth with a Pitot tube and the fractions of inspired and expired O_2 and CO_2 with fast responding gas analyzers (infrared and paramagnetic) integrated in the system. Ventilation (V'E) was derived from the flow signal and expressed in body temperature, pressure, saturated and per minute. The partial pressures of end-tidal O_2 ($PETO_2$) and CO_2 ($PETO_2$) were derived from the expired O_2 and CO_2 signals. Prior to each experimental session, the system was calibrated using a 3-L syringe (M9474; Medikro Oy, Finland) and precision gas mixtures of known O_2 and CO_2 concentrations (Carbogas AG, Gümligen, Switzerland).

Cerebrovascular and cardiovascular variables

Middle cerebral artery velocity (as an index of CBF) was measured bilaterally in the middle cerebral arteries using a 2-MHz pulsed Doppler ultrasound system (ST3; Spencer technology, Seattle, WA). The ultrasound probes were positioned over the temporal windows and held firmly in place with an adjustable headband (Marc 600 Head Frame; Spencer Technology). The signals were obtained by first locating the bifurcation of the middle and anterior cerebral artery; the angle and depth of insonation were then adjusted to obtain measurements from the MCA. The insonation depth and the velocity of MCA signals were recorded and compared to ensure within-subject repeatability of MCAv measurements between trials. In our hands, day-to-day reproducibility of MCAv has a coefficient of variation of <10%. The bilateral MCAv was averaged to represent global CBF during rest and exercise. Cerebral O_2 delivery (cerebral DO_2) was calculated using the equation: cerebral DO_2 = mean MCAv × arterial oxygen content (CaO_2, see below). Cerebral DO_2 was normalized to the room air baseline values prior to 5-min warmup during each trial and expressed as %.

Cerebral oxygenation in the left prefrontal lobe was assessed by monitoring changes in oxy- (O_2Hb), deoxy- (HHb), and delta (delta Hb: O_2Hb–HHb) hemoglobin obtained with spatially resolved, continuous wave near-infrared spectroscopy (NIRS; Artinis Oxymon, MKIII,

Zetten, the Netherlands). Source detector spacing was set at 4.1 cm and data obtained from the optode were used to calculate changes in O_2Hb and HHb with a differential pathlength factor (DPF) calculated using the formula: $DPF = 4.99 + 0.067 \times (age^{0.814})$ (Duncan et al. 1995). Muscle oxygenation in the left vastus lateralis (~15 cm proximal and 5 cm lateral to the superior border of the patella) was measured with an additional NIRS channel on the same instrument using a source detector spacing of 3.8 cm and DPF of 4.0 (Duncan et al. 1995). The cerebral and muscle O_2Hb and HHb are expressed at absolute changes from room air baseline prior to the 5-min warmup period. Beat-to-beat mean arterial blood pressure (MAP) was monitored using finger plethysmography (Finometer® MIDI, Finapress Medical Systems, Amsterdam, the Netherlands). Peripheral O_2 saturation (SpO_2) was measured using earlobe pulse oximetry (Radical-7; Masimo Corporation, Irvine, CA).

Blood gas variables

Arterialized earlobe capillary blood samples were taken at rest and every 5 km during exercise (Mollard et al. 2010). Vasodilating cream was applied to the earlobe 5 min prior to the sampling (Decontractyl, Sanofi Aventis, France). A lancet was used to pierce the earlobe and 60-μL capillary tubes (MultiCap; Siemens Healthcare Diagnostics Inc, Tarrytown, UK) were used to collect the samples. All samples were analyzed immediately (<5 sec) using an arterial blood gas analyzing system (Rapidlab™ 248; Siemens Healthcare Diagnostics Inc) for arterialized capillary pH, partial pressure of arterialized capillary O_2 (PcO_2) and CO_2 ($PcCO_2$), and arterialized O_2 saturation (ScO_2). Standard calibration was performed prior to each blood sample analysis. Arterialized hemoglobin concentration ([Hb]) was measured using an azidemethemoglobin double wavelength photometer method (HemoCue® Hb201+; HemoCue AB, Ängelholm, Sweden). Arterialized capillary O_2 content (CcO_2; index of CaO_2) was calculated using the equation: $1.36 \times [Hb] \times ScO_2/100) + 0.003 \times PaO_2$.

Electromyography

Quadriceps electromyogram (EMG) was recorded from the right vastus lateralis during exercise using electrodes with full-surface solid adhesive hydrogel. Following careful preparation of the skin (shaving, abrading, and cleaning with alcohol) to lower impedance <5 kΩ, pairs of circular silver chloride (recording diameter of 10 mm) surface electrodes (Medi-Trace™ 100; Tyco Healthcare Group, Mansfield, UK) with an interelectrode distance (center-to-center) of 20 mm were placed along the line from the superior lateral side of the patella to the anterior superior iliac spine at ~100 mm from the patella as described by Rainoldi et al. (2004). The position of the electrodes was marked on the skin for identical placement between exercise sessions. To minimize movement artifacts, the electrodes and cables were secured to the participant's leg using elastic bandage and netting. The EMG signals were amplified (Bio Amp Powerlab 26T; ADInstruments, Bella Vista, Australia) and filtered using a band-pass filter with low-pass cutoff frequency of 10 Hz and a high-pass cutoff frequency of 999 Hz (LabChart version 7.2; ADInstruments). The filtered EMG signals were sampled at 2 kHz by an analog-to-digital converter (PowerLab 26T; ADInstruments). As an index of motor drive, the EMG root mean square (RMS) was calculated for each single muscle contraction (LabChart version 7.2; ADInstruments).

Rate of perceived exertion (RPE)

During exercise, the participants were asked to score their RPE on the 0–10 Borg scale every 3 km (Borg 1982). The scale with descriptors was mounted in front of the subject at eye height. At regular intervals they were asked to activate a handle bar mounted switch, activating the appropriate led indicator next to the descriptor corresponding to their RPE, resulting in a corresponding voltage signal being fed into the analog-to-digital converter.

Data and statistical analysis

Resting values were obtained by averaging the data obtained in the last 30 sec of the 4 min resting period prior to exercise. During the time trial exercise, a single time-weighted mean value for each variable was obtained by averaging the means of the last 20 sec of each km. The effects of hypoxia and CO_2 clamping on cardiorespiratory, cerebrovascular, and blood gas responses at rest were assessed using a two-way repeated measures ANOVA with α-level of 0.05 (IBM SPSS Statistics version 20.0; IBM Corporation, Armonk, NY). Likewise, we performed two-way repeated measures ANOVA to isolate the effect of hypoxia and CO_2 clamping on the mean cardiorespiratory, cerebrovascular, and blood gas responses during the 15-km time trial cycling with a α-level of 0.05. Trends were considered when $P < 0.10$. For significant interactions between hypoxia and CO_2 clamping, four pairwise comparisons (Bonferroni corrected) were performed to isolate the effect of hypoxia and inspired CO_2 on the dependent measures within participants with a α level of 0.0125, indicated where appropriate with the superscript ^{B}. With the exception of RPE, which is reported as median and range, all data are reported as means ± SD.

With the exception of missing data at the end of exercise, which were replaced by repeated imputation using the missing data analysis function from the SPSS

program, individual missing data points were replaced by values derived from a linear interpolation procedure.

Results

All 10 participants completed the experimental protocol. Due to a technical problem, one participant could not complete the final 2 km during the CO_2 clamping in hypoxia. Accordingly, comparison of exercise time was carried out in nine participants. Due to technical issues, blood sample analysis could not be carried out in all the participants (see Table 2 for details). No participants reported any side effects such as headache or dyspnea following the experiments.

Resting variables

Respiratory variables

CO_2 clamping elevated resting V'E during normoxic and hypoxic conditions ($P < 0.001$) (Table 1). Meanwhile, acute hypoxia had a greater effect on V'E during the clamped condition compared to the unclamped condition (interaction: $P = 0.028$). Post hoc analysis showed that acute hypoxia elevated V'E during CO_2 clamping ($P = 0.001^B$ vs. normoxia), but not during the unclamped control condition ($P = 0.210^B$ vs. normoxia). There was an interaction between the effect of hypoxia and CO_2 clamping on both $PETCO_2$ and SpO_2 (interaction: $P < 0.001$ for both). As such, CO_2 clamping elevated $PETCO_2$ during both normoxic and hypoxic conditions (post hoc: $P = 0.001^B$ and $P < 0.001^B$, respectively), whereas acute hypoxia lowered $PETCO_2$ during the unclamped control condition ($P < 0.001^B$) and only tended to lower it during the clamped condition ($P = 0.098^B$). Post hoc analysis revealed that resting SpO_2 was lowered by acute hypoxia during both control and clamped conditions ($P < 0.001^B$ vs. normoxia), whereas CO_2 clamping selectively elevated SpO_2 in hypoxia ($P = 0.002^B$), but not during normoxia ($P = 0.713^B$). Acute hypoxia lowered resting $PETO_2$ ($P < 0.001$), whereas CO_2 clamping elevated it ($P < 0.001$).

Cerebrovascular variables

Both acute exposure to hypoxia and CO_2 clamping elevated resting MCAv (main effects: $P = 0.014$ and

Table 1. Effect of hypoxia and CO_2 clamping on resting respiratory, cerebrovascular, and cardiovascular variables.

	Normoxia		Hypoxia	
	Control	CO_2 clamp	Control	CO_2 clamp
Respiratory				
V'E (L/min)	15.7 ± 2.6	22.3 ± 3.3[1]	16.6 ± 5.9	30.4 ± 4.4[1,2]
$PETCO_2$ (mmHg)	42 ± 2	45 ± 2[1]	37 ± 1[2]	46 ± 1[1]
$PETO_2$ (mmHg)	108 ± 2	118 ± 5[1]	47 ± 2[2]	59 ± 2[1,2]
SpO_2 (%)	97.1 ± 1.6	97.2 ± 1.5	83.5 ± 3.7[2]	89.1 ± 3.1[1,2]
Cerebrovascular				
Middle cerebral artery velocity (cm/sec)	60 ± 6	61 ± 7[1]	61 ± 4[2]	69 ± 7[1,2]
Cerebral O_2Hb ($\Delta\mu$mol)	3.3 ± 3.4	3.4 ± 3.9	−1.4 ± 3.9[2]	1.3 ± 2.3[2]
Cerebral HHb ($\Delta\mu$mol)	−0.5 ± 0.7	−0.9 ± 1.8	4.9 ± 2.4[2]	2.3 ± 1.6[1,2]
Cerebral DO_2 (%) $n = 8$	101.7 ± 4.3	106.0 ± 8.3[1]	91.5 ± 8.7	109.3 ± 9.7[1]
Muscle oxygenation				
Muscle O_2Hb ($\Delta\mu$mol)	2.3 ± 4.1	1.8 ± 6.9	−3.7 ± 4.8[2]	−0.1 ± 5.0[2]
Muscle HHb ($\Delta\mu$mol)	−5.5 ± 2.7	−4.8 ± 3.9	−0.4 ± 6.4[2]	−2.2 ± 3.2[2]
Cardiovascular				
Mean arterial blood pressure (mmHg)	97 ± 6	101 ± 13	100 ± 8	106 ± 11
HR (b/min)	80 ± 10	81 ± 11[1]	92 ± 16[2]	86 ± 10[1,2]
Blood gases				
pH	7.46 ± 0.03	7.46 ± 0.05	7.48 ± 0.06	7.46 ± 0.04[1]
$PcCO_2$ (mmHg) $n = 9$	34 ± 3	36 ± 3[1]	29 ± 3[2]	37 ± 56[1]
PcO_2 (mmHg) $n = 9$	98 ± 5	107 ± 8[1]	46 ± 4[2]	57 ± 5[1,2]
ScO_2 (%) $n = 9$	97.9 ± 0.5	98.1 ± 0.3	87.2 ± 4.5[2]	90.9 ± 1.9[1,2]
CcO_2 (mL O_2/dL) $n = 9$	19.9 ± 1.2	19.9 ± 1.3	17.8 ± 1.7[2]	18.5 ± 1.1[2]

Values are mean±SD. Cerebral and muscle oxygenation data (O_2Hb and HHb) are expressed as delta change from baseline room air breathing values. Likewise, cerebral DO_2 is expressed as % of baseline room air breathing values.
[1]Different from control ($P < 0.05$).
[2]Different from normoxia ($P < 0.05$).

Table 2. Effect of hypoxia and CO_2 clamping on arterialized blood gas variables and cerebral O_2 delivery during 15-km time trial.

	Normoxia		Hypoxia	
	Control	CO_2 clamp	Control	CO_2 clamp
pH (n = 9)				
5 km	7.42 ± 0.08	7.38 ± 0.07	7.40 ± 0.10	7.40 ± 0.06
10 km	7.41 ± 0.07	7.39 ± 0.10	7.41 ± 0.08	7.43 ± 0.04
15 km	7.39 ± 0.06	7.37 ± 0.07	7.39 ± 0.09	7.35 ± 0.07
$PcCO_2$ (mmHg) n = 9				
5 km	32.0 ± 4.2	34.5 ± 3.2[1]	22.4 ± 2.5[2]	34.5 ± 3.1[1]
10 km	29.6 ± 2.5	33.7 ± 3.2[1]	19.8 ± 4.7[2]	34.2 ± 2.6[1]
15 km	27.6 ± 3.3	33.6 ± 4.1[1]	19.3 ± 3.1[2]	35.1 ± 3.0[1]
PcO_2 (mmHg) n = 8				
5 km	81.2 ± 8.2	86.9 ± 10.7[1]	32.7 ± 4.8[2]	37.7 ± 2.9[1,2]
10 km	82.1 ± 9.0	87.2 ± 9.3[1]	34.2 ± 3.3[2]	38.8 ± 3.0[1,2]
15 km	82.7 ± 9.7	93.0 ± 17.5[1]	33.6 ± 4.2[2]	39.0 ± 3.8[1,2]
ScO_2 (%) n = 7				
5 km	95.4 ± 1.6	95.5 ± 2.2	64.4 ± 5.3[2]	68.6 ± 7.3[1,2]
10 km	95.5 ± 1.8	96.4 ± 2.0	65.3 ± 3.4[2]	69.7 ± 6.0[1,2]
15 km	95.7 ± 1.8	96.6 ± 2.1	65.8 ± 6.7[2]	68.4 ± 8.5[1,2]
CcO_2 (mL O_2/dL) n = 7				
5 km	19.5 ± 1.3	19.3 ± 1.5	13.1 ± 1.6[2]	14.0 ± 1.0[2]
10 km	19.6 ± 1.5	19.5 ± 1.4	13.3 ± 1.1[2]	14.3 ± 0.8[2]
15 km	19.6 ± 1.2	19.4 ± 1.4	12.7 ± 1.1[2]	14.0 ± 1.4[2]
Cerebral DO_2 (%) n = 7				
5 km	115.5 ± 10.7	122.1 ± 13.2[1]	94.5 ± 9.7[2]	111.8 ± 11.8[1,2]
10 km	116.4 ± 11.4	137.8 ± 38.8[1]	102.0 ± 11.1[2]	117.8 ± 9.6[1,2]
15 km	117.4 ± 12.9	150.3 ± 37.3[1]	99.5 ± 7.7[2]	122.9 ± 13.6[1,2]

Values are mean±SD. Cerebral DO_2 expressed as % of baseline room air breathing values.
[1]Different from control ($P < 0.05$).
[2]Different from normoxia ($P < 0.05$).

$P < 0.001$, respectively; interaction: $P = 0.101$). Hypoxia at rest lowered cerebral O_2Hb ($P = 0.003$), whereas CO_2 clamping had no effect ($P = 0.222$). Hypoxia enhanced the effect of CO_2 clamping on resting cerebral HHb (interaction: $P = 0.047$). Post hoc tests showed that hypoxia elevated cerebral HHb during control and clamped condition ($P < 0.001^B$ and $P = 0.001^B$, respectively), whereas CO_2 clamp lowered resting cerebral HHb in hypoxia ($P = 0.001^B$) but not in normoxia ($P = 0.579^B$). Resting cerebral DO_2 was unaltered with hypoxia ($P = 0.227$), whereas CO_2 clamping elevated it ($P < 0.001$) with a trend for this increase to be greater in hypoxia compared to normoxia (interaction: $P = 0.090$). As a result, CO_2 clamping in hypoxia restored resting cerebral DO_2 to normoxic values (post hoc: $P = 0.821^B$ vs. normoxia).

Muscle oxygenation

Acute hypoxia lowered resting muscle O_2Hb and elevated muscle HHb (hypoxia: $P = 0.024$ and $P = 0.007$, respectively), whereas no effect was observed with CO_2 clamping (clamp: $P = 0.301$ and $P = 0.602$).

Cardiovascular variables

Resting HR was higher with acute hypoxia ($P = 0.001$) and lower with CO_2 clamping ($P = 0.050$, interaction: $P = 0.085$), whereas no differences were observed in MAP with either hypoxia or CO_2 clamping (main effects: $P = 0.128$ and $P = 0.136$, respectively).

Blood gas variables

No differences were observed in resting pH in hypoxia ($P = 0.508$), whereas there was an interaction between the effects of CO_2 clamping and hypoxia on pH (interaction: $P = 0.016$) despite a lack of effect of CO_2 clamp per se ($P = 0.489$). Post hoc analysis revealed that pH was lower with CO_2 clamping in hypoxia ($P < 0.001^B$ vs. control), but not during normoxia ($P = 0.112^B$). There was an interaction between hypoxia and CO_2 clamp on $PcCO_2$ (interaction: $P = 0.018$). Post hoc t-tests showed that hypoxia lowered $PcCO_2$ during the unclamped condition ($P = 0.010^B$ vs. normoxia) but not during the clamped condition ($P = 0.630^B$), whereas CO_2 clamping increased

$PcCO_2$ in hypoxia ($P = 0.001^B$ vs. control) but not in normoxia ($P = 0.035^B$). Meanwhile, both resting PcO_2 and ScO_2 were lowered with hypoxia ($P = 0.001$). CO_2 clamping elevated PcO_2 during both normoxia and hypoxia ($P < 0.002$) and elevated ScO_2 in hypoxia (post hoc: $P = 0.016^B$), but not in normoxia ($P = 0.115^B$, interaction: $P = 0.024$). Resting CcO_2 was lower with hypoxia ($P = 0.010$), but unaffected with CO_2 clamping ($P = 0.221$).

Time trial

Cycling performance

Exercise performance was impaired by 19 ± 7% in hypoxia (1899 ± 69 vs. 1584 ± 62 sec, hypoxia vs. normoxia, $P < 0.001$), while there was a nonsignificant trend for CO_2 clamping to exert a greater effect on exercise time in hypoxia compared to normoxia (CO_2: $P = 0.262$, interaction: $P = 0.097$). Nevertheless, post hoc analysis revealed no significant improvement in the exercise time with CO_2 clamping in hypoxia (1875 ± 72 vs. 1924 ± 5 sec, CO_2 clamp vs. control; $P = 0.108^B$) or normoxia (1589 ± 62 vs. 1579 ± 66 sec; $P = 0.586^B$). Hypoxia lowered mean power, speed, and cadence ($P < 0.001$, $P < 0.001$, and $P = 0.004$, respectively) compared to normoxia, whereas no difference was observed with CO_2 clamping ($P = 0.468$, $P = 0.0694$, and $P = 0.656$).

Rate of perceived exertion

The participants' RPE during the time trial, as indicated by the Borg score, was higher in hypoxia (hypoxia: $P = 0.009$), but no difference was observed with CO_2 clamping (clamp: $P = 0.459$).

Respiratory variables

V'E was elevated by both hypoxia and CO_2 clamping (hypoxia: $P = 0.048$, clamp: $P = 0.023$), whereas $PETO_2$ was lowered with hypoxia and elevated with CO_2 clamping throughout the exercise (main effects: $P < 0.001$ for both) (Fig. 1). Meanwhile, $PETCO_2$ was lower with hypoxia during control only ($P < 0.001^B$), whereas CO_2 clamping restored $PETCO_2$ to the values observed in the normoxic clamped condition ($P = 0.269^B$, interaction: $P < 0.001$). SpO_2 was lower during exercise in hypoxia during both control and clamped conditions ($P < 0.001^B$ for both), whereas SpO_2 was higher with CO_2 clamping during exercise in hypoxia ($P < 0.001^B$), but not normoxia ($P = 0.713^B$, interaction: $P < 0.001$).

In normoxia, the participants began cycling at ~63% VO_2max at the start of time trial, which progressively increased to 78% at the 14th km, before a final sprint reaching 85% VO_2max at the end of exercise. In contrast, during hypoxic conditions, the participants began cycling at 46% (normoxic) VO_2max, and progressively increased to 57% at the end of the time trial. Overall, both VO_2 and VCO_2 were lower in hypoxia ($P < 0.001$ for both), whereas CO_2 clamping tended to elevate VCO_2 but not VO_2 ($P = 0.064$ and $P = 0.603$, respectively).

Cerebrovascular variables

Both hypoxia and CO_2 clamping elevated MCAv during exercise (by 26% and 9%, respectively; hypoxia: $P < 0.001$, clamp: $P = 0.024$, interaction: $P = 0.963$) (Fig. 2 and Table 1). Hypoxia elevated cerebral HHb during exercise and lowered cerebral O_2Hb and delta Hb ($P < 0.001$ for all). In contrast, CO_2 clamping lowered HHb ($P = 0.015$) and elevated delta Hb during exercise ($P = 0.047$), whereas no difference was observed in O_2Hb with clamping ($P = 0.118$). During the time trial cycling, cerebral DO_2 was lower with hypoxia ($P = 0.004$) and elevated with CO_2 clamping ($P = 0.019$, interaction: $P = 0.911$).

Cardiovascular variables

There was a nonsignificant trend for MAP to be higher with CO_2 clamping ($P = 0.062$), whereas no effect was observed with hypoxia ($P = 0.535$). No differences were observed in HR with hypoxia ($P = 0.206$) or CO_2 clamping ($P = 0.196$).

Muscle oxygenation

The effect of CO_2 clamping on muscle O_2Hb and delta Hb was greater in hypoxia compared to normoxia (interaction: $P = 0.039$ and $P = 0.007$, respectively) and tended to be greater for muscle HHb (interaction: $P = 0.078$) (Fig. 3). As a result, post hoc analysis revealed that hypoxia lowered muscle O_2Hb and delta Hb during control condition ($P < 0.001^B$ vs. normoxia), and by a lesser extend during CO_2 clamped condition for muscle delta Hb ($P = 0.002^B$). Meanwhile hypoxia did not alter muscle O_2Hb in the CO_2 clamped condition ($P = 0.033^B$ vs. control). CO_2 clamping did not alter muscle O_2Hb in normoxia ($P = 0.554^B$), but tended to elevate it in hypoxia ($P = 0.013^B$). Hypoxia during exercise elevated muscle HHb in both control and clamped conditions ($P = 0.001^B$ and $P = 0.008^B$ vs. normoxia, respectively), whereas there was a tendency for CO_2 clamping to elevate muscle HHb during normoxia ($P = 0.067^B$ vs. control) but not during hypoxia ($P = 0.460^B$).

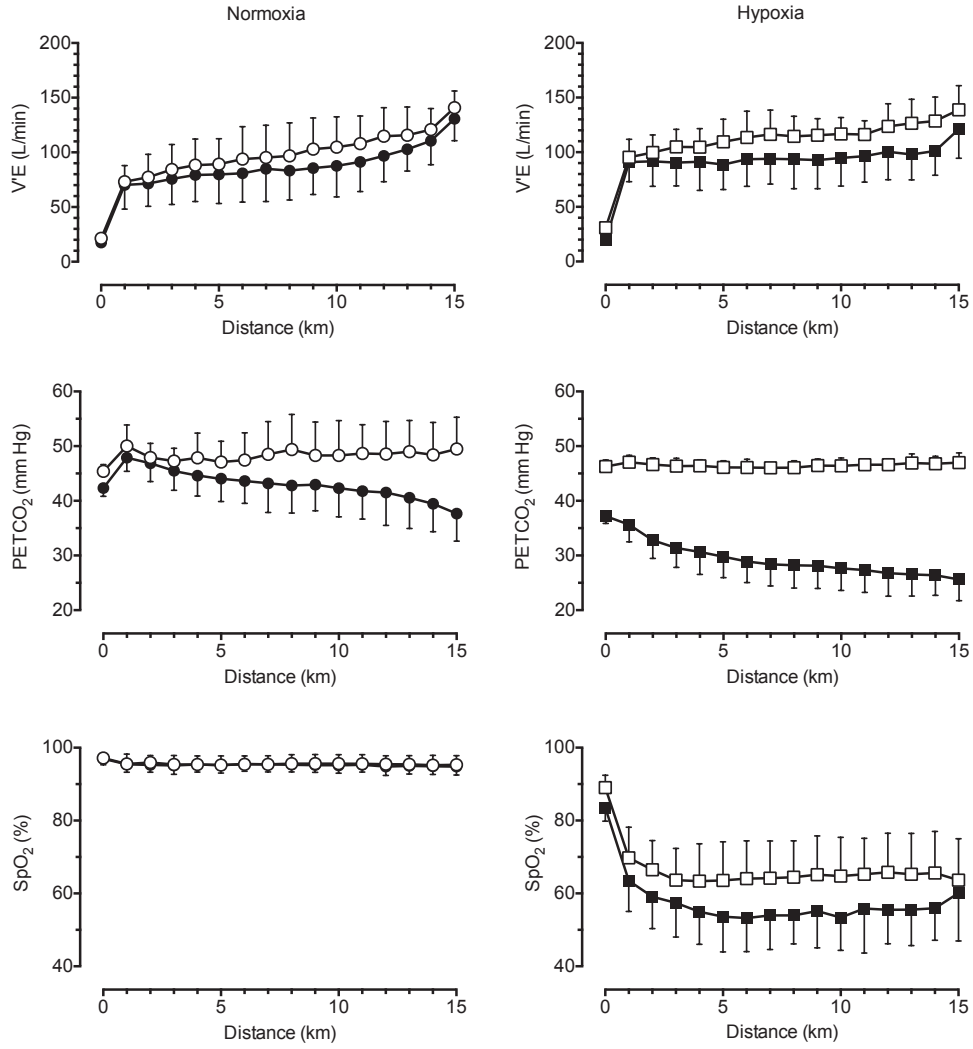

Figure 1. Effect of hypoxia and CO_2 clamping on respiratory variables during 15-km time trial cycling. Left panels, group data in normoxia (mean ± SD); right panels, group data in hypoxia. ●, normoxia control; ○, normoxia CO_2 clamp; ■, hypoxia control; □, hypoxia CO_2 clamp.

During exercise, EMG RMS increased slightly, in parallel with power output, showing a more pronounced increase during the end spurt ($P < 0.001$). EMG RMS was lower in hypoxia compared to normoxic exercise ($P = 0.002$), whereas no difference was observed with CO_2 clamping ($P = 0.628$).

Blood gas variables

CO_2 clamping during exercise elevated $PcCO_2$ by a greater extent in hypoxia compared to normoxia (interaction: $P < 0.001$) (Table 2). Accordingly, post hoc analysis revealed that CO_2 clamping elevated $PcCO_2$ during both normoxia ($P < 0.001^B$ vs. control) and hypoxia ($P = 0.004^B$), whereas hypoxia lowered $PcCO_2$ during control ($P = 0.001^B$ vs. normoxia) but no difference was observed between clamped values of $PcCO_2$ between normoxia and hypoxia ($P = 0.526^B$). Hypoxia lowered PcO_2 and CcO_2 during exercise (hypoxia: $P < 0.001$ for both), whereas CO_2 elevated PcO_2 ($P < 0.001$) and tended to elevate CcO_2 ($P = 0.089$). The effect of CO_2 clamping on ScO_2 was greater in hypoxia than normoxia (interaction: $P = 0.006$). Accordingly, hypoxia lowered ScO_2 during both control and clamped conditions (post hoc: $P < 0.001^B$ vs. normoxia), whereas CO_2 clamp elevated ScO_2 during hypoxia ($P = 0.005^B$ vs. control) but not during normoxia ($P = 0.754^B$). No changes were observed in pH with either hypoxia ($P = 0.568$) or CO_2 clamping during exercise ($P = 0.182$).

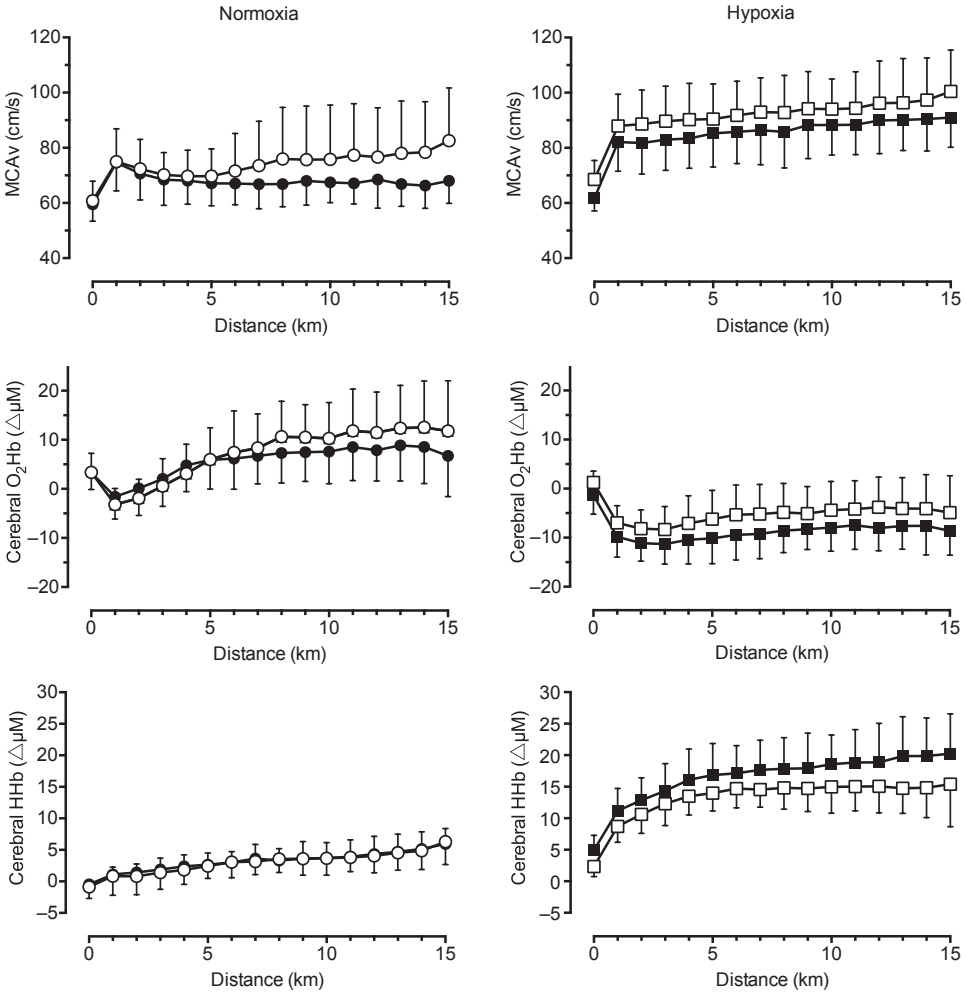

Figure 2. Effect of hypoxia and CO_2 clamping on cerebral variables during 15-km time trial cycling. Cerebral O_2Hb and HHb are expressed at delta changes from normoxia (room air baseline). Left panels, group data in normoxia (mean ± SD); right panels, group data in hypoxia. ●, normoxia control; ○, normoxia CO_2 clamp; ■, hypoxia control; □, hypoxia CO_2 clamp.

Discussion

The well-documented detrimental effect of severe hypoxia on exercise performance is not fully understood. Several studies found a relationship between exercise capacity or performance and a reduction in cerebral oxygenation during various exercise modes in hypoxia, such as time trial cycling (Amann 2006; Amann et al. 2006), repeated sprints (Smith and Billaut 2010), incremental exercise (Subudhi et al. 2007a; Peltonen et al. 2009), and static maximal muscle contraction to exhaustion (Rasmussen et al. 2006; Rupp and Perrey 2009; Vogiatzis et al. 2011; Goodall et al. 2012). These observations led to the hypothesis that the hyperventilation-induced hypocapnia and associated reduction in CBF and cerebral oxygenation may account for the impaired capacity and performance in hypoxia.

In this study, we tested the hypothesis that preventing the usual hyperventilation-induced hypocapnia and associated reduction in CBF and cerebral tissue oxygenation would improve 15-km time trial performance in severe normobaric hypoxia. Even though CBF was only increased by 9% during the hypoxic time trial with CO_2 clamping, it resulted, with the concomitant 9% increase in SpO_2 (Figs. 1, 2), in significantly increased cerebral DO_2 by 20% compared to the nonclamped control (Table 2). Importantly, this increase with CO_2 clamping in hypoxia was sufficient to *normalize* cerebral DO_2 to levels similar as observed during nonclamped normoxic exercise. As exercise performance in hypoxia was not significantly improved with clamping, it follows that the reduction in overall cerebral O_2 delivery is not a prime reason for the reduced exercise performance in hypoxia.

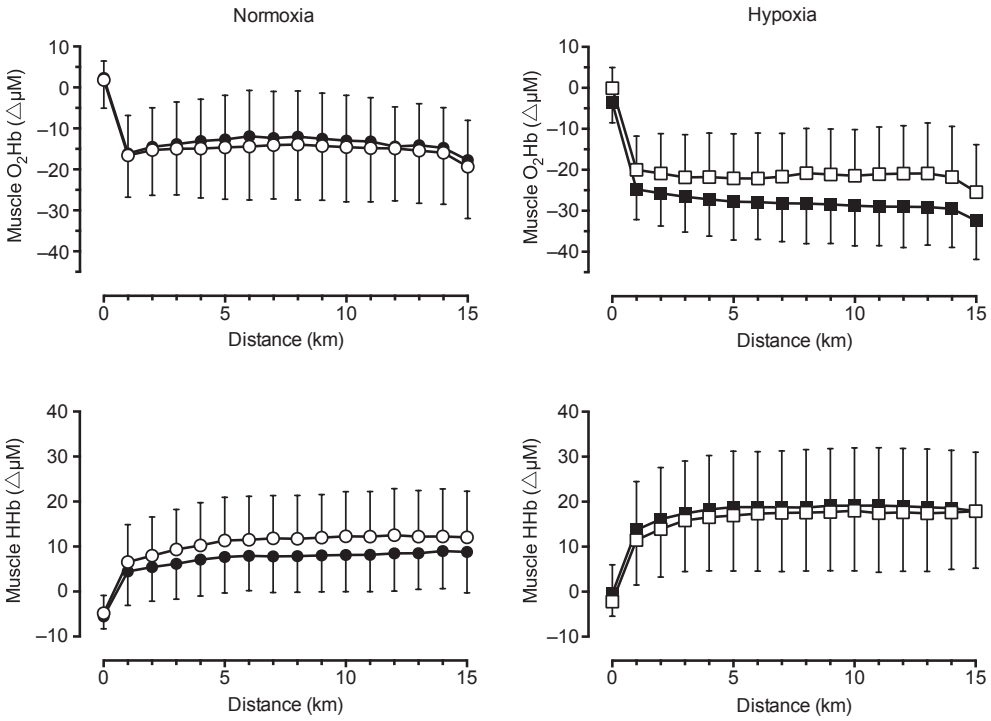

Figure 3. Effect of hypoxia and CO_2 clamping on muscle oxygenation during 15-km time trial cycling. Muscle O_2Hb and HHb are expressed at delta changes from normoxia (room air baseline). Left panels, group data in normoxia (mean ± SD); right panels, group data in hypoxia. ●, normoxia control; ○, normoxia CO_2 clamp; ■, hypoxia control; □, hypoxia CO_2 clamp.

Exercise in hypoxia

The detrimental effects of decreases in CaO_2 on endurance capacity of large muscle groups are well documented (Adams and Welch 1980; Koskolou and McKenzie 1994; Peltonen et al. 1995; Amann 2006; Amann et al. 2006, 2007). In this study, we found a 9–29% reduction in exercise performance during 15-km time trial cycling in severe hypoxia due to a decreased motor drive (~16% reduction in right vastus lateralis EMG RMS). In agreement with the results from Amann et al. (2006), we found that markedly different strategies were employed during time trial exercise in normoxia and hypoxia. Specifically, during the time trial in normoxia, the participants slightly increased their power output throughout the 15 km, reaching maximal power at the end of exercise in the form of an end spurt. In contrast, during the time trial in hypoxia, participants began cycling at a relatively high power output, slightly lowering work rate throughout the exercise test, only increasing power output again toward the end with a short sprint over the last 100 m. Interestingly, this progressive decline in central motor drive and power output occurred despite relatively stable CcO_2, ScO_2, and muscle [O_2Hb] during the time trial in hypoxia (Table 2 and Fig. 3). Meanwhile, both cerebral [O_2Hb] and cerebral DO_2 remained relatively stable despite parallel increases in MCAv and cerebral [HHb] (Fig. 2 and Table 2). As this decline in power coincided with an increase in perceived exertion, we tentatively interpret these findings as supporting the role of inhibitory feedback of muscle afferents in limiting central motor drive during exercise in severe hypoxia (Amann et al. 2006).

CO_2 clamping effects on CBF and prefrontal cerebral oxygenation

Our aim was to improve CBF during exercise by adding CO_2 to the inspirate, clamping $PETCO_2$ to 45 mmHg, preventing the normal hyperventilation-induced hypocapnia and associated cerebral vasoconstriction, while not influencing muscle oxygen supply. As expected, CO_2 clamping in normoxia prevented the development of hypocapnia during the second half of the time trial, thereby elevating CBF (Fig. 2). During CO_2 clamping in hypoxia, we were successful in elevating and maintaining $PETCO_2$ stable at levels similar to those in normoxia (Fig. 1), without any development of respiratory acidosis (Table 2). However, despite a relatively greater hypercapnic stimulus, we observed only modest elevations in

MCAv during exercise with CO_2 clamping in hypoxia (Fig. 2). In this study, MCAv increased despite relatively stable MAP, cardiac output (not reported), pH, and PcO_2 during exercise in hypoxia (Fig. 2, Table 2). Therefore, it seems unlikely that changes in perfusion pressure or circulating systemic stimuli (such as arterial hypoxemia) could account for the progressive rise in CBF during time trial cycling observed in hypoxia. Instead, we suspect that the increase in CBF is likely driven by other factors such as increased neural activity and cerebral metabolic demand associated with prolonged aerobic exercise in normobaric hypoxia.

Control of CBF during hypoxic exercise

The entire capillary network of the brain is covered by extensions of astrocytes, which constitutes the blood–brain barrier (Secher et al. 2007). As such, O_2 diffusion distance plays a vital role during increased neural activation and subsequent increased O_2 consumption. As there is no capillary recruitment in the brain, greater CBF is required to elevate the O_2 gradient (Secher et al. 2007). Acute hypoxic exposure at rest elevates right prefrontal cortex activity (Schneider and Strüder 2009), and enhances O_2 extraction in the brain (Ho et al. 2008). Meanwhile, studies of dynamically exercising humans have found that the increases in regional MCAv (and CBF) are driven by increased cortical activation of sensorimotor areas associated with neural input from the working limbs (Friedman et al. 1991, 1992; Jorgensen et al. 1992a). These observations support earlier findings in exercising dogs, of CBF redistribution to sensorimotor cerebral cortex, spinal cord, and cerebellum – primarily in cortical layers (Gross et al. 1980). Accordingly, we attribute the higher MCAv observed in this study to greater sensorimotor cortex activation associated with exercise in hypoxia. Despite a considerably lower power output, we observed higher rates of perceived effort during exercise in hypoxia when compared to normoxia, thereby confirming reports of a direct effect of hypoxia on effort perception during exercise in severe hypoxia (Amann et al. 2007). Taken together, we postulate that elevated cerebral metabolism associated with greater sensorimotor cortex activation – as indicated by higher perceived effort – could account for the elevated CBF observed during exercise in severe hypoxia.

Cerebral oxygenation, oxygen delivery, and performance

Mass cerebral oxygen delivery is the product of CBF and CaO_2. Therefore, it is important to consider the effect of CO_2 clamping on CaO_2. In this study, increased respiratory drive associated with CO_2 clamping elevated PcO_2 and ScO_2, which in term tended to elevate CcO_2 ($P = 0.089$, Table 2). Combined with modest elevations in MCAv during exercise (Fig. 2), we expected to see elevated mass O_2 delivery to the brain with CO_2 clamping in hypoxia. Instead, CO_2 clamping in hypoxia normalized the cerebral DO_2 to normoxic control values during time trial cycling (Table 2), which is supported by lower cerebral HHb and higher cerebral delta Hb (Fig. 2). Accordingly, we contend that we were successful in restoring cerebral DO_2 during the time trial exercise in hypoxia with our CO_2 clamping setup. Our data corroborate those of Subudhi et al. (2011), who found significant increases in cerebral oxygenation with CO_2 clamping during incremental exercise to capacity in hypoxia. We also found muscle oxygenation to be elevated with CO_2 clamping in hypoxia (Fig. 3) – presumably due to a higher SpO_2 associated with increased ventilation (Fig. 1). As the restoration of cerebral DO_2 with CO_2 clamping in our study was not accompanied by improvements in exercise time, motor drive or mean power output, it appears that cerebral deoxygenation associated with hyperventilation-induced hypocapnia cannot account for the impaired performance in hypoxia. Intriguingly, this lack of improvement was observed despite a tendency for improvement of muscle tissue oxygenation with CO_2 clamping in hypoxia. This lack of effect of increased cerebral O_2 delivery on performance may be attributed, at least in part, to increased cerebral nonoxidative glycolysis during exercise in hypoxia (Overgaard et al. 2012). Furthermore, it is important to acknowledge that changes in global cerebral DO_2 may not be representative of changes in local arterial and/or capillary P_{O_2} in the brain; therefore, it is possible that we were unable to restore oxygen diffusion drive to the mitochondria of especially active neuronal tissue with our CO_2 clamping setup.

Methodological considerations

Some technical considerations should be acknowledged when interpreting our data. Prefrontal lobe oxygenation was used to represent global changes in cerebrocortical oxygenation. Subudhi et al. (2009) have reported good correlations in oxygenation measurements between prefrontal, premotor, and motor cortices during maximal exercise. Therefore, we considered prefrontal oxygenation as an appropriate index of global changes in cerebrocortical oxygenation. Another important issue associated with NIRS is the influence of extracerebral and fat layers on the brain and muscle measurements, respectively (Cooper et al. 2010). Cerebral $[O_2Hb]$

measurements may be influenced by external carotid blood flow (Li et al. 2011) and forehead skin blood flow (Takahashi et al. 2011) during rest or a verbal fluency task, respectively. Nevertheless, Subudhi et al. (2011) reported significant elevations in cerebral [O_2Hb] and CBF with CO_2 clamping during exercise in normoxia and hypoxia. Likewise, we found cerebral [HHb] to be lower with CO_2 clamping, whereas cerebral [O_2Hb] tended to be elevated in the last 5 km of exercise during CO_2 clamping in normoxia and throughout exercise in hypoxia, coinciding with a higher MCAv (Fig. 2). Therefore, we contend that the NIRS methodology used in this study was sensitive enough to detect the changes in cerebral oxygenation associated with CO_2 clamping.

An important limitation of this study, as of many other studies, is the assumption that MCAv represents global CBF changes. Four lines of evidence support the use of MCAv as an index of global CBF during exercise: (i) MCAv reflects change in internal carotid blood flow during dynamic exercise in both normoxia (Sato et al. 2011) and hypoxia (Huang et al. 1991); (ii) increases in MCAv are matched by increases in cortical CBF during dynamic exercise (Jorgensen et al. 1992a,b); (iii) whereas cortical representation of leg muscle can be served from measuring blood flow velocity of the anterior cerebral artery (van der Zwan and Hillen 1991), cortical representation of muscle involved in cycling is served dominantly by MCA (Jorgensen et al. 1992a); (iv) the cross-sectional area of the MCA remains unchanged within a wide range of changes in $PETCO_2$ (Bradac et al. 1976; Giller et al. 1993; Valdueza et al. 1997; Serrador et al. 2000); and (v) the diameter of MCA, as measured using TCD, remains relatively unchanged at 5300 m (5.23 mm) compared to sea level (5.30 mm) (Wilson et al. 2011). Accordingly, we contend that MCAv is a reasonable index of changes in global CBF during heavy exercise in hypoxia. Nevertheless, we acknowledge that changes in CBF and cerebral metabolic activity during exercise and with hypoxia are not homogeneous. In this study, the assessment of CBF and cerebral tissue oxygenation was limited to the MCA and the left prefrontal cortex, respectively, which were taken as global indices. However, it is likely that certain brain regions are more metabolically active and others regions are less active during exercise in hypoxia.

Another limitation of this study is that we clamped end-tidal rather than arterial P_{CO_2} during exercise. As the end-tidal–arterial P_{CO_2} gradient (Robbins et al. 1990) varies with exercise (Jones et al. 1979; Liu et al. 1995), it is possible that we did not clamp arterial P_{CO_2} per se. In this study, we measured arterialized capillary blood samples, as a surrogate of arterial measurements, and were able to clamp and maintain capillary P_{CO_2} within 1 mmHg throughout exercise under both normoxic and hypoxic conditions. As Mollard et al. (2010) found good correlations between prewarmed capillary earlobe samples with radial artery measurements of P_{O_2} ($R^2 = 0.99$), P_{CO_2} ($R^2 = 0.86$), and SO_2 ($R^2 = 0.99$) during rest, submaximal, and near maximal exercise intensities, we are confident that our protocol was effective in clamping and maintaining arterial P_{CO_2}.

Finally, lack of power could potentially limit the interpretation of the tendency ($P = 0.108$) for performance to be improved with CO_2 clamping in hypoxia. However, this seems unlikely as power calculation using Gaussian approximation revealed a power of 0.95 for our post hoc *t*-test. Furthermore, given the results from the earlier studies using an incremental exercise paradigm (Subudhi et al. 2011; Siebenmann et al. 2012) and the rather small effect, if any, found in this study, the tested hypothesis should be refuted.

Conclusion

We were unable to improve exercise performance in severe hypoxia, despite a normalization of cerebral oxygen delivery to normoxia values and higher cerebral and muscle oxygenation. The hypothesis that the hyperventilation-induced hypocapnia and the subsequent drop in cerebral oxygenation are the cause of the reduced endurance exercise performance in hypoxia is thus refuted. Our data demonstrate that CBF is progressively elevated during time trial cycling in normobaric hypoxia, which appears to be independent of changes in perfusion pressure, cardiac output, or partial pressure of arterial CO_2 and O_2. We speculate that this increase in CBF during prolonged submaximal exercise in hypoxia may be due to either greater somatosensory input, increased motor drive output, or both.

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Author Contributions

J.F. contributed to the data collection, and led the data analysis, data interpretation, and writing of the manuscript. B.K. led the conception and designed the experiment, contributed in data collection, the interpretation of the data, and manuscript revision. N.B. contributed to the data collection, data interpretation, and manuscript revision. All authors approved the final version of this manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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Article seven

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Effect of inspired CO_2 on the ventilatory response to high intensity exercise

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ABSTRACT

We tested the hypothesis that preventing the poikilocapnic response to high intensity exercise would increase the ventilatory response to exercise. We measured ventilatory variables in 10 healthy men during incremental cycling with and without inspired CO_2 (randomised order). Inspired CO_2 elevated resting ventilation ($\dot{V}_E$), tidal volume (V_T), $P_{ET_{CO_2}}$ and $P_{ET_{O_2}}$ by 5 ± 5 L/min, 0.3 ± 0.2 L, 6 ± 3 mmHg and 11 ± 7 mmHg, respectively ($P < 0.05$); resting breathing frequency (f), expired CO_2 ($\dot{V}_{CO_2}$) and O_2 consumption ($\dot{V}_{O_2}$) remained similar ($P > 0.05$). During high intensity exercise, inspired CO_2 elevated $P_{ET_{CO_2}}$ by 7 ± 2, 10 ± 4 and 11 ± 4 mmHg at 80%, 90% and 100% $\dot{V}_{O_2\ max}$, respectively ($P < 0.01$), while $P_{ET_{O_2}}$ remained unchanged ($P > 0.05$). During high intensity exercise, inspired CO_2 elevated VT by 0.2 ± 0.3 L at 80%, 90% and 100% $\dot{V}_{O_2\ max}$, respectively ($P < 0.05$), while no differences were observed in $\dot{V}_E$, f, $\dot{V}_{O_2}$, or power output ($P > 0.05$). These data suggest a progressively diminishing role of CO_2 chemoreception in the control of ventilation during maximal incremental exercise.

1. Introduction

The regulation of exercise hyperpnoea has been extensively studied for more than a century but several aspects remain to be fully elucidated (see Flenley and Warren, 1983; Poon and Greene, 1985; Cunningham, 1987; Mateika and Duffin, 1995; Forster, 2000; Ward, 2000; Peronnet and Aguilaniu, 2006; Poon et al., 2007; Babb et al., 2010 for reviews). Among others, the role of chemoreception in the regulation of exercise hyperpnoea, especially during heavy exercise, remains controversial. Early animal studies found, using bilateral carotid denervation and altering cerebrospinal $[H^+]$, that exercise hyperpnoea would be independent of peripheral (Bisgard et al., 1982; Mitchell et al., 1984; Pan et al., 1986) and central (Smith et al., 1988) chemoreceptor input. On the other hand, Bisgard et al. (1982) found, in goats, that the peripheral chemoreceptors are nevertheless essential in modulating the neural drive for exercise hyperpnoea. Meanwhile, human studies reported either preserved (Lugliani et al., 1971; Wasserman et al., 1975) or blunted (Honda et al., 1979) ventilatory responses to moderate steady-state exercise in asthmatic patients with carotid body resection as compared to control subjects. Wasserman et al. (1975) also reported a lack of hyperventilation in response to metabolic acidosis during incremental exercise in patients with carotid resection, which led them to speculate that the peripheral chemoreceptors may be essential for ventilatory compensation of exercise-induced metabolic acidosis. However, these findings concerned asthmatic patients, either 3–9 yrs (Lugliani et al., 1971; Wasserman et al., 1975) or ~25 yrs (Honda et al., 1979) following bilateral or unilateral carotid body resection, and the role of chemoreception during exercise in healthy subjects remained to be better described. In intact humans, several studies investigated the effect of peripheral chemoreceptor inhibition by hyperoxia on ventilation ($\dot{V}_E$) during exercise below and above the respiratory compensation threshold (Dejours et al., 1958; Wasserman, 1976; Jeyaranjan et al., 1987; Miyamoto and Niizeki, 1995; Pianosi and Marchione, 1995; Kobayashi et al., 1996; St Croix et al., 1996). Those studies suggested that the peripheral chemoreceptors serve a minor role in the control of exercise hyperpnoea, contributing to approximately 10–25% of the overall $\dot{V}_E$ response. However, the role of CO_2 chemoception, which involves activation of both peripheral and central chemoreceptors, in the regulation of hyperpnoea during heavy and maximal exercise in humans remains to be investigated in greater detail.

Increasing the partial pressure of arterial CO_2 (Pa_{CO_2}) by inhalation of gas mixtures with a fixed increased $F_{I_{CO_2}}$ constitutes a stimulus for exercise hyperpnoea, particularly at low-to-moderate exercise intensities (Weil et al., 1972; Clark et al., 1980). Only few studies have examined the effect of inspired CO_2 on the ventilatory response to exercise. Most (Essfeld et al., 1990; St Croix et al., 1996; Hayashi et al., 2011), but not all (Olin et al., 2011) of these studies, only reported effects on $\dot{V}_E$ during steady-state exercise below the respiratory compensation threshold. Olin et al. (2011) did not study the ventilatory response to exercise but reported similar maximal $\dot{V}_E$ in two consecutive incremental exercise tests when end-tidal P_{CO_2} ($P_{ET_{CO_2}}$) was clamped at ~40 mmHg up to maximal exercise

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capacity in otherwise healthy, highly trained cyclists, suggesting that maximum aerobic exercise hyperpnoea may be independent of arterial P_{CO_2} tension. But their subjects reported increased respiratory effort to overcome an added inspiratory resistance from the breathing circuit during the $P_{ET_{CO_2}}$ condition compared to control, which may have limited the exercise performance and thus $\dot{V}_E$. In addition, the authors were unable to calculate O_2 uptake and expired CO_2 because of the particularities of their rebreathing circuit. As such, the effect of clamping of $P_{ET_{CO_2}}$ on $\dot{V}_E$ and other ventilatory variables during high intensity exercise remained to be described.

In the present study, we therefore examined the effect of inspired CO_2 on the ventilatory response to exercise during an incremental maximal exercise test. We tested the hypothesis that increasing $F_{I_{CO_2}}$ to prevent the normal decrease of $P_{ET_{CO_2}}$ above the respiratory compensation point (i.e., hypocapnia from exercise induced hyperventilation), would lead to increased $\dot{V}_E$ during incremental exercise, especially beyond the ventilatory threshold.

2. Methods

2.1.1. Participants

Ten healthy male subjects with a mean age of 24 ± 3 yr (mean ± SD), a body mass index of 22.8 ± 1.8 kg/m^2 and $\dot{V}_{O_2\ max}$ of 49.8 ± 5.5 ml/min/kg participated in this study. Participants were non-smokers, had no previous history of cardiovascular, cerebrovascular, or respiratory disease and were not taking any medication. All the participants partook in regular exercise, but none were especially trained. The study was approved by the Research Ethical Committee of the University Hospitals of Geneva and conformed to the standards set by the *Declaration of Helsinki*. All participants were informed regarding the purposes and procedures of this study, and informed consent was given prior to participation.

2.2. Experimental design

The participants visited the laboratory on three occasions. After a full familiarisation with the experimental procedures outlined below (visit one), participants underwent two experimental trials, control and inspired CO_2, in a randomised order. Before each experimental session, the participants were asked to abstain from caffeine for 12 h and heavy exercise and alcohol for 24 h. Each experimental testing session consisted of a 10 min instrumentation followed by a four min resting period data collection with the participant seated on an electronically braked cycle ergometer (Ergoselect 100, Ergoline GmbH, Bitz, Germany). At the end of the resting period, the participant was instructed to begin cycling at 0 W at a pedalling rate of 70 rpm. The work rate was then increased by 0.5 W every second until the participant reached exhaustion. Throughout each experimental session, the participants breathed through a leak-free respiratory mask (Hans-Rudolph 8980, Kansas City, MO, USA) attached to a low resistance one-way non-rebreathing valve (Hans-Rudolph 2700, Kansas City, MO, USA). During the control trial, participants breathed normal room air and $P_{ET_{CO_2}}$ was allowed to fluctuate freely and drop during the latter part of the trial leading to exercise hyperventilation-induced hypocapnia (Fig. 1). During the inspired CO_2 trial, the participants' $P_{ET_{CO_2}}$ during rest and throughout the exercise was controlled to values of ∼44 and ∼50 mmHg, at rest and during exercise, respectively, thus preventing the normal hyperventilation-induced hypocapnia at high intensity exercise (Fig. 1). We did not increase $P_{ET_{CO_2}}$ to 50 mmHg at rest to prevent an apparatus related overshoot at the beginning of the exercise protocol and aimed at keeping $P_{ET_{CO_2}}$ close

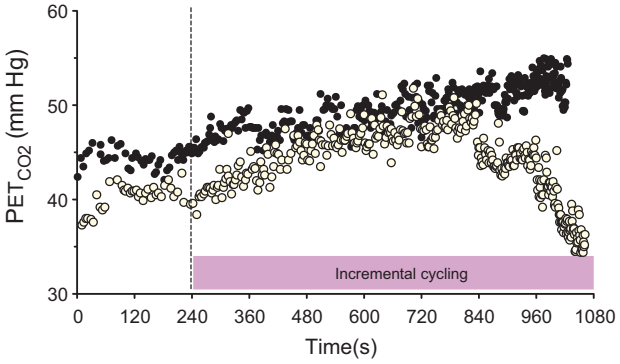

Fig. 1. Typical individual's end-tidal P_{CO_2} traces during control and inspired CO_2 at rest and during incremental cycling to exhaustion. (○) Control; (●) inspired CO_2.

to the maximum value observed during a control run, leaving it drift up slightly throughout the exercise test since end-tidal arterial differences increase with exercise intensity (Fig. 1) (Jones et al., 1979). The inspired CO_2 was achieved using a modified gas mixing system (Altitrainer, SMTec, Nyon, Switzerland), which was attached to the inspiratory valve of the facemask using a piece of large-bore low resistance tubing. The device consists of a reservoir in which air and experimental gases are mixed and from which the subject inspires. This setup enabled for bleeding additional CO_2 and O_2 into the inspired gas mixture, thereby increasing the $F_{I_{CO_2}}$ while keeping $F_{I_{CO_2}}$ constant. Under feedback of on-screen $P_{ET_{CO_2}}$ (Datex infrared CO_2 analyser, Finland), $F_{I_{CO_2}}$ was continuously adapted as to keep $P_{ET_{CO_2}}$ to its target value. The subjects breathed through the same low resistance circuit in the control and inspired CO_2 conditions and were kept unaware to what gas mixture they were breathing.

2.3. Measurements

Gas exchange was monitored on a breath-by-breath basis (Medgraphics CPX, Zürich, Switzerland) measuring flow at the mouth with a Pitot tube and the fractions of inspired and expired O_2 and CO_2 with fast responding gas analysers (infrared and paramagnetic) integrated in the system. $\dot{V}_E$ and its components tidal volume (V_T) and breathing frequency (f) were calculated from the flow signal and expressed in BTPS and per min, respectively. The partial pressure of end-tidal O_2 ($P_{ET_{CO_2}}$) and CO_2 ($P_{ET_{CO_2}}$), O_2 consumption ($\dot{V}_{O_2}$), expired CO_2 ($\dot{V}_{CO_2}$) and respiratory duty cycle (T_i/T_{tot}) were calculated by the gas analysis system; alveolar ventilation ($\dot{V}_A$) was estimated using the alveolar gas equation. Prior to each experimental session the system was calibrated using a 3-L syringe (Medikro M9474, Medikro Oy, Finland) and gas mixtures of known concentrations of O_2 and CO_2. Heart rate was measured using a thoracic belt and a watch (S610, Polar electro, Finland).

During exercise, the participants were asked to score their perceived sensation of respiratory and leg muscle exertion on the 0–10 Borg scale every minute (Borg, 1982).

2.4. Data and statistical analysis

Resting values were obtained by averaging the data obtained in the last minute of the four min resting period. During exercise, all the $\dot{V}_{O_2}$ data was normalised to the maximum value obtained for the control trial and expressed as percentages of maximal exercise aerobic capacity. Subsequently, we calculated 10–15 s averages at 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% $\dot{V}_{O_2\ max}$ for all variables during the incremental exercise. All the ventilatory variables were plotted against percentage $\dot{V}_{O_2\ max}$ with the exception

Table 1
Respiratory variables during rest and exercise under control and inspired CO_2 conditions.

Exercise	$P_{ET_{CO_2}}$ (mmHg)		$P_{ET_{O_2}}$ (mmHg)		$\dot{V}_E$ (L/min)		$\dot{V}_A$ (L/min)		f (breaths/min)		V_T (L)	
	Control	Inspired CO_2	Control	Inspired CO_2	Control	Inspired CO_2	Control	Inspired CO_2	Control	Inspired CO_2	Control	Inspired CO_2
Baseline	37 ± 2	44 ± 2*	99 ± 4	110 ± 6	11.7 ± 2.9	16.2 ± 6.2*	8.2 ± 2.2	12.6 ± 5.5*	13 ± 3	13 ± 4	0.9 ± 0.2	1.2 ± 0.2*
20%	42 ± 2	48 ± 3*	90 ± 6	114 ± 6*	18.4 ± 3.0	34.2 ± 9.2*	13.8 ± 2.1	30.8 ± 8*	18 ± 4	20 ± 4	1.1 ± 0.2	1.7 ± 0.4*
30%	43 ± 3	49 ± 4*	88 ± 5	107 ± 6*	25.9 ± 4.7	38.2 ± 7.8*	20.9 ± 3.5	33.9 ± 7.9*	18 ± 5	21 ± 4	1.5 ± 0.3	1.9 ± 0.3*
40%	46 ± 2	49 ± 5*	88 ± 4	101 ± 8*	33.8 ± 7.6	43.3 ± 8.6*	28.1 ± 6.3	38.0 ± 8.6*	20 ± 4	22 ± 4	1.7 ± 0.3	2.0 ± 0.3
50%	44 ± 2	49 ± 5	89 ± 4	98 ± 7*	42.8 ± 7.3	51.8 ± 9.8*	36.6 ± 6.4	46.1 ± 9.1*	21 ± 4	23 ± 5	2.0 ± 0.3	2.2 ± 0.2*
60%	47 ± 3	51 ± 5*	92 ± 6	98 ± 8*	54.4 ± 11.8	62.7 ± 16.4*	47.7 ± 10.6	56.8 ± 15.3*	24 ± 5	26 ± 7	2.3 ± 0.2	2.4 ± 0.2
70%	48 ± 4	52 ± 5*	95 ± 6	99 ± 8*	69.7 ± 16.2	78.3 ± 20.8	61.3 ± 14.7	61.3 ± 14.7*	29 ± 7	30 ± 7	2.4 ± 0.3	2.6 ± 0.3*
80%	47 ± 5	53 ± 5*	98 ± 7	101 ± 10	87.6 ± 20.4	94.3 ± 27.5	77.2 ± 18.8	77.2 ± 18.8	35 ± 8	34 ± 9	2.5 ± 0.3	2.7 ± 0.4*
90%	46 ± 7	53 ± 6*	102 ± 8	104 ± 10	106.0 ± 27.7	112.8 ± 30.5	93.7 ± 25.7	106.5 ± 32.5*	41 ± 8	41 ± 10	2.6 ± 0.3	2.8 ± 0.4*
100%	42 ± 8	53 ± 5*	104 ± 6	106 ± 9	117.1 ± 29.2	127.0 ± 35.6	103.7 ± 26.8	121.0 ± 38.2*	47 ± 9	46 ± 11	2.5 ± 0.3	2.7 ± 0.2*
End	40 ± 8	54 ± 6*	109 ± 6	106 ± 5*	116.2 ± 26.1	129.4 ± 33.0*	101.0 ± 22.6	122.9 ± 33.3*	50 ± 9	52 ± 12	2.3 ± 0.2	2.5 ± 0.3*

Values are mean ± SD.
* Different from Control ($P < 0.05$).

of $\dot{V}_E$ which was plotted against mechanical power output on the ergometer for the analysis of $\dot{V}_E$ slopes.

2.4.1. Respiratory compensation threshold and ventilatory response to exercise

The respiratory compensation threshold was obtained using the υ-slope method previously described by Beaver et al. (1986). The assessment of the ventilatory response to exercise (i.e., the slope of $\dot{V}_E$ rise during exercise) has been previously described (Babb, 1997a, 1997b) and will be outlined here. In brief, breath-by-breath $\dot{V}_E$ points were plotted against mechanical power output and the least squares regression was used to determine the ventilatory response to exercise above and below the respiratory compensation threshold (Babb, 1997a, 1997b).

Comparison of resting baseline variables as well as at the end of incremental exercise between the control and inspired CO_2 conditions was done using the paired t-test. Comparison of the ventilatory variables between control and inspired CO_2 conditions during incremental exercise were assessed using repeated-measures ANOVA with α-level of $P < 0.05$ (PASW Statistics 18, Chicago, USA). Post hoc analysis (by paired t-test) of significant results from the ANOVAs (either condition or exercise step effects) was performed with Bonferroni correction, to isolate the effects of condition on the dependent measures within participants.

3. Results

All 10 participants completed the control and the inspired CO_2 trials. There were no differences in $\dot{V}_{O_2\ max}$ [49.7 ± 4.3 (mean ± SD) vs. 49.8 ± 5.5 mL/min/kg] or maximal power output (327 ± 54 vs. 331 ± 49 W) between inspired CO_2 and control conditions ($P > 0.05$). The subjects did not report any side-effect from the inspired CO_2 procedure such as headache or dyspnoea.

3.1. Effect of inspired CO_2 on resting variables

During resting, inspired CO_2 elevated $P_{ET_{CO_2}}$ and $P_{ET_{O_2}}$ ($P < 0.05$ vs. control; Table 1). $P_{ET_{CO_2}}$ clamping elevated resting $\dot{V}_E$, $\dot{V}_A$ and V_T ($P < 0.05$), while f remained similar ($P > 0.05$ vs. control; Table 1). Inspired CO_2 also elevated T_i/T_{tot}, $\dot{V}_E/\dot{V}_{CO_2}$ and $\dot{V}_E/\dot{V}_{O_2}$, while $\dot{V}_{CO_2}$ was lowered ($P < 0.05$ vs. control; Table 1). No differences were observed in resting $\dot{V}_{O_2}$ between the $P_{ET_{CO_2}}$ clamped and control trial ($P > 0.05$; Table 1).

3.2. Effect of inspired CO_2 on ventilatory variables during incremental exercise

During incremental exercise, inspired CO_2 elevated $P_{ET_{CO_2}}$ at 20%, 30%, 40%, 60%, 70%, 80%, 90% and 100% $\dot{V}_{O_2\ max}$ ($P < 0.05$ vs. control), while no difference was observed at 50% $\dot{V}_{O_2\ max}$ ($P > 0.05$; Table 2). Inspired CO_2 elevated $P_{ET_{O_2}}$ at 20%, 30%, 40%, 50%, 60% and 70% $\dot{V}_{O_2\ max}$ ($P < 0.05$ vs. control); no difference was observed between 80% and 100% $\dot{V}_{O_2\ max}$ ($P < 0.05$; Table 1). During the inspired CO_2 trial, $\dot{V}_E$ was higher at 20%, 30%, 40%, 50% and 60% $\dot{V}_{O_2\ max}$ ($P < 0.05$ vs. control), while no significant differences were observed between 70% and 100% $\dot{V}_{O_2\ max}$ ($P > 0.05$; Table 1). Inspired CO_2 also elevated calculated $\dot{V}_A$ at all exercise intensities except at 80% $\dot{V}_{O_2\ max}$ ($P < 0.05$ vs. control; Table 1). Tidal volume was elevated at 20%, 30%, 50%, 70%, 80%, 90% and 100% $\dot{V}_{O_2\ max}$ ($P < 0.05$ vs.

Table 2
Metabolic and respiratory variables during rest and exercise under control and inspired CO_2 conditions.

Exercise	$\dot{V}_{CO_2}$ (mL/min)		$\dot{V}_{O_2}$ (mL/min)		$\dot{V}_E/\dot{V}_{CO_2}$		$\dot{V}_E/\dot{V}_{O_2}$		T_i/T_{tot}	
	Control	Inspired CO_2	Control	Inspired CO_2	Control	Inspired CO_2	Control	Inspired CO_2	Control	Inspired CO_2
Baseline	351 ± 84	283 ± 70*	368 ± 66	392 ± 80	33 ± 3	57 ± 11*	31 ± 4	41 ± 11*	0.28 ± 0.04	0.33 ± 05*
20%	646 ± 86	617 ± 136	767 ± 92	759 ± 100	29 ± 3	55 ± 9*	24 ± 3	45 ± 10*	0.34 ± 0.04	0.40 ± 0.05
30%	991 ± 132	927 ± 212	1181 ± 149	1138 ± 161	26 ± 2	42 ± 11*	22 ± 3	34 ± 5*	0.39 ± 0.03	0.41 ± 0.03
40%	1386 ± 280	1379 ± 283	1561 ± 233	1509 ± 220	24 ± 2	32 ± 6*	22 ± 3	29 ± 3*	0.40 ± 0.03	0.41 ± 0.05
50%	1856 ± 287	1938 ± 352	1936 ± 265	1882 ± 264	23 ± 2	27 ± 4*	22 ± 2	28 ± 4*	0.41 ± 0.03	0.43 ± 0.02
60%	2373 ± 412	2411 ± 451	2287 ± 304	2250 ± 284	23 ± 2	26 ± 5*	24 ± 3	28 ± 6*	0.43 ± 0.04	0.44 ± 0.03
70%	2983 ± 516	2921 ± 389	2663 ± 357	2626 ± 339	23 ± 2	27 ± 5*	26 ± 3	30 ± 7*	0.44 ± 0.04	0.45 ± 0.04
80%	3603 ± 554	3447 ± 599	3008 ± 390	3010 ± 425	24 ± 3	27 ± 5*	29 ± 5	31 ± 8	0.44 ± 0.04	0.46 ± 0.04
90%	4103 ± 616	3984 ± 645	3271 ± 473	3256 ± 459	26 ± 4	28 ± 6*	32 ± 6	35 ± 7	0.44 ± 0.04	0.44 ± 0.03
100%	4391 ± 581	4307 ± 589	3434 ± 443	3423 ± 516	26 ± 4	29 ± 6*	34 ± 6	37 ± 8*	0.44 ± 0.04	0.44 ± 0.04
End	4172 ± 437	4308 ± 687	3196 ± 403	3228 ± 486	28 ± 4	30 ± 5*	36 ± 5	40 ± 7*	0.43 ± 0.03	0.43 ± 0.04

Values are mean ± SD.
* Different from Control ($P < 0.05$).

control), while no difference was observed at 40% $\dot{V}_{O_2\ max}$ during the inspired CO_2 trial ($P > 0.05$; Table 1).

Inspired CO_2 elevated $\dot{V}_E/\dot{V}_{CO_2}$ at all exercise intensities ($P < 0.05$ vs. control; Table 2). Likewise, $\dot{V}_E/\dot{V}_{O_2}$ was higher during the $P_{ET_{CO_2}}$ clamp trial at 20%, 30%, 40%, 50%, 60%, 70% and 100% $\dot{V}_{O_2\ max}$ ($P < 0.05$ vs. control), but not at 90% $\dot{V}_{O_2\ max}$ ($P > 0.05$; Table 2). No differences were observed in f, $\dot{V}_{CO_2}$, $\dot{V}_{O_2}$ and T_i/T_{tot} between $P_{ET_{CO_2}}$ clamp and control trials ($P > 0.05$; Table 2).

3.3. Effect of inspired CO_2 on ventilatory variables at end of incremental exercise

Inspired CO_2 elevated $P_{ET_{CO_2}}$, $P_{ET_{O_2}}$, $\dot{V}_E$, $\dot{V}_A$, $\dot{V}_T$ $\dot{V}_E/\dot{V}_{CO_2}$ and $\dot{V}_E/\dot{V}_{O_2}$ at the end of incremental exercise ($P < 0.05$ vs. control), while no differences were observed in any other variables ($P > 0.05$ vs. control; Tables 1 and 2).

3.4. Respiratory compensation threshold and ventilatory response to exercise

Inspired CO_2 lowered the v-slope estimate of respiratory compensation threshold by 43 ± 38 W (200 ± 37 vs. 242 ± 43 W; $P < 0.05$ vs. control; Fig. 3). The ventilatory response to exercise below respiratory compensation threshold was lower with inspired CO_2 (0.15 ± 0.03 vs. 0.22 ± 0.04 L/min/W; $P < 0.05$ vs. control; Fig. 3). In contrast, the ventilatory response to high intensity exercise (i.e., above respiratory compensation threshold) was the same in both conditions ($P > 0.05$; Fig. 3).

3.5. Heart rate and sensation of exertion at maximal exercise

No difference was observed in the maximal HR (185 ± 8 vs. 185 ± 7 b/min; $P < 0.05$) between inspired CO_2 and control conditions. Likewise, no differences were observed in the sensation of respiratory (8.7 ± 1.1 vs. 8.3 ± 1.5) or leg exertion (9.7 ± 0.5 vs. 9.4 ± 1.1; $P < 0.05$ vs. control).

4. Discussion

We tested the hypothesis that preventing the usual exercise hyperventilation-induced hypocapnia above the respiratory compensation point during incremental exercise until exhaustion, by increasing $F_{I_{CO_2}}$ in order to elevate $P_{ET_{CO_2}}$ above the highest level observed during an incremental exercise test in control conditions, would lead to an elevated ventilatory response. We found that inspired CO_2 augmented $\dot{V}_E$ during rest, as expected, and also at low and moderate exercise intensities, and tended to increase $\dot{V}_E$ at maximal exercise (Table 1). But the main finding of the study was that despite these effects, inspired CO_2 did not augment ventilation during high intensity exercise (Fig. 3). We furthermore found inspired CO_2 *reduced* the respiratory compensation threshold during incremental exercise (Fig. 3). These findings indicate that above the respiratory compensation threshold CO_2 chemoreception does not increase $\dot{V}_E$ beyond an offset reached below the threshold.

4.1. Limitations

Although the present study provides the opportunity to examine the effect of inspired CO_2 on the ventilatory response during incremental exercise, there are limitations that should be taken into account when interpreting our findings. Firstly, we did not directly measure gases nor lactate concentration in arterial blood. It would have been useful to have arterial P_{CO_2}, P_{O_2}, pH, [HCO_3^-], [La^-] and [K^+] data to strengthen our results. As such, we do not have direct evidence that our intervention led to increases in both Pa_{CO_2} and [H^+]. Nevertheless, given the very large differences in $P_{ET_{CO_2}}$ between the two conditions, especially during high exercise intensities (Fig. 2), it seems very likely that we were able to elevate the body's CO_2 stores (i.e., central chemoreceptor P_{CO_2}) compared to control and exacerbate metabolic acidosis during incremental exercise with our inspired CO_2 protocol, thus potentially augmenting both central and peripheral chemoreceptor activation. In favour of that contention, in the study of Clark et al. (1980), the condition of inspired P_{CO_2} of 20 mmHg led to the stabilisation of Pa_{CO_2} towards peak exercise to similar levels as we found and was accompanied by a drop in arterial pH from 7.32 in the control condition to 7.24 in the inspired CO_2 condition.

We opted to keep $F_{I_{O_2}}$ constant throughout the protocol and did not attempt to clamp $P_{ET_{O_2}}$. Since during high intensity exercise $P_{ET_{O_2}}$ was not different between the trials (Fig. 2), it seems unlikely that this will have influenced our main finding of unaltered ventilation with inspired CO_2. We are aware of other setups (Tansley et al., 1997; Koehle et al., 2009), which allow clamping of both $P_{A_{CO_2}}$ and

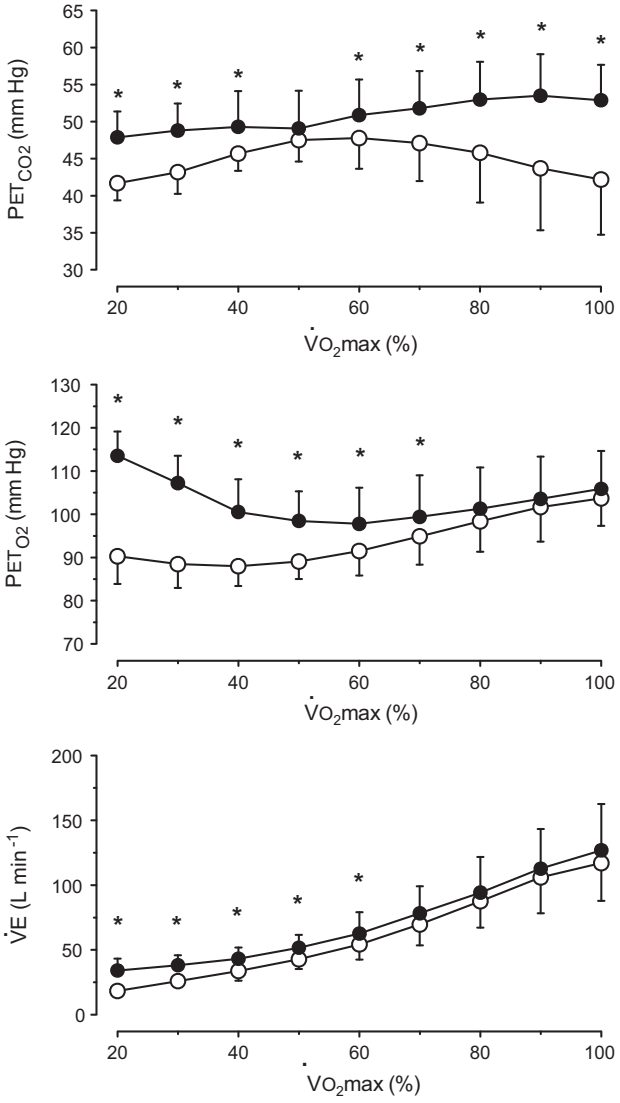

Fig. 2. Effect of inspired CO_2 on group end-tidal P_{CO_2}, end-tidal PO_2 and ventilation during incremental cycling to exhaustion. (○) Control; (●) inspired CO_2. Inspired CO_2 elevated $P_{ET_{CO_2}}$ during all exercise intensities except 50% $\dot{V}_{O_2\ max}$. As a result, elevated $P_{ET_{O_2}}$ and $\dot{V}_E$ at the lower to moderate exercise intensities and tended to elevate $\dot{V}_E$ at maximal exercise. *Different from Control ($P < 0.05$).

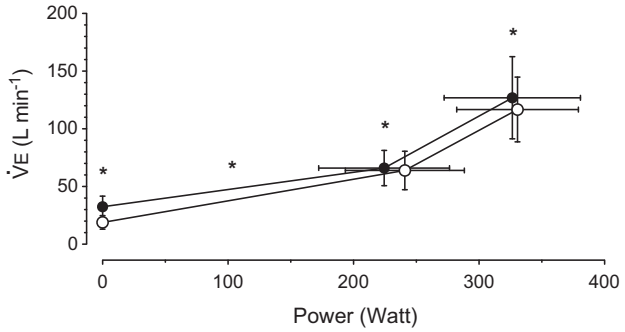

Fig. 3. Effect of inspired CO_2 on the respiratory compensation threshold and ventilatory response to exercise during incremental cycling to exhaustion. (○) Control; (●) inspired CO_2. Inspired CO_2 blunted the ventilatory response to exercise (slope) below the respiratory compensation threshold, while no difference was observed above the threshold. Inspired CO_2 also lowered the respiratory compensation threshold and elevated $\dot{V}_E$ during both the beginning and the end of exercise. *Different from control ($P < 0.05$).

$P_{A_{O_2}}$, but these have so far not been able to clamp at the very high rates of ventilation as observed during maximal exercise, which was the reason for the use of our setup.

4.2. Chemoreception of CO_2 and exercise hyperpnoea

Several studies have assessed the effect of chemoreceptor stimulation using inspired CO_2 on the ventilatory response to incremental exercise. In young sedentary subjects, increased inspired P_{CO_2} ($F_{I_{CO_2}} = 0.03$) has been shown to augment ventilatory response to exercise (i.e., *greater* rise in $\dot{V}_E$) below the respiratory compensation threshold (Babb, 1997a). Those authors reported that hypercapnia caused an offset of the ventilatory response to exercise to a higher value without any change in slope during exercise above the threshold – presumably due to an increasing influence of mechanical ventilatory constraints at high $\dot{V}_E$ levels (Babb, 1997a). Since they found a higher maximal $\dot{V}_E$ with hypercapnia compared to room-air breathing, they concluded that during near-maximal and maximal exercise in hypercapnia, the respiratory system, with regard to the expiratory airflow in particular, was not limiting. Several studies have examined the effect of $P_{ET_{CO_2}}$ clamping on the ventilatory response to exercise (Essfeld et al., 1990; St Croix et al., 1996; Hayashi et al., 2011; Olin et al., 2011), but these studies focused primarily on the effect of inspired CO_2 $P_{ET_{CO_2}}$ clamping during low and moderate intensity steady-state exercise, with the exception of Olin et al. (2011) who only reported maximal ventilation.

In contrast to our starting hypothesis, inspired CO_2 did not augment $\dot{V}_E$ during high intensity exercise in the present study (Fig. 2). Nevertheless, we found a tendency for $\dot{V}_E$ to be higher with inspired CO_2 at 100% $\dot{V}_{O_2\,max}$ (Fig. 2), while this difference reached significance at the end of exercise (Table 1). We observed a blunted $\dot{V}_E$ response to incremental exercise (i.e., a slower rise in $\dot{V}_E$) at lower exercise intensities with inspired CO_2 (Fig. 3). This attenuated $\dot{V}_E$ increase with inspired CO_2 was abolished at the respiratory compensation threshold (Fig. 3), whereby the slope of $\dot{V}_E$ rise became identical to control condition above the threshold. We attribute the apparent blunting of the ventilatory response to exercise below the compensatory threshold to the lack of rise in $P_{ET_{CO_2}}$ during the low and moderate exercise intensities with inspired CO_2 (Fig. 1). As a result, the difference in $P_{ET_{CO_2}}$ between the two conditions gradually decreased with increasing workload (Panel 3, Fig. 2). Meanwhile, since $\dot{V}_E$ was higher at the end of exercise with inspired CO_2 despite a lack of difference in the slope of $\dot{V}_E$ rise, it appears that increased CO_2 loading resulted in a fixed offset of the ventilatory response to higher values during heavy exercise. Our data agrees with those by Wasserman et al. (2011), which showed a dissociation between arterial [H^+] and ventilation during exercise above the respiratory compensation threshold. Accordingly, our findings do not appear to support the role of CO_2 chemoreception in the control of exercise hyperpnoea during high intensity, sub-maximal exercise.

An unexpected finding in the present study was a lowered respiratory compensation threshold with inspired CO_2 (Fig. 3). We speculate that this reduction could be attributed to either: (i) greater activation of the peripheral and central chemoreceptors (Takano, 2000); increased intramuscular metabo-receptor activation (Williamson et al., 1993; Smith et al., 1999); or (ii) altered ventilatory strategy associated with greater ventilation at low exercise intensities. Regardless of the mechanism(s), our data demonstrates inspired CO_2 leads to early onset of the respiratory compensation threshold.

4.3. Hypercapnia and perceived exertion

During exercise above the respiratory compensation threshold, cerebral blood flow decreases towards baseline because of the lower Pa_{CO_2} from a hyperventilation-induced cerebral vasoconstriction (Madsen et al., 1993; Ide et al., 1999; Olin et al., 2010). Cerebral blood flow thus decreases during heavy exercise despite an increased in cerebral demand and it is thought that this may accelerate, in some conditions, the advent of central fatigue (Rasmussen et al., 2010). As an auxiliary hypothesis of our study we therefore expected, by avoiding the hyperventilation induced drop in Pa_{CO_2}, to potentially modify the sensation of fatigue and exertion. In our set-up, such an effect seems not to have played any role since maximum exercise capacity, heart rate and the levels of perceived legs and respiratory exertion were the same between conditions. In contrast, Olin et al. (2010) reported ~6% reduction in maximal exercise capacity with $P_{ET_{CO_2}}$ clamping compared to control condition despite higher cerebral blood flow. However, this reduction in exercise capacity may have been due to the elevated respiratory resistance and dyspnoea associated with their particular rebreathing setup. It remains open if inspired CO_2 on cerebral blood flow would play a role in conditions of hypoxia, or during other types of exercise challenges like time-trials (Subudhi et al., 2008; Rasmussen et al., 2010).

To conclude, we demonstrated that elevating end-tidal P_{CO_2} during incremental exercise does not alter ventilation and respiratory rate beyond the ventilatory threshold. Our data add to the evidence against the classical concept of chemoreception of CO_2/pH in the regulation of breathing during high intensity exercise and highlights the need to develop better models to describe the ventilatory response to exercise, especially during high intensity exercise.

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